Neurodegeneration and microvascular dysfunction

Neurodegeneration and microvascular dysfunction: causes and consequences

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Neurodegeneration and microvascular dysfunction: causes and consequences

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CHAPTER 1

General introduction

1. Introduction

There is an imperative for novel strategies to in an early-stage prevent of major clinical diseases, such as dementia,[1] late-life depression,[2] and diabetic retinopathy.[3] Importantly, these diseases can have debilitating effects on quality of life for patients and their family; are associated a high societal burden in terms of health costs; and an epidemic increase in the number of patients with these diseases is expected in the next 25 years.[1-4] Epidemiologically, up to 1/3 and 1-7 women and men, respectively, are expected to develop dementia in their lifetime[5] (in the Netherlands in 2021 currently N=290,000 individuals suffer from dementia); up to 1/9 individuals is at risk for a lifetime episode of depressive symptoms;[6] and up to 1/2 individuals with diabetes, currently approximately 1 million individuals in The Netherlands, is expected to develop any sign of diabetic retinopathy during their life time (i.e. ~500,000 individuals).[3] Additionally, individuals with diabetes have an increased risk of developing dementia and late-life depression.[7,8]

Early prevention of major clinical disease may be possible via prevention of early changes which precede clinical symptoms of such disease. Biologically, dementia,[1] late-life depression,[2] and diabetic retinopathy,[3] are thought to be major clinical diseases that are (in part) of neuronal and microvascular origin. Mechanistically, increasing deterioration of neuronal and microvascular structures over time is thought to lead to, respectively, neurodegeneration and microvascular dysfunction, which can lead to impaired function of the brain and retina and ultimately the onset of clinical symptoms of major clinical disease.[9] Therefore, as postulated in the 'ticking clock hypothesis', there may be a window of opportunity (i.e. a new horizon) to prevent and/or slow the onset of major clinical disease via early prevention of neurodegeneration and microvascular dysfunction.[3,10-13]

However, the existing literature on early neurodegenerative changes and microvascular dysfunction has important knowledge gaps.[11,14]Specifically, two matters require further study. First, a better understanding is required of how potentially modifiable cardiovascular risk factors and lifestyle factors are associated with early neurodegenerative changes and microvascular dysfunction.[12,15] Modifiable cardiovascular risk factors and lifestyle factors may be potential early treatment targets.[3,10-13] Second, more knowledge is required on how early signs of neurodegenerative changes and microvascular dysfunction are associated with symptoms of (early-stage) major clinical disease.[11,12,15,16] Potentially, indices of early neurodegenerative changes and microvascular dysfunction may be biomarkers for the early identification of individuals at risk for major clinical disease and/or for the monitoring of early-stage disease progression.[11,12,16] Additionally, few data are available on whether the effects of potentially modifiable cardiovascular risk factors

and lifestyle factors on neuronal and microvascular structures differs by glucose metabolism status (i.e. between individuals with and without prediabetes or type 2 diabetes) or by sex (i.e. between men and women).[11,17] Possibly individuals with, versus without, prediabetes or type 2 diabetes may be more prone to neurodegenerative changes and microvascular dysfunction; and therefore the effects of certain cardiovascular and lifestyle factors on neuronal and microvascular structures may be stronger in individuals with, versus without, prediabetes or type 2 diabetes.[11] In addition, sex-differences may exist.[17,18]

Due to the recent development of novel techniques, which can non-invasively image early neuronal and microvascular changes, an opportunity has been created to better understand the early pathobiology of disease of neuronal and microvascular origin.[16,19] With novel techniques early neurodegenerative changes and microvascular dysfunction can be non-invasively assessed in various organ systems, including in the brain, the retina, the kidney, the skin, and in the blood.[16,19] Of these various organ systems in particular the retina is of interest, as in the retina neurodegenerative changes as well as microvascular dysfunction can be assessed; and these can be quantified with high (i.e. up to semi-histological) precision.[16] Additionally, retinal indices of neurodegenerative changes and microvascular dysfunction may be promising biomarkers for use in the clinic as retinal imaging has a number of practical advantages over imaging of other organs, e.g. retinal imaging is relatively fast and cheap.[16]

Hence, the main aims of this thesis are 1) to investigate whether potentially modifiable cardiovascular and lifestyle factors are determinants of early neurodegenerative changes and microvascular dysfunction; and 2) to investigate how early neurodegenerative changes and microvascular dysfunction are associated with (early-stage) symptoms of major clinical disease, which is of neuronal and microvascular origin. Additionally, the secondary aim of this thesis is investigate whether the strength of associations under study differs by glucose metabolism status or sex.

2. Background - key concepts

We provide background information on certain key concepts which underlie the research questions that are addressed in this thesis. First, we define neuronal tissue, the microvasculature, and the neurovascular coupling unit (section 2.1). Second, we discuss the biological rationale for the investigation of neurodegenerative changes and microvascular dysfunction in the early pathobiology of clinical disease and the presumed pathobiology of neurodegenerative changes and microvascular dysfunction (section 2.2). Third, we discuss the biological rationale for investigating how retinal neurodegenerative changes and microvascular dysfunction are associated with brain

disease (i.e. the biological rationale for the concept 'the retina as a window in the brain'; section 2.3).

2.1. Defining neuronal tissue, the microvasculature, and the neurovascular coupling unit

2.1.1. Neuronal tissue

The brain and the retina comprise part of the central nervous system, which serves to sense and integrate information, e.g. visual information, and execute certain functions, e.g. to regulate memory and mood.[20] Globally, the central nervous system consists of trillions of cells that are organized as localized collections of neuronal cell bodies (nuclei) and are connected via tracti.[20]

At a cellular level, the central nervous system can generally be divided into two main cell types, i.e. cells that serve to transmit information (i.e. neurons, e.g. ganglion cells) and cells that serve to support neuronal cells (i.e. glial cells, e.g. astrocytes).[20] Numerically, in the brain the ratio between neurons and glial cells is approximately 1:10.[20,21]

A neuron consists of a soma (i.e. cell body), multiple dendrites (most neurons), and an axon.[20] Mechanistically, dendrites transfer action potentials from synapses to the soma and, depending on the amount and strength of incoming action potentials via dendrites, the axonal hillock transmits an action potential into the axon and via this axon to the next synapse.[20]

Two main types of glial cells can be distinguished, i.e. microglia and macroglia (e.g. astrocytes).[1,20-22] Microglia are macrophage like cells that serve a phagocytic function and are involved in reaction to tissue damage.[1,20,21] Then, astrocytes are macroglial cells which are connected with both microvascular structures (i.e. endothelial cells of the capillaries) and neuronal structures (i.e. astrocytes enrap synapses).[1,20-22] Physiologically, astrocytes are required for an intact capillary endothelial cell network (so-called blood-brain or blood retina barrier in the brain and retina, respectively); and contribute to regulation of the concentrations of nutrients and waste products in the extracellular space (i.e. via exchange of nutrients and waste products between the blood supply and the active neuron); and communication between neighbouring neurons, where the communication between astrocytes and neurons is thought to be bidirectional.[1,20-22]

2.1.2. Microvasculature

The microvasculature, commonly defined as blood vessels with a diameter <150 μm, comprises >95% of the circulation and has a hemodynamic function, i.e. to maintain a

stable hydrostatic pressure, as well as a metabolic function, i.e. to enable optimal exchange of nutrients and waste products.[1,11,16] The microcirculation consists of arterioles, venules, capillaries, where arterioles and venules regulate the flow of blood to the capillaries and in the capillaries the exchange between nutrients and waste products takes place.[1,11,16] The arterioles receive blood with nutrients from the large arteriolar vessels of the macrocirculation and venules transport blood with waste products to larger venular vessels of the macrocirculation.[1,11,16]

Mechanistically, arteriolar and venular vessels control flow to the capillaries via two mechanisms. First, the arteriolar and venular diameters determine the extent of blood flow into the capillaries.[11,23,24] Biologically, endothelial cells, which line the interior side of blood vessels, to an important extent control diameters via regulating the tension of vascular smooth muscle cells, which are located adjacent to endothelial cells in arterioles and venules.[1,11,23,24] Second, arterioles regulate the number of capillaries that receive blood, where in a resting state (lower metabolic demand) less capillaries are 'open' to receive inflow than in an active state (higher metabolic demand).[11,25,26] Biologically, insulin is thought to be an important mediator that can recruit previously under-perfused capillaries by acting as an endothelium-dependent arteriolar dilator.[11,25,26]

In the capillaries of the brain the blood-brain barrier is located and in the capillaries of the retina the blood-retina barrier is located.[1,16] In the blood-brain and blood-retina barrier the exchange of nutrients and waste products is regulated in a highly selective manner in order to ensure optimal concentrations of extracellular compounds in brain and retinal neuronal tissue.[1,16] Biologically, the brain and inner retinal capillary endothelial can regulate uptake and excretion of compounds because the endothelium consists of tightly sealed cell-to-cell contacts and contains specialized receptors.[1,16] Mechanistically, capillary endothelial cells in the brain and inner retina can prevent trans-endothelial bulk flow via transcytosis, which occurs is most other organs, as in the brain and the retina endothelial cells have a high trans-endothelial electrical resistance and low paracellular and transcellular permeability.[1]

Endothelial cell function is to a strong extent determined by nitric oxide (NO) bioavailability.[11,27] Biologically, NO is synthesized by endothelial NO synthase (eNOS) from L-arginine.[27] Certain stimuli (e.g. shear stress or acetylcholine) can activate eNOS (via protein kinase A- or calcium calmodulin-dependent mechanisms) to synthesize NO.[27] In addition, for this enzymatic reaction co-factors are required (e.g. oxygen, haem, and tetrahydrobioptem [BH_4]).[27]

2.1.3. Neurovascular coupling unit

In the brain and in the retina neuronal and vascular structures together form the neurovascular coupling unit, which regulates the flow of blood (so-called

autoregulation)[28-30] and permeability of the blood-brain barrier. The function of the neurovascular coupling unit relies of the function of individual neuronal and microvascular structures as well as on the interaction between neuronal and microvascular structures.[28-30]

Mechanistically, the neurovascular coupling unit is thought to control autoregulation via three mechanisms. First, in response to an increase in neuronal activity neurons are thought to secrete vasorelaxant mediators (including NO), which can directly lead to microvascular smooth muscle cell relaxation and cause an increase in blood flow.[29,30] Second, in response to an increased metabolic activity, levels of local metabolic waste products in the capillaries increase, which can stimulate vasodilation (on the one hand waste products can generate local signals to stimulate capillary vasodilation; and on the other hand generates retrograde signals from the capillaries to the arterioles and venules can stimulate NO-mediated vascular smooth-muscle cell relaxation in the arterioles and venules).[29,30] Third, at a capillary level a cross-talk between neurons and microvascular structures exists (i.e. neurons, which are in close proximity of capillary endothelial cells, are thought to be able to activate retrograde endothelial cell signalling from the capillaries to the arteriolar and venular vessels, which [as described above] can cause arteriolar and venular smooth muscle cell relaxation and can cause an increase in blood flow).[29,30]

Biologically, the blood-brain barrier consists of astrocytic end feet and endothelial cells, which via effects of these structures on tight junctions between capillary endothelial cells regulate permeability of the blood-brain barrier.[1,31-33]

2.2. Biological rationale for neurodegenerative changes and microvascular dysfunction in the early pathobiology of clinical disease

Impairment of autoregulatory function is thought to be an important contributor to the early pathobiology of disease of neuronal and microvascular origin.[11,28-30] Pathomechanistically, impaired functioning of the autoregulation is thought to predispose to an enhanced capillary pressure and consequently capillary dilation; rupture; leakage; and non-perfusion, ultimately resulting in ischaemia, which can lead to brain and/or retinal injury.[11]

In the retina these pathobiological events can be observed. [11] For example, capillary dilation can be assessed as the formation of microaneurysms and capillary-rupture-induced leakage can be assessed as retinal hemorrhages.[11] Indeed, these hallmark features of non-proliferative diabetic retinopathy precede further structural deterioration of the retina (via angiogenesis-induced or macular oedema-induced degeneration of retinal architecture), and retinal dysfunction, i.e. visual impairment, which are hallmark features of proliferative diabetic retinopathy.[11]

Similarly, in the brain impairment of autoregulatory function is thought to lead to the formation of signs of cerebral small vessel disease (e.g. the presence of lacunar infarcts, microbleeds) and neuronal injury, which can manifest as brain connectivity dysfunction, cerebral neurodegeneration, and cognitive dysfunction, all of which are hallmark features of dementia.[11]

2.2.1. Presumed pathobiology of neuronal and microvascular deterioration

As postulated in "the vascular hypothesis", initial damage to the microvasculature is thought to cause secondary damage to neuronal structures in the brain and in the retina (Figure 1.1).[1,34] In the arterioles and venules, damage to the endothelium hampers haemodynamic autoregulation, which can lead to suboptimal distribution of blood flow to the capillaries (which can predispose to ischaemia), and a high intracapillary pressure, which is detrimental for the blood-brain and blood-retinal barrier.[11] In the capillaries, deterioration of the blood-brain and blood-retinal barrier enables toxic blood-derived molecules, cells and microbial agents to enter the brain or retina, respectively, resulting in an impaired interstitial fluid composition, and activation of inflammatory and immune-mediated pathways that can induce neuro-degeneration.[1] Pathobiologically, endothelial cell dysfunction in the blood-brain and blood-retinal barrier can lead to capillary leakage; loss of pericytes (which contribute to hemodynamic regulation at a capillary level); infiltration of leukocytes and red blood cells; and aberrant angiogenesis; all of which are pathobiological events that can cause an enhanced levels of reactive oxygen species; enhanced uptake of tau and amyloid beta; hypoxia; microglia and astrocyte-activation induced cytokine and chemokine activation; innate and adaptive immune system activation; and/or molecular changes (including lower glucose transporter 1 [GLUT1] expression and increased levels of receptors of advanced glycation endproducts [RAGE] expression); and via these processes, ultimately, induce neuronal injury, synaptic dysfunction, and neurodegeneration.[1]

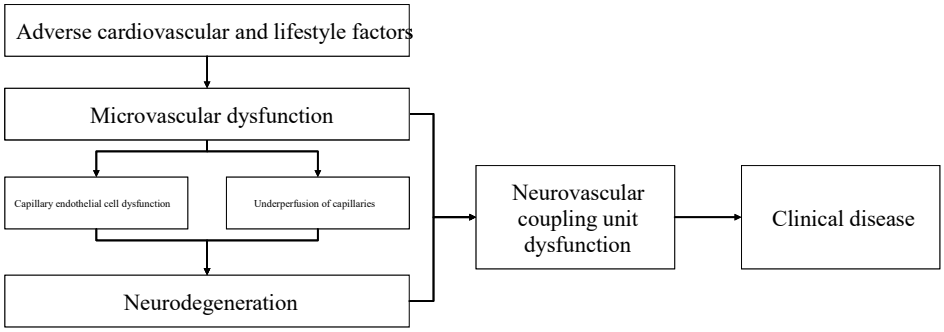


Figure 1.1 Theoretical framework of how adverse cardiovascular and lifestyle factors are associated with microvascular dysfunction, neurodegeneration, and clinical disease.

1.2.1.1. Microvascular dysfunction: presumed pathobiology

Microvascular dysfunction is thought to be to an important extent caused by endothelial cell dysfunction, which is caused by oxidative stress-induced eNOS uncoupling.[27] Mechanistically, eNOS uncoupling comprises that NO bioavailability is impaired via two mechanisms.[27] First, NO synthesis by the eNOS enzyme may be impaired due to the molecular changes of the eNOS enzyme (e.g. oxidative stress can cause monomerization of the ZnS_4 core of the eNOS enzyme and the eNOS enzyme requires availability of ZnS_4 to be able to synthesize NO); and due to the depletion of co-factors required for the synthesis of NO (e.g. oxidative stress can oxidate BH_4 in to BH_2 and BH_4 is a cofactor required for the synthesis of NO).[27] Second, free oxygen radicals and peroxide (i.e. forms of oxidative stress) can scavenge NO, which can lead to the production of oxidative stress (e.g. peroxynitrate).[27]
Additionally, a vicious circle may exist, the production of oxidative stress can lead to more impairment of NO bioavailability, which can, in turn, result in more eNOS uncoupling.[27]

1.2.1.1. Neurodegeneration: presumed pathobiology

Neuronal injury (caused by the factors described above) is thought to be mediated by oxidative stress.[1,35] Reactive oxygen species are thought to cause intracellular calcium accumulation, which can, consecutively, damage the mitochondria; lead to an impaired neuronal energy availability; and induce structural neuronal changes (including severe myelin damage and cytoskeletal disruption); resulting in the activation of pathways that lead to neuronal apoptosis.[35] Under physiological circumstances, glial cells uptake reactive oxidative species, such as peroxynitrate, in order to protect neurons.[35] However, under pathological circumstances, excessive and prolonged exposure to oxidative stress may induce glutamate release by glial cells, which can via inducing

excitotoxicity lead to a detrimentally high increase in intraneuronal calcium levels; and activate pathways that lead to apoptosis.[35,36]
Additionally, ischaemia (e.g. hypoxia) may also, independently of glutamate-toxicity mediated damage, activate pathways that lead to mitochondrial damage, and via mitochondrial damage lead to neuronal apoptosis.[37]
Last, neurodegenerative changes may spread in the central nervous system via retrograde degeneration, i.e. loss of neurons may lead to loss of connected neurons.[16,38]

2.3. The retina as a window in the brain

The retina has been proposed as a window into the brain as the retina and the brain have a shared embryology and similar anatomical and physiological features[16] Hence, the retina may provide a means to monitor (early) neurodegenerative changes and microvascular dysfunction in the brain.[16] Below, we globally discuss retinal function and anatomy and then we discuss how retinal and brain neuronal and microvascular embryology, anatomy, pathobiology, energy demand and metabolism are generally similar.

2.3.1. Retinal function

The function of the eye is to sense light and transmit visual information to the visual cortex, where signals from the retina are combined into 'vision'.[39] Mechanistically, light that enters the eye passes through the cornea, the lens, and the vitreous chamber, before it reaches the retina. In the retina light passes through several layers of neuronal tissue, all of which are transparent to enable light transmission, before it reaches the photoreceptors, which can convert incoming light signals (i.e. photons) into chemical signals.[39] In general, two types of photoreceptors can be distinguished, i.e. cones, which are most abundant in the fovea (i.e. in the centre of the macula), and rods, which are most abundant in retinal peripheral regions.[39] Globally, cones are required for colour vision and rods can distinguish light from dark (although many subtypes of cones and rods exist with specialized functions).[39] Physiologically, intact function of the foveal area is required for normal visual acuity and intact function of peripheral retinal regions is required for normal night vision.[39]
Within the retina electrochemical gradients from approximately 100 to 150 million photoreceptors are transmitted to approximately 1 million ganglion cells.[39] Biologically, photoreceptors transmit visual information via glutamate-mediated synapses to bipolar cells (a type of neuronal cell which in contrast to most neuronal cells have only one dendrite (instead of many dendrites) and bipolar cells transmit visual information to retinal ganglion cells via glutamate-mediated synapses.[39] In addition, horizontal and amacrine cells modulate how signals are transmitted between bipolar cells and ganglion cells and thereby contribute to 'selective filtering' of

information, strongly reducing the amount of information that is transmitted to the brain (i.e. input from up to 150 million photoreceptors is reduced to approximately 1 million signals that are transmitted via retinal ganglion cells).[39]

2.3.2. Retinal anatomy

Globally, the retina consists of 15 layers and can be anatomically be divided into the inner and the outer retina (Figure 1.2).[39]

The inner retina consists of six neuronal layers (i.e. the outer and inner nuclear layers, the ganglion cell layer, the outer and inner plexiform layers, and the retinal nerve fibre layer).[39] Biologically, the outer nuclear layer, the inner nuclear layer, and the ganglion cell layer, respectively, consist of the cell bodies of the photoreceptors, the bipolar cells, and the retinal ganglion cells.[39] In addition, besides the cell bodies of bipolar cells also horizontal cell bodies and amacrine cell bodies are part of the inner nuclear layer.[39] Next, the two plexiform layers consist of axons, dendrites and their synapses.[39] Specifically, the outer plexiform layer consists of the axons of the photoreceptor cells, the dendrites of the bipolar cells and their synapses; and the inner plexiform layer consists of the axons of the bipolar cells, the dendrites of the retinal ganglion cells and their synapses.[39] Last, the retinal nerve fibre layer consists of the axons of the retinal ganglion cells.[39]

The outer retina consists of seven layers (i.e. the photoreceptor layer, the retinal pigment epithelium layer, Bruch's membrane layer, the choriocapillaris layer, Sattler's layer, Haller's layers and the choroidal stroma).[39] Physiologically, in the photoreceptor layer photons are captured by the photoreceptors and converted in to electrochemical signals.[39] Then, the retinal pigment epithelium has several functions, including that the retinal pigment epithelium absorbs light to prevent scatter light and photo-oxidative stress; and that the retinal pigment epithelium tightly regulates the uptake of nutrients from the choroidal blood flow into the retina (i.e. the retinal pigment epithelium comprises part of the blood-retinal barrier).[39] Next, Bruch's membrane is the basal membrane of the retinal pigment epithelial cells. Last, the choriocapillaris consists of a single layer of capillaries; Sattler's and Haller's layers consist of medium- and larger-sized choroidal vessels; and the choroidal stromal layer is the boundary between the choroid and the sclera.[39]

Two retinal layers mark anatomical boundaries.[39] The inner limiting membrane is the boundary between the vitreous and the retina and the external limiting membrane marks the division between inner and outer retina.[39]

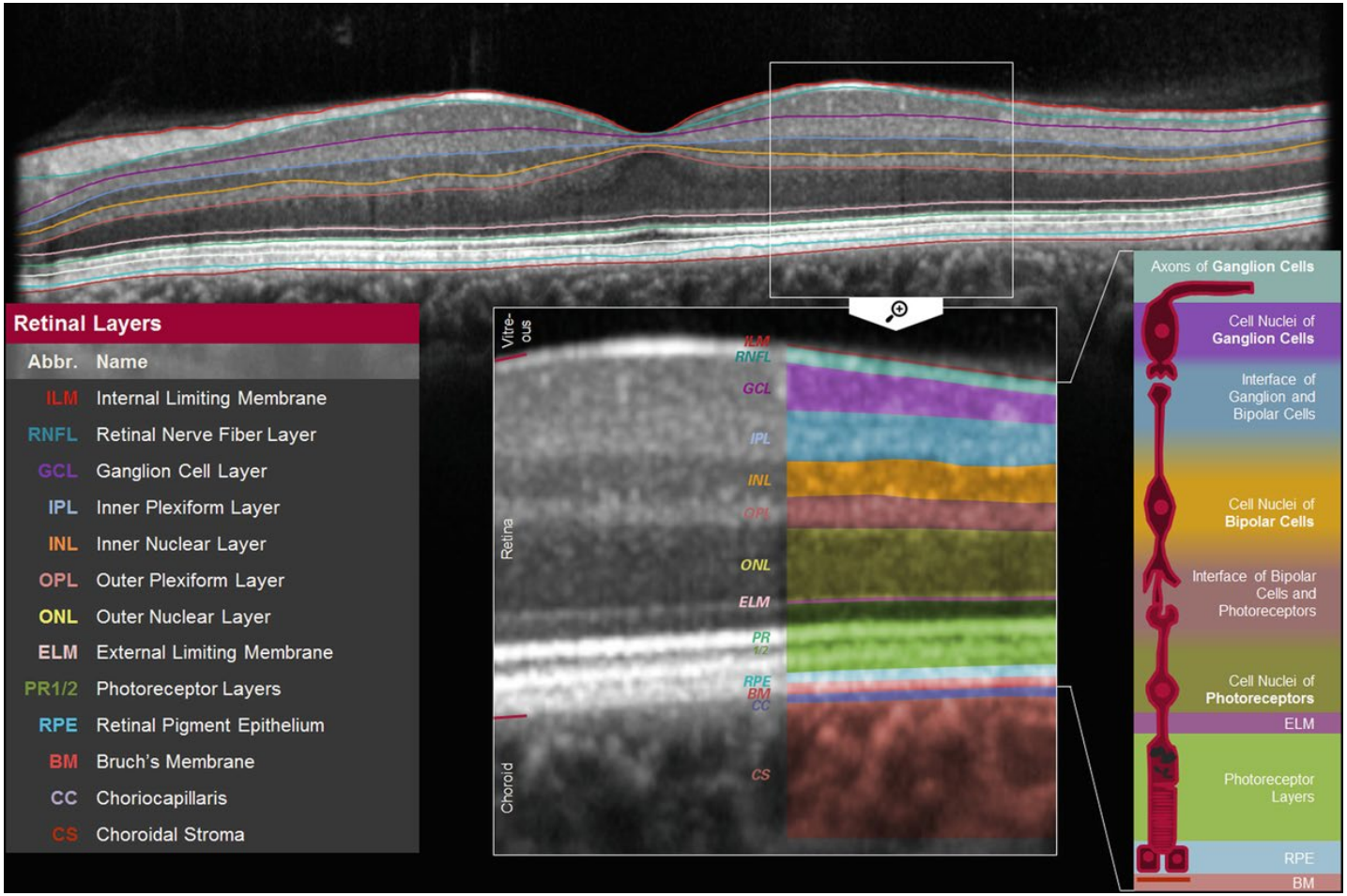


Figure 1.2 Shows a fovea-centred cross-sectional optical coherence tomography image of the retina with colours that show the names of individuals retinal layers an overview of cells per retinal layer (adapted from[40]).

2.3.3. Retinal blood supply

The inner and outer retina receive blood from separate vascular structures.
The inner retina receives blood from the ophthalmic artery, from which blood flows via the central retinal arteries (i.e. arterioles and venules) into the capillary network, which, anatomically, can be subdivided into the superficial network (which lies at the level of the retinal ganglion cells) and the deep capillary network (which lays at the level of the inner nuclear layer).[39] In addition, and only in the peripapillary region, a third capillary layer exists (i.e. the so-called peripapillary radial capillaries network, which supplies blood to the retinal nerve fibre layer).[39] The inner retinal circulation is characterized by a *low* blood flow and a high oxygen extraction (arteriovenous pO_2 differences are ~40%).[41] Retinal vascular endothelial cells form tight junctions that create a blood-retinal barrier; which is reminiscent of the blood-brain barrier and ensures selective exchanges between the circulation and the neural retina.[39,41]
The outer retina receives blood from the ophthalmic artery, from which blood flow via the posterior ciliary arteries to the choroidal circulation.[39,41] Biologically, the choriocapillaris is a *high*-flow capillary network, which consists of a single layer of anastomosing capillaries with wide lumina and fenestrations that are directed towards the retina.[39,41] The lumen is approximately three to four times that of ordinary capillaries, such that two or three red blood cells can pass through the capillary next to each other.[39]

2.3.4. Embryology

The retina and optic nerve are derived from the neural ectoderm around 23 days of gestation when they invaginate from the diencephalon.[16] Hence, the retina shares a common cellular origin with brain tissue and is considered part of the central nervous system.[16]

2.3.5. Anatomy and pathobiology

Neurons in the retina and brain have many structural and functional (patho)biological similarities.[16] For example, retinal and brain ganglion cells have axons that are (partially) enwrapped with an oligodendrocytic myelin sheath; and both have synaptic interconnections which use neurotransmitters such as glutamate, acetylcholine, dopamine, glycine, and Gaba-aminobutyric acid.[16] Globally, retinal axons can be considered biological equivalents of the white matter in the brain; and retinal somas and dendrites can be considered biological equivalents of the grey matter in the brain.[16] Further, in line with this concept, retinal neurodegeneration has been found to be associated brain neurodegeneration.[16] For example, lower retinal nerve fibre layer thickness and lower retinal ganglion cell layer thickness have been found to be

associated with lower brain volume.[16] Additionally, amyloid beta-plaques have found in the retina as well as in the brain.[16]

The microvasculature in the retina and brain share many (patho)biological similarities.[16] For example, retinal and cerebral blood vessels have a similar carrier-mediated transport function (i.e. in the blood-retina barrier and blood brain barrier); similar structural features of capillaries and tight junctions; similar capillary branching angles; and a similar pericyte-to-endothelial cell ratio.[16] Indeed, consistent with this concept, retinal microvascular dysfunction has been found to be associated with cerebral microvascular dysfunction.[16] For example, wider retinal venular diameters, increased tortuosity, reduced blood flow, and altered blood oxygen saturation have been found to be associated with more signs of cerebral small vessel disease.[16]

2.3.5. Energy demand and metabolism

The retina and the brain both have relatively high energy demands in comparison to other organs, albeit the energy demand of the retina is greater than the energy demand of the brain.[16]

Two biological explanations exist why the energy demand of the retinal neurons is higher than the energy demand of brain neurons.[41,42]

First, neurons located in the retina have axons that (in part) lack myelin enrapment.[42] Physiologically, myelin is not transparent and as transparent retinal neurons are required in order for light to be able to reach the photoreceptors, myelin is thought to lack myelin in the retinal part of neuronal cells (e.g. retinal nerve fibre).[42] Biologically, myelin facilitates energy-efficient neuronal communication.[42] Therefore, neurons without myelin are energetically inefficient (i.e. the energy required for a retinal neuron to transmit a signal is approximately 200 times greater than the energy required for a brain neuron to transmit a signal).[42]

Second, certain retina-specific processes have a high energy demand.[41] The high energy demand of the retina is thought to be determined by three processes which occur in the outer retina, i.e. 1) phototransduction; 2) detoxification of light-induced damage of lipids, proteins and nucleic acids; and 3) the continuous production of renewable photoreceptor outer segments (up to 10% turn over per day).[41] Notably, the energy demand of the retina is strongly determined by the presence of light, where the outer retina is much more strongly metabolically active in darkness.[41] Biologically, in the absence of light, open channels allow a steady flow of ions in and out of the cell resulting in a cellular depolarization known as the so-called ‘dark current’.[41] Upon light stimulation the ion channels are closed, neurotransmitters release is suppressed causing photoreceptor hyperpolarization, which leads to phototransduction.[41] Overall, more than 60% of the oxygen consumption of the retina is consumed by the outer retina.[41]

Retinal and brain neurons have a somewhat different metabolism. Biochemically, whereas brain neurons mainly only use glucose as energy substrate for the production of adenosine triphosphate (ATP), retinal neurons use relatively less glucose and relatively more free fatty acids for this process.[41] Importantly, in the retina only for up to 35% of retinal cell ATP is derived from glucose.[41]

3. Cardiovascular and lifestyle factors as potentially modifiable determinants of neuronal and microvascular changes used in this thesis

Early modification of adverse cardiovascular and lifestyle factors may be a potentially feasible strategy for the prevention of and/or slowing of the onset of major clinical disease.[10,11] First, cardiovascular and lifestyle factors are likely causes of early neurodegeneration and microvascular dysfunction as cardiovascular and lifestyle factors are associated with the incidence and progression of major clinical disease of neuronal and microvascular origin.[3,11,43] Second, modification of cardiovascular and lifestyle factors has been demonstrated to lower the incidence and/or slow the progression of major clinical disease of neuronal and microvascular origin.[3,11,43] Third, the pathobiology of cardiovascular and lifestyle factors has been relatively well-studied and (safe) therapeutic options to modify cardiovascular and lifestyle factors are already available.[44,45]

The investigation of cardiovascular risk factors and adverse lifestyle factors as determinants of early neurodegeneration and microvascular dysfunction will provide insight in how strongly certain determinants are associated with neurodegeneration and microvascular dysfunction. Importantly, quantitative knowledge enables discrimination between strong and weak determinants of early neurodegeneration and microvascular dysfunction. This is relevant because modification of stronger determinants may lead to a stronger reduction in the deterioration of neuronal and microvascular structures. For reasons of efficacy, in the clinic modification of strong determinants may be prioritized over the modification of weak determinants.

In this thesis we investigated seven cardiovascular risk factors and five lifestyle factors. Traditional cardiovascular risk factors under study are hyperglycaemia,[46] dyslipidaemia,[46] hypertension,[46] and obesity[46] and novel cardiovascular risk factors under study are daily glucose variability,[47] blood pressure variability,[48] and arterial stiffening.[49] Next, lifestyle factors under study are smoking,[43] cardiorespiratory fitness,[50] physical inactivity,[51] and an unhealthy diet (including alcohol consumption).[43]

The exact pathobiology of how individual cardiovascular and lifestyle factors lead to early neurodegeneration and microvascular dysfunction are discussed in the general discussion (chapter 10).

3.1. Hyperglycaemia and sex as effect modifiers in the associations of cardiovascular and lifestyle factors with neurodegeneration and microvascular dysfunction

The effects of certain cardiovascular or lifestyle factors on neurodegeneration and microvascular dysfunction may differ in strength in the presence of other risk factors, i.e. there may be effect modification by certain risk factors.
Hyperglycaemia may be such an effect modifier.[11] Biologically, as hyperglycaemia is detrimental for neuronal and microvascular structures, which hampers function of these individual structures and can lead to dysfunction of the neurovascular coupling unit, the effects of certain risk factors on the microvasculature and/or neurons may be stronger in individuals with elevated levels of glycaemia, e.g. individuals with prediabetes or type 2 diabetes.[11] For example, the detrimental effects of hypertension-induced haemodynamic stress may be stronger in individuals with, versus without, type 2 diabetes or prediabetes. Mechanistically, dysfunction of the neurovascular coupling unit impairs the ability to regulate capillary pressure and therefore hypertension is more likely to lead to a detrimentally high capillary pressure in individuals with prediabetes or type 2 diabetes.[11]
In addition, sex may be an effect modifier.[17,52] Due to biological differences between men and women the effects of certain cardiovascular or lifestyle factors on neuronal and microvascular structures may differ.[17,52]

3.2 Assessment of neuronal and microvascular measures used in this thesis

In this thesis we used data on neurodegeneration and microvascular dysfunction assessed in the retina and we used data on microvascular dysfunction assessed in various other organ systems (i.e. in the brain, skin, kidney, and blood).

3.2.1. Retinal measures

In the retina we assessed retinal nerve fibre layer (RNFL) thickness, retinal arteriolar and venular diameters, flicker light-induced increase in retinal arteriolar and venular diameter, and retinal sensitivity (Figure 1.3).

3.2.1.1. RNFL thickness

We used optical coherence tomography to assess peripapillary RNFL thickness (Figure 1.3).[53] Technically, optical coherence tomography transmits near-infrared light (i.e. laser light) in to the retina, and uses a technique called interferometry to, based on non-scattering reflecting light, determine the thickness of retinal layers (i.e. analogue to ultrasound, with as difference that ultrasound uses soundwaves instead of light-waves).[54] Biologically, lower RNFL thickness is thought to reflect lower number of retinal ganglion cell axons and astrocytes (i.e. indicating neurodegeneration).[54]

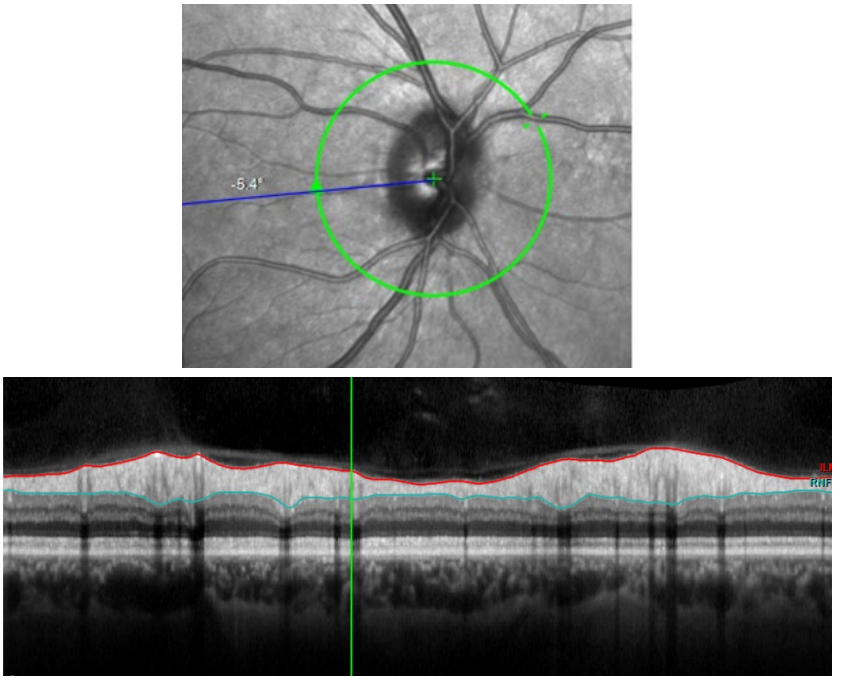


Figure 1.3A shows the location (i.e. around the optic nerve head) where retinal nerve fibre layer (RNFL) thickness is assessed (the green line indicates the exact location where RNFL thickness was assessed; shown for the right eye).
Figure 1.3B shows a cross-section of the retinal layers (assessed at 12 degrees from the optic nerve head). The red coloured line indicates the internal limiting membrane and the blue coloured line indicates the inferior boundary of the RNFL. RNFL thickness is calculated as the average distance between these layers.

3.2.1.2. Retinal microvascular diameters

We assessed peripapillary retinal microvascular diameters with fundoscopy (i.e. central retinal arteriolar equivalent [CRAE] and central retinal venular equivalent [CRVE]; Figure 1.4).[19] Technically, a conventional camera (analogue to a camera on a smart

phone) was used to take a flash photo.[55] Biologically, widening and narrowing of retinal arteriolar diameters are thought to reflect early-stage microvascular dysfunction (i.e. impairment of autoregulation) and late-stage microvascular dysfunction (i.e. microvascular remodelling), respectively; and widening of retinal venular diameters is thought to reflect worse microvascular function (in contrast to the pathobiology of arteriolar deterioration, the pathobiology of venular deterioration is not biphasic [i.e. with greater deterioration of microvascular function venules widen, but do not narrow]).[11]

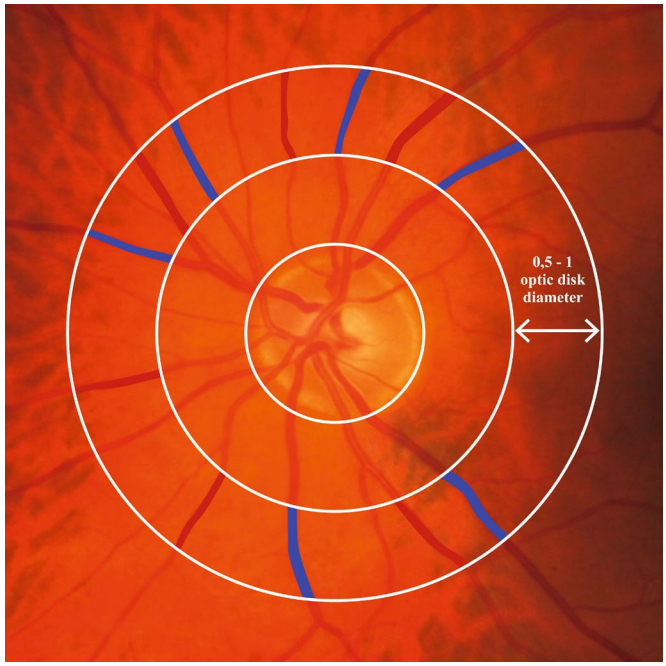


Figure 1.4 shows from where on the retina retinal arteriolar and venular diameters are calculated from the central retinal arteriolar (in red) and venular (in blue) vessels. Central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) are each calculated from six arteriolar or venular blood vessels.

3.2.1.3. Flicker light-induced increase in retinal microvascular diameters

We assessed flicker light-induced increase in retinal arteriolar and venular peripapillary diameters with the dynamic vessel analyser (Figure 3.1.5).[56] Technically, the dynamic vessel analyser exposes the retina to flicker light, which increases retinal metabolism, and provokes a dilation response, in order to meet the increased energy demand of the retina (i.e. to enable more exchange of nutrients and waste productions).[56] Biologically,

lower flicker light-induced increase in retinal arteriolar and venular diameters are thought to reflect worse function of the neurovascular coupling unit.[56]

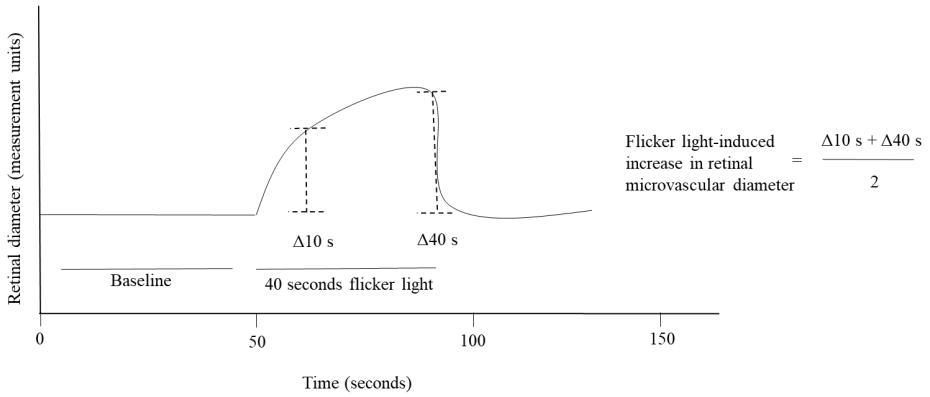


Figure 1.5 shows the retinal microvascular diameter during the assessment of the flicker light-induced increase in retinal microvascular diameter. During the first 50 seconds of the retinal diameter measurement the baseline diameter is assessed and during the 40 second of flicker light exposure the increase in retinal diameter from baseline is assessed as the mean increase from baseline at 10 and 40 seconds. The flicker light-induced increase in retinal arteriolar and venular diameters are determined from a central retinal arteriole and a central retinal venule at approximately 1-1.5 disk diameters from the optic nerve head (approximately the same location as where CRAE and CRVE are determined [Figure 1.4]).

3.2.1.4. Retinal sensitivity

We assessed retinal sensitivity with the an edge perimeter (Figure 3.1.6).[57] Technically, the Heidelberg edge perimeter presents visual stimuli of alternating strengths to determine the threshold at which the least strong stimulus can still be observed.[57] Biologically, lower retinal sensitivity is thought to reflect worse total retinal function and intact retinal function requires intact function of neuronal as well as microvascular structures.[58]

3.2.2. Non-retinal measures

We assessed various indices of microvascular dysfunction in the brain, the skin, the kidney and in the blood.[19]

3.2.2.1. Brain

In the brain we assessed features of cerebral small vessel disease.[19] We used magnetic resonance imaging to assess four features of cerebral small vessel disease, i.e. white matter hyperintensity volume, cerebral microbleeds, lacunar infarcts, and total brain volume.[19] Biologically, white matter hyperintensities are thought to reflect structural disruptions of fibre tracts in cerebral white matter and are thought to be caused by

perfusion deficits; cerebral microbleeds are thought to reflect microvascular leakage; lacunar infarcts are thought to reflect local ischaemia; and lower total brain volume are thought to reflect loss of neuronal tissue (i.e. neurodegeneration).[11]

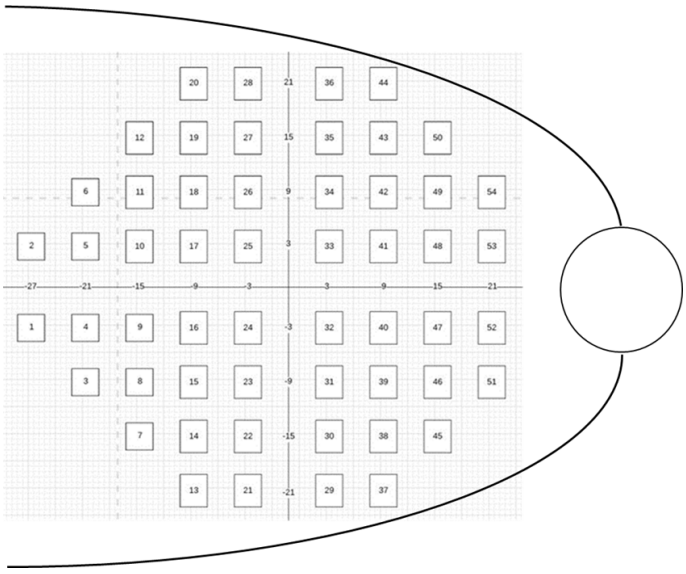


Figure 1.6 shows a schematic drawing of the retina of the right eyes with the an overview of the 54 coordinates where in the macular area retinal sensitivity was assessed with an edge perimeter. In addition, the optic nerve head and the superior and inferior central retinal microvasculature are schematically shown. Retinal sensitivity is assessed as the threshold at which a stimulus of the lowest strength that was presented (i.e. the lowest light intensity) can still be detected.

3.2.2.2. Skin

In the skin we assessed the skin hyperaemic response to heat.[19] Physiologically, the skin microvasculature regulates thermal control of the skin.[59] Therefore, in order to maintain optimal skin temperature, the skin microvasculature is thought to dilate in response to a local heat stimulus, in order to release excess heat.[59] We used laser-doppler flowmetry to quantify the difference in perfusion of the skin microvasculature in response to local heat.[52] Biologically, lower increase in skin hyperaemia in response to heat is thought to reflect worse microvascular function.[59]

3.2.2.3. Kidney

In urine we assessed albuminuria, a measure of microvascular dysfunction in the kidney.[11] Physiologically, the kidney filters blood in the glomerulus to control the composition and volume of blood.[60] Biologically, the capillaries of the glomerulus

consist of fenestrae (openings), which allows the passage of molecules (and the rapid passage of a large fluid volume), and the endothelial cells in the glomerular capillaries are negatively charged, which selectively regulates the passage of smaller molecules in to the glomerulus.[60] Under pathological circumstances, the selective regulation of the passage of molecules is impaired (due to endothelial cell dysfunction) and therefore larger molecules can enter the glomerulus and collect in the urine.[60] Hence, higher levels of albuminuria reflect worse microvascular function in the kidney.[60]

3.2.2.4. Blood

In the blood we assessed plasma biomarkers of microvascular dysfunction.[19] We assessed soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin], and Von Willebrand Factor [vWF]). Biologically, endothelial cells use molecules, including sICAM-1, sVCAM-1, sE-selectin and vWF, to regulate which cells and/or molecules can adhere to endothelial cells.[61] Mechanistically, via these molecules endothelial cells play an important role in the regulation of inflammation and haemostasis.[61] Pathobiologically, higher levels of sICAM-1, sVCAM-1, sE-selectin and vWF are thought to reflect worse endothelial cell function and a more inflammatory and pro-thrombotic phenotype.[61]

4. Limitations of the current literature

Here we provide a global overview of the limitations of the current literature (a detailed literature overview is provided in chapters 2-9).

The current literature on the associations between cardiovascular and life factors with neurodegeneration and microvascular dysfunction under study in this thesis has a number of important limitations. First, no large population-based studies (N>1,000) have yet reported the associations of cardiovascular and lifestyle factors with retinal sensitivity. This is an important limitation because results of smaller studies may not be valid in the general population.[62] Methodologically, findings of smaller, non-population-based studies are more likely to be prone to selection bias than larger, population-based studies.[62] Second, a large number of cardiovascular and lifestyle factors has not yet been investigated in relation to RNFL thickness, flicker light-induced increase in retinal microvascular diameters, and heat-induced skin hyperaemia. For example, the associations of physical activity and daily glucose variability with RNFL thickness have not yet been reported.[63,64] Third, many previous population-based studies that reported certain associations between cardiovascular and lifestyle factors with endpoints under study in this thesis did not account for a number of potential confounders, e.g. diet or physical activity. Therefore, (some extent of) residual

confounding may have occurred in these studies, and resulted in a (somewhat) spurious estimation of the strength of associations.[62] Fourth, other population-based have used measurement instruments of an inferior quality to investigate whether certain cardiovascular or lifestyle factors are associated with endpoints under study in this thesis.[62] This may have resulted in null findings via regression dilution bias or a loss in precision of estimation of betas, predisposing to a greater chance of a type 2 statistical error.[65] For example, previous population-based studies assessed blood pressure with office blood pressure measurements, instead of with 24-hour ambulatory blood pressure measurements, and 24-hour ambulatory blood pressure is a more precise and valid measure of blood pressure than office blood pressure.[63,64,66] 24-hour ambulatory blood pressure more precisely reflects blood pressure because 24-hour ambulatory blood pressure measurements consists of many more measurements than office blood pressure (i.e. >70 versus 3 measurements), and thus is less prone to measurement error.[65, 66] Then, 24-hour ambulatory blood pressure provides a more valid view on blood pressure over the day than office blood pressure as measurements are performed over a timeframe of 24-hours instead of within 5-10 minutes.[65,66] Fifth, only few population-based studies that investigated associations of cardiovascular and lifestyle factors with endpoints under study in this thesis have studied whether associations differ by sex (i.e. between men and women) or by glucose metabolism status (i.e. between individuals with type 2 diabetes, prediabetes, or normal glucose metabolism).[63,64] Sixth, few prospective data on retinal neurodegeneration and microvascular dysfunction with symptoms of early-stage major clinical brain disease of neuronal and microvascular origin are available in the current literature. At present, one population-based study has investigated the prospective association between lower RNFL thickness and cognitive function[67] and no population-based study has yet studied the prospective association between lower RNFL thickness and clinically-relevant depressive symptoms.[68] Then, one previous population-based study has investigated the prospective associations of (retinal) microvascular dysfunction with cognitive function[69] and one population-based study has investigated the prospective associations of (retinal) microvascular dysfunction with clinically-relevant depressive symptoms.[70] Last, no population-based study has yet reported whether neurodegeneration and microvascular dysfunction, assessed in the retina, modify the strength of each other's associations with cognitive performance.[71] Biologically, function of the neurovascular coupling unit may be considerably more strongly impaired in individuals with, versus without, both neurodegeneration and microvascular dysfunction or one or none of these factors.[29]

5. General methodological approach

5.1. Study design

To address these knowledge gaps, we used data from The Maastricht Study. The Maastricht Study is an observational population-based cohort study.[72] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of diabetes mellitus type 2 and is characterized by an extensive phenotyping approach.[72] Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands.[72] Participants were recruited through mass media campaigns, the municipal registries and the regional Diabetes Patient Registry via mailings.[72] Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes for reasons of efficiency.[72] The present report includes cross-sectional and prospective data from up to n=8,005 participants who completed the baseline survey between November 2010 and January 2019.[72]
All participants underwent a standardized set of measurements that were performed by trained research personnel with state-of-the-art measurement techniques.[72]

5.2. Statistical analyses

We used regression analyses to study the associations in this thesis and adjusted these associations for potential demographic, lifestyle, and cardiovascular factors. Generally, we showed crude results in model 1; adjusted for key demographic covariates (i.e. age, sex, and education status) and glucose metabolism status (because individuals with type 2 diabetes were oversampled by design in The Maastricht Study) in model 2; and in addition to covariates in model 2, adjusted for cardiovascular (i.e. blood pressure, antihypertensive medication, total cholesterol-high density lipid ratio, lipid-modifying medication, waist circumference) and lifestyle factors (i.e. smoking and alcohol consumption) in model 3. Next, we additionally adjusted for other lifestyle factors (i.e. diet and physical activity), although we performed these analyses in separate models as data on these covariates were generally available for relatively smaller numbers of individuals.
For all analyses, we tested for interaction by glucose metabolism status and sex.[7,11,17,52] A priori we hypothesized that certain cardiovascular and lifestyle factors may be more strongly associated with neuronal and microvascular changes in individuals with a more adverse glucose metabolism status.[7,11] A priori we did generally not expect sex-differences.[17,52] In addition, for certain analyses, if a priori data were available that suggested that certain cardiovascular or lifestyle factors may be effect modifiers in certain associations, we also tested for interaction by other covariates (e.g. in analyses

with alcohol consumption as main determinant we also tested for interaction by history of cardiovascular disease).[73]
We additionally adjusted for certain covariates which may (in part) be confounders as well as (in part) be mediators, and/or descendants of the outcome.[74] Because adjustment for these covariates may lead to overadjustment bias, we adjusted for these variables in separate models. We determined whether covariates where confounder, mediator, and/or based on theoretical reasoning. We considered the following covariates for these analyses: history of cardiovascular disease, low-grade inflammation (assessed from blood-based biomarkers), kidney variables (i.e. estimated glomerular filtration rate and urinary albumin excretion), and ocular variables (spherical equivalent, intraocular pressure, and certain retinal diseases).
We expressed associations as standardized betas (i.e. in standard deviations) so that the strength of the associations of individual determinants with outcomes under study could be compared between each other.

6. Aims, hypotheses and outline

6.1. Primary aims

This thesis has three main aims. First, to investigate how potentially modifiable cardiovascular and lifestyle factors are associated with early neurodegeneration and microvascular dysfunction. Second, to investigate whether early neurodegeneration precedes symptoms of clinical disease, which is presumed to be of neuronal and microvascular origin. Third, to investigate whether neurodegeneration and microvascular dysfunction modify how microvascular dysfunction and neurodegeneration, respectively, are associated with symptoms of clinical disease, which is presumed to be of neuronal and microvascular origin.

6.2. Secondary aim

To investigate whether associations under study differ in strength by glucose metabolism status or sex.

6.3. Hypotheses

6.3.1. Primary hypotheses

1. We hypothesized that cardiovascular and lifestyle factors are determinants of neurodegeneration and microvascular dysfunction.

2. We hypothesized that neurodegeneration precedes symptoms of clinical disease of presumed neuronal and microvascular origin.
3. We hypothesized that neurodegeneration is more strongly associated with lower cognitive performance in the presence of microvascular dysfunction; and that microvascular dysfunction is more strongly associated with lower cognitive performance in the presence of neurodegeneration.

6.3.2. Secondary hypotheses

We hypothesized that cardiovascular risk factors that can increase levels of haemodynamic stress may be more detrimental in individuals with a more adverse glucose metabolism status. In addition, the effects of alcohol consumption and physical activity on the microvasculature may be stronger in individuals with a more adverse glucose metabolism status. Last, individuals with, versus without, neurodegeneration as well as microvascular dysfunction may be more susceptible to haemodynamic stress, as occurs in hypertension.

6.4. Outline

Figure 1.7 summarizes the outline of the present thesis. In chapters 2 and 3 we investigated whether cardiovascular and lifestyle factors were determinants of neurodegeneration, estimated from retinal neuronal structure, and/or were determinants of retinal function. In chapters 4-7 we investigated cardiovascular and lifestyle determinants of microvascular dysfunction, assessed in the retina and various other organ systems. In chapters 8 and 9 we investigated how retinal neuronal structure and/or retinal microvascular dysfunction are associated with depressive symptoms and/or cognitive performance. Finally, in chapter 10, main findings of this thesis are summarized and further discussed.

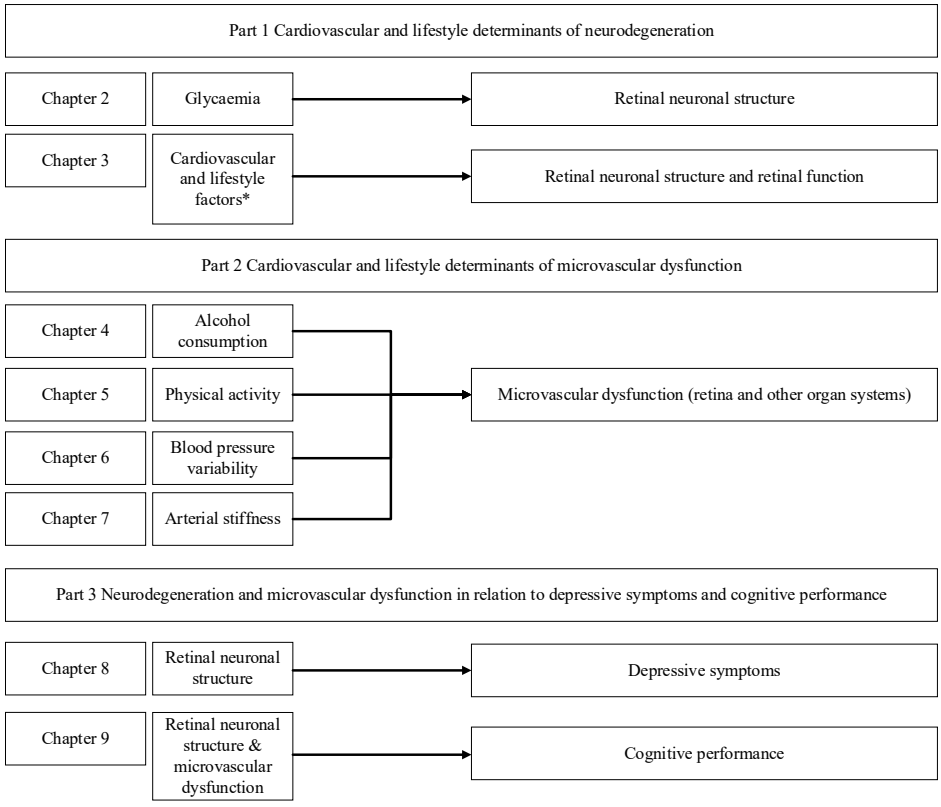


Figure 1.7 Overview of the associations investigated in this thesis. * Cardiovascular and lifestyle determinants include: blood pressure, cholesterol, obesity, glycaemia, diet, alcohol consumption, smoking, cardiorespiratory fitness, and physical activity.

References

1. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14:133-150.
2. Alexopoulos GS. Mechanisms and treatment of late-life depression. Transl Psychiatry. 2019;9:188.
3. Wong TY and Sabanayagam C. The War on Diabetic Retinopathy: Where Are We Now? Asia Pac J Ophthalmol (Phila). 2019;8:448-456.
4. Collaborators GBDN. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18:459-480.
5. van Bussel EF, Richard E, Arts DL, Nooyens AC, Coloma PM, de Waal MW, van den Akker M, Biermans MC, Nielen MM, van Boven K, Smeets H, Matthews FE, Brayne C, Busschers WB, van Gool WA and Moll van Charante EP. Dementia incidence trend over 1992-2014 in the Netherlands: Analysis of primary care data. PLoS Med. 2017;14:e1002235.
6. Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW and Ho RC. Prevalence of Depression in the Community from 30 Countries between 1994 and 2014. Sci Rep. 2018;8:2861.
7. Roy T and Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord. 2012;142 Suppl:S8-21.
8. Xue M, Xu W, Ou YN, Cao XP, Tan MS, Tan L and Yu JT. Diabetes mellitus and risks of cognitive impairment and dementia: A systematic review and meta-analysis of 144 prospective studies. Ageing Res Rev. 2019;55:100944.
9. Haffner SM, Stern MP, Hazuda HP, Mitchell BD and Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA. 1990;263:2893-8.
10. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, Brayne C, Burns A, Cohen-Mansfield J, Cooper C, Costafreda SG, Dias A, Fox N, Gitlin LN, Howard R, Kales HC, Kivimaki M, Larson EB, Ogunniyi A, Orgeta V, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbaek G, Teri L and Mukadam N. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396:413-446.
11. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67:1729-1741.
12. van Sloten TT, Sedaghat S, Carnethon MR, Launer LJ and Stehouwer CDA. Cerebral microvascular complications of type 2 diabetes: stroke, cognitive dysfunction, and depression. Lancet Diabetes Endocrinol. 2020;8:325-336.
13. Vyas CM and Okereke OI. Late-Life Depression: A Narrative Review on Risk Factors and Prevention. Harv Rev Psychiatry. 2020;28:72-99.
14. Perneczky R. Dementia prevention and reserve against neurodegenerative disease. Dialogues Clin Neurosci. 2019;21:53-60.
15. Han F. Cerebral microvascular dysfunction and neurodegeneration in dementia. Stroke Vasc Neurol. 2019;4:105-107.
16. Kashani AH, Asanad S, Chan JW, Singer MB, Zhang J, Sharifi M, Khansari MM, Abdolahi F, Shi Y, Biffi A, Chui H and Ringman JM. Past, present and future role of retinal imaging in neurodegenerative disease. Prog Retin Eye Res. 2021:100938.
17. Agarwala A, Michos ED, Samad Z, Ballantyne CM and Virani SS. The Use of Sex-Specific Factors in the Assessment of Women's Cardiovascular Risk. Circulation. 2020;141:592-599.
18. Podcasy JL and Epperson CN. Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin Neurosci. 2016;18:437-446.
19. Li W, Schram MT, Sorensen BM, van Agtmaal MJM, Berendschot T, Webers CAB, Jansen JFA, Backes WH, Gronenschild E, Schalkwijk CG, Stehouwer CDA and Houben A. Microvascular Phenotyping in the Maastricht Study: Design and Main Findings, 2010-2018. Am J Epidemiol. 2020;189:873-884.
20. Rosenberg GA. Molecular Physiology and Metabolism of the Nervous System. New York Oxford University Press; 2012.
21. Kurosinski P and Gotz J. Glial cells under physiologic and pathologic conditions. Arch Neurol. 2002;59:1524-8.

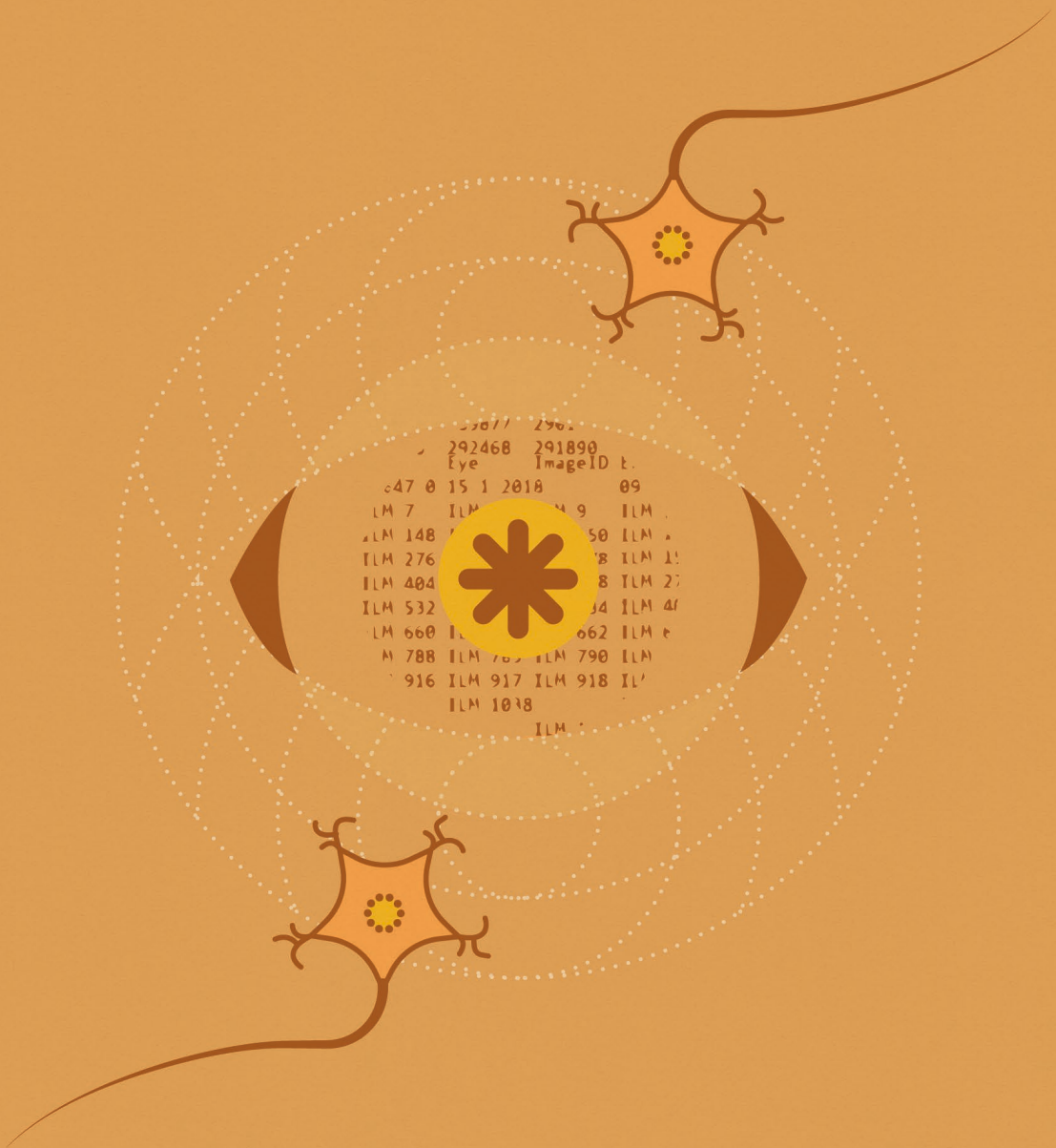
22. Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K, Xu J, Guo G, Tong A and Zhou L. The role of astrocytes in oxidative stress of central nervous system: A mixed blessing. Cell Prolif. 2020;53:e12781.
23. Grubb S, Cai C, Hald BO, Khennouf L, Murmu RP, Jensen AGK, Fordsmann J, Zambach S and Lauritzen M. Precapillary sphincters maintain perfusion in the cerebral cortex. Nat Commun. 2020;11:395.
24. Li W, Schram MT, Berendschot T, Webers CAB, Kroon AA, van der Kallen CJH, Henry RMA, Schaper NC, Huang F, Dashtbozorg B, Tan T, Zhang J, Abbasi-Sureshjani S, Ter Haar Romeny BM, Stehouwer CDA and Houben A. Type 2 diabetes and HbA1c are independently associated with wider retinal arterioles: the Maastricht study. Diabetologia. 2020;63:1408-1417.
25. Kellar D and Craft S. Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. Lancet Neurol. 2020;19:758-766.
26. Olver TD, Laughlin MH and Padilla J. Exercise and Vascular Insulin Sensitivity in the Skeletal Muscle and Brain. Exerc Sport Sci Rev. 2019;47:66-74.
27. Meza CA, La Favor JD, Kim DH and Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci. 2019;20.
28. Silverman A and Petersen NH. Physiology, Cerebral Autoregulation StatPearls Treasure Island (FL); 2021.
29. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. Neuron. 2017;96:17-42.
30. Guerra G, Lucariello A, Perna A, Botta L, De Luca A and Moccia F. The Role of Endothelial Ca(2+) Signaling in Neurovascular Coupling: A View from the Lumen. Int J Mol Sci. 2018;19.
31. Abbott NJ, Ronnback L and Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7:41-53.
32. Csiszar A, Tarantini S, Fulop GA, Kiss T, Valcarcel-Ares MN, Galvan V, Ungvari Z and Yabluchanskiy A. Hypertension impairs neurovascular coupling and promotes microvascular injury: role in exacerbation of Alzheimer's disease. Geroscience. 2017;39:359-372.
33. Iadecola C and Gottesman RF. Neurovascular and Cognitive Dysfunction in Hypertension. Circ Res. 2019;124:1025-1044.
34. Soto-Rojas LO, Pacheco-Herrero M, Martinez-Gomez PA, Campa-Cordoba BB, Apatiga-Perez R, Villegas-Rojas MM, Harrington CR, de la Cruz F, Garces-Ramirez L and Luna-Munoz J. The Neurovascular Unit Dysfunction in Alzheimer's Disease. Int J Mol Sci. 2021;22.
35. Rosso M and Chitnis T. Association Between Cigarette Smoking and Multiple Sclerosis: A Review. JAMA Neurol. 2020;77:245-253.
36. Benarroch EE. Glutamatergic synaptic plasticity and dysfunction in Alzheimer disease: Emerging mechanisms. Neurology. 2018;91:125-132.
37. Hardingham GE, Fukunaga Y and Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci. 2002;5:405-14.
38. Wang L and Mao X. Role of Retinal Amyloid-beta in Neurodegenerative Diseases: Overlapping Mechanisms and Emerging Clinical Applications. Int J Mol Sci. 2021;22.
39. Levin L, Nilsson S, Ver Hoeve J, Wu, M, Kaufman P, and Alm A, Adler's Physiology of the Eye: Saunders; 2011.
40. Aumann S, Donner S, Fischer J and Muller F. Optical Coherence Tomography (OCT): Principle and Technical Realization. In: J. F. Bille, ed. High Resolution Imaging in Microscopy and Ophthalmology: New Frontiers in Biomedical Optics Cham (CH); 2019: 59-85.
41. Joyal JS, Gantner ML and Smith LEH. Retinal energy demands control vascular supply of the retina in development and disease: The role of neuronal lipid and glucose metabolism. Prog Retin Eye Res. 2018;64:131-156.
42. Yu DY, Cringle SJ, Balaratnasingam C, Morgan WH, Yu PK and Su EN. Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. Prog Retin Eye Res. 2013;36:217-46.
43. Xu W, Tan L, Wang HF, Jiang T, Tan MS, Tan L, Zhao QF, Li JQ, Wang J and Yu JT. Meta-analysis of modifiable risk factors for Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2015;86:1299-306.
44. Bhatti GK, Reddy AP, Reddy PH and Bhatti JS. Lifestyle Modifications and Nutritional Interventions in Aging-Associated Cognitive Decline and Alzheimer's Disease. Front Aging Neurosci. 2019;11:369.

45. McKeever A and Agius M. Dementia risk assessment and risk reduction using cardiovascular risk factors. Psychiatr Danub. 2018;30:469-474.
46. Mahmood SS, Levy D, Vasan RS and Wang TJ. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet. 2014;383:999-1008.
47. Skrha J, Soupal J, Skrha J, Jr. and Prazny M. Glucose variability, HbA1c and microvascular complications. Rev Endocr Metab Disord. 2016;17:103-10.
48. Stevens SL, Wood S, Koshiaris C, Law K, Glasziou P, Stevens RJ and McManus RJ. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. BMJ. 2016;354:i4098.
49. Chirinos JA, Segers P, Hughes T and Townsend R. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;74:1237-1263.
50. Kurl S, Laukkanen JA, Lonnroos E, Remes AM and Soininen H. Cardiorespiratory fitness and risk of dementia: a prospective population-based cohort study. Age Ageing. 2018;47:611-614.
51. Brasure M, Desai P, Davila H, Nelson VA, Calvert C, Jutkowitz E, Butler M, Fink HA, Ratner E, Hemmy LS, McCarten JR, Barclay TR and Kane RL. Physical Activity Interventions in Preventing Cognitive Decline and Alzheimer-Type Dementia: A Systematic Review. Ann Intern Med. 2018;168:30-38.
52. Knowlton AA and Lee AR. Estrogen and the cardiovascular system. Pharmacol Ther. 2012;135:54-70.
53. Lains I, Wang JC, Cui Y, Katz R, Vingopoulos F, Staurenghi G, Vavvas DG, Miller JW and Miller JB. Retinal applications of swept source optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA). Prog Retin Eye Res. 2021:100951.
54. Drexler W and Fujimoto JG. State-of-the-art retinal optical coherence tomography. Prog Retin Eye Res. 2008;27:45-88.
55. Bekkers E DR BT, Romeny BT. A Multi-Orientation Analysis Approach to Retinal Vessel Tracking. J Math Imaging Vis. 2014;49:583-610.
56. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CD. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. Circulation. 2016;134:1339-1352.
57. Kaczorowski K, Mulak M, Szumny D and Misiuk-Hojlo M. Heidelberg Edge Perimeter: The New Method of Perimetry. Adv Clin Exp Med. 2015;24:1105-12.
58. Harwerth RS, Wheat JL, Fredette MJ and Anderson DR. Linking structure and function in glaucoma. Prog Retin Eye Res. 2010;29:249-71.
59. Low DA, Jones H, Cable NT, Alexander LM and Kenney WL. Historical reviews of the assessment of human cardiovascular function: interrogation and understanding of the control of skin blood flow. Eur J Appl Physiol. 2020;120:1-16.
60. Prasad RM and Tikaria R. Microalbuminuria StatPearls Treasure Island (FL); 2021.
61. Reglero-Real N, Colom B, Bodkin JV and Nourshargh S. Endothelial Cell Junctional Adhesion Molecules: Role and Regulation of Expression in Inflammation. Arterioscler Thromb Vasc Biol. 2016;36:2048-2057.
62. Rothman KJ GS, Lash TL. Modern epidemiology. Third edition ed: Lippincott Williams & Wilkin; 2008.
63. Khawaja AP, Chua S, Hysi PG, Georgoulas S, Currant H, Fitzgerald TW, Birney E, Ko F, Yang Q, Reisman C, Garway-Heath DF, Hammond CJ, Khaw PT, Foster PJ, Patel PJ, Strouthidis N, Eye UKB and Vision C. Comparison of Associations with Different Macular Inner Retinal Thickness Parameters in a Large Cohort: The UK Biobank. Ophthalmology. 2020;127:62-71.
64. Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, Loeffler M, Colijn JM, Ikram MA, Vingerling JR, Williams KM, Hammond CJ, Creuzot-Garcher C, Bron AM, Silva R, Nunes S, Delcourt C, Cougnard-Gregoire A, Holz FG, Klaver CCW, Breteler MMB, Finger RP and European Eye Epidemiology C. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. Ophthalmology. 2018;125:1526-1536.
65. Hutcheon JA, Chiolero A and Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.
66. Myers MG and Kaczorowski J. Office blood pressure is lower than awake ambulatory blood pressure at lower targets for treatment. J Clin Hypertens (Greenwich). 2017;19:1210-1213.

67. Ko F, Muthy ZA, Gallacher J, Sudlow C, Rees G, Yang Q, Keane PA, Petzold A, Khaw PT, Reisman C, Strouthidis NG, Foster PJ, Patel PJ, Eye UKB and Vision C. Association of Retinal Nerve Fiber Layer Thinning With Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. JAMA Neurol. 2018;75:1198-1205.
68. Garcia-Portilla MP, Garcia-Alvarez L, de la Fuente-Tomas L, Velasco-Iglesias A, Saiz PA, Gonzalez-Blanco L, Bobes Bascaran MT, Baamonde B, Alcalde I, Merayo-Lloves J and Bobes J. Could structural changes in the retinal layers be a new biomarker of mental disorders? A systematic review and thematic synthesis. Rev Psiquiatr Salud Ment (Engl Ed). 2019;12:116-129.
69. Lesage SR, Mosley TH, Wong TY, Szklo M, Knopman D, Catellier DJ, Cole SR, Klein R, Coresh J, Coker LH and Sharrett AR. Retinal microvascular abnormalities and cognitive decline: the ARIC 14-year follow-up study. Neurology. 2009;73:862-8.
70. Geraets AFJ, van Agtmaal MJM, Stehouwer CDA, Sorensen BM, Berendschot T, Webers CAB, Schaper NC, Henry RMA, van der Kallen CJH, Eussen S, Koster A, van Sloten TT, Kohler S, Schram MT and Houben A. Association of Markers of Microvascular Dysfunction With Prevalent and Incident Depressive Symptoms: The Maastricht Study. Hypertension. 2020;76:342-349.
71. Ravi Teja KV, Tos Berendschot T, Steinbusch H, Carroll Webers AB, Praveen Murthy R and Mathuranath PS. Cerebral and Retinal Neurovascular Changes: A Biomarker for Alzheimer's Disease. J Gerontol Geriatr Res. 2017;6.
72. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
73. Fragopoulou E, Choleva M, Antonopoulou S and Demopoulos CA. Wine and its metabolic effects. A comprehensive review of clinical trials. Metabolism. 2018;83:102-119.
74. Schisterman EF, Cole SR and Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20:488-95.

Part I

Determinants of neurodegeneration



CHAPTER 2

(Pre)diabetes, greater glycaemia, and greater daily glucose variability are associated with lower retinal nerve fibre layer thickness, an index of neurodegeneration – The Maastricht Study

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Submitted

Abstract

Objective

Retinopathy and neuropathy in type 2 diabetes are preceded by retinal nerve fiber layer (RNFL) thinning, an index of neurodegeneration. We investigated whether glucose metabolism status (GMS), measures of glycemia, and daily glucose variability (GV) are associated with RNFL thickness over the entire range of glucose tolerance.

Research design and methods

We used cross-sectional data from The Maastricht Study (up to 5,455 participants, 48.9% men, mean age 59.5 years and 22.7% with type 2 diabetes) to investigate the associations of GMS, measures of glycaemia (fasting plasma glucose [FPG], 2-hour post-load glucose [2-h PG], HbA1c, advanced glycation endproducts [AGEs] assessed as skin autofluorescence [SAF]) and indices of daily GV (incremental glucose peak [IGP] and continuous glucose monitoring [CGM]-assessed standard deviation [SD]) with mean RNFL thickness. We used linear regression analyses and, for GMS, P for trend analyses. We adjusted associations for demographic, cardiovascular risk and lifestyle factors, and, only for measures of GV, for indices of mean glycaemia.

Results

After full adjustment, type 2 diabetes and prediabetes (versus normal glucose metabolism) were associated with lower RNFL thickness (standardized beta [95%CI], respectively -0.16 [-0.25;-0.08]; -0.05 [-0.13;0.03]; P_{trend}=0.001). Greater FPG, 2-h PG, HbA1c, SAF, IGP and CGM-assessed SD were also associated with lower RNFL thickness (per SD, respectively -0.05 [-0.08; -0.01]; -0.06 [-0.09; -0.02]; -0.05 [-0.08; -0.02]; -0.04 [-0.07; -0.01]; -0.06 [-0.12; -0.01]; and -0.07 [-0.21; 0.07]).

Conclusion

In this population-based study, a more adverse GMS and, over the entire range of glucose tolerance, greater glycaemia and daily GV were associated with lower RNFL thickness. Hence, early identification of individuals with hyperglycaemia, early glucose-lowering treatment, and early monitoring of daily GV may contribute to the prevention of RNFL thinning, an index of neurodegeneration and precursor of retinopathy and neuropathy.

Introduction

Retinopathy and neuropathy, both hallmark microvascular complications of type 2 diabetes, are preceded by subtle neurodegenerative changes. Such changes include retinal nerve fibre layer (RNFL) thinning, which can be non-invasively assessed.[1-3] RNFL thinning reflects a gradual loss of retinal ganglion cell axons, which transmit visual information from the retina to the brain.[1,2] Mechanistically, elevated glucose concentrations are thought to be toxic for retinal ganglion cells as well as for retinal endothelial and glia cells, which contribute to local metabolic regulation.[4,5] Thus, hyperglycaemia can lead to retinal ganglion cell apoptosis and loss of retinal ganglion cell axons both directly and through impairment of endothelial and glia cell function.[6]

As postulated in the "ticking clock hypothesis", hyperglycaemia-mediated damage is thought to be a continuous (i.e. linear) process that already starts before the onset of type 2 diabetes.[7,8] Indeed, our group observed linear associations of more adverse glucose metabolism status (GMS) and higher glycaemia (estimated by various measures) with lower heart rate variability,[9] more structural brain abnormalities,[10,11] and worse peripheral nerve function,[12] all of which are measures of neurodegeneration.

The current literature on the associations of hyperglycaemia with RNFL thickness has some important limitations. First, in previous population-based studies, several important confounders were not included, such as age,[13-15] sex,[14,15] socioeconomic status,[13-23] cardiovascular risk factors,[13-15,19,21-24] and lifestyle factors (e.g., alcohol use,[13,16-19,21] diet,[13-24] and physical activity).[13-19,21-24] Second, no population-based studies have yet investigated whether advanced glycation endproducts (AGEs) or daily glucose variability are associated with RNFL thickness. It is important to establish to what extent hyperglycaemia (including AGEs) and daily glucose variability contribute to RNFL thinning over the entire range of glucose tolerance because early diagnosis and treatment of hyperglycaemia as well as novel strategies to monitor glycaemic exposure may contribute to the early prevention of hyperglycaemia-mediated RNFL thinning.[5,25,26]

In view of above, we investigated, using a large, well-characterized population-based cohort study, whether more adverse GMS, greater glycaemia, and greater daily glucose variability are associated with lower RNFL thickness.

Methods

Study population and design

We used data from The Maastricht Study, a prospectively designed, population-based observational cohort study. The rationale and methodology have been described previously.[27] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency.[27] The present report includes cross-sectional data of 8,005 participants who completed the baseline survey between November 2010 and September 2018. The examinations of each participant were performed within a time window of three months. From 19 September 2016 until 13 September 2018, participants were invited to also undergo CGM.[26] During this period, a selected group of recently included participants was invited to return for CGM ('catch-up visit'). For these participants only there was a median time interval ('visit interval') of 2.1 years between CGM and all other measurements (more details are provided in the Supplemental Material). The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.[27]

Glucose metabolism status

After an overnight fast, participants underwent a standardized seven-point oral glucose tolerance test (OGTT) as part of which venous samples were collected at 15, 30, 45, 60, 90, and 120 minutes post ingestion of a 75g glucose drink. All participants underwent an OGTT except for the participants who used insulin or had a fasting plasma glucose (FPG) concentration above 11.0 mmol/L. Based on FPG and 2-hour post-load glucose, GMS was determined as normal glucose metabolism (NGM), prediabetes (impaired fasting glucose, impaired glucose tolerance, or both), type 2 diabetes, or other types of diabetes (including type 1 diabetes) in accordance with the World Health Organization 2006 criteria.[28]

Measures of glycaemia

FPG (mmol/L) and haemoglobin A_{1c} (HbA1c; mmol/mol; %) were determined in venous plasma samples collected after an overnight fast. Two-hour post-load glucose (mmol/L) was determined in venous plasma collected at 120 minutes post glucose drink ingestion. AGEs were assessed with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). In brief, the AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to quantify their accumulation in the skin as skin autofluorescence (SAF; arbitrary units [AU]).[29] The AGE Reader illuminates a skin surface of 4 cm^2, shielded from other light, and uses the ratio of the reflection of fluorescent light (wavelength 420 to 600 nm) to non-fluorescent light (300–420 nm) to calculate SAF (more details are provided in the Supplemental Materials).

Indices of daily glucose variability

The incremental glucose peak (IGP; mmol/L), a recently validated OGTT-based index of daily glucose variability, was calculated by subtracting FPG from the maximum glucose peak value measured during a complete seven-point OGTT.[30] At the time of analysis, data on IGP were available in a subset of participants (n=2,407). We used CGM (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) to assess daily glucose variability during a 1-week period, which was expressed as standard deviation (mmol/L) of mean sensor glucose (mmol/L).[26] CGM-assessed data were available in a subset of participants (n=622). More details on the assessment of glycaemic indices with OGTT and CGM are provided in the Supplemental Methods.

RNFL thickness

We assessed peripapillary RNFL thickness (μm) in both eyes using optical coherence tomography (OCT; Spectralis unit and Eye Explorer version 5.7.5.0 software; Heidelberg Engineering, Heidelberg, Germany; 3.45-mm-diameter—circle scan, manually centred on optic nerve head, 12°, 768 voxels, 100 automatic real-time tracking). Intra- and interindividual reliability, expressed as intraclass correlation coefficients, are 0.97 and 0.96 respectively.[31] At least 15 minutes before the examination, pupils were dilated with topical 0.5% tropicamide and 2.5% phenylephrine. Experienced graders masked to clinical information on the participants reviewed the OCT scans and graded their quality. OCT images were excluded if one of the following criteria was present: scan error (i.e. incomplete scan, poor centring of the circular scan on the optic nerve head, RNFL layer incorrectly defined, or technical problem with the OCT device) or poor imaging quality (signal-to-noise ratio<15 dB).[23]

If data from both eyes were available (n=2,796 participants) we averaged RNFL thickness in order to reduce measurement error. If data from only one eye were available (n=2,755 participants), we used the RNFL thickness of that eye in the analyses. More details, including on quality criteria, are shown in the Supplemental Methods.

Covariates

As described previously,[27] we assessed educational level (low, intermediate, high), socio-economic status (income level and occupational status),[32] smoking status (never, former, current), alcohol use (none, low, high), history of cardiovascular disease, and duration of diabetes by questionnaire; assessed dietary habits ("dietary intake") with the Dutch Healthy Diet index sum score, a measure of adherence to the Dutch dietary guidelines 2015,[33] based on a validated food frequency questionnaire;[34] assessed lipid-modifying, antihypertensive, intraocular pressure-lowering, and glucose-lowering medication use as part of a medication interview; assessed weight, height, and waist circumference during a physical examination; calculated body mass index (BMI) based on body weight and height; measured office and 24-hour ambulatory blood pressure (BP); measured total daily physical activity (hours/day) with an accelerometer;[35] measured lipid profile and plasma biomarkers of low-grade inflammation[36] (i.e. high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8 and tumour necrosis factor alpha) in fasting venous blood samples; measured urinary albumin excretion in two 24-hour urine collections; calculated the estimated glomerular filtration rate (eGFR) based on serum creatinine only, since cystatin C was not presently available in all study participants; assessed presence of retinopathy in both eyes via fundus photography; and used an automated refractor and noncontact tonometer (Tonoref II; Nidek, Gamagordi, Japan) to assess spherical equivalent and intraocular pressure in both eyes. Glaucoma was defined as use of intraocular pressure-lowering medication, intraocular pressure higher than 21 mm Hg in any eye (91.3% of all participants had data on intraocular pressure available for at least 1 eye), or both. Spherical equivalent was defined as the mean spherical equivalent of both eyes (available for 91.1% of all participants) or as the spherical equivalent of the eye for which data were available.

Statistical analysis

We used multivariable linear regression analyses to investigate the associations of GMS (entered as dummy variables of prediabetes, type 2 diabetes, or other types of diabetes versus NGM) and standardized FPG, 2-hour post-load glucose, HbA1c, SAF,

IGP, and CGM-assessed standard deviation (determinants) with standardized mean RNFL thickness (outcome).

We performed P for trend analyses to test for linear trend with more adverse GMS. To test for trend, we entered GMS into the model as an ordinal variable (i.e. GMS was coded as NGM=0, prediabetes=1, type 2 diabetes=2). In P for trend analyses we excluded participants with other types of diabetes because other types of diabetes (such as type 1 diabetes) do not constitute part of the spectrum of deterioration of GMS from NGM to prediabetes and type 2 diabetes. Then, we checked whether we could assume a linear trend by comparing the statistical variance explained by the statistical model in which GMS was entered as dummy variables to the statistical model in which GMS was entered as an ordinal variable. We used a likelihood ratio test to assess whether the amount of variance explained by both models differed statistically significantly. A P-value >0.05 indicates that both models are not different and, thus, that a linear trend can be assumed. For all analyses under study, the P-value for the likelihood ratio test was >0.05 (data not shown) and therefore a linear trend could be assumed.

Model 1 shows crude results. In model 2, we adjusted for age, sex, educational status (low, medium, high). We chose these variables because they are key potential confounders.[17] In model 3, we additionally adjusted for variables of which their status as confounder has been less firmly established (office systolic BP, use of antihypertensive medication [yes/no], waist circumference, total cholesterol / HDL cholesterol ratio, lipid-modifying medication [yes/no], smoking status [current, former, never], and alcohol consumption status [none, low, high]).[17] Then, and only for IGP and CGM-assessed standard deviation, we additionally adjusted for HbA1c or mean sensor glucose, respectively (model 3 + HbA1c; model 3 + mean sensor glucose), so that we could differentiate between daily glucose variability and average glycaemia, both of which are strongly related.[37] Additionally, and only for CGM-assessed indices, we entered 'visit interval' in model 1. The associations were expressed as standardized regression coefficient (stβ) and corresponding 95% confidence interval (95%CI). Collinearity diagnostics (i.e. tolerance <0.10 and/or variance inflation factor >10) were used to detect excessive multicollinearity between covariates.

We tested for interaction to assess whether associations differed by GMS (i.e. between individuals with type 2 diabetes, individuals with prediabetes, and individuals with NGM) or by sex. For interaction analyses with GMS, we excluded participants with other types of diabetes from the interaction analyses because the number of these participants was small.

To assess the robustness of our findings we performed several sensitivity analyses. First, we repeated the analyses with additional adjustment for lifestyle factors (dietary intake, physical activity) or ocular variables (spherical equivalent, intraocular pressure).[38-40] Adjustment for these potential confounders was not included in the main

analyses because data were missing for a relatively large number of participants (up to n=768 had missing data on one or more of these variables). Second, we additionally adjusted for kidney variables (eGFR and urinary albumin excretion), history of cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy, and glaucoma. We adjusted for these covariates in a separate model because they may be confounders but may also (in part) be mediators or descendants of the outcome.[41] Third, we performed analyses in which participants with retinopathy, glaucoma, or other types of diabetes were excluded. Fourth, we replaced IGP with other OGTT-based indices of daily glucose variability (i.e. maximum glucose peak and 1-hour post-load glucose). We did not include maximum glucose peak and 1-hour post-load glucose in the main analyses because they are known to correlate less strongly than IGP with CGM-assessed indices of daily glucose variability in GMS-stratified groups.[30] Fifth, we replaced CGM-assessed standard deviation with other CGM-assessed measures of daily glucose variability (more details are provided in the Supplemental Methods section). Sixth, we studied the association between mean sensor glucose and retinal nerve fibre layer thickness. Although the sample size of the CGM study population was relatively small (n=622), CGM-based glycaemic mean sensor glucose may be less susceptible to measurement error than other measures of glycaemia under study, as mean sensor glucose constitutes the average of a large number of glucose concentrations.[30,42] Seventh, we replaced waist circumference with BMI; educational status with occupational status or income level; and office systolic BP with office diastolic BP, systolic or diastolic 24-hour ambulatory BP. Eighth, and only for SAF, we additionally adjusted the association between SAF and RNFL thickness for FPG, 2-hour post-load glucose, or HbA_{1c}. We additionally adjusted for these measures to investigate whether the association between SAF and RNFL thickness was independent of short to middle long-term exposure to hyperglycaemia. Ninth, and only for IGP and CGM-assessed standard deviation, we respectively replaced HbA_{1c} with FPG or SAF, and mean sensor glucose with FPG, SAF, or HbA_{1c}. Tenth, and only for CGM-assessed standard deviation, we repeated the analyses after exclusion of individuals with insufficient CGM recording days, with CGM recording data gaps, or with a visit interval. Eleventh, because of the strong correlation between CGM-assessed standard deviation and mean sensor glucose,[37] we repeated the main analysis using ridge regression, which is a valid method to counter potential instability caused by multicollinearity (additional information is provided in the Supplemental Methods).[43] Last, we studied the association between duration of diabetes and RNFL thickness. Data on duration of diabetes were only available for individuals with type 2 diabetes (n=982).

All analyses were performed with Statistical Package for Social Sciences version 25.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). For all analyses, a P-value <0.05 was considered statistically significant.

Results

Selection and characteristics of the study population

Figure 2.1 shows an overview of the study population selection. Participants in whom OCT data were missing or of insufficient quality were excluded first (n=2,454). Next, individuals with missing data on confounders were excluded (n=96). The sample size of the final study populations depended on the availability of data on the main determinant (n=5,455 for GMS, n=982 to 5,454 for measures of glycaemia, and n=622 to 2,407 for indices of daily glucose variability).

Table 2.1 and Supplemental Table S2.1 show general participant characteristics according to tertiles of RNFL thickness. Overall, participants with a thinner RNFL were more often men, were older, and had a more adverse cardiovascular risk profile. General characteristics of participants included in the study were comparable to those of participants with missing data (Supplemental Table S2.2).

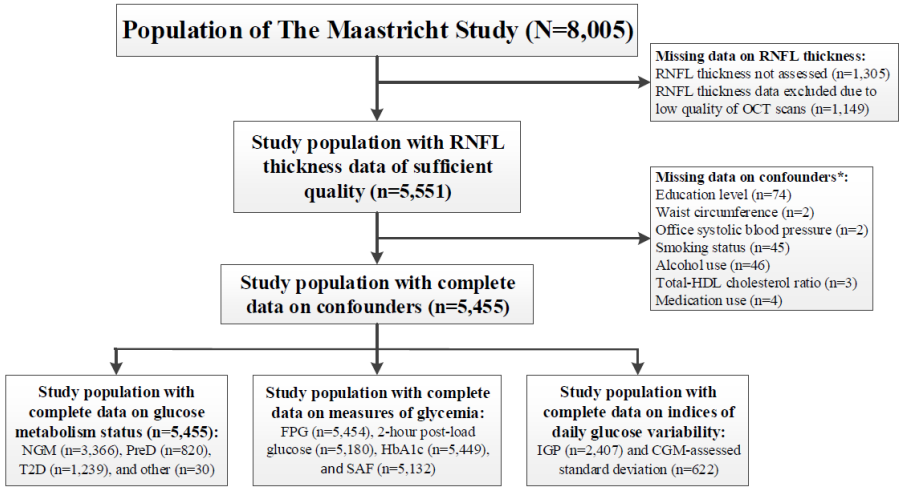


Figure 2.1 RNFL study population selection. OCT, optical coherence tomography; RNFL, retinal nerve fibre layer; HDL, high density lipoprotein; NGM, normal glucose metabolism; PreD, prediabetes; T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1c, haemoglobin A1C; SAF, skin autofluorescence; IGP, incremental glucose peak; CGM, continuous glucose monitoring. * Not mutually exclusive.

Table 2.1 General study population characteristics according to tertiles of the retinal nerve fibre layer thickness in the study population with complete data on glucose metabolism status.

Characteristic	RNFL thickness			
	Total study population (n=5,455)	Tertile 1 (high) (n=1,818)	Tertile 2 (middle) (n=1,819)	Tertile 3 (low) (n=1,818)
Age (years)	59.5 ± 8.6	59.1 ± 8.7	59.3 ± 8.7	60.0 ± 8.5
Men	2,665 (48.9)	805 (48.9)	847 (46.6)	1,013 (55.7)
Educational level				
Low	1,914 (35.1)	669 (36.8)	651 (35.8)	594 (32.7)
Medium	1,519 (27.8)	525 (28.9)	505 (27.8)	489 (26.9)
High	2,022 (37.1)	624 (34.3)	663 (36.4)	735 (40.4)
Glucose metabolism status				
NGM	3,366 (61.7)	1,174 (64.6)	1,144 (62.9)	1,048 (57.6)
Prediabetes	820 (15.0)	266 (14.6)	280 (15.4)	274 (15.1)
Type 2 diabetes	1,239 (22.7)	370 (20.4)	383 (21.1)	486 (26.7)
Other type of diabetes	30 (0.5)	8 (0.4)	12 (0.7)	10 (0.6)
Measures of glycaemia				
Fasting plasma glucose (mmol/L)*	5.9 ± 1.5	5.8 ± 1.4	5.8 ± 1.5	6.0 ± 1.7
2-hour post-load glucose (mmol/L)*	6.2 [4.9-8.6]	6.1 [4.9-8.2]	6.1 [4.9-8.5]	6.3 [5.1-9.2]
HbA1c (mmol/mol)*	39.2 ± 9.1	38.7 ± 8.5	39.1 ± 9.3	39.9 ± 9.5
HbA1c (%)*	5.7 ± 0.8	5.7 ± 0.8	5.7 ± 0.8	5.8 ± 0.9
Skin autofluorescence (AU)*	2.2 ± 0.5	2.1 ± 0.5	2.2 ± 0.5	2.2 ± 0.5
Indices of daily glucose variability				
Incremental glucose peak (mmol/L)*	4.1 [2.7-6.5]	3.9 [2.6-5.9]	4.1 [2.8-6.5]	4.4 [2.8-7.0]
CGM-assessed standard deviation (mmol/L)*	0.86 [0.68-1.21]	0.86 [0.67-1.18]	0.85 [0.67-1.17]	0.88 [0.70-1.29]
Use of glucose-modifying medication, yes vs. no	927 (17.0)	266 (14.6)	289 (15.9)	372 (20.5)
Waist circumference, men (cm)	100.4 ± 11.6	100.2 ± 11.7	99.9 ± 11.7	101.0 ± 11.5
Waist circumference, women (cm)	89.1 ± 12.5	89.2 ± 12.8	88.9 ± 12.1	89.2 ± 12.8
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	3.3 [2.7-4.1]	3.3 [2.8-4.2]	3.4 [2.8-4.2]
Use of lipid-modifying medication, yes vs. no	1,687 (30.9)	535 (29.4)	541 (29.7)	611 (33.6)
Office systolic blood pressure (mmHg)	133.2 ± 17.7	132.0 ± 18.0	133.0 ± 17.6	134.5 ± 17.5
Office diastolic blood pressure (mmHg)	75.5 ± 9.8	75.0 ± 9.8	75.3 ± 9.7	76.4 ± 9.9
Use of antihypertensive medication, yes vs. no	1,983 (36.4)	601 (33.1)	637 (35.0)	745 (41.0)
Smoking status				
Never	2,101 (38.5)	717 (39.4)	684 (37.6)	700 (38.5)
Former	2,666 (48.9)	843 (44.4)	911 (50.1)	912 (50.2)
Current	688 (12.6)	258 (14.2)	224 (12.3)	206 (11.3)
Alcohol consumption				
None	995 (18.2)	344 (18.9)	356 (19.6)	295 (16.2)
Moderate	3,181 (58.3)	1,070 (58.9)	1,042 (57.3)	1,069 (58.8)
High	1,279 (23.4)	404 (22.2)	421 (23.1)	454 (25.0)
RNFL thickness (μm)	94.8 ± 10.8	106.0 ± 6.3	95.2 ± 2.5	83.2 ± 6.8

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). * Data shown in the study population with complete data on fasting plasma glucose (n=5,454), 2-hour post-load glucose (n=5,180), HbA1c (n=5,449), skin autofluorescence (n=5,132), incremental glucose peak (n=2,407), and CGM-assessed standard deviation (n=622). CGM, continuous glucose monitoring; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fibre layer; AU, arbitrary units.

Glucose metabolism status and RNFL thickness

After full adjustment (model 3), a more adverse GMS was associated with lower RNFL thickness (standardized beta [95%CI], type 2 diabetes versus NGM -0.16 [-0.25; -0.08]; prediabetes versus NGM -0.05 [-0.13; 0.03], P for trend =0.001; Table 2.2 and Figure 2.2).

Measures of glycaemia and RNFL thickness

After full adjustment (model 3), greater FPG, 2-hour post-load glucose, HbA1c, and SAF were statistically significantly associated with lower RNFL thickness (per SD, respectively -0.05 [-0.08; -0.01]; -0.06 [-0.09; -0.02]; -0.05 [-0.08; -0.02]; and -0.04 [-0.07; -0.01]; Table 2.2 and Figure 2.2).

Indices of daily glucose variability and RNFL thickness

After full adjustment (model 3), greater IGP was statistically significantly associated with lower RNFL thickness (per SD, -0.06 [-0.11; -0.01]; Figure 2.2 and Table 2.2). The association remained statistically significant after additional adjustment for HbA1c (per SD, -0.06 [-0.12; -0.01]). After full adjustment (model 3), CGM-assessed standard deviation was also negatively associated with RNFL thickness, but not statistically significantly (per SD, -0.08 [-0.17; 0.01]). The association was similar after further adjustment for mean sensor glucose.

Interaction analyses

GMS and sex did not modify any of the associations under study. All P-values for interaction are shown in Supplemental Table S2.4.

Additional analyses

Quantitatively similar results were observed in a range of sensitivity analyses and are presented in the Supplemental Results section.

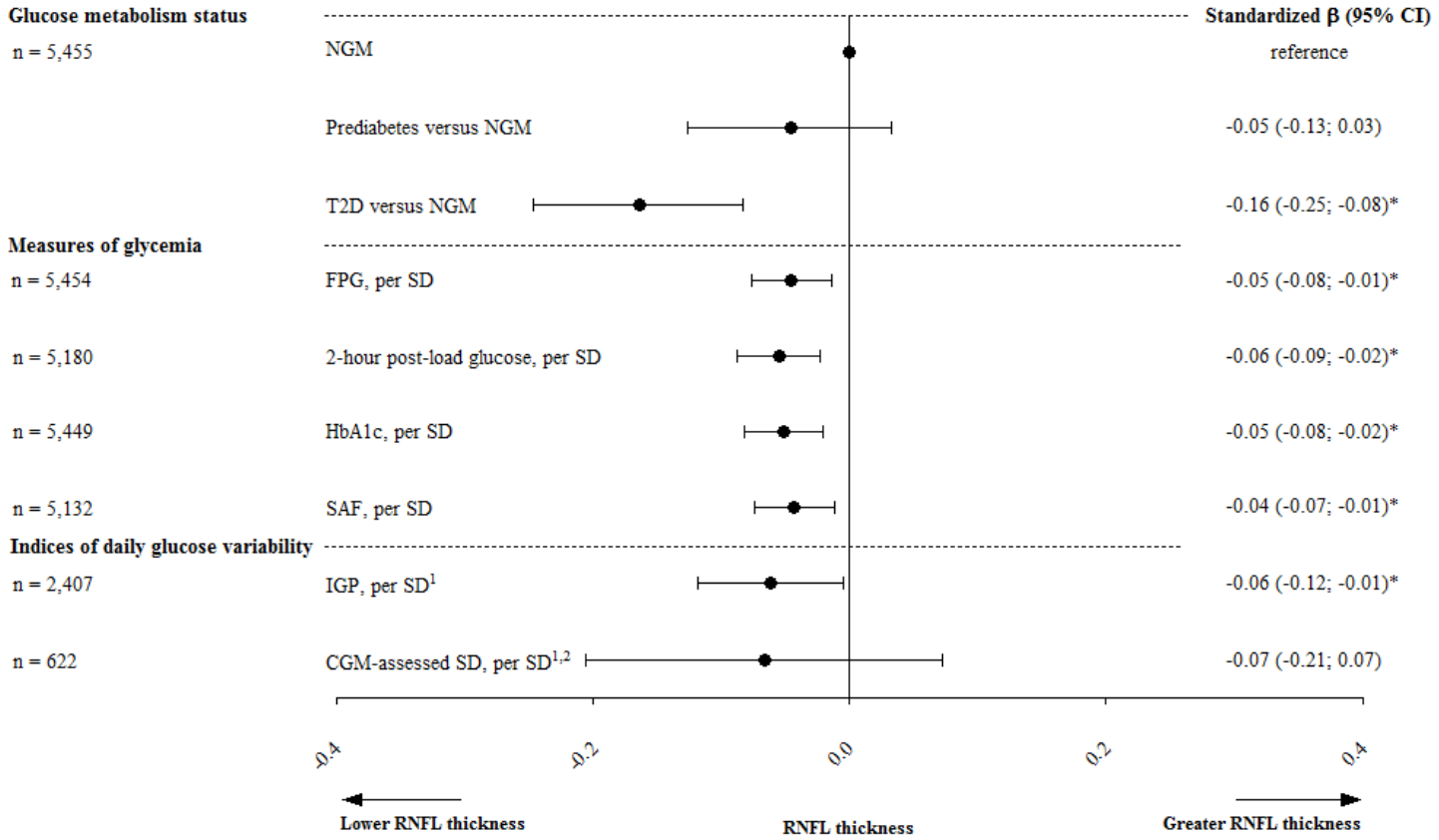

Glucose metabolism status
Standardized β (95% CI)
n = 5,455
NGM
reference
Prediabetes versus NGM
-0.05 (-0.13; 0.03)
T2D versus NGM
-0.16 (-0.25; -0.08)*
Measures of glycemia
n = 5,454
FPG, per SD
-0.05 (-0.08; -0.01)*
n = 5,180
2-hour post-load glucose, per SD
-0.06 (-0.09; -0.02)*
n = 5,449
HbA1c, per SD
-0.05 (-0.08; -0.02)*
n = 5,132
SAF, per SD
-0.04 (-0.07; -0.01)*
Indices of daily glucose variability
n = 2,407
IGP, per SD[1]
-0.06 (-0.12; -0.01)*
n = 622
CGM-assessed SD, per SD[1,2]
-0.07 (-0.21; 0.07)
-0.4
-0.2
0.0
0.2
0.4
Lower RNFL thickness
RNFL thickness
Greater RNFL thickness

Figure 2.2 Associations between glucose metabolism status, measures of glycaemia and indices of daily glucose variability with RNFL thickness (per SD). Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for type 2 diabetes or prediabetes status versus NGM status, or per SD greater measure of glycaemia or index of daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and SAF study populations, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for SAF. For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak. For CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation. Variables entered in the models in addition to glucose metabolism status, measures of glycaemia, or indices of daily glucose variability: age, sex, and educational status (low, medium, high), office systolic blood pressure, total cholesterol to HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), waist circumference, smoking status (current, ever, never), and alcohol consumption status (none, low, high). SD, standard deviation; CI, confidence interval; NGM, normal glucose metabolism; T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; SAF, skin autofluorescence; IGP, incremental glucose peak; CGM, continuous glucose monitoring. * Indicates statistically significant ($p<0.05$). [1] The associations of indices of daily glucose variability with RNFL thickness were additionally adjusted for HbA1c (IGP) or mean sensor glucose (CGM-assessed SD). [2] Additionally, and

Table 2.2 Associations of glucose metabolism status, measures of glycaemia, and indices of daily glucose variability with RNFL thickness.

		RNFL, per SD			
	Number of participants	**Model 1 stβ (95% CI)**	**Model 2 stβ (95% CI)**	**Model 3 stβ (95% CI)**	**Model 3 + HbA1c/MSG stβ (95% CI)**
Glucose metabolism status					
Prediabetes versus NGM	5,455	-0.07 (-0.15; 0.003)	-0.05 (-0.12; 0.03)	-0.05 (-0.13; 0.03)	NA
Type 2 diabetes versus NGM	5,455	**-0.19 (-0.25; -0.12)**	**-0.16 (-0.26; -0.09)**	**-0.16 (-0.25; -0.08)**	NA
Measures of glycaemia					
Fasting plasma glucose, per SD	5,454	**-0.07 (-0.09; -0.04)**	**-0.05 (-0.08; -0.02)**	**-0.05 (-0.08; -0.01)**	NA
2-hour post-load glucose, per SD	5,180	**-0.07 (-0.09; -0.04)**	**-0.06 (-0.09; -0.03)**	**-0.06 (-0.09; -0.02)**	NA
HbA1c, per SD	5,449	**-0.06 (-0.09; -0.03)**	**-0.05 (-0.08; -0.02)**	**-0.05 (-0.08; -0.02)**	NA
Skin autofluorescence, per SD	5,132	**-0.06 (-0.08; -0.03)**	**-0.04 (-0.04; -0.01)**	**-0.04 (-0.07; -0.01)**	NA
Indices of daily glucose variability					
Incremental glucose peak, per SD	2,407	**-0.09 (-0.13; -0.05)**	**-0.07 (-0.11; -0.03)**	**-0.06 (-0.11; -0.01)**	**-0.06 (-0.12; -0.01)**
CGM-assessed standard deviation, per SD	622	**-0.09 (-0.18; -0.01)**	**-0.09 (-0.17; -0.001)**	-0.08 (-0.17; 0.01)	-0.07 (-0.21; 0.07)

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for type 2 diabetes or prediabetes versus NGM or per SD greater measure of glycaemia or index of daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations, 1 SD corresponds with 10.8 μm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence. For incremental glucose peak, 1 SD corresponds with 11.1 μm for RNFL thickness and 2.9 mmol/L for incremental glucose peak. For the CGM-assessed standard deviation, 1 SD corresponds with 10.8 μm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation. Bold denotes P<0.05. Variables entered in the models in addition to glucose metabolism status, measures of glycaemia, or indices of daily glucose variability: model 1: none (crude results); model 2: age, sex, and educational status (low, medium, high); model 3: model 2 + office systolic blood pressure, total cholesterol to HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), waist circumference, smoking status (current, ever, never), and alcohol consumption status (none, low, high). In addition, only for incremental glucose peak, model 3 was additionally adjusted for HbA1c (model 3 + HbA1c), and only for CGM-assessed standard deviation, model 3 was additionally adjusted for MSG (model 3 + MSG). Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. AU, arbitrary unit; CI, confidence interval; CGM, continuous glucose monitoring; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; NA, not applicable; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer; MSG, mean sensor glucose.

Discussion

The present population-based study had two main findings. First, a more adverse GMS, greater glycaemia (estimated from FPG, 2-hour post-load glucose, HbA1c, and SAF) and greater daily glucose variability (estimated from IGP and CGM-assessed standard deviation) were all linearly and—except for CGM-assessed standard deviation—statistically significantly associated with lower RNFL thickness. Second, the associations between indices of daily glucose variability and RNFL thickness did not materially change after additional adjustment for measures of glycaemia.

Our findings are in line with and extend observations from most previous studies.[13,19-24] The present study is the first large population-based study to comprehensively report associations of GMS, measures of glycaemia, and indices of daily glucose variability with RNFL thickness, and also adjust for an extensive set of potential confounders. Additionally, the present study is the first to present associations of SAF, duration of diabetes, and indices of daily glucose variability with RNFL thickness.

Mechanistically, the linearity of the associations of GMS, measures of glycaemia, and indices of daily glucose variability with RNFL thickness most likely reflects the increasing loss of retinal ganglion cells due to both hyperglycaemia-induced neurotoxicity and impairment of functioning of retinal cells that contribute to metabolic regulation.[4,5] Such impairment of metabolic regulation can predispose retinal ganglion cells to ischemia.[5,44] Importantly, retinal ganglion cells are thought to be highly susceptible to ischemia, since they are highly active and have an energy demand that exceeds that of brain cells.[44]

These findings extend our previous work on the "ticking clock hypothesis",[5,9-12] which postulates that hyperglycaemia-induced microvascular and neuronal deterioration is a continuous, gradual process that starts in prediabetes, progresses with the onset of type 2 diabetes, and continues during type 2 diabetes.[8] Indeed, we observed that the regression estimate for prediabetes was in between the estimate for type 2 diabetes and the reference category (i.e. NGM), and was directionally and numerically comparable to our previous findings.[9-12] However, the association between prediabetes and RNFL thickness was not statistically significant, which is most likely due to insufficient statistical power. We, therefore, additionally tested for a linear trend with GMS deterioration by using the statistically more powerful P for trend analysis,[45] which was consistent with a linear decrease in RNFL thickness with more adverse GMS. In support, all measures of glycaemia, regardless of whether they reflect shorter (i.e. FPG, 2-h PG, and HbA1c) or longer (i.e. SAF and duration of diabetes) exposure, were consistently linearly associated with RNFL thickness.

Similarly, a likely explanation why the association between CGM-assessed standard deviation and RNFL thickness was not statistically significant is that the statistical

power to detect any such association was too low.[46] Indeed, we observed that the association between IGP and RNFL thickness, which included almost fourfold the number of participants (n=2,407 versus n=622), was statistically significant. Moreover, the strength of the associations of IGP and CGM-assessed standard deviation with RNFL thickness were numerically analogous.

A probable explanation why the association between daily glucose variability and RNFL thickness was not materially altered after additional adjustment for measures of average glycaemia is that daily glucose variability, measures of glycaemia, and GMS represent different underlying constructs.[26] While daily glucose variability reflects oscillating glucose levels, other measures under study reflect exposure to average chronic levels of glycaemia. Mechanistically, substantial glucose fluctuations entail hyperglycaemic peaks, hypoglycaemic nadirs (in individuals with type 2 diabetes treated with agents that can induce hypoglycaemia), or both, which are thought to be potent inducers of retinal ganglion cell apoptosis.[26,44] Whereas hyperglycaemic peaks may be highly neurotoxic, hypoglycaemic nadirs likely hamper retinal ganglion cell metabolism as their key nutrient is glucose.[44]

Our findings can have several implications for clinical practice. First, the strength of the association between type 2 diabetes and RNFL thickness corresponds with 15 years of aging and, thus, indicates that with respect to neurodegeneration substantial "additional aging" occurs in individuals with type 2 diabetes (Supplemental Table S7.14 shows how this comparison was calculated). Second, RNFL thickness may be a biomarker for the identification of individuals at risk of retinopathy and neuropathy. Use of RNFL thickness measurement is feasible because RNFL thickness assessment is non-invasive,[2] relatively inexpensive[2] and easier to perform than other tests of early neuronal dysfunction such as 24-hour electrocardiogram,[9] magnetic resonance imaging,[10,11] or electromyography.[12] Indeed, RNFL thickness has been found to be a promising early biomarker for other neurodegenerative diseases (e.g., multiple sclerosis).[47] Third, early glycaemic control, possibly already in prediabetes, is likely crucial in the early prevention of microvascular and neuronal complications.[5] Last, our findings add to growing evidence that control of daily glucose variability besides mean glucose concentrations may be important to prevent microvascular complications.[48]

Strengths of this study are 1) the large size of this population-based cohort with oversampling of individuals with type 2 diabetes, which enabled accurate comparison of individuals with and without diabetes; 2) the extensive number of potential confounders that were considered; 3) the use of state-of-the-art and novel methods (e.g., CGM)[26] to assess all variables included in this study; and 4) the considerable number of additional analyses, which generally yielded consistent findings.

The study has certain limitations. First, due to the cross-sectional nature of the study, causal inferences should be made with caution.[49] Mechanistically, hyperglycaemia may

not only lead to neurodegeneration but the reverse may also be true, thus causing a vicious cycle. Intact neurovascular interaction is required for normal microvascular function and impaired microvascular function may aggravate hyperglycaemia.[5, 50] Second, we may have underestimated the strength of the associations of GMS, measures of glycaemia, and daily glucose variability with RNFL thickness if such an association was similar or stronger in participants that were excluded from the study population (who generally tend to be less healthy).[51] The 2-hour post-load glucose and IGP results are most susceptible to this form of selection bias, as no data was available in individuals with the most therapy-intensive diabetes because they were excluded from undergoing an OGTT. Such range restriction may lead to underestimated associations.[51] Third, a single OGTT may misclassify GMS, especially in individuals with prediabetes. Because individuals classified with prediabetes based on their first OGTT are relatively more prone to receive a NGM classification based on their second OGTT,[52] this would likely lead to an underestimation of the association with RNFL thickness in the prediabetes group. Fourth, although we took an extensive set of confounders into account, we cannot fully exclude bias due to unmeasured confounding (e.g., environmental factors such as air pollution).[53] Fifth, due to the relatively low numbers of participants with data on CGM-based glycaemic indices (n=622)), and—to a lesser extent—IGP (n=2,407), statistical power of analyses with these determinants was reduced compared to statistical power of analyses with GMS and measures of glycaemia (n=5,132 to n=5,455).[46] Last, we studied Caucasian individuals aged 40-75 years with access to high-quality diabetes care. Therefore, the generalizability of our results to other populations requires further study.

Conclusions

In summary, the present population-based study demonstrated that adverse GMS, greater glycaemia, and greater daily glucose variability are associated with lower RNFL thickness, i.e. neurodegeneration, independent of demographics, cardiovascular risk factors, and lifestyle risk factors. Hence, these results suggest that RNFL thickness may be an early biomarker for the identification of individuals at risk of retinopathy and neuropathy. Additionally, the combination of early glycaemic monitoring and early glucose-lowering treatment, possibly already in prediabetes, may contribute to the prevention of RNFL thinning and, ultimately, retinopathy and neuropathy.

References

1. Zakin E, Abrams R, Simpson DM. Diabetic Neuropathy. Semin Neurol. 2019;39(5):560-9.
2. Srinivasan S, Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, et al. Optical coherence tomography predicts 4-year incident diabetic neuropathy. Ophthalmic Physiol Opt. 2017;37(4): 451-9.
3. De Clerck EE, Schouten JS, Berendschot TT, Kessels AG, Nuijts RM, Beckers HJ, et al. New ophthalmologic imaging techniques for detection and monitoring of neurodegenerative changes in diabetes: a systematic review. Lancet Diabetes Endocrinol. 2015;3(8):653-63.
4. Tomlinson DR, Gardiner NJ. Glucose neurotoxicity. Nat Rev Neurosci. 2008;9(1):36-45.
5. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67(9):1729-41.
6. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54(6):1615-25.
7. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA. 1990;263(21):2893-8.
8. Wong MS, Gu K, Heng D, Chew SK, Chew LS, Tai ES. The Singapore impaired glucose tolerance follow-up study: does the ticking clock go backward as well as forward? Diabetes Care. 2003;26(11):3024-30.
9. Coopmans C, Zhou TL, Henry RMA, Heijman J, Schaper NC, Koster A, et al. Both Prediabetes and Type 2 Diabetes Are Associated With Lower Heart Rate Variability: The Maastricht Study. Diabetes Care. 2020;43(5):1126-33.
10. van Agtmaal MJM, Houben A, de Wit V, Henry RMA, Schaper NC, Dagnelie PC, et al. Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. Diabetes Care. 2018;41(12):2535-43.
11. Vergoossen LW, Schram MT, de Jong JJ, Stehouwer CD, Schaper NC, Henry RM, et al. White Matter Connectivity Abnormalities in Prediabetes and Type 2 Diabetes: The Maastricht Study. Diabetes Care. 2020;43(1):201-8.
12. van der Velde J, Koster A, Strotmeyer ES, Mess WH, Hilkman D, Reulen JPH, et al. Cardiometabolic risk factors as determinants of peripheral nerve function: the Maastricht Study. Diabetologia. 2020;63(8):1648-58.
13. Huru J, Leiviska I, Saarela V, Liinamaa MJ. Prediabetes influences the structure of the macula: thinning of the macula in the Northern Finland Birth Cohort. Br J Ophthalmol. 2020.
14. Hashimoto S, Yasuda M, Ninomiya T, Hata J, Yoshida D, Tahara-Asakuma T, et al. Foveal and Macular Thickness in a Japanese Population: The Hisayama Study. Ophthalmic Epidemiol. 2016;23(3):202-8.
15. Cheung CY, Chen D, Wong TY, Tham YC, Wu R, Zheng Y, et al. Determinants of quantitative optic nerve measurements using spectral domain optical coherence tomography in a population-based sample of non-glaucomatous subjects. Invest Ophthalmol Vis Sci. 2011;52(13):9629-35.
16. Mauschitz MM, Holz FG, Finger RP, Breteler MMB. Determinants of Macular Layers and Optic Disc Characteristics on SD-OCT: The Rhineland Study. Transl Vis Sci Technol. 2019;8(3):34.
17. Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, et al. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. Ophthalmology. 2018;125(10):1526-36.
18. Lamparter J, Schmidtmann I, Schuster AK, Siouli A, Wasielica-Poslednik J, Mirshahi A, et al. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One. 2018;13(5):e0197682.
19. Ho H, Tham YC, Chee ML, Shi Y, Tan NYQ, Wong KH, et al. Retinal Nerve Fiber Layer Thickness in a Multiethnic Normal Asian Population: The Singapore Epidemiology of Eye Diseases Study. Ophthalmology. 2019;126(5):702-11.
20. Fujiwara K, Yasuda M, Hata J, Hirakawa Y, Hashimoto S, Ueda E, et al. Glucose Tolerance Levels and Circumpapillary Retinal Nerve Fiber Layer Thickness in a General Japanese Population: The Hisayama Study. Am J Ophthalmol. 2019;205:140-6.

21. Duan XR, Liang YB, Friedman DS, Sun LP, Wong TY, Tao QS, et al. Normal macular thickness measurements using optical coherence tomography in healthy eyes of adult Chinese persons: the Handan Eye Study. Ophthalmology. 2010;117(8):1585-94.
22. De Clerck EEB, Schouten J, Berendschot T, Goezinne F, Dagnelie PC, Schaper NC, et al. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. Acta Ophthalmol. 2018;96(2):174-82.
23. De Clerck EEB, Schouten JSAG, Berendschot TTJM, Beckers HJM, Schaper NC, Schram MT, et al. Loss of Temporal Peripapillary Retinal Nerve Fibers in Prediabetes or Type 2 Diabetes Without Diabetic Retinopathy: The Maastricht Study. Invest Ophth Vis Sci. 2017;58(2).
24. Khawaja AP, Chua S, Hysi PG, Georgoulas S, Currant H, Fitzgerald TW, et al. Comparison of Associations with Different Macular Inner Retinal Thickness Parameters in a Large Cohort: The UK Biobank. Ophthalmology. 2020;127(1):62-71.
25. Jud P, Sourij H. Therapeutic options to reduce advanced glycation end products in patients with diabetes mellitus: A review. Diabetes Res Clin Pract. 2019;148:54-63.
26. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care. 2017;40(12):1631-40.
27. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29(6):439-51.
28. World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. . Geneva, Switzerland, . 2006.
29. van Eupen MG, Schram MT, van Sloten TT, Scheijen J, Sep SJ, van der Kallen CJ, et al. Skin Autofluorescence and Pentosidine Are Associated With Aortic Stiffening: The Maastricht Study. Hypertension. 2016;68(4):956-63.
30. Foreman YD, Brouwers MCGJ, van der Kallen CJH, Pagen DME, van Greevenbroek MMJ, Henry RMA, et al. Glucose Variability Assessed with Continuous Glucose Monitoring: Reliability, Reference Values, and Correlations with Established Glycemic Indices-The Maastricht Study. Diabetes Technol Ther. 2020;22(5):395-403.
31. Hong JT, Sung KR, Cho JW, Yun SC, Kang SY, Kook MS. Retinal nerve fiber layer measurement variability with spectral domain optical coherence tomography. Korean J Ophthalmol. 2012;26(1): 32-8.
32. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N, et al. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16(8).
33. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, et al. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20(13):2289-99.
34. van Dongen MC, Wijckmans-Duysens NEG, den Biggelaar LJ, Ocke MC, Meijboom S, Brants HA, et al. The Maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the Maastricht study. Nutrition. 2019;62:39-46.
35. van der Berg JD, Willems PJ, van der Velde JH, Savelberg HH, Schaper NC, Schram MT, et al. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. J Sports Sci. 2016;34(19):1867-73.
36. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, et al. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-6.
37. Rodbard D. The challenges of measuring glycemic variability. J Diabetes Sci Technol. 2012;6(3): 712-5.
38. Bikbov MM, Kazakbaeva GM, Gilmanshin TR, Zainullin RM, Arslangareeva, II, Salavatova VF, et al. Axial length and its associations in a Russian population: The Ural Eye and Medical Study. PLoS One. 2019;14(2):e0211186.
39. Haeger A, Costa AS, Schulz JB, Reetz K. Cerebral changes improved by physical activity during cognitive decline: A systematic review on MRI studies. Neuroimage Clin. 2019;23:101933.
40. Poulose SM, Miller MG, Scott T, Shukitt-Hale B. Nutritional Factors Affecting Adult Neurogenesis and Cognitive Function. Adv Nutr. 2017;8(6):804-11.

41. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20(4):488-95.
42. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. Diabetes Care. 2019;42(8):1593-603.
43. Vatcheva KP, Lee M, McCormick JB, Rahbar MH. Multicollinearity in Regression Analyses Conducted in Epidemiologic Studies. Epidemiology (Sunnyvale). 2016;6(2).
44. Yu DY, Cringle SJ, Balaratnasingam C, Morgan WH, Yu PK, Su EN. Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. Prog Retin Eye Res. 2013;36:217-46.
45. Greenland S. Avoiding power loss associated with categorization and ordinal scores in dose-response and trend analysis. Epidemiology. 1995;6(4):450-4.
46. Jones SR, Carley S, Harrison M. An introduction to power and sample size estimation. Emerg Med J. 2003;20(5):453-8.
47. Britze J, Frederiksen JL. Optical coherence tomography in multiple sclerosis. Eye (Lond). 2018;32(5):884-8.
48. Ceriello A. Glucose Variability and Diabetic Complications: Is It Time to Treat? Diabetes Care. 2020;43(6):1169-71.
49. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 2005;95 Suppl 1:S144-50.
50. Toth P, Tarantini S, Csiszar A, Ungvari Z. Functional vascular contributions to cognitive impairment and dementia: mechanisms and consequences of cerebral autoregulatory dysfunction, endothelial impairment, and neurovascular uncoupling in aging. Am J Physiol Heart Circ Physiol. 2017;312(1):H1-H20.
51. Bland JM, Altman DG. Correlation in restricted ranges of data. BMJ. 2011;342:d556.
52. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, et al. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia. 1996;39(3):298-305.
53. Sharma S, Wakode S, Sharma A, Nair N, Dhobi M, Wani MA, et al. Effect of environmental toxicants on neuronal functions. Environ Sci Pollut Res Int. 2020;27(36):44906-21.

Supplemental materials

Supplemental methods

Performance of optical coherence tomography (OCT) scans

For a subset of participants (n=227) OCT scans were performed as part of a catch up visit. For these participants only there was a median time interval of 4.4 (interquartile range 4.0-5.9) years between OCT and all other measurements. We checked whether this affected the associations and this was not the case (data not shown).

Grading of OCT circle scans

OCT scans were considered of sufficient quality if all the following criteria were met: good centring of the circular scan on the optic nerve head (examples of good, poor and very poor centring are shown in Supplemental Figure S27.1); complete (data of all 768 voxels was available); automatic quality ≥15 dB (an example of a scan with poor quality imaging is shown in Supplemental Figure S2); no measurement error present (examples of all assessed measurement errors are shown in Supplemental Figure S2.2). The percentage of agreement for selection of scans with sufficient quality ranged between 90% and 94% for four trained graders and was 70% for one grader (n=50 OCT scans per comparison).

Assessment of advanced glycation endproducts

Advanced glycation endproducts (AGEs) were assessed with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). In brief, the AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to quantify their accumulation in the skin as skin autofluorescence (SAF; arbitrary units [AU]).[1] The AGE Reader illuminates a skin surface of 4 cm^2, shielded from other light, and uses the ratio of the reflection of fluorescent light (wavelength 420 to 600 nm) to non-fluorescent light (300–420 nm) to calculate SAF.
Due to the use of different versions of calibration software absolute SAF values assessed before the 4th September 2012 were 0.5 arbitrary units higher than the SAF values assessed after this date. To realign absolute SAF values we recalculated the SAF values assessed before the 4th of September 2012 by subtracting 0.5 arbitrary units from the SAF values assessed before the 4th September 2012.

Assessment of OGTT-based and CGM-based glycaemic indices

Incremental glucose peak (IGP), maximum glucose peak (i.e. the highest of the seven oral glucose tolerance test [OGTT] time points) and 1-h post-load glucose value were

assessed in venous OGTT-derived samples. We have recently shown that the 1-hour post-load glucose value, maximum glucose peak, and IGP correlated most strongly with continuous glucose monitoring (CGM)-based indices of daily glucose variability[2]. Of these indices, the association between IGP and CGM-measured daily glucose variability indices was most consistent across different GMS strata, and, hence, IGP is considered the preferred OGTT-based index of daily glucose variability.

The rationale and methodology of CGM (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) in The Maastricht Study have been described previously.[2] From 19 September 2016 until 13 September 2018, all new participants were invited to undergo CGM as part of their regular measurements at The Maastricht Study. To accelerate the inclusion process and to ensure the inclusion of a sufficient number of participants with prediabetes and type 2 diabetes, a selected group of participants who had recently visited The Maastricht Study was re-invited to undergo CGM as a separate research visit ('catch-up visit'). For individuals who participated in the catch-up visit, there was an average time period ("visit interval") of 2.1 years between CGM and other measurements. The CGM device was worn on the lower abdomen and recorded subcutaneous interstitial glucose values (range: 2.2-22.2 mmol/L) every five minutes for a 7-day period. Participants were asked to perform self-measurements of blood glucose four times daily (Contour Next; Ascensia Diabetes Care, Mijdrecht, the Netherlands) for retrospective calibration of the CGM device. Participants were blinded to the CGM recording, but not to the self-measured values. Diabetes medication use was allowed during the CGM period, and no dietary or physical activity instructions were given.

The first 24 hours of CGM were excluded, because of insufficient calibration. Next, we excluded individuals of whom less than 24 hours of recording (less than one data day) remained. Then, we calculated mean sensor glucose (mmol/L), standard deviation (mmol/L), coefficient of variation (standard deviation / mean sensor glucose * 100%), and time in range (TIR; i.e. % of time between 3.9–10.0 mmol/L) for the total recording period. Based on international consensus, we used standard deviation and coefficient of variation as indices of daily glucose variability.[3] The CGM-assessed coefficient of variation, which is intrinsically adjusted for mean sensor glucose, was not used as main determinant, as use of a ratio variable may introduce bias.[4,5] TIR is an emerging glycaemic index that is partly determined by daily glucose variability.[6]

Ridge regression

Because we presumed the reliability of 'model 3 + mean sensor glucose' to be negatively impacted by multicollinearity, due to the strong correlation between CGM-assessed standard deviation and mean sensor glucose (rho=0.69),[7] we additionally performed ridge regression. It is a L2-regularized form of linear regression and a valid

statistical method to counter a degree of model instability caused by multicollinearity.[8] Ridge regression estimates are computed according to the combination of the residual sum of squares characteristic of regular linear regression and predefined penalization of the coefficients (i.e. $Ridge = RRS + \frac{1}{n} * \lambda * \sum_{j=1}^{p} \beta_j^2$ where RSS is the residual sum of squares, n is the sample size, λ is the chosen amount of penalization, and $\sum_{j=1}^{p} \beta_j^2$ represents the sum of all squared regression coefficients). As such, it slightly biases the regression coefficients and can strongly reduce inflated variances that arise when high multicollinearity is present. We pragmatically chose the level of penalization based on the lambda (λ) required to reduce the variance inflation factor (VIF) of model 3.1 back to the VIF of model 3 (or halfway back). The ridge regression results are presented as: standardized regression coefficient (stβ) (95%CI), P-value. The median stβs (95%CIs) were estimated with use of resampling (1,000 bootstraps).

Supplemental results

Additional analyses

Quantitatively similar results were observed in a range of sensitivity analyses. First, associations remained similar after we additionally adjusted for physical activity and dietary habits, or spherical equivalent and intraocular pressure (Supplemental Table S2.5). Second, associations were generally comparable when we additionally adjusted for kidney variables, history of cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy, and glaucoma (Supplemental Table S27.5). Third, associations were similar when we excluded participants with retinopathy, glaucoma, or other types of diabetes (Supplemental Table S2.6). Fourth, associations were numerically comparable when we replaced IGP with other OGTT-based indices of glucose variability and CGM-assessed standard deviation with other CGM-assessed measures of daily glucose variability (Supplemental Table S2.7). Fifth, the strength of the association of CGM-assessed mean sensor glucose with RNFL thickness was numerically comparable to the strength of the associations of measures of glycaemia with RNFL thickness (Supplemental Table S2.7). Sixth, associations remained similar after replacement of waist circumference with BMI; of educational status with occupational status or income level; and of office systolic BP with office diastolic BP, or systolic or diastolic 24-hour ambulatory BP (Supplemental Tables S2.8 and S2.9). Seventh, the association between SAF was not altered after additional adjustment for FPG, 2-hour post-load glucose, or HbA1c (Supplemental Table S2.10). Eighth,

associations of IGP and CGM-assessed standard deviation with RNFL thickness were similar after replacement of HbA1c or mean sensor glucose with other measures of glycaemia (Supplemental Table S2.11). Ninth, the associations between CGM-assessed standard deviation and RNFL thickness were similar after exclusion of individuals with insufficient recording days or recording data gaps (Supplemental Table S2.12). Exclusion of participants with a visit interval, most of whom had type 2 diabetes,[2] strongly attenuated the association between CGM-assessed standard deviation and RNFL thickness. Tenth, ridge regression did not yield materially different results for the association of CGM-assessed standard deviation with RNFL thickness (Supplemental Table S2.13). Last, after full adjustment (model 3), longer duration of diabetes was significantly associated with lower RNFL thickness (per SD, -0.07 [-0.13; -0.001]; Supplemental Table S2.14).

References

1. van Eupen MG, Schram MT, van Sloten TT, Scheijen J, Sep SJ, van der Kallen CJ, et al. Skin Autofluorescence and Pentosidine Are Associated With Aortic Stiffening: The Maastricht Study. Hypertension. 2016;68(4):956-63.
2. Foreman YD, Brouwers MCGJ, van der Kallen CJH, Pagen DME, van Greevenbroek MMJ, Henry RMA, et al. Glucose Variability Assessed with Continuous Glucose Monitoring: Reliability, Reference Values, and Correlations with Established Glycemic Indices-The Maastricht Study. Diabetes Technol Ther. 2020;22(5):395-403.
3. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care. 2017;40(12):1631-40.
4. Curran-Everett D. Explorations in statistics: the analysis of ratios and normalized data. Adv Physiol Educ. 2013;37(3):213-9.
5. Rodbard D. Glycemic variability: challenges in interpretation. Diabetes Technol Ther. 2015;17(6):370-2.
6. Rodbard D. Glucose Time In Range, Time Above Range, and Time Below Range Depend on Mean or Median Glucose or HbA_{1c}, Glucose Coefficient of Variation, and Shape of the Glucose Distribution. Diabetes Technol Ther. 2020.
7. Vatcheva KP, Lee M, McCormick JB, Rahbar MH. Multicollinearity in Regression Analyses Conducted in Epidemiologic Studies. Epidemiology (Sunnyvale). 2016;6(2).
8. Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carre G, et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography. 2013;36(1):27-46.

Supplemental figures

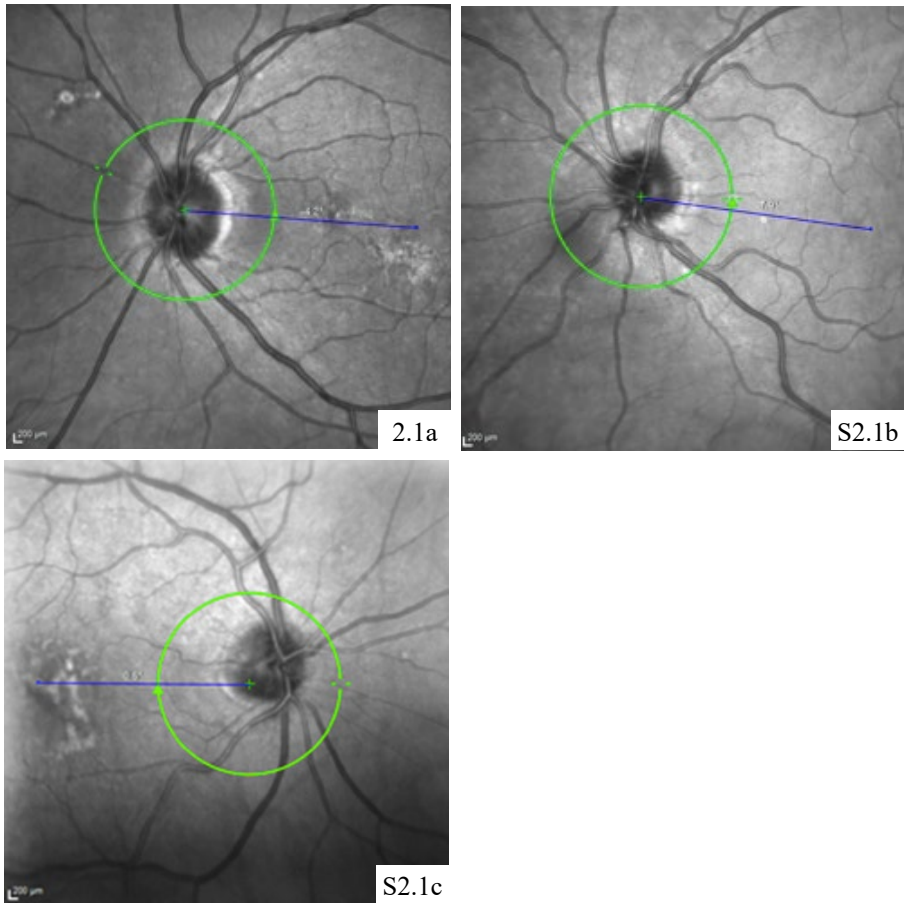
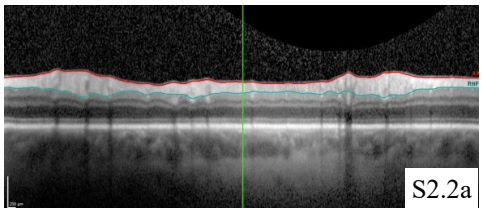
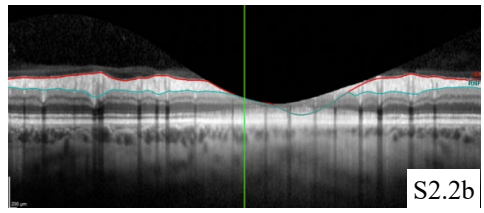
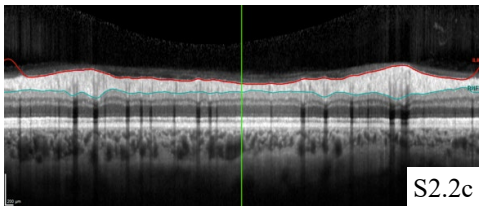
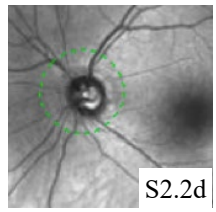


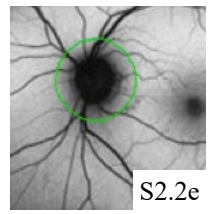
Figure S2.1 Examples of quality of centring of circular scans on the optic nerve head. Supplemental Figure S2.1 shows examples of quality of centring of the circular scan on the optic nerve head: S2.1a shows good quality, S2.1b shows poor quality, and S2.1c shows very poor quality.

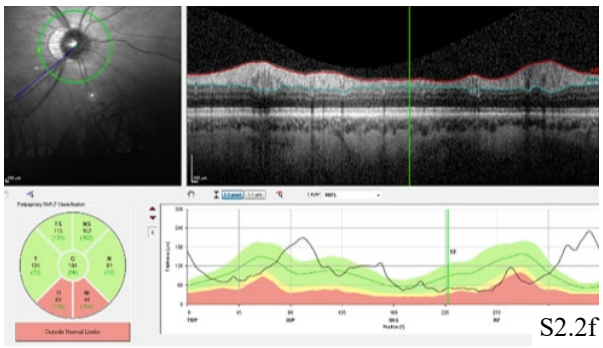












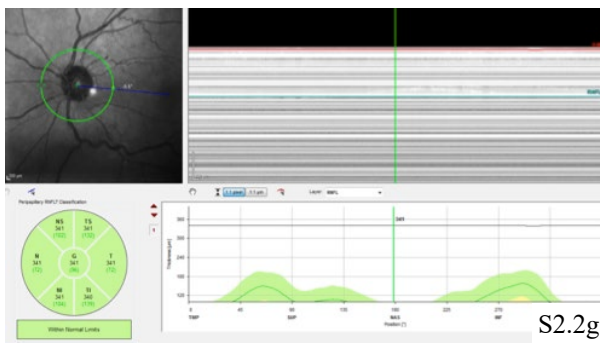


Figure S2.2 Examples of poor quality and scan errors. S2.2a: Example of poor imaging quality (Signal-to-noise ratio<15 dB); S2.2b: OCT device too close to the eye; S2.2c: RNFL layer incorrectly defined; S2.2d: incorrect circle position (dashed line); S2.2e: autofluorescence on; S2.2f: participant does not look in the correct direction; S2.2g: technical problem with OCT device. OCT, optical coherence tomography; RNFL, retinal nerve fibre layer thickness.

Table S2.1 Additional general study population characteristics according to tertiles of retinal nerve fibre layer thickness in the study population with complete data on glucose metabolism status.

Characteristic	Total study population	RNFL thickness			Number of participants with missing data
		Tertile 1 (high)	Tertile 2 (middle)	Tertile 3 (low)	
	(N=5,455)	(N=1,818)	(N=1,819)	(N=1,818)	
BMI (kg/m^2)	26.8 ± 4.4	26.8 ± 4.5	26.7 ± 4.3	26.9 ± 4.3	1
Income level (euro)	1,856 [1,502-2,386]	1,856 [1,502-2,386]	1,875 [1,503-2,386]	1,856 [1,503-2,386]	1,288
Occupational status					3,618
Low	572 (31.1)	195 (34.2)	190 (31.2)	187 (28.4)	
Intermediate	666 (36.3)	207 (36.3)	231 (37.9)	228 (34.7)	
High	599 (32.6)	168 (29.5)	188 (30.9)	243 (36.9)	
Systolic ambulatory 24-hour blood pressure (mmHg)	118.5 ± 11.3	117.8 ± 11.6	118.6 ± 11.2	119.3 ± 11.3	535
Diastolic ambulatory 24-hour blood pressure (mmHg)	72.9 ± 7.1	72.4 ± 7.2	73.0 ± 7.1	73.3 ± 6.9	535
Dutch Healthy diet score (points)	84.0 ± 15.0	84.5 ± 15.1	84.6 ± 15.1	82.8 ± 14.9	388
Physical activity (hours/day)	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.7	768
Spherical equivalent (diopter)	0.13 [-1.19-1.06]	0.6 [-0.4-1.6]	0.1 [-1.1-1.1]	-0.5 [-2.9-0.6]	275
Intraocular pressure (mmHg)	14.0 [11.7-16.0]	13.5 [11.5-15.7]	13.8 [11.5-15.7]	14.3 [12.0-16.5]	319
Glaucoma	234 (4.6)	63 (3.7)	53 (3.1)	118 (6.9)	319
Retinopathy	79 (1.5)	8 (1.3)	5 (0.8)	12 (1.7)	137
History of CVD	1,337 (16.9)	279 (15.4)	293 (16.2)	324(17.9)	101
eGFR, ml/min/1.73m^2	82.3 ± 14.0	83.0 ± 13.8	82.1 ± 13.9	81.9 ± 14.1	3
Urinary albumin excretion (mg/24 hours)	5.4 [3.4-10.0]	5.2 [3.4-9.8]	5.4 [3.4-9.5]	5.5 [3.4-10.6]	23
Albuminuria	424 (7.8)	130 (7.2)	137 (7.6)	157 (8.7)	23
Biomarkers of low-grade inflammation					3,049
C-reactive protein, µg/ml	1.2 [0.6-2.7]	1.2 [0.6-2.7]	1.2 [0.6-2.6]	1.2 [0.7-2.8]	
Serum amyloid A, µg/ml	3.2 [2.0-5.4]	3.2 [2.0-5.5]	3.2 [2.1-5.2]	3.3 [2.1-5.6]	
Interleukin-6, pg/ml	0.6 [0.4-0.9]	0.6 [0.4-0.9]	0.5 [0.4-0.8]	0.6 [0.4-1.0]	
Interleukin-8, pg/ml	4.0 [3.2-5.2]	3.9 [3.2-4.9]	4.1 [3.2-5.2]	4.2 [3.2-5.3]	
Tumor necrosis factor α, pg/ml	2.2 [1.9-2.6]	2.1 [1.8-2.6]	2.2 [1.9-2.5]	2.2 [1.9-2.6]	
Duration of diabetes (years)	4.0 [0-9.0]	3.0 [0-9.0]	3.0 [0-8.0]	4.0 [1.0-10.8]	257
Maximum glucose peak (mmol/L)	9.5 [7.9-12.4]	9.3 [7.8-11.7]	9.5 [7.9-12.2]	9.8 [8.1-13.4]	3,048
1-hour post-load glucose (mmol/L)	8.6 [6.5-11.9]	8.4 [6.3-11.2]	8.6 [6.6-11.7]	8.8 [6.7-12.6]	2,924

Table S2.1 (continued)

Characteristic	Total study population (N=5,455)	RNFL thickness Tertile 1 (high) (N=1,818)	Tertile 2 (middle) (N=1,819)	Tertile 3 (low) (N=1,818)	Number of participants with missing data
CGM: indices and methodology					4,833
Mean sensor glucose, mmol/L	6.1 [5.9-6.8]	6.1 [5.7-6.6]	6.0 [5.7-6.7]	6.1 [5.7-6.9]	
Coefficient of variation, %	14.5 [11.7-17.8]	14.6 [11.6-17.4]	14.1 [11.5-17.6]	14.8 [11.9-18.7]	
Time in range, %	99.7 [96.8-100.0]	99.7 [96.8-100.0]	99.7 [97.8-100.0]	99.7 [96.1-100.0]	
CGM during regular visit	409 (65.8)	123 (63.4)	135 (65.9)	151 (67.7)	
CGM during 'catch-up visit'	213 (34.2)	71 (36.6)	70 (34.1)	72 (32.2)	
Insufficient recording day, yes vs. no	8 (1.3)	1 (0.5)	2 (1.0)	5 (2.2)	
CGM recording data gap, yes vs. no	49 (7.9)	18 (9.3)	14 (6.8)	17 (7.6)	
CGM visit interval* (years)	2.1 [2.0-2.2]	2.1 [2.0-2.2]	2.1 [2.1-2.2]	2.1 [2.0-2.2]	

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). BMI, body-mass index; CGM, continuous glucose monitoring, CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate. * Of 'catch-up visit' participants only (n=213).

Table S2.2 General study population characteristics of the included and excluded participants for the study population of glucose metabolism status.

	Included study population (n= 5,455)	Missing data in/excluded	Excluded study population (n=2,550)
Characteristic			
Age (years)	59.5 ± 8.6	0/0	60.6 ± 8.7
Men	2,665 (48.9)	0/0	1356 (53.2)
Educational level		0/116	
Low	1,914 (35.1)		821 (33.7)
Medium	1,519 (27.8)		647 (26.6)
High	2,022 (37.1)		966 (39.7)
Glucose metabolism status		0/0	
NGM	3,366 (61.7)		1,479 (58.0)
Prediabetes	820 (15.0)		370 (14.5)
Type 2 diabetes	1,239 (22.7)		681 (26.7)
Other type of diabetes	30 (0.5)		20 (0.8)
Measures of glycaemia			
Fasting plasma glucose (mmol/L)	5.9 ± 1.5	1/0	6.0 ± 1.8
2-hour post-load glucose (mmol/L)	6.2 [4.9-8.6]	275/212	6.2 [4.9-8.8]
HbA1c (mmol/mol)	39.2 ± 9.1	6/8	40.6 ± 10.4
HbA1c (%)	5.7 ± 0.8	6/8	5.9 ± 1.0
SAF (AU)	2.2 ± 0.5	323/296	2.2 ± 0.5
Indices of daily glucose variability			
Incremental glucose peak (mmol/L)	4.1 [2.7-6.5]	3048/1368	4.2 [2.7-6.6]
CGM-assessed standard deviation (mmol/L)	0.86 [0.68-1.21]	4833/2319	0.79 [0.68-1.08]
Use of glucose-modifying medication, yes vs. no	927 (17.0)	0/6	536 (21.1)
Duration of diabetes (years)	4.0 [0-9.0]	257/155	4.5 [1.0-10.0]
Waist circumference, men (cm)	100.4 ± 11.6	0/2	102.1 ± 12.5
Waist circumference, women (cm)	89.1 ± 12.5	0/3	90.7 ± 13.7
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	0/4	3.5 [2.8-4.4]
Use of lipid-modifying medication, yes vs. no	1,687 (30.9)	0/6	864 (34.0)
Office systolic blood pressure (mmHg)	133.2 ± 17.7	0/3	134.6 ± 18.3
Office diastolic blood pressure (mmHg)	75.5 ± 9.8	1/3	75.4 ± 9.8
Use of antihypertensive medication, yes vs. no	1,983 (36.4)	0/6	1039 (40.8)
Smoking status		0/65	
Never	2,101 (38.5)		858 (34.5)
Former	2,666 (48.9)		1253 (50.4)
Current	688 (12.6)		374 (15.1)
Alcohol consumption		0/66	
None	995 (18.2)		478 (19.2)
Moderate	3,181 (58.3)		1,447 (58.3)
High	1,279 (23.4)		559 (22.5)
RNFL thickness (μm)	94.8 ± 10.8	0/2454*	95.1 ± 11.5

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). * Number of participants with missing data represents the number of participants that missed RNFL thickness assessment in both eyes or had a RNFL thickness assessment of insufficient quality for both eyes. CGM, continues glucose monitoring; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fibre layer; SAF, skin autofluorescence AU, arbitrary units; NA, not applicable.

Table S2.3 General study population characteristics of the included and excluded participants for the study populations of indices of daily glucose variability (incremental glucose peak and continuous glucose monitoring-assessed standard deviation).

	Incremental glucose peak			Continuous glucose monitoring-assessed standard deviation		
	Included study Population (N=2,407)	**Missing data in/excluded**	**Excluded study population (N=5,598)**	**Included study population (N=622)**	**Missing data in/excluded**	**Excluded study population (N=7383)**
Characteristic						
Age (years)	59.5 ± 8.2	0/0	60.0 ± 8.9	59.8 ± 8.5	0/0	59.8 ± 8.7
Men	1,211 (50.3)	0/0	2810 (50.2)	318 (51.1)	0/0	3703 (50.2)
Educational level		0/116			0/116	
Low	794 (33.0)		1941 (35.4)	205 (33.0)		2530 (34.8)
Medium	691 (28.7)		1475 (26.9)	168 (27.0)		1998 (27.5)
High	922 (38.3)		2066 (37.7)	249 (40.0)		2739 (37.7)
Glucose metabolism status		0/0			0/0	
NGM	1,455 (60.4)		3,390 (60.6)	328 (52.7)		4517 (61.2)
Prediabetes	430 (17.9)		760 (13.6)	140 (22.5)		1050 (14.2)
Type 2 diabetes	522 (21.7)		1398 (25.0)	152 (24.4)		1768 (23.9)
Other type of diabetes	0 (0.0)		50 (0.9)	2 (0.3)		48 (0.7)
Measures of glycaemia						
Fasting plasma glucose (mmol/L)	5.8 ± 1.1	0/1	6.0 ± 1.8	5.9 ± 1.5	0/1	5.9 ± 1.6
2-hour post-load glucose (mmol/L)	6.4 [5.1-9.1]	0/487	6.1 [4.9-8.4]	6.8 [5.3-9.4]	29/458	6.1 [4.9-8.6]
HbA1c (mmol/mol)	38.9 ± 6.9	0/14	40.0 ± 10.5	39.6 ± 8.8	0/14	39.7 ± 9.6
HbA1c (%)	5.7 ± 0.6	0/14	5.8 ± 1.0	5.8 ± 0.8	0/14	5.8 ± 0.9
SAF (AU)	2.2 ± 0.4	112/507	2.2 ± 0.5	2.1 ± 0.5	49/570	2.2 ± 0.5
Indices of daily glucose variability						
Incremental glucose peak (mmol/L)	4.1 [2.7-6.5]	0/4416	4.2 [2.7-6.6]	4.4 [3.1-6.9]	75/4341	4.1 [2.7-6.4]
CGM-assessed standard deviation (mmol/L)	0.84 [0.68-1.16]	1860/5292	0.84 [0.69-1.23]	0.86 [0.68-1.21]	0/7152	0.79 [0.68-1.08]
Use of glucose-modifying medication, yes vs. no	350 (14.5)	0/6	1113 (19.9)	88 (14.1)	0/6	1375 (18.6)
Duration of diabetes	2.0 [0.0-6.0]	97/315	5.0 [1.0-11.0]	1.0 [0.0-4.0]	22/390	4.0 [1.0-10.0]
Waist circumference, men (cm)	100.7 ± 11.3	0/2	101.1 ± 12.2	102.6 ± 11.8	0/2	100.8 ± 12.0
Waist circumference, women (cm)	89.4 ± 12.2	0/3	89.6 ± 13.2	90.8 ± 12.4	0/3	89.4 ± 12.9
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	0/4	3.4 [2.8-4.3]	3.5 [2.8-4.4]	0/4	3.4 [2.8-4.2]

Table S2.3 (continued)

	Incremental glucose peak			Continuous glucose monitoring-assessed standard deviation		
	Included study Population (N=2,407)	**Missing data in/excluded**	**Excluded study population (N=5,598)**	**Included study population (N=622)**	**Missing data in/excluded**	**Excluded study population (N=7383)**
Use of lipid-modifying medication, yes vs. no	735 (30.5)	0/6	1816 (32.5)	159 (25.6)	0/6	2392 (32.4)
Office systolic blood pressure (mmHg)	134.0 ± 17.8	0/3	133.5 ± 18.0	134.1 ± 18.3	0/3	133.6 ± 17.9
Office diastolic blood pressure (mmHg)	76.3 ± 10.0	0/4	75.2 ± 9.7	75.7 ± 10.2	0/4	75.5 ± 9.8
Use of antihypertensive medication, yes vs. no	878 (36.5)	0/6	2144 (38.3)	231 (37.1)	0/6	2791 (37.8)
Smoking status		0/65			0/65	
Never	863 (35.9)		2096 (37.9)	238 (38.3)		2721 (37.2)
Former	1,238 (51.4)		2681 (48.5)	308 (49.5)		3611 (49.3)
Current	306 (12.7)		756 (13.7)	76 (12.2)		986 (13.5)
Alcohol consumption		0/66			0/66	
None	401 (16.7)		1072 (19.4)	104 (16.7)		1369 (18.7)
Moderate	399 (58.1)		3,229 (58.4)	400 (64.3)		4228 (57.8)
High	607 (25.2)		1231 (22.3)	118 (19.0)		1720 (23.5)
RNFL thickness (µm)	94.5 ± 11.1	0/2454*	95.1 ± 10.6	94.5 ± 10.8	0/2454*	94.9 ± 10.9

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). * Number of participants with missing data represents the number of participants that missed RNFL thickness assessment in both eyes or had a RNFL thickness assessment of insufficient quality for both eyes. CGM, continues glucose monitoring; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fibre layer; SAF, skin autofluorescence AU, arbitrary units; NA, not applicable.

Table S2.4 P-values of interaction terms with glucose metabolism status and sex.

	Number of participants	Prediabetes P-value	Type 2 diabetes P-value	Sex P-value
Determinant				
Prediabetes versus NGM	5,455	NA	NA	0.07
Type 2 diabetes versus NGM	5,455	NA	NA	0.49
Fasting plasma glucose	5,454	0.81	0.85	0.69
2-hour post-load glucose	5,180	0.61	0.77	0.26
HbA1c	5,449	0.27	0.77	0.98
Skin autofluorescence	5,132	0.97	0.90	0.35
Incremental glucose peak	2,407	0.30	0.76	0.76
CGM-assessed standard deviation	622	0.59	0.55	0.85

P-values represent the P-values for the interaction terms of sex, glucose metabolism status (i.e. prediabetes versus normal glucose metabolism status or type 2 diabetes versus normal glucose metabolism status) with determinants (e.g., sex*HbA1c) in the associations of glucose metabolism status, measures of glycaemia, incremental glucose peak, and CGM-assessed standard deviation with RNFL thickness. Variables in the model in addition to determinants and interaction term(s) with sex, glucose metabolism status and are: age, sex, educational status, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, waist circumference, smoking status, and alcohol consumption status. In addition, for interaction analyses with glucose metabolism status, glucose metabolism status was also entered in the model. Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. P-value <0.05 denotes statistically significant interaction. CGM, continues glucose monitoring; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; NA, not applicable; NGM, normal glucose metabolism; RNFL, retinal nerve fibre layer.

Table S2.5 Associations of GMS, measures of glycaemia, and indices of daily glucose variability with RNFL thickness after additional adjustment for dietary intake and physical activity (model 4A), for spherical equivalent and intraocular pressure (model 4B), or for eGFR, urinary albumin excretion, history of CVD, plasma biomarkers of low-grade inflammation, retinopathy, and glaucoma (model 4C).

	RNFL thickness, per SD					
	Model 4A*		Model 4B		Model 4C	
	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)
Glucose metabolism status						
Prediabetes versus NGM	4,353	-0.07 (-0.16; 0.02)	5,107	-0.06 (-0.14; 0.02)	2,148	-0.08 (-0.20; 0.05)
Type 2 diabetes versus NGM	4,353	**-0.17 (-0.26; -0.08)**	5,107	**-0.15 (-0.23; -0.07)**	2,148	**-0.20 (-0.32; -0.07)**
Measures of glycaemia						
Fasting plasma glucose, per SD	4,352	**-0.04 (-0.07; -0.002)**	5,106	**-0.03 (-0.06; -0.002)**	2,147	-0.04 (-0.08; 0.01)
2-hour post-load, per SD	4,130	**-0.06 (-0.10; -0.02)**	4,873	**-0.05 (-0.08; -0.02)**	2,015	**-0.07 (-0.12; -0.02)**
HbA1c, per SD	4,348	**-0.04 (-0.08; -0.01)**	5,102	**-0.04 (-0.07; -0.01)**	2,145	-0.04 (-0.09; 0.01)
Skin autofluorescence, per SD	4,091	**-0.04 (-0.08; -0.01)**	4,805	**-0.04 (-0.07; -0.01)**	2,071	**-0.07 (-0.12; -0.02)**
Indices of daily glucose variability						
Incremental glucose peak, per SD	1,187	**-0.08 (-0.13; -0.02)**	2,251	-0.03 (-0.08; 0.02)	1,637	-0.05 (-0.10; 0.01)
Model 4 + HbA1c	1,187	**-0.08 (-0.15; -0.01)**	2,251	-0.03 (-0.09; 0.02)	1,637	-0.06 (-0.13; 0.01)
CGM- assessed standard deviation, per SD	439	-0.09 (-0.20; 0.02)	615	-0.06 (-0.15; 0.02)	592	-0.06 (-0.15; 0.03)†
Model 4 + MSG	439	-0.08 (-0.24; 0.08)	615	-0.07 (-0.19; 0.06)	592	-0.07 (-0.21; 0.07)†

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for individuals with type 2 diabetes or prediabetes versus NGM or per SD greater measure of glycaemia or daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations 1 SD corresponds with 10.8 µm for RNFL thickness, and (respectively) 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence (model 4A). For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak (model 4A). For CGM-assessed standard deviation, 1 SD corresponds with 10.6 µm for RNFL thickness and 0.62 mmol/L for CGM-assessed standard deviation (model 4A). In models 4B and 4C values per SD were numerically comparable. Bold denotes P<0.05. Variables in model 3: age, sex, educational status, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, waist circumference, smoking status, and alcohol consumption status. Incremental glucose peak was additionally adjusted for HbA1c. CGM-assessed standard deviation was additionally adjusted for MSG. Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. *Diet intake was entered in the model as diet score minus the alcohol component to avoid multicollinearity. †Plasma biomarkers of low-grade inflammation were not available in the CGM study population, and were, thus, not included in the analyses. AU, arbitrary unit; CGM, continuous glucose monitoring; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; MSG, mean sensor glucose; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.6 Associations of glucose metabolism status, measures of glycaemia, and indices of daily glucose variability with RNFL thickness after exclusion of individuals with retinopathy (model 3A), glaucoma (model 3B), or other types of diabetes (model 3C).

	RNFL thickness, per SD					
	Model 3A		Model 3B		Model 3C	
	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)
Glucose metabolism status						
Prediabetes versus NGM	5,239	-0.06 (-0.14; 0.02)	4,902	-0.02 (-0.11; 0.06)	5,425	-0.04 (-0.12; 0.04)
Type 2 diabetes versus NGM	5,239	**-0.17 (-0.26; -0.09)**	4,902	**-0.19 (-0.27; -0.10)**	5,425	**-0.16 (-0.24; -0.08)**
Measures of glycaemia						
Fasting plasma glucose, per SD	5,238	**-0.05 (-0.08; -0.02)**	4,901	**-0.05 (-0.08; -0.01)**	5,424	**-0.04 (-0.07; -0.01)**
2-hour post-load glucose, per SD	5,010	**-0.06 (-0.09; -0.02)**	4,689	**-0.06 (-0.10; -0.03)**	5,180	**-0.06 (-0.09; -0.02)**
HbA1c, per SD	5,233	**-0.06 (-0.09; -0.03)**	4,897	**-0.05 (-0.08; -0.02)**	5,419	**-0.05 (-0.08; -0.02)**
Skin autofluorescence, per SD	4,927	**-0.05 (-0.08; -0.01)**	4,616	**-0.04 (-0.07; -0.01)**	5,103	**-0.04 (-0.07; -0.01)**
Indices of daily glucose variability						
Incremental glucose peak, per SD	2,322	**-0.06 (-0.11; -0.01)**	1,542	**-0.05 (-0.11; -0.01)**	2,407	**-0.06 (-0.11; -0.01)**
Model 3 + HbA1c	2,322	**-0.06 (-0.12; -0.01)**	1,542	-0.05 (-0.12; 0.02)	2,407	**-0.06 (-0.12; -0.01)**
CGM- assessed standard deviation, per SD	594	-0.08 (-0.18; 0.01)	564	-0.07 (-0.17; 0.03)	620	-0.08 (-0.17; 0.01)
Model 3 + MSG	594	-0.06 (-0.20; 0.08)	564	-0.07 (-0.22; 0.07)	620	-0.06 (-0.19; 0.08)

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for individuals with type 2 diabetes or prediabetes versus individuals with NGM or per SD greater measure of glycaemia or daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations, 1 SD corresponds with 10.8 µm for RNFL thickness,1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence (model 3A). For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak (model 3A). For CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.57 mmol/L for CGM-assessed standard deviation (model 3A). In models 3B and 3C values per SD were numerically comparable. Bold denotes $p<0.05$. Variables in model 3: age, sex, educational status, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, waist circumference, smoking status, and alcohol consumption status. Incremental glucose peak was additionally adjusted for HbA1c (model 3 + HbA1c). CGM-assessed standard deviation was additionally adjusted for MSG (model 3 + MSG). Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. AU, arbitrary unit; CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; MSG, mean sensor glucose; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.7 Associations or OGTT-based indices of daily glucose variability and CGM-based measures with RNFL thickness.

		RNFL thickness, per SD				
	Number of participants	**Model 1 stβ (95% CI)**	**Model 2 stβ (95% CI)**	**Model 3 stβ (95% CI)**	**Model 3 + HbA1c stβ (95% CI)**	**Model 3 + SD stβ (95% CI)**
Other OGTT-based measures						
Maximum glucose peak, per SD	2,407	**-0.09 (-0.13; -0.05)**	**-0.07 (-0.11; -0.03)**	**-0.05 (-0.10; -0.01)**	-0.06 (-0.12; 0.004)	NA
1-hour post-load glucose, per SD	2,531	**-0.07 (-0.11; -0.03)**	**-0.05 (-0.10; -0.01)**	-0.04 (-0.08; 0.01)	-0.03 (-0.09; 0.03)	NA
CGM-based measures						
Mean sensor glucose, per SD	622	**-0.10 (-0.18; -0.01)**	-0.08 (-0.17; 0.004)	-0.07 (-0.17, 0.03)	NA	-0.02 (-0.16; 0.13)
Coefficient of variation, per SD	622	-0.07 (-0.15; 0.01)	-0.07 (-0.15; 0.02)	-0.06 (-0.15; 0.02)	NA	NA
Time in range, per SD	622	0.06 (-0.03; 0.14)	0.05 (-0.04; 0.13)	0.03 (-0.06; 0.12)	NA	NA

Results (β [95% confidence interval]) represent the difference in retinal nerve fibre layer thickness (in SD) for one SD greater exposure to a determinant. For incremental glucose peak and maximum glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness, 2.9 mmol/L for incremental glucose peak and 3.9 mmol/L for maximum glucose peak. For 1-hour post-load glucose, 1 SD corresponds with 11.0 µm for RNFL thickness and 4.0 mmol/L for 1-hour post-load glucose. For the CGM-based measures, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.3 mmol/L for mean sensor glucose, 5.6% for coefficient of variation, and 12.9% for time in range. Model 1 was not adjusted for potential confounders (crude); model 2 was adjusted for age, sex, and education level [low, middle, high]; model 3 was additionally adjusted for waist circumference, office systolic blood pressure, antihypertensive medication, total cholesterol to HDL cholesterol ratio, use of lipid-modifying medication, smoking status [current, ever, never], and alcohol consumption status [none, low, high]; model 3 + HbA1c was additionally adjusted for HbA1c (in case of maximum glucose peak, 1-hour post-load glucose). Model 3 + SD was additionally adjusted for CGM-assessed standard deviation (only applicable for mean sensor glucose). Additionally, and only for CGM-assessed indices, we entered 'visit interval' in model 1. AU, arbitrary unit; CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.8 Associations of glucose metabolism status, measures of glycaemia, and indices of daily glucose variability with RNFL thickness, where waist circumference was replaced with BMI (model 3A) or educational level was replaced with occupational status (model 3B) or income level (model 3C).

	RNFL thickness, per SD					
	Model 3A		Model 3B		Model 3C	
	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)
Glucose metabolism status						
Prediabetes versus NGM	5,456	-0.05 (-0.13; 0.03)	1,847	-0.10 (-0.23; 0.04)	4,189	-0.06 (-0.15; 0.04)
Type 2 diabetes versus NGM	5,456	**-0.17 (-0.25; -0.09)**	1,847	**-0.20 (-0.34; -0.06)**	4,189	**-0.16 (-0.26; -0.07)**
Measures of glycaemia						
Fasting plasma glucose, per SD	5,455	**-0.05 (-0.08; -0.02)**	1,847	-0.04 (-0.09; 0.01)	4,188	**-0.05 (-0.08; -0.01)**
2-hour post-load glucose , per SD	5,181	**-0.06 (-0.09; -0.03)**	1,727	**-0.08 (-0.14; -0.03)**	3,998	**-0.05 (-0.09; -0.01)**
HbA1c, per SD	5,450	**-0.05 (-0.09; -0.02)**	1,843	-0.04 (-0.09; 0.02)	4,184	**-0.05 (-0.08; -0.01)**
Skin autofluorescence, per SD	5,133	**-0.04 (-0.07; -0.01)**	1,781	**-0.06 (-0.11; -0.01)**	3,942	**-0.05 (-0.08; -0.01)**
Indices of daily glucose variability						
Incremental glucose peak, per SD	2,407	**-0.06 (-0.11; -0.02)**	1,541	-0.06 (-0.11; 0.003)	1,860	-0.05 (-0.10; 0.004)
Model 3 + HbA1c	2,407	**-0.06 (-0.12; -0.01)**	1,541	-0.06 (-0.13; 0.01)	1,860	-0.04 (-0.11; 0.02)
CGM- assessed standard deviation, per SD	622	-0.08 (-0.17; 0.01)	0	N/A	483	**-0.13 (-0.13; -0.02)**
Model 3 + MSG	622	-0.07 (-0.21; 0.07)	0	N/A	483	-0.11 (-0.27; 0.05)

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for individuals with type 2 diabetes or prediabetes versus individuals with NGM or per SD greater measure of glycaemia or daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence (model 3A). For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak (model 3A). For CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation (model 3A). In models 3B and 3C values per SD were numerically comparable. Bold denotes $p<0.05$. Variables in model 3: age, sex, educational status (where applicable), office systolic blood pressure, total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, waist circumference (where applicable), smoking status, and alcohol consumption status. Incremental glucose peak was additionally adjusted for HbA1c (model 3+ HbA1c). CGM-assessed standard deviation was additionally adjusted for MSG. Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. AU, arbitrary unit; BMI, body mass index; CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; MSG, mean sensor glucose; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.9 Associations of glucose metabolism status, measures of glycaemia, and indices of daily glucose variability with RNFL thickness where office systolic blood pressure was replaced with office diastolic blood pressure (model 3A), 24-hour ambulatory systolic blood pressure (model 3B), or 24-hour ambulatory diastolic blood pressure (model 3C).

	RNFL thickness, per SD					
	Model 3A		Model 3B		Model 3C	
	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)	Number of participants	RNFL, per SD stβ (95% CI)
Glucose metabolism status						
Prediabetes versus NGM	5,454	-0.05 (-0.13; 0.03)	4,921	-0.04 (-0.13; 0.04)	4,921	-0.04 (-0.13; 0.04)
Type 2 diabetes versus NGM	5,454	**-0.17 (-0.25; -0.09)**	4,921	**-0.16 (-0.25; -0.08)**	4,921	**-0.17 (-0.25; -0.08)**
Measures of glycaemia						
Fasting plasma glucose, per SD	5,453	**-0.05 (-0.08; -0.02)**	4,920	**-0.05 (-0.08; -0.02)**	4,920	**-0.05 (-0.08; -0.02)**
2-hour post-load glucose , per SD	5,179	**-0.06 (-0.09; -0.02)**	4,683	**-0.05 (-0.09; -0.02)**	4,683	**-0.05 (-0.09; -0.02)**
HbA1c, per SD	5,448	**-0.05 (-0.08; -0.02)**	4,915	**-0.05 (-0.08; -0.02)**	4,915	**-0.05 (-0.08; -0.02)**
Skin autofluorescence, per SD	5,131	**-0.04 (-0.08; -0.01)**	4,627	**-0.04 (-0.07; -0.01)**	4,627	**-0.04 (-0.07; -0.01)**
Indices of daily glucose variability						
Incremental glucose peak, per SD	2,407	**-0.06 (-0.10; -0.01)**	2,134	-0.05 (-0.10; 0.002)	2,134	-0.05 (-0.10; 0.001)
Model 3 + HbA1c	2,407	**-0.06 (-0.12; -0.01)**	2,134	-0.06 (-0.12; 0.003)	2,134	-0.06 (-0.12; 0.001)
CGM- assessed standard deviation, per SD	622	-0.08 (-0.17; 0.01)	562	-0.08 (-0.17; 0.02)	562	-0.07 (-0.17; 0.02)
Model 3 + MSG	622	-0.07 (-0.21; 0.07)	562	-0.08 (-0.22; 0.06)	562	-0.08 (-0.22; 0.06)

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for individuals with type 2 diabetes or prediabetes versus individuals with NGM or per SD greater measure of glycaemia or daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence (model 3A). For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak (model 3A). For CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation (model 3A). In models 3B and 3C values per SD were numerically comparable. Bold denotes $p<0.05$. Variables in model 3: age, sex, educational status, office diastolic blood pressure (where applicable), total cholesterol/HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication, waist circumference (where applicable), smoking status, and alcohol consumption status. Incremental glucose peak was additionally adjusted for HbA1c (model 3+ HbA1c). CGM-assessed standard deviation was additionally adjusted for MSG. Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; HbA1c, haemoglobin A1c; MSG, mean sensor glucose; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.10 Standardized regression coefficients of skin autofluorescence with retinal nerve fibre thickness after additional adjustment for fasting plasma glucose, 2-hour post-load glucose, or HbA1c.

	RNFL thickness, per SD					
	Model 3 + fasting plasma glucose		Model 3 + 2-hour post-load glucose		Model 3 + HbA1c	
	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)
SAF, per SD	5,131	**-0.04 (-0.07; -0.01)**	4,872	**-0.04 (-0.07; -0.01)**	5,126	**-0.04 (-0.07; -0.01)**

Results (β [95% confidence interval]) represent the difference in retinal nerve fibre layer thickness (in SD) for one SD greater exposure to a determinant. One SD corresponds with 10.9 µm for the RNFL and 0.5 AU for SAF in model 3+fasting plasma glucose. In models 3B and 3C values per SD were numerically comparable. Bold denotes p<0.05. The associations were adjusted for age, sex, education level [low, middle, high], waist circumference, office systolic blood pressure, antihypertensive medication, total cholesterol to HDL cholesterol ratio, use of lipid-modifying medication, smoking status [current, ever, never], alcohol consumption status [none, low, high], and fasting plasma glucose (model 3 + fasting plasma glucose), 2-hour post load glucose (model 3 + 2-hour post load glucose), or HbA1c (model 3 + HbA1c). Abbreviations: SAF, skin autofluorescence; CI, confidence interval; HbA1c, haemoglobin A1c; SD, standard deviation; RNFL, retinal nerve fibre layer; SAF, skin autofluorescence.

Table S2.11 Standardized regression coefficients of incremental glucose peak and CGM-assessed standard deviation with retinal nerve fibre thickness after replacement of HbA1c with fasting plasma glucose or skin autofluorescence, or mean sensor glucose with fasting plasma glucose, skin autofluorescence, or HbA1c.

	Retinal nerve fibre layer thickness, per SD					
	Model 3 + fasting plasma glucose		Model 3 + SAF		Model 3 + HbA1c	
	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)
Incremental glucose peak, per SD	2,407	**-0.07 (-0.13; -0.01)**	2,295	**-0.05 (-0.10; -0.01)**	N/A	N/A
CGM-assessed standard deviation, per SD	622	-0.06 (-0.17; 0.04)	573	-0.07 (-0.17; 0.03)	622	-0.05 (-0.16; 0.07)

Results (β [95% confidence interval]) represent the difference in retinal nerve fibre layer thickness (in SD) for 1 SD greater exposure to a determinant. For the incremental glucose peak results, 1 SD corresponds with 11.1 µm for the RNFL and 2.9 mmol/L for incremental glucose peak. For the CGM-assessed standard deviation results, 1 SD corresponds with 10.7 µm for the RNFL and 0.58 mmol/L for CGM-assessed standard deviation. The associations were adjusted for age, sex, education level [low, middle, high], waist circumference, office systolic blood pressure, antihypertensive medication, total cholesterol to HDL cholesterol ratio, use of lipid-modifying medication, smoking status [current, ever, never], alcohol consumption status [none, low, high], and fasting plasma glucose (model 3 + fasting plasma glucose), skin autofluorescence (model 3 + SAF), or HbA1c (model 3 + HbA1c). Additionally, and only for CGM-assessed SD, we entered 'visit interval' in model 1. Abbreviations: CGM, continues glucose monitoring; CI, confidence interval; HbA1c, haemoglobin A1c; SD, standard deviation; RNFL, retinal nerve fibre layer; SAF, skin autofluorescence.

Table S2.12 Associations of continuous glucose monitoring-assessed standard deviation with retinal nerve fibre thickness after exclusion of individuals with less than 3 days of CGM data available (model 3A), individuals with CGM data gaps (model 3B), or with a 'visit interval' (Model 3C).

	RNFL thickness, per SD					
	Model 3A		Model 3B		Model 3C*	
	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)	Number of participants	stβ (95% CI)
CGM-assessed standard deviation, per SD	596	-0.09 (-0.18; 0.01)	573	**-0.10 (-0.19; -0.001)**	409	0.01 (-0.10; 0.12)
Model 3 + MSG	596	-0.08 (-0.23; 0.06)	573	-0.08 (-0.23; 0.07)	409	0.02 (-0.12; 0.17)

Results (β [95% confidence interval]) represent the difference in retinal nerve fibre layer thickness (in SD) for 1 SD greater exposure to a determinant. For the results, 1 SD corresponds with 10.7µm for the RNFL and 0.58 mmol/L for CGM-assessed standard deviation (model 3A). In models 3B and 3C values per SD were numerically comparable. Bold denotes p<0.05. The associations were adjusted for visit interval, age, sex, education level [low, middle, high], waist circumference, office systolic blood pressure, antihypertensive medication, total cholesterol to HDL cholesterol ratio, use of lipid-modifying medication, smoking status [current, ever, never], alcohol consumption status [none, low, high], and mean sensor glucose. * Of note, model 3C was not adjusted for visit interval, as all individuals had an interval of 0 years. CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; MSG, mean sensor glucose; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.13 Associations of CGM-assessed standard deviation and mean sensor glucose with retinal nerve fibre thickness estimated with ridge regression and presented for different degrees of penalization.

		RNFL thickness , per SD	
VIF	**Number of participants**	**CGM-assessed standard deviation, per SD (st.β, 95% CI)**	**CGM-assessed mean sensor glucose, per SD (st.β, 95% CI)**
Model 3 + MSG (λ=0)	622	-0.063 (-0.183; 0.058)	-0.021 (-0.158; 0.121)
Halfway (λ=0.04)	622	-0.062 (-0.183; 0.056)	-0.023 (-0.156; 0.114)
Model 3 (λ=0.10)	622	-0.063 (-0.186; 0.053)	-0.020 (-0.152; 0.103)

Standardized regression coefficients (st.β) indicate the median difference (95% confidence interval) associated with 1 SD higher SD or MSG. All coefficients were estimated by use of ridge regression. We pragmatically chose the level of penalization based on the λ required to reduce the variance inflation factor (VIF) of model 3 + MSG back to the VIF of model 3 (or halfway back). Point estimates and 95% confidence intervals were calculated by use of 1,000 bootstraps estimates. The associations were adjusted for visit interval, age, sex, education level [low, middle, high], waist circumference, office systolic blood pressure, antihypertensive medication, total cholesterol to HDL cholesterol ratio, use of lipid-modifying medication, smoking status [current, ever, never], alcohol consumption status [none, low, high], and mean sensor glucose. CGM, continues glucose monitoring; CI, confidence interval; GMS, glucose metabolism status; MSG, mean sensor glucose; SD, standard deviation; RNFL, retinal nerve fibre layer.

Table S2.14 Associations of duration of diabetes and age with retinal nerve fibre layer thickness.

		RNFL thickness, per SD		
	Number of participants	**Model 1 stβ (95% CI)**	**Model 2 stβ (95% CI)**	**Model 3 stβ (95% CI)**
Duration of diabetes, per SD	982	**-0.07 (-0.13; -0.004)**	-0.06 (-0.13; 0.001)	**-0.07 (-0.13; -0.001)**
Age, per SD	5,180	**-0.06 (-0.08; -0.03)**	**-0.11 (-0.13; -0.08)**	**-0.09 (-0.12; -0.06)**

Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD per SD longer duration of diabetes or age. One SD corresponds with 7.4 years for duration of diabetes or 8.7 years for age and 11.2 µm or 10.9 µm for RNFL thickness in respectively the duration of diabetes study population and the age study population. Bold denotes $p<0.05$. Variables entered in the models in addition to duration of diabetes or age: model 1: none (crude results); model 2: age (where applicable), sex, and educational status (low, medium, high); model 3: model 2 + office systolic blood pressure, total cholesterol to HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), waist circumference, smoking status (current, ever, never), and alcohol consumption status (none, low, high). Additionally, and only for analyses with age as determinant, glucose metabolism status and spherical equivalent were entered in model 2. We compared the standardized betas of type 2 diabetes versus NGM (i.e. -0.16SD) with the standardized beta for age to calculate that beta of type 2 diabetes [stβ, -0.16] corresponds with approximately 15 years of aging (calculated as stβtype 2 diabetes/ stβage * number of years per SD = [0.16/0.09]* 8.7 years = 15 years). CI, confidence interval; HDL, high-density lipoprotein; SD, standard deviation; RNFL, retinal nerve fibre layer; NGM, normal glucose metabolism; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c.

CHAPTER 3

Retinal functional and structural neural indices: potential biomarkers for the monitoring of cerebral neurodegeneration – The Maastricht Study

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Submitted

Abstract

Objective
Retinal biomarkers of neurodegeneration may be biomarkers for the monitoring of therapeutic strategies that aim to prevent early neurodegenerative changes in the brain in the absence of clinical dementia. To further establish this concept, it is important to investigate whether risk factors for dementia are associated with retinal neurodegenerative changes. We investigated whether potentially modifiable risk factors for dementia are associated with retinal functional and structural neurodegenerative changes.

Research design and methods
We used cross-sectional data from The Maastricht Study (up to 5,666 participants, 50.5% men, mean ± SD age 59.7±8.7 years, and 22.6% with type 2 diabetes [the latter oversampled by design]) to investigate the associations of potentially modifiable risk factors for dementia with retinal sensitivity, an index of retinal neural function, and retinal nerve fibre layer (RNFL) thickness, an index of retinal neural structure. We used linear regression analyses with adjustment for potential confounders and tested for interaction by sex and glucose metabolism status.

Results
After full adjustment, greater HbA1c was significantly associated with lower retinal sensitivity and lower RNFL thickness (standardized betas [stβs] per SD higher HbA1c -0.05 and -0.05, respectively); lower healthy diet score was significantly associated with lower retinal sensitivity and lower RNFL thickness (stβs per SD lower diet score -0.06 and -0.03, respectively); lower cardiorespiratory fitness was significantly associated with lower retinal sensitivity, but not RNFL thickness (stβs per SD lower cardiorespiratory fitness -0.05 and -0.03, respectively); and high versus light alcohol consumption was significantly associated with lower RNFL thickness, but not retinal sensitivity (stβs for high versus light alcohol consumption -0.08 and 0.04, respectively). Then, after full adjustment, current smoking was significantly associated with lower retinal sensitivity, but not associated with greater RNFL thickness (stβs for current versus never smoking -0.14 and 0.09, respectively); greater 24-hour ambulatory blood pressure was in individuals with, but not without, type 2 diabetes significantly associated with lower retinal sensitivity and numerically similar, though not statistically significantly, with lower RNFL thickness (in individuals with type 2 diabetes, stβs per SD greater 24-hour ambulatory systolic blood pressure -0.06 and -0.06, respectively); greater total cholesterol was significantly associated with greater retinal sensitivity (stβs per SD greater total cholesterol was 0.05) and was significantly associated with greater RNFL thickness, though the latter only in individuals with, but not without, type 2 diabetes (in individuals with type 2 diabetes, stβ per SD greater total cholesterol was 0.09). Last, after full adjustment, waist circumference and physical activity were not associated with outcomes; and sex did not modify any of the associations under study.

Conclusion
This population-based study found that most potentially modifiable risk factors for dementia were independently associated with lower retinal sensitivity and lower RNFL thickness. Hence, retinal indices of neural function and structure may be biomarkers for the monitoring of therapeutic strategies that aim to prevent early-stage cerebral neurodegeneration and, ultimately, dementia.

Introduction

Clinical dementia, the end stage of cerebral neurodegeneration, is preceded by a gradual loss of neurons over time.[1-3] Mechanistically, deterioration of microvascular and neuronal structures is thought to hamper the function of the neurovascular coupling unit, which can impair autoregulation, and subsequently result in neural ischaemia and neural exposure to toxins, both of which can induce neurodegeneration.[1,2] As postulated in the ticking clock hypothesis,[4,5] there may be an opportunity to prevent the onset and/or progression of early microvascular and neuronal deterioration via reducing early exposure to potentially modifiable risk factors for these detrimental changes.[3-5] Such risk factors include hyperglycaemia, an unhealthy diet, lower cardiorespiratory fitness, excessive alcohol consumption, smoking, hypertension, dyslipidaemia, obesity, and lower levels of physical activity.[6-13]

Currently, there are no clinical tools available to, at an individual level, monitor the efficacy of therapeutic strategies that aim to prevent early-stage cerebral neurodegeneration in the absence of clinical dementia.[14,15] This is an important issue because early monitoring may facilitate personalized, targeted prevention of early-stage cerebral neurodegeneration.[14,15]

The retina may provide an opportunity to monitor therapeutic strategies that aim to prevent early-stage neurodegenerative changes.[16] Retinal measures of neuronal function and structure are biologically plausible biomarkers for the monitoring of cerebral neurodegenerative changes because the retina and the brain have a shared embryology, and both have many anatomical and physiological similarities.[16] Indeed, early structural retinal neurodegenerative changes have been found to be associated with magnetic resonance imaging (MRI)-assessed markers of cerebral neurodegeneration (i.e. lower grey and white matter volume),[17] cognitive decline,[18] and dementia.[19]

In the retina early functional and structural neurodegenerative changes can, respectively, be non-invasively and accurately assessed as lower retinal sensitivity and lower retinal nerve fibre layer (RNFL) thickness.[16,20] Loss of retinal sensitivity reflects greater dysfunction of the neural networks which perceive, filter, and transmit visual information from the retina to the brain.[20] RNFL thinning reflects loss of retinal ganglion cell axons, which transmit visual information from the retina to the brain.[16]

Current literature on whether potentially modifiable risk factors for dementia may be determinants of retinal sensitivity or RNFL thickness has important limitations.[21-32] First, no large population-based studies have investigated the associations of potentially modifiable risk factors for dementia with retinal sensitivity. Second, previous studies have not yet investigated the associations with RNFL thickness of a number of

important potentially modifiable risk factors for dementia, i.e. adherence to a healthy diet, cardiorespiratory fitness, and accelerometer-assessed lower physical activity.[22-32]

In view of above, we investigated, using a large, well-characterized population-based cohort study, whether potentially modifiable risk factors for dementia are associated with retinal sensitivity and RNFL thickness.

Materials and methods

Here key elements of the Material and Methods are provided, more details are provided in the Supplemental Methods.

Study population and design

We used data from The Maastricht Study, a population-based observational cohort study. The rationale and methodology have been described previously.[33] In brief, the study focuses on the aetiology, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency.[33] The present report includes cross-sectional data of 7,689 participants who completed the baseline survey between November 2010 and December 2017.

Retinal sensitivity

We assessed retinal sensitivity of both eyes in the central and peri macular area with the Heidelberg Edge Perimeter (Heidelberg Engineering, Heidelberg, Germany. In brief, light stimuli varying in strength between 0 and 35 decibel were presented at 54 coordinates on the retina; at each coordinate the threshold of visual perception (i.e. the threshold at which the weakest presented visual stimulus could be perceived) was determined; and results were averaged into ‘retinal sensitivity’. The intra-observer reliability for the assessment of the retinal sensitivity is 0.95.[34]

RNFL thickness

We assessed RNFL thickness with optical coherence tomography (OCT; Spectralis unit; Heidelberg Engineering, Heidelberg, Germany). The RNFL thickness (μm) of

both eyes was measured within a 3.45 mm diameter circular scan (12°, 768 voxels, 100 automatic real-time tracking) centred on the optic nerve head. All OCT scans were reviewed and their quality was scored. Intra- and interindividual reliability, expressed as intraclass correlation coefficients, were 0.97 and 0.96, respectively.[35]

Assessment of risk factors

We determined haemoglobin A1c (HbA1c; % or mmol/mol) and total cholesterol (mmol/L) in fasting venous plasma samples;[33] assessed dietary intake, including alcohol consumption, with a validated food frequency questionnaire,[36] and calculated the Dutch Healthy Diet index sum score (without alcohol consumption);[36,37] and categorized alcohol consumption into none, light, moderate, and high (definitions in the Supplemental Material).[38] Then, we assessed cardiorespiratory fitness, defined as the maximum power output adjusted for body mass (i.e. $W_{max} \cdot kg^{-1}$), with a graded cycle ergometer-, submaximal exercise test;[39] assessed smoking status (current, former, never smoking) via a questionnaire;[33] assessed antihypertensive medication use, an index of past exposure to a relatively high blood pressure, as part of an interview, assessed 24-hour ambulatory blood pressure (mm Hg) with an oscillometric device;[40] calculated mean arterial pressure from the 24-hour ambulatory blood pressure measurements as ([1/3*systolic 24-hour ambulatory blood pressure] + [2/3*diastolic 24-hour ambulatory blood pressure]); assessed waist circumference (cm) as part of a physical examination;[33] and measured 8-day physical activity (hours/day) with an accelerometer.[41]

Covariates

As described previously,[33] we assessed educational level (low, intermediate, high) by questionnaire,[42] high-density lipoprotein (HDL) and fasting plasma glucose in fasting venous blood samples;[33] assessed medication use as part of an interview, and assessed glucose metabolism status based on fasting plasma glucose and oral glucose tolerance test-derived 2-hour post load glucose.[33]

Statistical analysis

We used multivariable regression analysis to investigate the associations of potentially modifiable risk factors for dementia (determinants) with retinal sensitivity and RNFL thickness (outcomes). We standardized determinants and outcomes of a continuous nature (i.e. expressed as z-score) and entered categorical variables into models as dummy variables. Next, we inversed (i.e. multiplied by -1) the healthy diet score, cardiorespiratory fitness, and physical activity so that higher values indicate lower

healthy diet score, lower cardiorespiratory fitness, or lower physical activity. Last, we used complete case analysis, where individuals were included in the main analyses if data were available on the main determinant, the outcome, and potential confounders required for the fully adjusted model (model 3; more details in the next paragraph).

We adjusted for potential confounders. In model 1 we did not adjust for any confounders ("crude"). In model 2 we adjusted for demographic confounders (i.e. age, sex, and educational status) and for glucose metabolism status.[32] In model 3 we additionally adjusted for cardiovascular and lifestyle variables (i.e. office systolic blood pressure, antihypertensive medication use [yes/no], waist circumference, total cholesterol / HDL cholesterol ratio, lipid-lowering medication use [yes/no], smoking status [current, former, never], and alcohol consumption status [none, moderate, high]).[32] For associations where HbA1c was the determinant, glucose metabolism status was not entered into the model to prevent collinearity. The associations were expressed as standardized regression coefficient (stβ) and corresponding 95% confidence interval (95%CI).

We tested for interaction by sex and glucose metabolism status to assess whether associations, respectively, differed between men and women or between individuals with type 2 diabetes, prediabetes, and normal glucose metabolism. For interaction analyses with glucose metabolism status, we excluded participants with other types of diabetes because the number of these participants was small.

To assess the robustness of our findings we performed a range of additional analyses (we report one additional analysis in the main manuscript; and report all additional analyses in the Supplemental Methods). We studied the associations of age with retinal sensitivity and RNFL thickness. We performed these analyses so that we could compare with how many years of "ageing" the betas for determinants under study correspond.

All analyses were performed with Statistical Package for Social Sciences version 25.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). For all analyses, a P-value <0.05 was considered statistically significant.

Results

Selection and characteristics of the study population

Figure 3.1 shows an overview of the study population selection.

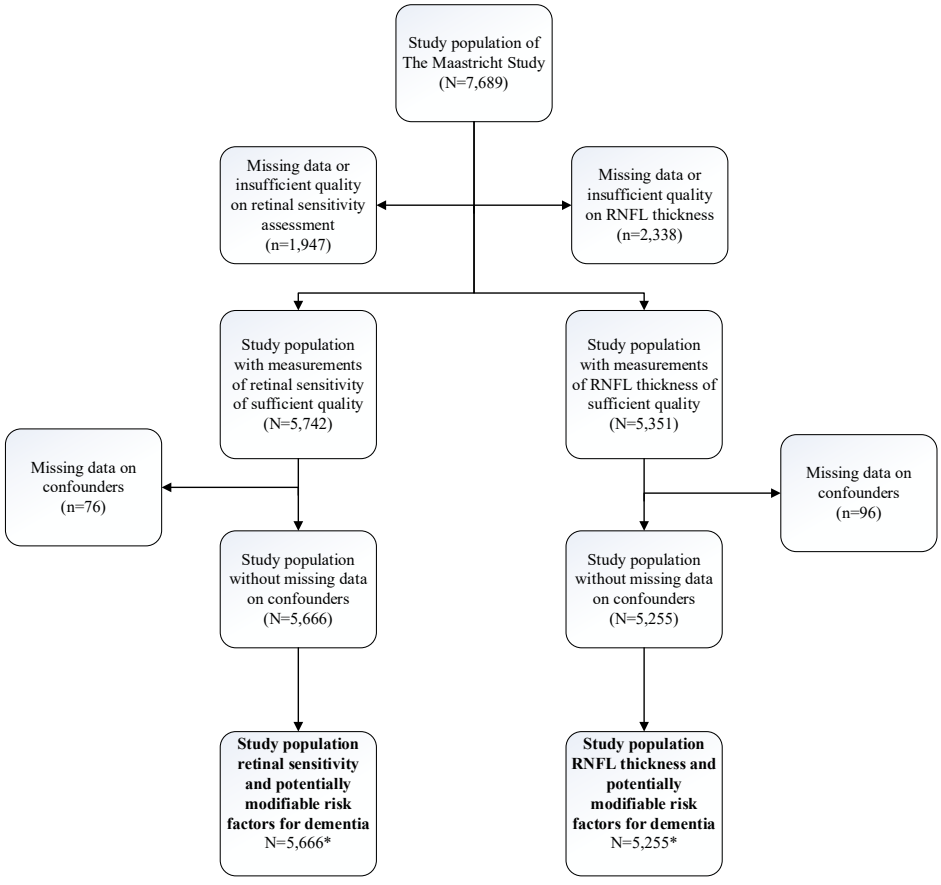


Figure 3.1 delineates the selection of participants for inclusion in analyses.
* For retinal sensitivity and RNFL thickness, respectively, the numbers of participants with complete data for analyses with HbA1c were n=5,662 and n=5,249; for analyses with the healthy diet score were n=5,369 and n=4,981; for analyses with cardiorespiratory fitness were n=4,899 and n=4,452; for analyses with alcohol consumption were n=5,377 and n=4,989; for analyses with smoking were n=5,666 and n=5,255; for analyses with antihypertensive medication use were n=5,666 and n=5,255; for analyses with 24-hour ambulatory blood pressure were n=5,074 and n=4,746; for analyses with total cholesterol were n=5,664 and n=5,255; for analyses with waist circumference were n=5,666 and n=5,255; and for analyses with physical activity were n=5,027 and n=4,510. Confounders are: age, sex, glucose metabolism status, educational level, office systolic blood pressure (where applicable), antihypertensive medication use, waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). RNFL, retinal nerve fibre layer; HbA1c, glycated haemoglobin.

Table 3.1 and Supplemental Tables S3.1 and S3.2 show general participant characteristics according to tertiles of retinal sensitivity and RNFL thickness. Overall, participants with a lower retinal sensitivity and thinner RNFL were older, and generally

had a more adverse risk factor profile. General characteristics of participants included in the study were comparable to those of participants with missing data (Supplemental Table S3.3).

Table 3.1 General study population characteristics according to tertiles of retinal sensitivity in the study population with complete data on waist circumference.

	Retinal sensitivity			
Characteristic	**Total study group (N=5,666)**	**Tertile 1 (low) (N=1,877)**	**Tertile2 (middle) (N=1,901)**	**Tertile 3 (high) (N=1,888)**
Demographic characteristics				
Age (years)	59.7 ± 8.7	64.2 ± 7.9	59.6 ± 8.2	55.4 ± 7.8
Men	2862 (50.5)	936 (49.%)	960 (50.5)	966 (51.2)
Educational level				
Low	1927 (34.0)	809 (43.1)	618 (32.5)	500 (26.5)
Middle	1571 (27.7)	463 (24.7)	525 (27.6)	583 (30.9)
High	2168 (38.3)	605 (32.2)	758 (39.9)	805 (42.6)
Potentially modifiable risk factors for dementia				
HbA1c (mmol/mol)*	39.1 ± 9.3	40.9 ± 9.8	38.7 ± 9.1	37.9 ± 8.8
HbA1c (%)*	5.7 ± 0.9	5.9 ± 0.9	5.7 ± 0.8	5.6 ± 0.8
Dutch Healthy Diet score (points)*	76.6 ± 14.6	77.1 ± 14.6	76.9 ± 14.6	75.8 ± 14.5
Cardiorespiratory fitness (Wmax·kg−1)	2.1 ± 0.6	2.0 ± 0.6	2.2 ± 0.6	2.3 ± 0.6
Alcohol consumption				
None	814 (15.1)	305 (17.1)	240 (13.2)	269 (15.2)
Light	1656 (17.6)	533 (29.8)	548 (30.2)	574 (32.4)
Moderate	1186 (22.1)	358 (20.0)	396 (21.8)	430 (24.3)
High	1721 (32.0)	592 (33.1)	631 (34.8)	497 (28.1)
Smoking status				
Never	2170 (38.3)	662 (35.3)	732 (38.5)	776 (41.1)
Former	2785 (49.2)	981 (52.3)	934 (49.1)	870 (46.1)
Current	711 (12.5)	234 (12.5)	235 (12.4)	242 (12.8)
Antihypertensive medication use	2074 (36.6)	895 (47.7)	665 (35.0)	514 (27.0)
Ambulatory 24-h systolic blood pressure (mmHg)*	118.9 ± 11.6	120.1 ± 12.1	118.6 ± 11.2	117.7 ± 11.3
Ambulatory 24-h diastolic blood pressure(mmHg)*	72.9 ± 7.2	72.0 ± 7.1	73.1 ± 7.1	73.3 ± 7.3
Mean arterial pressure (mm Hg)	88.2 ± 8.0	88.1 ± 8.0	88.2 ± 7.8	88.4 ± 8.1
Total cholesterol (mmol/L)*	5.2 ± 1.1	5.1 ± 1.1	5.2 ± 1.1	5.3 ± 1.1
Waist circumference (cm)	94.8 ± 13.4	96.3 ± 13.5	94.4 ± 13.2	93.5 ± 13.3
Physical activity (minutes per day)*	118.9 ± 40.9	115.0 ± 39.7	121.4 ± 41.3	120.3 ± 41.2
Other				
Glucose metabolism status				
Normal glucose metabolism	3514 (62.0)	995 (53.0)	1199 (63.1)	1320 (69.9)
Prediabetes	840 (14.8)	319 (17.0)	292(15.4)	229 (12.1)
Type 2 diabetes	1278 (22.6)	552 (29.4)	400 (21.0)	326 (17.3)
Other type of diabetes	34 (0.6)	11 (0.6)	10 (0.5)	13 (0.7)
Glucose-lowering medication use	957 (16.9)	414 (21.1)	299 (15.7)	244 (12.9)
Lipid-lowering medication use	1744 (30.8)	781 (41.6)	550 (28.9)	413 (21.9)
Total/HDL cholesterol ratio (no unit)	3.6 ± 1.2	3.5 ± 1.1	3.6 ± 1.2	3.6 ± 1.2

Table 3.1 (continued)

Characteristic	Retinal sensitivity			
	Total study group (N=5,666)	**Tertile 1 (low) (N=1,877)**	**Tertile2 (middle) (N=1,901)**	**Tertile 3 (high) (N=1,888)**
Outcomes				
Retinal sensitivity (dB)	27.7 ± 1.6	26.1 ± 1.6	27.9 ± 0.3	29.1 ± 0.5
RNFL thickness (µm)*	94.9 ± 10.8	83.3 ± 6.8	95.3 ± 2.5	106.1 ± 6.3

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). * Data shown in the study population with complete data on ambulatory 24-hour blood pressure (n=5,074), total cholesterol (n=5,664), HbA1c (n=5,662), Dutch Healthy Diet score (n=5,369), alcohol consumption (n=5,377 [shown for alcohol consumption assessed with the food frequency questionnaire]), cardiorespiratory fitness (n=4,899), physical activity (n=5,027), and RNFL thickness (n=5,255). HDL, high-density lipoprotein; HbA1c, glycated haemoglobin A1c.

Associations of risk factors with retinal sensitivity and RNFL thickness

HbA1c

After full adjustment (model 3), greater HbA1c was significantly associated with lower retinal sensitivity and lower RNFL thickness (per SD, standardized beta [95% CI], -0.05 [-0.08; -0.02], and -0.05 [-0.08; -0.02], respectively; Table 3.2 and Figure 3.2).

Healthy diet score without alcohol consumption

After full adjustment (model 3), lower healthy diet score was significantly associated with lower retinal sensitivity and lower RNFL thickness (per SD, standardized beta [95% CI], -0.06 [-0.09; -0.03], and -0.03 [-0.06; -0.00], respectively).

Cardiorespiratory fitness

After full adjustment (model 3), lower cardiorespiratory fitness was significantly associated with lower retinal sensitivity, but not with lower RNFL thickness (per SD, standardized beta [95% CI], -0.05 [-0.08; -0.01], and -0.03 [-0.07; 0.01], respectively).

Alcohol consumption

After full adjustment (model 3), high versus light alcohol consumption was not associated with lower retinal sensitivity, but was significantly associated with lower RNFL thickness (standardized beta [95% CI], 0.04 [-0.03; 0.10], and -0.08 [-0.16; -0.01], respectively).

Table 3.2 Associations of potentially modifiable risk factors for dementia with retinal sensitivity and RNFL thickness.

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	Number of participants in analyses	stβ (95% CI)	Number of participants in analyses	stβ (95% CI)
Potentially modifiable risk factors for dementia					
HbA1c, per SD	1	N=5,662	**-0.15 (-0.17;-0.12)**	N=5,249	**-0.06 (-0.09; -0.04)**
	2		**-0.07 (-0.10;-0.05)**		**-0.05 (-0.08; -0.02)**
	3		**-0.05 (-0.08;-0.02)**		**-0.05 (-0.08; -0.02)**
Lower healthy diet score, per SD	1	N=5,369	-0.01 (-0.03; 0.02)	N=4,981	**-0.04 (-0.07; -0.01)**
	2		**-0.07 (-0.09;-0.04)**		-0.03 (-0.06; 0.00)
	3		**-0.06 (-0.09;-0.03)**		**-0.03 (-0.06;-0.00)**
Lower cardiorespiratory fitness, per SD	1	N=4,899	**-0.21 (-0.23; -0.18)**	N=4,542	-0.01 (-0.04; 0.02)
	2		**-0.05 (-0.08; -0.02)**		-0.02 (-0.05; 0.02)
	3		**-0.05 (-0.08; -0.01)**		-0.03 (-0.07; 0.01)
Alcohol consumption		N=5,377		N=4,989	
- None versus light	1		**-0.10 (-0.19; -0.02)**		0.05 (-0.04; 0.13)
	2		-0.05 (-0.13; 0.03)		0.01 (-0.08; 0.10)
	3		-0.03 (-0.11; 0.05)		0.01 (-0.08; 0.09)
- Moderate versus light	1		0.07 (-0.01; 0.14)		0.03 (-0.05; 0.11)
	2		**0.07 (0.00; 0.14)**		0.04 (-0.04; 0.11)
	3		0.06 (-0.01; 0.13)		0.04 (-0.04; 0.11)
- High versus light	1		-0.04 (-0.11; 0.03)		**-0.10 (-0.17; -0.03)**
	2		0.05 (-0.02; 0.11)		**-0.09 (-0.16; -0.01)**
	3		0.04 (-0.03; 0.10)		**-0.08 (-0.16; -0.01)**
Smoking		N=5,666		N=5,255	
-Former versus never	1		**-0.09 (-0.15; -0.04)**		-0.05 (-0.11; 0.01)
	2		**0.06 (0.01; 0.11)**		-0.02 (-0.08; 0.04)
	3		**0.05 (0.00; 0.11)**		-0.01 (-0.07; 0.05)
-Current versus never	1		**-0.14 (-0.22; -0.06)**		0.08 (-0.01; 0.17)
	2		**-0.15 (-0.23; -0.07)**		0.09 (-0.00; 0.17)
	3		**-0.14 (-0.22;-0.06)**		0.09 (-0.00;0.18)
Antihypertensive medication use	1	N=5,666	**-0.33 (-0.39;-0.28)**	N=5,255	**-0.16 (-0.21; -0.10)**
	2		-0.05 (-0.10; 0.00)		**-0.09 (-0.15;-0.03)**
	3		-0.03 (-0.09; 0.03)		**-0.12 (-0.19;-0.05)**
24-hour ambulatory systolic blood pressure, per SD	1	N=5,074	**-0.09 (-0.12;-0.06)**	N=4,746	**-0.06 (-0.09; -0.03)**
	2		-0.01 (-0.04; 0.01)		-0.01 (-0.04; 0.02)
	3		-0.01 (-0.04; 0.02)		-0.01 (-0.04; 0.02)
24-hour ambulatory diastolic blood pressure, per SD	1	N=5,074	**0.09 (0.07; 0.12)**	N=4,746	**-0.05 (-0.08; -0.02)**
	2		0.03 (-0.00; 0.05)		**-0.03 (-0.06; -0.00)**
	3		0.03 (-0.00; 0.05)		**-0.03 (-0.06; -0.00)**
Mean arterial pressure, per SD	1	N=5,074	0.01 (-0.03; 0.03)	N=4,746	**-0.06 (-0.09; -0.03)**
	2		0.01 (-0.02; 0.04)		-0.02 (-0.05; 0.01)
	3		0.01 (-0.02; 0.04)		-0.03 (-0.06; 0.01)
Total cholesterol, per SD	1	N=5,664	**0.08 (0.05; 0.10)**	N=5,255	**0.06 (0.03; 0.08)**
	2		**0.06 (0.04; 0.09)**		0.02 (-0.01; 0.05)
	3		**0.05 (0.02; 0.08)**		0.03 (-0.00; 0.06)
Waist circumference, per SD	1	N=5,666	**-0.10 (-0.12;-0.07)**	N=5,255	**-0.05 (-0.07; -0.02)**
	2		-0.02 (-0.05; 0.01)		0.02 (-0.01;0.05)
	3		-0.01 (-0.05; 0.02)		0.03 (-0.00;0.07)
Lower physical activity, per SD	1	N=5,027	**-0.06 (-0.08; -0.03)**	N=4,510	-0.02 (-0.05; 0.01)
	2		-0.01 (-0.03; 0.02)		-0.00 (-0.03; 0.03)
	3		0.01 (-0.02; 0.04)		-0.01 (-0.04; 0.02)

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity or RNFL thickness (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus

without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. One SD corresponds with 11.6 and 7.2 mm Hg for systolic and diastolic 24-hour ambulatory blood pressure, respectively, 8.0 mm Hg for mean arterial pressure, 1.1 mmol/L for total cholesterol, 13.4 cm for waist circumference, 0.9% for HbA1c, 14.6 points for the healthy diet score, 0.6 W/kg for cardiorespiratory fitness, 40.9 minutes/day for physical activity, 1.6 dB for retinal sensitivity (all determined in the study population with complete data on retinal sensitivity and waist circumference), or 10.8 micrometres for RNFL thickness (RNFL thickness was determined in the study population with complete data on antihypertensive medication use [n=5,666]; values per SD for other variables were numerically similar in the retinal sensitivity and RNFL thickness study populations). Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; HDL, high density lipoprotein.

Smoking

After full adjustment (model 3), current versus never smoking was significantly associated with lower retinal sensitivity, but was not associated with RNFL thickness (standardized beta [95% CI], -0.14 [-0.22; -0.06], and 0.09 [-0.00; 0.18], respectively).
In contrast, after full adjustment (model 3), former versus never smoking was significantly associated with greater retinal sensitivity, and was not associated with lower RNFL thickness (standardized beta [95% CI], 0.05 [0.00; 0.11], and -0.01 [-0.07; 0.05], respectively).

Blood pressure

After full adjustment (model 3), antihypertensive medication use was not associated with retinal sensitivity, but was significantly associated with lower RNFL thickness (per SD, standardized beta [95% CI], -0.03 [-0.09; 0.03], and -0.12 [-0.19; -0.05], respectively).
After full adjustment (model 3), greater 24-hour ambulatory systolic, diastolic, and mean arterial blood pressure were not associated with retinal sensitivity (per SD, standardized beta [95% CI], -0.01 [-0.04; 0.02], 0.03 [-0.00; 0.05], and 0.01 [-0.02; 0.04], respectively).
After full adjustment (model 3), greater 24-hour ambulatory diastolic, but not 24-hour ambulatory systolic blood pressure or mean arterial blood pressure, was significantly associated with lower RNFL thickness (per SD, standardized beta [95% CI], -0.03 [-0.06; -0.00], -0.01 [-0.04; 0.02], and -0.03 [-0.06; 0.01], respectively).

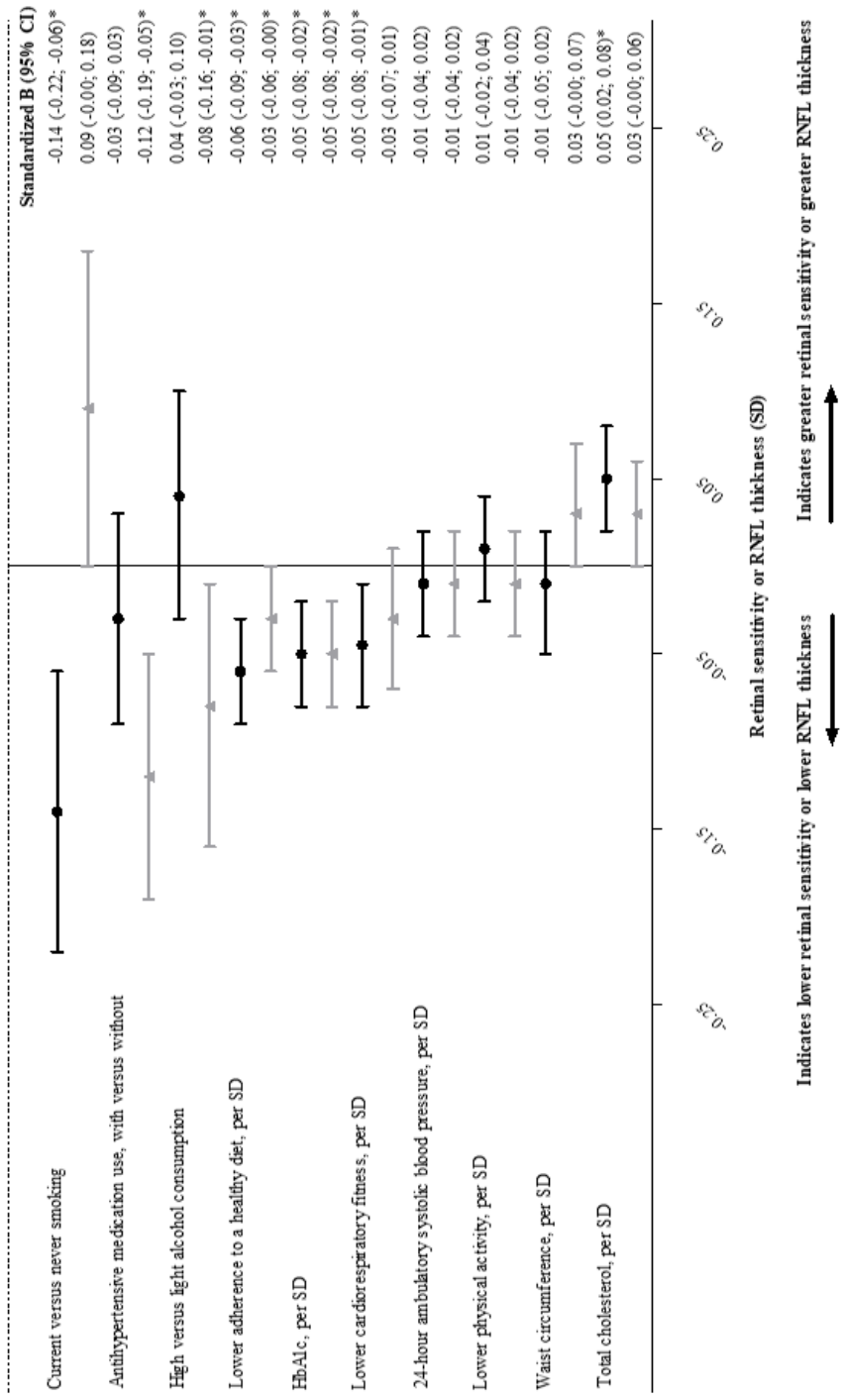

Associations of potentially modifiable risk factors for dementia with retinal sensitivity and RNFL thickness (in SD; model 3), ranked by strength and direction of associations
Standardized B (95% CI)
Current versus never smoking
-0.14 (-0.22; -0.06)*
Antihypertensive medication use, with versus without
0.09 (-0.00; 0.18)
High versus light alcohol consumption
-0.03 (-0.09; 0.03)
Lower adherence to a healthy diet, per SD
-0.12 (-0.19; -0.05)*
HbA1c, per SD
0.04 (-0.03; 0.10)
Lower cardiorespiratory fitness, per SD
-0.08 (-0.16; -0.01)*
24-hour ambulatory systolic blood pressure, per SD
-0.06 (-0.09; -0.03)*
Lower physical activity, per SD
-0.03 (-0.06; -0.00)*
Waist circumference, per SD
-0.05 (-0.08; -0.02)*
Total cholesterol, per SD
-0.05 (-0.08; -0.02)*
-0.05 (-0.08; -0.01)*
-0.03 (-0.07; 0.01)
-0.01 (-0.04; 0.02)
-0.01 (-0.04; 0.02)
0.01 (-0.02; 0.04)
-0.01 (-0.04; 0.02)
-0.01 (-0.05; 0.02)
0.03 (-0.00; 0.07)
0.05 (0.02; 0.08)*
0.03 (-0.00; 0.06)
-0.25
-0.15
-0.05
0.05
0.15
0.25
Retinal sensitivity or RNFL thickness (SD)
Indicates lower retinal sensitivity or lower RNFL thickness
Indicates greater retinal sensitivity or greater RNFL thickness

Figure 3.2 shows the associations of potentially modifiable risk factors for dementia with retinal sensitivity and RNFL thickness, ranked by strength and direction of associations. Standardized regression coefficients (stβ) represent the difference in retinal sensitivity (black circular point estimates with error bars) or RNFL thickness (grey triangular point estimated with error bars) in SD for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for high versus light total alcohol consumption, for current versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. Numerical values with which 1 SD corresponds are presented in the legend of Table 3.2. * denotes P<0.05. Variables in models: age, sex, glucose metabolism status (where applicable), educational level, office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, haemoglobin A1c; HDL, high density lipoprotein.

Cholesterol

After full adjustment (model 3), greater total cholesterol was significantly associated with *greater* retinal sensitivity, but was not associated with RNFL thickness (per SD, standardized beta [95% CI], 0.05 [0.02; 0.08], and 0.03 [-0.00; 0.06], respectively).

Waist circumference

After full adjustment (model 3), greater waist circumference was neither associated with retinal sensitivity, nor with RNFL thickness (per SD, standardized beta [95% CI], -0.01 [-0.05; 0.02], and 0.03 [-0.00; 0.07], respectively).

Physical activity

After full adjustment (model 3), lower physical activity was neither associated with retinal sensitivity, nor with RNFL thickness (per SD, standardized beta [95% CI], 0.01 [-0.02; 0.04], and -0.01 [-0.04; 0.02], respectively).

Tests for interaction and stratified analyses

Sex did not modify any of the associations, but glucose metabolism status did (all P-values for interaction are shown in Supplemental Table S3.4). Type 2 diabetes modified the association of 24-hour ambulatory systolic blood pressure with retinal sensitivity ($P_{interaction}<0.001$); the association of 24-hour ambulatory systolic blood pressure with RNFL thickness ($P_{interaction}=0.049$); and the association of total cholesterol with RNFL thickness ($P_{interaction}=0.04$). Additionally, prediabetes inconsistently modified the associations of healthy diet score and physical activity with retinal sensitivity.

In individuals with, but not without, type 2 diabetes, greater 24-hour ambulatory systolic blood pressure was significantly associated with lower retinal sensitivity, but not with lower RNFL thickness (model 3; in individuals with type 2 diabetes, per SD, standardized beta [95% CI], -0.06 [-0.12; -0.04], and -0.06 [-0.13; 0.00], respectively; Supplemental Table S3.5). In addition, we observed a similar pattern for 24-hour ambulatory diastolic and mean arterial blood pressure. Then, in individuals with, but not without, type 2 diabetes, greater total cholesterol was significantly associated with *greater* RNFL thickness (model 3; in individuals with type 2 diabetes, per SD, standardized beta [95% CI], 0.09 [0.03; 0.16]). However, we did not observe this pattern for retinal sensitivity.

Additional analyses

We generally observed quantitatively similar results in a range of additional analyses (all results are reported in the Supplemental Results section and are shown in Supplemental Tables S3.6-S3.12). Here we highlight one main finding. After full adjustment (model 3), greater age was significantly associated with lower retinal sensitivity and lower RNFL thickness (standardized beta [95% confidence interval], per year -0.04 [-0.05; -0.04] and -0.01 [-0.01; -0.01], respectively; Supplemental Table S3.8). Hence, the beta for 1 SD greater HbA1c corresponds with approximately 1.3 years of ageing for retinal sensitivity and 5.0 years of ageing for RNFL thickness; the beta of 1 SD lower adherence to a healthy diet corresponds with approximately 1.5 years of ageing for retinal sensitivity and 3.0 years of ageing for RNFL thickness; and the beta of 1 SD lower cardiorespiratory fitness corresponds with approximately 1.3 years of ageing for retinal sensitivity and 3.0 years of ageing for RNFL thickness. Therefore, added up, the combination of these three adverse factors corresponds with approximately 4.1 and 11.0 years of "ageing" for, respectively, retinal sensitivity and RNFL thickness.

Discussion

The present population-based study has four main findings. First, we found significant associations with lower retinal sensitivity of greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, current versus never smoking, and greater 24-hour ambulatory blood pressure, though the latter only in individuals with, but not without, type 2 diabetes. Second, we found significant associations with lower RNFL thickness of greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, high versus light alcohol consumption, current versus never smoking, and antihypertensive medication use. Third, greater total cholesterol was associated with *greater* retinal sensitivity and *greater* RNFL thickness, though the latter only in individuals with, but not without, type 2 diabetes. Fourth, waist circumference and physical activity were not associated with outcomes under study.

Our findings on RNFL thickness are in line with findings from most previous population-based studies.[22-32] Importantly, the present study is the first population-based study to investigate the associations of potentially modifiable risk factors for dementia with retinal sensitivity.[21] In addition, the present study is the first study to report associations of 24-hour ambulatory-assessed blood pressure levels, waist circumference, adherence to a healthy diet, cardiorespiratory fitness, and accelerometer-assessed physical activity with RNFL thickness.

Mechanistically, most risk factors that were significantly associated with outcomes can increase levels of oxidative stress in the retina, which can lead to retinal microvascular dysfunction, loss of retinal neural structures (including RNFL thinning), and retinal dysfunction (i.e. resulting in lower retinal sensitivity).[2,43] Additionally, antihypertensive medication use, lower cardiorespiratory fitness and a less healthy diet may be associated with more retinal neurodegenerative changes as they, respectively, reflect past exposure to higher levels of blood pressure over time (i.e. hypertension), lower exposure to neuroprotective factors in the retina;[44] and lower exposure to certain nutrients which can reduce levels of oxidative stress.[45] Biologically, impaired microvascular function likely predisposes the retinal capillaries to a high intracapillary pressure, which is detrimental for the capillaries and can lead to ischaemia and a hampered clearance of toxins from the neuronal tissue, both of which can activate pathways that lead to neurodegeneration.[2,43] Physiologically, up to 20-50% of retinal ganglion cells can be lost before worse retinal function is detectable.[46] In addition, progressive loss of neural structures can also increasingly predispose retinal neurons to ischaemia as neuronal structures contribute to function of the neurovascular coupling unit, which tightly regulates the supply of nutrients (e.g. oxygen) to retinal neurons.[1,2,43]
In participants with, but not without, type 2 diabetes greater 24-hour ambulatory systolic blood pressure was significantly associated with lower retinal sensitivity and numerically similar in strength, though not statistically significant, with lower RNFL thickness. Biologically, as hyperglycaemia is detrimental for neuronal and microvascular structures, which regulate capillary pressure, individuals with, versus without, type 2 diabetes may be more susceptible to hypertension.[43]
Directionally inconsistent with our hypothesis, *greater* total cholesterol was associated with *greater* retinal sensitivity and *greater* RNFL thickness, though the latter only in individuals with, but not without, type 2 diabetes. Mechanistically, a greater total cholesterol level, which reflects higher levels of circulating cholesterol, may be beneficial for the retina as cholesterol is an important contributor to the formation of neuronal synapses.[47] Then, as under hyperglycaemic circumstances neuronal and microvascular structures (i.e. neurovascular coupling unit) are functionally impaired, the ability to ensure a continuous supply of cholesterol to the retina is likely reduced, resulting in lower levels of retinal cholesterol, and subsequently less synaptogenesis, in individuals with, versus without, type 2 diabetes.[47]
Waist circumference was not associated with outcomes, possibly because the effects of adiposity on neural retina tissue are bidirectional. Mechanistically, higher levels of visceral (white) adipose tissue, which greater waist circumference represents, likely leads to higher levels of adipokines in the circulation, which may have both neuroprotective as well as neurodegenerative effects on retinal neurons.[48] For example,

directionally opposing effects have been reported for tumour necrosis factor-alpha [TNF-alpha]), a relatively well-studied adipokine.[48]

Lower physical activity was not associated with outcomes under study, possibly because other factors than the amount of physical activity determine the extent of the neuroprotective physiological response to physical activity.[49] Indeed, besides the amount of physical activity, also genetic factors and the type, frequency, and intensity of activity have been found to determine the physiological response to physical activity.[49]

Our findings contribute to the increasing evidence that there may be an opportunity to improve clinical care via the use of retinal neural biomarkers. First, our findings are consistent with the concept that retinal sensitivity and RNFL thickness may be biomarkers for the monitoring of therapeutic strategies that aim to prevent cerebral neurodegeneration in the absence of clinical dementia.[16,20] However, in order to able to move from a research setting to a clinical setting, future trials are warranted to further investigate whether modification of potentially modifiable risk factors for dementia can actually lead to a detectable increase in retinal sensitivity and RNFL thickness.[50] Second, the modification of a combination of risk factors may result in a considerable reduction of retinal neuronal ageing. Indeed, added up, 1 SD greater HbA1c, 1 SD lower adherence to a healthy diet, and 1 SD lower cardiorespiratory fitness correspond with approximately 4.1 and 11.0 years of "ageing" for, respectively, retinal sensitivity and RNFL thickness.

Strengths of this study are 1) the large size of this population-based cohort with oversampling of individuals with type 2 diabetes, which enabled accurate comparison of individuals with and without diabetes; 2) the extensive number of potential confounders that were considered; 3) the use of state-of-the-art methods to assess all variables included in this study.[50]

The study has certain limitations. First, due to the cross-sectional nature of the study, causal inferences should be made with caution.[50] Mechanistically, hyperglycaemia and hypertension may lead to neurodegeneration but the reverse may also be true, i.e. there may be a vicious cycle.[43] Intact neurovascular interaction is required for normal microvascular function and impaired microvascular function may aggravate hyperglycaemia and hypertension.[43] Second, we may have underestimated the strength of the associations under study if such associations were stronger in participants that were not included in the study population (who generally tended to be less healthy).[50] Third, although we took an extensive set of confounders into account, we cannot fully exclude bias due to unmeasured confounding (e.g. due to environmental factors such as air pollution).[50] Fourth, we may have underestimated the strength of certain associations under study (e.g. diet) as we adjusted for certain variables which may (in part) reflect the effects on the outcomes of certain potentially modifiable risk factors

for dementia (e.g. we adjusted for glucose metabolism status and blood pressure; and a healthy dietary intake may in part protect against neurodegeneration via reducing the risk of hyperglycaemia and hypertension).[50] Last, we studied Caucasian individuals aged 40-75 years and therefore our results may be generalizable to such a population; whether these results also apply to other populations requires further study.[50]

In summary, the present population-based study demonstrated that most potentially modifiable risk factors for dementia were independently associated with indices of retinal neuronal function (i.e. retinal sensitivity) and structure (i.e. RNFL thickness). Hence, retinal indices of neural function and structure may be biomarkers for the monitoring of therapeutic strategies that aim to prevent early-stage cerebral neurodegeneration and, ultimately, dementia.

References

1. Soto-Rojas LO, Pacheco-Herrero M, Martinez-Gomez PA, Campa-Cordoba BB, Apatiga-Perez R, Villegas-Rojas MM, Harrington CR, de la Cruz F, Garces-Ramirez L and Luna-Munoz J. The Neurovascular Unit Dysfunction in Alzheimer's Disease. Int J Mol Sci. 2021;22.
2. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14:133-150.
3. Yu JT, Xu W, Tan CC, Andrieu S, Suckling J, Evangelou E, Pan A, Zhang C, Jia J, Feng L, Kua EH, Wang YJ, Wang HF, Tan MS, Li JQ, Hou XH, Wan Y, Tan L, Mok V, Tan L, Dong Q, Touchon J, Gauthier S, Aisen PS and Vellas B. Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. J Neurol Neurosurg Psychiatry. 2020;91:1201-1209.
4. Haffner SM, Stern MP, Hazuda HP, Mitchell BD and Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA. 1990;263:2893-8.
5. Wong MS, Gu K, Heng D, Chew SK, Chew LS and Tai ES. The Singapore impaired glucose tolerance follow-up study: does the ticking clock go backward as well as forward? Diabetes Care. 2003;26:3024-30.
6. Rodrigues B, Asamane EA, Magalhaes R, Sousa N, Thompson JL and Santos NC. The association of dietary patterns with cognition through the lens of neuroimaging-a Systematic review. Ageing Res Rev. 2020;63:101145.
7. Cox SR, Lyall DM, Ritchie SJ, Bastin ME, Harris MA, Buchanan CR, Fawns-Ritchie C, Barbu MC, de Nooij L, Reus LM, Alloza C, Shen X, Neilson E, Alderson HL, Hunter S, Liewald DC, Whalley HC, McIntosh AM, Lawrie SM, Pell JP, Tucker-Drob EM, Wardlaw JM, Gale CR and Deary IJ. Associations between vascular risk factors and brain MRI indices in UK Biobank. Eur Heart J. 2019;40:2290-2300.
8. Ou YN, Tan CC, Shen XN, Xu W, Hou XH, Dong Q, Tan L and Yu JT. Blood Pressure and Risks of Cognitive Impairment and Dementia: A Systematic Review and Meta-Analysis of 209 Prospective Studies. Hypertension. 2020;76:217-225.
9. Cancela-Carral JM, Lopez-Rodriguez A and Mollinedo-Cardalda I. Effect of physical exercise on cognitive function in older adults' carriers versus noncarriers of apolipoprotein E4: systematic review and meta-analysis. J Exerc Rehabil. 2021;17:69-80.
10. Geijselaers SLC, Sep SJS, Schram MT, van Boxtel MPJ, Henry RMA, Verhey FRJ, Kroon AA, Schaper NC, Dagnelie PC, van der Kallen CJH, Stehouwer CDA and Biessels GJ. Insulin resistance and cognitive performance in type 2 diabetes - The Maastricht study. J Diabetes Complications. 2017;31:824-830.
11. van Agtmaal MJM, Houben A, de Wit V, Henry RMA, Schaper NC, Dagnelie PC, van der Kallen CJ, Koster A, Sep SJ, Kroon AA, Jansen JFA, Hofman PA, Backes WH, Schram MT and Stehouwer CDA. Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. Diabetes Care. 2018;41:2535-2543.
12. Lombardi G, Crescioli G, Cavedo E, Lucenteforte E, Casazza G, Bellatorre AG, Lista C, Costantino G, Frisoni G, Virgili G and Filippini G. Structural magnetic resonance imaging for the early diagnosis of dementia due to Alzheimer's disease in people with mild cognitive impairment. Cochrane Database Syst Rev. 2020;3:CD009628.
13. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, Stensvold D, Selbaek G and Wisloff U. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. Lancet Public Health. 2019;4:e565-e574.
14. Cano A, Turowski P, Ettcheto M, Duskey JT, Tosi G, Sanchez-Lopez E, Garcia ML, Camins A, Souto EB, Ruiz A, Marquie M and Boada M. Nanomedicine-based technologies and novel biomarkers for the diagnosis and treatment of Alzheimer's disease: from current to future challenges. J Nanobiotechnology. 2021;19:122.

15. Alawode DOT, Heslegrave AJ, Ashton NJ, Karikari TK, Simren J, Montoliu-Gaya L, Pannee J, A OC, Weston PSJ, Lantero-Rodriguez J, Keshavan A, Snellman A, Gobom J, Paterson RW, Schott JM, Blennow K, Fox NC and Zetterberg H. Transitioning from cerebrospinal fluid to blood tests to facilitate diagnosis and disease monitoring in Alzheimer's disease. J Intern Med. 2021.
16. Kashani AH, Asanad S, Chan JW, Singer MB, Zhang J, Sharifi M, Khansari MM, Abdolahi F, Shi Y, Biffi A, Chui H and Ringman JM. Past, present and future role of retinal imaging in neurodegenerative disease. Prog Retin Eye Res. 2021:100938.
17. Mutlu U, Bonnemaijer PWM, Ikram MA, Colijn JM, Cremers LGM, Buitendijk GHS, Vingerling JR, Niessen WJ, Vernooij MW, Klaver CCW and Ikram MK. Retinal neurodegeneration and brain MRI markers: the Rotterdam Study. Neurobiol Aging. 2017;60:183-191.
18. Ko F, Muthy ZA, Gallacher J, Sudlow C, Rees G, Yang Q, Keane PA, Petzold A, Khaw PT, Reisman C, Strouthidis NG, Foster PJ, Patel PJ, Eye UKB and Vision C. Association of Retinal Nerve Fiber Layer Thinning With Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. JAMA Neurol. 2018;75:1198-1205.
19. Gupta VB, Chitranshi N, den Haan J, Mirzaei M, You Y, Lim JK, Basavarajappa D, Godinez A, Di Angelantonio S, Sachdev P, Salekdeh GH, Bouwman F, Graham S and Gupta V. Retinal changes in Alzheimer's disease- integrated prospects of imaging, functional and molecular advances. Prog Retin Eye Res. 2020:100899.
20. Ciudin A, Simo-Servat O, Hernandez C, Arcos G, Diego S, Sanabria A, Sotolongo O, Hernandez I, Boada M and Simo R. Retinal Microperimetry: A New Tool for Identifying Patients With Type 2 Diabetes at Risk for Developing Alzheimer Disease. Diabetes. 2017;66:3098-3104.
21. Gella L, Raman R, Kulothungan V, Saumya Pal S, Ganesan S and Sharma T. Retinal sensitivity in subjects with type 2 diabetes mellitus: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS II, Report No. 4). Br J Ophthalmol. 2016;100:808-13.
22. Cheung CY, Chen D, Wong TY, Tham YC, Wu R, Zheng Y, Cheng CY, Saw SM, Baskaran M, Leung CK and Aung T. Determinants of quantitative optic nerve measurements using spectral domain optical coherence tomography in a population-based sample of non-glaucomatous subjects. Invest Ophthalmol Vis Sci. 2011;52:9629-35.
23. Fujiwara K, Yasuda M, Hata J, Hirakawa Y, Hashimoto S, Ueda E, Iwase A, Araie M, Yoshitomi T, Ninomiya T and Sonoda KH. Glucose Tolerance Levels and Circumpapillary Retinal Nerve Fiber Layer Thickness in a General Japanese Population: The Hisayama Study. Am J Ophthalmol. 2019;205:140-146.
24. Ho H, Tham YC, Chee ML, Shi Y, Tan NYQ, Wong KH, Majithia S, Cheung CY, Aung T, Wong TY and Cheng CY. Retinal Nerve Fiber Layer Thickness in a Multiethnic Normal Asian Population: The Singapore Epidemiology of Eye Diseases Study. Ophthalmology. 2019;126:702-711.
25. Khawaja AP, Chan MP, Garway-Heath DF, Broadway DC, Luben R, Sherwin JC, Hayat S, Khaw KT and Foster PJ. Associations with retinal nerve fiber layer measures in the EPIC-Norfolk Eye Study. Invest Ophthalmol Vis Sci. 2013;54:5028-34.
26. Khawaja AP, Chua S, Hysi PG, Georgoulas S, Currant H, Fitzgerald TW, Birney E, Ko F, Yang Q, Reisman C, Garway-Heath DF, Hammond CJ, Khaw PT, Foster PJ, Patel PJ, Strouthidis N, Eye UKB and Vision C. Comparison of Associations with Different Macular Inner Retinal Thickness Parameters in a Large Cohort: The UK Biobank. Ophthalmology. 2020;127:62-71.
27. Lamparter J, Schmidtmann I, Schuster AK, Siouli A, Wasielica-Poslednik J, Mirshahi A, Hohn R, Unterrainer J, Wild PS, Binder H, Lackner K, Beutel ME, Munzel T, Pfeiffer N and Hoffmann EM. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One. 2018;13:e0197682.
28. Mauschitz MM, Holz FG, Finger RP and Breteler MMB. Determinants of Macular Layers and Optic Disc Characteristics on SD-OCT: The Rhineland Study. Transl Vis Sci Technol. 2019;8:34.
29. Myers CE, Klein BE, Meuer SM, Swift MK, Chandler CS, Huang Y, Gangaputra S, Pak JW, Danis RP and Klein R. Retinal thickness measured by spectral-domain optical coherence tomography in eyes without retinal abnormalities: the Beaver Dam Eye Study. Am J Ophthalmol. 2015;159:445-56 e1.
30. Wang YX, Pan Z, Zhao L, You QS, Xu L and Jonas JB. Retinal nerve fiber layer thickness. The Beijing Eye Study 2011. PLoS One. 2013;8:e66763.

31. Zhang Q, Wang D, Wang A, Zhang S, Pan Y, Li Y, Chen S, Wu S, Wei W and Zhao X. Relationship of ideal cardiovascular health metrics with retinal vessel calibers and retinal nerve fiber layer thickness: a cross-sectional study. BMC Cardiovasc Disord. 2018;18:187.
32. Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, Loeffler M, Colijn JM, Ikram MA, Vingerling JR, Williams KM, Hammond CJ, Creuzot-Garcher C, Bron AM, Silva R, Nunes S, Delcourt C, Cougnard-Gregoire A, Holz FG, Klaver CCW, Breteler MMB, Finger RP and European Eye Epidemiology C. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. Ophthalmology. 2018;125:1526-1536.
33. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
34. Cui Q, Gogt P, Lam J, Siraj S, Hark L, Myers J, Katz L and Waisbourd M. Validation and reproducibility of the Heidelberg Edge Perimeter in the detection of glaucomatous visual field defects. International journal of ophthalmology. 2019;12:577-581.
35. Hong JT, Sung KR, Cho JW, Yun SC, Kang SY and Kook MS. Retinal nerve fiber layer measurement variability with spectral domain optical coherence tomography. Korean J Ophthalmol. 2012;26:32-8.
36. van Dongen MC, Wijckmans-Duysens NEG, den Biggelaar LJ, Ocke MC, Meijboom S, Brants HA, de Vries JH, Feskens EJ, Bueno-de-Mesquita HB, Geelen A, Stehouwer CD, Dagnelie PC and Eussen SJ. The Maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the Maastricht study. Nutrition. 2019;62:39-46.
37. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, de Vries J and Geelen A. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20:2289-2299.
38. Davis BJ, Vidal JS, Garcia M, Aspelund T, van Buchem MA, Jonsdottir MK, Sigurdsson S, Harris TB, Gudnason V and Launer LJ. The alcohol paradox: light-to-moderate alcohol consumption, cognitive function, and brain volume. J Gerontol A Biol Sci Med Sci. 2014;69:1528-35.
39. JHPM VDV, Koster A, JD VDB, Sep SJS, CJH VDK, Dagnelie PC, Schram MT, Henry RMA, Eussen S, MCJM VAND, Stehouwer CDA, Schaper NC and Savelberg H. Sedentary Behavior, Physical Activity, and Fitness-The Maastricht Study. Med Sci Sports Exerc. 2017;49:1583-1591.
40. Zhou TL, Kroon AA, Reesink KD, Schram MT, Koster A, Schaper NC, Dagnelie PC, van der Kallen CJH, Sep SJS, Stehouwer CDA and Henry RMA. Blood pressure variability in individuals with and without (pre)diabetes: The Maastricht Study. J Hypertens. 2018;36:259-267.
41. van der Berg JD, Willems PJ, van der Velde JH, Savelberg HH, Schaper NC, Schram MT, Sep SJ, Dagnelie PC, Bosma H, Stehouwer CD and Koster A. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. J Sports Sci. 2016;34:1867-73.
42. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N, Stehouwer C and Bosma H. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16.
43. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67:1729-1741.
44. Di Liegro CM, Schiera G, Proia P and Di Liegro I. Physical Activity and Brain Health. Genes (Basel). 2019;10.
45. Man AWC, Li H and Xia N. Impact of Lifestyles (Diet and Exercise) on Vascular Health: Oxidative Stress and Endothelial Function. Oxid Med Cell Longev. 2020;2020:1496462.
46. Harwerth RS, Wheat JL, Fredette MJ and Anderson DR. Linking structure and function in glaucoma. Prog Retin Eye Res. 2010;29:249-71.
47. Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A and Pfrieger FW. CNS synaptogenesis promoted by glia-derived cholesterol. Science. 2001;294:1354-7.
48. Parimisetty A, Dorsemans AC, Awada R, Ravanan P, Diotel N and Lefebvre d'Hellencourt C. Secret talk between adipose tissue and central nervous system via secreted factors-an emerging frontier in the neurodegenerative research. J Neuroinflammation. 2016;13:67.

49. Raichlen DA, Klimentidis YC, Bharadwaj PK and Alexander GE. Differential associations of engagement in physical activity and estimated cardiorespiratory fitness with brain volume in middle-aged to older adults. Brain Imaging Behav. 2020;14:1994-2003.
50. Lash TL VT, Haneuse S, Rothman KJ. . Modern epidemiology. 4. . Philadelphia: Wolters Kluwer; 2021.

Supplemental methods

Study population and design

We used data from The Maastricht Study, a prospectively designed, population-based observational cohort study. The rationale and methodology have been described previously.[1] In brief, the study focuses on the aetiology, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency.[1] The present report includes cross-sectional data of 7,689 participants who completed the baseline survey between November 2010 and December 2017. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.[1]

Retinal sensitivity

We used the Heidelberg Edge Perimeter (Heidelberg Engineering, Heidelberg, Germany), a static flicker perimeter, to assess retinal sensitivity.[2] Measurements were performed in a dimly lit room under supervision of a trained examiner. Any refractive error was corrected for with external lenses. For each eye, retinal sensitivity was measured at 54 coordinates in the central and peri macular area (between 48° in the transverse plane and 42° in the sagittal plane) and results were averaged in to “retinal sensitivity”. In brief, the participant was instructed to fixate their vision on a focus point and to indicate when they observed a static white light stimulus by pressing a joystick button. Light stimuli varying in strength between 0 and 35 decibel (dB) and sized 0.43° in diameter (Goldmann perimeter size III) were presented on an isoluminant background of 10 candela per square meter. To, per coordinate, determine the threshold of visual perception (i.e. the threshold at which the weakest presented visual stimulus could be perceived), we used the adaptive staircase thresholding algorithm standard automated perimetry 24-2 pattern setting. The intra-observer reliability for the assessment of the retinal sensitivity is 0.95.[3]

The device automatically calculated the following indices of measurement quality: the percentage of false positive entries, the percentage of false negative entries, and the

number of fixation errors. A false positive entry indicates that the participant responded when no stimulus was presented.[4] A false negative entry indicates that the participant did not respond to a stimulus that should be visible based on an earlier response.[4] A fixation error indicates that the fixation of the eye deviated more than 5° from the central fixation point.[4] We defined sufficient measurement quality as ≤ 15% false positive responses and ≤ 30% false negative responses.[4] To reduce measurement error, we averaged retinal sensitivity of both eyes when data of sufficient quality from both eyes were available (n=5,087 participants [89.8%]). When data from only one eye were available (n=579 participants [10.2%]) we used the retinal sensitivity of that eye in the analyses. More details are provided in the Supplemental Methods.

RNFL thickness

We assessed peripapillary RNFL thickness (μm) in both eyes using optical coherence tomography (OCT; Spectralis unit and Eye Explorer version 5.7.5.0 software; Heidelberg Engineering, Heidelberg, Germany; 3.45-mm-diameter—circle scan, manually centered on the optic nerve head, 12°, 768 voxels, 100 automatic real-time tracking). Intra- and interindividual reliability, expressed as intraclass correlation coefficients, are 0.97 and 0.96, respectively. At least 15 minutes before the examination pupils were dilated with topical 0.5% tropicamide and 2.5% phenylephrine. Experienced graders masked to clinical information on the participants reviewed the OCT scans and graded their quality. OCT images were excluded if one of the following criteria was present: scan error (i.e., incomplete scan, poor centring of the circular scan on the optic nerve head, RNFL layer incorrectly defined, or technical problem with the OCT device) or poor imaging quality (signal-to-noise ratio<15 dB).[5] If data from both eyes were available (n=2,711 participants) we averaged RNFL thickness of both eyes in order to reduce measurement error. If data from only one eye were available (n=2,544 participants), we used the RNFL thickness of that eye in the analyses. More details, including on quality criteria, are shown in the Supplemental Methods.

Grading of OCT circle scans

OCT scans were considered of sufficient quality if all the following criteria were met: good centring of the circular scan on the optic nerve head (examples of good, poor and very poor centring are shown in Supplemental Figure S3.1); complete (data of all 768 voxels was available); automatic quality ≥15 dB (an example of a scan with poor quality imaging is shown in Supplemental Figure S3.2); and no measurement error present (examples of all assessed measurement errors are shown in Supplemental Figure S3.2). The percentage of agreement for selection of scans with sufficient quality

ranged between 90% and 94% for four trained graders and was 70% for one grader (n=50 OCT scans per comparison).

Time lag between assessment of retinal sensitivity or RNFL thickness and other covariates

For a subset of participants retinal sensitivity and RNFL thickness measurements were performed as part of a catch up visit (n=227 and n=305, respectively). We checked whether exclusion of these participants or additional adjustment for the lag time between measurements altered associations under study and this was not the case (data not shown).

Healthy diet and alcohol consumption

We assessed dietary intake, including alcohol consumption, with a validated food frequency questionnaire,[6] and calculated the Dutch Healthy Diet index sum score, a measure of adherence to the Dutch dietary guidelines 2015.[6,7] The Dutch Healthy diet index sum score was developed based on 15 components of a Dutch diet, however as data on coffee intake (one of the 15 items) were presently not available, for this study we calculated the Dutch Healthy Diet index sum score based on 14 components (i.e. all 15 components except coffee intake). Next, as we investigated alcohol consumption separately from other components of a healthy diet, we recalculated the Dutch Healthy diet index sum score so that alcohol consumption was left out of this total score (therefore the Dutch Healthy diet index sum score used in the main analyses to study healthy diet as a main determinant consisted out of 13 components in total).
We categorized alcohol consumption into none (<1 unit/week [for both men and women]), light (≥1 unit/ week to 1 unit/day for men, ≥1 unit/ week to 0.5 unit/day for women), moderate (>1 to 2 units/day for men, >0.5 to 1 unit/day for women), and high (>2 units/day for men, >1 units/day for women) where 1 unit was defined as 10 gram/day (g/d) of total alcohol (i.e. ethanol) consumption, as previously described.[8] In analyses we used light drinkers as a reference group because we cannot distinguish so-called sick quitters from never drinkers (i.e. life-long abstainers) within the none consumers.[9]
We also assessed alcohol consumption with a different questionnaire than the validated food frequency questionnaire.[1] We used data on alcohol consumption from this different questionnaire in analyses where alcohol consumption was not the main determinant because data from this questionnaire were available in a larger number of participants (i.e. n=5,666 instead of n=5,377). For analyses where alcohol consumption was not the main determinant, we categorized alcohol consumption as none (for women and men, 0 units/week), moderate (for women and men, respectively, 1-7 units

and 1-14 units of alcohol consumption/week), and high (for women and men, respectively, >7 units and >14 units of alcohol consumption/week). We did not calculate a light alcohol consumption group because this questionnaire did not allow to distinguish between light and moderate alcohol consumption.

Cardiorespiratory fitness

We assessed cardiorespiratory fitness from a graded submaximal exercise protocol performed on a cycle ergometer system (CASE^{TM} version 6.6 in combination with e-bike; GE Healthcare, Milwaukee, WI), as described previously.[10] Cardiorespiratory fitness was defined as the maximum power output W_{max} and was adjusted for body mass (i.e. $W_{max} \cdot kg^{-1}$). During the submaximal exercise protocol blood pressure and electrical activity of the heart rhythm were monitored as described previously. Participants were excluded from the submaximal cycle ergometer test if they had experienced cardiovascular complications in the preceding three months; had an abnormal resting electrocardiogram; had a medical history of certain cardiovascular complications (e.g. pericarditis or hypertrophic cardiomyopathy); had severe hypertension (systolic blood pressure ≥180 mmHg and/or diastolic blood pressure ≥110 mmHg); had a history of kidney failure; or had an implantable cardioverter-defibrillator or a pacemaker.

The protocol consisted of a short warm-up period and at most seven stages with increasing work load. Participants were instructed to cycle at a cadence of 60–70 rotation per minute (rpm) during a short familiarization period without any external workload. For the first exercise stage, external workload was set at 25 W. Then, every 2 minutes the external workload was consecutively increased with 25 W. At the end of each stage, heart rate and blood pressure were measured. Further, the participant was asked to provide a rating of perceived exertion based on the 15-point Borg-scale, which is an interval scale that ranges from 6 points ("no exertion at all") up to 20 points ("maximal exertion"). The exercise protocol was considered as "completed" when heart rate reached ≥85% of the estimated maximum heart rate (220 minus the age) or when a rating of perceived exertion ≥17 was scored by the participant. Then, the test was also terminated by the end of stage 7 (work load of 175 W) if the heart rate was <85% and rating of perceived exertion was <17); or (prematurely) for medical reasons or when the participant was unwilling to continue. More details can be found elsewhere.[10]

Blood pressure

We assessed 24-hour ambulatory blood pressure (mm Hg) with an oscillometric device (WatchBP O3; Microlife, Widnau, Switzerland).[11] We assessed antihypertensive

medication use, an index of past exposure to relatively higher levels of blood pressure, via an interview.

Cholesterol

We determined total cholesterol (mmol/L) in fasting venous plasma sample.[1]

Physical activity

We measured daily activity levels (hours/day) with the activPAL3™ physical activity monitor (PAL technologies, Glasgow, UK), as previously described.[12] Participants were asked to wear the accelerometer for 8 consecutive days, without removing the device at any time. The total amount of physical activity (stepping time) was based on the stepping posture and calculated as the mean time (minutes) spent stepping during waking time per day, where standing time was not included. The method used to determine waking time has been previously described.[12]

Covariates

As described previously,[1] we assessed educational level (low, intermediate, high), socio-economic status (income level and occupational status; both presently available only in a subset of participants),[13] history of cardiovascular disease, mobility limitation, and age-related macular degeneration by questionnaire;[1] high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, fasting plasma glucose, and serum creatinine in fasting venous blood samples;[1] assessed glucose metabolism status based on fasting plasma glucose and oral glucose tolerance test-derived 2-hour post load glucose;[1] assessed office blood pressure and body-mass index as part of a physical examination;[1] and current presence of cataract and medication use as part of an interview; calculated the estimated glomerular filtration rate (eGFR) based on serum creatinine only,[14] since cystatin C was not presently available in all study participants; measured urinary albumin excretion in two 24-hour urine collections; assessed presence of retinopathy in both eyes via fundus photography; and used an automated refractor and noncontact tonometer (Tonoref II; Nidek, Gamagordi, Japan) to assess spherical equivalent and intraocular pressure in both eyes. Glaucoma was defined as use of intraocular pressure-lowering medication, intraocular pressure higher than 21 mm Hg in any eye (91.3% of all participants had data on intraocular pressure available for at least 1 eye), or both. Spherical equivalent was defined as the mean spherical equivalent of both eyes (available for 91.1% of all participants) or as the spherical equivalent of the eye for which data were available.

Statistical analyses

Collinearity diagnostics (i.e., tolerance <0.10 and/or variance inflation factor >10) were used to detect multicollinearity between covariates.

Additional analyses

To assess the robustness of our findings we performed a range of additional analyses. First, we repeated the analyses with additional adjustment for lifestyle factors (dietary intake, physical activity). Adjustment for these potential confounders was not included in the main analyses because data were missing for a relatively large number of participants (up to n=904 had missing data on one or more of these variables). Second, we studied the associations of office systolic and diastolic blood pressure with the outcomes under study; and associations of mean arterial pressure, estimated from office blood pressure (using the same formula as for 24-hour ambulatory blood pressure), with the outcomes under study. We did not use these covariates in the main analyses because they are less precise estimates of blood pressure than 24-hour ambulatory blood pressure.[15, 16] Third, we studied the association of lipid-lowering medication with outcomes under study. Fourth, we performed additional analyses in which we omitted antihypertensive medication use from the model for associations of 24-hour ambulatory systolic, diastolic, and mean arterial pressure with the outcomes under study to investigate how strongly antihypertensive medication use affected these associations. In addition, to investigate how strongly office systolic blood pressure affected the association of antihypertensive medication use with the outcomes under study, we performed additional analyses without adjustment for office systolic blood pressure. Fifth, to obtain a more detailed insight into how lipids are associated with outcomes under study, we studied associations of individual types of lipids (i.e. HDL, LDL, triglycerides) with outcomes under study (all three covariates were entered in the same model). Sixth, we studied the associations of body-mass index with outcomes under study. We did not use body-mass index in the main analyses because waist circumference is a more precise measure of visceral fat than body-mass index.[17] Seventh, to more robustly investigate whether hyperglycaemia may be a determinant of outcomes under study, we investigated whether fasting plasma glucose and 2-hour post load glucose were associated with retinal sensitivity and RNFL thickness. Eighth, to check whether both alcohol questionnaires that we used were similar, we studied the associations of alcohol consumption with the outcomes under study using data from both questionnaires. For this comparison only, we categorized alcohol consumption assessed with the food frequency questionnaire as none, moderate, and high alcohol consumption. Nineth, as non-linear associations of alcohol intake with cerebral neural measures have previously been described,[18] we tested for a quadratic association by

entering alcohol consumption (continuous variable) and a quadratic term for alcohol consumption in the model (i.e. alcohol consumption[continuous variable]*alcohol consumption[continuous variable]; we used the formula $y=x^2+x$). If the P-value of the quadratic term was <0.05, the association was considered to be statistically better described by a non-linear, quadratic association than by a linear association.[19] Tenth, we re-analysed the associations of current and never smoking with outcomes under study and used former smoking as a reference group. Eleventh, and only for cardiorespiratory fitness and physical activity, we adjusted associations for mobility limitation. We entered mobility limitation into a separate model because adjustment for this covariate may be overadjustment (i.e. mobility limitation is strongly associated with cardiorespiratory fitness and physical activity but the association with retinal sensitivity and RNFL thickness is less clear).[20] Twelfth, we additionally adjusted for kidney variables (eGFR and urinary albumin excretion), history of cardiovascular disease, intraocular pressure, spherical equivalent, cataract, retinopathy, glaucoma, and age-related macular degeneration. We adjusted for these covariates in separate models because they may be confounders but may also (in part) be mediators or descendants of the outcome.[20] Thirteenth, we performed additional analyses in which we excluded participants with either retinopathy, cataract, age-related macular degeneration, glaucoma, or any combination of these morbidities. Fourteenth, we repeated the analyses applying more strict quality criteria (i.e. when we in addition to the other criteria excluded measurements with >20% fixation errors entries). We did not include individuals with >20% fixation errors in the main analyses as this would have led to a strong reduction in the size of the study population (up to n=2,254 would not have been included in the analyses). Fifteenth, we replaced educational status with occupational status or income level; and we replaced glucose metabolism status with fasting plasma glucose, 2-hour post load glucose, or HbA1c. Sixteenth, to test robustness of interaction findings for glucose metabolism status we performed tests of interactions with continuous measures of glycaemia (i.e. fasting plasma glucose, 2-hour post load glucose, or HbA1c) if associations were modified by glucose metabolism status. Last, we studied the associations of age with retinal sensitivity and RNFL thickness. We performed these analyses so that we could compare with how many years of “ageing” the betas for determinants under study correspond.

References

1. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
2. Kaczorowski K, Mulak M, Szumny D and Misiuk-Hojło M. Heidelberg Edge Perimeter: The New Method of Perimetry. Adv Clin Exp Med. 2015;24:1105-12.
3. Cui Q, Gogt P, Lam J, Siraj S, Hark L, Myers J, Katz L and Waisbourd M. Validation and reproducibility of the Heidelberg Edge Perimeter in the detection of glaucomatous visual field defects. International journal of ophthalmology. 2019;12:577-581.
4. Germany HEG. Heidelberg Engineering User Manual. Software Version 3.1. 2014.
5. De Clerck EEB, Schouten JSAG, Berendschot TTJM, Beckers HJM, Schaper NC, Schram MT, Stehouwer CDA and Webers CAB. Loss of Temporal Peripapillary Retinal Nerve Fibers in Prediabetes or Type 2 Diabetes Without Diabetic Retinopathy: The Maastricht Study. Invest Ophth Vis Sci. 2017;58.
6. van Dongen MC, Wijckmans-Duysens NEG, den Biggelaar LJ, Ocke MC, Meijboom S, Brants HA, de Vries JH, Feskens EJ, Bueno-de-Mesquita HB, Geelen A, Stehouwer CD, Dagnelie PC and Eussen SJ. The Maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the Maastricht study. Nutrition. 2019;62:39-46.
7. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, de Vries J and Geelen A. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20:2289-2299.
8. Davis BJ, Vidal JS, Garcia M, Aspelund T, van Buchem MA, Jonsdottir MK, Sigurdsson S, Harris TB, Gudnason V and Launer LJ. The alcohol paradox: light-to-moderate alcohol consumption, cognitive function, and brain volume. J Gerontol A Biol Sci Med Sci. 2014;69:1528-35.
9. Tsubono Y, Yamada S, Nishino Y, Tsuji I and Hisamichi S. Choice of comparison group in assessing the health effects of moderate alcohol consumption. JAMA. 2001;286:1177-8.
10. JHPM VDV, Koster A, JD VDB, Sep SJS, CJH VDK, Dagnelie PC, Schram MT, Henry RMA, Eussen S, MCJM VAND, Stehouwer CDA, Schaper NC and Savelberg H. Sedentary Behavior, Physical Activity, and Fitness-The Maastricht Study. Med Sci Sports Exerc. 2017;49:1583-1591.
11. Zhou TL, Kroon AA, Reesink KD, Schram MT, Koster A, Schaper NC, Dagnelie PC, van der Kallen CJH, Sep SJS, Stehouwer CDA and Henry RMA. Blood pressure variability in individuals with and without (pre)diabetes: The Maastricht Study. J Hypertens. 2018;36:259-267.
12. van der Berg JD, Willems PJ, van der Velde JH, Savelberg HH, Schaper NC, Schram MT, Sep SJ, Dagnelie PC, Bosma H, Stehouwer CD and Koster A. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. J Sports Sci. 2016;34:1867-73.
13. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N, Stehouwer C and Bosma H. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16.
14. Shahbaz H and Gupta M. Creatinine Clearance StatPearls Treasure Island (FL); 2021.
15. Hutcheon JA, Chiolero A and Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.
16. Myers MG and Kaczorowski J. Office blood pressure is lower than awake ambulatory blood pressure at lower targets for treatment. J Clin Hypertens (Greenwich). 2017;19:1210-1213.
17. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, Cuevas A, Hu FB, Griffin BA, Zambon A, Barter P, Fruchart JC, Eckel RH, Matsuzawa Y and Despres JP. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol. 2020;16:177-189.
18. McEvoy LK, Fennema-Notestine C, Elman JA, Eyler LT, Franz CE, Hagler DJ, Jr., Hatton SN, Lyons MJ, Panizzon MS, Dale AM and Kremen WS. Alcohol intake and brain white matter in middle aged men: Microscopic and macroscopic differences. Neuroimage Clin. 2018;18:390-398.
19. Goel S, Sharma A and Garg A. Effect of Alcohol Consumption on Cardiovascular Health. Curr Cardiol Rep. 2018;20:19.

20. Schisterman EF, Cole SR and Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20:488-95.

Supplemental results

We generally observed quantitatively similar results in a range of additional analyses. First, we had numerically similar results after we additionally adjusted associations under study for lifestyle factors (i.e. dietary intake and physical activity; Supplemental Tables S3.6 and S3.7). Second, systolic, diastolic, and mean arterial blood pressure, estimated from office blood pressure measurements, were associated with greater retinal sensitivity and with lower RNFL thickness (Supplemental Table S3.8). Third, after full adjustment (model 3), lipid-lowering medication use was significantly associated with lower retinal sensitivity, but was not associated with RNFL thickness (per SD, standardized beta [95% CI], -0.08 [-0.14; -0.01], and 0.05 [-0.02; 0.13], respectively; Supplemental Table S3.8). Fourth, associations of systolic, diastolic, and mean arterial blood pressure (estimated from 24-hour ambulatory blood pressure measurements) with outcomes under study did not materially change when we omitted antihypertensive medication use from the model (Supplemental Table S3.8). Similarly, associations of antihypertensive medication use with outcomes remained similar when we did not adjust for office systolic blood pressure (Supplemental Table S3.8). Fifth, greater HDL and LDL, but not triglycerides, were associated with greater retinal sensitivity and greater RNFL thickness (Supplemental Table S3.8). Sixth, greater body-mass index was associated with greater RNFL thickness, but not with retinal sensitivity (Supplemental Table S3.8). Seventh, fasting plasma glucose and 2-hour post load glucose were associated with lower retinal sensitivity and lower RNFL thickness (Supplemental Table S3.8). Eighth, we had similar findings when we studied the associations of alcohol consumption with the outcomes under study using data from both questionnaires (both questionnaires are explained in the Supplemental Methods; Supplemental Table S3.8). Ninth, the association of alcohol consumption with retinal sensitivity was non-linear (i.e. dome shaped; $P_{quadratic}<0.01$) and the association of alcohol consumption with RNFL thickness was linear ($P_{quadratic}>0.05$; Supplemental Figure S3.3). Tenth, current versus former smoking, and, less strongly, never versus former smoking were associated with lower retinal sensitivity and greater RNFL thickness (Supplemental Table S3.8). Eleventh, we had numerically similar findings when we additionally adjusted associations of lower cardiorespiratory fitness and lower physical activity with outcomes under study for mobility limitation (Supplemental Table S3.8). Twelfth, results did generally not materially change after additional adjustment for kidney variables (eGFR and urinary albumin excretion), history of cardiovascular disease, intraocular pressure, spherical equivalent, cataract, retinopathy, glaucoma, and age-related macular degeneration (Supplemental Tables S3.6 and S3.7). There was one exception, when we additionally adjusted for spherical equivalent, the association of current versus never smoking with RNFL thickness was strongly

attenuated (when we adjusted for intraocular pressure instead of both intraocular pressure and spherical equivalent [which is shown in Supplemental Table S3.7] we did not observe this strong attenuation). Thirteenth, results were numerically similar after exclusion of participants with cataract, retinopathy, glaucoma, and age-related macular degeneration, except for the association between total cholesterol and RNFL thickness which was strongly attenuated after exclusion of these participants (Supplemental Table S3.9). Fourteenth, we had similar findings when we applied more strict quality criteria for analyses in which retinal sensitivity was the outcome (Supplemental Table S3.9). Fifteenth, we had similar findings when we replaced educational status with occupational status or income level; and when we replaced glucose metabolism status with fasting plasma glucose, 2-hour post load glucose, or HbA1c (Supplemental Tables S3.10 and S3.11). There were some inconsistent exceptions: when we replaced educational level with occupational level, we found that the association of waist circumference with retinal sensitivity became stronger; that the association of total cholesterol with RNFL thickness became less strong; and that the association of antihypertensive medication with RNFL thickness became less strong. Then, when we replaced educational level with occupational level or income level, we found less strong associations of cardiorespiratory fitness with RNFL thickness. Sixteenth, for the three associations under study that were modified by glucose metabolism status, when we tested whether interaction terms composed of continuous measures of glycaemia also modified these associations, we found that two associations were modified by all three continuous measures and one associations was modified by two out of three continuous measures (Supplemental Table S3.12). Last, after full adjustment (model 3), greater age was significantly associated with lower retinal sensitivity and lower RNFL thickness (standardized beta [95% confidence interval], per year greater age -0.04 [-0.05; -0.04] and -0.01 [-0.01;-0.01], respectively; Supplemental Table S3.8). Hence, the beta for 1 SD greater HbA1c corresponds with approximately 1.3 years of ageing for retinal sensitivity and 5.0 years of ageing for RNFL thickness; the beta of 1 SD lower adherence to a healthy diet corresponds with approximately 1.5 years of ageing for retinal sensitivity and 3.0 years of ageing for RNFL thickness; and the beta of 1 SD lower cardiorespiratory fitness corresponds with approximately 1.3 years of ageing for retinal sensitivity and 3.0 years of ageing for RNFL thickness. Therefore, added up, the combination of these three adverse factors corresponds with approximately 4.1 and 11.0 years of ageing for, respectively, retinal sensitivity and RNFL thickness.

Supplemental figures and tables

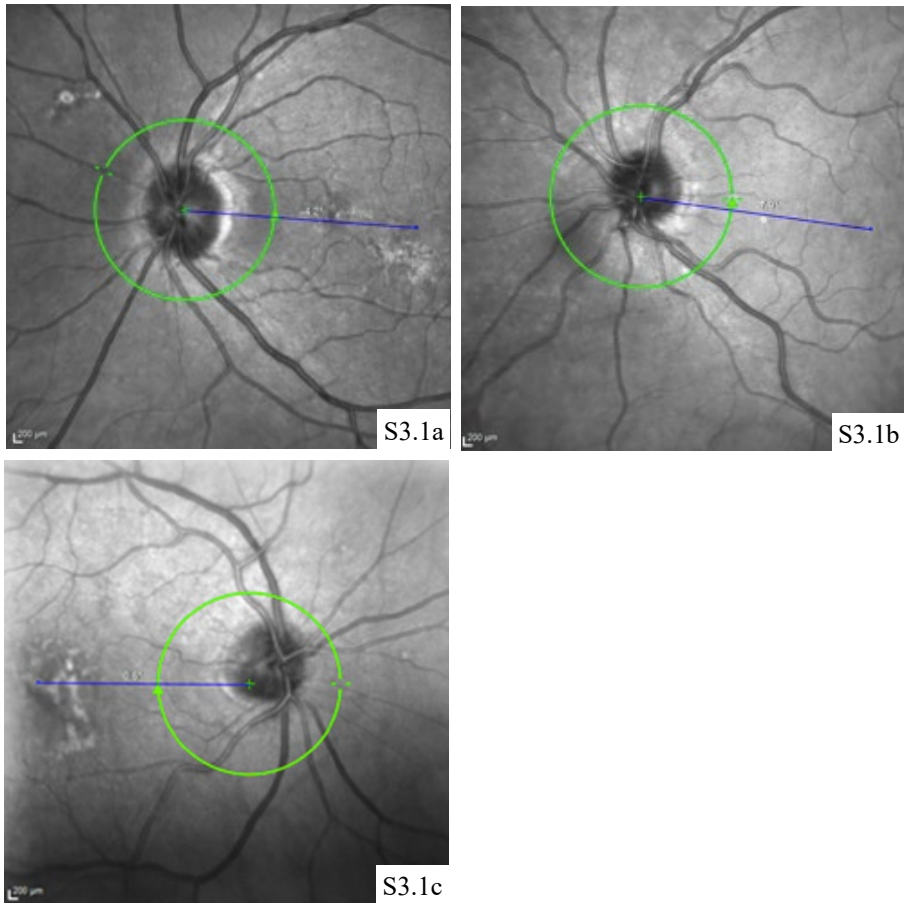
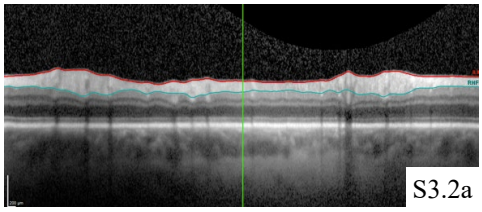


Figure S3.1 Examples of quality of centring of circular scans on the optic nerve head. Figure S3.1 shows examples of quality of centring of the circular scan on the optic nerve head: S3.1a shows good quality, S3.1b shows poor quality, and S3.1c shows very poor quality.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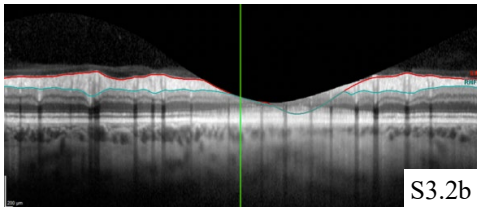
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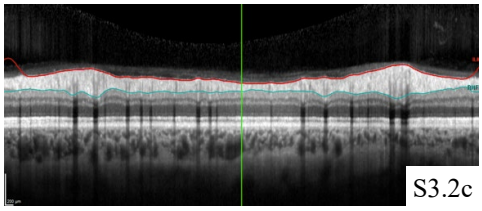
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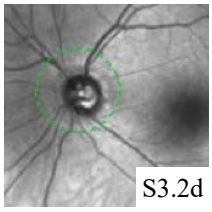
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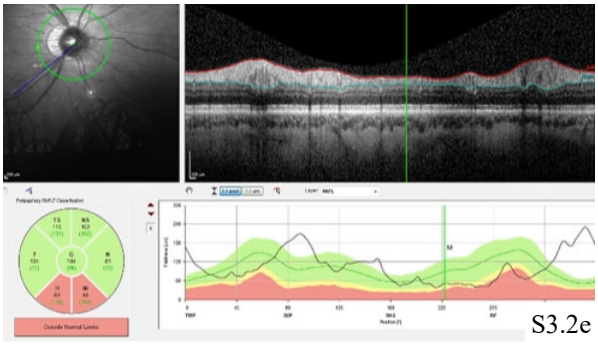
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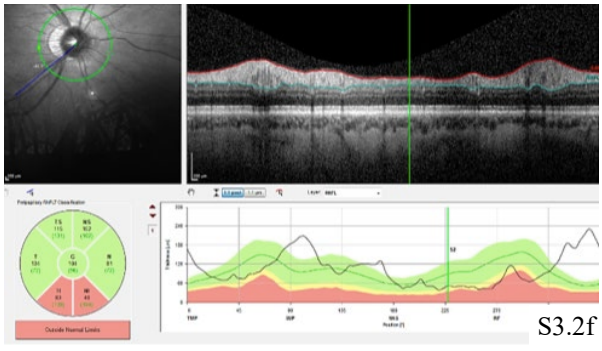

S3.2f

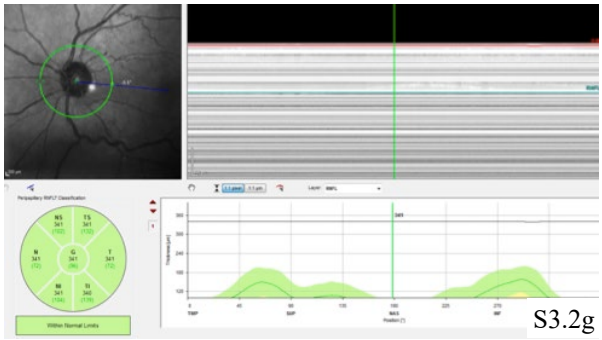


Figure S3.2 Examples of poor quality and scan errors. S3.2a: Example of poor imaging quality (Signal-to-noise ratio<15 dB); S3.2b: OCT device too close to the eye; S3.2c: RNFL layer incorrectly defined; S3.2d: incorrect circle position (dashed line); S3.2e: participant does not look in the correct direction; S3.2f: technical problem with OCT device; S3.2g: autofluorescence on. Abbreviations: OCT, optical coherence tomography; RNFL, retinal nerve fibre layer thickness.

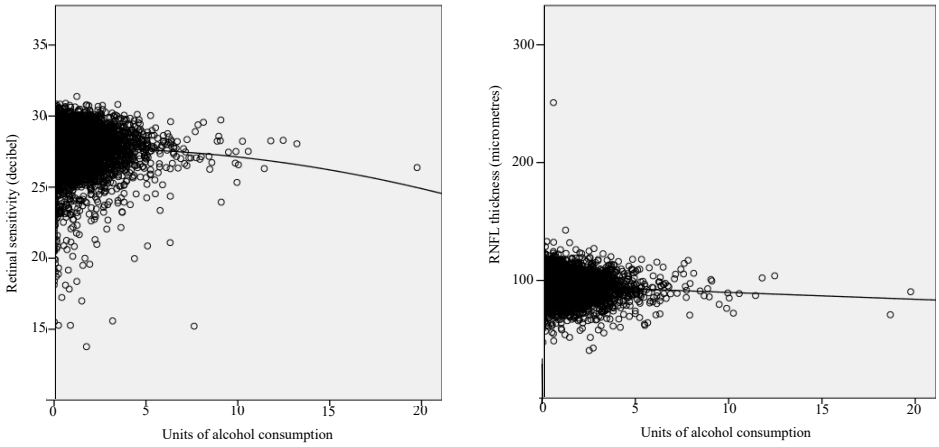


Figure S3.3 shows the quadratic (i.e. non-linear) association of alcohol consumption with retinal sensitivity and shows the linear association of alcohol consumption with RNFL thickness. One unit of alcohol consumption corresponds with 10 grams of ethanol intake/ day.
Abbreviations: RNFL, retinal nerve fibre layer.

Table S3.1 Additional general study population characteristics according to tertiles of retinal sensitivity in the study population with complete data on waist circumference.

	Retinal sensitivity				
Characteristic	**Total study population (n=5,666)**	**Tertile 1 (high) (n=1,877)**	**Tertile 2 (middle) (n=1,901)**	**Tertile 3 (low) (n=1,888)**	
Additional characteristics					**Number of participants with missing data**
Income level (euro)	2,038 ± 831	1,983 ± 846	2,071 ± 831	2,056 ± 816	1,332
Educational status					4,135
Low	450 (29.4)	139 (34.2)	134 (28.1)	177 (27.2)	
Intermediate	576 (37.6)	134 (33.2)	181 (37.9)	261 (40.2)	
High	505 (33.0)	131 (32.4)	162 (34.0)	212 (32.6)	
Office systolic blood pressure (mmHg)	133.2 ± 17.7	136.1 ± 18.7	132.3 ± 17.1	131.2 ± 16.8	0
Office diastolic blood pressure (mmHg)	75.3 ± 9.7	74.6 ± 9.8	75.2 ± 9.5	76.2 ± 9.9	1
Mean arterial pressure, estimated from office blood pressure (mmHg)	94.6 ± 11.2	95.1 ± 11.4	94.2 ± 11.0	94.6 ± 11.3	1
HDL (mmol/L)	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	2
LDL (mmol/L)	3.0 ± 1.0	2.9 ± 1.0	3.1 ± 1.0	3.1 ± 1.0	4
Triglycerides (mmol/L)	1.2 [0.9-1.7]	1.2 [0.9-1.7]	1.2 [0.9-1.7]	1.2 [0.9-1.7]	2
Measures of glycaemia					
Fasting plasma glucose (mmol/L)	5.9 ± 1.5	6.0 ± 1.7	5.8 ± 1.5	5.7 ± 1.4	0
2-hour post-load glucose (mmol/L)	6.1 [4.9-8.5]	6.7 [5.2-9.8]	6.0 [4.8-8.4]	5.3 [4.9-5.9]	3
Body-mass index (kg/m^2)	26.9 ± 4.4	27.2 ± 4.5	26.7 ± 4.3	26.6 ± 4.4	0
Mobility limitation	944 (16.7)	408 (21.8)	311 (16.4)	225 (12.0)	18
Spherical equivalent (dioptre)	0.1 [-1.4-1.1]	0.1 [-1.5-1.3]	0.1 [-1.4-1.1]	-0.1 [-1.5-0.9]	268
Intraocular pressure (mmHg)	14.0 [11.7-16.0]	14.0 [11.5-16.0]	13.9 [11.5-16.0]	13.9 [11.7-15.7]	310
Retinopathy	82 (1.5)	20 (2.8)	19 (1.0)	13 (0.7)	227
Cataract	298 (7.4)	195 (13.6)	78 (5.6)	25 (2.1)	1,618
Age-related macular degeneration	65 (1.3)	43 (2.7)	15 (0.9)	7 (0.4)	656
Glaucoma	223 (4.2)	103 (5.8)	62 (3.4)	58 (3.3)	310
History of CVD	915 (16.2)	404 (21.6)	305 (16.1)	206 (11.0)	19
eGFR, ml/min/1.73m2	81.6 ± 14.0	78.2 ± 14.4	81.5 ± 13.6	85.2 ± 13.0	4
Urinary albumin excretion (mg/24 hours)	5.4 [3.3-10.0]	5.5 [3.8-11.0]	5.1 [3.3-9.4]	5.2 [3.3-9.6]	23
Albuminuria*	462 (8.2)	183 (9.8)	156 (8.2)	123 (6.5)	23

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). Abbreviations: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table S3.2 General study population characteristics according to tertiles of RNFL thickness in the study population with complete data on waist circumference.

	RNFL thickness				
Characteristic	**Total study population (n=5,255)**	**Tertile 1 (low) (n=1,751)**	**Tertile 2 (middle) (n=1,752)**	**Tertile 3 (high) (n=1,752)**	
Demographic characteristics					**Number of participants with missing data**
Age (years)	59.5 ± 8.7	60.1 ± 8.5	59.3 ± 8.7	59.1 ± 8.7	0
Men	2,576 (49.0)	978 (55.9)	819 (46.7)	779 (44.5)	0
Educational level					0
Low	1,848 (35.2)	576 (32.9)	626 (35.7)	646 (36.9)	
Middle	1,472 (28.0)	477 (27.2)	484 (27.6)	511 (29.2)	
High	1,935 (36.8)	698 (39.9)	642 (36.6)	595 (34.0)	
Potentially modifiable risk factors for dementia					
HbA1c (mmol/mol)	39.3 ± 9.2	40.0 ± 9.6	39.2 ± 9.4	38.8 ± 8.6	6
HbA1c (%)	5.8 ± 0.8	5.8 ± 0.9	5.7 ± 0.9	5.7 ± 0.8	6
Dutch Healthy Diet score (points)	76.6 ± 14.5	75.7 ± 14.3	77.2 ± 14.6	76.9 ± 14.5	274
Cardiorespiratory fitness (Wmax·kg−1)	2.2 ± 0.6	2.1 ± 0.6	2.2 ± 0.6	2.1 ± 0.6	713
Alcohol consumption					266
None	787 (15.8)	237 (14.3)	280 (16.8)	270 (16.3)	
Light	1,546 (31.0)	517 (31.1)	501 (31.1)	528 (31.8)	
Moderate	1,068 (21.4)	340 (20.5)	353 (21.2)	373 (22.5)	
High	1,588 (31.8)	568 (34.2)	530 (31.9)	488 (29.4)	
Smoking status					0
Never	2,016 (38.4)	668 (38.1)	654 (37.3)	694 (39.6)	
Former	2,567 (48.8)	882 (50.4)	881 (50.3)	804 (45.9)	
Current	672 (12.8)	201 (11.5)	217 (12.4)	254 (14.5)	
Antihypertensive medication use	1,933 (36.8)	733 (41.9)	615 (35.1)	585 (33.4)	0
Ambulatory 24-h diastolic blood pressure(mmHg)	72.9 ± 7.1	73.2 ± 6.8	73.0 ± 7.1	72.4 ± 7.2	509
Mean arterial pressure, estimated from 24-h ambulatory blood pressure	88.1 ± 7.8	88.6 ± 7.6	88.2 ± 7.8	87.5 ± 8.0	509
Total cholesterol (mmol/L)	5.3 ± 1.1	5.1 ± 1.1	5.3 ± 1.1	5.3 ± 1.1	2
Waist circumference (cm)	94.7 ± 13.4	95.8 ± 13.5	94.1 ± 13.0	94.1 ± 13.5	0
Physical activity (minutes per day)	118.9 ± 40.7	117.0 ± 40.2	120.6 ± 40.4	119.2 ± 41.5	745

Table S3.2 (continued)

Characteristic	RNFL thickness				
	Total study population (n=5,255)	**Tertile 1 (low) (n=1,751)**	**Tertile 2 (middle) (n=1,752)**	**Tertile 3 (high) (n=1,752)**	
Demographic characteristics					**Number of participants with missing data**
Additional characteristics					
Glucose metabolism status					0
Normal glucose metabolism	3,213 (61.1)	990 (56.5)	1,100 (62.8)	1,123 (64.1)	
Prediabetes	788 (15.0)	271 (15.5)	261 (14.9)	256 (14.6)	
Type 2 diabetes	1,224 (23.3)	480 (27.4)	379 (21.6)	365 (20.8)	
Other type of diabetes	30 (0.6)	10 (0.6)	12 (0.7)	8 (0.5)	
Glucose-lowering medication use	921 (17.5)	370 (21.1)	286 (16.3)	265 (15.1)	0
Lipid-lowering medication use	1,658 (31.6)	606 (34.6)	526 (30.0)	526 (30.0)	0
Income level (euro)	2,019 ± 829	2,012 ± 845	2,043 ± 835	2,003 ± 806	1243
Occupational status					
Low	572 (31.1)	188 (28.4)	190 (31.3)	194 (11.1)	
Intermediate	666 (36.3)	232 (35.0)	229 (37.7)	205 (36.2)	
High	599 (32.6)	243 (36.7)	189 (31.1)	167 (29.5)	
Measures of glycaemia					
Fasting plasma glucose (mmol/L)	5.9 ± 1.5	6.0 ± 1.7	5.8 ± 1.5	5.8 ± 1.4	1
2-hour post-load glucose (mmol/L)	6.2 [4.9-8.7]	6.1 [5.1-9.4]	6.1 [4.9-8.5]	6.1 [4.9-8.3]	273
Office systolic blood pressure (mmHg)	133.3 ± 17.7	134.8 ± 17.6	133.0 ± 17.5	132.1 ± 18.0	0
Office diastolic blood pressure (mmHg)	75.6 ± 9.8	76.4 ± 9.9	75.3 ± 9.7	75.0 ± 9.8	1
Mean arterial pressure, estimated from office blood pressure (mmHg)	94.8 ± 11.3	95.9 ± 11.3	94.5 ± 11.1	94.0 ± 11.4	1
Total/HDL cholesterol ratio (no unit)	3.6 ± 1.2	3.6 ± 1.1	3.6 ± 1.3	3.5 ± 1.1	0
HDL (mmol/L)	1.6 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	2
LDL (mmol/L)	3.0 ± 1.0	3.0 ± 1.0	3.1 ± 1.0	3.1 ± 1.0	4
Triglycerides (mmol/L)	1.2 [0.9-1.7]	1.2 [0.9-1.7]	1.2 [0.9-1.7]	1.2 [0.9-1.6]	2
Body-mass index (kg/m^2)	26.8 ± 4.4	26.9 ± 4.3	26.7 ± 4.4	26.8 ± 4.5	1

Table S3.2 (continued)

Characteristic	RNFL thickness				
	Total study population (n=5,255)	**Tertile 1 (low) (n=1,751)**	**Tertile 2 (middle) (n=1,752)**	**Tertile 3 (high) (n=1,752)**	
Demographic characteristics					**Number of participants with missing data**
Mobility limitation	870 (16.6)	310 (17.8)	275 (15.8)	285 (16.3)	19
Spherical equivalent (dioptre)	0.1 [-1.2-1.1]	-0.5 [-2.8-0.6]	0.1 [-1.1-1.1]	0.6 [-0.3-1.6]	274
Intraocular pressure (mmHg)	14.0 [11.8-16.0]	14.5 [12.0-16.5]	13.9 [11.7-15.9]	13.5 [11.5-15.9]	316
Retinopathy	76 (1.5)	31 (1.8)	17 (1.0)	28 (1.6)	125
Cataract	231 (7.0)	87 (8.3)	75 (6.8)	69 (6.1)	1,963
Age-related macular degeneration	60 (1.3)	24 (1.6)	21 (1.4)	15 (1.0)	625
Glaucoma	230 (4.7)	116 (7.1)	51 (3.1)	63 (3.8)	316
History of CVD	872 (16.7)	317 (18.2)	285 (16.4)	270 (15.5)	22
eGFR, ml/min/1.73m^2	82.4 ± 13.9	81.9 ± 14.2	82.2 ± 13.9	83.1 ± 13.7	3
Urinary albumin excretion (mg/24 hours)	5.4 [3.4-10.0]	5.5 [3.5-10.7]	5.4 [3.4-9.6]	5.2 [3.4-9.8]	22
Albuminuria	411 (7.9)	153 (8.8)	133 (7.6)	125 (7.2)	22

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). Abbreviations: RNFL, retinal nerve fibre layer thickness, HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated haemoglobin A1c; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate.

Table S3.3 General study population characteristics of the included and excluded participants, for the study populations with complete data on waist circumference.

	Retinal sensitivity			RNFL thickness		
Characteristic	**Included study population (n=5,666)**	**Missing data in/excluded**	**Excluded study population (n=2,023)**	**Included study population (n=5,255)**	**Missing data in/excluded**	**Excluded study population (n=2,434)**
Demographic						
Age (years)	59.7 ± 8.7	0/0	60.1 ± 8.6	59.5 ± 8.7	0/0	60.6 ± 8.7
Men	2862 (50.5)	0/0	1,012 (50.0)	2,576 (49.0)	0/0	1,298 (53.3)
Educational level		0/115			0/115	
Low	1927 (34.0)		703 (36.8)	1,848 (35.2)		782 (33.7)
Medium	1571 (27.7)		521 (27.3)	1,472 (28.0)		620 (26.7)
High	2168 (38.3)		684 (35.8)	1,935 (36.8)		917 (39.5)
Potentially modifiable risk factors for dementia						
HbA1c (mmol/mol)*	39.1 ± 9.3	4/10	41.7 ± 10.4	39.3 ± 9.2	6/8	40.9 ± 10.5
HbA1c (%)*	5.7 ± 0.9	4/10	6.0 ± 1.0	5.8 ± 0.8	6/8	5.9 ± 1.0
Dutch Healthy Diet score (points)	76.6 ± 14.6	297/208	75.7 ± 14.3	76.6 ± 14.5	274/231	75.8 ± 14.7
Cardiorespiratory fitness (Wmax·kg−1)	2.1 ± 0.6	767/429	2.1 ± 0.6	2.2 ± 0.6	713/483	2.1 ± 0.6
Alcohol consumption		289/206			266/229	
None	814 (15.1)		330 (18.2)	787 (15.8)		357 (16.2)
Light	1656 (17.6)		614 (33.8)	1,546 (31.0)		723 (32.8)
Moderate	1186 (22.1)		346 (19.0)	1,068 (21.4)		464 (21.0)
High	1721 (32.0)		527 (29.0)	1,588 (31.8)		661 (30.0)
Smoking status		0/64			0/64	
Never	2170 (38.3)		665 (33.9)	2,016 (38.4)		819 (34.6)
Former	2785 (49.2)		979 (50.0)	2,567 (48.8)		1,197 (50.5)
Current	711 (12.5)		315 (16.1)	672 (12.8)		354 (14.9)
Antihypertensive medication use	2074 (36.6)	0/6	862 (42.7)	1,933 (36.8)	0/6	1003 (41.3)
Ambulatory 24-h systolic blood pressure (mmHg)	118.9 ± 11.6	592/285	119.0 ± 11.9	118.6 ± 11.4	509/367	119.7 ± 12.2
Ambulatory 24-h diastolic blood pressure(mmHg)	72.9 ± 7.2	592/285	73.2 ± 7.0	72.9 ± 7.1	509/367	73.2 ± 7.4
Mean arterial pressure (mm Hg)	88.2 ± 8.0	592/285	88.5 ± 7.9	88.1 ± 7.8	509/367	88.7 ± 8.3
Total cholesterol (mmol/L)	5.2 ± 1.1	2/4	5.2 ± 1.2	5.3 ± 1.1	2/4	5.2 ± 1.1
Waist circumference (cm)	94.8 ± 13.4	0/5	96.9 (14.5)	94.7 ± 13.4	0/5	96.8 ± 14.3
Physical activity (minutes per day)	118.9 ± 40.9	639/593	115.4 ± 41.1	118.9 ± 40.7	745/487	116.4 ± 41.3

Table S3.3 (continued)

	Retinal sensitivity			RNFL thickness		
Characteristic	**Included study population (n=5,666)**	**Missing data in/excluded**	**Excluded study population (n=2,023)**	**Included study population (n=5,255)**	**Missing data in/excluded**	**Excluded study population (n=2,434)**
Other						
Glucose metabolism status		0/0			0/0	
Normal glucose metabolism	3514 (62.0)		1,091 (53.9)	3,213 (61.1)		1,392 (57.2)
Prediabetes	840 (14.8)		301 (14.9)	788 (15.0)		353 (14.5)
Type 2 diabetes	1278 (22.6)		615 (30.4)	1,224 (23.3)		669 (27.5)
Other type of diabetes	34 (0.6)		16 (0.8)	30 (0.6)		20 (0.8)
Glucose-lowering medication use	957 (16.9)	0/6	498 (24.7)	921 (17.5)	0/6	534 (22.0)
Total/HDL cholesterol ratio (no unit)	3.6 ± 1.2	0/4	3.8 ± 1.2	3.6 ± 1.2	0/4	3.7 ± 1.2
Lipid-lowering medication use	1744 (30.8)	0/6	760 (37.7)	1,658 (31.6)	0/6	846 (34.8)
Outcomes						
Retinal sensitivity (dB)	27.7 ± 1.6	0/1947*	27.5 ± 1.7	NA		NA
RNFL thickness (µm)	NA		NA	94.9 ± 10.8	0/2,338*	95.1 ± 11.5

Data are presented as mean ± standard deviation, median [interquartile range] or number (%). *number of participants with missing data or with retinal sensitivity or RNFL thickness assessments of insufficient quality in both eyes. Abbreviations: RNFL, retinal nerve fibre layer; HDL, high-density lipoprotein; NGM, normal glucose metabolism; Hba1c, glycated haemoglobin.; NA, not applicable.

Table S3.4 P-values for interaction of sex and glucose metabolism status with potentially modifiable risk factors for dementia in associations with retinal sensitivity and RNFL thickness as the outcomes.

	Retinal sensitivity			RNFL thickness		
	Sex	Prediabetes	Type 2 diabetes	Sex	Prediabetes	Type 2 diabetes
	P-value	P-value	P-value	P-value	P-value	P-value
Potentially modifiable risk factors for dementia						
HbA1c	0.57	0.55	0.94	0.99	0.15	0.37
Healthy Diet score	0.54	**0.04***	0.31	0.18	0.78	0.23
Cardiorespiratory fitness	0.70	0.44	0.38	0.70	0.98	0.78
Alcohol consumption						
None versus light	0.78	0.65	0.21	0.53	0.40	0.81
Moderate versus light	0.21	0.29	0.18	0.95	0.75	0.50
High versus light	0.25	0.81	0.25	0.36	0.57	0.38
Smoking						
Former smoker	0.27	0.51	0.15	0.57	0.50	0.80
Current smoker	0.38	0.76	0.36	0.51	0.46	0.46
Antihypertensive medication use	0.90	0.93	0.91	0.31	0.71	0.44
24-hour ambulatory systolic blood pressure	0.76	0.06	**0.00**	0.65	0.65	**0.049**
24-hour ambulatory diastolic blood pressure	0.83	0.16	0.47	0.84	0.15	0.19
Mean arterial pressure	0.73	0.07	0.06	0.91	0.29	0.07
Total cholesterol	0.21	0.69	0.88	0.55	0.17	**0.04**
Waist circumference	0.60	0.85	0.59	0.25	0.62	0.45
Lower physical activity	0.09	**0.04***	0.40	0.71	0.11	0.42

P-values represents the P-values for the interaction terms of sex and glucose metabolism status (i.e. dummy variables of prediabetes versus normal glucose metabolism status and type 2 diabetes versus normal glucose metabolism status) with the determinant under study (e.g. sex*total cholesterol) in the associations of determinants with retinal sensitivity or RNFL thickness. * In glucose metabolism status-stratified analyses we did not observe a consistent pattern (data not shown). Variables in the model in addition to determinants and interaction term(s) with sex or glucose metabolism status are: age, sex, glucose metabolism status (where applicable) educational status, office systolic blood pressure, total cholesterol/HDL cholesterol ratio, antihypertensive or lipid-lowering medication use, waist circumference, smoking status, and alcohol consumption status (a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Bold indicates P<0.05. Abbreviations: RNFL, retinal nerve fibre layer; HbA1c, glycated haemoglobin A1c; HDL, high density lipoprotein.

Table S3.5 Associations with retinal sensitivity and RNFL thickness of 24-hour ambulatory blood pressure and total cholesterol, stratified by glucose metabolism status.

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	Number of participants in analyses	stβ (95% CI)	Number of participants in analyses	stβ (95% CI)
24-hour ambulatory systolic blood pressure, per SD					
Type 2 diabetes	1	N=1,132	**-0.08 (-0.14; -0.02)**	N=1,100	**-0.10 (-0.15; -0.04)**
	2		**-0.06 (-0.12; -0.01)**		**-0.07 (-0.13; -0.00)**
	3		**-0.06 (-0.12; -0.00)**		-0.06 (-0.13; 0.00)
Prediabetes	1	N=753	**-0.11 (-0.18; -0.04)**	N=721	**-0.04 (-0.11; 0.03)**
	2		-0.05 (-0.12; 0.02)		0.02 (-0.06; 0.09)
	3		-0.06 (-0.13; 0.01)		0.01 (-0.07; 0.09)
Normal glucose metabolism	1	N=3,163	**-0.05 (-0.08; -0.01)**	N=2,903	**-0.03 (-0.06; -0.01)**
	2		0.02 (-0.02; 0.05)		0.01 (-0.03; 0.05)
	3		0.02 (-0.02; 0.06)		0.01 (-0.03; 0.05)
24-hour ambulatory diastolic blood pressure, per SD					
Type 2 diabetes	1	N=1,132	**0.08 (0.01; 0.15)**	N=1,100	**-0.07 (-0.13; -0.01)**
	2		-0.00 (-0.06; 0.06)		-0.06 (-0.12; 0.01)
	3		0.00 (-0.06; 0.06)		-0.06 (-0.12; 0.01)
Prediabetes	1	N=753	**0.08 (0.01; 0.15**	N=721	**-0.09 (-0.16; -0.02)**
	2		-0.03 (-0.10; 0.04)		-0.05 (-0.12; 0.03)
	3		-0.05 (-0.12; 0.02)		-0.06 (-0.13; 0.02)
Normal glucose metabolism	1	N=3,163	**0.08 (0.05; 0.12)**	N=2,903	-0.03 (-0.07; 0.01)
	2		**0.04 (0.01; 0.08)**		-0.02 (-0.06; 0.02)
	3		**0.05 (0.01; 0.08)**		-0.02 (-0.06; 0.02)
Mean arterial pressure, per SD					
Type 2 diabetes	1	N=1,132	0.03 (-0.03; 0.09)	N=1,100	**-0.09 (-0.15; -0.03)**
	2		-0.09 (-0.09; 0.03)		**-0.07 (-0.12; -0.00)**
	3		-0.03 (-0.09; 0.03)		-0.06 (-0.13; 0.00)
Prediabetes	1	N=753	-0.01 (-0.08; -0.06)	N=721	**-0.07 (-0.15; -0.00)**
	2		-0.04 (-0.11; 0.03)		-0.02 (-0.10; 0.06)
	3		-0.06 (-0.13; 0.01)		-0.03 (-0.11; 0.05)
Normal glucose metabolism	1	N=3,163	0.03 (-0.01; 0.06)	N=2,903	**-0.03 (-0.07; 0.01)**
	2		**0.04 (0.00; 0.07)**		-0.01 (-0.05; 0.03)
	3		**0.04 (0.00; 0.07)**		-0.01 (-0.05; 0.03)

Table S3.5 (continued)

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	**Number of participants in analyses**	**stβ (95% CI)**	**Number of participants in analyses**	**stβ (95% CI)**
Total cholesterol, per SD					
Type 2 diabetes	1	N=1,278	**0.07 (0.01; 0.13)**	N=1,224	**0.07 (0.01; 0.12)**
	2		0.05 (-0.01; 0.11)		0.05 (-0.01; 0.11)
	3		0.05 (-0.02; 0.12)		**0.09 (0.03; 0.16)**
Prediabetes	1	N=840	**0.11 (0.04; 0.18)**	N=788	0.06 (-0.01; 0.13)
	2		**0.09 (0.02; 0.15)**		0.04 (-0.03; 0.11)
	3		0.05 (-0.02; 0.12)		0.05 (-0.03; 0.14)
Normal glucose metabolism	1	N=3,512	-0.01 (-0.04; 0.03)	N=3,211	0.00 (-0.03; 0.04)
	2		**0.05 (0.02; 0.08)**		0.00 (-0.03; 0.04)
	3		**0.05 (0.01; 0.08)**		0.00 (-0.04; 0.04)

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity or RNFL thickness (in SD) for 1 SD greater 24-hour ambulatory blood pressure or total cholesterol, in analyses stratified by glucose metabolism status. For individuals with type 2 diabetes, prediabetes, and normal glucose metabolism, respectively, one SD corresponds with 11.7 , 11.6, and 11.1 mm Hg of 24-h systolic ambulatory blood pressure in the retinal sensitivity study population, respectively; 11.5 , 11.7, and 10.9 mm Hg of 24-h diastolic ambulatory blood pressure in the retinal sensitivity study population, respectively; 7.7 , 7.6, and 8.1 mm Hg of 24-h ambulatory mean arterial blood pressure in the retinal sensitivity study population, respectively; 1.0, 1.1,1.0 mmol/L total cholesterol, respectively; 1.8 , 1.5, and 1.5 dB of retinal sensitivity (estimated in the population with complete data on 24-hour ambulatory blood pressure), respectively; or 11.4, 10.6, and 10.7 micrometres of RNFL thickness (estimated in the population with complete data on 24-hour ambulatory blood pressure), respectively. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use, waist circumference, total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking, and alcohol consumption (a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval.

Table S3.6 Associations with retinal sensitivity of potentially modifiable risk factors for dementia, additionally adjusted for lifestyle factors (dietary intake and physical activity; Model 3A); for kidney variables (model 3B); history of cardiovascular disease (model 3C); for intraocular pressure and spherical equivalent (model 3D); or for cataract, retinopathy, glaucoma, and age-related macular degeneration (model 3E).

	Retinal sensitivity, per SD				
	Model 3A stβ (95% CI)	**Model 3B stβ (95% CI)**	**Model 3C stβ (95% CI)**	**Model 3D stβ (95% CI)**	**Model 3E stβ (95% CI)**
Potentially modifiable risk factors for dementia					
HbA1c, per SD	-0.04 (-0.07;-0.01)	-0.05 (-0.07; -0.02)	-0.05 (-0.08;-0.02)	-0.06 (-0.08;-0.03)	-0.06 (-0.09;-0.02)
Lower healthy diet score, per SD	-0.06 (-0.09;-0.03)	-0.06 (-0.08;-0.03)	-0.06 (-0.09;-0.03)	-0.06 (-0.09;-0.03)	-0.05 (-0.09;-0.02)
Lower cardiorespiratory fitness, per SD	-0.05 (-0.09; -0.02)	-0.05 (-0.08; -0.01)	-0.05 (-0.08; -0.01)	-0.04 (-0.08; -0.01)	-0.05 (-0.09; -0.00)
Alcohol consumption					
-None versus light	-0.01 (-0.09; 0.07)	-0.03 (-0.10; 0.05)	-0.03 (-0.11; 0.05)	-0.04 (-0.12; 0.04)	-0.07 (-0.17; 0.04)
-Moderate versus light	0.09 (0.02; 0.16)	0.06 (-0.00; 0.13)	0.06 (-0.01; 0.13)	0.04 (-0.03; 0.11)	0.05 (-0.04; 0.13)
-High versus light	0.05 (-0.02; 0.12)	0.03 (-0.03; 0.10)	0.04 (-0.03; 0.10)	0.01 (-0.05; 0.08)	0.02 (-0.07; 0.10)
Smoking					
-Former versus never	0.07 (0.01; 0.12)	0.06 (0.00; 0.11)	0.06 (0.00; 0.11)	0.04 (-0.01; 0.10)	0.05 (-0.02; 0.12)
-Current versus never	-0.07 (-0.17; 0.01)	-0.14 (-0.22; -0.06)	-0.14 (-0.22;-0.06)	-0.17 (-0.25;-0.09)	-0.09 (-0.19;0.01)
Antihypertensive medication use	-0.02 (-0.09; 0.04)	-0.02 (-0.08; 0.05)	-0.02 (-0.08; 0.04)	-0.01 (-0.10; 0.10)	-0.01 (-0.07; 0.05)
24-hour ambulatory systolic blood pressure, per SD	-0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.02)	-0.02 (-0.04; 0.01)	0.01 (-0.04; 0.02)	0.00 (-0.04; 0.04)
24-hour ambulatory diastolic blood pressure, per SD	0.02 (-0.01; 0.05)	0.03 (0.00; 0.05)	0.02 (-0.00; 0.05)	0.02 (-0.00; 0.05)	0.03 (-0.01; 0.06)
Mean arterial pressure, per SD	0.04 (0.01; 0.07)	0.01 (-0.02; 0.04)	0.01 (-0.02; 0.04)	0.01 (-0.02; 0.04)	0.02 (-0.02; 0.05)
Total cholesterol, per SD	0.06 (0.03; 0.09)	0.05 (0.02; 0.08)	0.05 (0.02; 0.08)	0.04 (0.01; 0.07)	0.05 (0.01; 0.09)
Waist circumference, per SD	-0.00 (-0.04; 0.03)	-0.01 (-0.04; 0.02)	-0.01 (0.04; 0.02)	-0.01 (-0.04; 0.02)	-0.01 (-0.05; 0.03)
Lower physical activity, per SD	0.01 (-0.02; 0.04)	0.02 (-0.01; 0.04)	0.01 (-0.02; 0.04)	-0.00 (-0.03; 0.03)	-0.01 (-0.04; 0.03)

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. For all models, the values per SD are numerically similar to the values per SD that are shown in the legend of Table 3.2. Numbers of participants in models: Model 3A: n=4,762 for HbA1c; n=4,661 for cardiorespiratory fitness; n=4,298 for 24-hour ambulatory blood pressure and mean arterial pressure; n=4,768 for alcohol consumption; n=4,764 for total cholesterol; n=4,766 for waist circumference, smoking, and antihypertensive medication use; and n=4,766 for physical activity. Model 3B: n=5,636 for HbA1c; n=5,348 for the healthy diet score; n=4,876 for cardiorespiratory fitness; n=5,348 for alcohol consumption; n=5,054 for 24-hour ambulatory blood pressure and mean arterial pressure; n=5,639 for total cholesterol; n=5,639 for waist circumference, smoking, and antihypertensive medication use; and n=5,004 for physical activity. Model 3C: n=5,643 for HbA1c; n=5,351 for the healthy diet score; n=4,883 for cardiorespiratory fitness; n=5,356 for alcohol consumption;

n=5,058 for 24-hour ambulatory blood pressure and mean arterial pressure; n=5,645 for total cholesterol; n=5,647 for waist circumference, smoking, and antihypertensive medication use; and n=5,012 for physical activity. Model 3D: n=5,313 for HbA1c; n=5,030 for the healthy diet score; n=4,602 for cardiorespiratory fitness; and n=5,038 for alcohol consumption; n=4,753 for 24-hour ambulatory blood pressure and mean arterial pressure; n=5,316 for total cholesterol; n=5,317 for waist circumference, smoking, and antihypertensive medication use; n=4,716 for physical activity. Model 3E: n=3,357 for HbA1c; n=3,199 for the healthy diet score; n=2,978 for cardiorespiratory fitness; n=3,206 for alcohol consumption; n=3,068 for 24-hour ambulatory blood pressure and mean arterial pressure; n=3,360 for total cholesterol; n=3,360 for waist circumference, smoking, and antihypertensive medication use; and n=3,059 for physical activity. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Additionally, for model 3A, we only entered dietary intake into the models where physical activity or cardiorespiratory fitness were main determinants and we only entered physical activity into the models where healthy diet score was the main determinant. Abbreviations: SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; NA, not applicable; HDL, high-density lipoprotein.

Table S3.7 Associations with RNFL thickness of potentially modifiable risk factors for dementia, additionally adjusted for lifestyle factors (dietary intake, physical activity; Model 3A); for kidney variables (model 3B); history of cardiovascular disease (model 3C); for intraocular pressure and spherical equivalent (model 3D); or for cataract, retinopathy, glaucoma, and age-related macular degeneration (model 3E).

	RNFL thickness, per SD				
	Model 3A stβ (95% CI)	**Model 3B** stβ (95% CI)	**Model 3C** stβ (95% CI)	**Model 3D** stβ (95% CI)	**Model 3E** stβ (95% CI)
Potentially modifiable risk factors for dementia					
HbA1c, per SD	**-0.04 (-0.07; 0.00)**	**-0.05 (-0.08; -0.02)**	**-0.05 (-0.08; -0.02)**	**-0.03 (-0.06; -0.00)**	**-0.06 (-0.10; -0.01)**
Lower healthy diet score, per SD	-0.03 (-0.06; 0.00)	-0.03 (-0.06; 0.00)	**-0.03 (-0.06; -0.00)**	-0.03 (-0.06; 0.00)	-0.02 (-0.06; 0.03)
Lower cardiorespiratory fitness, per SD	-0.04 (-0.09; 0.00)	-0.03 (-0.07; 0.01)	-0.03 (-0.07; 0.01)	-0.02 (-0.06; 0.02)	-0.05 (-0.10; 0.01)
Smoking					
-Former versus never	-0.02 (-0.09; 0.04)	-0.01 (-0.07; 0.05)	-0.01 (-0.07; 0.05)	-0.05 (-0.10; 0.01)	0.02 (-0.06; 0.10)
-Current versus never	0.10 (-0.00; 0.20)	0.09 (-0.00; 0.18)	0.09 (0.00; 0.18)	0.03 (-0.06; 0.11)	**0.14 (0.01; 0.26)**
Alcohol consumption					
-None versus light	0.01 (-0.08; 0.10)	0.01 (-0.07; 0.10)	0.01 (-0.08; 0.10)	-0.03 (-0.11; 0.06)	-0.12 (-0.24; 0.01)
-Moderate versus light	0.06 (-0.03; 0.14)	0.04 (-0.04; 0.12)	0.04 (-0.04; 0.11)	0.02 (-0.05; 0.10)	-0.03 (-0.14; 0.07)
-High versus light	**-0.08 (-0.16; -0.00)**	**-0.08 (-0.16; -0.01)**	**-0.08 (-0.15; -0.01)**	**-0.10 (-0.17; -0.03)**	**-0.11 (-0.21; -0.01)**
Antihypertensive medication use	**-0.16 (-0.23; -0.08)**	**-0.12 (-0.19; -0.05)**	**-0.11 (-0.18; -0.04)**	**-0.09 (-0.15; -0.02)**	**-0.20 (-0.30; -0.11)**
24-hour ambulatory systolic blood pressure, per SD	-0.01 (-0.05; 0.02)	-0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.02)	0.00 (-0.03; 0.04)	-0.01 (-0.05; 0.03)
24-hour ambulatory diastolic blood pressure, per SD	**-0.04 (-0.07; -0.00)**	**-0.03 (-0.07; -0.00)**	**-0.03 (-0.07; -0.00)**	-0.02 (-0.05; 0.01)	-0.03 (-0.07; 0.01)
Mean arterial pressure, per SD	-0.03 (-0.06; 0.01)	-0.03 (-0.06; 0.01)	-0.03 (-0.06; 0.01)	-0.01 (-0.03; 0.02)	-0.02 (-0.06; 0.02)
Total cholesterol, per SD	0.04 (-0.00; 0.07)	0.03 (-0.00; 0.06)	0.03 (-0.01; 0.06)	0.02 (-0.01; 0.05)	0.02 (-0.03; 0.06)
Waist circumference, per SD	**0.06 (0.01; 0.10)**	0.04 (0.00; 0.07)	0.03 (-0.00; 0.07)	**0.04 (0.00; 0.07)**	**0.06 (0.01; 0.11)**
Lower physical activity, per SD	-0.02 (-0.05; 0.02)	-0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.02)	-0.02 (-0.06; 0.02)

Standardized regression coefficients (stβ) represent the difference in RNFL thickness (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. For all models, the values per SD are numerically similar to the values per SD that are shown in the legend of Table 3.2. Numbers of participants in models: Model 3A: n=4,275 for HbA1c; n=3,734 for cardiorespiratory fitness; n=4,282 for alcohol consumption; n=3,882 for 24-hour ambulatory blood pressure and mean arterial pressure; n=4,278 for total cholesterol; n=4,280 for waist circumference, smoking, and antihypertensive medication use; and n=4,280 for physical activity. Model 3B: n=5,226 for HbA1c; n=4,961 for the healthy diet score; n=4,521 for cardiorespiratory fitness; n=4,969 for alcohol consumption; n=4,728 for 24-hour ambulatory blood

pressure and mean arterial pressure; n=5,230 for total cholesterol; n=5,230 for waist circumference, smoking, and antihypertensive medication use; and n=4,486 for physical activity. Model 3C: n=5,227 for HbA1c; n=4,961 for the healthy diet score; n=4,521 for cardiorespiratory fitness; n=4,966 for alcohol consumption; n=4,726 for 24-hour ambulatory blood pressure and mean arterial pressure; n=5,231 for total cholesterol; n=5,233 for waist circumference, smoking, and antihypertensive medication use; and n=4,493 for physical activity. Model 3D: n=4,905 for HbA1c; n=4,650 for the healthy diet score; n=4,254 for cardiorespiratory fitness; n=4,658 for alcohol consumption; n=4,429 for 24-hour ambulatory blood pressure and mean arterial pressure; n=4,909 for total cholesterol; n=4,910 for waist circumference, smoking, and antihypertensive medication use; and n=4,213 for physical activity. Model 3E: n=2,727 for HbA1c; n=2,599 for the healthy diet score; n=2,418 for cardiorespiratory fitness; n=2,605 for alcohol consumption; n=2,512 for 24-hour ambulatory blood pressure and mean arterial pressure; n=2,729 for total cholesterol; n=2,729 for waist circumference, smoking, and antihypertensive medication use; and n=2,476 for physical activity. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Additionally, for model 3A, we only entered dietary intake into the models where physical activity or cardiorespiratory fitness were determinants and we only entered physical activity into the models where healthy diet score was the main determinant. Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; NA, not applicable; HDL, high-density lipoprotein.

Table S3.8 Associations with retinal sensitivity and RNFL thickness of systolic and diastolic office blood pressure; mean arterial pressure estimated from office blood pressure; 24-hour ambulatory systolic and diastolic blood pressure (without adjustment for antihypertensive medication use [model 3A]); mean arterial pressure estimated from 24-hour ambulatory blood pressure measurements (without adjustment for antihypertensive medication use [model 3A]); antihypertensive medication use (without adjustment for office systolic blood pressure [model 3A]), total cholesterol (without adjustment for lipid-lowering medication use [model 3A]); HDL; LDL; triglycerides; lipid-lowering medication use; body-mass index; fasting plasma glucose; 2-hour post load glucose; alcohol consumption (assessed with the food frequency questionnaire or with another questionnaire); smoking (where former smoking was the reference group); lower cardiorespiratory fitness (with additional adjustment for mobility limitation in models 1B-3B); lower physical activity (with additional adjustment for mobility limitation in models 1B-3B); and greater age.

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	**Number of participants in analyses**	**stβ (95% CI)**	**Number of participants in analyses**	**stβ (95% CI)**
Systolic office blood pressure, per SD	1	N=5,666	**-0.11 (-0.13;-0.08)**	N=5,254	**-0.07 (-0.09; -0.04)**
	2		0.01 (-0.01; 0.04)		-0.03 (-0.06; 0.00)
	3		0.01 (-0.02; 0.04)		-0.02 (-0.05; 0.01)
Diastolic office blood pressure, per SD	1	N=5,666	**0.07 (0.04; 0.09)**	N=5,254	**-0.06 (-0.08; -0.03)**
	2		**0.04 (0.01; 0.06)**		**-0.04 (-0.07; -0.01)**
	3		**0.04 (0.02; 0.07)**		**-0.04 (-0.07; -0.01)**
Mean arterial pressure, per SD	1	N=5,666	-0.02 (-0.04; 0.01)	N=5,254	**-0.07 (-0.09; -0.04)**
	2		**0.03 (0.00; 0.05)**		**-0.04 (-0.07; -0.01)**
	3		**0.03 (0.01; 0.06)**		**-0.03 (-0.06; -0.00)**
Antihypertensive medication use	1	N=5,666	**-0.33 (-0.39;-0.28)**	N=5,255	**-0.16 (-0.21; -0.10)**
	2		-0.05 (-0.10; 0.00)		**-0.09 (-0.15;-0.03)**
	3A		-0.03 (-0.09; 0.04)		**-0.12 (-0.19;-0.05)**
24-hour ambulatory systolic blood pressure, per SD	1	N=5,074	**-0.09 (-0.12;-0.06)**	N=4,746	**-0.06 (-0.09; -0.03)**
	2		-0.01 (-0.04; 0.01)		-0.01 (-0.04; 0.02)
	3A		-0.01 (-0.04; 0.01)		-0.01 (-0.04; 0.02)
24-hour ambulatory diastolic blood pressure, per SD	1	N=5,074	**0.09 (0.07; 0.12)**	N=4,746	**-0.05 (-0.08; -0.02)**
	2		0.03 (-0.00; 0.05)		**-0.03 (-0.06; -0.00)**
	3A		0.03 (-0.00; 0.05)		**-0.03 (-0.06; -0.00)**
Mean arterial pressure (estimated from 24-hour blood pressure measurements), per SD	1	N=5,074	0.01 (-0.03; 0.03)	N=4,746	**-0.06 (-0.09; -0.03)**
	2		0.01 (-0.02; 0.04)		-0.02 (-0.05; 0.01)
	3A		0.01 (-0.02; 0.04)		-0.03 (-0.06; 0.01)

Table S3.8 (continued)

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	**Number of participants in analyses**	**stβ (95% CI)**	**Number of participants in analyses**	**stβ (95% CI)**
Total cholesterol	1	N=5,664	**0.08 (0.05; 0.10)**	N=5,255	**0.06 (0.03; 0.08)**
	2		**0.06 (0.04; 0.09)**		0.02 (-0.01; 0.05)
	3A		**0.06 (0.03; 0.08)**		0.02 (-0.01; 0.05)
HDL, per SD	1	N=5,664	**0.04 (0.02; 0.07)**	N=5,253	**0.05 (0.03; 0.08)**
	2		**0.07 (0.04; 0.09)**		0.01 (-0.02; 0.04)
	3		**0.05 (0.02; 0.08)**		0.03 (-0.01; 0.06)
LDL, per SD	1	N=5,662	**0.08 (0.05; 0.11)**	N=5,251	**0.05 (0.02; 0.07)**
	2		**0.04 (0.02; 0.07)**		0.02 (-0.01; 0.05)
	3		**0.03 (0.01; 0.06)**		0.03 (-0.01; 0.06)
Triglycerides, per SD	1	N=5,664	-0.03 (-0.06; 0.01)	N=5,253	-0.02 (-0.05; 0.01)
	2		-0.00 (-0.03; 0.02)		0.01 (-0.02; 0.03)
	3		0.00 (-0.02; 0.03)		0.00 (-0.03; 0.03)
Lipid-lowering medication use	1	N=5,666	**-0.37 (-0.42;-0.31)**	N=5,255	**-0.09 (-0.15; -0.04)**
	2		**-0.09 (-0.15;-0.03)**		0.02 (-0.05; 0.09)
	3		**-0.08 (-0.14; -0.01)**		0.05 (-0.02; 0.13)
Body-mass index, per SD	1	N=5,666	**-0.06 (-0.09;-0.04)**	N=5,254	0.00 (-0.03; 0.03)
	2		-0.01 (-0.03; 0.02)		**0.03 (0.00; 0.06)**
	3		0.00 (-0.03; 0.03)		**0.04 (0.01; 0.08)**
Fasting plasma glucose, per SD	1	N=5,666	**-0.11 (-0.14;-0.09)**	N=5,254	**-0.07 (-0.10; -0.04)**
	2		**-0.05 (-0.08; -0.03)**		**-0.05 (-0.08; -0.03)**
	3		**-0.03 (-0.06; -0.01)**		**-0.04 (-0.07; -0.01)**
2-hour post load glucose, per SD	1	N=5,363	**-0.13 (-0.16;-0.11)**	N=4,982	**-0.07 (-0.10; -0.04)**
	2		-0.03 (-0.06; -0.01)		**-0.06 (-0.09; -0.03)**
	3		-0.01 (-0.04; 0.02)		**-0.05 (-0.08; -0.02)**
Alcohol consumption					
-Moderate versus none (assessed with the FFQ)	1	N=5,377	**0.13 (0.05; 0.21)**	N=4,989	-0.03 (-0.11; 0.05)
	2		**0.08 (0.00; 0.15)**		0.00 (-0.08; 0.08)
	3		0.06 (-0.02; 0.13)		0.01 (-0.07; 0.09)

Table S3.8 (continued)

		Retinal Sensitivity, per SD		RNFL thickness, per SD	
	Model	**Number of participants in analyses**	**stβ (95% CI)**	**Number of participants in analyses**	**stβ (95% CI)**
-Moderate versus none (assessed with another questionnaire than the FFQ)	1	N=5,666	**0.15 (0.08; 0.22)**	N=5,255	-0.05 (-0.12; 0.03)
	2		**0.08 (0.01; 0.15)**		-0.01 (-0.09; 0.06)
	3		0.06 (-0.01; 0.13)		-0.00 (-0.08; 0.07)
-High versus none (assessed with the FFQ)	1	N=5,377	0.06 (-0.02; 0.14)	N=4,989	**-0.14 (-0.23; -0.06)**
	2		**0.09 (0.01; 0.17)**		**-0.10 (-0.19; -0.01)**
	3		0.07 (-0.02; 0.15)		**-0.09 (-0.18; -0.00)**
-High versus none (assessed with another questionnaire than the FFQ)	1	N=5,666	**0.09 (0.01; 0.17)**	N=5,255	**-0.10 (-0.19;-0.02)**
	2		**0.10 (0.02; 0.18)**		-0.06 (-0.15; 0.03)
	3		0.08 (-0.00; 0.16)		-0.06 (-0.15; 0.03)
Smoking		N=5,666		N=5,255	
-Current versus former	1		-0.05 (-0.13; 0.04)		**0.13 (0.04; 0.21)**
	2		**-0.28 (-0.21;-0.13)**		**0.10 (0.02; 0.19)**
	3		**-0.19 (-0.27; -0.12)**		**0.10 (0.01; 0.19))**
-Never versus former	1		**0.09 (0.04; 0.15)**		0.05 (-0.01; 0.11)
	2		**-0.06 (-0.11; -0.01)**		0.02 (-0.04; 0.08)
	3		**-0.05 (-0.11; -0.00)**		0.01 (-0.05; 0.07)
Lower cardiorespiratory fitness, per SD	1B	N=4,887	**-0.20 (-0.22; -0.17)**	N=4,530	-0.00 (-0.03; 0.03)
	2B		**-0.04 (-0.08; -0.01)**		-0.02 (-0.05; 0.02)
	3B		**-0.04 (-0.08; -0.01)**		-0.03 (-0.07; 0.01)
Lower physical activity, per SD	1B	N=5,011	**-0.04 (-0.06; -0.01)**	N=4,494	-0.02 (-0.05; 0.01)
	2B		0.00 (-0.02; 0.03)		0.00 (-0.03; 0.03)
	3B		0.01 (-0.01; 0.04)		-0.01 (-0.04; 0.02)
Age, per year	1	N=5,666	**-0.04 (-0.05;-0.04)**	N=4,981	**-0.02 (-0.02; -0.01)**
	2		**-0.04 (-0.05;-0.04)**		**-0.01 (-0.02; -0.01)**
	3		**-0.04 (-0.05; -0.04)**		**-0.01 (-0.01; -0.01)**

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity or RNFL thickness (in SD) for one SD greater systolic or diastolic office blood pressure, greater total cholesterol, greater HDL, greater LDL, greater triglycerides, greater body-mass index, greater fasting plasma glucose, greater 2-hour post load glucose, higher age, lower cardiorespiratory fitness, lower physical activity, or for moderate or high versus none alcohol consumption, current or former versus never smoking, or for with versus without antihypertensive use. One SD corresponds with 17.7 and 9.7 mmHg for systolic and diastolic office blood pressure, respectively;

11.2 mmHg for mean arterial office blood pressure; 11.6 and 7.2 mm Hg for systolic and diastolic 24-hour ambulatory blood pressure, respectively; 8.0 mmHg for mean arterial 24-hour ambulatory blood pressure; 1.1 mmol/L for total cholesterol; 0.5 mmol/L for HDL; 1.0 mmol/L for LDL; 0.9 mmol/L for triglycerides; 4.4 kg/m^2 for body-mass index; 1.5 mmol/L for fasting plasma glucose; 4.0 mmol/L for 2-hour post load glucose; 0.6 W/kg for cardiorespiratory fitness; 40.9 minutes/day for physical activity; 1.6 dB for retinal sensitivity (all determined in the study population with complete data on retinal sensitivity and waist circumference) or 10.9 micrometres for RNFL thickness (RNFL thickness was determined in the study population with complete data on RNFL thickness and waist circumference). For all continuous determinants, values per SD were numerically similar in the retinal sensitivity and RNFL thickness study populations. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age (where applicable), sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio where applicable, lipid-lowering medication use, smoking, and alcohol consumption (a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Glucose metabolism status was not entered in the models where fasting plasma glucose or 2-hour post load glucose were the main determinants; and waist circumference was not entered in the model where body-mass index was the main determinant. Additionally, we entered mobility limitation into model 1 (model 1B) for the associations of lower cardiorespiratory fitness and lower physical activity with both outcomes under study; and we entered spherical equivalent into model 1 for the association between age and RNFL thickness. Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; DL, high-density lipoprotein; LDL, low-density lipoprotein.

Table S3.9 Associations with retinal sensitivity and RNFL thickness of potentially modifiable risk factors for dementia, where participants with cataract, retinopathy, glaucoma, and age-related macular degeneration were excluded (model 4A), or where individuals with measurements with >20% fixation errors were excluded (model 4B; only applicable for retinal sensitivity).

	Retinal Sensitivity, per SD				RNFL thickness, per SD	
	Number of participants in analyses	Model 4A	Number of participants in analyses	Model 4B	Number of participants in analyses	Model 4A
		stβ (95% CI)		stβ (95% CI)		stβ (95% CI)
Potentially modifiable risk factors for dementia						
HbA1c, per SD	N=2,943	-0.04 (-0.08;0.00)	N=3,408	-0.02 (-0.06; 0.01)	N=2,397	**-0.06 (-0.11; -0.02)**
Lower healthy diet score, per SD	N=2,800	**-0.06 (-0.09;-0.02)**	N=3,222	-0.03 (-0.07;0.00)	N=2,281	-0.02 (-0.07; 0.03)
Lower cardiorespiratory fitness, per SD	N=2,618	**-0.06 (-0.11;-0.01)**	N=3,012	-0.02 (-0.06; 0.03)	N=2,132	-0.05 (-0.10; 0.01)
Smoking	N=2,945		N=3,412		N=2,398	
-Former versus never		0.07 (-0.01; 0.14)		0.04 (-0.03; 0.11)		0.01 (-0.08; 0.10)
-Current versus never		**-0.12 (-0.23; -0.01)**		-0.08 (-0.18;0.03)		**0.13 (0.00; 0.26)**
Alcohol consumption	N=2,805		N=3,226		N=2,285	
-None versus light		**-0.06 (-0.17; 0.05)**		-0.07 (-0.18; 0.03)		-0.07 (-0.20; 0.06)
-Moderate versus light		0.07 (-0.03; 0.16)		0.03 (-0.06; 0.12)		-0.03 (-0.15; 0.08)
-High versus light		0.03 (-0.06; 0.12)		0.04 (-0.05; 0.12)		**-0.11 (-0.22; 0.00)**
Antihypertensive medication use	N=2,945	-0.04 (-0.13; 0.05)	N=3,412	-0.04 (-0.12; 0.04)	N=2,398	**-0.21 (-0.32; -0.10)**
24-hour ambulatory systolic blood pressure, per SD	N=2,684	-0.01 (-0.05; 0.03)	N=3,059	-0.01 (-0.05; 0.02)	N=2,202	-0.01 (-0.06; 0.04)
24-hour ambulatory diastolic blood pressure, per SD	N=2,684	0.02 (-0.01; 0.06)	N=3,059	**0.04 (0.01; 0.08)**	N=2,202	-0.03 (-0.08; 0.01)
Mean arterial pressure, per SD	N=2,684	0.01 (-0.03; 0.05)	N=3,059	0.02 (-0.02; 0.06)	N=2,202	-0.03 (-0.07; 0.02)
Total cholesterol, per SD	N=2,945	**0.05 (0.01; 0.09)**	N=3,411	**0.05 (0.01; 0.08)**	N=2,398	-0.00 (-0.05; 0.04)
Waist circumference, per SD	N=2,945	-0.02 (-0.07; 0.02)	N=3,412	0.01 (-0.03; 0.05)	N=2,398	**0.06 (0.01; 0.12)**
Lower physical activity, per SD	N=2,676	-0.00 (-0.04;0.03)	N=3,012	0.01 (-0.02; 0.05)	N=2,168	-0.01 (-0.06; 0.03)

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity or RNFL thickness (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. For all models, the values per SD are numerically similar to the values per SD that are shown in the legend of Table 3.2. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; NA, not applicable; HDL, high-density lipoprotein.

Table S3.10 Associations with retinal sensitivity of potentially modifiable risk factors for dementia, where educational status was replaced with occupational status (model 5A) or income level (model 5B); or glucose metabolism status was replaced with fasting plasma glucose (model 6A), 2-hour post load glucose (model 6B), or HbA1c (model 6C).

	Retinal sensitivity, per SD									
	Number of participants in analyses	Model 5A stβ (95% CI)	Number of participants in analyses	Model 5B stβ (95% CI)	Number of participants in analyses	Model 6A stβ (95% CI)	Number of participants in analyses	Model 6B stβ (95% CI)	Number of participants in analyses	Model 6C stβ (95% CI)
Potentially modifiable risk factors for dementia										
HbA1c, per SD	N=1,529	-0.04 (-0.09; 0.02)	N=4,330	**-0.04 (-0.08;-0.01)**	NA	NA	NA	NA	NA	NA
Lower healthy diet score, per SD	N=1,460	**-0.09 (-0.14;-0.04)**	N=4,132	**-0.06 (-0.09;-0.03)**	N=5,639	**-0.06 (-0.09;-0.03)**	N=5,091	**-0.05 (-0.08;-0.02)**	N=5,369	**-0.06 (-0.09;-0.03)**
Lower cardiorespiratory fitness, per SD	N=1,299	-0.05 (-0.11; 0.02)	N=3,760	**-0.05 (-0.09; -0.01)**	N=4,899	**-0.05 (-0.08; -0.01)**	N=4,699	**-0.05 (-0.08; -0.01)**	N=4,895	**-0.05 (-0.08; -0.01)**
Smoking	N =1,531		N=4,334		N=5,666		N=5,363		N=5,662	
-Former versus never		0.03 (-0.07; 0.13)		0.09 (0.03; 0.15)		**0.06 (0.00; 0.11)**		0.05 (-0.01; 0.10)		**0.06 (0.00; 0.11)**
-Current versus never		-0.06 (-0.22; 0.10)		**-0.11 (-0.20 ;-0.02)**		**-0.14 (-0.22;-0.06)**		**-0.14 (-0.22; -0.06)**		**-0.13 (-0.21; -0.05)**
Alcohol consumption	N=1,461		N=4,138		N=5,377		N=5,097		N=5,373	
-None versus light		0.03 (-0.13; 0.19)		0.01 (-0.09; 0.10)		-0.03 (-0.11; 0.05)		-0.02 (-0.11; 0.06)		-0.03 (-0.11; 0.05)
-Moderate versus light		0.05 (-0.09; 0.18)		0.05 (-0.03; 0.13)		0.07 (-0.01; 0.13)		**0.07 (0.00; 0.14)**		0.06 (-0.01; 0.13)
-High versus light		0.07 (-0.05; 0.19)		0.03 (-0.05; 0.10)		0.04 (-0.03; 0.10)		0.04 (-0.03; 0.11)		0.03 (-0.03; 0.10)
Antihypertensive medication use	N=1,531	-0.02 (-0.13; 0.10)	N=4,334	0.01 (-0.06; 0.08)	N=5,666	-0.03 (-0.09; 0.03)	N=5,363	-0.03 (-0.09; 0.04)	N=5,662	-0.03 (-0.09; 0.03)
24-hour ambulatory systolic blood pressure, per SD	N=1,331	-0.01 (-0.07; 0.04)	N=3,914	0.00 (-0.03; 0.03)	N=5,074	-0.01 (-0.04; 0.02)	N=4,821	-0.01 (-0.04; 0.02)	N=5,070	-0.01 (-0.04; 0.02)
24-hour ambulatory diastolic blood pressure, per SD	N=1,331	0.02 (-0.03; 0.08)	N=3,914	**0.04 (0.01; 0.07)**	N=5,074	0.03 (-0.00; 0.05)	N=4,821	0.03 (-0.00; 0.05)	N=5,070	0.03 (0.00; 0.05)
Mean arterial pressure, per SD	N=1,331	0.01 (-0.05; 0.06)	N=3,914	0.02 (-0.01; 0.06)	N=5,074	0.01 (-0.00; 0.04)	N=4,821	0.01 (-0.02; 0.04)	N=5,070	0.01 (-0.02; 0.04)
Total cholesterol, per SD	N=1,531	**0.07 (0.01; 0.12)**	N=4,332	**0.06 (0.03; 0.10)**	N=5,664	0.05 (0.03; 0.08)	N=5,361	**0.05 (0.02; 0.08)**	N=5,661	**0.05 (0.03; 0.08)**
Waist circumference, per SD	N=1,531	**0.06 (0.00; 0.12)**	N=4,334	-0.01 (-0.05; 0.02)	N=5,666	-0.01 (-0.04; 0.02)	N=5,363	-0.01 (-0.04; 0.02)	N=5,662	-0.01 (-0.04; 0.02)
Lower physical activity, per SD	N=1,312	0.01 (-0.05; 0.06)	N=3,834	0.00 (-0.03; 0.03)	N=5,027	0.01 (-0.02; 0.04)	N=4,764	0.02 (-0.01; 0.05)	N=5,023	0.01 (-0.02; 0.04)

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. For all models, the values per SD are numerically similar to the values per SD that are shown in the legend of Table 3.2. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Abbreviations: SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; NA, not applicable; HDL, high-density lipoprotein.

Table S3.11 Associations with RNFL thickness of potentially modifiable risk factors for dementia, where educational status was replaced with occupational status (model 5A) or income level (model 5B); or glucose metabolism status was replaced with fasting plasma glucose (model 6A), 2-hour post load glucose (model 6B), or HbA1c (model 6C).

	RNFL thickness, per SD									
	Number of participants in analyses	Model 5A stβ (95% CI)	Number of participants in analyses	Model 5B stβ (95% CI)	Number of participants in analyses	Model 6A stβ (95% CI)	Number of participants in analyses	Model 6B stβ (95% CI)	Number of participants in analyses	Model 6C stβ (95% CI)
Potentially modifiable risk factors for dementia										
HbA1c, per SD	N=1,833	**-0.03 (-0.08;-0.02)**	N=4,007	**-0.05 (-0.08;-0.01)**	NA	NA	NA	NA	NA	NA
Lower healthy diet score, per SD	N=1,745	**-0.05 (-0.10;-0.01)**	N=3,826	-0.03 (-0.06; 0.01)	N=4,980	**-0.03 (-0.06;-0.00)**	N=4,721	**-0.03 (-0.06; 0.00)**	N=4,975	**-0.03 (-0.06;-0.00)**
Lower cardiorespiratory fitness, per SD	N=1,565	-0.01 (-0.07; 0.06)	N=3,486	-0.01 (-0.05; 0.04)	N=4,541	-0.04 (-0.08; 0.00)	N=4,363	-0.03 (-0.07; 0.01)	N=4,538	-0.04 (-0.08; 0.00)
Smoking	N=1,837		N=4,012		N=5,254		N=4,982		N=5,249	
-Former versus never		-0.05 (-0.15; 0.05)		-0.02 (-0.09; 0.05)		-0.01 (-0.07; 0.05)		-0.02 (-0.08; 0.05)		-0.01 (-0.07; 0.05)
- Current versus never		0.07 (-0.08; 0.22)		0.08 (-0.02; 0.18)		0.08 (-0.01; 0.17)		0.07 (-0.02; 0.16)		**0.09 (0.00;0.18)**
Alcohol consumption	N=1,746		N=3,832		N=4,988		N=4,728		N=4,983	
-None versus light		0.02 (-0.13; 0.17)		-0.03 (-0.14; 0.07)		0.00 (-0.09; 0.09)		-0.01 (-0.10; 0.08)		0.00 (-0.09; 0.09)
-Moderate versus light		0.06 (-0.08; 0.19)		0.02 (-0.07; 0.11)		0.04 (-0.03; 0.12)		0.02 (-0.06; 0.10)		0.04 (-0.04; 0.12)
-High versus light		-0.08 (-0.20; 0.04)		**-0.11 (-0.19; -0.03)**		**-0.08 (-0.15; -0.01)**		**-0.09 (-0.17; -0.02)**		**-0.09 (-0.16; -0.02)**
Antihypertensive medication use	N=1,837	0.02 (-0.10; 0.14)	N=4,012	**-0.08 (-0.16; -0.00)**	N=5,254	**-0.12 (-0.19;-0.06)**	N=4,982	**-0.13 (-0.20; -0.06)**	N=5,249	**-0.13 (-0.20;-0.06)**
24-hour ambulatory systolic blood pressure, per SD	N=1,615	-0.03 (-0.09; 0.02)	N=3,645	-0.01 (-0.04; 0.03)	N=4,745	-0.01 (-0.04; 0.02)	N=4,510	0.00 (-0.03; 0.03)	N=4,740	-0.01 (-0.04; 0.02)
24-hour ambulatory diastolic blood pressure, per SD	N=1,615	**-0.07 (-0.12; -0.01)**	N=3,645	**-0.04 (-0.07; 0.00)**	N=4,745	**-0.03 (-0.06; 0.00)**	N=4,510	-0.03 (-0.06; 0.01)	N=4,740	**-0.03 (-0.06; 0.00)**
Mean arterial pressure, per SD	N=1,615	**-0.06 (-0.11; -0.00)**	N=3,645	-0.03 (-0.06; 0.01)	N=4,745	-0.02 (-0.06; 0.01)	N=4,510	-0.02 (-0.05; 0.02)	N=4,740	-0.02 (-0.05; 0.01)
Total cholesterol, per SD	N=1,837	0.05 (-0.01; 0.10)	N=4,010	0.02 (-0.02; 0.06)	N=5,252	**0.04 (0.00; 0.07)**	N=4,980	0.03 (-0.01; 0.06)	N=5,248	**0.04 (0.00; 0.07)**
Waist circumference, per SD	N=1,837	0.00 (-0.06; 0.06)	N=4,012	**0.05 (0.01; 0.09)**	N=5,254	0.03 (-0.01; 0.06)	N=4,982	0.03 (-0.01; 0.06)	N=5,249	0.03 (-0.01; 0.06)
Lower physical activity, per SD	N=1,466	0.03 (-0.02; 0.09)	N=3,440	-0.01 (-0.05; 0.03)	N=4,509	-0.01 (-0.04; 0.02)	N=4,277	-0.01 (-0.04; 0.02)	N=4,505	-0.01 (-0.05; 0.02)

Standardized regression coefficients (stβ) represent the difference in RNFL thickness (in SD) for one SD greater HbA1c, lower healthy diet score, lower cardiorespiratory fitness, for none, moderate, or high versus light total alcohol consumption, for current or former versus never smoking, for with versus without antihypertensive medication use, greater 24-hour ambulatory systolic blood pressure, greater total cholesterol, greater waist circumference, or lower physical activity. For all models, the values per SD are numerically similar to the values per SD that are shown in the legend of Table 3.2. Bold denotes P<0.05. Variables in models: Model 1: crude; Model 2: model 1 + age, sex, glucose metabolism status (where applicable), educational level; Model 3: model 2 + office systolic blood pressure (where applicable), antihypertensive medication use (where applicable), waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-lowering medication use, smoking (where applicable), and alcohol consumption (where applicable; a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Abbreviations: RNFL, retinal nerve fibre layer; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; NA, not applicable/

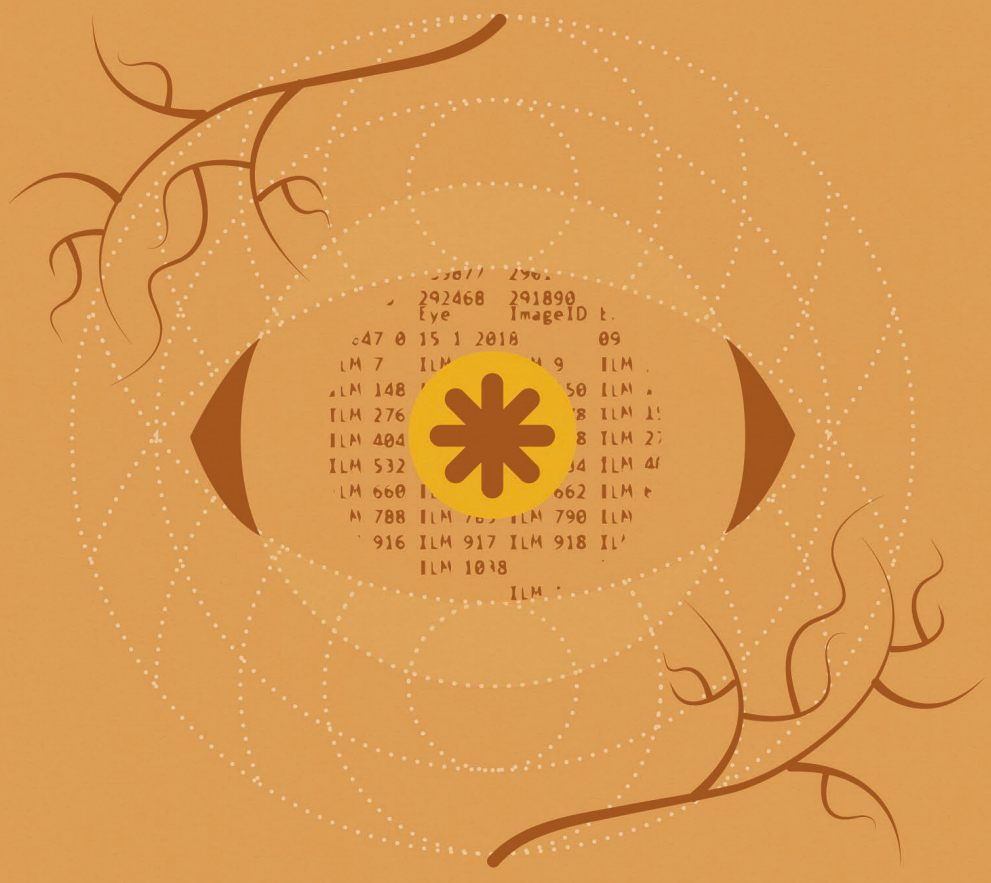
Table S3.12 P-values for interaction tests of continuous measures of glycaemia with 24-hour ambulatory systolic blood pressure and total cholesterol in the associations with retinal sensitivity and RNFL thickness as outcomes.

	Retinal sensitivity			RNFL thickness		
	Fasting plasma glucose	**2-hour post load glucose**	**HbA1c**	**Fasting plasma glucose**	**2-hour post load glucose**	**HbA1c**
	P-value	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**
24-hour ambulatory systolic blood pressure	**<0.001**	**<0.001**	**<0.001**	**0.01**	0.31	**0.03**
Total cholesterol	NA	NA	NA	**0.03**	**0.04**	**0.001**

P-values represents the P-values for the interaction terms of continuous measures of glycaemia (i.e. fasting plasma glucose, 2-hour post load glucose, and HbA1c) with 24-hour ambulatory systolic blood pressure (e.g., 24-hour systolic blood pressure*HbA1c) or total cholesterol in the associations with retinal sensitivity and/or retinal nerve fibre layer thickness as outcomes. For 24-hour ambulatory systolic blood pressure, the numbers of individuals included in the analyses, for retinal sensitivity and RNFL thickness respectively, were n=5,048 and n=4,723 for interaction tests with fasting plasma glucose; n=4,821 and n=4,510 for interaction tests with 2-hour post load glucose; and n=5,044 and n=4,718 for interaction tests with HbA1c. For total cholesterol, the numbers of individuals included in the analyses with RNFL thickness as endpoints, were n=5,222 for interaction tests with fasting plasma glucose; n=4,980 for interaction tests with 2-hour post load glucose; and n=5,218 for interaction tests with HbA1c. Variables in the model in addition to determinants, any interaction term, and any continuous measure of glycaemia are: age, sex, educational status, office systolic blood pressure, total cholesterol/HDL cholesterol ratio (where applicable), antihypertensive medication use, lipid-lowering medication use, waist circumference, smoking status, and alcohol consumption status (a precise overview of covariates entered per model [for all analyses] is presented in the Methods). Bold indicates P<0.05. Abbreviations: RNFL, retinal nerve fibre layer; HbA1c, glycated haemoglobin A1c; HDL, high density lipoprotein; NA, not applicable.

Part II

Determinants of microvascular dysfunction



CHAPTER 4

Alcohol consumption and microvascular dysfunction: a J-shaped association – The Maastricht Study

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Submitted

Abstract

Background

Microvascular dysfunction (MVD) is an important contributor to major clinical disease, such as stroke, dementia, depression, retinopathy, and chronic kidney disease. Alcohol consumption may be a determinant of MVD.

Objective and design

Main objectives were 1) to study whether alcohol consumption was associated with MVD, where MVD was assessed in the brain, retina, skin, kidney and in the blood; and 2) to investigate whether associations differed by history of cardiovascular disease or sex.

We used cross-sectional data from The Maastricht Study (N=3,120 participants, 50.9% men, mean age 60 years, 16.8% with a history of cardiovascular disease, and 27.5% with type 2 diabetes [oversampled by design]). We used regression analyses to study the association between total alcohol, wine, beer, and spirits consumption (per unit and in the categories, i.e. none, light [reference], moderate, high) and MVD, where all measures of MVD were combined into a total MVD composite score. We adjusted all associations for demographic, cardiovascular, and lifestyle factors. We tested for interaction by history of cardiovascular disease and sex ($P_{interaction}<0.05$).

Results

The association between total alcohol consumption and MVD was non-linear, i.e. J-shaped. Moderate versus light total alcohol consumption was significantly associated with less MVD, after adjustment for demographic, cardiovascular, and lifestyle factors (moderate versus light total alcohol consumption, standardized beta [95% confidence interval], -0.10 [-0.19; -0.01]). We had similar findings for wine, beer, and spirits consumption. History of cardiovascular disease ($P_{interaction}<0.001$) and sex ($P_{interaction}=0.03$) modified associations under study, where in stratified analyses the quantity of alcohol consumption at which this minimum was located was at higher amounts of alcohol consumption in individuals with, versus without, a history of cardiovascular disease and in men versus women; and the depth of the mathematical minimum of the J-curve (indicating stronger associations) was considerably lower in individuals with, versus without a history of cardiovascular disease and somewhat lower in women versus men.

Conclusions

The present population-based study found a J-shaped association between alcohol consumption and MVD, where the exact shape of the association differed by history of cardiovascular disease and sex. Therefore, alcohol consumption may be a determinant of MVD. Hence, although increasing alcohol consumption cannot be recommended as a policy, this study suggests that prevention of MVD may be possible through dietary interventions.

Introduction

Major clinical diseases such as stroke,[1] dementia,[1] depression,[1] retinopathy,[2] and chronic kidney disease[3] are thought to be (in part) caused by microvascular dysfunction (MVD). Mechanistically, MVD is thought to hamper haemodynamic autoregulation, which can predispose capillaries to a detrimentally high pressure, leading to capillary dilation, leakage, rupture, nonperfusion (i.e. ischaemia), and, ultimately, clinical symptoms of disease that (in part) have a microvascular origin.[2] Biologically, MVD is thought to be to an important extent caused by endothelial cell dysfunction as a consequence of impaired endothelial cell nitric oxide (NO) bioavailability.[2]

Subtle functional and structural changes of the microvasculature, which reflect (more) MVD, can be non-invasively assessed in various organs.[2] First, MVD in the brain can be assessed as the presence of features of cerebral small vessel disease (CSVD; i.e. greater white matter hyperintensity volume, more cerebral microbleeds, and more lacunar infarcts).[2] Second, MVD in the retina can be inferred from wider or narrower retinal arteriolar diameters, wider retinal venular diameters, or as lower flicker light-induced increase in retinal microvascular diameters.[2] The interpretation of the retinal arteriolar diameter is thought to depend on the stage of MVD, with widening as an early-stage and narrowing as a later-stage feature of MVD.[2,4] Third, MVD in skin, kidney, and blood can respectively be assessed as lower heat-induced skin hyperaemia, higher urinary albumin excretion (UAE), and higher levels of plasma biomarkers of MVD (i.e. higher levels of soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin] and Von Willebrand Factor [vWF]).[2]

Alcohol consumption may be a potentially modifiable determinant of MVD and the association between alcohol consumption and MVD may be J-shaped.[2,5,6] Mechanistically, at certain lower levels of alcohol consumption, ethanol and polyphenols, the main bioactive constituents in alcoholic beverages, may be able to reduce MVD via increasing endothelial cell NO bioavailability. First, ethanol can increase NO bioavailability via stimulating NO synthesis by the endothelial cell NO synthase enzyme (eNOS).[7,8] Second, polyphenols are thought to increase NO bioavailability via reducing oxidative stress, which is a potent reductor of NO bioavailability.[2,9,10] Additionally, as wine and beer contain more polyphenols than spirits, wine and beer may be stronger stimulators of NO bioavailability than spirits.[5] Then, opposingly, at certain higher levels of alcohol consumption, ethanol can induce oxidative stress.[5] Therefore, there may be a threshold where NO bioavailability is more impaired by ethanol than increased by polyphenols and ethanol, resulting in more instead of less MVD.[5]

In addition, at which levels of alcohol consumption this threshold occurs and how strong the effects of alcohol consumption on MVD are may differ by background levels of oxidative stress (which are presumably higher in e.g. individuals with, versus without, a history of cardiovascular disease) [10-12] and by sex.[13,14]
Indeed, there is some evidence that alcohol consumption may be a determinant of MVD, however, this evidence has important limitations.[15-53] First, many population-based studies did not quantify the amount of alcohol consumption[15,18,21,27,28,32,33,37,40,44,47,50,52]; did not take potential cardiovascular[16-20,22,25,26,29,34,35,38,39,41,45,46,48,49,51,53] and/or lifestyle[23,24,31,32,42,43] confounders in to account; and/or did not account for sick quitters[19,22,30,34,36,42,43] (i.e. individuals who quit drinking and are thought to have an increased cardiovascular risk).[54,55] Second, only few studies investigated the associations of wine, beer, and spirits consumption with MVD.[23,26,27,42,43,45] Third, no population-based studies have yet reported the association between alcohol consumption and MVD in individuals with and without a history of cardiovascular disease.
In view of the above, we investigated, using a large, well-characterized population-based cohort study, whether total alcohol, wine, beer, and spirits consumption were associated with MVD, estimated from features of CSVD, retinal microvascular diameters, flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. In addition, we tested whether associations were modified by history of cardiovascular disease or sex.

Methods

Study population and design

The present study used data from The Maastricht Study, an observational population-based cohort study. The rationale and methodology have been described previously.[56] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of diabetes mellitus type 2 and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns, the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes for reasons of efficiency. The present report includes cross-sectional data from 3,451 participants who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time

window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare, and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Alcohol consumption

Habitual alcohol consumption over the past 12 months was assessed via a self-administered validated food frequency questionnaire (FFQ).[57] Total alcohol consumption was calculated from the questionnaire-assessed average consumption of individual types of wine (i.e. red wine, white wine, strong wine [such as sherry]), individual types of beer (i.e. pilsner, light alcoholic beer, high alcoholic beer) and spirits.[57] The intraclass correlation coefficient for alcohol consumption assessed by FFQ versus (up to 5) 24-hour recalls was 0.78 (95% confidence interval, 0.70–0.83; n=135).[57]

We categorized alcohol consumption into none (<1 unit per week [for both men and women]), light (≥1 unit/ week to 1 unit/day for men, ≥1 unit/ week to 0.5 unit/day for women), moderate (>1 to 2 units/day for men, >0.5 to 1 unit/day for women), and high (>2 units/day for men, >1 units/day for women) where 1 unit was defined as 10 gram/day (g/d) of total alcohol (i.e. ethanol) consumption, 100 g/d of red or white wine consumption, 50 g/day of strong wine consumption, 225 g/d of pilsner, 320 g/d light alcoholic beer consumption, 160 g/d of high alcoholic beer consumption, or 35 g/d of spirits consumption.[58]

Features of CSVD, microvascular retinal diameters, and measures of MVD

Here, we briefly describe the methods used; a detailed description is provided in the Extended Methods (Supplementary Material).

Features of CSVD

We evaluated three CSVD features, i.e. white matter hyperintensity volume, cerebral microbleeds, and lacunar infarcts with a 3T brain magnetic resonance imaging scanner (Siemens Magnetom Prisma-fit Syngo MR D13D, Erlangen, Germany).

Retinal microvascular diameters

We measured retinal microvascular diameters with static retinal vessel analysis from an optic disk-centred fundus photograph with the retinal health information and notification system (RHINO) software, as described previously.[59] In brief, we

measured the diameter (expressed in measurement units [MU]) of the six largest retinal vessels at 0.5–1.0-disc diameter away from the optic disc margin. Diameters of arteriolar or venular vessels were combined into an average arteriolar retinal diameter (i.e. central retinal arteriolar equivalent [CRAE]) or venular retinal diameter (i.e. central retinal venular equivalent [CRVE]).

Flicker light-induced increase in retinal arteriolar and venular diameter

We assessed the flicker light-induced increase in retinal arteriolar and venular diameters (in MU) with the Dynamic Vessel Analyzer (Imedos, Jena, Germany), as previously described.[60,61] Briefly, a 50 second-baseline recording was consecutively followed by a 40-second flicker light exposure and a 60-second recovery period. Baseline diameter was calculated as the average diameter between 20 seconds and 50 seconds of the baseline recording. The diameter during flicker light exposure was calculated as the mean of the diameters assessed at time points 10 and 40 seconds of flicker light stimulation exposure. Flicker light-induced increase in retinal diameter was calculated as the diameter during flicker light exposure minus the baseline diameter.

Heat-induced skin hyperaemia

We measured heat-induced skin hyperaemia by laser Doppler flowmetry (Perimed, Järfälla, Sweden), as previously described.[60] Briefly, at the wrist skin blood flow, expressed in arbitrary perfusion units (PU), was recorded unheated for 2 minutes to serve as a baseline. After 2 minutes, the temperature of the laser Doppler probe was rapidly and locally increased to 44°C and was kept constant until the end of the registration. Heat-induced increase in skin blood flow was expressed as the increase in skin blood flow during the 23 minutes heating phase. We calculated heat-induced increase in skin blood flow as the average skin blood flow during the 23 minutes heating phase minus the baseline skin blood flow (i.e. average skin blood flow during the first 2 minutes).

Urinary albumin excretion

Urinary albumin excretion (UAE) was calculated as the average UAE of two 24-hour urine collections (two collections were available for 91.3% of participants). We used an automatic analyser to measure urinary albumin concentration with a standard immunoturbidimetric assay. We multiplied urinary albumin concentration by collection volume to obtain 24-hour UAE. The detection limit for assessment of urinary albumin concentration was set at 1.5 mg/L.

Plasma biomarkers of microvascular dysfunction

We evaluated four plasma biomarkers of microvascular dysfunction (MVD) i.e. soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin], and Von Willebrand Factor [vWF].[62] sICAM-1, sVCAM-1, and sE-selectin were measured in EDTA plasma samples with commercially available 4-plex sandwich immunoassay kits with different standards and antibodies (Meso Scale Discovery, Rockville, Maryland, United States of America). vWF was quantified in citrate plasma using ELISA (Dako, Glostrup, Denmark).

Covariates

As described previously,[56] we determined glucose metabolism status according to the World Health Organization 2006 criteria as normal glucose metabolism, prediabetes, type 2 diabetes, or other types of diabetes than type 2;[63] assessed educational level (low, intermediate, high), socio-economic status (income level and occupational status),[64] smoking status (never, former, current), and history of cardiovascular disease by questionnaire; assessed dietary habits with the Dutch Healthy Diet index sum score, a measure of adherence to the Dutch dietary guidelines 2015,[65] based on a validated food frequency questionnaire;[57] assessed lipid-modifying, antihypertensive, and glucose-lowering medication use as part of a medication interview; assessed weight, height, waist circumference, office and 24-hour ambulatory blood pressure during a physical examination; calculated body-mass index (BMI) based on body weight and height; measured total daily physical activity (hours/day) with an accelerometer;[66] measured fasting plasma glucose, 2-hour post load glucose, haemoglobin A1c (HbA1c), lipid profile, serum creatinine, serum cystatin C, and plasma biomarkers of low-grade inflammation[67] (i.e. high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8 and tumour necrosis factor alpha) in fasting venous blood samples; calculated the estimated glomerular filtration rate (eGFR) with the CKD-EPI (Chronic Kidney Disease Epidemiology collaboration) formula using serum creatinine and cystatin C;[68] and assessed presence of retinopathy in both eyes via fundus photography.

Statistical analyses

We used a total MVD composite score as endpoint. In order to perform analyses we recalculated several variables. First, we inversed (i.e. multiplied by -1) flicker light-induced increase in retinal arteriolar and venular diameters and heat-induced skin hyperaemia so that higher values indicate more MVD. Second, we logarithmically transformed white matter hyperintensity volume, cerebral microbleeds, lacunar infarcts,

and UAE as these covariates were not normally distributed. Third, to reduce noise (i.e. measurement error) we calculated composite scores for CSVD features, retinal microvascular diameters, flicker light-induced increase in retinal microvascular diameters, and plasma biomarkers of MVD; and we composed a total MVD composite score.[69] We composed these composite scores because we assume that all measures of MVD under study represent a similar underlying measure of MVD.[2] Before we computed the total MVD composite score we checked whether associations of alcohol consumption with individual measures were not directionally inconsistent. This was the case for all these measures (including for retinal arteriolar and venular diameters). Then, we standardized all measures of MVD (i.e. expressed as z-score) and calculated the total MVD composite z-score. We composed the total MVD composite z-score in two steps. First, we combined z-scores for individual CSVD features, retinal arteriolar and venular diameters, flicker light-induced increase in retinal arteriolar and venular diameters, and individual plasma biomarkers of MVD into composite z-scores. Second, we computed the total MVD composite score by averaging the composite z-scores for CSVD features, retinal microvascular diameters, flicker light-induced increase in retinal microvascular diameters, and plasma biomarkers of MVD and the z-scores for heat-induced skin hyperaemia and UAE. To maximize the number of participants that we could use in the main analyses we included participants in the main analyses if data were available for at least two out of six measures of MVD. Last, we recalculated the Dutch Healthy Diet score so that the "diet score" reflects dietary intake without alcohol consumption.

As the association between alcohol consumption and MVD may be non-linear and J-shaped (e.g. quadratic), as previously described, we tested a quadratic association.[70] To test for a quadratic association, we entered a quadratic term of total alcohol consumption in to the model. If the P-value of the quadratic term was <0.05, we considered the association under study statistically better described by a quadratic association than by a linear association. We performed this test for total alcohol consumption instead of individual types of alcohol for reasons of statistical power, i.e. as the range of total alcohol consumption is greater than the range of individual types of alcohol consumption, the statistical power to detect a non-linear association is likely greater.[71] In these analyses we did not exclude zero alcohol consumers (more details in the next paragraph).

We used multivariable regression analyses to analyse non-linear and linear associations of total alcohol consumption, wine, beer, and spirits consumption with the total MVD composite score.

We analysed both linear and non-linear associations for all associations under study to comprehensively provide insight in the data. For non-linear analyses, we entered total alcohol consumption, wine, beer, and spirits consumption in to the statistical model as

dummies of none, moderate, or high, versus light, alcohol consumption. Wine, beer, and spirits consumption were entered in the same model to allow mutual adjustment for consumption of other alcoholic beverages. In these analyses we used light drinkers as a reference group as we cannot distinguish so-called sick quitters from never drinkers (i.e. life-long abstainers) within the none consumers.[55] For linear analyses, we entered alcohol in the model as a continuous variable (per unit); and for P for trend analyses we entered alcohol consumption in the model as a categorical variable (coded 0=none, 1=light, 2=moderate, and 3=high alcohol consumption). For all linear analyses, we used zero drinkers as reference group. We did not use light drinkers as a reference group for linear analyses because in order to perform such analyses zero drinkers should be excluded, a methodological choice which will lead to a substantial reduction in the size of the study population and a considerable loss of statistical power. [71]

Model 1 shows crude results. In model 2 we adjusted for age, sex, glucose metabolism status (entered as dummies of prediabetes, type 2 diabetes, or other types of diabetes versus normal glucose metabolism status[reference]) and educational level (low [reference], middle, high). We chose these variables as they are key potential confounders (all) or were oversampled (type 2 diabetes). In model 3A we additionally adjusted for potential confounders (waist circumference, smoking status [current, ever, never {reference}], and diet score). In model 3B we additionally adjusted for variables which are potential confounders and may additionally also be potential mediators (office systolic blood pressure, use of antihypertensive medication [yes/no], total cholesterol / HDL cholesterol ratio, lipid-modifying medication [yes/no], and history of cardiovascular disease [yes/no]). Additionally, and only in analyses with heat-induced skin hyperaemia as a separate outcome, we entered baseline skin blood flow in model 1 (more details are presented in the Supplemental Methods section). Data were expressed as regression coefficients and corresponding 95% confidence intervals. Collinearity diagnostics (i.e. tolerance <0.10 and/or variance inflation factor >10) were used to detect multicollinearity between covariates.

We tested for interaction by history of cardiovascular disease and sex. We a priori hypothesized that the shape of the association may differ between individuals with and without a history of cardiovascular disease[10-12] and between men and women.[13,14]

We used a likelihood ratio test to test for interaction. The likelihood ratio test compares the goodness in fit between the fully adjusted model (model 3B) with and without an interaction term (e.g. history of cardiovascular disease*total alcohol consumption). A statistically significant P-value from the likelihood ratio test indicates that the shape of the association under study is (statistically) different between subgroups (e.g. between individuals with and without a history of cardiovascular disease).

To test robustness of our observations we performed several additional analyses. First, we tested whether the association of total alcohol consumption with the total MVD

composite score was modified by cardiovascular risk factors (i.e. glucose metabolism status, hypertension, dyslipidaemia, and current smoking). For tests of interaction by glucose metabolism status, we excluded individuals with other types of diabetes than type 2 diabetes because the number of participants with other types of diabetes was small. Presence of hypertension was defined as office systolic blood pressure ≥140 mm Hg and/or use of antihypertensive medication. Current smokers were defined as smokers and both former and never smokers were considered non-smokers. Presence of dyslipidaemia was defined as LDL>3.5 mmol/L (i.e. median LDL level in the general population) and/or use of lipid-modifying medication. Second, we investigated the association between total alcohol consumption and the total MVD composite score in individuals with zero, one, two, three, or four of the above cardiovascular risk factors to investigate whether associations were stronger in individuals with presumed increasingly higher levels of oxidative stress. Third, we studied the association between total alcohol consumption and a total MVD composite score based on five instead of six measures of MVD, where we, one by one, left out one of measures of MVD. Fourth, we investigated the associations of total alcohol, wine, beer, and spirits consumption with individual measures used in the total MVD composite scores as endpoint. Fifth, we additionally adjusted for accelerometer-assessed physical activity. We did not include accelerometer-assessed physical activity in the main analyses because data was missing for a relatively large number of participants (up to n=699 had missing data).[72] Sixth, we repeated analyses with additional adjustment for diabetic retinopathy, eGFR, or plasma biomarkers of low-grade inflammation. We entered these covariates in a separate model instead of in model 3B because up to n=497 participants had missing data on one or more of these variables. Seventh, if associations under study were modified by glucose metabolism status we tested whether interaction terms composed of continuous glycaemic measures (i.e. fasting plasma glucose, 2-hour post load glucose, and HbA1c) instead of glucose metabolism status also modified these associations (e.g. HbA1c*total alcohol consumption). Last, we repeated analyses after replacement of educational level with income level and occupational status; after replacement of glucose metabolism status with fasting plasma glucose, 2-hour post load glucose, or HbA1c; and after replacement of office systolic blood pressure with office diastolic blood pressure, 24-hour ambulatory systolic blood pressure, or 24-hour diastolic blood pressure.

We performed all regression analyses with Statistical Package for Social Sciences version 23.0 (IBM SPSS, IBM Corp, Armonk, NY, USA) and likelihood ratio tests with Software for Statistics and Data Sciences version 14.0 (StataCorp, Texas, USA). For all analyses, including interaction analyses, a P-value <0.05 was considered statistically significant.

Results

Selection and characteristics of the study population

Figure 4.1 shows an overview of the study population selection and Table 4.1 shows general characteristics according to total alcohol consumption (shown for in individuals with complete data on UAE [n=3,107]). General characteristics of individuals in the study population are: mean age 60 years old, 51% men, 27.5% type 2 diabetes. Next, 16%, 31%, 20%, and 33% of participants were, respectively, none, light, moderate, and high total alcohol consumers and 59%, 43%, and 10% of participants, respectively, were wine, beer, or spirits consumers. Overall, participants with a higher level of alcohol consumption were older and less likely to have type 2 diabetes. General characteristics of participants included in the study were highly comparable to those of participants with missing data (Supplemental Tables S4.1 and S4.2).

Associations between alcohol consumption and measures of MVD

The association between total alcohol consumption and MVD was non-linear, i.e. J-shaped (model 3B; $P_{quadratic}$-value=0.01; Figure 4.2). The mathematical minimum of the J-curve ("minimum") was at approximately 4 units/day (i.e. the amount of total alcohol consumption where the association becomes directionally different; Figure 4.2).

After full adjustment (model 3B), moderate versus light total alcohol, wine, beer, and spirits consumption was statistically significantly associated with less MVD (model 3B; moderate versus light total alcohol, wine, beer, and spirits consumption, respectively; standardized betas [95% confidence interval], -0.10 [-0.19; -0.01]; -0.15 [-0.25; -0.05]; -0.13 [-0.25; -0.02]; and -0.16 [-0.29; -0.04]; Table 4.2 and Figure 4.3).

Interaction analyses

History of cardiovascular disease and sex modified the association between total alcohol consumption and the total MVD composite score (P-for-interaction values; $P_{interaction}<0.001$ and $P_{interaction}=0.03$, respectively). Supplemental Table S4.3 shows all P-for-interaction values.

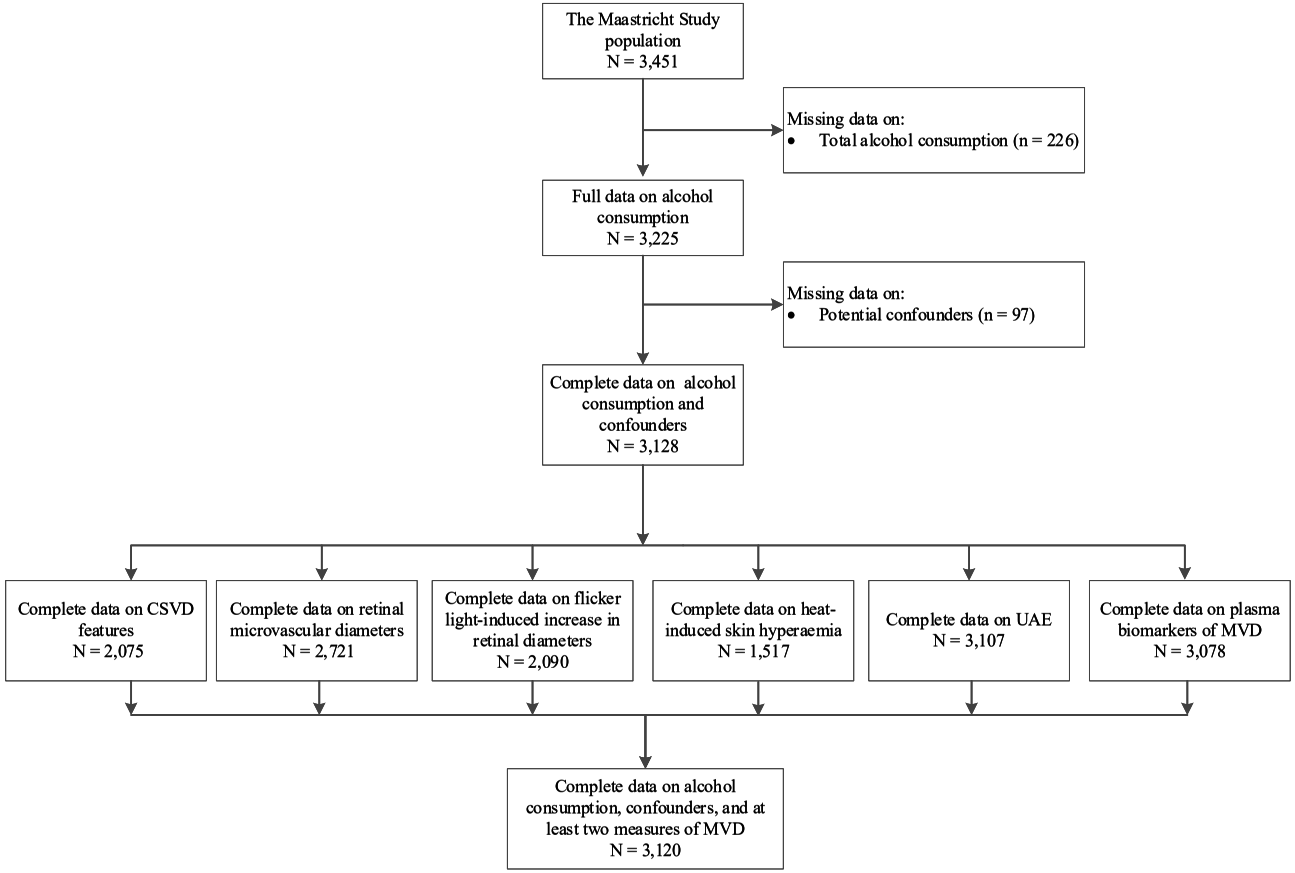

Figure 4.1 Delineates the selection of participants for inclusion. Abbreviations: CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction.

Table 4.1 General characteristics of the MVD study population in the UAE study population.

Characteristic	Total study population (n=3107)	Alcohol consumption			
		None (n=498)	Light (n=964)	Moderate (n=620)	High (n=1025)
Demographics					
Age, years	59.9 ± 8.2	59.0 ± 8.6	59.1 ± 8.8	60.1 ± 8.2	61.0 ± 7.2
Men	1,582 (50.9)	163 (32.7)	555 (57.6)	357 (57.6)	507 (49.5)
Lifestyle factors					
Smoking status:					
Never	1088 (35.0)	214 (43.0)	379 (39.3)	236 (38.1)	259 (25.3)
Former	1,624 (52.3)	196 (39.4)	480 (49.8)	313 (50.5)	635 (62.0)
Current	395 (12.7)	88 (17.7)	105 (10.9)	71 (11.5)	131 (12.8)
Body mass index*, kg/m^2	27.0 ± 4.5	28.2 ± 5.5	27.5 ± 4.6	26.4 ± 3.9	26.3 ± 4.0
Waist circumference, cm	95.8 ± 13.7	97.4 ± 15.7	97.3 ± 14.0	94.5 ± 12.7	94.2 ± 12.7
Physical activity*, hours/day	2.0 ± 0.7	1.9 ± 0.8	1.9 ± 0.7	2.0 ± 0.6	2.1 ± 0.7
Dutch Healthy Diet score, points	83.2 ± 14.7	84.8 ± 14.7	84.9 ± 14.1	85.7 ± 14.4	79.4 ± 14.8
Cardiovascular risk factors					
Glucose metabolism status					
Normal glucose metabolism	1,760 (56.6)	209 (42.0)	538 (55.8)	385 (62.1)	628 (61.3)
Prediabetes	460 (14.8)	59 (11.8)	137 (14.2)	90 (14.5)	174 (17.0)
Type 2 diabetes	854 (27.5)	225 (45.2)	282 (29.3)	137 (22.1)	210 (20.5)
Other types of diabetes	33 (1.1)	5 (1.0)	7 (0.7)	8 (1.3)	13 (1.3)
Fasting plasma glucose*, mmol/l	5.5 [5.1 – 6.5]	5.8 [5.1 – 7.3]	5.5 [5.0 – 6.7]	5.5 [5.0 – 6.4]	5.5 [5.1 – 6.2]
2 hour post load plasma glucose*, mmol/l	9.3 [6.3 – 9.3]	7.4 [5.5 – 13.0]	6.3 [5.0 – 9.4]	6.1 [4.9 – 8.3]	6.1 [5.1 – 8.4]
HbA1c*, %	5.7 [5.4 – 6.2]	5.9 [5.5 – 6.7]	5.7 [5.3 – 6.3]	5.6 [5.3 – 6.1]	5.6 [5.3 – 6.0]
Use of glucose-lowering medication	697 (22.4)	196 (39.4)	244 (25.3)	102 (16.5)	155 (15.1)
Total/HDL cholesterol ratio	3.7 ± 1.2	3.8 ± 1.2	3.8 ± 1.2	3.6 ± 1.1	3.5 ± 1.2
Use of lipid-modifying medication	1,137 (36.6)	231 (46.4)	347 (36.0)	222 (35.8)	337 (32.9)
Office systolic blood pressure, mm Hg	135.0 ± 18.3	134.4 ± 18.3	134.3 ± 18.1	135.9 ± 18.4	135.5 ± 18.3
Office diastolic blood pressure, mm Hg	76.1 ± 9.9	75.0 ± 9.2	76.4 ± 10.0	76.3 ± 10.6	76.4 ± 9.8
Ambulatory systolic blood pressure, mm Hg	118.9 ± 11.7	116.7 ± 11.1	118.6 ± 11.7	119.8 ± 11.8	119.7 ± 11.7
Ambulatory diastolic blood pressure, mm Hg	73.4 ± 7.1	71.9 ± 9.0	73.7 ± 7.2	73.6 ± 7.3	73.7 ± 7.1
Use of antihypertensive medication	1,252 (40.3)	261 (52.4)	387 (40.1)	236 (38.1)	368 (35.9)

Table 4.1 (continued)

Characteristic	Total study population (n=3107)	Alcohol consumption			
		None (n=498)	Light (n=964)	Moderate (n=620)	High (n=1025)
History of cardiovascular disease	522 (16.8)	115 (23.1)	175 (18.2)	101 (16.3)	131 (12.8)
Diabetic retinopathy*	41 (1.6)	9 (2.1)	20 (2.5)	6 (1.1)	6 (0.6)
eGFR, ml/min/1.73^2	88.0 ± 14.9	86.4 ± 17.3	87.3 ± 15.5	88.6 ± 14.6	89.0 ± 13.2
Biomarkers of low-grade inflammation*					
C-reactive protein, µg/ml	1.2 [6.1 – 2.8]	1.7 [0.7 – 3.8]	1.4 [0.7 – 3.0]	1.2 [0.6 – 2.5]	1.0 [0.6 – 2.3]
Serum amyloid A, µg/ml	3.3 [2.1 – 5.4]	3.7 [2.3 –6.4]	3.3 [1.9 – 5.7]	3.2 [2.0 – 5.3]	3.2 [2.1 – 5.1]
Tumour necrosis factor alpha, pg/ml	2.2 [1.9 – 2.6]	2.3 [1.9 – 2.7]	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.5]	2.1 [1.8 – 2.5]
Interleukin-6, pg/ml	4.1 [3.3 – 5.3]	0.7 [0.5 – 1.0]	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.8]	0.6 [0.4 – 0.9]
Interleukin-8, pg/ml	4.1 [3.3 – 5.3]	4.2 [3.4 – 5.4]	4.2 [3.3 – 5.4]	3.9 [3.2 – 5.3]	4.2 [3.3 – 5.3]
Other					
Educational status					
Low	1041 (33.5)	225 (45.2)	331 (34.3)	190 (30.6)	295 (28.8)
Medium	877 (28.2)	158 (31.7)	285 (29.6)	172 (27.7)	262 (25.6)
High	1189 (38.3)	115 (23.1)	348 (36.1)	258 (41.6)	468 (45.7)
Occupational status*					
Low	801 (31.0)	177 (46.6)	265 (32.5)	151 (28.8)	208 (20.3)
Middle	922 (35.7)	130 (34.2)	289 (35.5)	198 (37.7)	305 (35.3)
High	860 (33.3)	73 (19.2)	261 (32.0)	176 (33.5)	350 (40.6)
Income per month*, euros	2028 ± 818	1653 ± 704	1919 ± 725	2123 ± 823	2229 ± 869
Alcohol consumption					
Total alcohol consumption, units/day	0.85 [0.2 – 1.9]	0.0 ± 0.0	0.3 [0.1 – 0.5]	1.1 [0.8 – 1.5]	2.3 [1.8 – 3.1]
Total wine consumption, units/day	0.3 [0.0 – 1.1]	0.0 ± 0.0	0.1 [0.0 – 0.3]	0.6 [0.3 – 0.9]	1.7 [0.9 – 2.1]
Total beer consumption, gram/day	0.1 [0.0 – 0.5]	0.0 ± 0.0	0.1 [0.0 – 0.3]	0.3 [0.0 – 0.8]	0.3 [0.0 – 1.6]
Total spirits consumption, units/day	0.0 [0.0 – 0.0]	0.0 ± 0.0	0.0 [0.0 – 0.0]	0.0 [0.0 – 0.0]	0.0 [0.0 – 0.1]
Endpoints					
CSVD features					
White matter hyperintensity volume, ml†	0.0 [0.0 – 0.1]	0.0 [0.0 – 0.1]	0.0 [0.0 – 0.0]	0.0 [0.0 – 0.1]	0.0 [0.0 – 0.1]
Presence of cerebral microbleeds†	245 (11.8)	26 (8.3)	74 (11.8)	60 (14.2)	85 (11.9)
Presence of lacunar infarcts†	114 (5.5)	20 (6.4)	37 (5.9)	21 (5.0)	36 (5.0)
Composite score†	0.0± 1.0	-0.0± 1.0	-0.0± 1.0	0.0± 1.0	0.0± 1.0

Table 4.1 (continued)

Characteristic	Total study population (n=3107)	Alcohol consumption			
		None (n=498)	Light (n=964)	Moderate (n=620)	High (n=1025)
Retinal microvascular diameters					
Arteriolar diameter, µm†	142.3 ± 20.2	145.3 ± 19.9	143.0 ± 20.1	140.9 ± 20.7	141.1 ± 20.1
Venular diameter, µm†	214.6 ± 31.4	218.2 ± 31.3	215.6 ± 32.2	211.0 ± 31.4	214.0 ± 30.4
Composite score†	0.0± 1.0	-0.0± 1.0	-0.0± 1.0	0.1± 1.0	0.0± 1.0
Flicker light-induced increase in retinal microvascular diameters					
Arteriolar flicker light-induced dilation, µm †	4.4 ± 3.6	4.1 ± 3.7	4.3 ± 3.4	4.8 ± 3.8	4.3 ± 3.5
Venular flicker light-induced dilation, µm †	7.6 ± 4.1	7.7 ± 4.2	7.5 ± 4.0	7.5 ± 4.1	7.7 ± 4.1
Composite score†	0.0± 1.0	0.0± 1.0	0.0± 1.0	-0.1± 1.0	-0.0± 1.0
Heat-induced skin hyperaemia, PU†	112.1 ± 57.3	113.1 ± 61.4	107.7 ± 53.0	109.9 ± 55.5	116.9 ± 59.8
UAE, mg/24 hours†	6.7 [4.0 – 11.9]	7.3 [4.4 – 14.2]	6.7 [4.2 – 12.3]	6.4 [3.8 – 11.0]	6.5 [3.9 – 11.4]
≥30 mg/24h†	270 (18.7)	59 (11.8)	86 (8.9)	47 (7.6)	78 (7.6)
Plasma biomarkers of MVD composite score					
sICAM-1, ng/ml†	353.9 ± 99.8	390.0 ± 134.2	349.2 ± 89.6	347.2 ± 95.5	344.6 ± 87.2
sVCAM-1, ng/ml†	428 ± 101.0	450.1 ± 125.9	432.8 ± 99.2	427.8 ± 96.3	412.9 ± 88.6
sE-selectin, ng/ml†	117.8 ± 65.7	131.8 ± 90.7	117.9 ± 64.2	119 ± 58.2	114.3 ± 55.0
vWF, %†	132.6 ± 48.4	140.4 ± 52.0	133.7 ± 47.1	131.5 ± 48.4	128.4 ± 47.3
Composite score†	0.0± 1.0	0.4± 1.3	0.0± 0.9	-0.1± 1.0	-0.1± 0.9

Data are presented as mean ± standard deviation, median [interquartile range] or n (%). * Data were available for: ambulatory blood pressure, n= 1,345; BMI, n= 3,106; physical activity, n=2,408; fasting plasma glucose, n=3,106; 2-hour post load glucose, n=2,868; HbA1c, n=3,100; diabetic retinopathy, n=2,610; eGFR, n=3,082; biomarkers of low grade-inflammation, n=2,079; occupational status, n=2,583; income, n=2,368. † value shown for study population with complete data on cerebral small vessel disease, or retinal arteriolar and venular diameters, or flicker light-induced increase in retinal arteriolar and venular diameter, or heat-induced skin hyperaemia, or UAE, or plasma biomarkers of microvascular dysfunction i.e. for features of cerebral small vessel disease n=2,075; for retinal arteriolar and venular diameters n=2,721; for flicker light-induced increase in retinal arteriolar and venular diameter n=2,090; for heat-induced skin hyperaemia n=1,517; for urinary albumin excretion n=3,107; and for plasma biomarkers of microvascular dysfunction n=3,078. Abbreviations: HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; CSVD, cerebral small vessel disease; SD, standard deviation; MVD, microvascular dysfunction; PU, perfusion units; ICAM, soluble intercellular adhesin molecule-1; sVCAM, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; UAE, urinary albumin excretion.

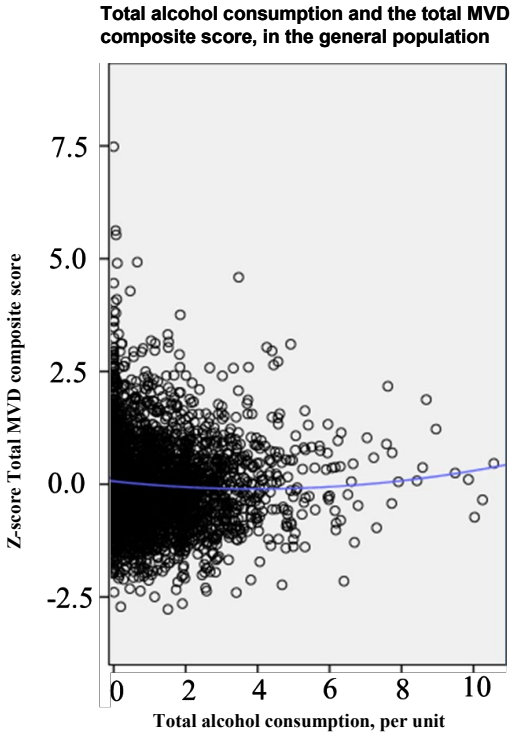

Figure 4.2 General population (N=3,120; minimum of the J-curve at 4 units/day). The Scatter plot shows data points for total alcohol consumption (x-axis; per unit) and the total MVD composite score (y-axis; in SD) where a quadratic association was modelled (blue line). In the general population the minimum of the J-curve was located at approximately 4 units/day.

Stratified analyses

History of cardiovascular disease

In individuals with and without a history of cardiovascular disease the shapes of the association of total alcohol consumption with the total MVD composite score were different, both with regard to the location of the minimum of the J-curve as well as the depth of the minimum of the J-curve. The minimum of the J-curve, was at approximately 6 units/day in individuals with a history of cardiovascular disease and at approximately 2 units/day in individuals without a history of cardiovascular disease (Supplemental Figure S4.1); and the minimum of the J-curve was lower in individuals with, versus without, a history of cardiovascular disease (indicating that higher than light total alcohol consumption was more strongly associated with less MVD in individuals with, versus without, a history of cardiovascular disease; Figure 4.3 and Supplemental Table S4.4).

Table 4.2 Associations of total alcohol, wine, beer, and spirits consumption with the total MVD composite score in the general population.

		Alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
General population, n=3,120						
Total alcohol consumption	1	-0.02 (-0.05; 0.00)	**0.23 (-0.12; 0.33)**	**-0.15 (-0.25; -0.05)**	**-0.15 (-0.24; -0.06)**	**0.00**
	2	**-0.05 (-0.07; -0.02)**	**0.16 (0.06; 0.26)**	**-0.12 (-0.22; -0.03)**	**-0.13 (-0.21; -0.05)**	**0.00**
	3A	**-0.05 (-0.08; -0.03)**	**0.15 (0.05; 0.25)**	**-0.10 (-0.19; -0.01)**	**-0.14 (-0.22; -0.06)**	**0.00**
	3B	**-0.04 (-0.07; -0.02)**	**0.12 (0.02; 0.22)**	**-0.10 (-0.19; -0.01)**	**-0.11 (-0.19; -0.03)**	**0.00**
Wine consumption	1	**-0.11 (-0.15; -0.08)**	**0.24 (0.15; 0.33)**	**-0.20 (-0.31; -0.09)**	**-0.12 (-0.23; -0.01)**	**0.00**
	2	**-0.10 (-0.13; -0.06)**	**0.15 (0.07; 0.24)**	**-0.17 (-0.27; -0.06)**	-0.06 (-0.17; 0.04)	**0.00**
	3A	**-0.09 (-0.12; -0.06)**	**0.11 (0.03; 0.20)**	**-0.16 (-0.26; -0.06)**	-0.07 (-0.17; 0.03)	**0.00**
	3B	**-0.08 (-0.11; -0.04)**	**0.10 (0.02; 0.18)**	**-0.15 (-0.25; -0.05)**	-0.05 (-0.15; 0.05)	**0.00**
Beer consumption	1	**0.04 (0.01; 0.08)**	**-0.08 (-0.17; -0.00)**	**-0.14 (-0.27; -0.01)**	0.00 (-0.15; 0.15)	0.57
	2	-0.01 (-0.04; 0.03)	-0.01 (-0.10; 0.07)	-0.11 (-0.23; 0.01)	-0.05 (-0.19; 0.08)	0.30
	3A	-0.02 (-0.05; 0.02)	-0.02 (-0.10; 0.07)	**-0.14 (-0.25; -0.02)**	-0.10 (-0.23; 0.04)	0.09
	3B	-0.01 (-0.04; 0.03)	-0.02 (-0.10; 0.06)	**-0.13 (-0.25; -0.02)**	-0.07 (-0.20; 0.06)	0.21
Spirits consumption	1	**0.17 (0.03; 0.31)**	-0.05 (-0.13; 0.04)	**-0.24 (-0.39; -0.10)**	**-0.33 (-0.58; -0.08)**	**0.01**
	2	0.05 (-0.08; 0.17)	0.00 (-0.09; 0.09)	**-0.15 (-0.28; -0.02)**	-0.18 (-0.41; 0.05)	0.40
	3A	-0.00 (-0.13; 0.12)	-0.01 (-0.09; 0.08)	**-0.17 (-0.30; -0.04)**	-0.19 (-0.42; 0.03)	0.83
	3B	-0.01 (-0.13; 0.11)	-0.01 (-0.09; 0.07)	**-0.16 (-0.29; -0.04)**	-0.17 (-0.40; 0.05)	0.69

Betas and 95% confidence intervals indicate the strength of the association between total alcohol, wine, beer, and spirits consumption with the total MVD composite score where a negative beta indicates less MVD. Total alcohol, wine, beer, and spirits consumption were entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). One SD corresponds with 1.6 ml white matter hyperintensity volume (logarithmic scale), 2.4 cerebral microbleeds (logarithmic scale), 1.6 lacunar infarcts (logarithmic scale; all three combined in the CSVD features composite score); 20.2 µm of CRAE, 31.4 µm of CRVE (combined in the retinal microvascular diameter composite score); 3.6 µm of flicker light-induced increase in retinal arteriolar diameter, 4.1 µm of flicker light-induced increase in retinal venular diameter (combined in the flicker light-induced increase in retinal microvascular diameter composite score); 0.98 mg/24 hours of logarithmically transformed UAE; 57.3 PU of heat-induced skin hyperaemia; or 99.8 ng/ml sICAM-1, 101.0 ng/ml of sVCAM-1, 65.7 ng/ml of sE-selectin, or 48.4% vWF (combined in the plasma biomarkers of MVD composite score). The numbers of participants with complete data on CSVD features, retinal microvascular diameters, flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD respectively are n=2,075; n=2,721; n=2,090; n=1,517; n=3,107; and n=3,078. Model 1: crude; Model 2: age, sex, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), education level [low, middle, high]; model 3A: model 2 + waist circumference, smoking status [current, ever, never], diet score; model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Additionally, for associations with heat-induced skin hyperaemia baseline skin blood flow was entered in model 1. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; CSVD, cerebral small vessel disease; CRAE, central retina arteriolar equivalent; CRVE, central retinal venular equivalent; SD: standard deviation; PU, perfusion units; UAE, urinary albumin excretion; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; MVD, microvascular dysfunction.

Associations of moderate versus light total alcohol consumption with the total MVD composite score in the general population and stratified by history of cardiovascular disease and sex (in SD; model 3B)

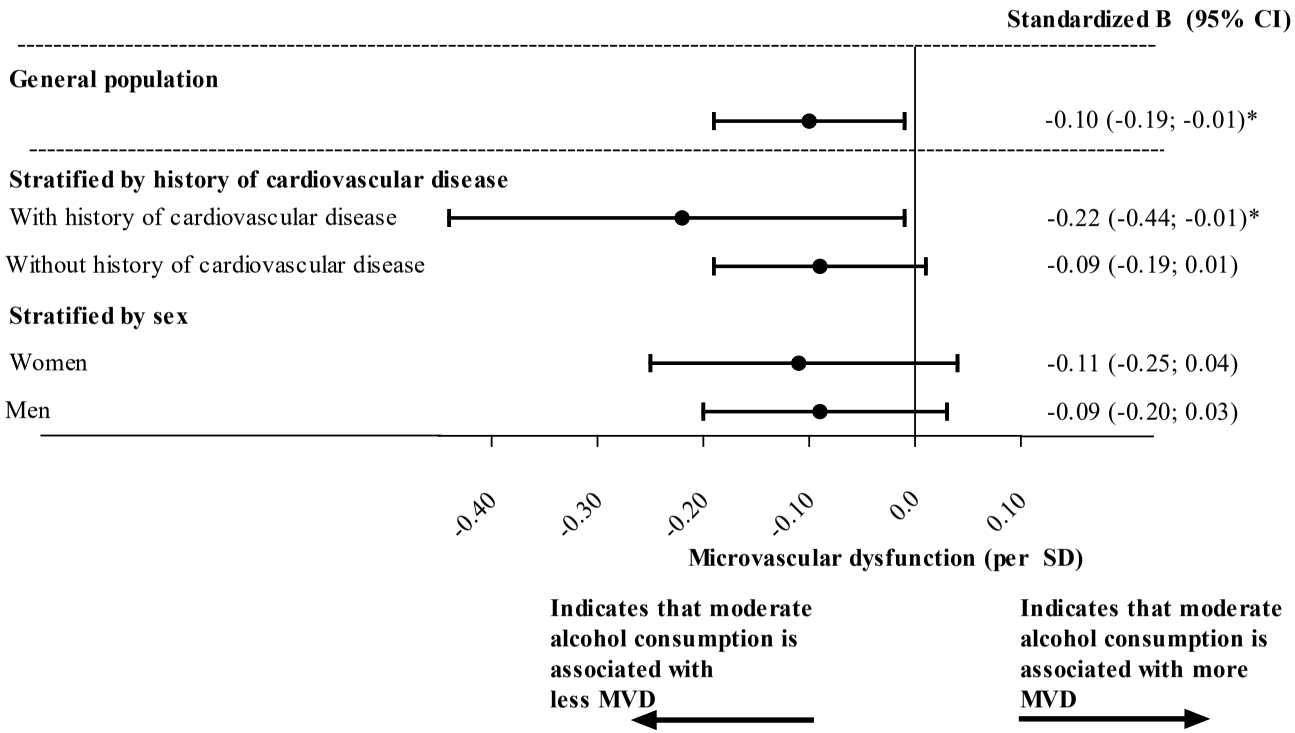

Figure 4.3 Associations of moderate versus light total alcohol consumption with the total MVD composite score in the general population. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (moderate versus light) with total MVD composite score (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legends of Table 4.2 (general population), Supplemental Table S4.4 (history of cardiovascular disease strata) and Supplemental Table S4.5 (sex strata). Variables included in model 3B are age, sex (where applicable), glucose metabolism status, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and history of cardiovascular disease (where applicable). * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Then, in individuals with a history of cardiovascular disease wine and beer consumption were considerably more strongly associated with less MVD than spirits consumption (for wine, beer, and spirits, per unit [zero drinkers as reference], -0.16 [-0.25; -0.08]; -0.14 [-0.22; -0.06]; and -0.00 [-0.28; 0.28], respectively; Supplemental Table S4.4 and Supplemental Figure S4.2).

Sex

In men and women the shapes of the associations of total alcohol consumption with the total MVD composite score differed with regard to the location of the minimum of the J-curve and somewhat, but not materially, with regard to the depth of the minimum of the J-curve. To start, the minimum of the J-curve for total alcohol consumption in the association with the total MVD composite score was at approximately 5 units/day in men and at approximately 3 units per/day in women (Supplemental Figure S4.1). Next, the strength of this association was numerically somewhat stronger in women than in men (e.g. model 3B; moderate versus light total alcohol consumption -0.11 [-0.25; 0.04] in women versus -0.09 [-0.20; 0.03] in men; Figure 4.3 and Supplemental Table S4.5).
Additionally, in both men and women, wine consumption was somewhat more strongly associated with less MVD, estimated from the total MVD composite score, than beer or spirits consumption, where wine, but not beer or spirits, consumption was somewhat more strongly associated with less MVD in women than in men (-0.09 [-0.14; -0.01] in women versus -0.06 [-0.11; -0.02] in men; Supplemental Table S4.5).

Additional analyses

We observed quantitatively similar results in a range of additional analyses (all results are reported in the Extended Results section in the Supplemental Material). In addition, we found that the minimum in the J-curve was at increasingly higher levels of total alcohol consumption in individuals with increasingly more cardiovascular risk factors (Supplemental Table S4.7 and Supplemental Figure S4.5); and that when we left wine, beer, or spirits out of the total alcohol consumption index the location and depth of the minimum of the J-curve were different (Supplemental Figure S4.7).

Discussion

The present population-based study has three main findings. First, in the general population we found a J-shaped association between total alcohol consumption with

MVD, where moderate versus light total alcohol consumption was associated with less MVD and higher than moderate versus light total alcohol consumption was associated with more MVD. Additionally, results were similar for wine, beer, and spirits. Second, in individuals with, versus without, a history of the cardiovascular disease, the minimum of the J-curve was at higher levels of total alcohol consumption and the depth of the minimum of the J-curve was considerably lower (indicating stronger associations in individuals with, versus without, a history of cardiovascular disease). In addition, in individuals with a history of cardiovascular disease, the depth of the minimum of the J-curve was considerably lower for wine and beer consumption than for spirits consumption. Third, in men, versus women, the minimum was at higher, versus lower, levels of alcohol consumption and, alternatively, in women, versus men, the depth of the minimum of the J-curve was somewhat lower (indicating somewhat stronger associations in women than in men). In addition, wine, but not beer or spirits, consumption was somewhat more strongly associated with less MVD in women than in men.

Our findings are in line with observations from most previous studies.[15-53] Importantly, the present study is the first large population-based study to comprehensively report associations of total alcohol, wine, beer, and spirits consumption with MVD assessed in various organs, both in the general population as well as in substrata of individuals with a history of cardiovascular disease or a cardiovascular risk factor. Further, the present study is the first study to report the associations of total alcohol, wine, beer, and spirits consumption with flicker light-induced increase in retinal diameters and heat-induced skin hyperaemia.

Our observations support the concept that alcohol consumption is a determinant of MVD. All measures of MVD under study likely (in part) reflect endothelial cell function, which relies on NO bioavailability, and NO bioavailability can be modified by alcohol consumption.[2,6,7] Mechanistically, the J-shaped association between alcohol and MVD is thought to reflect a triphasic balance, where in the first phase (descending part of the curve) alcohol consumption induces a net increase in NO bioavailability (reducing MVD); in the second phase (minimum of the curve) there is an equilibrium between increasing and reducing effects of alcohol on NO bioavailability (net no effect on MVD); and in the third phase (ascending part of the curve) alcohol consumption induces a net reduction in NO bioavailability (increasing MVD).[5,9,73-75] Biologically, at lower levels of alcohol consumption ethanol likely increases NO bioavailability via stimulation of NO synthesis by the enzyme eNOS;[5,9,73-75] and polyphenols likely increase NO bioavailability via reducing eNOS uncoupling and scavenging of NO by oxidative stress.[5,9,73-75] Then, at higher levels of alcohol consumption ethanol likely reduces NO bioavailability by inducing oxidative stress.[5,9,73-75]

In individuals with, versus without, a history of the cardiovascular disease, the minimum of the J-curve in the association of total alcohol consumption with the total MVD composite score was at higher levels of total alcohol consumption, likely because levels of background oxidative stress are higher in individuals with, versus without, a history of cardiovascular disease.[5,73] Biologically, the quantity of alcohol consumption at which there is equilibrium between net NO bioavailability increasing and net NO bioavailability reducing effects of alcohol consumption may be at higher, versus lower, levels of alcohol consumption in individuals with, versus without, a history of cardiovascular disease because higher levels of ethanol-induced oxidative stress may be required to (substantially) induce more oxidative stress than already present in the background.[5,27] Indeed, consistent with this concept, we found that the minimum in the J-curve was at increasingly higher levels of total alcohol consumption in individuals with increasingly more cardiovascular risk factors.

In individuals with, versus without, a history of the cardiovascular disease the depth of the minimum of the J-shaped association of total alcohol consumption with MVD was considerably lower (indicating a stronger association), likely because at higher, versus lower, levels of background oxidative stress polyphenols can more potently increase NO bioavailability.[5,27,73] Biologically, polyphenols can both increase NO bioavailability via preventing the scavenging of co-factors that are required for NO synthesis and via inhibiting a vicious circle in which oxidative stress scavenges NO and oxidizes NO into more oxidative stress (i.e. peroxynitrate, a reactive nitrogen species).[5,27] Indeed, consistent with this concept, in individuals with a history of cardiovascular disease wine and beer consumption, which reflect greater intake of polyphenols than spirits consumption, were more strongly associated with less MVD than spirits consumption.

In men, versus women, the minimum of the J-curve was located at higher levels of total alcohol consumption, likely due to sex differences in the pharmacokinetics of ethanol.[76,77] Biologically, as in women, versus men, the gastric activity of the antidiuretic hormone (ADH) is lower, which regulates the clearance of ethanol (first-pass metabolism), the consumption of a comparable quantity of ethanol likely leads to a higher level of ethanol in the blood of women than men.[76,77] Additionally, as women on average have a lower volume of body water than men and ethanol is distributed in water in the body, the consumption of a comparable quantity of ethanol likely leads to higher blood concentrations of ethanol in women than in men.[76,77]

In women the depth of the minimum of the J-curve was somewhat, but not materially, lower than in men, possibly because certain small polyphenol-based pharmacodynamic sex differences exist.[14,78] Biologically, as certain polyphenols in alcoholic beverages (e.g. resveratrol) can, via binding to the oestrogen receptor, in a sex-specific manner alter intra-endothelial cell signalling pathways that regulate NO bioavailability, alcohol

consumption may more strongly lead to an increase in endothelial cell NO bioavailability in women than in men.[14,78,79] Indeed, consistent with this concept, we found that wine, which contains resveratrol (mainly derived from red wine), was somewhat more strongly associated with less MVD in women than in men.[14,78]

In analyses with individual measures of MVD we observed that higher alcohol consumption was associated with narrower retinal microvascular diameters. Retinal arteriolar widening is thought to occur in early stages of MVD; thus, a narrower arteriolar diameter may represent less widening (i.e. indicating less MVD).[2,4] Biologically, widening of retinal arteriolar diameter is thought to reflect impairment of autoregulation, which is (in part) thought to be caused by endothelial cell dysfunction, as well as focal downstream ischaemia.[2,4] Indeed, human and animal data from observational and experimental studies in the retina, as well as in other organs such as the kidney, support this concept.[2,4]

Our findings do not imply that alcohol can be used to prevent MVD, because greater consumption of alcohol is associated with increased risk of all-cause mortality.[80] Nevertheless, our findings add to the increasing body of evidence that it may be possible to reduce MVD via dietary interventions.[81] Therefore, future research is warranted to investigate what dietary components or supplements may be able to reduce MVD.

Main strengths of this study are the large size of this population-based cohort study with oversampling of individuals with type 2 diabetes, which enables accurate comparison of individuals with and without diabetes;[71] the large number of potential confounders that was considered;[82] and the use of state-of-the-art techniques to assess CSVD features, retinal microvascular diameters, and MVD in various organ beds.[59] In addition, a strength of this study is that light drinkers were used as reference group to account for sick quitters.[55]

Limitations include the following. First, due to the cross-sectional nature of the study causal inferences should be made with considerable caution. Second, some misclassification of high drinkers may have occurred as high drinkers may be more likely to self-underreport their alcohol consumption.[36] This may have led to an underestimation of strength of the associations in this study.[69] Third, even though we took an extensive set of confounders into account, we cannot fully exclude unmeasured confounding. For example, we did not take binge drinking into account and binge drinking may be more detrimental than chronic high levels of drinking.[6] Fourth, there were relatively low numbers of high beer consumers (≤7% of participants) and moderate or high spirits consumers (≤2% of participants) in this study and this may resulted in a too low statistical power to be able to detect statistically significant associations of beer and spirits consumption with endpoints under study (i.e. type 2 statistical error).[71] Fifth, we studied Caucasian individuals aged 40-75 years and

therefore our results may be generalizable to such a population; whether these results also apply to other populations requires further study.[83]

The present population-based study found a J-shaped association between total alcohol, wine, beer, and spirits consumption and MVD, where moderate versus light alcohol consumption was associated with less MVD and higher than moderate versus light alcohol consumption was associated with more MVD. Additionally, the location and the depth of the minimum of the J-curve differed by history of cardiovascular disease and sex. Therefore, alcohol consumption may be a determinant of MVD and via MVD mitigate microvascular clinical disease such as stroke, dementia, depression, retinopathy, and chronic kidney disease.[1-3] Although increasing alcohol consumption cannot be recommended as a policy, this study suggests that prevention of MVD may be possible through dietary interventions.

References

1. Rensma SP, van Sloten TT, Launer LJ and Stehouwer CDA. Cerebral small vessel disease and risk of incident stroke, dementia and depression, and all-cause mortality: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2018;90:164-173.
2. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67:1729-1741.
3. Bidani AK, Polichnowski AJ, Loutzenhiser R and Griffin KA. Renal microvascular dysfunction, hypertension and CKD progression. Curr Opin Nephrol Hypertens. 2013;22:1-9.
4. Li W, Schram MT, Berendschot T, Webers CAB, Kroon AA, van der Kallen CJH, Henry RMA, Schaper NC, Huang F, Dashtbozorg B, Tan T, Zhang J, Abbasi-Sureshjani S, Ter Haar Romeny BM, Stehouwer CDA and Houben A. Type 2 diabetes and HbA1c are independently associated with wider retinal arterioles: the Maastricht study. Diabetologia. 2020;63:1408-1417.
5. Phillips SA, Osborn K, Hwang CL, Sabbahi A and Piano MR. Ethanol Induced Oxidative Stress in the Vasculature: Friend or Foe. Curr Hypertens Rev. 2020;16:181-191.
6. Piano MR. Alcohol's Effects on the Cardiovascular System. Alcohol Res. 2017;38:219-241.
7. Phillips SA, Osborn K, Hwang CL, Sabbahi A and Piano MR. Ethanol induced oxidative stress in the vasculature: Friend or Foe. Curr Hypertens Rev. 2019.
8. Kaewphaleuk T, Watanapa WB and Panich U. Ethanol enhances endothelial ionic currents and nitric oxide release via intermediate-conductance calcium-activated potassium channel. Life Sci. 2019;228:21-29.
9. Oak MH, Auger C, Belcastro E, Park SH, Lee HH and Schini-Kerth VB. Potential mechanisms underlying cardiovascular protection by polyphenols: Role of the endothelium. Free Radic Biol Med. 2018;122:161-170.
10. Fragopoulou E, Choleva M, Antonopoulou S and Demopoulos CA. Wine and its metabolic effects. A comprehensive review of clinical trials. Metabolism. 2018;83:102-119.
11. Blomster JI, Zoungas S, Chalmers J, Li Q, Chow CK, Woodward M, Mancia G, Poulter N, Williams B, Harrap S, Neal B, Patel A and Hillis GS. The relationship between alcohol consumption and vascular complications and mortality in individuals with type 2 diabetes. Diabetes Care. 2014;37:1353-9.
12. Huang C, Zhan J, Liu YJ, Li DJ, Wang SQ and He QQ. Association between alcohol consumption and risk of cardiovascular disease and all-cause mortality in patients with hypertension: a meta-analysis of prospective cohort studies. Mayo Clin Proc. 2014;89:1201-10.
13. White SL, Polkinghorne KR, Cass A, Shaw JE, Atkins RC and Chadban SJ. Alcohol consumption and 5-year onset of chronic kidney disease: the AusDiab study. Nephrol Dial Transplant. 2009;24:2464-72.
14. Louis XL, Raj P, Chan L, Zieroth S, Netticadan T and Wigle JT. Are the cardioprotective effects of the phytoestrogen resveratrol sex-dependent? (1). Can J Physiol Pharmacol. 2019;97:503-514.
15. Chudek J, Wieczorowska-Tobis K, Zejda J, Broczek K, Skalska A, Zdrojewski T and Wiecek A. The prevalence of chronic kidney disease and its relation to socioeconomic conditions in an elderly Polish population: results from the national population-based study PolSenior. Nephrol Dial Transplant. 2014;29:1073-82.
16. Daviglus ML, Greenland P, Stamler J, Elliott P, Appel LJ, Carnethon MR, Chan Q, Claeys G, Kesteloot H, Miura K, Nakagawa H, Pirzada A, Steffen L, Yan LL, Zhou B and Dyer AR. Relation of nutrient intake to microalbuminuria in nondiabetic middle-aged men and women: International Population Study on Macronutrients and Blood Pressure (INTERMAP). Am J Kidney Dis. 2005;45:256-66.
17. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, van Duijn CM, Hofman A and Breteler MM. Alcohol intake in relation to brain magnetic resonance imaging findings in older persons without dementia. Am J Clin Nutr. 2004;80:992-7.
18. Dervenis N, Coleman AL, Harris A, Wilson MR, Yu F, Anastasopoulos E, Founti P, Pappas T, Kilintzis V and Topouzis F. Factors Associated With Retinal Vessel Diameters in an Elderly Population: the Thessaloniki Eye Study. Invest Ophthalmol Vis Sci. 2019;60:2208-2217.

19. Ding J, Eigenbrodt ML, Mosley TH, Jr., Hutchinson RG, Folsom AR, Harris TB and Nieto FJ. Alcohol intake and cerebral abnormalities on magnetic resonance imaging in a community-based population of middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. Stroke. 2004;35:16-21.
20. Ding J, Sigurdsson S, Garcia M, Phillips CL, Eiriksdottir G, Gudnason V, van Buchem MA and Launer LJ. Risk Factors Associated With Incident Cerebral Microbleeds According to Location in Older People: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. JAMA Neurol. 2015;72:682-8.
21. Duan J, Wang C, Liu D, Qiao Y, Pan S, Jiang D, Zhao Z, Liang L, Tian F, Yu P, Zhang Y, Zhao H and Liu Z. Prevalence and risk factors of chronic kidney disease and diabetic kidney disease in Chinese rural residents: a cross-sectional survey. Sci Rep. 2019;9:10408.
22. Dunkler D, Kohl M, Heinze G, Teo KK, Rosengren A, Pogue J, Gao P, Gerstein H, Yusuf S, Oberbauer R, Mann JF and Investigators O. Modifiable lifestyle and social factors affect chronic kidney disease in high-risk individuals with type 2 diabetes mellitus. Kidney Int. 2015;87:784-91.
23. Gu Y, Scarmeas N, Short EE, Luchsinger JA, DeCarli C, Stern Y, Manly JJ, Schupf N, Mayeux R and Brickman AM. Alcohol intake and brain structure in a multiethnic elderly cohort. Clin Nutr. 2014;33:662-7.
24. Hara M, Yakushiji Y, Nannri H, Sasaki S, Noguchi T, Nishiyama M, Hirotsu T, Nakajima J and Hara H. Joint effect of hypertension and lifestyle-related risk factors on the risk of brain microbleeds in healthy individuals. Hypertens Res. 2013;36:789-94.
25. Ikram MK, de Jong FJ, Vingerling JR, Witteman JC, Hofman A, Breteler MM and de Jong PT. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. Invest Ophthalmol Vis Sci. 2004;45:2129-34.
26. Jespersen T, Kruse N, Mehta T, Kuwabara M, Noureddine L and Jalal D. Light wine consumption is associated with a lower odd for cardiovascular disease in chronic kidney disease. Nutr Metab Cardiovasc Dis. 2018;28:1133-1139.
27. Karatzi K, Aissopou EK, Katsana K, Moiragia M, Tentolouris N, Sfikakis PP and Protogerou AD. Retinal microcirculation in association with caffeinated and alcoholic drinks in subjects at increased cardiovascular risk. Microcirculation. 2016;23:591-596.
28. Kaze FF, Meto DT, Halle MP, Ngogang J and Kengne AP. Prevalence and determinants of chronic kidney disease in rural and urban Cameroonians: a cross-sectional study. BMC Nephrol. 2015;16:117.
29. Klein R, Klein BE and Moss SE. Prevalence of microalbuminuria in older-onset diabetes. Diabetes Care. 1993;16:1325-30.
30. Klein R, Myers CE, Knudtson MD, Lee KE, Gangnon R, Wong TY and Klein BE. Relationship of blood pressure and other factors to serial retinal arteriolar diameter measurements over time: the beaver dam eye study. Arch Ophthalmol. 2012;130:1019-27.
31. Koning SH, Gansevoort RT, Mukamal KJ, Rimm EB, Bakker SJ, Joosten MM and Group PS. Alcohol consumption is inversely associated with the risk of developing chronic kidney disease. Kidney Int. 2015;87:1009-16.
32. Liew G, Sharrett AR, Wang JJ, Klein R, Klein BE, Mitchell P and Wong TY. Relative importance of systemic determinants of retinal arteriolar and venular caliber: the atherosclerosis risk in communities study. Arch Ophthalmol. 2008;126:1404-10.
33. Lin CC, Liu CS, Li TC, Chen CC, Li CI and Lin WY. Microalbuminuria and the metabolic syndrome and its components in the Chinese population. Eur J Clin Invest. 2007;37:783-90.
34. McEvoy LK, Fennema-Notestine C, Elman JA, Eyler LT, Franz CE, Hagler DJ, Jr., Hatton SN, Lyons MJ, Panizzon MS, Dale AM and Kremen WS. Alcohol intake and brain white matter in middle aged men: Microscopic and macroscopic differences. Neuroimage Clin. 2018;18:390-398.
35. Metcalf P, Baker J, Scott A, Wild C, Scragg R and Dryson E. Albuminuria in people at least 40 years old: effect of obesity, hypertension, and hyperlipidemia. Clin Chem. 1992;38:1802-8.
36. Mukamal KJ, Jadhav PP, D'Agostino RB, Massaro JM, Mittleman MA, Lipinska I, Sutherland PA, Matheney T, Levy D, Wilson PW, Ellison RC, Silbershatz H, Muller JE and Tofler GH. Alcohol consumption and hemostatic factors: analysis of the Framingham Offspring cohort. Circulation. 2001;104:1367-73.
37. Rohde LE, Hennekens CH and Ridker PM. Cross-sectional study of soluble intercellular adhesion molecule-1 and cardiovascular risk factors in apparently healthy men. Arterioscler Thromb Vasc Biol. 1999;19:1595-9.

38. Rudnicka AR, Rumley A, Whincup PH, Lowe GD and Strachan DP. Sex differences in the relationship between inflammatory and hemostatic biomarkers and metabolic syndrome: British 1958 Birth Cohort. J Thromb Haemost. 2011;9:2337-44.
39. Sacanella E, Badia E, Nicolas JM, Fernandez-Sola J, Antunez E, Urbano-Marquez A and Estruch R. Differential effects of moderate or heavy alcohol consumption on circulating adhesion molecule levels. Thromb Haemost. 2002;88:52-5.
40. Tano T, Ono K, Hiratsuka Y, Otani K, Sekiguchi M, Konno S, Kikuchi S, Onishi Y, Takegami M, Yamada M, Fukuhara S and Murakami A. Retinal vessel diameters in a Japanese population: the Locomotive Syndrome and Health Outcome in Aizu Cohort Study. Acta Ophthalmol. 2016;94: e432-41.
41. Thorand B, Baumert J, Doring A, Schneider A, Chambless L, Lowel H, Kolb H and Koenig W. Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. Results from the MONICA/KORA Augsburg Study. Thromb Haemost. 2006;95:134-41.
42. van Bussel BC, Henry RM, Ferreira I, van Greevenbroek MM, van der Kallen CJ, Twisk JW, Feskens EJ, Schalkwijk CG and Stehouwer CD. A healthy diet is associated with less endothelial dysfunction and less low-grade inflammation over a 7-year period in adults at risk of cardiovascular disease. J Nutr. 2015;145:532-40.
43. van Bussel BCT, Henry RMA, Schalkwijk CG, Dekker JM, Nijpels G, Feskens EJM and Stehouwer CDA. Alcohol and red wine consumption, but not fruit, vegetables, fish or dairy products, are associated with less endothelial dysfunction and less low-grade inflammation: the Hoorn Study. Eur J Nutr. 2018;57:1409-1419.
44. Villa-Zapata L, Warholak T, Slack M, Malone D, Murcko A, Runger G and Levengood M. Predictive modeling using a nationally representative database to identify patients at risk of developing microalbuminuria. Int Urol Nephrol. 2016;48:249-56.
45. Wannamethee SG, Lowe GD, Shaper G, Whincup PH, Rumley A, Walker M and Lennon L. The effects of different alcoholic drinks on lipids, insulin and haemostatic and inflammatory markers in older men. Thromb Haemost. 2003;90:1080-7.
46. Williamson W, Lewandowski AJ, Forkert ND, Griffanti L, Okell TW, Betts J, Boardman H, Siepmann T, McKean D, Huckstep O, Francis JM, Neubauer S, Phellan R, Jenkinson M, Doherty A, Dawes H, Frangou E, Malamateniou C, Foster C and Leeson P. Association of Cardiovascular Risk Factors With MRI Indices of Cerebrovascular Structure and Function and White Matter Hyperintensities in Young Adults. JAMA. 2018;320:665-673.
47. Wong TY, Islam FM, Klein R, Klein BE, Cotch MF, Castro C, Sharrett AR and Shahar E. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). Invest Ophthalmol Vis Sci. 2006;47:2341-50.
48. Yao H, Takashima Y, Hashimoto M, Uchino A and Yuzuriha T. Subclinical cerebral abnormalities in chronic kidney disease. Contrib Nephrol. 2013;179:24-34.
49. Yarnell JW, Sweetnam PM, Rumley A and Lowe GD. Lifestyle and hemostatic risk factors for ischemic heart disease : the Caerphilly Study. Arterioscler Thromb Vasc Biol. 2000;20:271-9.
50. Nagy I and Mandi Y. Serum and ascitic levels of soluble intercellular adhesion molecule-1 in patients with alcoholic liver cirrhosis: relation to biochemical markers of disease activity and alcohol intake. Alcohol Clin Exp Res. 1996;20:929-33.
51. Davis BJ, Vidal JS, Garcia M, Aspelund T, van Buchem MA, Jonsdottir MK, Sigurdsson S, Harris TB, Gudnason V and Launer LJ. The alcohol paradox: light-to-moderate alcohol consumption, cognitive function, and brain volume. J Gerontol A Biol Sci Med Sci. 2014;69:1528-35.
52. Li F, Chen QX, Chen Y, Wang G, Peng B and Yao T. Prevalence and risk factors of microalbuminuria in patients with lacunar infarction. Postgrad Med. 2019;131:342-347.
53. Dunkler D, Kohl M, Teo KK, Heinze G, Dehghan M, Clase CM, Gao P, Yusuf S, Mann JF, Oberbauer R and Investigators O. Population-Attributable Fractions of Modifiable Lifestyle Factors for CKD and Mortality in Individuals With Type 2 Diabetes: A Cohort Study. Am J Kidney Dis. 2016;68:29-40.
54. Fillmore KM, Stockwell T, Chikritzhs T, Bostrom A and Kerr W. Moderate alcohol use and reduced mortality risk: systematic error in prospective studies and new hypotheses. Ann Epidemiol. 2007;17:S16-23.
55. Tsubono Y, Yamada S, Nishino Y, Tsuji I and Hisamichi S. Choice of comparison group in assessing the health effects of moderate alcohol consumption. JAMA. 2001;286:1177-8.

56. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
57. van Dongen MC, Wijckmans-Duysens NEG, den Biggelaar LJ, Ocke MC, Meijboom S, Brants HA, de Vries JH, Feskens EJ, Bueno-de-Mesquita HB, Geelen A, Stehouwer CD, Dagnelie PC and Eussen SJ. The Maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the Maastricht study. Nutrition. 2019;62:39-46.
58. Kennis Instituut Bier; website Kennis Instituut bier [www.kennisinstituutbier.nl]; Wageningen, The Netherlands; Kennis Instituut Bier; [2011]; URL: https://www.kennisinstituutbier.nl/sites/default/files/Glazenstandaard%20downloaden.pdf.
59. Li W, Schram MT, Sorensen BM, Agtmaal MJM, Berendschot T, Webers CAB, Jansen JFA, Backes WH, Gronenschild E, Schalkwijk CG, Stehouwer CDA and Houben A. Microvascular Phenotyping in The Maastricht Study: Design, and Main findings, 2010-2018. Am J Epidemiol. 2020.
60. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CD. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. Circulation. 2016;134:1339-1352.
61. Houben A, Martens RJH and Stehouwer CDA. Assessing Microvascular Function in Humans from a Chronic Disease Perspective. J Am Soc Nephrol. 2017;28:3461-3472.
62. Muris DM, Houben AJ, Schram MT and Stehouwer CD. Microvascular dysfunction: an emerging pathway in the pathogenesis of obesity-related insulin resistance. Rev Endocr Metab Disord. 2013;14:29-38.
63. Correction: Neighbourhood socioeconomic position and risks of major chronic diseases and all-cause mortality: a quasi-experimental study. BMJ Open. 2019;9:e018793corr1.
64. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N, Stehouwer C and Bosma H. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16.
65. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, de Vries J and Geelen A. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20:2289-2299.
66. van der Berg JD, Willems PJ, van der Velde JH, Savelberg HH, Schaper NC, Schram MT, Sep SJ, Dagnelie PC, Bosma H, Stehouwer CD and Koster A. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. J Sports Sci. 2016;34:1867-73.
67. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, Schaper NC, van der Kallen CJ, Koster A, Sep SJ, Denollet J, Verhey FR and Pouwer F. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-6.
68. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS and Investigators C-E. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367:20-9.
69. Hutcheon JA, Chiolero A and Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.
70. Goel S, Sharma A and Garg A. Effect of Alcohol Consumption on Cardiovascular Health. Curr Cardiol Rep. 2018;20:19.
71. Jones SR, Carley S and Harrison M. An introduction to power and sample size estimation. Emerg Med J. 2003;20:453-8.
72. Sorensen BM, van der Heide FCT, Houben A, Koster A, T TJMB, J SAGS, Kroon AA, van der Kallen CJH, Henry RMA, van Dongen M, S JPME, H HCMS, van der Berg JD, Schaper NC, Schram MT and Stehouwer CDA. Higher levels of daily physical activity are associated with better skin microvascular function in type 2 diabetes-The Maastricht Study. Microcirculation. 2020;27:e12611.
73. Meza CA, La Favor JD, Kim DH and Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci. 2019;20.
74. Osorio-Paz I, Brunauer R and Alavez S. Beer and its non-alcoholic compounds in health and disease. Crit Rev Food Sci Nutr. 2020;60:3492-3505.

75. Yamagata K, Tagami M and Yamori Y. Dietary polyphenols regulate endothelial function and prevent cardiovascular disease. Nutrition. 2015;31:28-37.
76. Ely M, Hardy R, Longford NT and Wadsworth ME. Gender differences in the relationship between alcohol consumption and drink problems are largely accounted for by body water. Alcohol Alcohol. 1999;34:894-902.
77. Baraona E, Abittan CS, Dohmen K, Moretti M, Pozzato G, Chayes ZW, Schaefer C and Lieber CS. Gender differences in pharmacokinetics of alcohol. Alcohol Clin Exp Res. 2001;25:502-7.
78. Arnal JF, Fontaine C, Billon-Gales A, Favre J, Laurell H, Lenfant F and Gourdy P. Estrogen receptors and endothelium. Arterioscler Thromb Vasc Biol. 2010;30:1506-12.
79. Boese AC, Kim SC, Yin KJ, Lee JP and Hamblin MH. Sex differences in vascular physiology and pathophysiology: estrogen and androgen signaling in health and disease. Am J Physiol Heart Circ Physiol. 2017;313:H524-H545.
80. Collaborators GBDA. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2018;392:1015-1035.
81. Man AWC, Li H and Xia N. Impact of Lifestyles (Diet and Exercise) on Vascular Health: Oxidative Stress and Endothelial Function. Oxid Med Cell Longev. 2020;2020:1496462.
82. Schisterman EF, Cole SR and Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20:488-95.
83. Shi Y and Wardlaw JM. Update on cerebral small vessel disease: a dynamic whole-brain disease. Stroke Vasc Neurol. 2016;1:83-92.

Supplemental materials

Extended methods

Assessment of cerebral small vessel disease (CSVD) features, retinal microvascular diameters, and microvascular dysfunction (MVD) measures

Participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement.[1] A light meal was allowed until ≥90 minutes prior to the examination. For retinal measurements, pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine ≥15 minutes before the start of the examination. Skin blood flow measurements were performed in a climate-controlled room at 24°C.[2]

Brain magnetic resonance imaging

We evaluated three CSVD features, i.e. white matter hyperintensity volume, lacunar infarcts and cerebral microbleeds with a 3T brain magnetic resonance imaging (MRI) scanner (Siemens Magnetom Prisma-fit Syngo MR D13D, Erlangen, Germany) and used a 64-element head/neck coil for parallel imaging. The MRI protocol consisted of a 3D T_1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR/TI/TE 2300/900/2.98 ms, 176 slices, 256×240 matrix size, 1.00 mm cubic voxel size); a fluid-attenuated inversion recovery (FLAIR) sequence (TR/TI/TE 5000/1800/394 ms, 176 slices, 512×512 matrix size, 0.49×0.49×1.00 mm voxel size); a combined proton density (PD) and T_2-weighted turbo spin echo (TSE) pulse sequence (TR/TE1/TE2 3200/9.4/94 ms, 30 slices, 640×540 matrix size, 0.36×0.36×4.00 mm voxel size); and a susceptibility-weighted imaging (SWI) sequence (TR/TE 28/20 ms, 144 slices, 384×312 matrix size, 0.57×0.57×1.00 mm voxel size).
Contra-indications for MRI assessment were presence of: a cardiac pacemaker or implantable cardioverter-defibrillator, a neurostimulator, a non-detachable insulin pump, metallic vascular clips or stents in the head, a cochlear implant, a metal-containing intra-uterine device, metal splinters or shrapnel, dentures with magnetic clip, an inside bracket, pregnancy, epilepsy, or claustrophobia.
T_1-weighted images and FLAIR images were analysed with an ISO-13485:2012 certified automated method (which included visual inspection).[3,4] T_1-weighted images were segmented into grey matter, white matter and cerebrospinal fluid volumes (1 voxel = 1.00 mm^3 = 0.001 ml).[3] Intracranial volume was calculated as the sum of grey matter, white matter (including white matter hyperintensity volume), and cerebrospinal fluid volumes. White matter hyperintensity volume was summed to assess total white matter hyperintensity burden, and expressed relative to intracranial volume. Lacunar

infarcts were defined as focal brain parenchyma defects of ≥3 mm and <15 mm in size with a similar signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on T_2 and FLAIR images.[5] Cerebral microbleeds were rated on T_2-weighted and SWI images by use of the Microbleed Anatomical Rating Scale,[6] and were defined as focal lesions of ≥2 mm and ≤10 mm in size with a hypointense signal.[5] The number of lacunar infarcts and cerebral microbleeds was rated manually by three neuroradiologists. The two-way mixed effects, consistency, intraclass correlation coefficients for the presence of lacunar infarcts and cerebral microbleeds for the three raters based on 50 randomly selected scans were 0.84 (95% confidence interval 0.74; 0.91) and 0.83 (0.72; 0.90), respectively.

Retinal microvascular diameters

We measured retinal arteriolar and venular diameters with static retinal vessel analysis from an optic disk-centred fundus photograph. All fundus photographs were taken with an auto-focus, auto-shot, and auto-tracking fundus camera (Model AFC-230; Nidek, Gamagori, Japan) in an optic disc-centred field of view of 45° in a darkened room. We manually evaluated quality of fundus photographs and excluded images of insufficient quality (e.g. images in which lashes obstructed the view on the optic nerve head or images that were defocused). Per participant we randomly selected one fundus photo from one eye (either left or right eye) to calculate retinal vessel diameters. We used the retinal health information and notification system (RHINO) software developed by the RetinaCheck group of the Technical University of Eindhoven (Eindhoven, the Netherlands) to assess the diameters of the six largest retinal vessels at 0.5–1.0-disc diameter away from the optic disc margin (expressed in measurement units [MU]).[7,8] We manually checked the automatically detected optic disc position and arteriole/venule classification and adjusted if necessary. Diameters of arteriolar or venular vessels were combined into an average arteriolar diameter (i.e. central retinal arteriolar equivalent [CRAE]) or venular diameter (i.e. central retinal venular equivalent [CRVE]).The scale factor, based on the optic disc diameter, was assumed to be 1800 μm,[9] i.e. 1 MU = 1 pixel size× 1800 μm/pixel size of optic disc diameter. The calculations were based on the improved Knudtson–Parr–Hubbard formula.[10] We calculated the intraclass correlation coefficients for CRAE and CRVE to assess the agreement between analyses of the RHINO software with vs without manual identification of arterioles and venules using 2556 images. The intraclass correlation coefficients for CRAE and CRVE respectively were 0.910 and 0.897.

Retinal flicker light-induced increase in retinal arteriolar and venular diameters

The flicker light-induced increase in retinal arteriolar and venular diameters, which is thought to be related to nutritive demands of activated retinal neurons,[11] was measured in a dimly lit room with the Dynamic Vessel Analyzer (DVA) (Imedos, Jena, Germany). For safety reasons, participants with an intraocular pressure exceeding 30 mmHg were excluded from retinal measurements. Per participant, we randomly measured the left or right eye.

During the measurement, the participant was instructed and encouraged to focus on the tip of a fixated needle inside the retinal camera (FF450; Carl Zeiss GmbH, Jena, Germany) while the fundus of the eye was examined under green measuring light (530-600 nm, illumination of fundus approximately 6500 lux). A straight arteriolar or venular segment of approximately 1.5 mm in length located 0.5 to 2.0-disc diameter from the margin of the optic disc in the temporal section was examined. When the specific vessel profile was recognized the vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker-light exposure period (flicker frequency 12.5Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The DVA automatically corrected for alterations in luminance caused by e.g. slight eye movements. During blinks and small eye movements the registration stopped and restarted once the vessel segments were automatically re-identified.[11]

The integrated DVA software (version 4.51, Imedos) automatically calculated the baseline arteriolar and venular diameter and the increase in arteriolar and venular diameters during flicker light exposure. Baseline diameter was calculated as the average diameter during the 20-50 seconds baseline recording and was expressed in measurement units (MU), where 1 MU is equal to 1µm of the Gullstrand eye.[12] Flicker light-induced increase in retinal microvascular diameter was calculated as the diameter during flicker light exposure minus the baseline diameter. The diameter during flicker light exposure was defined as the mean of the diameter assessed at time points 10 and 40 seconds of flicker light stimulation exposure. The purpose of taking the average increase in diameter at two time points during flicker light stimulation was to account for the inter-individual variation in increase in diameter during exposure to flicker light. For 95.4% of the measurements the assessment of flicker light-induced increase in arteriolar and venular diameter was based on the arteriolar and venular diameters assessed at both 10 and 40 seconds of flicker light stimulation. For 4.6% of the measurements the assessment of flicker light-induced increase in arteriolar and venular diameters was based on the arteriolar and venular diameter assessed at either 10 or 40 seconds of flicker light stimulation.

The measurement of retinal flicker light-induced increase in arteriolar and venular diameters was done by different trained observers. The inter-observer reliability

intraclass coefficients for the assessment of baseline retinal microvascular diameter and flicker-light induced increase in retinal microvascular diameter between two randomly selected observers were, respectively, 0.980 and 0.796 for arteriolar vessels and 0.972 and 0.871 for venular vessels (n=9 participants). The quality of the flicker light-induced increase in retinal arteriolar and venular response curves was analysed by one observer. Retinal response curves of insufficient measurement quality, e.g. insufficient measurement points or movement artifacts, were evaluated and discussed with a second observer and excluded on mutual agreement. To assess the inter-observer reliability of retinal response curves quality decisions, 50 curves were evaluated by two observers (inter-observer reliability =0.883).

Heat-induced skin hyperaemia

Skin blood flow was measured with a laser-Doppler system (Periflux 5000; Perimed, Järfalla, Sweden) equipped with a thermostatic laser-Doppler probe (PF457; Perimed) at the dorsal side of the wrist of the left hand. The laser-Doppler output was recorded for 25 minutes with a sample rate of 32 Hz, which gives semiquantitative assessment of skin blood flow expressed in arbitrary perfusion units. Skin blood flow at the wrist, expressed in arbitrary perfusion units (PU), was recorded unheated for 2 minutes to serve as a baseline. After 2 minutes, the temperature of the laser Doppler probe was rapidly and locally increased to 44°C and was kept constant until the end of the registration. In 596 individuals, the recording of skin blood flow was incomplete between 20 and 25 minutes of the recording. For these individuals data were extrapolated to 25 minutes using the last-minute average as reference, with a weighted correction factor of 1.017. This correction factor is the ratio of the increase in average PU in the 20-25-minute interval as compared to the average PU in the 19-20-minute interval.

For analyses where heat-induced skin hyperaemia was included in the total MVD composite score, we expressed heat-induced increase in skin blood as the increase in heat-induced increase in skin blood flow during the 23 minutes heating phase. We calculated heat-induced increase in skin blood flow as the average skin blood flow during the 23 minutes heating phase minus the baseline skin blood flow (i.e. average skin blood flow during the first 2 minutes). For other analyses, we expressed heat-induced increase in skin blood flow as the average skin blood flow during the 23 minutes heating phase and adjusted for the baseline skin blood flow to account for inter-individual differences in baseline skin blood flow, as previously described.[13] We checked whether both approaches yielded the same results and this was the case (data not shown).

Urinary albumin excretion

Urinary albumin excretion (UAE) was calculated as the average UAE of two 24-hour urine collections (two collections were available for 91.3% of participants). We measured urinary albumin concentration with a standard immunoturbidimetric assay by an automatic analyser (due to a change of supplier, by the Beckman Synchron LX20 and the Roche Cobas 6000). We multiplied urinary albumin concentration by collection volume to obtain 24-hour UAE. A urinary albumin concentration below the detection limit of the assay was set at 1.5 mg/L (2 mg/L for the Beckman Synchron LX20 and 3 mg/L for the Roche Cobas 6000) before multiplying by collection volume. Only urine collections with a collection time between 20 and 28 hours were considered valid. If needed, UAE was extrapolated to 24-hour excretion.

Plasma biomarkers of microvascular dysfunction

Four plasma biomarkers of MVD were evaluated (soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], sE-selectin [sE-selectin] and Von Willebrand Factor [vWF]). sICAM-1, sVCAM-1 and sEselectin of the first 866 individuals of The Maastricht Study were measured in ethylenediaminetetraacetic acid (EDTA) plasma samples with commercially available 4-plex sandwich immunoassay kits (Meso Scale Discovery (MSD), Rockville, Maryland, United States of America) as described previously.[14] From individual 867 onwards, plasma biomarkers were measured in EDTA plasma samples with renewed commercially available 4-plex sandwich immunoassay kits with different standards and antibodies (Meso Scale Discovery (MSD), Rockville, Maryland, United States of America). For this technique in this study, the intra- and inter-assay coefficients of variation were respectively 10.3% and 8.4% for sICAM-1, 5.0% and 4.7% for sVCAM-1, and 2.9% and 7.4% for sE-selectin. Absolute values of plasma biomarkers differed between the individuals measured with the old and renewed 4-plex sandwich immunoassay kits. To realign the absolute values of individuals measured with the old 4-plex sandwich immunoassay to individuals measured with the renewed 4-plex sandwich immunoassay, realign formulas were calculated with Deeming regression analyses.[15] In order to do so, the first 419 out of 866 individuals, who were measured with the old 4-plex sandwich immunoassay, were measured with the renewed 4-plex sandwich immunoassay as well. The biomarker of endothelial dysfunction vWF was quantified in citrate plasma using ELISA (Dako, Glostrup, Denmark). The intra- and inter-assay coefficients of variation were 3.0% and 4.3%, respectively.

Extended results

Additional analyses

Quantitatively similar results were observed in a range of additional analyses. First, all cardiovascular risk factors statistically significantly modified the association of total alcohol consumption with the total MVD composite score ($P_{interaction}$=0.02 for glucose metabolism status, $P_{interaction}$<0.001 for hypertension, $P_{interaction}$=0.046 for dyslipidaemia, and $P_{interaction}$=0.045 for current smoking; Supplemental Table S4.3 shows all P-for-interaction values). In stratified analyses, higher than light total alcohol consumption, wine, beer, and spirits consumption was generally more strongly associated with less MVD in individuals with, versus without, a cardiovascular risk factor (Supplemental Table S4.6 and Supplemental Figures S4.3 and S4.4). In addition, wine consumption was generally more strongly associated with less MVD than, consecutively, beer or spirits consumption (Supplemental Table S4.6 and Supplemental Figures S4.3 and S4.4). Second, we found that the strength of the association between total alcohol consumption and the total MVD composite score was increasingly stronger in individuals with increasingly more cardiovascular risk factors (Supplemental Table S4.7 and Supplemental Figure S4.5). Additionally, the minimum of the J-curve was at approximately 1 unit/day of total alcohol consumption in individuals without any cardiovascular risk factors and between 2 and 6 units/day in individuals with any risk factor, e.g. 4 units in individuals with type 2 diabetes (Supplemental Figure S4.6). Next, when we left wine or beer consumption out of the total alcohol consumption index the minimum of the J-curve was, respectively, higher and lower (Supplemental Figure S4.7). Further, the shape of the J-curve changed somewhat when wine, beer, or spirits consumption was left out of the total alcohol consumption index: without wine the sharp U-curve (at the minimum of the J curve) disappeared; without beer this U-curve became sharper; and without spirits the ascending part of the J-curve was much less steep (Supplemental Figure S4.7).

Third, we observed numerically similar associations when we analysed the associations of total alcohol consumption with the total MVD composite score, when such a composite score was composed out of any five instead of six endpoints (Supplemental Table S4.8). Fourth, we overall had similar findings for associations of total alcohol, wine, beer, and spirits consumption with individual measures of MVD, both in the general population (Supplemental Tables S4.9-S4.11 and Supplemental Figure S8; only shown for total alcohol consumption) as well as in substrata of individuals with and without a history of cardiovascular disease (Supplemental Tables S4.11 and S4.12 and Supplemental Figure S4.9; only shown for total alcohol consumption). In addition, we generally had similar findings in analyses with individual measures of MVD that were stratified by cardiovascular risk factor status (Supplemental Tables S4.13-S4.16 and

Supplemental Figures S4.10-S4.13; only shown for total alcohol consumption). Fifth, associations did not materially change after additional adjustment for accelerometer-assessed physical activity, diabetic retinopathy, eGFR, or plasma biomarkers of low-grade inflammation (Supplemental Table S4.17). Sixth, HbA1c ($P_{interaction}$=0.01), fasting plasma glucose ($P_{interaction}$=0.07), and 2-hour post load glucose ($P_{interaction}$=0.08) (borderline) significantly modified associations of alcohol consumption with the total MVD composite score (Supplemental Table S4.3). Last, associations under study were numerically nearly identical when educational status was replaced with income level or occupational status; or when glucose metabolism status was replaced with fasting plasma glucose, 2-hour post load glucose, or HbA1c; or when office systolic blood pressure was replaced with office diastolic blood pressure, or systolic or diastolic 24-hour ambulatory blood pressure (Supplemental Table S4.18).

References

1. Garhofer G, Resch H, Sacu S, Weigert G, Schmidl D, Lasta M and Schmetterer L. Effect of regular smoking on flicker induced retinal vasodilatation in healthy subjects. Microvasc Res. 2011;82:351-5.
2. Pettersson A, Bostrom KB, Gustavsson P and Ekselius L. Which instruments to support diagnosis of depression have sufficient accuracy? A systematic review. Nord J Psychiatry. 2015;69:497-508.
3. Vrooman HA, Cocosco CA, van der Lijn F, Stokking R, Ikram MA, Vernooij MW, Breteler MM and Niessen WJ. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. Neuroimage. 2007;37:71-81.
4. de Boer R, Vrooman HA, van der Lijn F, Vernooij MW, Ikram MA, van der Lugt A, Breteler MM and Niessen WJ. White matter lesion extension to automatic brain tissue segmentation on MRI. Neuroimage. 2009;45:1151-61.
5. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M and nEuroimaging STfRVco. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-38.
6. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR and Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. Neurology. 2009;73:1759-66.
7. Bart M. ter Haar Romeny EJB, Jiong Zhang, Samaneh Abbasi-Sureshjani, Fan Huang, Remco Duits, Behdad Dashtbozorg, Tos T. J. M. Berendschot, Iris Smit-Ockeloen, Koen A. J. Eppenhof, Jinghan Feng, Julius Hannink, Jan Schouten, Mengmeng Tong, Hanhui Wu, Han W. van Triest, Shanshan Zhu, Dali Chen, Wei He, Ling Xu, Ping Han & Yan Kang Brain-inspired algorithms for retinal image analysis. Machine Vision and Applications. 2016;27: 1117–1135.
8. Erik Bekkers RD, Tos Berendschot, and Romeny BtH. A Multi-Orientation Analysis Approach to Retinal Vessel Tracking. J Math Imaging Vis. 2013.
9. Jonas JB, Gusek GC and Naumann GO. Optic disc, cup and neuroretinal rim size, configuration and correlations in normal eyes. Invest Ophthalmol Vis Sci. 1988;29:1151-8.
10. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R and Klein BE. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res. 2003;27:143-9.
11. Nagel E and Vilser W. Flicker observation light induces diameter response in retinal arterioles: a clinical methodological study. Br J Ophthalmol. 2004;88:54-6.

12. Nagel E, Vilser W, Fink A and Riemer T. [Variance of retinal vessel diameter response to flicker light. A methodical clinical study]. Ophthalmologe. 2006;103:114-9.
13. Sorensen BM, van der Heide FCT, Houben A, Koster A, T TJMB, J SAGS, Kroon AA, van der Kallen CJH, Henry RMA, van Dongen M, S JPME, H HCMS, van der Berg JD, Schaper NC, Schram MT and Stehouwer CDA. Higher levels of daily physical activity are associated with better skin microvascular function in type 2 diabetes-The Maastricht Study. Microcirculation. 2020;27:e12611.
14. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, Schaper NC, van der Kallen CJ, Koster A, Sep SJ, Denollet J, Verhey FR and Pouwer F. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-6.
15. van Bussel BC, Ferreira I, van de Waarenburg MP, van Greevenbroek MM, van der Kallen CJ, Henry RM, Feskens EJ, Stehouwer CD and Schalkwijk CG. Multiple inflammatory biomarker detection in a prospective cohort study: a cross-validation between well-established single-biomarker techniques and an electrochemiluminescense-based multi-array platform. PLoS One. 2013;8:e58576.

Supplemental figures and tables

Total alcohol consumption and the total MVD composite score, in individuals with a history of cardiovascular disease

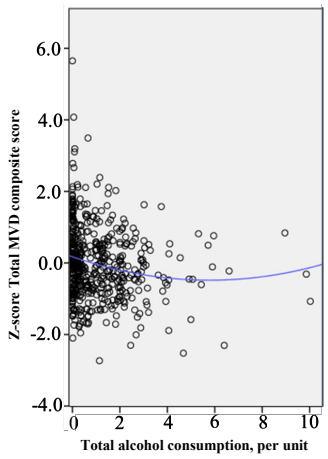

Figure S4.1.1 With history of cardiovascular disease (N=595; minimum at 6 units/day).

Total alcohol consumption and the total MVD composite score, in individuals without a history of cardiovascular disease

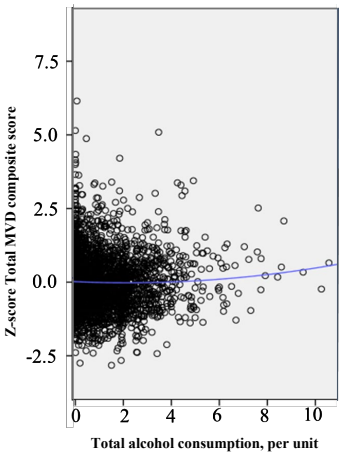

Figure S4.1.2 Without history of cardiovascular disease (N=2,595; minimum at 2 units/day).

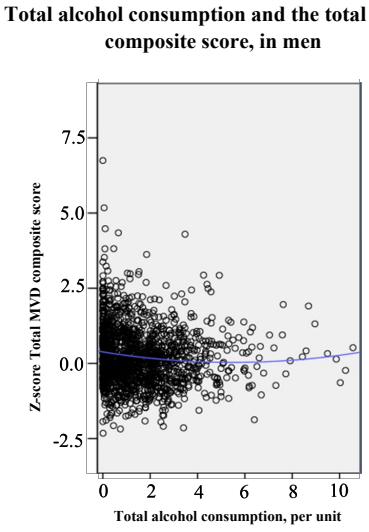

Figure S4.1.3 Men (N=1,592; minimum at 5 units/day).

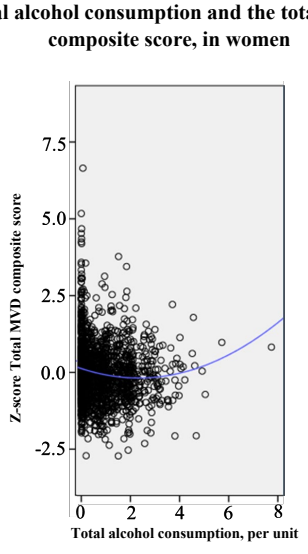

Figure S4.1.4 Women (N=1,528; minimum at 3 units per day). Supplemental Figure S4.1.shows scatter plots with data points for total alcohol consumption (x-axis; per unit) and the total MVD composite score (y-axis; per SD) where a quadratic association was modeled (blue line). In individuals with a history of cardiovascular disease the minimum of the J-curve was at approximately 6 units/day (Supplemental Figure 4.1.1). In individuals without a history of cardiovascular disease the minimum of the J-curve was at approximately 2 units/day (Supplemental Figure 4.1.2). In men the minimum of the J-curve was at approximately 5 units/day (Supplemental Figure 4.1.3). In women the minimum of the J-curve was at approximately 2 units/day (Supplemental Figure 4.1.4). Abbreviations: MVD, microvascular dysfunction; SD, standard deviation.

Associations of total alcohol consumption, wine, beer, and spirits consumption (per unit) with the total MVD composite score (in SD) in the general population and stratified by history of cardiovascular disease (model 3B)

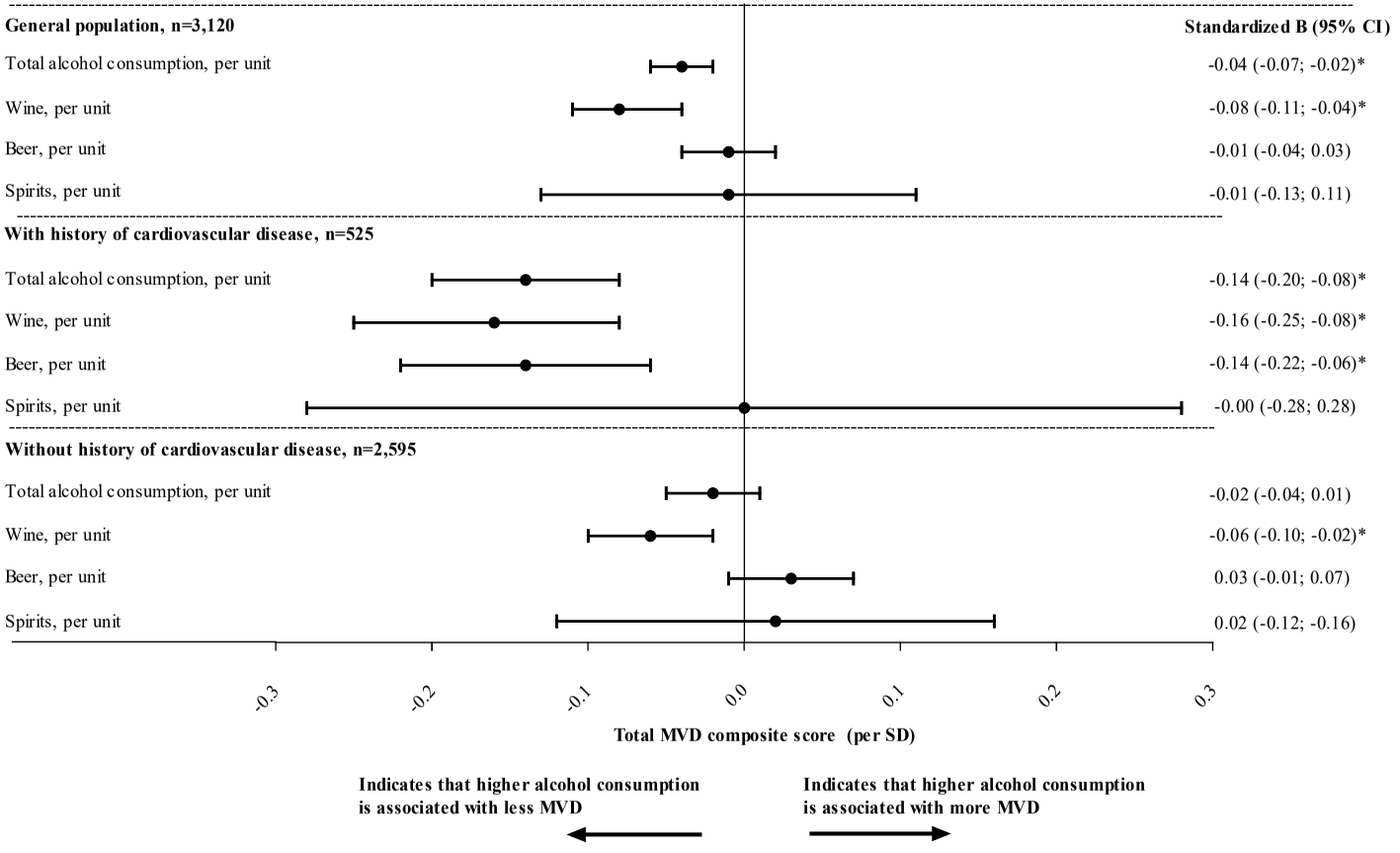

Figure S4.2 Associations of total alcohol, wine, beer, and spirits consumption with the total MVD composite score, in the general population and stratified by history of cardiovascular disease. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption, wine, beer, or spirits consumption (per unit) with the total MVD composite score (per SD) where a negative beta indicates less MVD. The numbers included in the analyses and the numerical values per SD for all endpoints are reported in legends of Table 4.2 (general population) and Supplemental Table S4 (history of cardiovascular disease strata). Variables included in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and history of cardiovascular disease (where applicable). * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Associations of total alcohol consumption (high versus light) with the total MVD composite score (in SD) in the general population and stratified by history of cardiovascular disease or cardiovascular risk factor status (model 3B)

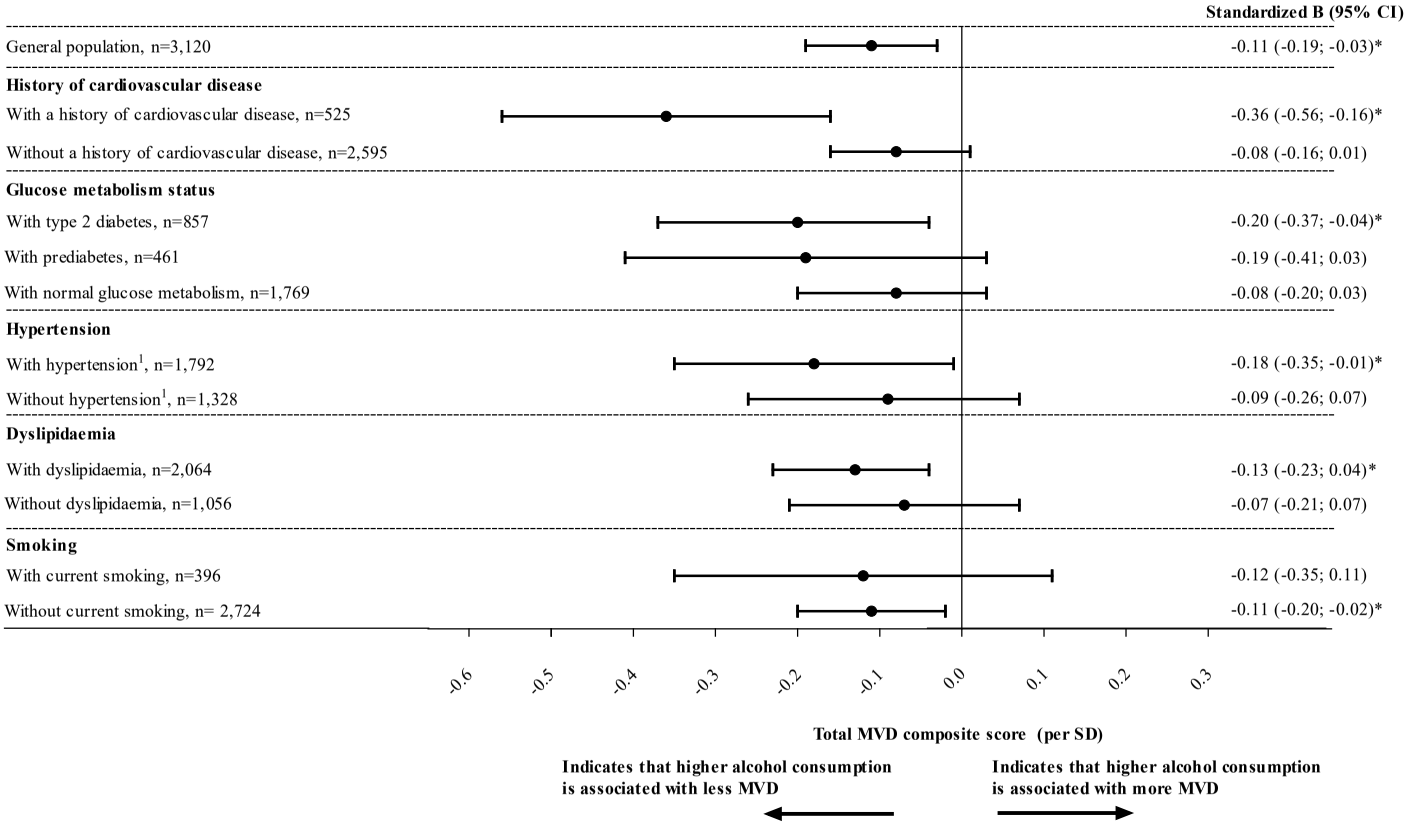

Figure S4.3 Associations of total alcohol consumption with the total MVD composite score, in the general population and stratified by cardiovascular risk factor status. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (high versus light) with the total MVD composite score (per SD) where a negative beta indicates less MVD. The numbers included in the analyses and the numerical values per SD for all endpoints are reported in the legends of Table 4.2 (general population) and Supplemental Table S6 (cardiovascular risk factors). Variables included in model 3B are age, sex, glucose metabolism status (where applicable), education level, waist circumference, smoking status (where applicable), diet score, office systolic blood pressure, use of antihypertensive medication (where applicable), total cholesterol / HDL cholesterol ratio, lipid-modifying medication (where applicable), and history of cardiovascular disease. * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Associations of wine, beer, and spirits consumption (per unit) with the total MVD composite score (in SD) stratified by cardiovascular risk factor status (model 3B)

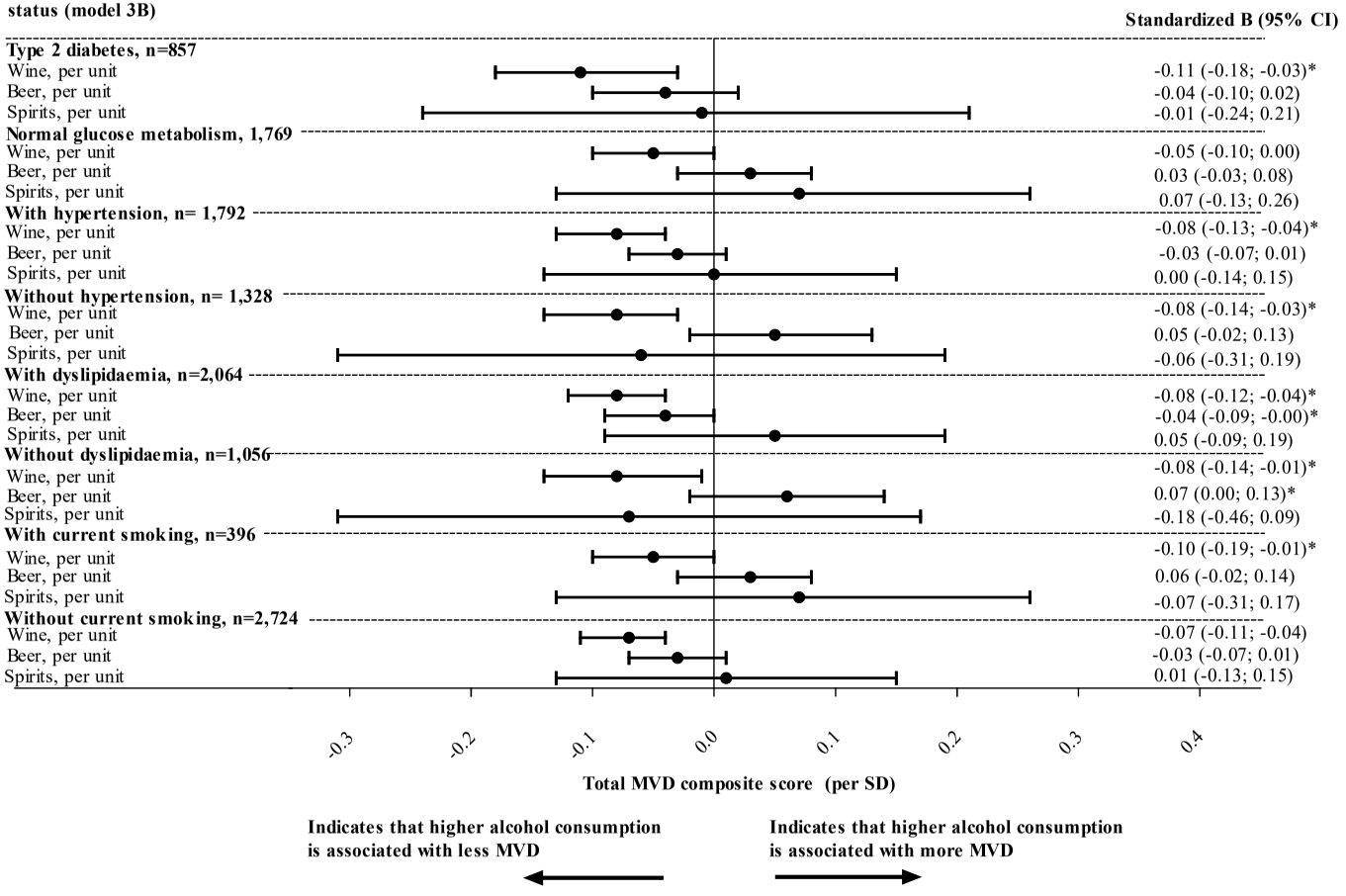

Figure S4.4 Associations of wine, beer, and spirits consumption with the total MVD composite score, stratified by cardiovascular risk factor status. Betas and 95% confidence intervals indicate the strength of the associations wine, beer, or spirits consumption (per unit) with the total MVD composite score (per SD) where a negative beta indicates less MVD. The numbers included in the analyses and the numerical values per SD for all endpoints are reported in the legends of Table 4.2 (general population) and Supplemental Table S6 (cardiovascular risk factors). Variables included in model 3B are age, sex, glucose metabolism status (where applicable), education level, waist circumference, smoking status (where applicable), diet score, office systolic blood pressure, use of antihypertensive medication (where applicable), total cholesterol / HDL cholesterol ratio, lipid-modifying medication (where applicable), and history of cardiovascular disease. * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Associations of total alcohol consumption (high versus light) with the total MVD composite score (in SD) with up to four cardiovascular risk factors or with a history of cardiovascular disease (model 3B)

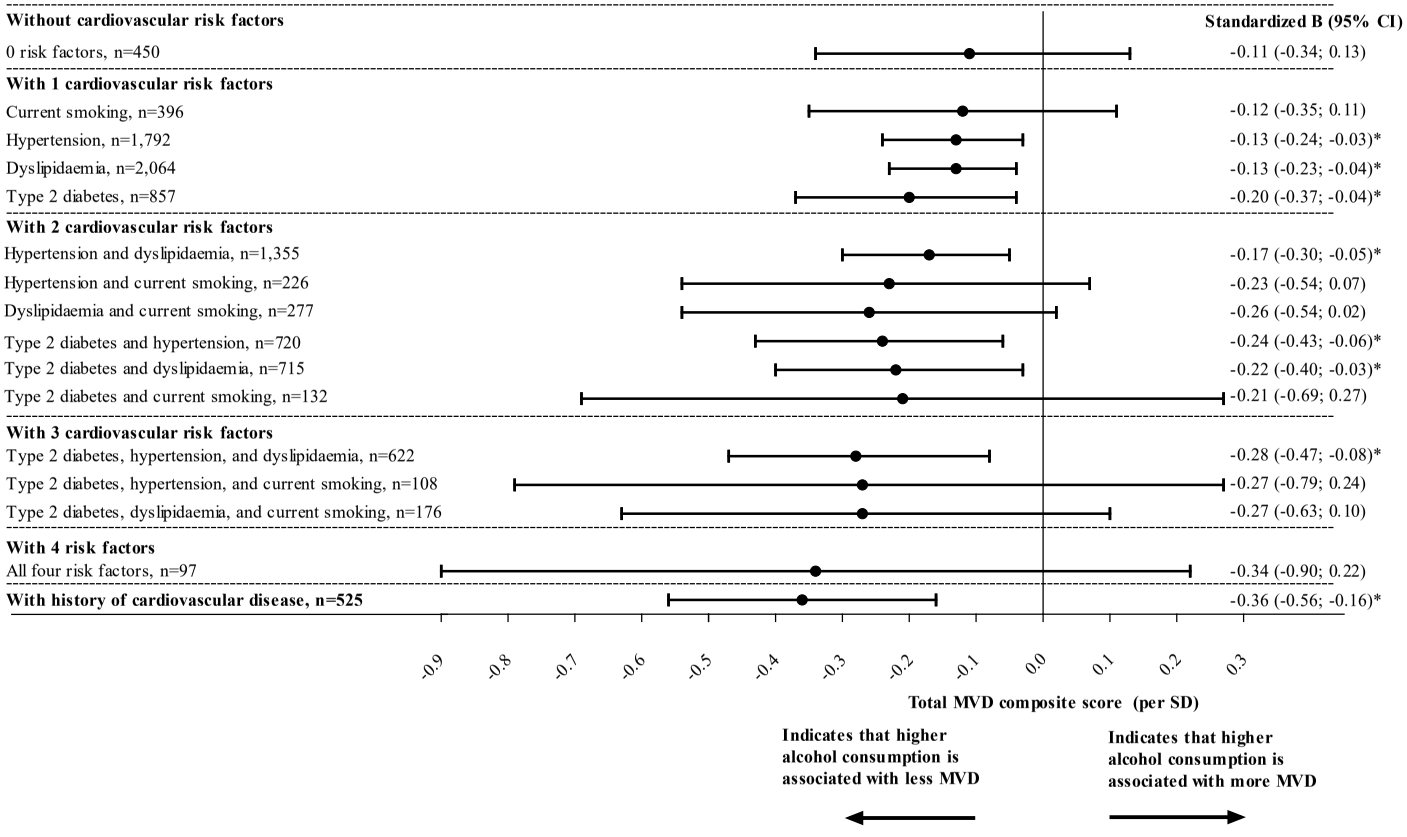

Figure S4.5 Associations of total alcohol consumption with the total MVD composite score, stratified by number of cardiovascular risks or history of cardiovascular disease (model 3B). Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (high versus light) with the total MVD composite score (per SD) where a negative beta indicates less MVD. The numbers included in the analyses and the numerical values per SD for all endpoints are reported in legends of Supplemental Table S4.4 (history of cardiovascular disease) and Supplemental Table S6 (cardiovascular risk factors). Variables included in model 3B are age, sex, glucose metabolism status (where applicable), education level, waist circumference, smoking status (where applicable), diet score, office systolic blood pressure, use of antihypertensive medication (where applicable), total cholesterol / HDL cholesterol ratio, lipid-modifying medication (where applicable), and history of cardiovascular disease (where applicable). * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Total alcohol consumption and the total MVD composite score, in individuals with normal glucose metabolism and without hypertension, without dyslipidaemia, and without current smoking

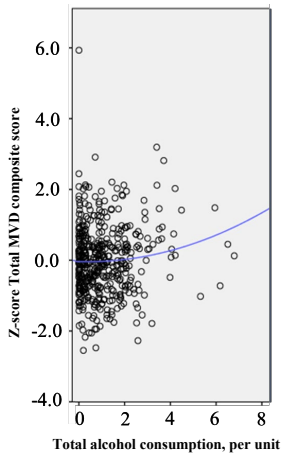

Figure S4.6.1

Total alcohol consumption and the total MVD composite score, stratified by glucose metabolism status

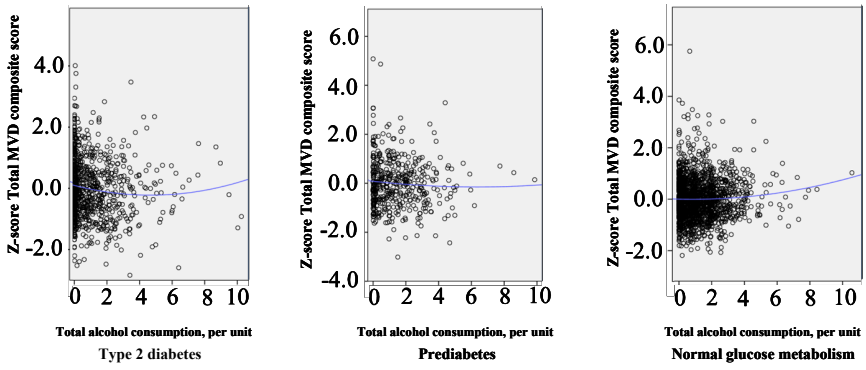

Figure S4.6.2

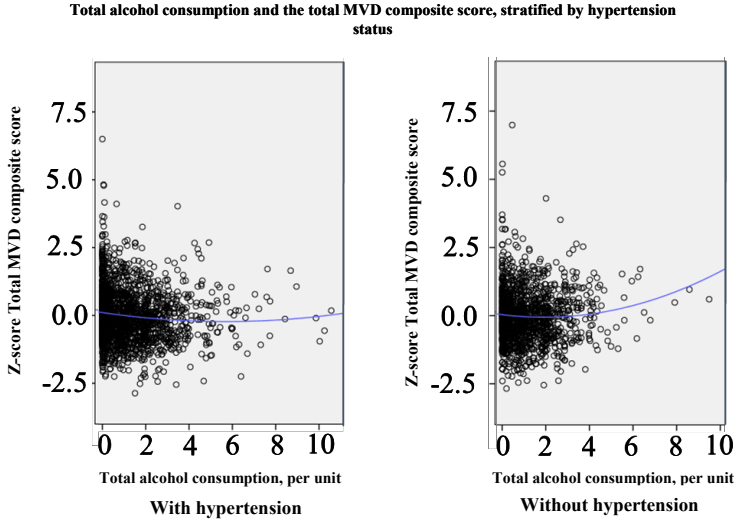

Figure S4.6.3

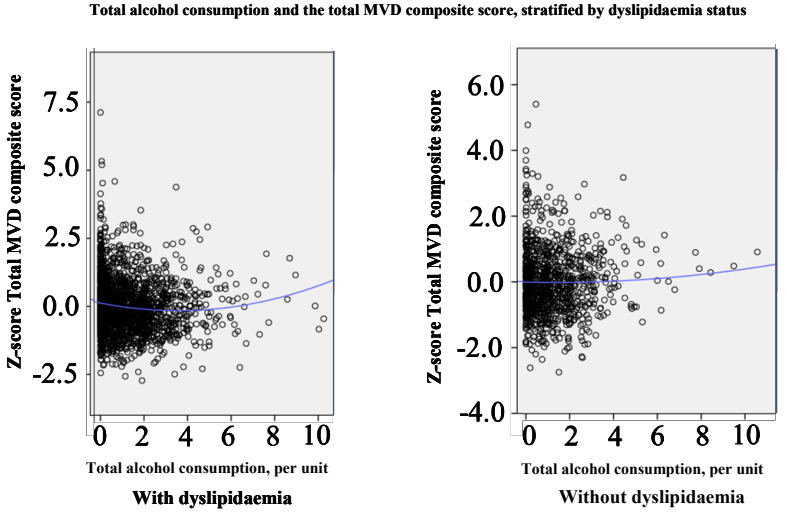

Figure S4.6.4

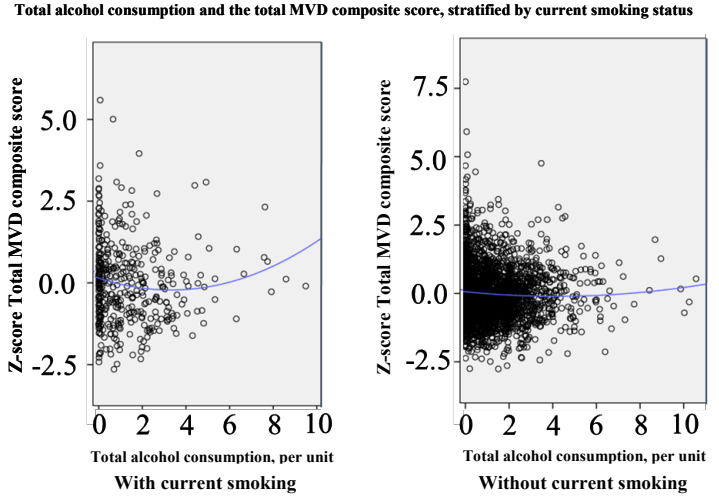

Figure 4.6.5

Figure S4.6 shows Scatter plots with data points for total alcohol consumption (x-axis; per unit) and the total MVD composite score (y-axis; per SD) where a quadratic association was modelled (blue line). Supplemental Figure S4.6 shows the scatter plot in individuals without any risk factors (n=450; S4.6.1), or stratified by glucose metabolism status (S4.6.2), hypertension status (S4.6.3), dyslipidaemia status (S4.6.4), or current smoking status (S4.6.5). The number of participants per stratum is reported in the legend of Supplemental Table S4.6. Abbreviations: MVD, microvascular dysfunction; SD, standard deviation.

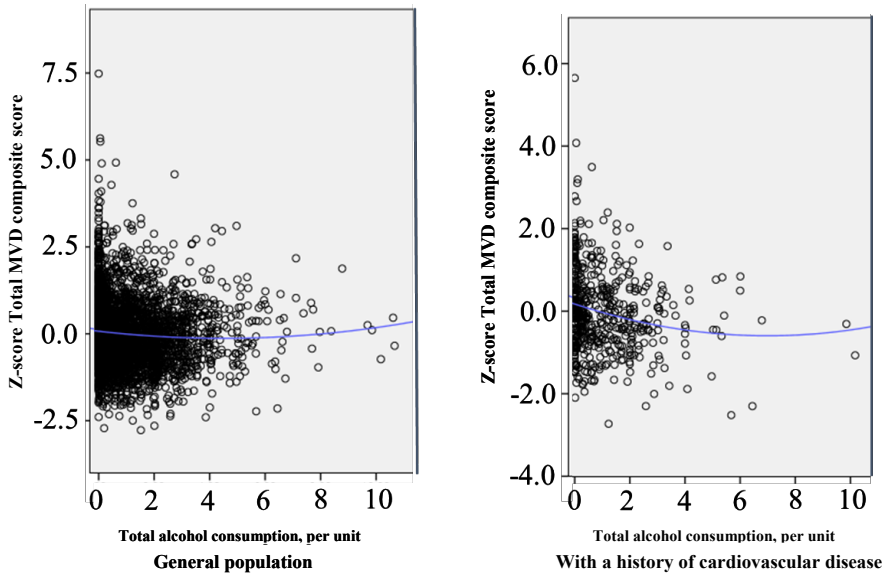

Figure S4.7.1 Total alcohol consumption without spirits (i.e. only wine and beer).

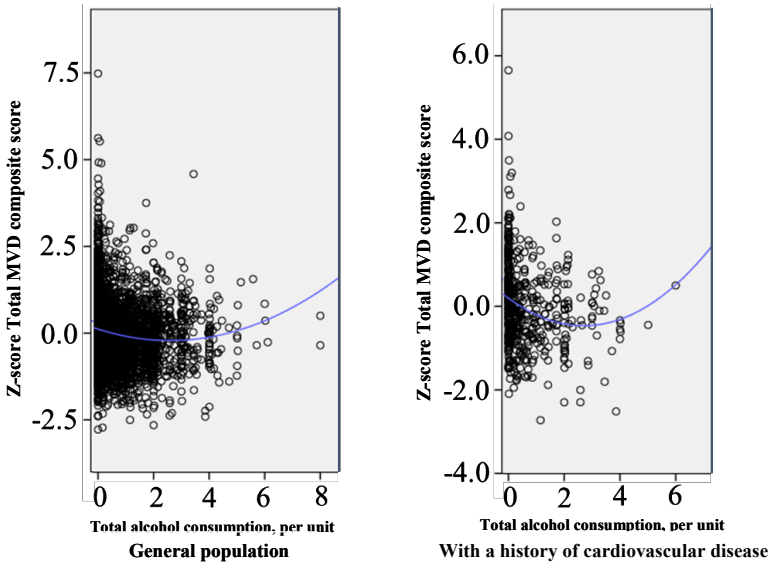

Figure S4.7.2 Total alcohol consumption without beer (i.e. only wine and spirits).

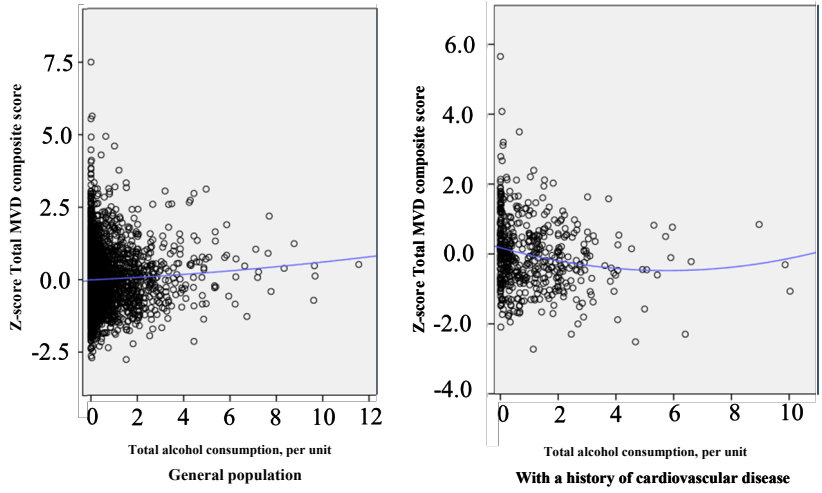

Figure S4.7.3 Total alcohol consumption without wine (i.e. only beer and spirits).

Figure S4.7 Scatter plots show data points for total alcohol consumption (x-axis; per unit) and the total MVD composite score (y-axis; per SD) where a quadratic association was modelled (blue line). Figure S7.7 shows the scatter plots in the general population and in individuals with a history of cardiovascular disease for total alcohol consumption without spirits (S4.7.1), without beer (S4.7.2), and without wine (S4.7.3). The number of participants per stratum are reported in the legend of Table 4.2 (general population) and Supplemental Table S4 (history of cardiovascular disease strata). Abbreviations: MVD, microvascular dysfunction; SD, standard deviation.

Associations of total alcohol consumption (per unit) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD) (model 3B)

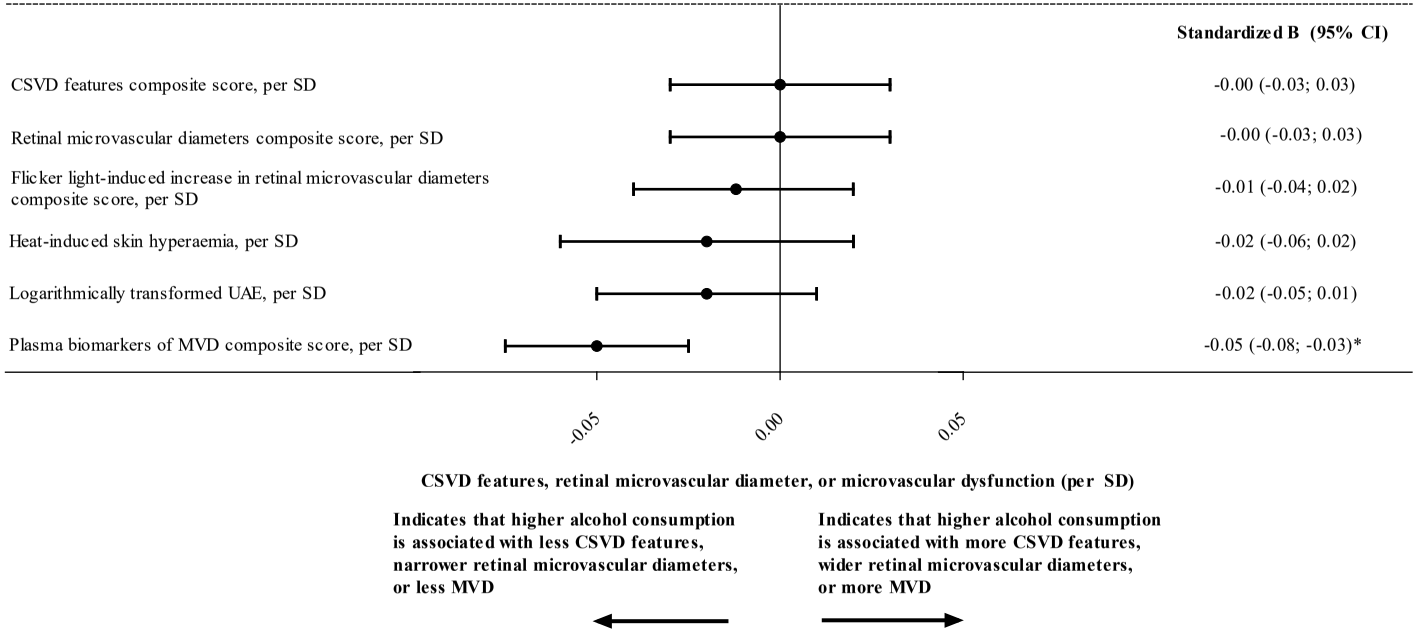

Figure S4.8 Associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table 4.2. Variables included in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and history of cardiovascular disease. Additionally, for associations with heat-induced skin hyperaemia baseline skin blood flow was entered in the model. For UAE, the beta per unit of total alcohol consumption corresponds with an odds ratio of 0.98 (95% CI, 0.96; 1.01) for 30mg/24-hour greater urinary albumin excretion. * indicates P-value<0.05. Abbreviations: B, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Associations of total alcohol consumption (high versus light) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD), stratified by history of cardiovascular disease (model 3B)

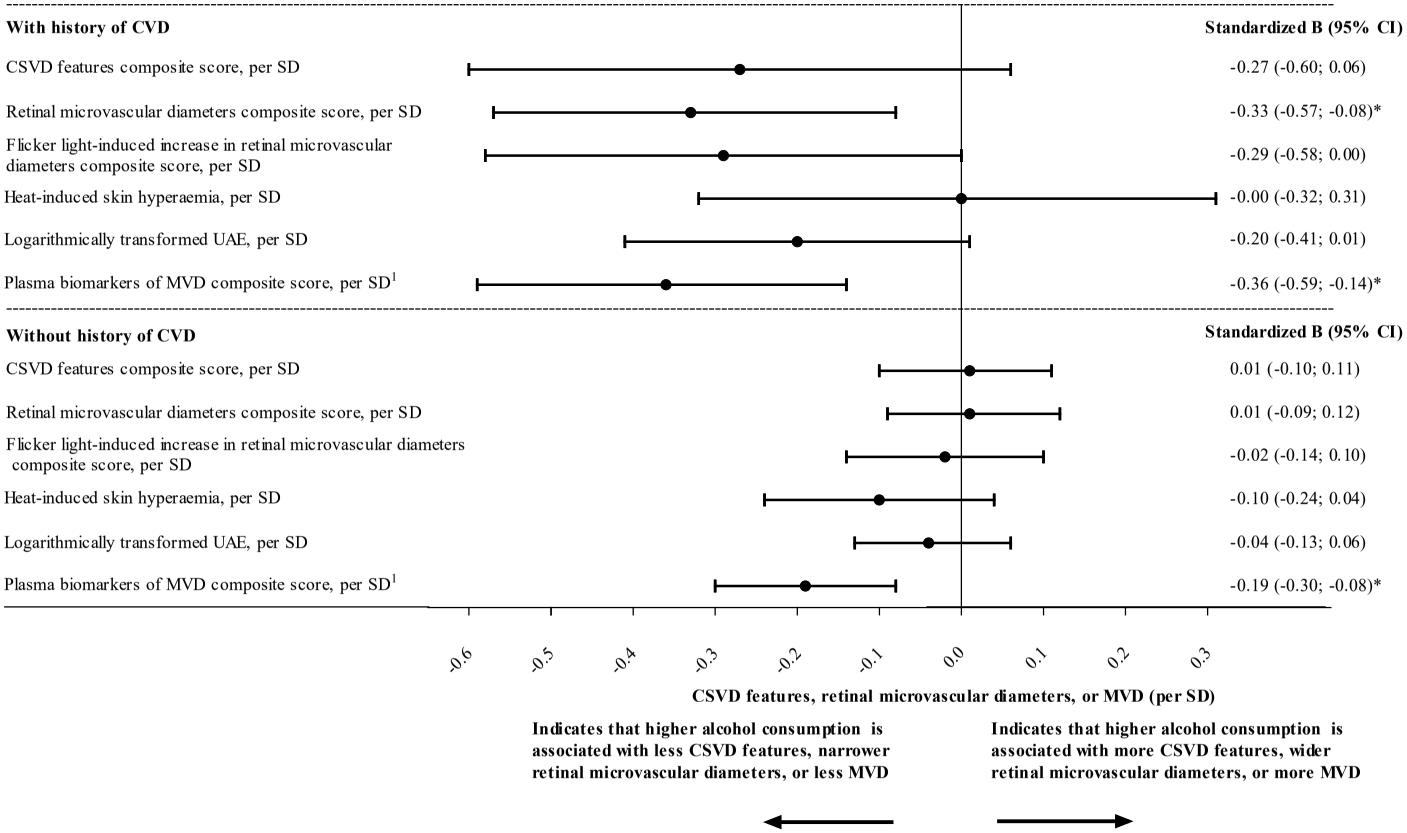

Figure S4.9 History of cardiovascular disease-stratified associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table S4.4. Variables included in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, and lipid-modifying medication. Additionally, for associations with heat-induced skin hyperaemia baseline skin blood flow was entered in the model. [1] For the associations with plasma biomarkers of MVD composite score, the association for light versus none total alcohol consumption is presented instead of the association for high versus light total alcohol consumption. For UAE, the beta of high versus light total alcohol consumption corresponds with an odds ratio of 0.79 (95% CI, 0.62; 1.01) and 0.97 (95% CI, 0.89; 1.05) for 30mg/24-hour greater urinary albumin excretion for individuals with and without a history of cardiovascular disease. * indicates P-value<0.05. Abbreviations: B, beta; CI, confidence interval; SD: standard deviation; UAE, urinary albumin excretion; MVD, microvascular dysfunction.

Associations of total alcohol consumption (high versus light) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD), stratified by glucose metabolism status (model 3B)

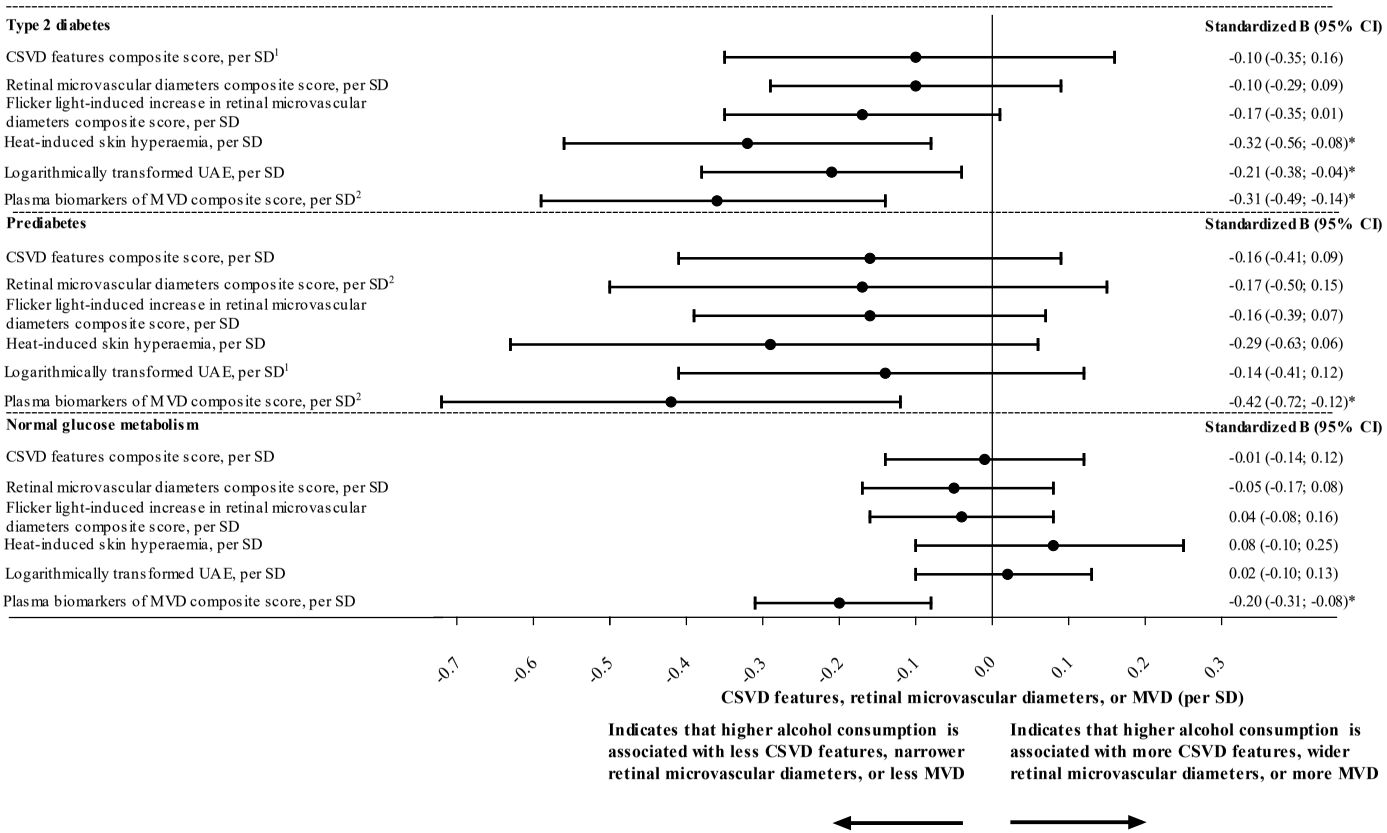

Figure S4.10 Glucose metabolism status-stratified associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table S4.6. Variables entered in model 3B are age, sex, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and prior cardiovascular disease. Additionally, for associations with heat-induced skin hyperaemia baseline skin blood flow was entered in the model. [1] indicates that the association for moderate versus light total alcohol consumption is presented instead of the association for high versus light total alcohol consumption; [2] indicates that the association for light versus none total alcohol consumption is presented instead of the association for high versus light total alcohol consumption. For UAE, the beta for total alcohol consumption (high versus light) corresponds with an odds ratio of 0.79 (95% CI, 0.65; 0.96) for 30mg/24-hour greater urinary albumin excretion in individuals with type 2 diabetes, 0.88 (95% CI, 0.69; 1.12) for 30mg/24-hour greater urinary albumin excretion in individuals with prediabetes (for moderate versus light instead of high versus light), and 1.01 (95% CI, 0.92; 1.12) for 30mg/24-hour greater urinary albumin excretion in individuals with normal glucose metabolism (for high versus light). * indicates P-value<0.05. Abbreviations: B, beta; CI, confidence interval; SD: standard deviation; UAE, urinary albumin excretion; MVD, microvascular

Associations of total alcohol consumption (high versus light) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD), stratified by hypertension status (model 3B)

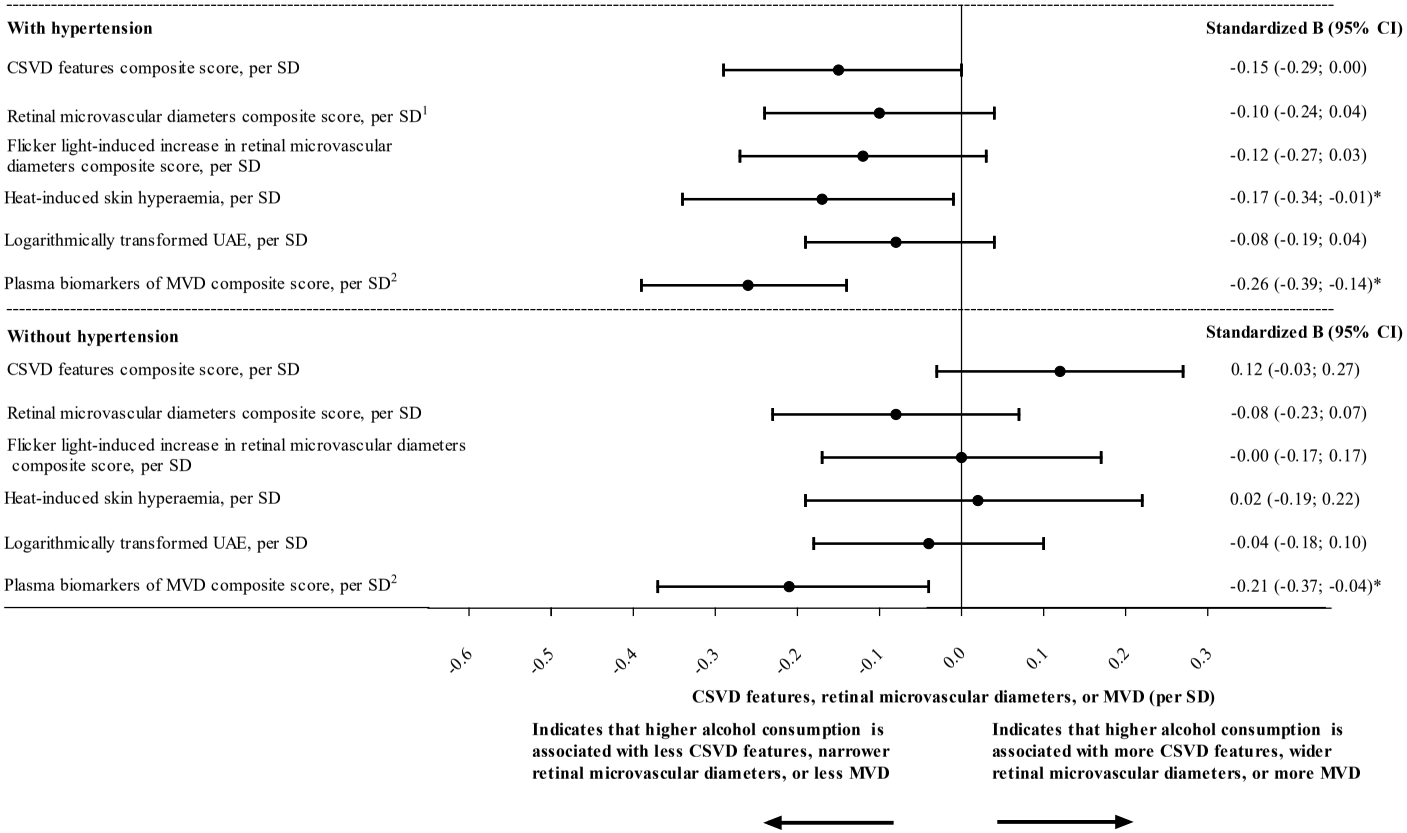

Figure S4.11 Hypertension status-stratified associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table S4.6. Variables entered in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication (where applicable), total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and history of cardiovascular disease. Additionally, for associations with heat-induced skin hyperaemia baseline skin blood flow was entered in the model. [1] indicates that the association for moderate versus light alcohol consumption is presented instead of the association for high versus light alcohol consumption; [2] indicates that the association for light versus none alcohol consumption is presented instead of the association for high versus light alcohol consumption. For UAE, the beta for high versus light total alcohol consumption corresponds with an odds ratio of 0.92 (95% CI, 0.81; 1.04) and the beta for moderate versus light total alcohol consumption corresponds with an odds ratio of 0.92 (95% CI, 0.81; 1.02) for 30mg/24-hour greater urinary albumin excretion for individuals with and without hypertension. Bold denotes P-value<0.05. Abbreviations: B, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE,

Associations of total alcohol consumption (high versus light) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD), stratified by dyslipidemia status (model 3B)

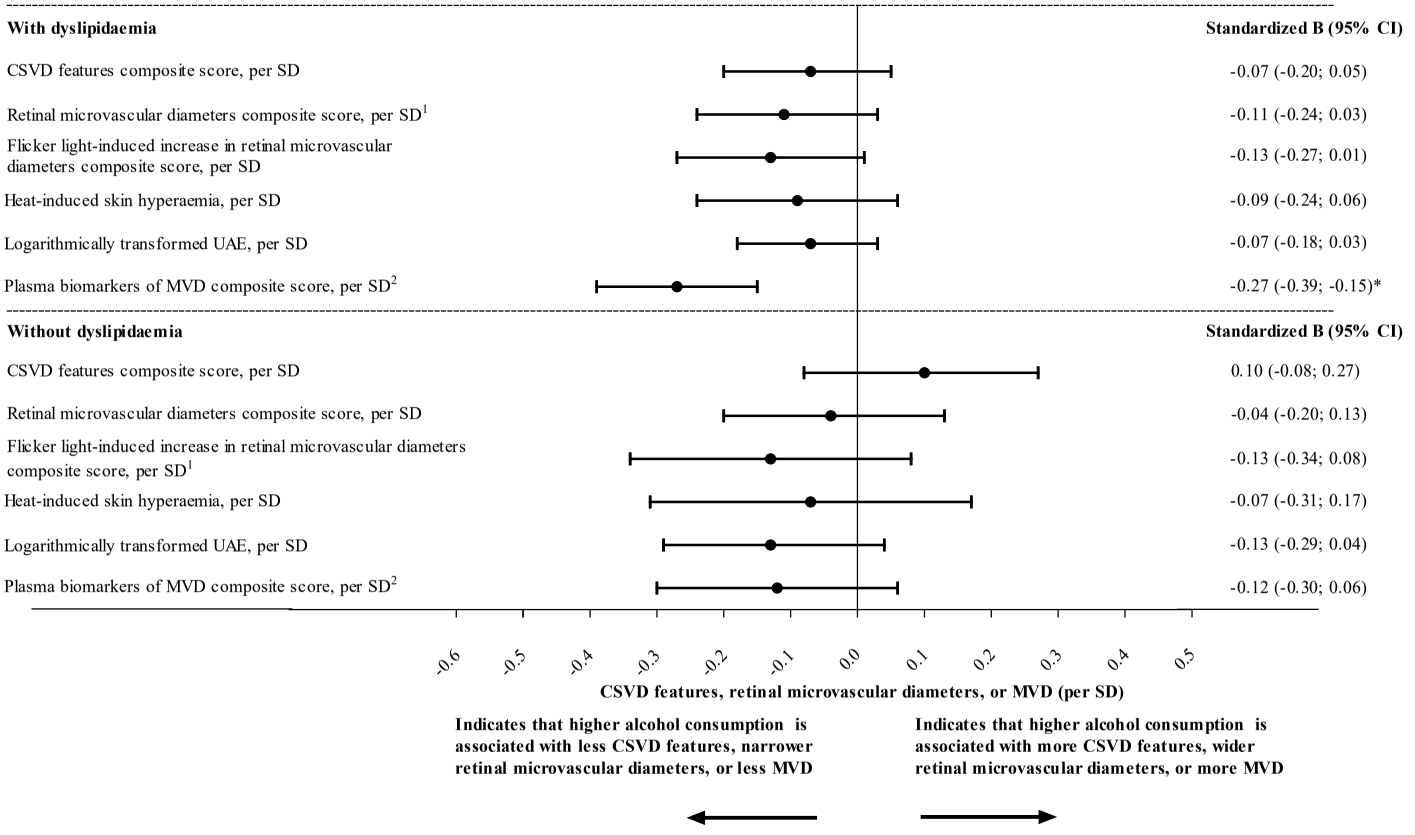

Figure S4.12 Dyslipidaemia status-stratified associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table S4.6. Variables entered in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status (former versus never smoker; only applicable in the analyses in individuals without [current] smoking), diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication (where applicable), and history of cardiovascular disease. [1] indicates that the association for moderate versus light alcohol consumption is presented instead of the association for high versus light alcohol consumption; [2] indicates that the association for light versus none alcohol consumption is presented instead of the association for high versus light alcohol consumption. For UAE, the beta of total alcohol consumption (for high versus light) corresponds with an odds ratio of 0.93 (95% CI, 0.84; 1.03) and the beta of total alcohol consumption (for moderate versus light) 0.89 (95% CI, 0.77; 1.04) for 30mg/24-hour greater urinary albumin excretion for individuals with and without dyslipidaemia. Bold denotes P-value<0.05. Abbreviations: B, beta; CI, confidence interval; SD: standard deviation; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

Associations of total alcohol consumption (high versus light) with CSVD features, retinal microvascular diameters, and measures of MVD (in SD), stratified by current smoking status (model 3B)

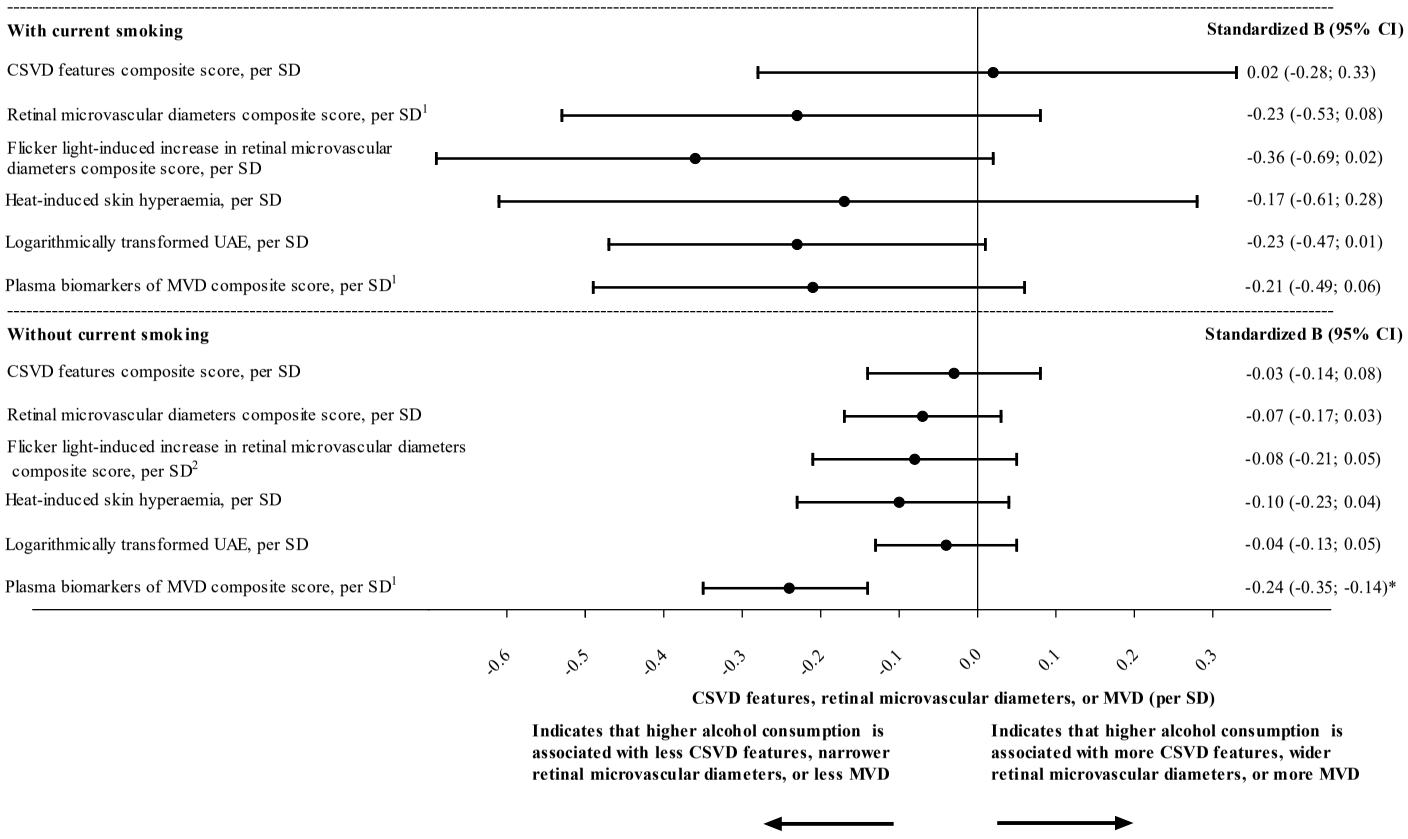

Figure S4.13 Current smoking status-stratified associations of total alcohol consumption with CSVD features, retinal microvascular diameters and MVD measures, where MVD was estimated from flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD. Betas and 95% confidence intervals indicate the strength of the associations of total alcohol consumption (per unit) with measures of MVD (per SD) where a negative beta indicates less MVD. The number of participants in analyses and the numerical values per SD for all endpoints are reported in the legend of Table S4.6. Variables entered in model 3B are age, sex, glucose metabolism status, education level, waist circumference, smoking status (former versus never smoker; only applicable in the analyses in individuals without current smoking), diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and history of cardiovascular disease. [1] indicates that the association for light versus none alcohol consumption is presented instead of the association for high versus light alcohol consumption; [2] indicates that the association for moderate versus light alcohol consumption is presented instead of the association for high versus light alcohol consumption. Bold denotes P-value<0.05. For UAE, the beta of total alcohol consumption (for moderate versus light) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE of 0.74 (0.55; 1.01) in individuals with current smoking and 0.95 (0.86; 1.05) in individuals without current smoking. Abbreviations: B, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

Table S4.1 Characteristics of the study population and individuals excluded from the analyses due to missing values for the CSVD features, retinal microvascular diameters, and flicker light-induced increase in retinal microvascular diameters study populations.

	CSVD features			Retinal microvascular diameters			Flicker light-induced increase in retinal microvascular diameters		
	Complete (n=2,075)	Number of participants with missing data in complete / missing	Missing (n=1,376)	Complete (n=2,721)	Number of participants with missing data in complete / missing	Missing (n=730)	Complete (n=2,090)	Number of participants with missing data in complete / missing	Missing (n=1,361)
Demographics									
Age, years	59.4 ± 8.1	0/0	60.4 ± 8.4	59.8 ± 8.2	0/0	59.5 ± 8.7	59.6 ± 8.2	0/0	60.0 ± 8.4
Men	1,051 (50.7)	0/0	724 (52.6)	1,383 (50.8)	0/0	392 (53.7)	1,043 (49.9)	0/0	732 (53.8)
Lifestyle factors									
Smoking status:		0/50			0/50			0/50	
Never	780 (37.6)		392 (29.6)	957 (35.2)		215 (31.6)	744 (35.6)		428 (32.6)
Former	1,059 (51.0)		700 (52.8)	1,431 (52.6)		328 (48.2)	1,097 (52.5)		662 (50.5)
Current	236 (11.4)		234 (17.6)	333 (12.2)		137 (20.1)	249 (11.9)		221 (16.9)
Body mass index, kg/m^2	26.5 ± 4.1	0/3	27.9 ± 5.0	27.0 ± 4.5	1/2	27.4 ± 4.8	26.9 ± 4.5	1/2	27.4 ± 4.7
Waist circumference, cm	94.2 ± 12.6	0/4	98.6 ± 15.0	95.7 ± 13.7	0/4	96.8 ± 14.1	95.3 ± 13.7	0/4	97.0 ± 14.0
Physical activity, hours/day	2.1 ± 0.7	461/345	1.9 ± 0.7	2.0 ± 0.7	508/298	2.0 ± 0.7	2.0 ± 0.7	300/506	1.9 ± 0.7
Dutch Healthy diet score, points	84.0 ± 14.6	0/226	81.9 ± 14.8	83.6 ± 14.7	0/226	81.4 ± 14.9	83.8 ± 14.7	0/226	82.2 ±14.8
Cardiovascular risk factors									
Glucose metabolism status		0/0			0/0			0/0	
Normal glucose metabolism	1,274 (61.4)		650 (47.2)	1,533 (56.3)		391 (53.6)	1,197 (57.3)		727 (53.4)
Prediabetes	321 (15.5)		190 (13.8)	403 (14.8)		108 (14.8)	307 (14.7)		204 (15.0)
Type 2 diabetes	459 (22.1)		516 (37.5)	758 (27.9)		217 (29.7)	563 (26.9)		412 (30.3)
Other types of diabetes	21 (1.0)		20 (1.5)	27 (1.0)		14 (1.9)	23 (1.1)		18 (1.3)
Fasting plasma glucose, mmol/l	5.5 [5.0 – 6.2]	0/1	5.7 [5.1 – 7.1]	5.5 [5.0 – 6.5]	1/0	5.6 [5.1 – 6.7]	5.5 [5.0 – 6.5]	0/1	5.6 [5.1 – 6.7]
2-hour post load plasma glucose, mmol/l	6.0 [4.9 – 8.5]	114/178	6.8 [5.3 – 11.4]	6.3 [5.1 – 9.4]	206/86	6.3 [5.0 – 9.2]	6.3 [5.1 – 9.1]	148/144	6.3 [5.0 – 9.5]
HbA1c, %	5.6 [5.3 – 6.0]	4/9	5.8 [5.4 – 6.5]	5.6 [5.3 – 6.2]	6/7	5.8 [5.5 – 6.3]	5.6 [5.3 – 6.2]	3/10	5.8 [5.5 – 6.3]
Use of glucose-lowering medication	361 (17.4)	0/0	446 (32.4)	624 (22.9)	0/0	183 (25.1)	463 (22.2)	0/0	344 (25.3)
Total/HDL cholesterol ratio	3.6 ± 1.2	0/4	3.6 ± 4.6	3.6 ± 1.1	0/4	3.9 ± 1.3	3.6 ± 1.1	0/4	3.8 ± 1.2
Use of lipid-modifying medication	647 (31.2)	0/0	612 (44.5)	999 (36.7)	0/0	260 (35.6)	745 (35.6)	0/0	514 (37.8)
Office systolic blood pressure, mm Hg	133.9 ± 17.3	0/2	136.8 ± 19.3	134.9 ± 18.1	0/2	135.6 ± 18.5	134.9 ± 18.1	0/2	135.4 ± 18.4
Office diastolic blood pressure, mm Hg	75.9 ± 9.8	0/2	76.6 ± 10.0	76.1 ± 9.9	0/2	76.2 ± 9.7	76.2 ± 9.9	0/2	76.1 ± 9.8
Ambulatory systolic blood pressure, mm Hg	118.4 ± 11.2	224/180	120.1 ± 12.7	118.9 ± 11.5	299/105	119.9 ± 12.8	118.9 ± 11.5	230/174	119.3 ± 12.3
Ambulatory diastolic blood pressure, mm Hg	73.4 ± 7.0	224/180	73.6 ± 7.4	73.4 ± 7.1	299/105	74.1 ± 7.2	73.3 ± 7.1	230/174	73.7 ± 7.2
Use of antihypertensive medication	728 (35.1)	0/0	664 (48.3)	1,105 (40.6)	0/0	287 (39.3)	804 (38.5)	0/0	588 (43.2)

Table S4.1 (continued)

	CSVD features			Retinal microvascular diameters			Flicker light-induced increase in retinal microvascular diameters		
	Complete (n=2,075)	Number of participants with missing data in complete / missing	Missing (n=1,376)	Complete (n=2,721)	Number of participants with missing data in complete / missing	Missing (n=730)	Complete (n=2,090)	Number of participants with missing data in complete / missing	Missing (n=1,361)
History of cardiovascular disease	253 (12.2)	0/67	315 (24.1)	459 (16.9)	0/67	109 (16.4)	330 (15.8)	0/67	238 (18.4)
Diabetic retinopathy	22 (1.3)	322/262	24 (2.2)	41 (1.6)	169/425	5 (1.6)	33 (1.6)	60/534	13 (1.6)
eGFR, ml/min/1.73^2	88.7 ± 14.3	14/19	87.3 ± 15.8	88.2 ± 14.8	20/13	88.0 ± 15.5	88.2 ± 14.6	12/21	88.0 ± 15.4
Plasma biomarkers of low-grade inflammation		14/25			25/14			16/23	
C-reactive protein, µg/ml	1.2 [0.6 – 2.5]		1.4 [0.7 – 3.2]	1.2 [0.6 – 2.7]		1.4 [0.6 – 3.1]	1.2 [0.6 – 2.7]		1.3 [0.6 – 3.0]
Serum amyloid A, µg/ml	3.2 [2.0 – 5.4]		3.3 [2.1 – 5.6]	3.3 [2.1 – 5.5]		3.1 [2.0 – 5.5]	3.3 [2.1 – 5.4]		3.2 [2.0 – 5.5]
Tumor necrosis factor alpha, pg/ml	2.2 [1.9 – 2.5]		2.3 [1.9 – 2.7]	2.2 [1.9 – 2.6]		2.2 [1.9 – 2.5]	2.2 [1.9 – 2.6]		2.2 [1.9 – 2.5]
Interleukin-6, pg/ml	0.6 [0.4 – 0.8]		0.7 [0.4 – 1.0]	0.6 [0.4 – 0.9]		0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]		0.6 [0.4 – 0.9]
Interleukin-8, pg/m	4.0 [3.2 – 5.1]		4.4 [3.4 – 5.7]	4.1 [3.3 – 5.3]		5.5 [4.3 – 7.4]	4.1 [3.2 – 5.3]		4.3 [3.4 – 5.5]
Other									
Educational status		0/74			0/74			0/74	
Low	630 (30.4)		505 (38.8)	910 (33.4)		225 (34.3)	676 (32.3)		459 (35.7)
Middle	606 (29.2)		348 (26.7)	777 (28.6)		177 (27.0)	601 (28.8)		353 (27.4)
High	839 (40.4)		449 (34.5)	1034 (38.0)		254 (38.7)	813 (38.9)		475 (36.9)
Occupational status		313/314			456/171			345/282	
Low	508 (22.8)		385 (36.3)	708 (31.3)		185 (33.1)	539 (30.9)		354 (32.8)
Middle	651 (36.9)		344 (32.4)	808 (35.7)		187 (33.5)	642 (36.8)		353 (32.7)
High	603 (34.2)		333 (31.4)	749 (33.1)		187 (33.5)	564 (32.3)		372 (34.5)
Income per month, euros	2,070 ± 821	452/416	1,922 ± 808	2012 ± 806	641/227	2,024 ± 873	2034 ± 804	471/397	1,982 ± 843
Alcohol consumption									
Total alcohol consumption, units/day	0.9 [0.2 – 1.9]	0/226	0.7 [0.1 – 1.7]	0.9 [0.1 – 1.9]	0/226	0.7 [0.1 – 1.7]	0.9 [0.2 – 1.9]	0/226	0.8 [0.1 – 1.8]
None	313 (15.1)		207 (18.0)	439 (16.1)		81 (16.1)	329 (15.7)		191 (16.8)
Light	627 (30.2)		387 (33.7)	833 (30.6)		181 (35.9)	640 (30.6)		374 (33.0)
Moderate	422 (20.3)		220 (19.1)	544 (20.0)		98 (19.4)	418 (20.0)		224 (19.7)
High	713 (34.4)		336 (24.4)	905 (33.3)		144 (28.6)	703 (33.6)		346 (30.5)
Total wine consumption, units/day	0.3 [0.0 – 1.2]		0.2 [0.0 – 1.0]	0.3 [0.0 – 1.2]		0.1 [0.0 – 0.9]	0.3 [0.0 – 1.2]		0.2 [0.0 – 1.0]
None	772 (37.2)		559 (48.6)	1070 (39.3)		261 (51.8)	791 (37.8)		540 (47.6)
Light	527 (25.4)		239 (20.8)	669 (24.6)		97 (19.2)	519 (24.8)		247 (21.8)
Moderate	347 (16.7)		162 (14.1)	443 (16.3)		66 (13.1)	349 (16.7)		160 (14.1)
High	429 (20.7)		190 (16.5)	539 (19.8)		80 (15.9)	431 (20.6)		188 (16.6)

Table S4.1 (continued)

	CSVD features			Retinal microvascular diameters			Flicker light-induced increase in retinal microvascular diameters		
	Complete (n=2,075)	**Number of participants with missing data in complete / missing**	**Missing (n=1,376)**	**Complete (n=2,721)**	**Number of participants with missing data in complete / missing**	**Missing (n=730)**	**Complete (n=2,090)**	**Number of participants with missing data in complete / missing**	**Missing (n=1,361)**
Total beer consumption, units/day	0.1 [0.0 – 0.5]		0.1 [0.0 – 0.5]	0.1 [0.0 – 0.5]		0.1 [0.0 – 0.6]	0.1 [0.0 – 0.4]		0.1 [0.0 – 0.6]
None	1,184 (57.1)		672 (58.4)	1,580 (58.1)		276 (54.8)	1,227 (58.7)		629 (55.4)
Light	543 (26.2)		280 (24.3)	692 (25.4)		131 (26.0)	543 (26.0)		280 (24.7)
Moderate	206 (10.0)		115 (10.0)	269 (9.9)		54 (10.7)	192 (9.2)		131 (11.5)
High	140 (6.7)		83 (7.2)	180 (6.6)		43 (8.5)	128 (6.1)		95 (8.4)
Total spirits consumption, units /day	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]
None	1,870 (90.1)		1,032 (89.7)	2,443 (89.8)		459 (91.1)	1,877 (89.8)		1,025 (90.3)
Light	173 (8.3)		93 (8.1)	231 (8.5)		35 (6.9)	175 (8.4)		91 (8.0)
Moderate	25 (1.2)		20 (1.7)	35 (1.3)		10 (2.0)	28 (1.3)		17 (1.5)
High	7 (0.3)		5 (0.4)	12 (0.4)		0 (0.0)	10 (0.5)		2 (0.2)
Endpoints									
CSVD features		0/1194			-			-	
White matter hyperintensity volume, ml	0.0 [0.0 – 0.01]		0.0 [0.0 – 0.01]	-		-	-		-
Participants with cerebral microbleeds	245 (11.8)		26 (14.3)	-		-	-		-
Number of cerebral microbleeds	0.0 [0.0-0.0]		0.0 [0.0-0.0]	-		-	-		-
Number of participants with lacunar infarcts	114 (5.5)		10 (4.4)						
Number of lacunar infarcts	0.0 [0.0-0.0]		0.0 [0.0-0.0]						
Retinal microvascular diameters					0/493			-	
Arteriolar diameter, µm	-	-	-	142.3 ± 20.2		140.7 ± 122.9	-		-
Venular diameter, µm	-		-	214.6 ± 31.4		214.0 ± 31.9	-		-
Flicker light-induced increase in retinal microvascular diameters		-			-		-	0/1161	-
Arteriolar, µm	-		-	-		-	4.4 ± 3.6		3.8 ± 3.5
Venular, µm	-		-	-		-	7.6 ± 4.1		7.5 ± 3.9

Data are presented as mean ± standard deviation, median [interquartile range] or n(%). Abbreviations: CSVD, cerebral small vessel disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; SD, standard deviation; MVD, microvascular dysfunction.

Table S4.2 Characteristics of the study population and individuals excluded from the analyses due to missing values for the heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD study populations.

	Heat-induced skin hyperaemia			UAE			Plasma biomarkers of MVD		
	Complete (n=1,517)	Number of participants with missing data in complete / missing	Missing (n=1,934)	Complete (n=3,107)	Number of participants with missing data in complete / missing	Missing (n=344)	Complete (n=3,078)	Number of participants with missing data in complete / missing	Missing (n=373)
Demographics									
Age, years	60.2 ± 8.1	0/0	59.4 ± 8.4	59.9 ± 8.2	0/0	58.5 ± 8.9	59.9 ± 8.2	0/0	58.8 ± 9.0
Men	786 (51.8)	0/0	989 (51.1)	1,582 (50.9)	0/0	193 (56.1)	1,577 (51.2)	0/0	198 (53.1)
Lifestyle factors									
Smoking status:		0/50			0/50			0/50	
Never	500 (33.0)		672 (35.7)	1,088 (35.0)		84 (28.6)	1,075 (34.9)		97 (30.0)
Former	841 (55.4)		918 (48.7)	1,624 (52.3)		135 (45.9)	1,612 (52.4)		147 (45.5)
Current	176 (11.6)		294 (15.6)	395 (12.7)		75 (25.5)	391 (12.7)		79 (24.5)
Body mass index, kg/m^2	27.0 ± 4.4	1/2	27.1 ± 4.6	27.0 ± 4.5	1/2	27.6 ± 5.1	27.1 ± 4.5	1/2	27.4 ± 4.9
Waist circumference, cm	96.0 ± 13.4	0/4	95.9 ± 14.1	95.8 ± 13.7	0/4	97.6 ± 14.7	95.8 ± 13.7	0/4	97.0 ± 14.2
Physical activity, hours/day	2.0 ± 0.7	250/556	2.0 ± 0.7	2.0 ± 0.7	699/107	2.0 ± 0.7	2.0 ± 0.7	686/120	1.9 ± 0.7
Dutch Healthy diet score, points	83.1 ± 14.6	0/226	83.4 ± 14.8	83.2 ± 14.7	0/226	83.6 ± 14.8	83.2 ± 14.7	0/226	83.7 ± 14.2
Cardiovascular risk factors									
Glucose metabolism status		0/0			0/0			0/0	
Normal glucose metabolism	825 (54.4)		1,099 (56.8)	1,760 (56.6)		164 (47.7)	1,742 (56.6)		182 (48.8)
Prediabetes	230 (15.2)		281 (14.5)	460 (14.8)		51 (14.8)	455 (14.8)		56 (15.0)
Type 2 diabetes	439 (28.9)		536 (27.7)	854 (27.5)		121 (35.2)	849 (27.6)		126 (33.8)
Other types of diabetes	23 (1.5)		18 (0.9)	33 (1.1)		8 (2.3)	32 (1.0)		9 (2.4)
Fasting plasma glucose, mmol/l	5.6 [5.1 – 6.6]	0/1	5.6 [5.1 – 6.5]	5.5 [5.1 – 6.5]	1/0	5.7 [5.1 – 7.1]	5.5 [5.1 – 6.5]	1/0	5.6 [5.0 – 7.0]
2- hour post load plasma glucose, mmol/l	6.3 [5.1 – 9.8]	122/170	6.3 [5.1 – 9.0]	9.3 [6.3 – 9.3]	239/53	6.4 [5.0 – 9.6]	6.3 [5.1 – 9.2]	240/52	6.5 [5.0 – 9.8]
HbA1c, %	5.7 [5.4 – 6.3]	1/12	5.6 [5.3 – 6.2]	5.7 [5.4 – 6.2]	7/6	5.8 [5.5 – 6.5]	5.7 [5.4 – 6.2]	8/5	5.8 [5.5 – 6.4]
Use of glucose-lowering medication	366 (24.1)	0/0	441 (22.8)	697 (22.4)	0/0	110 (32.0)	694 (22.5)	0/0	113 (30.3)
Total/HDL cholesterol ratio	3.7 ± 1.1	0/4	3.7 ± 1.2	3.7 ± 1.2	0/4	3.8 ± 1.2	3.7 ± 1.2	0/4	3.8 ± 1.2
Use of lipid-modifying medication	592 (39.0)	0/0	667 (34.5)	1,137 (36.6)	0/0	122 (35.5)	1,128 (36.6)	0/0	131 (35.1)
Office systolic blood pressure, mm Hg	135.8 ± 18.3	0/2	134.5 ± 18.1	135.0 ± 18.3	0/2	135.3 ± 17.6	135.0 ± 18.2	0/2	135.5 ± 18.0
Office diastolic blood pressure, mm Hg	76.5 ± 9.6	0/2	75.9 ± 10.0	76.1 ± 9.9	0/2	76.3 ± 9.2	76.1 ± 9.9	0/2	76.1 ± 9.0
Ambulatory systolic blood pressure, mm Hg	119.5 ± 11.4	183/2	118.7 ± 12.2	118.9 ± 11.7	349/55	120.9 ± 13.2	119.0 ± 11.7	348/56	120.0 ± 12.7
Ambulatory diastolic blood pressure, mm Hg	73.5 ± 6.9	183/2	73.5 ± 7.4	73.4 ± 7.1	349/55	74.6 ± 7.2	73.4 ± 7.2	348/56	74.2 ± 7.2

Table S4.2 (continued)

	Heat-induced skin hyperaemia			UAE			Plasma biomarkers of MVD		
	Complete (n=1,517)	**Number of participants with missing data in complete / missing**	**Missing (n=1,934)**	**Complete (n=3,107)**	**Number of participants with missing data in complete / missing**	**Missing (n=344)**	**Complete (n=3,078)**	**Number of participants with missing data in complete / missing**	**Missing (n=373)**
Use of antihypertensive medication	638 (42.1)	0/0	754 (39.0)	1,252 (40.3)	0/0	140 (40.7)	1,246 (40.5)	0/0	146 (39.1)
History of cardiovascular disease	265 (17.5)	0/67	303 (16.2)	522 (16.8)	0/67	46 (16.6)	519 (16.9)	0/67	49 (16.0)
Diabetic retinopathy	22 (1.6)	144/450	24 (1.6)	41 (1.6)	497/97	5 (2.0)	42 (1.6)	488/106	4 (1.5)
eGFR, ml/min/1.73^2	88.1 ± 14.6	10/23	88.1 ± 15.2	88.0 ± 14.9	25/8	89.5 ± 15.3	88.0 ± 14.9	7/26	89.2 ± 15.3
Plasma biomarkers of low-grade inflammation		11/28			28/11			1/38	
C-reactive protein, µg/ml	1.2 [0.6 – 2.7]		1.3 [0.6 – 2.9]	1.2 [6.1 – 2.8]		1.3 [0.6 – 3.1]	1.2 [0.6 – 2.8]		1.4 [0.6 – 3.1]
Serum amyloid A, µg/ml	3.3 [2.1 – 5.5]		3.2 [2.0 – 5.5]	3.3 [2.1 – 5.4]		3.1 [2.0 – 5.5]	3.2 [2.0 – 5.5]		3.1 [2.0 – 5.4]
Tumor necrosis factor alpha, pg/ml	2.2 [1.9 – 2.6]		2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]		2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]		2.2 [1.9 – 2.6]
Interleukin-6, pg/ml	0.6 [0.4 – 0.9]		0.6 [0.4 – 0.9]	4.1 [3.3 – 5.3]		0.6 [0.4 – 1.0]	0.6 [0.4 – 0.9]		0.6 [0.4 – 1.0]
Interleukin-8, pg/ml	4.1 [3.3 – 5.3]		4.2 [3.3 – 5.4]	4.1 [3.3 – 5.3]		4.3 [3.3 – 5.6]	4.1 [3.3 – 5.3]		4.3 [3.3 – 5.5]
Other									
Educational status		0/74			0/74			0/74	
Low	504 (33.2)		631 (33.9)	1041 (33.5)		94 (34.8)	1027 (33.4)		108 (36.1)
Middle	428 (28.2)		526 (28.3)	877 (28.2)		77 (28.5)	867 (28.2)		87 (29.1)
High	585 (38.6)		703 (37.8)	1189 (38.3)		99 (36.7)	1184 (38.5)		104 (34.8)
Occupational status		250/377			524/103			519/108	
Low	412 (32.5)		481 (30.9)	801 (31.0)		92 (38.2)	797 (31.1)		96 (36.2)
Middle	432 (34.1)		563 (36.2)	922 (35.7)		73 (30.3)	908 (35.5)		87 (32.8)
High	423 (33.4)		513 (32.9)	860 (33.3)		76 (31.5)	854 (33.4)		82 (30.9)
Income per month, euros	2,029 ± 813	353/515	2,002 ± 824	2,028 ± 818	739/129	1,871 ± 819	2,028 ± 821	734/134	1,886 ± 787
Alcohol consumption									
Total alcohol consumption, units/day	0.9 [0.1 – 1.9]	0/226	0.8 [0.1 – 1.8]	0.9 [0.1 – 1.9]	0/226	0.4 [0.0 – 1.3]	0.9 [0.1 – 1.9]	0/226	0.5 [0.1 – 1.4]
None	239 (15.8)		281 (16.5)	498 (16.0)		22 (18.6)	498 (16.2)		22 (15.0)
Light	459 (30.3)		555 (32.5)	964 (31.0)		50 (42.4)	951 (30.9)		63 (42.9)
Moderate	313 (20.6)		329 (19.3)	620 (20.0)		22 (18.6)	615 (20.0)		27 (18.4)
High	506 (33.4)		543 (31.8)	1025 (33.0)		24 (20.3)	1014 (32.9)		35 (23.8)

Table S4.2 (continued)

	Heat-induced skin hyperaemia			UAE			Plasma biomarkers of MVD		
	Complete (n=1,517)	**Number of participants with missing data in complete / missing**	**Missing (n=1,934)**	**Complete (n=3,107)**	**Number of participants with missing data in complete / missing**	**Missing (n=344)**	**Complete (n=3,078)**	**Number of participants with missing data in complete / missing**	**Missing (n=373)**
Total wine consumption, units/day	0.3 [0.0 – 1.2]		0.3 [0.0 – 1.1]	0.3 [0.0 – 1.1]		0.1 [0.0 – 0.6]	0.3 [0.0 – 1.1]		0.1 [0.0 – 0.6]
None	628 (41.4)		703 (41.2)	1265 (40.7)		66 (55.9)	1253 (40.7)		78 (53.1)
Light	338 (22.3)		428 (25.1)	743 (23.9)		23 (19.5)	734 (23.8)		32 (21.8)
Moderate	248 (16.3)		261 (15.3)	497 (16.0)		12 (10.2)	495 (16.1)		14 (9.5)
High	303 (20.0)		316 (18.5)	602 (19.4)		17 (14.4)	596 (19.4)		23 (15.6)
Total beer consumption, units/day	0.1 [0.0 – 0.5]		0.1 [0.0 – 0.5]	0.1 [0.0 – 0.5]		0.0 [0.0 – 0.3]	0.1 [0.0 – 0.5]		0.0 [0.0 – 0.4]
None	864 (57.0)		992 (58.1)	1775 (57.1)		81 (68.6)	1763 (57.3)		93 (63.3)
Light	390 (25.7)		433 (25.4)	802 (25.8)		21 (17.8)	793 (25.8)		30 (20.4)
Moderate	159 (10.5)		164 (9.6)	312 (10.0)		11 (9.3)	309 (10.0)		14 (9.5)
High	104 (6.9)		119 (7.0)	218 (7.0)		5 (4.2)	213 (6.9)		10 (6.8)
Total spirits consumption, units /day	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]	0.0 [0.0 – 0.0]		0.0 [0.0 – 0.0]
None	1358 (89.5)		1544 (90.4)	2789 (89.8)		113 (95.8)	2764 (89.8)		138 (93.9)
Light	133 (8.8)		133 (7.8)	262 (8.4)		4 (3.4)	260 (8.4)		6 (4.1)
Moderate	17 (1.1)		28 (1.6)	44(1.4)		1 (0.8)	42 (1.4)		3 (2.0)
High	9 (0.6)		3 (0.2)	12 (0.4)		0 (0.0)	12 (0.4)		(0.0)
Endpoints									
Heat-induced skin hyperaemia, PU	112.1 ± 57.3	0/1,775	109.9 ± 55.8	-	-	-	-	-	-
Urinary albumin excretion, mg/24 hours	-	-	-	6.7 [4.0 – 11.9]	0/42	7.2 [4.3 – 12.9]	-	-	-
≥30 mg/24h	-		-	270 (18.7)		29 (9.6)	-		-
Plasma biomarkers of MVD		-			-		-	0/40	-
sICAM-1, ng/ml	-		-	-		-	353.9 ± 99.8		365.0 ± 98.8
sVCAM-1, ng/ml	-		-	-		-	428.1 ± 101.0		433.0 ± 108.2
sE-selectin, ng/ml	-		-	-		-	117.8 ± 65.7		125.6 ± 59.6
vWF, %	-		-	-		-	132.6 ± 48.4		135.9 ± 49.3

Data are presented as mean ± standard deviation, median [interquartile range] or n(%). Abbreviations: HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; SD, standard deviation; MVD, microvascular dysfunction; PU, perfusion units; sICAM-1, soluble intercellular adhesin molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; UAE, urinary albumin excretion.

Table S4.3 P-values from likelihood ratio tests for interaction.

	Main analyses			Additional analyses					
	History of cardiovascular disease status	**Sex**	**Glucose metabolism status**	**Hypertension status**	**Smoking status**	**Dyslipidaemia status**	**Fasting plasma glucose**	**2-hour post load glucose**	**HbA1c**
	Total alcohol consumption entered as a continuous/ categorical variable(s)	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**	**Total alcohol consumption entered as a continuous/ categorical variable(s)**
Determinants	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**
Total alcohol consumption	<0.001/<0.001	0.87/0.03	0.24/0.12	<0.001/0.01	0.45/0.87	0.06/0.21	NA	NA	NA
Individual types of alcohol consumption	<0.001/<0.001	0.60/0.09	0.18/0.02	0.33/<0.001	0.24/0.045	0.046/0.19	0.31/0.07	0.46/0.08	0.69/0.01

P-values are derived from the likelihood ratio test in which the goodness in fit between the model with and without interaction term(s) was tested. A significant P-value (P<0.05) indicates that the models with and without addition of the interaction term statistically significantly differ. Total alcohol consumption was either entered as continuous variable or as a categorical variable (i.e. dummies of none, moderate, or high versus light alcohol consumption). The following variables were entered in the model in addition to total alcohol consumption or and the interaction term(s) of alcohol consumption with prior cardiovascular disease, blood pressure, glucose metabolism status, continuous measures of glucose, cholesterol, or smoking status: age, sex, glucose metabolism status), educational level, waist circumference, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, smoking status, prior cardiovascular disease, diet score, and, only for heat-induced skin hyperaemia, baseline skin blood. For blood pressure and cholesterol level we entered interaction terms with both blood pressure and antihypertensive or total cholesterol / HDL cholesterol ratio and lipid-modifying medication, respectively. Individuals with other types of diabetes than type 2 diabetes were excluded when interaction terms with glucose metabolism status or continuous measures of glycaemia were tested. Interaction by continuous measures of glycaemia was only investigated if glucose metabolism status modified an association (in these additional analyses glucose metabolism status was not entered in the model but replaced with the applicable continuous measure of glycaemia). Bold denotes P<0.05. Abbreviations: NA, not applicable; CI: confidence interval; SD: standard deviation; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein.

Table S4.4 Associations of alcohol consumption and the total microvascular dysfunction composite score, stratified by history of cardiovascular disease.

		Alcohol consumption				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
With a history of cardiovascular disease, n=525						
Total alcohol	1	**-0.12 (-0.18; -0.06)**	**0.40 (0.17; 0.62)**	-0.09 (-0.33; 0.14)	**-0.35 (-0.57; -0.13)**	**0.00**
consumption	2	**-0.15 (-0.21; -0.09)**	**0.23 (0.02; 0.45)**	**-0.23 (-0.45; -0.01)**	**-0.35 (-0.56; -0.15)**	**0.00**
	3A	**-0.15 (-0.21; -0.09)**	0.21 (-0.01; 0.42)	-0.21 (-0.43; 0.00)	**-0.35 (-0.55; -0.15)**	**0.00**
	3B	**-0.14 (-0.20; -0.08)**	0.17 (-0.05; 0.39)	**-0.22 (-0.44; -0.01)**	**-0.36 (-0.56; -0.16)**	**0.00**
Wine consumption	1	**-0.23 (-0.32; -0.14)**	**0.42 (0.22; 0.63)**	-0.25 (-0.54; 0.04)	-0.28 (-0.56; 0.01)	**0.00**
	2	**-0.18 (-0.27; -0.09)**	0.22 (0.02; 0.43)	**-0.28 (-0.54; -0.01)**	-0.22 (-0.49; 0.05)	**0.00**
	3A	**-0.17 (-0.26; -0.08)**	0.16 (-0.04; 0.36)	**-0.27 (-0.54; -0.01)**	-0.24 (-0.50; 0.03)	**0.00**
	3B	**-0.16 (-0.25; -0.08)**	0.14 (-0.06; 0.34)	**-0.29 (-0.56; -0.03)**	-0.23 (-0.49; 0.04)	**0.00**
Beer consumption	1	-0.06 (-0.15; 0.02)	-0.10 (-0.30; 0.10)	-0.19 (-0.50; 0.12)	-0.39 (-0.80; 0.01)	0.52
	2	**-0.14 (-0.22; -0.05)**	0.06 (-0.14; 0.25)	-0.23 (-0.52; 0.05)	**-0.42 (-0.79; -0.06)**	**0.02**
	3A	**-0.15 (-0.23; -0.07)**	0.06 (-0.13; 0.25)	-0.27 (-0.54; 0.01)	**-0.50 (-0.86; -0.14)**	**0.00**
	3B	**-0.14 (-0.22; -0.06)**	0.05 (-0.14; 0.24)	-0.28 (-0.55; 0.00)	-0.48 (-0.84; 0.12)	**0.01**
Spirits consumption	1	0.14 (-0.18; 0.45)	-0.11 (-0.32; 0.10)	-0.19 (-0.53; 0.15)	-0.35 (-0.99; 0.30)	0.94
	2	0.02 (-0.27; 0.31)	0.02 (-0.18; 0.22)	-0.15 (-0.46; 0.16)	-0.13 (-0.72; 0.45)	0.50
	3A	0.02 (-0.26; 0.30)	0.03 (-0.17; 0.23)	-0.20 (-0.50; 0.11)	-0.24 (-0.82; 0.34)	0.39
	3B	-0.00 (-0.28; 0.28)	0.02 (-0.17; 0.22)	-0.21 (-0.51; 0.10)	-0.24 (-0.82; 0.34)	0.32
Without a history of cardiovascular disease, n=2,595						
Total alcohol	1	0.01 (-0.02; 0.04)	0.11 (-0.01; 0.23)	**-0.15 (-0.26; -0.04)**	-0.09 (-0.18; 0.01)	**0.00**
consumption	2	-0.04 (-0.04; -0.01)	0.08 (-0.04; 0.19)	**-0.11 (-0.21; -0.01)**	-0.08 (-0.17; 0.01)	**0.00**
	3A	-0.02 (-0.05; 0.01)	0.07 (-0.05; 0.18)	-0.09 (-0.19; 0.01)	-0.09 (-0.17; 0.00)	**0.00**
	3B	-0.02 (-0.04; 0.01)	0.06 (-0.06; 0.17)	-0.09 (-0.19; 0.01)	-0.08 (-0.16; 0.01)	**0.01**
Wine consumption	1	**-0.07 (-0.11; -0.03)**	**0.16 (0.06; 0.26)**	**-0.18 (-0.30; -0.06)**	-0.07 (-0.19; 0.04)	**0.00**
	2	**-0.07 (-0.11; -0.03)**	**0.10 (0.01; 0.20)**	**-0.16 (-0.28; -0.05)**	-0.05 (-0.16; 0.06)	**0.00**
	3A	**-0.07 (-0.10; -0.03)**	0.07 (-0.02; 0.17)	**-0.16 (-0.27; -0.05)**	-0.05 (-0.16; 0.06)	**0.00**
	3B	**-0.06 (-0.10; -0.02)**	0.07 (-0.03; 0.16)	**-0.15 (-0.27; -0.04)**	-0.04 (-0.15; 0.07)	**0.00**
Beer consumption	1	**0.08 (0.04; 0.12)**	-0.08 (-0.17; 0.01)	-0.13 (-0.27; 0.01)	0.13 (-0.03; 0.29)	0.11
	2	0.04 (0.00; 0.08)	-0.04 (-0.13; 0.06)	-0.10 (-0.23; 0.03)	0.06 (-0.09; 0.20)	0.56
	3A	0.03 (-0.01; 0.07)	-0.04 (-0.13; 0.05)	-0.12 (-0.25; 0.01)	0.03 (-0.12; 0.17)	0.87
	3B	0.03 (-0.01; 0.07)	-0.04 (-0.13; 0.05)	-0.12 (-0.25; 0.01)	0.03 (-0.12; 0.18)	0.77

Table S4.4 (continued

		Alcohol consumption				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
Spirits consumption	1	**0.19 (0.04; 0.34)**	-0.03 (-0.13; 0.07)	**-0.26 (-0.42; -0.10)**	**-0.30 (-0.58; -0.03)**	**0.00**
	2	0.08 (-0.06; 0.22)	-0.01 (-0.11; 0.08)	**-0.19 (-0.33; -0.04)**	-0.22 (-0.48; 0.04)	0.12
	3A	0.03 (-0.11; 0.17)	-0.02 (-0.11; 0.08)	**-0.20 (-0.34; -0.05)**	-0.21 (-0.46; 0.04)	0.55
	3B	0.02 (-0.12; 0.16)	-0.02 (-0.11; 0.08)	**-0.19 (-0.34; -0.05)**	-0.20 (-0.46; 0.05)	0.69

Betas and 95% confidence intervals indicate the strength of the association between total alcohol, wine, beer, and spirits consumption with the total MVD composite score where a negative beta indicates less MVD. Total alcohol, wine, beer, and spirits consumption were entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For individuals with and without a history of cardiovascular disease, respectively, 1 SD corresponds with 1.6 and 1.6 ml white matter hyperintensity volume; 3.1 and 2.3 cerebral microbleeds; 2.5 and 1.5 lacunar infarcts (all logarithmically transformed and standardized per stratum and summed in to the composite score for CSVD features, where the numbers of participants per stratum respectively are n=253 and n=1,822); 21.1 and 20.1 µm of CRAE; 32.8 and 31.1 µm of CRVE (per stratum summed in to the composite score for retinal diameters, where the numbers of participants per stratum respectively are n=459 and n=2262); 3.7 and 3.5 µm of flicker light-induced increase in retinal arteriolar diameter and 4.3 and 4.1 µm of flicker light-induced increase in retinal venular diameter (per stratum summed in to the composite score for flicker light-induced increase in retinal diameters, where the numbers of participants per stratum respectively are n=330 and n=1760); 54.2 PU and 57.7 PU of heat-induced skin-hyperaemia (where the numbers of participants per stratum respectively are n=265 and n=1,252); 1.16 and 0.93 mg/24 hours of logarithmically transformed UAE (where the numbers of participants per stratum respectively are n=522 and n=2585); or 122.2 and 93.9 ng/ml sICAM-1; 119.8 and 95.4 ng/ml of sVCAM-1; 91.1 ng/ml and 58.8 ng/ml of sE-selectin; or 51.4 and 47.2% vWF (per stratum summed in to the composite score for plasma biomarkers of MVD, where the numbers of participants per stratum respectively are n=519 and n=2,559). Model 1: crude; Model 2: age, sex, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high]; model 3A: model 2 + waist circumference, smoking status [current, ever, never], diet score; model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; CSVD, cerebral small vessel disease; CRAE, central retina arteriolar equivalent; CRVE, central retinal venular equivalent; SD: standard deviation; PU, perfusion units; UAE, urinary albumin excretion; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; MVD, microvascular dysfunction.

Table S4.5 Associations of total alcohol, wine, beer, and spirits consumption with the total MVD composite score, stratified by sex.

		Men					Women				
	Model	Continuous	None vs. light	Moderate vs. light	High vs. light	P for trend	Continuous	None vs. light	Moderate vs. light	High vs. light	P for trend
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P-value	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P-value
Total alcohol consumption	1	**-0.05 (-0.08; -0.02)**	**0.46 (0.29; 0.63)**	-0.12 (-0.25; 0.01)	**-0.15 (-0.27; -0.03)**	**0.00**	**-0.09 (-0.14; -0.03)**	**0.26 (0.11; 0.40)**	**-0.18 (-0.32; -0.02)**	-0.10 (-0.23; 0.03)	**0.00**
	2	**-0.04 (-0.07; -0.01)**	**0.30 (0.14; 0.45)**	-0.10 (-0.22; 0.02)	**-0.14 (-0.24; -0.03)**	**0.00**	**-0.07 (-0.12; -0.02)**	0.11 (-0.03; 0.25)	**-0.16 (-0.30; -0.01)**	-0.12 (-0.25 0.00)	**0.00**
	3A	**-0.05 (-0.07; -0.02)**	**0.27 (0.11; 0.42)**	-0.08 (-0.19; 0.04)	**-0.15 (-0.26; -0.04)**	**0.00**	**-0.08 (-0.13; -0.03)**	0.11 (-0.02; 0.25)	-0.13 (-0.27; 0.02)	-0.12 (-0.24; 0.00)	**0.00**
	3B	**-0.04 (-0.06; -0.01)**	**0.22 (0.07; 0.37)**	-0.09 (-0.20; 0.03)	**-0.13 (-0.23; -0.02)**	**0.00**	**-0.07 (-0.12; -0.01)**	0.09 (-0.04; 0.23)	-0.11 (-0.25; 0.04)	-0.10 (-0.22; 0.03)	**0.00**
Wine	3B	**-0.06 (-0.11; -0.02)**	**0.13 (0.03; 0.23)**	**-0.17 (-0.31; -0.04)**	-0.00 (-0.16; 0.15)	**0.00**	**-0.09 (-0.14; -0.01)**	0.03 (-0.11; 0.18)	**-0.18 (-0.34; -0.02)**	-0.14 (-0.28; 0.01)	**0.00**
Beer	3B	-0.02 (-0.05; 0.02)	0.03 (-0.07; 0.13)	-0.11 (-0.24; 0.02)	-0.07 (-0.22; 0.08)	0.09	0.07 (-0.05; 0.19)	**-0.11 (-0.26; 0.04)**	-0.17 (-0.43; 0.09)	-0.02 (-0.32; 0.29)	0.40
Spirits	3B	-0.02 (-0.15; 0.11)	0.05 (-0.06; 0.15)	-0.15 (-0.30; -0.01)	-0.20 (-0.47; 0.07)	0.62	0.02 (-0.32; 0.37)	-0.07 (-0.23; 0.09)	-0.23 (-0.51; 0.05)	-0.26 (-0.78; 0.25)	0.98

Betas and 95% confidence intervals represent the difference in total MVD composite score (per SD) per unit of total alcohol, wine, beer, or spirits consumption or for none, moderate, or high versus light total alcohol, wine, beer, or spirits consumption where a negative beta indicates less MVD. Total alcohol, wine, beer and spirits consumption were entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For all analyses the value of one SD was numerically comparable to the value of one SD that was presented in the legend of Table 2. The number of men and women, respectively are n=1,592 and n=1,528. Model 1: crude; Model 2: age, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high]; model 3A: model 2 + waist circumference, smoking status [current, ever, never], diet score; model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Bold denotes P-value<0.05. Abbreviations: β, beta; CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

Table S4.6 Associations of total alcohol, wine, beer, and spirits consumption with the total MVD composite score, stratified by cardiovascular risk factor status.

		Alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P- value**
With type 2 diabetes, n=857						
Total alcohol consumption	1	**-0.05 (-0.10; -0.01)**	0.04 (-0.13; 0.21)	**-0.21 (-0.41; -0.01)**	**-0.25 (-0.43; -0.07)**	**0.00**
	2	**-0.09 (-0.13; -0.04)**	0.17 (-0.01; 0.34)	**-0.20 (-0.40; -0.00)**	**-0.24 (-0.41; -0.06)**	**0.00**
	3A	**-0.08 (-0.13; -0.04)**	0.15 (-0.03; 0.32)	-0.16 (-0.35; 0.03)	**-0.23 (-0.40; -0.06)**	**0.00**
	3B	**-0.07 (-0.11; -0.02)**	0.09 (-0.08; 0.26)	-0.18 (-0.37; 0.01)	**-0.20 (-0.37; -0.04)**	**0.00**
Wine	3B	**-0.11 (-0.18; -0.03)**	0.08 (-0.08; 0.25)	**-0.35 (-0.59; -0.12)**	0.01 (-0.23; 0.26)	**0.03**
Beer	3B	-0.04 (-0.10; 0.02)	0.15 (-0.01; 0.32)	-0.03 (-0.28; 0.21)	-0.09 (-0.33; 0.16)	**0.02**
Spirits	3B	-0.01 (-0.24; 0.21)	**0.17 (0.00; 0.33)**	-0.08 (-0.34; 0.19)	-0.22 (-0.63; 0.20)	0.94
With prediabetes, n=461						
Total alcohol consumption	1	-0.02 (-0.08; 0.03)	0.31 (0.00; 0.61)	0.00 (-0.27;0.26)	-0.10 (-0.32; 0.12)	**0.02**
	2	-0.06 (-0.11; 0.00)	**0.43 (0.12; 0.73)**	-0.08 (-0.34; 0.18)	-0.18 (-0.40; 0.04)	**0.00**
	3A	-0.06 (-0.11; 0.00)	**0.42 (0.12; 0.72)**	-0.07 (-0.33; 0.19)	-0.17 (-0.39; 0.05)	**0.00**
	3B	-0.06 (-0.12; 0.00)	**0.38 (0.08; 0.68)**	-0.11 (-0.37; 0.15)	-0.19 (-0.41; 0.03)	**0.00**
Wine	3B	**-0.12 (-0.20; -0.04)**	**0.31 (0.08; 0.54)**	-0.15 (-0.45; 0.14)	-0.11 (-0.38; 0.16)	**0.00**
Beer	3B	0.02 (-0.07; 0.10)	**-0.25 (-0.48; -0.02)**	-0.44 (-0.74; 0.13)	-0.30 (-0.64; 0.04)	0.63
Spirits	3B	-0.16 (-0.48; 0.16)	-0.21 (-0.44; 0.03)	-0.59 (-0.92; 0.26)	**-0.77 (-1.29; -0.25)**	**0.13**
With normal glucose metabolism, n=1,769						
Total alcohol consumption	1	0.03 (-0.01; 0.07)	0.02 (-0.14; 0.18)	-0.09 (-0.22; 0.04)	-0.03 (-0.15; 0.08)	0.40
	2	-0.02 (-0.06; 0.02)	0.07 (-0.09; 0.22)	**-0.14 (-0.27; -0.01)**	-0.10 (-0.22; 0.01)	**0.01**
	3A	-0.03 (-0.07; 0.01)	0.06 (-0.10; 0.22)	-0.12 (-0.25; 0.01)	-0.11 (-0.23; 0.00)	**0.01**
	3B	-0.01 (-0.05; 0.03)	0.04 (-0.11; 0.20)	-0.10 (-0.22; 0.03)	-0.08 (-0.20; 0.03)	0.05
Wine	3B	-0.05 (-0.10; 0.00)	0.03 (-0.09; 0.15)	**-0.15 (-0.28; -0.01)**	-0.08 (-0.21; 0.06)	**0.02**
Beer	3B	0.03 (-0.03; 0.08)	**-0.12 (0.24; 0.00)**	-0.15 (-0.32; 0.01)	0.05 (-0.16; 0.26)	0.22
Spirits	3B	0.07 (-0.13; 0.26)	-0.12 (0.25; -0.00)	-0.15 (-0.34; 0.04)	0.06 (-0.31; 0.43)	0.65

Table S4.6 (continued)

		Alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P- value**
With hypertension, n=1,792						
Total alcohol consumption	1	**-0.05 (-0.08; -0.02)**	**0.22 (0.08; 0.35)**	**-0.14 (-0.27; -0.00)**	**-0.23 (-0.35; -0.11)**	**0.00**
	2	**-0.06 (-0.09; -0.03)**	**0.21 (0.08; 0.34)**	-0.09 (-0.21; 0.03)	**-0.16 (-0.26; -0.05)**	**0.00**
	3A	**-0.06 (-0.09; -0.03)**	**0.19 (0.06; 0.32)**	-0.06 (-0.18; 0.06)	**-0.15 (-0.26; -0.04)**	**0.00**
	3B	**-0.05 (-0.08; -0.02)**	**0.15 (0.02; 0.28)**	-0.06 (-0.18; 0.06)	**-0.13 (-0.24; -0.03)**	**0.00**
Wine	3B	**-0.08 (-0.13; -0.04)**	**0.17 (0.06; 0.28)**	-0.11 (-0.24; 0.03)	0.01 (-0.13; 0.15)	**0.01**
Beer	3B	-0.03 (-0.07; 0.01)	0.06 (-0.05; 0.17)	-0.00 (-0.15; 0.15)	-0.16 (-0.22; 0.01)	**0.02**
Spirits	3B	0.00 (-0.14; 0.15)	0.08 (-0.03; 0.18)	-0.05 (-0.22; 0.11)	**-0.33 (-0.61; -0.05)**	0.75
Without hypertension, n=1,328						
Total alcohol consumption	1	0.01 (-0.03; 0.06)	**0.18 (0.01; 0.35)**	**-0.16 (-0.32; -0.01)**	-0.02 (-0.15; 0.12)	0.05
	2	-0.02 (-0.07; 0.02)	0.11 (-0.06; 0.28)	**-0.18 (-0.33; -0.04)**	-0.10 (-0.23; 0.04)	**0.01**
	3A	-0.04 (-0.09; 0.01)	0.11 (-0.06; 0.28)	**-0.18 (-0.33; -0.03)**	-0.12 (-0.25; 0.01)	**0.00**
	3B	-0.03 (-0.08; 0.01)	0.09 (-0.07; 0.26)	**-0.17 (-0.32; -0.02)**	-0.11 (-0.24; 0.02)	**0.01**
Wine	3B	**-0.08 (-0.14; -0.03)**	-0.02 (-0.16; 0.12)	**-0.27 (-0.44; -0.11)**	**-0.18 (-0.34; -0.03)**	**0.00**
Beer	3B	0.05 (-0.02; 0.13)	**-0.15 (-0.29; -0.01)**	**-0.34 (-0.54; -0.15)**	0.18 (-0.06; 0.43)	0.21
Spirits	3B	-0.06 (-0.31; 0.19)	**-0.14 (-0.29; 0.00)**	**-0.37 (-0.60; -0.14)**	0.12 (-0.32; 0.56)	0.89
With dyslipidaemia, n=2,064						
Total alcohol consumption	1	**-0.05 (-0.08; -0.02)**	**0.20 (0.07; 0.33)**	**-0.16 (-0.28; -0.03)**	**-0.21 (-0.31; -0.10)**	**0.00**
	2	**-0.06 (-0.09; -0.03)**	**0.17 (0.05; 0.29)**	**-0.12 (-0.23; -0.00)**	**-0.15 (-0.25; -0.05)**	**0.00**
	3A	**-0.07 (-0.10; -0.04)**	**0.16 (0.04; 0.28)**	-0.10 (-0.21; 0.01)	**-0.15 (-0.25; -0.05)**	**0.00**
	3B	**-0.06 (-0.09; -0.03)**	0.12 (-0.00; 0.24)	-0.10 (-0.21; 0.01)	**-0.13 (-0.23; -0.04)**	**0.00**
Wine	3B	**-0.08 (-0.12; -0.04)**	**0.12 (0.02; 0.22)**	**-0.15 (-0.27; -0.02)**	-0.02 (-0.14; 0.11)	**0.00**
Beer	3B	**-0.04 (-0.09; -0.00)**	0.05 (-0.05; 0.15)	-0.07 (-0.21; 0.07)	-0.15 (-0.21; 0.07)	**0.01**
Spirits	3B	0.05 (-0.09; 0.19)	0.05 (-0.05; 0.15)	-0.09 (-0.24; 0.07)	-0.20 (-0.48; 0.09)	0.42

Table S4.6 (continued)

		Alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P- value**
Without dyslipidaemia, n=1,056						
Total alcohol consumption	1	0.03 (-0.02; 0.07)	**0.26 (0.06; 0.45)**	-0.12 (-0.29; 0.05)	-0.02 (-0.17; 0.13)	**0.02**
	2	-0.02 (-0.06; 0.03)	0.16 (-0.03; 0.34)	-0.14 (-0.30; 0.02)	-0.10 (-0.24; 0.04)	**0.01**
	3A	-0.02 (-0.07; 0.02)	0.15 (-0.03; 0.33)	-0.11 (-0.27; 0.05)	-0.10 (-0.24; 0.04)	**0.01**
	3B	-0.01 (-0.05; 0.04)	0.13 (-0.06; 0.31)	-0.09 (-0.25; 0.07)	-0.07 (-0.21; 0.07)	**0.04**
Wine	3B	**-0.08 (-0.14; -0.01)**	0.07 (-0.08; 0.22)	**-0.19 (-0.37; -0.02)**	-0.11 (-0.29; 0.06)	**0.01**
Beer	3B	**0.07 (0.00; 0.13)**	-0.16 (-0.30; 0.02)	-0.28 (-0.49; 0.07)	0.12 (-0.12; 0.36)	0.12
Spirits	3B	-0.18 (-0.46; 0.09)	-0.14 (-0.28; 0.01)	**-0.35 (-0.59; -0.13)**	-0.11 (-0.48; -0.27)	**0.03**
With current smoking, n=396						
Total alcohol consumption	1	-0.03 (-0.09; 0.04)	0.24 (-0.04; 0.52)	-0.24 (-0.54; 0.06)	-0.19 (-0.45; 0.06)	**0.00**
	2	-0.04 (-0.10; 0.03)	0.09 (-0.18; 0.36)	-0.20 (-0.48; 0.08)	-0.19 (-0.42; 0.05)	**0.02**
	3A	-0.02 (-0.08; 0.03)	0.11 (-0.15; 0.37)	-0.15 (-0.41; 0.12)	-0.16 (-0.38; 0.07)	**0.02**
	3B	-0.01 (-0.07; 0.05)	0.11 (-0.14; 0.37)	-0.14 (-0.40; 0.13)	-0.12 (-0.35; 0.11)	0.06
Wine	3B	**-0.10 (-0.19; -0.01)**	0.04 (-0.20; 0.27)	**-0.52 (-0.83; -0.20)**	-0.12 (-0.41; 0.17)	**0.03**
Beer	3B	0.06 (-0.02; 0.14)	-0.10 (-0.33; 0.12)	-0.14 (-0.46; 0.18)	0.07 (-0.25; 0.38)	0.35
Spirits	3B	-0.07 (-0.31; 0.17)	-0.07 (-0.30; 0.15)	-0.23 (-0.57; 0.11)	-0.24 (-0.75; 0.27)	0.40
Without current smoking, n=2,724						
Total alcohol consumption	1	-0.02 (-0.05; 0.00)	**0.20 (0.08; 0.32)**	-0.14 (-0.24; -0.03)	**-0.15 (-0.24; -0.06)**	**0.00**
	2	**-0.06 (-0.08; -0.03)**	**0.16 (0.05; 0.26)**	**-0.12 (-0.22; -0.02)**	**-0.13 (-0.22; -0.04)**	**0.00**
	3A	**-0.06 (-0.08; -0.03)**	**0.16 (0.05; 0.27)**	-0.10 (-0.19; 0.00)	-0.13 (-0.22; 0.05)	**0.00**
	3B	**-0.05 (-0.08; -0.02)**	0.13 (0.02; 0.24)	-0.09 (-0.19; 0.01)	**-0.11 (-0.20; -0.02)**	**0.00**
Wine	3B	**-0.07 (-0.11; -0.04)**	**0.11 (0.02; 0.20)**	-0.10 (-0.21; 0.00)	-0.04 (-0.15; 0.07)	**0.00**
Beer	3B	-0.03 (-0.07; 0.01)	-0.00 (-0.09; 0.08)	-0.12 (-0.25; 0.00)	-0.10 (-0.25; 0.05)	0.09
Spirits	3B	0.01 (-0.13; 0.15)	0.00 (-0.09; 0.09)	-0.13 (-0.27; 0.01)	-0.14 (-0.39; 0.11)	0.98

Betas and 95% confidence intervals indicate the strength of the association between total alcohol, wine, beer, and spirits consumption with the total MVD composite score where a negative beta indicates less MVD. Total alcohol, wine, beer, and spirits consumption were entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For individuals with type 2 diabetes, individuals with prediabetes, and individuals with normal glucose metabolism, respectively, one SD corresponds with 1.6, 1.7 and 1.5 ml white matter hyperintensity volume; 2.6, 2.4 and 2.3 cerebral microbleeds; 2.0, 1.9 and 1.4 lacunar infarcts (all logarithmically

transformed and standardized per stratum and summed in to the composite score for CSVD features, where the numbers of participants per stratum respectively are n=459, n=321, and n=1274); 20.9, 20.2 and 19.9 µm of CRAE; 32.7, 31.5 and 30.5 µm of CRVE (per stratum summed in to the composite score for retinal diameters, where the numbers of participants per stratum respectively are n=758, n=403, and n=1533); 3.5, 3.4 and 3.6 µm of flicker light-induced increase in retinal arteriolar diameter and 4.2, 4.4 and 4.0 µm of flicker light-induced increase in retinal venular diameter (per stratum summed in to the composite score for flicker light-induced increase in retinal diameters, where the numbers of participants per stratum respectively are n=563, n=307, and n=1197); 48.8, 53.2, and 60.7 PU of heat-induced skin hyperaemia (where the numbers of participants per stratum respectively are n= 439, n=230, n=825); 1.1 mg/24 hours, 0.9 mg/24 hours, 0.81 mg/24 hours of logarithmically transformed UAE (where the numbers of participants per stratum respectively are n= 854, n=460, n=1760); 127.7, 95.7, and 78.6 ng/ml of sICAM-1; 119.6, 101.1, and 85.6 ng/ml of sVCAM-1; 88.3, 55.3, and 46.8 ng/ml of sE-selectin; and 53.3, 47.8, and 44.2 % vWF (per stratum combined in the plasma biomarkers of MVD composite score, where the numbers of participants per stratum respectively are n= 849, n=455, n=1742). For individuals with and without hypertension, respectively, 1 SD corresponds with 1.6 and 1.4 ml white matter hyperintensity volume; 2.6 and 2.1 cerebral microbleeds; 1.8 and 1.4 lacunar infarcts (all logarithmically transformed and standardized per stratum and summed in to the composite score for CSVD features, where the numbers of participants per stratum respectively are n=1101 and n=974); 20.4 and 19.6 µm of CRAE, 32.3 and 30.0 µm of CRVE (per stratum summed in to the composite score for retinal diameters, where the numbers of participants per stratum respectively are n=1559 and n=1162); 3.5 and 3.6 µm of flicker light-induced increase in retinal arteriolar diameter and 4.0 and 4.2 µm of flicker light-induced increase in retinal venular diameter (per stratum summed in to the composite score for flicker light-induced increase in retinal diameters, where the numbers of participants per stratum respectively are n=1173 and n=917); 55.7 and 58.6 PU of heat-induced skin hyperaemia, where the numbers of participants per stratum respectively are n=898 and n=619; and 1.2 and 0.7 mg/24hours of logarithmically transformed UAE, where the numbers of participants per stratum respectively are n=1788 and n=1319; or 107.3 and 86.1 ng/ml sICAM-1; 107.5 and 88.3 ng/ml of sVCAM-1; 74.8 ng/ml and 46.7 ng/ml of sE-selectin; or 49.5 and 46.1% vWF (per stratum summed in to the composite score for plasma biomarkers of MVD, where the numbers of participants per stratum respectively are n=1772 and n=1306). For individuals with and without dyslipidaemia, respectively, 1 SD corresponds with 1.6 and 1.5 ml white matter hyperintensity volume; 2.5 and 2.2 cerebral microbleeds; 1.8 and 1.4 lacunar infarcts (all logarithmically transformed and standardized per stratum and summed in to the composite score for CSVD features, where the numbers of participants per stratum respectively are n=1301 and n=774); 19.8 and 21.0 µm of CRAE; 31.4 and 31.3 µm of CRVE (per stratum summed in to the composite score for retinal diameters, where the numbers of participants per stratum respectively are n=1780 and n=941); 3.6 and 3.5 µm of flicker light-induced increase in retinal arteriolar diameter and 4.1 and 4.1 µm of flicker light-induced increase in retinal venular diameter (per stratum summed in to the composite score for flicker light-induced increase in retinal diameters, where the numbers of participants per stratum respectively are n=1350 and n=740); 57.5 and 56.7 PU of heat-induced skin hyperaemia, where the numbers of participants per stratum respectively are n=1055 and n=462; 1.0 and 0.9 mg/24-hours of UAE, where the numbers of participants per stratum respectively are n=2060 and n=1047); or 104.1 and 89.7 ng/ml sICAM-1; 101.8 and 99.3 ng/ml of sVCAM-1; 70.7 ng/ml and 52.6 ng/ml of sE-selectin; or 48.3 and 48.4% vWF (per stratum summed in to the composite score for plasma biomarkers of MVD, where the numbers of participants per stratum respectively are n=2038 and n=1040). For current and non-current smokers, respectively, 1 SD corresponds with 1.6 and 1.6 ml white matter hyperintensity volume; 2.3 and 2.4 cerebral microbleeds; 1.8 and 1.6 lacunar infarcts (all logarithmically transformed and standardized per stratum and summed in to the composite score for CSVD features, where the numbers of participants per stratum respectively are n=236 and n=1839); 20.7 and 20.1 µm of CRAE; 32.5 and 31.0 µm of CRVE (per stratum summed in to the composite score for retinal diameters, where the numbers of participants per stratum respectively are n=333 and n=2388); 3.6 and 3.6 µm of flicker light-induced increase in retinal arteriolar diameter and 4.8 and 4.0 µm of flicker light-induced increase in retinal venular diameter (per stratum summed in to the composite score for flicker light-induced increase in retinal diameters, where the numbers of participants per stratum respectively are n=249 and n=1841); 48.7 and 58.1 PU of heat-induced skin hyperaemia, where the numbers of participants per stratum respectively are n=176 and n=1341; 1.1 and 1.0 mg/24-hours of UAE, where

the numbers of participants per stratum respectively are n=395 and n=2712; or 127.0 and 93.4 ng/ml sICAM-1; 105.7 and 99.9 ng/ml of sVCAM-1; 66.4 ng/ml and 65.6 ng/ml of sE-selectin; or 48.7 and 48.3% vWF (per stratum summed in to the composite score for plasma biomarkers of MVD, where the numbers of participants per stratum respectively are n=391 and n=2687). Model 1: crude; Model 2: age, sex, glucose metabolism status (where applicable; entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high]; model 3A: model 2 + waist circumference, smoking status (where applicable; current, ever, never), diet score; model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Table S4.7 Associations of total alcohol consumption with the total MVD composite score, stratified by number of cardiovascular risk factors.

		Alcohol consumption				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P- value**
Total MVD composite score, per SD						
0 risk factors, n= 450	3B	0.00 (-0.09; 0.09)	0.12 (-0.19; 0.43)	-0.18 (-0.43; 0.07)	-0.11 (-0.34; 0.13)	0.12
One risk factor						
- Type 2 diabetes, n= 857	3B	**-0.07 (-0.11; 0.02)**	0.09 (-0.08; 0.26)	-0.18 (-0.37; 0.01)	**-0.20 (-0.37; -0.04)**	**0.00**
- Hypertension, n= 1,792	3B	**-0.05 (-0.08; -0.02)**	**0.15 (0.02; 0.28)**	-0.06 (-0.18; 0.06)	**-0.13 (-0.24; -0.03)**	**0.00**
- Dyslipidaemia, n=2,064	3B	**-0.06 (-0.09; -0.03)**	0.12 (-0.00; 0.24)	-0.10 (-0.21; 0.01)	**-0.13 (-0.23; -0.04)**	**0.00**
- Smoking, n= 396	3B	-0.01 (-0.07; 0.05)	0.11 (-0.14; 0.37)	-0.14 (-0.40; 0.13)	-0.12 (-0.35; 0.11)	0.06
Two risk factors						
- Type 2 diabetes and hypertension, n=720	3B	**-0.07 (-0.12; -0.03)**	0.08 (-0.11; 0.27)	-0.18 (-0.39; 0.03)	**-0.24 (-0.43; -0.06)**	**0.00**
- Type 2 diabetes and dyslipidaemia, n=715	3B	**-0.07 (-0.12; -0.02)**	0.05 (-0.15; 0.24)	-0.16 (-0.37; 0.05)	**-0.22 (-0.40; -0.03)**	**0.00**
- Type 2 diabetes and smoking, n= 132	3B	0.01 (-0.09; 0.12)	-0.07 (-0.53; 0.40)	-0.20 (-0.74; 0.35)	-0.21 (-0.69; 0.27)	0.44
- Hypertension and dyslipidaemia, n= 1,355	3B	**-0.07 (-0.10; -0.03)**	0.13 (-0.02; 0.28)	-0.09 (-0.22; 0.05)	**-0.17 (-0.30; -0.05)**	**0.00**
- Hypertension and smoking, n= 226	3B	-0.00 (-0.08; 0.08)	0.05 (-0.30; 0.39)	-0.10 (-0.46; 0.25)	-0.23 (-0.54; 0.07)	0.07
- Dyslipidaemia and smoking, n=277	3B	-0.03 (-0.10; 0.05)	0.09 (-0.20; 0.39)	0.20 (-0.52; 0.11)	-0.26 (-0.54; 0.02)	**0.01**
Three risk factors						
- Type 2 diabetes, hypertension, and dyslipidaemia, n=622	3B	**-0.08 (-0.13; -0.03)**	0.02 (-0.18; 0.23)	-0.19 (-0.41;0.04)	**-0.28 (-0.47; -0.08)**	**0.00**
- Type 2 diabetes, hypertension, and smoking, n=108	3B	0.00 (-0.12; 0.13)	-0.15 (-0.67; 0.13)	-0.16 (-0.75; 0.42)	-0.27 (-0.79; 0.24)	0.51
-Hypertension, dyslipidaemia, and smoking, n=176	3B	0.00 (-0.09; 0.09)	-0.06 (-0.45; 0.34)	-0.16 (-0.61; 0.28)	-0.27 (-0.63; 0.10)	0.19
Four risk factors, n=97	3B	0.01 (-0.13; 0.15)	-0.28 (0.84; 0.29)	-0.24 (-0.90; 0.42)	-0.34 (-0.90; 0.22)	0.64

Betas and 95% confidence intervals indicate the strength of the association between total alcohol consumption and the total MVD composite score where a negative beta indicates less MVD. Total alcohol consumption was entered in the model as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). Numerical values per SD in individual with and without a risk factor are presented in the legend of Table S4.6. Variables in model 3B: age, sex, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high], waist circumference, smoking status (current, ever, never), diet score, office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and prior cardiovascular disease. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Table S4.8 Associations of total alcohol consumption with the total MVD composite score composed out of five instead of six measures of MVD.

		Alcohol consumption				
	Model	Continuous	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P- value
Total MVD composite score, per SD						
Without CSVD features						
Total alcohol consumption, n=3,114	3B	**-0.04 (-0.07; -0.02)**	**0.11 (0.01; 0.21)**	**-0.11 (-0.20; -0.01)**	**-0.11 (-0.19; -0.03)**	**0.00**
Without retinal microvascular diameters						
Total alcohol consumption, n=3,116	3B	**-0.04 (-0.07; -0.02)**	**0.12 (0.03; 0.21)**	-0.06 (-0.14; 0.03)	**-0.11 (-0.18; -0.03)**	**0.00**
Without flicker light-induced retinal microvascular diameters						
Total alcohol consumption, n=3,120	3B	**-0.04 (-0.07; -0.02)**	**0.14 (0.05; 0.24)**	-0.09 (-0.17; 0.00)	**-0.10 (-0.18; -0.02)**	**0.00**
Without heat-induced skin hyperaemia						
Total alcohol consumption, n=3,120	3B	**-0.04 (-0.06; -0.02)**	**0.13 (0.03; 0.23)**	**-0.10 (-0.18; -0.01)**	**-0.11 (-0.19; -0.03)**	**0.00**
Without UAE						
Total alcohol consumption, n=3,009	3B	**-0.04 (-0.06; -0.02)**	**0.09 (-0.02; 0.19)**	-0.07 (-0.17; 0.02)	**-0.12 (-0.20; -0.03)**	**0.00**
Without plasma biomarkers of MVD						
Total alcohol consumption, n=3,017	3B	**-0.03 (-0.05; 0.00)**	0.01 (-0.10; 0.12)	**-0.11 (-0.21; -0.02)**	**-0.11 (-0.19; -0.02)**	**0.01**

Betas and 95% confidence intervals indicate the strength of the association between total alcohol consumption and the total MVD composite score where a negative beta indicates less MVD. Total alcohol consumption was entered in the model as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). Numerical values per SD were similar to the values per SD for the total MVD composite score based on six measures of MVD in the general population, which are presented in the legend of Table 4.2. Variables in model 3B: age, sex, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high], waist circumference, smoking status (current, ever, never), diet score, office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, and prior cardiovascular disease. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; SD: standard deviation; MVD, microvascular dysfunction.

Table S4.9 Associations of total alcohol consumption with measures of MVD, in the general population.

		Total alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P- value**
CSVD features composite score, per SD	1	0.04 (0.01; 0.07)	-0.01 (-0.15; 0.12)	0.04 (-0.08; 0.17)	0.06 (-0.05; 0.17)	0.01
	2	-0.00 (-0.03; 0.03)	-0.01 (-0.13; 0.12)	-0.00 (-0.12; 0.11)	-0.02 (-0.12; 0.08)	0.93
	3A	-0.01 (-0.04; 0.02)	-0.00 (-0.13; 0.12)	-0.05 (-0.12; 0.11)	-0.04 (-0.14; 0.06)	0.56
	3B	-0.00 (-0.03; 0.03)	-0.02 (-0.15; 0.11)	-0.00 (-0.12; 0.11)	-0.02 (-0.13; 0.08)	0.86
Retinal microvascular diameters composite score, per SD	1	-0.04 (0.07; -0.02)	0.11 (-0.01; 0.22)	-0.14 (-0.24; 0.03)	-0.06 (-0.15; 0.04)	0.00
	2	-0.01 (-0.03; 0.02)	0.03 (-0.09; 0.14)	-0.11 (-0.21; 0.00)	-0.06 (-0.15; 0.04)	0.09
	3A	-0.01 (-0.04; -0.02)	0.01 (-0.11; 0.13)	-0.10 (-0.21; 0.00)	-0.06 (-0.16; 0.03)	0.10
	3B	-0.00 (-0.03; 0.03)	0.02 (-0.10; 0.13)	-0.08 (-0.19; 0.02)	-0.04 (-0.14; 0.06)	0.23
Flicker light-induced increase in retinal diameter composite score, per SD	1	0.01 (-0.02; 0.04)	-0.00 (-0.14; 0.13)	-0.09 (-0.21; 0.04)	-0.04 (-0.14; 0.07)	0.40
	2	-0.01 (-0.04; 0.02)	-0.02 (-0.16; 0.11)	-0.09 (-0.21; 0.04)	-0.06 (-0.16; 0.05)	0.37
	3A	-0.01 (-0.04; 0.02)	-0.02 (-0.16; 0.12)	-0.08 (-0.20; 0.04)	-0.06 (-0.17; 0.05)	0.33
	3B	-0.01 (-0.04; 0.02)	-0.02 (-0.16; 0.11)	-0.09 (-0.21; 0.04)	-0.06 (-0.16; 0.06)	0.38
Heat-induced skin hyperaemia, per SD	1	0.02 (-0.02; 0.05)	-0.09 (-0.24; 0.07)	-0.04 (-0.18;0.10)	-0.14 (-0.27; -0.02)	0.15
	2	-0.02 (-0.06; 0.02)	-0.04 (-0.20; 0.11)	0.00 (-0.14; 0.14)	-0.08 (-0.20;0.04)	0.39
	3A	-0.03 (-0.06; 0.01)	-0.06 (-0.22; 0.09)	-0.01 (-0.15; 0.13)	-0.10 (-0.22;0.03)	0.37
	3B	-0.02 (-0.06; 0.02)	-0.07 (-0.22; 0.09)	-0.01 (-0.15; 0.13)	-0.09 (-0.21; 0.04)	0.48
Logarithmically transformed UAE, per SD	1	0.01 (-0.02; 0.30)	0.09 (-0.02; 0.20)	-0.10 (-0.21; -0.00)	-0.09 (-0.18; 0.00)	0.00
	2	-0.02 (-0.05; 0.01)	0.07 (-0.04; 0.17)	-0.09 (-0.18; 0.01)	-0.06 (-0.14; 0.03)	0.02
	3A	-0.02 (-0.05; 0.00)	0.06 (-0.05; 0.16)	-0.07 (-0.16; 0.03)	-0.06 (-0.14; 0.03)	0.02
	3B	-0.02 (-0.05; 0.01)*	0.04 (-0.07; 0.14)	-0.08 (-0.18; 0.01)	-0.06 (-0.15; 0.02)	0.04
Plasma biomarkers of MVD composite score, per SD	1	-0.05 (-0.07; -0.02)	0.34 (0.23; 0.45)	-0.08 (-0.18; 0.03)	-0.15 (-0.24; -0.06)	0.00
	2	-0.06 (-0.09; -0.04)	0.27 (0.17; 0.38)	-0.05 (-0.14; 0.05)	-0.12 (-0.20; -0.03)	0.00
	3A	-0.06 (-0.09; -0.04)	0.26 (0.16; 0.36)	-0.00 (-0.10; 0.08)	-0.10 (-0.18; -0.02)	0.00
	3B	-0.05 (-0.08; -0.03)	0.24 (0.14; 0.34)	0.0 (-0.09; 0.09)	-0.08 (-0.16; 0.00)	0.00

Betas and 95% confidence intervals indicate the strength of the association between total alcohol consumption and measures of MVD, where a negative beta indicates less MVD (i.e. less CSVD features, narrower diameters, higher flicker light-induced increase in retinal microvascular diameters, higher heat-induced skin hyperaemia, lower UAE, or lower levels of plasma biomarkers of MVD). Total alcohol consumption was entered in the model as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). Numerical values per SD are presented in the legend of Table 4.2. The numbers of participants with complete data on CSVD features, retinal

microvascular diameters, flicker light-induced increase in retinal microvascular diameters, heat-induced skin hyperaemia, UAE, and plasma biomarkers of MVD respectively are n=2,075, n=2,721, n=2,090, n=1,517, n=3,107, and n=3,078. *For urinary albumin excretion, the beta in model 3B for total alcohol consumption, investigated as a continuous variable, corresponds with an odds ratio of 0.55 (95% CI, 0.26; 1.16) for 30mg/24-hour greater urinary albumin excretion. Model 1: crude; Model 2: age, sex, glucose metabolism status (entered as dummies of type 2 diabetes, prediabetes, or other types of diabetes versus normal glucose metabolism status), educational level [low, middle, high]; model 3A: model 2 + waist circumference, smoking status [current, ever, never], diet score; model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication [yes/no] total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood flow was entered in model 1. Bold denotes P-value<0.05. Abbreviations: CI: confidence interval; CSVD, cerebral small vessel disease; CRAE, central retina arteriolar equivalent; CRVE, central retinal venular equivalent; SD: standard deviation; PU, perfusion units; UAE, urinary albumin excretion; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; MVD, microvascular dysfunction.

Table S4.10 Associations of total alcohol consumption with individual measures used in composite scores, in the general population.

	Model	Total alcohol consumption				
		Continuous β (95% CI)	None vs. light β (95% CI)	Moderate vs. light β (95% CI)	High vs. light β (95% CI)	P for trend P-value
CSVD features						
Logarithmically transformed white matter hyperintensity volume, per SD	1	**0.05 (0.02; 0.08)**	0.05 (-0.08; 0.19)	0.06 (-0.07; 0.18)	0.15 (-0.04; 0.26)	**0.02**
	2	0.01 (-0.02; 0.04)	0.01 (-0.11; 0.13)	0.00 (-0.11; 0.11)	0.03 (-0.06; 0.13)	0.59
	3A	0.00 (-0.03; 0.03)	0.01 (-0.11; 0.13)	0.00 (-0.11; 0.11)	0.02 (-0.08; 0.11)	0.79
	3B	0.01 (-0.02; 0.04)	-0.00 (-0.12; 0.12)	0.01 (-0.11; 0.11)	0.03 (-0.07; 0.12)	0.58
Logarithmically transformed number of cerebral microbleeds, per SD	1	**0.03 (0.00; 0.07)**	-0.10 (-0.24; 0.04)	0.07 (-0.05; 0.19)	0.01 (-0.10; 0.11)	0.17
	2	0.01 (-0.03; 0.04)	-0.07 (-0.20; 0.07)	0.05 (-0.07; 0.18)	-0.01 (-0.12; 0.10)	0.54
	3A	0.01 (-0.03; 0.04)	-0.07 (-0.21; 0.07)	0.06 (-0.07; 0.18)	-0.02 (-0.13; 0.09)	0.60
	3B	0.01 (-0.03; 0.04)	-0.08 (-0.21; 0.06)	0.06 (-0.07; 0.18)	-0.01 (-0.12; 0.10)	0.44
Logarithmically transformed number of lacunar infarcts, per SD	1	0.00 (-0.03; 0.04)	0.02 (-0.11; 0.16)	-0.04 (-0.17; 0.08)	-0.04 (-0.15; 0.07)	0.28
	2	-0.02 (-0.05; 0.01)	0.05 (-0.09; 0.18)	-0.06 (-0.18; 0.07)	-0.06 (-0.17; 0.05)	0.10
	3A	-0.03 (-0.06; 0.01)	0.05 (-0.09; 0.19)	-0.06 (-0.18; 0.06)	-0.08 (-0.19; 0.03)	0.05
	3B	-0.02 (-0.06; 0.01)	0.04 (-0.10; 0.17)	-0.06 (-0.18; 0.06)	-0.07 (-0.18; 0.04)	0.11
Retinal microvascular diameters						
CRAE, per SD	1	**-0.06 (-0.09; -0.03)**	**0.12 (0.00; 0.23)**	-0.10 (-0.21; 0.01)	**-0.10 (-0.19; 0.00)**	**0.00**
	2	-0.02 (-0.04; 0.01)	0.03 (-0.08; 0.15)	-0.07 (-0.18; 0.04)	-0.07 (-0.16; 0.03)	0.05
	3A	-0.02 (-0.04; 0.01)	0.02 (-0.10; 0.14)	-0.07 (-0.18; 0.03)	-0.07 (-0.16; 0.03)	0.08
	3B	-0.01 (-0.04; 0.02)	0.03 (-0.09; 0.14)	-0.05 (-0.15; 0.06)	-0.04 (-0.14; 0.05)	0.20
CRVE, per SD	1	-0.02 (-0.05; 0.01)	**0.08 (0.03; 0.20)**	**-0.15 (-0.26; -0.04)**	-0.05 (-0.15; 0.04)	**0.01**
	2	0.01 (-0.02; 0.04)	0.02 (-0.10; 0.14)	**-0.13 (-0.23; -0.02)**	-0.03 (-0.13; 0.06)	0.24
	3A	0.00 (-0.03; 0.03)	0.00 (-0.12; 0.12)	**-0.12 (-0.23; -0.01)**	-0.05 (-0.14; 0.05)	0.22
	3B	0.01 (-0.02; 0.04)	0.01 (-0.11; 0.12)	-0.11 (-0.21; 0.00)	-0.03 (-0.13; 0.06)	0.34
Flicker light-induced increase in retinal diameters						
Arteriolar diameter, per SD	1	0.01 (-0.02; 0.04)	0.03 (-0.10; 0.17)	**-0.14 (-0.26;-0.02)**	-0.02 (-0.13; 0.08)	0.26
	2	-0.00 (-0.04; 0.03)	-0.01 (-0.14; 0.13)	**-0.14 (-0.26; -0.02)**	-0.04 (-0.15; 0.07)	0.32
	3A	-0.01 (-0.04; 0.03)	-0.01 (-0.14;0.13)	**-0.14 (-0.26; -0.01)**	-0.05 (-0.15; 0.06)	0.33
	3B	-0.00 (-0.04; 0.03)	-0.01 (-0.15; 0.12)	**-0.14 (-0.26; -0.02)**	-0.04 (-0.15; 0.07)	0.38
Venular diameter, per SD	1	-0.00 (-0.03; 0.03)	-0.04 (-0.17; 0.10)	-0.01 (-0.12; 0.13)	-0.04 (-0.14; 0.07)	0.82
	2	-0.01 (-0.04; 0.02)	-0.03 (-0.17; 0.10)	-0.01 (-0.12; 0.12)	-0.05 (-0.15; 0.06)	0.66
	3A	-0.01 (-0.05; 0.02)	-0.02 (-0.16; 0.11)	-0.00 (-0.12; 0.13)	-0.05 (-0.16; 0.06)	0.56
	3B	-0.01 (-0.05; 0.02)	-0.03 (-0.16; 0.11)	0.00 (-0.12; 0.12)	-0.05 (-0.16; 0.06)	0.59

Table S4.10 (continued)

	Model	Total alcohol consumption				
		Continuous β (95% CI)	None vs. light β (95% CI)	Moderate vs. light β (95% CI)	High vs. light β (95% CI)	P for trend P-value
Plasma biomarkers of MVD						
sICAM-1, per SD	1	**-0.06 (-0.08; -0.03)**	**0.41 (0.30; 0.52)**	-0.02 (-0.12; 0.08)	-0.05 (-0.13; 0.04)	**0.00**
	2	**-0.04 (-0.07; -0.02)**	**0.30 (0.19; 0.41)**	0.01 (-0.09; 0.11)	-0.02 (-0.11; 0.06)	**0.00**
	3A	**-0.05 (-0.07; -0.02)**	**0.27 (0.16; 0.37)**	0.03 (-0.06; 0.13)	-0.05 (-0.11; 0.06)	**0.00**
	3B	**-0.04 (-0.06; -0.01)**	**0.25 (0.15; 0.36)**	0.04 (-0.05; 0.14)	-0.01 (-0.09; 0.08)	**0.00**
sVCAM-1, per SD	1	**-0.06 (-0.09; -0.04)**	**0.17 (0.06; 0.28)**	-0.05 (-0.15; 0.05)	**-0.20 (-0.28; -0.11)**	**0.00**
	2	**-0.09 (-0.12; -0.07)**	**0.17 (0.07; 0.28)**	-0.05 (-0.14; 0.05)	**-0.19 (-0.28; -0.11)**	**0.00**
	3A	**-0.09 (-0.12; -0.06)**	**0.19 (0.08; 0.29)**	-0.03 (-0.13; 0.07)	**-0.18 (-0.27; -0.09)**	**0.00**
	3B	**-0.09 (-0.11; -0.06)**	**0.18 (0.07; 0.28)**	-0.03 (-0.13; 0.07)	**-0.17 (-0.26; -0.09)**	**0.00**
sE-selectin, per SD	1	0.00 (-0.03; 0.03)	**0.21 (0.10;0.32)**	-0.09 (-0.19; 0.01)	-0.06 (-0.14; 0.03)	**0.00**
	2	-0.00 (-0.03; 0.03)	**0.14 (0.04; 0.25)**	-0.04 (-0.13;0.06)	0.02 (-0.07; 0.11)	0.12
	3A	0.00 (-0.02; 0.03)	**0.13 (0.03; 0.23)**	0.01 (-0.09; 0.10)	0.04 (- 0.04; 0.13)	0.42
	3B	0.02 (-0.01; 0.04)	**0.11 (0.01; 0.21)**	0.01 (-0.08; 0.11)	0.07 (-0.01; 0.15)	0.88
vWF, per SD	1	-0.01 (-0.03; 0.02)	**0.14 (0.03; 0.25)**	-0.05 (- 0.15; 0.06)	**-0.11 (-0.20; -0.02)**	**0.00**
	2	**-0.04 (-0.06; -0.01)**	**0.13 (0.03; 0.24)**	-0.05 (-0.15; 0.05)	**-0.13 (-0.21; -0.04)**	**0.00**
	3A	**-0.04 (-0.06; -0.01)**	**0.12 (0.02; 0.23)**	-0.03 (-0.13; 0.07)	**-0.12 (-0.20; -0.03)**	**0.00**
	3B	**-0.03 (-0.06; -0.01)**	**0.12 (0.02; 0.23)**	-0.03 (-0.12; 0.07)	**-0.11 (-0.19; -0.02)**	**0.00**

Betas and 95% confidence intervals represent the difference in CSVD features, retinal microvascular diameters, or measures of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption where a negative beta indicates less CSVD features, narrower retinal microvascular diameter, or less MVD (i.e. for flicker light-induced increase in retinal arteriolar or venular diameter and individual plasma biomarkers of MVD). Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). Numerical values per SD are presented in the legend of Table 2. The numbers of participants with complete data on CSVD features, retinal microvascular diameters and plasma biomarkers of MVD respectively are n=2,075; n=2,721; and n=3,078. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, glucose metabolism status, educational level; Model 3A: model 2 + waist circumference, smoking status, diet score; Model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Bold denotes P-value<0.05. Abbreviations: β, beta; CI: confidence interval; CSVD, cerebral small vessel disease; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; SD: standard deviation; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

Table S4.11 Associations of wine, beer and spirits consumption with MVD measures in the general population and stratified by history of cardiovascular disease status.

		Wine consumption					Beer consumption					Spirits consumption				
	Model	Continuous	None vs. light	Moderate vs. light	High vs. light	P for trend	Continuous	None vs. light	Moderate vs. light	High vs. light	P for trend	Continuous	None vs. light	Moderate vs. light	High vs. light	P for trend
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P-value	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P-value	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	P-value
CSVD features composite score, per SD																
General population, n=2,075	3B	-0.01 (-0.06; 0.03)	0.08 (-0.02; 0.19)	-0.02 (-0.15; 0.11)	0.07 (-0.06; 0.19)	0.49	0.02 (-0.03; 0.07)	-0.01 (-0.11; 0.10)	-0.06 (-0.20; 0.09)	0.07 (-0.10; 0.24)	0.76	-0.08 (-0.24; 0.08)	0.01 (-0.07; 0.15)	-0.08 (-0.25 0.08)	-0.02 (-0.32; 0.28)	0.80
With CVD, n=253	3B	-0.07 (-0.20; 0.07)	-0.07 (-0.38; 0.23)	-0.21 (-0.63; 0.20)	-0.15 (-0.55; 0.25)	0.71	-0.12 (-0.34; 0.10)	0.18 (-0.14; 0.50)	-0.15 (-0.57; 0.27)	-0.03 (-0.72; 0.66)	0.17	-0.45 (-0.95; 0.06)	0.20 (-0.18; 0.58)	-0.12 (-0.75; 0.50)	-0.06 (-1.45; 1.33)	**0.04**
Without CVD, n=1822	3B	-0.00 (-0.05; 0.04)	0.10 (-0.02; 0.21)	0.01 (-0.13; 0.14)	0.11 (-0.03; 0.24)	0.73	0.03 (-0.01; 0.08)	-0.04 (-0.15; 0.07)	-0.05 (-0.21; 0.11)	0.08 (-0.10; 0.26)	0.33	0.00 (-0.17; 0.18)	-0.03 (-0.14; 0.08)	-0.09 (-0.26; 0.09)	-0.03 (-0.34; 0.28)	0.38
Retinal microvascular diameters composite score, per SD																
General population, n=2,721	3B	-0.03 (-0.07; 0.01)	0.01 (-0.09; 0.11)	**-0.13 (-0.25; -0.01)**	-0.08 (-0.20; 0.04)	**0.03**	0.02 (-0.02; 0.07)	0.00 (-0.10; 0.10)	0.03 (-0.11; 0.17)	0.02 (-0.15; 0.18)	0.61	0.07 (-0.08; 0.21)	0.02 (-0.08; 0.12)	-0.02 (-0.18; 0.13)	-0.16 (-0.43; 0.11)	0.78
With CVD, n=459	3B	**-0.13 (-0.23; -0.02)**	0.01 (-0.24; 0.25)	**-0.44 (-0.76; -0.12)**	-0.28 (-0.59; 0.03)	**0.01**	-0.05 (-0.15; 0.04)	-0.07 (-0.30; 0.16)	-0.20 (-0.54; 0.15)	-0.23 (-0.66; 0.20)	0.70	**-0.37 (-0.02; -0.73)**	-0.07 (-0.30; 0.16)	-0.20 (-0.54; 0.15)	-0.23 (-0.66; 0.20)	0.15
Without CVD, n= 2,262	3B	-0.02 (-0.06; 0.03)	0.02 (-0.09; 0.12)	-0.08 (-0.20; 0.05)	-0.04 (-0.17; 0.09)	0.23	0.04 (-0.01; 0.08)	0.03 (-0.08; 0.13)	0.07 (-0.08; 0.23)	0.07 (-0.11; 0.24)	0.48	0.01 (-0.15; 0.17)	0.05 (-0.06; 0.16)	0.00 (0.17; 0.17)	-0.17 (-0.47; 0.13)	0.77
Flicker light-induced increase in retinal diameters composite score, per SD																
General population, n=2,090	3B	-0.02 (-0.06; 0.03)	0.02 (-0.10; 0.13)	-0.05 (-0.19; 0.08)	0.04 (-0.10; 0.17)	0.99	0.00 (-0.05; 0.05)	-0.02 (-0.13; 0.10)	-0.11 (-0.28; 0.05)	0.07 (-0.12; 0.27)	0.88	-0.02 (-0.19; 0.14)	-0.02 (-0.14; 0.09)	-0.09 (-0.27; 0.09)	0.15 (-0.16; 0.46)	0.29
With CVD, n=330	3B	-0.12 (-0.24; 0.00)	0.15 (-0.14; 0.45)	-0.21 (-0.59; 0.18)	-0.17 (-0.55; 0.20)	**0.04**	-0.13 (-0.25; -0.01)	0.10 (-0.18; 0.38)	-0.13 (-0.56; 0.30)	-0.30 (-0.83; 0.23)	0.21	0.19 (-0.23; 0.60)	0.06 (-0.23; 0.34)	-0.04 (-0.50; 0.42)	0.07 (-0.69; 0.82)	0.48
Without CVD, n=1,760	3B	0.00 (-0.05; 0.05)	-0.02 (-0.14; 0.10)	-0.04 (-0.19; 0.10)	0.05 (-0.09; 0.20)	0.46	0.03 (-0.03; 0.08)	-0.04 (-0.16; 0.09)	-0.12 (-0.30; 0.06)	0.14 (-0.07; 0.35)	0.42	-0.06 (-0.24; 0.12)	-0.04 (-0.16; 0.09)	-0.12 (-0.31; 0.08)	0.15 (-0.19; 0.50)	0.39
Heat-induced skin hyperaemia, per SD																
General population, n=1,517	3B	-0.03 (-0.08; 0.03)	-0.10 (-0.24; 0.03)	-0.05 (-0.21; 0.11)	-0.12 (-0.28; 0.04)	0.98	-0.03 (-0.08; 0.03)	-0.04 (-0.16; 0.09)	-0.07 (-0.25; 0.11)	-0.17 (-0.38; 0.04)	0.29	0.03 (-0.15; 0.20)	-0.03 (-0.16; 0.10)	-0.09 (-0.28; 0.11)	-0.21 (-0.56; 0.14)	0.88
With CVD, n=265	3B	0.11 (-0.02; 0.24)	-0.18 (-0.51; 0.14)	0.10 (-0.33;0.53)	-0.01 (-0.41; 0.40)	0.20	-0.04 (-0.15;0.07)	-0.10 (-0.40 ;0.20)	-0.06 (-0.48; 0.35)	-0.43 (-0.99; 0.13)	0.49	-0.03 (-0.45; 0.39)	-0.09 (-0.39; 0.21)	-0.17 (-0.61; 0.27)	-0.57 (-1.43; 0.28)	0.66
Without CVD, n=1252	3B	-0.05 (-0.10; 0.01)	-0.10 (-0.25; 0.04)	-0.08 (-0.26; 0.09)	-0.15 (-0.32; 0.03)	0.64	-0.02 (-0.08; 0.04)	-0.03 (-0.18; 0.11)	-0.07 (-0.27; 0.13)	-0.12 (-0.35; 0.11)	0.48	0.04 (-0.16; 0.23)	-0.03 (-0.18; 0.12)	-0.08 (-0.31; 0.15)	-0.16 (-0.56; 0.25)	0.71
Logarithmically transformed UAE, per SD																
General population, n=3,107	3B	-0.03 (-0.06; 0.01)	**0.11 (0.02; 0.20)**	-0.01 (-0.12; 0.10)	0.04 (-0.07; 0.14)	0.07	-0.01 (-0.04; 0.03)	-0.01 (-0.10; 0.08)	-0.09 (-0.21; 0.04)	-0.04 (-0.18; 0.10)	0.42	-0.07 (-0.20; 0.06)	-0.00 (-0.09; 0.09)	-0.11 (-0.24; 0.03)	-0.10 (-0.34; 0.13)	0.61
With CVD, n=522	3B	-0.07 (-0.16; 0.03)	0.16 (-0.05; 0.38)	0.10 (-0.18; 0.37)	-0.05 (-0.33; 0.23)	0.18	**-0.11 (-0.19; -0.02)**	0.02 (-0.18; 0.22)	-0.22 (-0.51; 0.07)	**0.40 (-0.78; -0.03)**	**0.03**	-0.16 (-0.45; 0.14)	0.00 (-0.20; 0.21)	-0.17 (-0.49; 0.14)	-0.26 (-0.87; 0.34)	0.30
Without CVD, n=2,585	3B	-0.02 (-0.06; 0.02)	0.07 (-0.03; 0.17)	-0.05 (-0.17; 0.07)	0.03 (-0.09; 0.14)	0.18	-0.03 (-0.02; 0.07)	-0.02 (-0.12; 0.08)	-0.06 (-0.20; 0.07)	0.05 (-0.11; 0.20)	0.73	-0.03 (-0.18; 0.12)	-0.00 (-0.10; 0.10)	-0.12 (-0.27; 0.04)	-0.12 (-0.38; 0.15)	0.91

Tabel S4.11 (continued)

	Model	Wine consumption					Beer consumption					Spirits consumption				
		Continuous	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
Plasma biomarkers of MVD composite score, per SD																
General population, n=3,078	3B	**-0.08** **(-0.12; -0.05)**	0.08 (0.00; 0.17)	**-0.14** **(-0.24; -0.04)**	**-0.11** **(-0.21; -0.01)**	**0.00**	-0.03 (-0.06; 0.01)	-0.01 (-0.09; 0.08)	-0.06 (-0.18; 0.06)	-0.11 (-0.25; 0.02)	0.16	0.06 (-0.07; 0.18)	0.01 (-0.09; 0.08)	-0.06 (-0.19; 0.07)	0.11 (-0.34; 0.12)	0.46
With CVD, n=519	3B	**-0.11** **(-0.20; -0.02)**	0.12 (-0.09; 0.34)	-0.19 (-0.47; 0.09)	-0.09 (-0.37; 0.19)	**0.03**	-0.04 (-0.13; 0.05)	0.02 (-0.18; 0.23)	-0.00 (-0.30; 0.29)	-0.14 (-0.52; 0.24)	0.63	-0.01 (-0.31; 0.28)	0.01 (-0.20; 0.22)	0.03 (-0.29; 0.35)	-0.03 (-0.64; 0.59)	0.95
Without CVD, n=2,559	3B	**-0.08** **(-0.12; -0.04)**	0.06 (-0.03; 0.15)	**-0.14** **(-0.26; -0.03)**	**-0.12** **(-0.23; -0.01)**	**0.00**	-0.02 (-0.06; 0.02)	-0.02 (-0.11; 0.07)	-0.07 (-0.20; 0.06)	-0.11 (-0.26; 0.04)	0.26	0.09 (-0.06; -0.23)	-0.02 (-0.12; 0.08)	-0.08 (-0.23; 0.07)	-0.13 (-0.38; 0.13)	0.30

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of wine, beer, or spirits consumption or for none, moderate, or high versus light total wine, total beer, or total spirits consumption where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Wine, beer, and spirits consumption were entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For all analyses the value of one SD was numerically comparable to the value of one SD that was presented in the legend of Table 2 (for the general population) or Table S4.4 (stratified by history of cardiovascular disease). Variables in model 3B: age, sex, glucose metabolism status (where applicable), educational level, waist circumference, smoking status, diet score, office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease (where applicable) and, only for heat-induced skin hyperaemia, for baseline skin blood flow. Bold denotes P-value<0.05. Abbreviations: β, beta; CI: confidence interval; CSVD, cerebral small vessel disease; CVD, cardiovascular disease; SD: standard deviation; UAE, urinary albumin excretion; MVD, microvascular dysfunction; NGM, normal glucose metabolism; HDL, high-density lipoprotein.

Table S4.12 Associations of total alcohol consumption with MVD measures, stratified by history of cardiovascular disease status.

		History of cardiovascular disease					No history of cardiovascular disease				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. Light β (95% CI)**	**P for trend P-value**	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
CSVD features composite score, per SD	1	-0.05 (-0.15; 0.06)	0.22 (-0.13; 0.56)	0.08 (-0.27; 0.43)	-0.09 (-0.42; 0.24)	0.17	**0.07 (0.03; 0.10)**	-0.12 (-0.27; 0.03)	0.05 (-0.09; 0.18)	0.11 (-0.00; 0.22)	**0.00**
	2	-0.10 (-0.21; 0.01)	0.13 (-0.22; 0.48)	-0.04 (-0.38; 0.31)	-0.19 (-0.52; 0.14)	0.08	0.02 (-0.02; 0.05)	-0.08 (-0.22; 0.06)	0.00 (-0.12; 0.13)	0.02 (-0.09; 0.12)	0.27
	3A	**-0.11 (-0.22; 0.00)**	0.10 (-0.25; 0.45)	-0.06 (-0.40; 0.28)	-0.22 (-0.54; 0.11)	0.08	0.01 (-0.02; 0.05)	-0.08 (-0.21; 0.07)	0.01 (-0.12; 0.13)	0.00 (-0.11; 0.11)	0.41
	3B	**-0.12 (-0.23; -0.01)**	0.02 (-0.34; 0.37)	-0.12 (-0.46; 0.22)	-0.27 (-0.60; 0.06)	0.08	0.01 (-0.02; 0.05)	-0.07 (-0.21; 0.07)	0.01 (-0.12; 0.13)	0.01 (-0.10; 0.11)	0.40
Retinal microvascular diameters composite score, per SD	1	**-0.09 (0.16; 0.03)**	-0.13 (-0.38; 0.13)	**-0.38 (-0.64; -0.13)**	**-0.38 (-0.61; -0.14)**	**0.01**	**-0.03 (-0.06; -0.00)**	**0.16 (0.03; 0.29)**	-0.08 (-0.20; 0.04)	-0.02 (-0.12; 0.08)	**0.02**
	2	-0.06 (-0.13; 0.01)	-0.17 (-0.44; 0.09)	**-0.29 (-0.55; -0.04)**	**-0.35 (-0.59; -0.11)**	**0.03**	0.01 (-0.02; 0.04)	0.08 (-0.05; 0.21)	-0.06 (-0.18; 0.06)	0.01 (-0.10; 0.11)	0.41
	3A	-0.05 (-0.12; 0.02)	-0.19 (-0.46; 0.07)	**-0.29 (-0.55; -0.04)**	**-0.34 (-0.58; -0.10)**	0.06	0.01 (-0.03; 0.04)	0.06 (-0.07; 0.20)	-0.06 (-0.18; 0.06)	0.00 (-0.10; 0.10)	0.41
	3B	-0.05 (-0.12; 0.02)	-0.17 (-0.44; 0.09)	**-0.27 (-0.53; 0.01)**	**-0.33 (-0.57; 0.08)**	0.06	0.01 (-0.02; 0.04)	0.06 (-0.07; 0.19)	-0.04 (-0.16; 0.08)	0.01 (-0.09; 0.12)	0.62
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.06 (-0.14; 0.02)	0.15 (-0.15; 0.46)	-0.09 (-0.40; 0.22)	-0.20 (0.48; 0.08)	**0.02**	0.03 (-0.01; 0.06)	-0.05 (-0.20; 0.10)	-0.08 (-0.21; 0.06)	0.00 (-0.12; 0.12)	0.73
	2	**-0.09 (-0.17; -0.01)**	0.10 (-0.22; 0.42)	-0.18 (-0.49; 0.14)	-0.24 (0.52; 0.04)	**0.02**	0.01 (-0.03; 0.05)	-0.06 (-0.21; 0.09)	-0.07 (-0.20; 0.06)	-0.02 (-0.14; 0.10)	0.95
	3A	**-0.09 (-0.18; -0.01)**	0.10 (-0.22; 0.41)	-0.17 (-0.49; 0.15)	-0.26 (-0.54; 0.03)	**0.02**	0.01 (-0.03; 0.04)	-0.05 (-0.21; 0.10)	-0.06 (-0.20; 0.07)	-0.02 (-0.14; 0.10)	0.99
	3B	**-0.10 (-0.19; -0.02)**	0.09 (-0.23; 0.41)	-0.18 (-0.50; 0.14)	-0.29 (0.58; 0.00)	**0.01**	0.01 (-0.03; 0.05)	-0.06 (-0.21; 0.10)	-0.07 (-0.20; 0.07)	-0.02 (-0.14; 0.10)	0.93
Heat-induced skin hyperaemia	1	0.02 (-0.06; 0.09)	-0.25 (-0.59; 0.09)	-0.03 (-0.38; 0.32)	-0.14 (-0.46; -0.18)	0.71	0.02 (-0.02; 0.06)	-0.06 (-0.24; 0.12)	-0.04 (-0.20; 0.12)	-0.14 (-0.27; 0.00)	0.13
	2	0.02 (-0.06; 0.10)	**-0.37 (0.70; -0.04)**	**-0.07 (-0.40; -0.27)**	0.01 (-0.30; 0.31)	0.10	-0.03 (-0.07; 0.02)	0.02 (-0.15; 0.20)	0.01 (-0.15; 0.16)	-0.09 (-0.22; 0.05)	0.16
	3A	0.02 (-0.06; 0.10)	**-0.36 (-0.67; -0.03)**	-0.06 (-0.40; 0.28)	-0.02 (-0.33; 0.29)	0.15	-0.03 (-0.07; 0.01)	-0.01 (-0.19; 0.17)	-0.01 (-0.17; 0.14)	-0.10 (-0.24; 0.04)	0.17
	3B	0.02 (-0.06; 0.10)	**-0.36 (-0.69; -0.03)**	-0.03 (-0.37; 0.32)	-0.00 (-0.32; 0.31)	0.12	-0.03 (-0.07; 0.01)	-0.01 (-0.19; 0.17)	-0.01 (-0.16; 0.15)	-0.10 (-0.24; 0.04)	0.19

Table S4.12 (continued)

		History of cardiovascular disease					No history of cardiovascular disease				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Logarithmically transformed UAE, per SD	1	**-0.08 (-0.14; -0.02)**	**0.37 (0.14; 0.60)**	-0.11 (-0.35; 0.13)	**-0.23 (-0.45; -0.01)**	**0.00**	**0.03 (0.00; 0.06)**	-0.03 (-0.15; 0.10)	-0.10 (-0.21; 0.01)	-0.05 (-0.14; 0.05)	0.39
	2	**-0.11 (-0.17; -0.05)**	**0.25 (0.03; 0.48)**	**-0.23 (-0.46; -0.01)**	-0.21 (-0.41; 0.01)	**0.00**	0.01 (-0.02; 0.03)	-0.03 (-0.15; 0.10)	-0.07 (-0.17; 0.04)	-0.03 (-0.13; 0.06)	0.58
	3A	**-0.10 (-0.16; -0.04)**	**0.23 (0.01; 0.45)**	-0.21 (-0.44; 0.02)	-0.19 (-0.40; 0.02)	**0.00**	0.00 (-0.03; 0.03)	-0.03 (-0.15; 0.09)	-0.05 (-0.16; 0.05)	-0.04 (-0.13; 0.06)	0.61
	3B*	**-0.10 (-0.16; -0.04)**	0.18 (-0.05; 0.40)	**-0.24 (-0.46; -0.01)**	-0.20 (-0.41; 0.01)	**0.00**	0.00 (-0.03; 0.03)	-0.04 (-0.15; 0.09)	-0.06 (-0.17; 0.04)	-0.04 (-0.13; 0.06)	0.62
Plasma biomarkers of MVD composite score, per SD	1	**-0.06 (-0.12; 0.00)**	**0.54 (0.31; 0.77)**	0.16 (-0.08; 0.40)	-0.09 (-0.31; 0.13)	**0.00**	**-0.04 (-0.06; -0.01)**	**0.24 (0.12; 0.36)**	**-0.13 (-0.24; -0.02)**	**-0.15 (-0.24; -0.05)**	**0.00**
	2	**-0.08 (-0.14; -0.02)**	**0.38 (0.16; 0.60)**	0.06 (-0.17; 0.29)	-0.09 (-0.30; 0.12)	**0.00**	**-0.05 (-0.08; -0.03)**	**0.20 (0.09; 0.32)**	-0.08 (-0.19; 0.02)	**-0.12 (-0.21; -0.03)**	**0.00**
	3A	**-0.08 (-0.14; -0.02)**	**0.36 (0.14; 0.59)**	0.08 (-0.14; 0.31)	-0.10 (-0.31; 0.11)	**0.00**	**-0.05 (-0.08; -0.02)**	**0.19 (0.08; 0.30)**	-0.04 (-0.15; 0.06)	**-0.10 (-0.19; -0.01)**	**0.00**
	3B	**-0.07 (-0.13; -0.01)**	**0.38 (0.15; 0.60)**	0.12 (-0.11; 0.35)	-0.06 (-0.27; 0.16)	**0.01**	**-0.05 (-0.07; -0.02)**	**0.18 (0.07; 0.30)**	-0.04 (-0.14; 0.07)	-0.09 (-0.18; 0.00)	**0.00**

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption stratified by history of cardiovascular disease status where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). Numerical values per SD are presented in the legend of Table S4.6. The number of participants with and without a history of cardiovascular disease respectively are n=253 and n=1822 for CSVD features, n=459 and n=2,262 for retinal microvascular diameters, n=330 and n=1,760 for flicker light-induced increase in retinal microvascular diameters, n=265 and n=1,252 for heat-induced skin hyperaemia, n=522 and n=2,585 for UAE, and n=519 and n=2,559 for plasma biomarkers of MVD. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, glucose metabolism status, educational level; Model 3A: model 2 + waist circumference, smoking status, diet score; Model 3B: model 3A + office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood flow was entered in model 1. Bold denotes P-value<0.05. * For respectively individuals with and without a history of cardiovascular disease the betas of total alcohol consumption (per unit) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE: 0.89 (0.83; 0.96) and 1.00 (0.98; 1.03). Abbreviations: β, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction. HDL, high-density lipoprotein.

Table S4.13 Associations of total alcohol consumption with MVD measures, stratified by glucose metabolism status.

		Total alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Type 2 diabetes						
CSVD features composite score, per SD	1	0.04 (-0.03; 0.11)	-0.03 (-0.27; -0.22)	-0.04 (-0.31; 0.23)	0.12 (-0.12; 0.36)	0.31
	2	-0.00 (-0.07; 0.06)	0.06 (-0.19; 0.30)	-0.07 (-0.33; 0.19)	0.04 (-0.19; 0.27)	0.84
	3A	-0.01 (-0.07; 0.06)	0.04 (-0.20; 0.29)	-0.08 (-0.34; 0.18)	0.02 (-0.21; 0.25)	0.79
	3B	0.00 (-0.06; 0.07)	-0.01 (-0.26; 0.23)	-0.10 (-0.35; 0.16)	0.02 (-0.21; 0.26)	0.93
Retinal microvascular diameters composite score, per SD	1	-0.05 (0.10; -0.01)	0.10 (-0.09; 0.28)	-0.06 (-0.28; 0.16)	-0.13 (-0.32; 0.06)	0.02
	2	-0.03 (-0.08; 0.02)	0.01 (-0.19; 0.20)	-0.07 (-0.28; 0.15)	-0.10 (-0.29; 0.09)	0.23
	3A	-0.03 (-0.08; 0.02)	0.00 (-0.19; 0.20)	-0.05 (-0.27; 0.16)	-0.10 (-0.29; 0.09)	0.28
	3B	-0.01 (-0.06; 0.04)	-0.02 (-0.21; 0.18)	-0.04 (-0.26; 0.18)	-0.06 (-0.25; 0.13)	0.63
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.01 (-0.06; 0.03)	-0.05 (-0.22; 0.12)	-0.05 (-0.25; 0.15)	-0.17 (-0.35; 0.01)	0.15
	2	-0.02 (-0.07; 0.02)	-0.01 (-0.19; 0.16)	-0.04 (-0.24; 0.16)	-0.18 (-0.35; 0.00)	0.07
	3A	-0.02 (-0.07; 0.02)	-0.01 (-0.18; 0.17)	-0.04 (-0.24; 0.17)	-0.17 (-0.35; 0.01)	0.07
	3B	-0.03 (-0.07; 0.02)	0.02 (-0.16; 0.19)	-0.04 (-0.25; 0.16)	-0.17 (-0.35; 0.01)	0.05
Heat-induced skin hyperaemia, per SD	1	-0.01 (-0.07; 0.05)	-0.29 (-0.53; -0.05)	-0.38 (-0.67; -0.09)	-0.36 (-0.60; -0.11)	0.18
	2	-0.06 (-0.11; 0.00)	-0.19 (-0.34; 0.15)	-0.34 (-0.63; -0.05)	-0.33 (-0.57; -0.10)	0.01
	3A	-0.06 (-0.12; -0.00)	-0.16 (-0.40; 0.08)	-0.33 (-0.62; -0.05)	-0.35 (-0.59; -0.11)	0.03
	3B	-0.05 (-0.11; 0.01)	-0.19 (-0.44; 0.05)	-0.33 (-0.62; -0.04)	-0.32 (-0.56; -0.08)	0.08
Logarithmically transformed UAE, per SD	1	-0.03 (-0.08; 0.01)	-0.11 (-0.28; 0.07)	-0.24 (-0.44; -0.04)	-0.21 (-0.39; -0.03)	0.08
	2	-0.07 (-0.12; -0.03)	0.03 (-0.15; 0.21)	-0.24 (-0.44; -0.04)	-0.20 (0.37; -0.02)	0.00
	3A	-0.07 (-0.12; -0.03)	0.00 (-0.17; 0.18)	-0.22 (-0.42; -0.02)	-0.21 (0.38; -0.03)	0.01
	3B*	-0.07 (-0.12; -0.03)	-0.02 (-0.20; 0.15)	-0.25 (-0.45; -0.06)	-0.21 (-0.38; -0.04)	0.01
Plasma biomarkers of MVD composite score, per SD	1	-0.04 (-0.08; 0.01)	0.32 (0.15; 0.49)	0.08 (-0.13; 0.28)	-0.03 (-0.20; 0.15)	0.00
	2	-0.04 (-0.09; 0.01)	0.37 (0.19; 0.55)	0.08 (-0.12; 0.29)	-0.01 (-0.19; 0.16)	0.00
	3A	-0.03 (-0.08; 0.01)	0.37 (0.20; 0.54)	0.14 (-0.06; 0.33)	0.02 (-0.15; 0.20)	0.01
	3B	-0.02 (-0.06; 0.03)	0.31 (0.14; 0.49)	0.14 (-0.05; 0.34)	0.06 (-0.12; 0.23)	0.05

Table S4.13 (continued)

		Total alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Prediabetes						
CSVD features composite score, per SD	1	-0.00 (-0.07; 0.06)	-0.06 (-0.42; 0.29)	0.08 (-0.25; 0.40)	-0.02 (-0.29; 0.25)	0.89
	2	-0.02 (-0.09; 0.04)	0.03 (-0.31; 0.37)	0.01 (-0.30; 0.31)	-0.10 (-0.35; 0.15)	0.34
	3A	-0.02 (-0.09; 0.04)	0.03 (-0.32; 0.37)	-0.06 (-0.37; 0.25)	-0.14 (-0.39; 0.11)	0.22
	3B	-0.02 (-0.09; 0.04)	0.00 (-0.34; 0.34)	-0.11 (-0.41; 0.20)	-0.16 (-0.41; 0.09)	0.20
Retinal microvascular diameters composite score, per SD	1	-0.00 (-0.06; 0.06)	0.17 (-0.16; 0.50)	-0.20 (-0.48; 0.09)	-0.04 (-0.28; 0.21)	0.26
	2	0.04 (-0.03; 0.10)	0.12 (-0.22; 0.45)	-0.15 (-0.44; 0.14)	0.00 (-0.24; 0.24)	0.61
	3A	0.02 (-0.04; 0.08)	0.17 (-0.16; 0.49)	-0.05 (-0.34; 0.23)	0.01 (-0.23; 0.24)	0.51
	3B	0.03 (-0.03; 0.09)	0.17 (-0.15; 0.50)	-0.04 (-0.32; 0.25)	0.02 (-0.22; 0.26)	0.59
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.02 (-0.07; 0.03)	-0.02 (-0.33; 0.28)	-0.00 (-0.27; 0.27)	-0.07 (-0.29; 0.15)	0.61
	2	-0.03 (-0.08; 0.02)	0.03 (-0.28; 0.34)	-0.06 (-0.33; 0.22)	-0.14 (-0.36; 0.08)	0.17
	3A	-0.04 (-0.09; 0.02)	-0.00 (-0.32; 0.31)	-0.03 (-0.31; 0.24)	-0.13 (-0.36; 0.09)	0.24
	3B	-0.05 (-0.10; 0.01)	0.02 (-0.30; 0.34)	-0.04 (-0.32; 0.24)	-0.16 (-0.39; 0.07)	0.14
Heat-induced skin hyperaemia, per SD	1	-0.07 (-0.15; 0.02)	0.06 (-0.41; 0.53)	0.00 (-0.36; 0.36)	-0.26 (-0.59; 0.06)	0.07
	2	-0.13 (-0.22; -0.04)	0.13 (-0.34; 0.60)	-0.10 (-0.47; 0.26)	-0.33 (-0.66; 0.00)	0.02
	3A	-0.13 (-0.23; -0.04)	0.13 (-0.33; 0.60)	-0.07 (-0.43; 0.30)	-0.31 (-0.64; 0.03)	0.03
	3B	-0.13 (-0.23; -0.03)	0.13 (-0.35; 0.60)	-0.05 (-0.42; 0.32)	-0.29 (-0.63; 0.06)	0.05
Logarithmically transformed UAE, per SD	1	0.05 (-0.01; 0.11)	-0.09 (-0.40; 0.21)	0.01 (-0.26; 0.28)	0.05 (-0.18; 0.27)	0.40
	2	0.01 (-0.04; 0.07)	0.03 (-0.28; 0.34)	-0.06 (-0.33; 0.20)	-0.02 (-0.24; 0.20)	0.73
	3A	0.02 (-0.04; 0.08)	0.01 (-0.30; 0.32)	-0.07 (-0.34; 0.19)	-0.01 (-0.23; 0.22)	0.89
	3B*	0.01 (-0.04; 0.07)	-0.04 (-0.35; 0.26)	-0.14 (-0.41; 0.12)	-0.04 (-0.27; 0.18)	0.80
Plasma biomarkers of MVD composite score, per SD	1	-0.03 (-0.08; 0.03)	0.41 (0.10; 0.71)	0.12 (-0.15; 0.39)	-0.04 (-0.26; 0.18)	0.03
	2	-0.05 (-0.11; 0.01)	0.48 (0.18; 0.79)	0.07 (-0.19; 0.33)	-0.07 (-0.29; 0.15)	0.01
	3A	-0.04 (-0.10; 0.01)	0.44 (0.14; 0.73)	0.05 (-0.20; 0.31)	-0.05 (-0.27; 0.16)	0.02
	3B	-0.04 (-0.10; 0.02)	0.42 (0.12; 0.72)	0.05 (-0.21; 0.31)	-0.04 (-0.67; 0.18)	0.03

Table S4.13 (continued)

		Total alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Normal glucose metabolism						
CSVD features composite score, per SD	1	0.08 (0.04; 0.12)	-0.06 (-0.25; 0.12)	0.11 (-0.04; 0.27)	0.14 (0.00; 0.28)	0.01
	2	0.02 (-0.03; 0.06)	-0.03 (-0.21; 0.15)	0.02 (-0.13; 0.17)	0.00 (-0.13; 0.13)	0.77
	3A	0.00 (-0.04; 0.05)	-0.03 (-0.21; 0.15)	0.02 (-0.13; 0.17)	-0.03 (-0.16; 0.11)	0.92
	3B	0.01 (-0.04; 0.05)	-0.04 (-0.22; 0.14)	0.03 (-0.12; 0.17)	-0.01 (-0.14; 0.12)	0.84
Retinal microvascular diameters composite score, per SD	1	-0.07 (-0.11; -0.03)	0.11 (-0.06; 0.28)	-0.14 (-0.28; -0.00)	-0.09 (-0.21; 0.04)	0.02
	2	-0.02 (-0.06; 0.03)	0.03 (-0.14; 0.20)	-0.11 (-0.25; 0.03)	-0.07 (-0.19; 0.06)	0.14
	3A	-0.02 (-0.06; 0.03)	0.02 (-0.15; 0.19)	-0.11 (-0.25; 0.03)	-0.07 (-0.19; 0.06)	0.19
	3B	-0.01 (-0.05; 0.03)	0.05 (-0.12; 0.22)	-0.09 (-0.23; 0.05)	-0.05 (-0.17; 0.08)	0.22
Flicker light-induced increase in retinal diameters composite score, per SD	1	0.05 (0.01; 0.09)	-0.08 (-0.24; 0.09)	-0.06 (-0.18; 0.07)	0.06 (-0.05; 0.17)	0.11
	2	0.02 (-0.02; 0.07)	-0.05 (-0.21; 0.11)	-0.07 (-0.20; 0.06)	0.03 (-0.08; 0.15)	0.40
	3A	0.02 (-0.02; 0.07)	-0.05 (-0.21; 0.11)	-0.08 (-0.20; 0.05)	0.03 (-0.09; 0.14)	0.46
	3B	0.03 (-0.02; 0.07)	-0.06 (-0.22; 0.11)	-0.07 (-0.20; 0.06)	0.04 (-0.08; 0.16)	0.31
Heat-induced skin hyperaemia, per SD	1	0.08 (0.02; 0.14)	-0.14(-0.38; 0.10)	0.15 (-0.04; 0.38)	0.05 (-0.12; -0.22)	0.12
	2	0.04 (-0.02; 0.10)	-0.04 (-0.27; 0.20)	0.15 (-0.04; 0.34)	0.09 (-0.08; 0.26)	0.17
	3A	0.03 (-0.03; 0.10)	-0.05 (-0.29; 0.19)	0.13 (-0.06; 0.32)	0.07 (-0.10; 0.25)	0.22
	3B	0.04 (-0.02; 0.10)	-0.06 (-0.30; 0.18)	0.13 (-0.06; 0.32)	0.08 (-0.10; 0.25)	0.19
Logarithmically transformed UAE, per SD	1	0.05 (0.01; 0.09)	0.06 (-0.10; 0.22)	0.02 (-0.11; 0.15)	0.05 (-0.06; 0.17)	0.67
	2	0.02 (-0.02; 0.06)	0.10 (-0.07; 0.26)	-0.01 (-0.14; 0.12)	0.02 (-0.09; 0.14)	0.65
	3A	0.02 (-0.02; 0.06)	0.10 (-0.06; 0.26)	0.01 (-0.13; 0.14)	0.02 (-0.10; 0.14)	0.62
	3B*	0.02 (-0.02; 0.06)	0.07 (-0.09; 0.23)	-0.00 (-0.13; 0.13)	0.02 (-0.10; 0.13)	0.75
Plasma biomarkers of MVD composite score, per SD	1	-0.04 (-0.08; 0.00)	0.02 (-0.14; 0.18)	-0.17 (-0.30; -0.04)	-0.18 (-0.30; -0.06)	0.00
	2	-0.09 (-0.13; -0.05)	0.07 (-0.09; 0.22)	-0.20 (-0.33; -0.07)	-0.22 (-0.34; -0.11)	0.00
	3A	-0.10 (-0.14; -0.06)	0.06 (-0.10; 0.21)	-0.17 (-0.30; -0.04)	-0.22 (-0.33; -0.10)	0.00
	3B	-0.09 (-0.13; -0.05)	0.05 (-0.11; 0.20)	-0.15 (-0.28; -0.03)	-0.20 (-0.31; -0.08)	0.00

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption stratified by glucose metabolism status where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Total alcohol

consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). The numerical values per SD are presented in the legend of Supplemental Table S4.6. The number of participants with type 2 diabetes, prediabetes, and normal glucose metabolism respectively are n=459, n=321, and n=1274 for CSVD features, n=758, n=403, and n=1533 for retinal microvascular diameters, n=563, n=307, and n=1197for flicker light-induced increase in retinal microvascular diameters, n= 439, n=230, n=825 for heat-induced skin hyperaemia, n= 854, n=460, n=1760 for UAE, and n= 849, n=455, n=1742 for plasma biomarkers of MVD. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, educational level; Model 3A: model 2 + waist circumference, smoking status, diet score; Model 3B: model 3A+ office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood flow was entered in model 1. Bold denotes P-value<0.05. * For individuals with respectively type 2 diabetes, prediabetes, or normal glucose metabolism the betas of total alcohol consumption (per unit) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE: 0.92 (0.88; 0.97), 1.01 (0.96; 1.07), and 1.02 (0.98; 1.05). Abbreviations: β, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction. HDL, high-density lipoprotein.

Table S4.14 Associations of total alcohol consumption with MVD measures, stratified hypertension status.

		With hypertension					Without hypertension				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
CSVD features composite score, per SD	1	-0.02 (-0.06; 0.03)	-0.06 (-0.24; 0.12)	-0.04 (-0.21; 0.13)	-0.09 (-0.24; 0.06)	0.45	**0.15 (0.10; 0.20)**	0.03 (-0.17; 0.23)	**0.21 (0.03; 0.39)**	**0.32 (0.17; 0.48)**	**0.00**
	2	-0.03 (-0.07; 0.01)	-0.04 (-0.22; 0.14)	-0.08 (-0.24; 0.09)	-0.13 (-0.27; 0.02)	0.13	**0.08 (0.03; 0.12)**	0.04 (-0.16; 0.23)	0.12 (-0.05; 0.28)	**0.15 (0.00; 0.30)**	0.06
	3A	-0.04 (-0.08; 0.00)	-0.05 (-0.22; 0.13)	-0.07 (-0.23; 0.09)	**-0.15 (-0.29; -0.00)**	0.09	**0.06 (0.01; 0.12)**	0.05 (-0.14; 0.24)	0.11 (-0.06; 0.28)	0.12 (-0.03; 0.27)	0.19
	3B	-0.04 (-0.08; 0.01)	-0.05 (-0.23; 0.13)	-0.08 (-0.24; 0.08)	**-0.15 (-0.29; -0.00)**	0.17	**0.06 (0.01; 0.12)**	0.04 (-0.15; 0.24)	0.11 (-0.06; 0.28)	0.12 (-0.03; 0.27)	0.19
Retinal microvascular diameters composite score, per SD	1	**-0.04 (-0.00; -0.07)**	**0.24 (0.10; 0.39)**	**-0.15 (-0.29; -0.01)**	-0.05 (-0.17; 0.08)	**0.00**	**-0.05 (-0.10; -0.01)**	-0.08 (-0.27; 0.10)	-0.11 (-0.27; 0.06)	-0.12 (-0.27; 0.02)	0.22
	2	-0.01 (-0.04; 0.03)	**0.16 (0.01; 0.31)**	-0.13 (-0.27; 0.02)	-0.03 (-0.15; 0.10)	**0.03**	-0.00 (-0.05; 0.05)	-0.15 (-0.34; 0.03)	-0.07 (-0.23; 0.09)	-0.08 (-0.23; 0.06)	0.93
	3A	-0.01 (-0.04; 0.03)	0.14 (0.01; 0.30)	-0.12 (-0.26; 0.04)	-0.03 (-0.16; 0.10)	**0.04**	-0.01 (-0.06; 0.04)	-0.18 (-0.36; 0.01)	-0.07 (-0.24; 0.09)	-0.09 (-0.24; 0.05)	0.96
	3B	0.00 (-0.03; 0.04)	0.14 (0.01; 0.29)	-0.10 (-0.24; 0.04)	-0.00 (-0.13; 0.12)	0.11	-0.00 (-0.05; 0.05)	-0.16 (-0.35; 0.03)	-0.05 (-0.22; 0.11)	-0.08 (-0.23; 0.07)	0.96
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.02 (-0.06; 0.02)	-0.01 (-0.19; 0.16)	0.00 (-0.17; 0.17)	-0.13 (-0.28; 0.01)	0.09	0.05 (-0.00; 0.11)	-0.02 (-0.22; 0.19)	**-0.19 (-0.38; -0.01)**	0.07 (-0.09; 0.23)	0.48
	2	-0.03 (-0.07; 0.01)	-0.03 (-0.21; 0.15)	0.03 (-0.14; 0.19)	-0.12 (-0.27; 0.03)	0.18	0.03 (-0.03; 0.09)	-0.02 (-0.23; 0.19)	**-0.22 (-0.41; -0.04)**	0.01 (-0.16; 0.17)	0.94
	3A	-0.03 (-0.07; 0.01)	-0.04 (-0.22; 0.14)	0.04 (-0.13; 0.20)	-0.11 (-0.26; 0.03)	0.27	0.02 (-0.04; 0.07)	-0.02 (-0.23; 0.20)	**-0.24 (-0.42; -0.05)**	-0.02 (-0.19; 0.14)	0.64
	3B	-0.03 (-0.07; 0.01)	-0.05 (-0.23; 0.13)	0.02 (-0.14; 0.19)	-0.12 (-0.27; 0.03)	0.25	0.02 (-0.04; 0.08)	-0.01 (-0.22; 0.21)	**-0.22 (-0.41; -0.03)**	-0.00 (-0.17; 0.17)	0.78
Heat-induced skin hyperaemia, per SD	1	-0.01 (-0.05; 0.04)	**-0.25 (-0.44; -0.05)**	-0.08 (-0.27; 0.11)	**-0.26 (0.43; -0.09)**	0.24	0.05 (-0.01; 0.11)	0.09 (-0.17; 0.35)	-0.01 (-0.23; 0.21)	-0.01 (-0.20; 0.19)	0.59
	2	**-0.05 (-0.09; -0.00)**	-0.10 (-0.29; 0.10)	-0.02 (-0.20; 0.17)	**-0.17 (-0.33; -0.01)**	0.20	0.04 (-0.03; 0.10)	0.06 (-0.20; 0.32)	0.02 (-0.20; 0.25)	0.03 (-0.16; 0.23)	0.97
	3A	**-0.05 (-0.10; -0.01)**	-0.10 (-0.30; 0.09)	-0.02 (-0.20; 0.16)	**-0.18 (-0.34; -0.02)**	0.18	0.03 (-0.04; 0.10)	0.03 (-0.24; 0.29)	0.00 (-0.22; 0.22)	0.02 (-0.18; 0.22)	0.97
	3B	**-0.05 (-0.10; -0.01)**	-0.13 (-0.32; 0.07)	-0.02 (-0.20; 0.16)	**-0.17 (-0.34; -0.01)**	0.29	0.03 (-0.04; 0.10)	0.02 (-0.25; 0.29)	-0.01 (-0.23; 0.22)	0.02 (-0.19; 0.22)	0.96

Table S4.14 (continued)

		With hypertension					Without hypertension				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
Logarithmically transformed UAE, per SD	1	-0.01 (-0.04; 0.02)	0.05 (-0.09; 0.19)	-0.11 (-0.24; 0.02)	**-0.14 (-0.26; -0.03)**	**0.00**	0.02 (-0.03; 0.06)	0.08 (-0.09; 0.26)	-0.11 (-0.27; 0.04)	0.03 (-0.11; 0.16)	0.63
	2	-0.02 (-0.06; 0.01)	0.08 (0.06; 0.22)	-0.08 (-0.20; 0.05)	-0.08 (-0.19; 0.04)	**0.02**	0.00 (-0.05; 0.05)	0.05 (-0.13; 0.22)	-0.12 (-0.28; 0.03)	-0.01 (-0.15; 0.13)	0.47
	3A	-0.02 (-0.06; 0.01)	0.06 (-0.07; 0.20)	-0.05 (-0.18; 0.08)	-0.07 (-0.19; 0.04)	0.05	-0.01 (-0.06; 0.04)	0.06 (-0.12; 0.24)	-0.12 (-027; 0.03)	-0.04 (-0.17; 0.10)	0.24
	3B*	-0.03 (-0.06; 0.01)	0.05 (-0.09; 0.19)	-0.07 (-0.19; 0.06)	-0.08 (-0.19; 0.04)	0.05	-0.01 (-0.06; 0.04)	0.04 (-0.14; 0.21)	-0.13 (-0.28; 0.03)	-0.04 (-0.18; 0.10)	0.30
Plasma biomarkers of MVD composite score, per SD	1	**-0.05 (-0.08; -0.02)**	**0.34 (0.21; 0.48)**	-0.06 (-0.19; 0.08)	**-0.13 (0.25; -0.02)**	**0.00**	**-0.07 (-0.12; -0.03)**	**0.27 (0.10; 0.44)**	-0.13 (-0.28; 0.03)	**-0.18 (-0.31; -0.05)**	**0.00**
	2	**-0.04 (-0.01; -0.01)**	**0.31 (0.17; 0.44)**	-0.01 (-0.13; 0.12)	-0.06 (-0.17; 0.05)	**0.00**	**-0.12 (-0.16; -0.06)**	**0.23 (0.06; 0.40)**	-0.13 (-0.28; 0.02)	**-0.22 (-0.36; -0.09)**	**0.00**
	3A	**-0.04 (-0.01; -0.01)**	**0.29 (0.16; 0.42)**	0.04 (-0.08; 0.16)	-0.04 (-0.15; 0.07)	**0.00**	**-0.12 (-0.16; -0.07)**	**0.22 (0.05; 0.38)**	-0.11 (-0.26; 0.04)	**-0.21 (-0.34; -0.08)**	**0.00**
	3B	**-0.03 (-0.06; -0.00)**	**0.26 (0.13; 0.39)**	0.05 (-0.07; 0.18)	-0.01 (-0.12; 0.10)	**0.00**	**-0.11 (-0.16; -0.07)**	**0.21 (0.04; 0.37)**	-0.11 (-0.26; 0.04)	**-0.20 (-0.33; -0.07)**	**0.00**

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption stratified by glucose metabolism status where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). The numerical values per SD are presented in the legend of Supplemental Table S4.6. The number of participants with and without hypertension respectively are n=1101 and n=974 for CSVD features, n=1559 and n=1162for retinal microvascular diameters, n=1173 and n=917 for flicker light-induced increase in retinal microvascular diameters, n=898 and n=619 for heat-induced skin hyperaemia, n=1788 and n=1319 for UAE, and n=1772 and n=1306 for plasma biomarkers of MVD. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, glucose metabolism status, educational level; Model 3A: model 2 + waist circumference, smoking status, diet score; Model 3B: model 3A + office systolic blood pressure, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, history of cardiovascular disease. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood was entered in model 1. Bold denotes P-value<0.05. * For respectively individuals with and without hypertension the betas of total alcohol consumption (per unit) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE: 0.97 (0.94; 1.01) and 0.99 (0.94; 1.04). Abbreviations: β, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction. HDL, high-density lipoprotein.

Table S4.15 Associations of total alcohol consumption with MVD measures, stratified by dyslipidaemia status.

		With hypercholesterolemia					Without hypercholesterolemia				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
CSVD features composite score, per SD	1	0.02 (-0.02; 0.06)	-0.02 (-0.19; 0.15)	-0.03 (-0.19; 0.13)	-0.05 (-0.18; 0.09)	0.62	**0.09 (0.04; 0.14)**	0.01 (-0.21; 0.24)	**0.22 (0.02; 0.42)**	**0.29 (0.12; 0.47)**	**0.00**
	2	-0.01 (-0.05; 0.03)	0.02 (-0.15; 0.18)	-0.04 (-0.19; 0.10)	-0.07 (-0.19; 0.06)	0.23	0.02 (-0.03; 0.07)	-0.02 (-0.23; 0.19)	0.10 (-0.09; 0.28)	0.10 (-0.07; 0.26)	0.16
	3A	-0.02 (-0.06; 0.02)	0.02 (-0.14; 0.18)	-0.05 (-0.20; 0.10)	-0.09 (-0.21; 0.04)	0.13	0.02 (-0.04; 0.07)	-0.02 (-0.23; 0.19)	0.12 (-0.07; 0.30)	0.08 (-0.09; 0.25)	0.23
	3B	-0.01 (-0.06; 0.03)	-0.02 (-0.18; 0.15)	-0.06 (-0.20; 0.09)	-0.07 (-0.20; 0.05)	0.29	0.02 (-0.03; 0.07)	-0.04 (-0.26; 0.17)	0.12 (-0.07; 0.31)	0.10 (-0.08; 0.27)	0.13
Retinal microvascular diameters composite score, per SD	1	**-0.05 (0.09; -0.02)**	**0.15 (0.01; 0.29)**	**-0.17 (-0.30; -0.03)**	-0.07 (-0.19; 0.05)	**0.00**	-0.03 (-0.07; 0.02)	0.03 (-0.17; 0.24)	-0.08 (-0.26; 0.10)	-0.10 (-0.25; 0.06)	0.13 0.82
	2	-0.02 (-0.06; 0.02)	0.06 (-0.09; 0.20)	**-0.14 (-0.27; -0.01)**	-0.06 (-0.18; 0.05)	0.06	0.02 (-0.02; 0.07)	-0.02 (-0.23; 0.18)	-0.05 (-0.23; 0.13)	-0.03 (-0.19; 0.14)	0.15 0.72
	3A	-0.02 (-0.06; 0.02)	0.04 (-0.11; 0.18)	**-0.13 (-0.27; -0.00)**	-0.06 (-0.18; 0.06)	0.10	0.02 (-0.03; 0.06)	-0.03 (-0.24; 0.18)	-0.06 (-0.24; 0.13)	-0.06 (-0.22; 0.11)	
	3B	-0.01 (-0.05; 0.02)	0.05 (-0.10; 0.19)	-0.11 (-0.24; 0.03)	-0.04 (-0.15; 0.08)	0.19	0.02 (-0.03; 0.07)	-0.02 (-0.23; 0.19)	-0.05 (-0.23; 0.14)	-0.04 (-0.20; 0.13)	
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.02 (-0.06; 0.02)	0.01 (-0.16; 0.17)	-0.06 (-0.22; 0.09)	-0.13 (-0.26; 0.01)	**0.04**	**0.05 (0.00; 0.11)**	-0.07 (-0.31; 0.16)	-0.14 (-0.34; 0.07)	0.11 (-0.07; 0.29)	0.14
	2	-0.03 (-0.07; 0.01)	0.01 (-0.16; 0.17)	-0.05 (-0.21; 0.10)	-0.12 (-0.25; 0.02)	0.06	0.03 (-0.02; 0.09)	-0.09 (-0.33; 0.15)	-0.15 (-0.35; 0.06)	0.07 (-0.12; 0.25)	0.31
	3A	-0.04 (-0.08; 0.01)	0.01 (-0.15; 0.18)	-0.05 (-0.20; 0.10)	-0.13 (-0.26; 0.01)	0.05	0.03 (-0.02; 0.09)	-0.09 (-0.33; 0.16)	-0.14 (-0.34; 0.07)	0.08 (-0.11; 0.26)	0.29
	3B	-0.03 (-0.08; 0.01)	-0.00 (-0.17; 0.16)	-0.06 (-0.22; 0.09)	-0.13 (-0.27; 0.01)	0.06	0.03 (-0.03; 0.09)	-0.07 (-0.31; 0.17)	-0.13 (-0.34; 0.08)	0.07 (-0.12; 0.26)	0.34
Heat-induced skin hyperaemia, per SD	1	0.02 (-0.02; 0.06)	-0.16 (-0.35; 0.02)	-0.05 (-0.22; 0.12)	-0.14 (-0.30; 0.01)	0.57	-0.01 (-0.08; 0.06)	0.10 (-0.20; 0.39)	-0.04 (-0.30; 022)	-0.15 (-0.38; 0.07)	0.07
	2	-0.02 (-0.06; 0.02)	-0.06 (-0.24; 0.12)	-0.00 (-0.17; 0.16)	-0.07 (-0.22; 0.08)	0.65	-0.03 (-0.10; 0.05)	0.06 (-0.24; 0.35)	-0.01 (-0.28; 0.25)	-0.11 (-0.34; 0.12)	0.23
	3A	-0.03 (-0.07; 0.02)	-0.08 (-0.26; 0.10)	-0.02 (-0.19; 0.14)	-0.10 (-0.24; 0.05)	0.53	-0.02 (-0.09; 0.06)	0.02 (-0.28; 0.32)	-0.06 (-0.32; 0.21)	-0.10 (-0.33; 0.14)	0.34
	3B	-0.03 (-0.07; 0.02)	-0.09 (-0.27; 0.09)	-0.02 (-0.18; 0.15)	-0.09 (-0.24; 0.06)	0.61	-0.01 (-0.09; 0.07)	0.01 (-0.29; 0.31)	-0.05 (-0.32; 0.22)	-0.07 (-0.31; 0.17)	0.52

Table S4.15 (continued)

		With hypercholesterolemia					Without hypercholesterolemia				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
Logarithmically transformed UAE, per SD	1	-0.01 (-0.04; 0.02)	0.04 (-0.09; 0.17)	-0.09 (-0.21; 0.04)	**-0.13 (-0.24; -0.02)**	**0.00**	0.03 (-0.01; 0.08)	**0.20 (0.00; 0.39)**	-0.15 (-0.32; 0.02)	0.00 (-0.15; 0.15)	0.11
	2	-0.03 (-0.06; 0.00)	0.03 (-0.09; 0.16)	-0.05 (-0.17; 0.07)	-0.07 (-0.17; 0.04)	0.08	0.00 (-0.04; 0.05)	0.16 (-0.04; 0.35)	-0.16 (-0.32; 0.01)	-0.03 (-0.18; 0.12)	0.07
	3A	-0.03 (-0.06; 0.00)	0.02 (-0.11; 0.15)	-0.04 (-0.16; 0.08)	-0.07 (-0.17; 0.04)	0.12	0.00 (-0.05; 0.05)	0.16 (-0.03; 0.35)	-0.13 (-0.30; 0.04)	-0.03 (-0.18; 0.12)	0.08
	3B	-0.03 (-0.06; 0.00)	-0.01 (-0.14; 0.12)	-0.06 (-0.18; 0.06)	-0.07 (-0.18; 0.03)	0.17	0.00 (-0.05; 0.05)	0.14 (-0.05; 0.33)	-0.13 (-0.29; 0.04)	-0.04 (-0.19; 0.12)	0.10
Plasma biomarkers of MVD composite score, per SD	1	**-0.06 (-0.09; -0.03)**	**0.35 (0.23; 0.48)**	-0.06 (-0.18; 0.06)	**-0.14 (-0.25; -0.03)**	**0.00**	-0.03 (-0.07; 0.02)	**0.27 (0.08; 0.47)**	-0.11 (-0.28; 0.06)	-0.16 (-0.31; 0.02)	**0.00**
	2	**-0.06 (-0.09; -0.03)**	**0.31 (0.18; 0.44)**	-0.01 (-0.13; 0.10)	-0.08 (-0.18; 0.03)	**0.00**	**-0.07 (-0.11; -0.03)**	0.17 (-0.02; 0.35)	-0.11 (-0.27; 0.05)	**-0.22 (-0.37; -0.08)**	**0.00**
	3A	**-0.06 (-0.09; -0.03)**	**0.30 (0.18; 0.42)**	0.01 (-0.10; 0.13)	-0.07 (-0.17; 0.03)	**0.00**	**-0.07 (-0.11; -0.03)**	0.15 (-0.03; 0.33)	-0.06 (-0.22; 0.10)	**-0.19 (-0.33; -0.05)**	**0.00**
	3B	**-0.05 (-0.08; -0.02)**	**0.27 (0.15; 0.39)**	0.02 (-0.09; 0.14)	-0.04 (-0.14; 0.06)	**0.00**	**-0.05 (-0.09; -0.01)**	0.12 (-0.06; 0.30)	-0.04 (-0.20; 0.11)	**-0.16 (-0.30; 0.02)**	**0.00**

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption stratified by dyslipidaemia status where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). The numerical values per SD are presented in the legend of Supplemental Table S4.6. The number of participants with and without hypertension respectively are n=1301 and n=774 for CSVD features, n=1780 and n=941 for retinal microvascular diameters, n=1350 and n=740 for flicker light-induced increase in retinal microvascular diameters, n=1055 and n=462 for heat-induced skin hyperaemia, n=2060 and n=1047 for UAE, and n=2038 and n=1040 for plasma biomarkers of MVD. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, glucose metabolism status, educational level; Model 3A: model 2 + waist circumference, smoking status, diet score; Model 3B: model 3A + office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, history of cardiovascular disease. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood was entered in model 1. Bold denotes P-value<0.05. * For respectively individuals with and without dyslipidaemia the betas of total alcohol consumption (per unit) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE: 0.97 (0.94; 1.00) and 1.00 (0.96; 1.04). Abbreviations: β, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction. HDL, high-density lipoprotein.

Table S4.16 Associations of total alcohol consumption with MVD measures, stratified by current smoking status.

		Current smoker					Former or never smoker				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
CSVD features composite score, per SD	1	0.05 (-0.03; 0.13)	0.12 (-0.25; 0.50)	**0.49 (0.09; 0.90)**	0.09 (-0.25; 0.42)	0.76	**0.04 (0.01; 0.08)**	-0.03 (-0.18; 0.12)	-0.01 (-0.14; 0.12)	0.06 (-0.05; 0.17)	0.20
	2	0.03 (-0.05; 0.11)	-0.02 (-0.37; 0.34)	**0.44 (0.07; 0.81)**	0.02 (-0.29; 0.33)	0.62	-0.01 (-0.04; 0.03)	-0.01 (-0.14; 0.13)	-0.06 (-0.18; 0.06)	-0.03 (-0.13; 0.08)	0.61
	3A	0.03 (-0.05; 0.11)	-0.00 (-0.35; 0.34)	**0.44 (0.07; 0.81)**	0.02 (-0.29; 0.32)	0.67	-0.02 (-0.05; 0.02)	0.00 (-0.13; 0.14)	-0.06 (-0.18; 0.06)	-0.05 (-0.15; 0.06)	0.33
	3B	0.04 (-0.04; 0.12)	-0.10 (-0.44; 0.25)	**0.41 (0.05; 0.77)**	0.02 (-0.28; 0.33)	0.37	-0.01 (-0.04; 0.03)	-0.01 (-0.15; 0.13)	-0.06 (-0.18; 0.06)	-0.03 (-0.14; 0.08)	0.58
Retinal microvascular diameters composite score, per SD	1	-0.00 (-0.07; 0.07)	0.22 (-0.09; 0.52)	-0.14 (-0.47; 0.19)	0.15 (-0.13; 0.43)	0.76	**-0.05 (-0.08; -0.02)**	0.07 (-0.06; 0.20)	**-0.14 (-0.25; -0.02)**	**-0.12 (-0.21; -0.02)**	**0.00**
	2	0.02 (-0.05; 0.08)	0.25 (-0.06; 0.55)	-0.05 (-0.36; 0.27)	0.21 (-0.06; 0.48)	0.92	-0.01 (-0.05; 0.02)	-0.02 (-0.14; 0.11)	-0.11 (-0.22; 0.01)	-0.09 (-0.19; 0.01)	0.07
	3A	0.02 (-0.05; 0.08)	0.25 (-0.06; 0.55)	-0.03 (-0.35; 0.29)	0.21 (-0.06; 0.48)	0.89	-0.01 (-0.05; 0.02)	-0.01 (-0.14; 0.11)	-0.11 (-0.22; 0.01)	-0.09 (-0.19; 0.01)	0.07
	3B	0.03 (-0.04; 0.10)	0.23 (-0.08; 0.53)	-0.00 (-0.32; 0.32)	0.25 (-0.03; 0.52)	0.58	-0.01 (-0.04; 0.02)	-0.00 (-0.13; 0.13)	-0.08 (-0.20; 0.03)	-0.07 (-0.17; 0.03)	0.14
Flicker light-induced increase in retinal diameters composite score, per SD	1	-0.01 (-0.08; 0.07)	-0.15 (-0.51; 0.21)	-0.15 (-0.53; 0.23)	-0.32 (-0.64; 0.00)	0.16	0.01 (-0.02; 0.05)	0.02 (-0.12; 0.16)	-0.08 (-0.21; 0.06)	0.00 (-0.10; 0.12)	0.75
	2	-0.02 (-0.10; 0.06)	-0.18 (0.55; 0.20)	-0.12 (-0.51; 0.27)	-0.32 (-0.65; 0.00)	0.19	-0.01 (-0.04; 0.03)	-0.00 (-0.15; 0.15)	-0.08 (-0.21; 0.05)	-0.01 (-0.13; 0.10)	0.70
	3A	-0.02 (-0.10; 0.06)	-0.17 (-0.55; 0.21)	-0.11 (-0.50; 0.28)	-0.32 (-0.64; 0.01)	0.20	-0.01 (-0.05; 0.03)	0.00 (-0.15; 0.15)	-0.08 (-0.20; 0.06)	-0.02 (-0.14; 0.10)	0.64
	3B	-0.02 (-0.11; 0.06)	-0.17 (-0.55; 0.21)	-0.13 (-0.52; 0.27)	-0.36 (-0.69; 0.02)	0.15	-0.01 (-0.05; 0.03)	-0.01 (-0.15; 0.14)	-0.08 (-0.21; 0.05)	-0.02 (-0.13; 0.10)	0.72
Heat-induced skin hyperaemia, per SD	1	0.01 (-0.07; 0.10)	0.10 (-0.32; 0.53)	-0.29 (-0.74; 0.17)	-0.09 (-0.50; 0.31)	0.25	0.01 (-0.03; 0.05)	-0.15 (-0.32; 0.02)	-0.02 (-0.17; 0.13)	-0.16 (-0.29; 0.03)	0.31
	2	-0.02 (-0.10; 0.07)	0.08 (0.35; 0.50)	-0.14 (-0.58; 0.30)	-0.05 (-0.43; 0.33)	0.51	-0.03 (-0.07; 0.01)	-0.10 (-0.26; 0.07)	0.00 (-0.15; 0.15)	-0.10 (-0.24; 0.03)	0.48
	3A	-0.02 (-0.10; 0.06)	0.07 (-0.35; 0.50)	-0.14 (-0.58; 0.30)	-0.06 (-0.44; 0.33)	0.50	-0.03 (-0.07; 0.01)	-0.09 (-0.26; 0.07)	-0.00 (-0.15; 0.15)	-0.11 (-0.24; 0.03)	0.46
	3B	-0.03 (-0.12; 0.06)	-0.12 (-0.31; 0.55)	-0.17 (-0.61; 0.28)	-0.09 (-0.49; 0.31)	0.30	-0.03 (-0.07; 0.02)	-0.10 (-0.27; 0.07)	0.01 (-0.14; 0.15)	-0.10 (-0.23; 0.04)	0.57

Table S4.16 (continued)

		Current smoker					Former or never smoker				
	Model	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**	**Continuous**	**None vs. light**	**Moderate vs. light**	**High vs. light**	**P for trend**
		β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**P-value**
Logarithmically transformed UAE, per SD	1	-0.03 (-0.09; 0.03)	0.07 (-0.21; 0.35)	**-0.31 (-0.61; -0.01)**	**-0.24 (-0.49; -0.02)**	**0.01**	0.01 (-0.02; 0.04)	0.07 (-0.04; 0.19)	-0.08 (-0.18; 0.03)	-0.07 (-0.16; 0.03)	**0.01**
	2	-0.05 (-0.11; 0.01)	-0.08 (0.34; 0.20)	**-0.29 (-0.56; -0.01)**	**-0.24 (-0.48; -0.01)**	0.06	-0.02 (-0.04; 0.01)	0.07 (-0.05; 0.18)	-0.06 (-0.16; 0.05)	-0.04 (-0.13; 0.05)	0.08
	3A	-0.04 (-0.10; 0.02)	-0.07 (-0.33; 0.20)	-0.25 (-0.52; 0.02)	-0.22 (-0.45; 0.01)	0.08	-0.02 (-0.05; 0.01)	0.07 (-0.04; 0.19)	-0.04 (-0.14; 0.06)	-0.04 (-0.13; 0.06)	0.08
	3B*	-0.04 (-0.10; 0.02)	-0.06 (-0.32; 0.21)	-0.27 (-0.54; 0.01)	-0.23 (-0.47; 0.01)	0.06	-0.02 (-0.04; 0.01)	0.04 (-0.07; 0.15)	-0.05 (-0.16; 0.05)	-0.04 (-0.13; 0.05)	0.16
Plasma biomarkers of MVD composite score, per SD	1	-0.04 (-0.19; 0.02)	**0.35 (0.07; 0.64)**	-0.02 (-0.32; 0.28)	-0.10 (-0.36; 0.16)	**0.00**	**-0.05 (-0.08; -0.02)**	**0.33 (0.21; 0.44)**	-0.09 (-0.19; 0.02)	**-0.16 (-0.25; -0.07)**	**0.00**
	2	-0.04 (-0.11; 0.02)	0.22 (-0.06; 0.51)	0.01 (-0.28; 0.30)	-0.10 (-0.35; 0.15)	**0.03**	**-0.07 (-0.10; -0.04)**	**0.27 (0.16; 0.39)**	-0.06 (-0.16; 0.04)	**-0.13 (-0.22; -0.04)**	**0.00**
	3A	-0.03 (-0.09; 0.03)	0.23 (-0.04; 0.50)	0.06 (-0.22; 0.36)	-0.08 (-0.31; 0.17)	0.05	**-0.07 (-0.10; -0.04)**	**0.27 (0.16; 0.38)**	-0.02 (-0.12; 0.07)	**-0.11 (-0.20; -0.02)**	**0.00**
	3B	-0.02 (-0.08; 0.04)	0.21 (-0.06; 0.49)	0.08 (-0.20; 0.36)	-0.04 (-0.28; 0.21)	0.13	**-0.06 (-0.09; -0.03)**	**0.24 (0.14; 0.35)**	-0.01 (-0.11; 0.08)	-0.09 (-0.17; 0.00)	**0.00**

Betas and 95% confidence intervals represent the difference in CSVD features composite score, retinal microvascular diameters composite score, or (composite score of) measure(s) of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption stratified by smoking status where a negative beta indicates less CSVD features, narrower retinal microvascular diameters, or less MVD (i.e. for the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score). Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). The numerical values per SD are presented in the legend of Supplemental Table S4.6. The number of participants with and without current smoking respectively are 236 and n=1,839 for CSVD features, n=333 and n=2,388 for retinal microvascular diameters, n=249 and n=1,841 for flicker light-induced increase in retinal microvascular diameters, n=176 and n=1,341 for heat-induced skin hyperaemia, n=395 and n=2,712 for UAE, and n=391 and n=2,687 for plasma biomarkers of MVD. Variables per model: Model 1: crude; Model 2: model 1+ age, sex, glucose metabolism status, educational level; Model 3A: model 2 + waist circumference, smoking status (former/never; only applicable for former or never smoker stratum), diet score; Model 3B: model 3A + office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, history of cardiovascular disease. Additionally, and only for heat-induced skin hyperaemia, baseline skin blood was entered in model 1. * For respectively current and non-current smokers the betas of total alcohol consumption (per unit) in model 3B correspond with the following odds ratios (95% CI) for 30mg/24-hour greater UAE: 0.96 (0.90; 1.02) and 0.98 (0.96; 1.01). Abbreviations: β, beta; CI, confidence interval; SD: standard deviation; CSVD, cerebral small vessel disease; UAE, urinary albumin excretion; MVD, microvascular dysfunction. HDL, high-density lipoprotein.

Table S4.17 Associations of total alcohol consumption with the total MVD composite score and MVD measures, additionally adjusted for physical activity, diabetic retinopathy, eGFR, and plasma biomarkers of low-grade inflammation.

		Total alcohol consumption				
	Model	Continuous β (95% CI)	None vs. light β (95% CI)	Moderate vs. light β (95% CI)	High vs. light β (95% CI)	P for trend P-value
Total MVD composite score, per SD	3B+ physical activity, n=2422	**-0.04 (-0.07; -0.02)**	**0.12 (0.01; 0.23)**	**-0.10 (-0.20;- 0.00)**	**-0.11 (-0.20; -0.02)**	**0.00**
	3B+ diabetic retinopathy, n=2903	**-0.04 (-0.07; -0.02)**	0.08 (-0.02; 0.18)	**-0.10 (-0.19; -0.01)**	**-0.13 (-0.21; -0.05)**	**0.00**
	3B + eGFR, n=3096	**-0.04 (-0.07; -0.02)**	**0.13 (0.03; 0.22)**	-0.08 (-0.17; -0.00)	**-0.08 (-0.16; -0.00)**	**0.00**
	3B + biomarkers of LGI, n=3092	**-0.04 (-0.07; -0.02)**	**0.11 (0.02; 0.21)**	**-0.10 (-0.18; -0.01)**	**-0.11 (-0.19; -0.03)**	**0.00**
CSVD features composite score, per SD	3B+ physical activity, n=1614	-0.01 (-0.05; 0.03)	-0.01 (-0.16; 0.13)	-0.03 (-0.16; 0.10)	-0.04 (-0.13; 0.10)	0.80
	3B+ diabetic retinopathy, n=1743	-0.00 (-0.04; 0.03)	-0.01 (-0.14; 0.12)	-0.01 (-0.13; 0.10)	-0.03 (-0.13; 0.08)	0.73
	3B + eGFR, n=2061	0.00 (-0.03; 0.03)	-0.02 (-0.14; 0.11)	-0.00 (-0.12; 0.11)	-0.02 (-0.12; 0.09)	0.92
	3B + biomarkers of LGI, n=2061	-0.00 (-0.03; 0.03)	-0.03 (-0.16; 0.10)	0.00 (-0.11; 0.11)	-0.02 (-0.12; 0.08)	0.96
Retinal microvascular diameters composite score, per SD	3B+ physical activity, n=2213	-0.00 (-0.04; 0.03)	0.03 (-0.10; 0.16)	-0.05 (-0.17; 0.07)	-0.03 (-0.14; 0.07)	0.31
	3B+ diabetic retinopathy, n=2552	0.00 (-0.03; 0.03)	0.02 (-0.10; 0.14)	-0.08 (-0.19; 0.02)	-0.03 (-0.13; 0.06)	0.28
	3B + eGFR, n=2701	0.00 (-0.03; 0.03)	0.02 (-0.10; 0.14)	-0.09 (-0.19; 0.02)	-0.04 (-0.13; 0.06)	0.24
	3B + biomarkers of LGI, n=2696	-0.00 (-0.03; 0.03)	0.02 (-0.10; 0.14)	-0.08 (-0.19; 0.03)	-0.04 (-0.13; 0.06)	0.24
Flicker light-induced increase in retinal diameters composite score, per SD	3B+ physical activity, n=1790	-0.02 (-0.05; 0.02)	-0.03 (-0.18; 0.12)	-0.06 (-0.20; 0.07)	-0.05 (-0.17; 0.06)	0.46
	3B+ diabetic retinopathy, n=2030	-0.01 (-0.04; 0.03)	-0.02 (-0.16; 0.11)	-0.08 (-0.20; 0.05)	-0.04 (-0.15; 0.07)	0.52
	3B + eGFR, n=2078	-0.01 (-0.05; 0.02)	-0.03 (-0.16; 0.11)	-0.09 (-0.22; 0.03)	-0.06 (-0.17; 0.05)	0.33
	3B + biomarkers of LGI, n=2074	-0.01 (-0.04; 0.03)	-0.02 (-0.16; 0.11)	-0.09 (-0.21; 0.04)	-0.05 (-0.16; 0.06)	0.44
Heat-induced skin hyperaemia, per SD	3B+ physical activity, n=1267	-0.02 (-0.06; 0.02)	-0.13 (-0.30; 0.04)	-0.07 (-0.22; 0.09)	-0.12 (-0.26; 0.02)	0.46
	3B+ diabetic retinopathy, n=1373	-0.03 (-0.07; 0.01)	-0.07 (-0.23; 0.09)	-0.02 (-0.16; 0.13)	-0.12 (-0.25; 0.01)	0.25
	3B + eGFR, n=1507	-0.02 (-0.06; 0.02)	-0.07 (-0.22; 0.09)	0.00 (-0.14; 0.14)	-0.08 (-0.21; 0.05)	0.53
	3B + biomarkers of LGI, n=1506	-0.02 (-0.06; 0.02)	-0.08 (-0.23; 0.08)	-0.01 (-0.15; 0.13)	-0.08 (-0.21; 0.04)	0.55
Logarithmically transformed UAE, per SD	3B+ physical activity, n=2408	-0.02 (-0.04; 0.01)	0.08 (-0.04; 0.19)	**-0.11 (-0.22; -0.01)**	-0.03 (-0.13; 0.07)	0.08
	3B+ diabetic retinopathy, n=2610	-0.02 (-0.04; 0.01)	0.03 (-0.08; 0.14)	-0.06 (-0.16; 0.04)	-0.05 (-0.14; 0.04)	0.10
	3B + eGFR, n=3082	-0.01 (-0.04; 0.01)	0.04 (-0.07; 0.14)	-0.08 (-0.17; 0.02)	-0.05 (-0.13; 0.04)	0.09
	3B + biomarkers of LGI, n=3079	-0.02 (-0.05; 0.01)	0.03 (-0.07; 0.14)	-0.08 (-0.18; 0.01)	-0.06 (-0.15; 0.02)	**0.04**
Plasma biomarkers of MVD composite score, per SD	3B+ physical activity, n=2392	**-0.05 (-0.08; -0.02)**	**0.25 (0.14; 0.37)**	0.02 (-0.08; 0.13)	-0.08 (-0.17; 0.02)	**0.00**
	3B+ diabetic retinopathy, n=2590	**-0.05 (-0.08; -0.03)**	**0.21 (0.11; 0.32)**	-0.00 (-0.10; 0.09)	**-0.09 (-0.18; -0.01)**	**0.00**
	3B + eGFR, n=3071	**-0.04 (-0.06; -0.01)**	**0.25 (0.15; 0.35)**	0.02 (-0.07; 0.11)	-0.03 (-0.11; 0.05)	**0.00**
	3B + biomarkers of LGI, n=3077	**-0.05 (-0.08; -0.03)**	**0.23 (0.13; 0.32)**	-0.00 (-0.09; 0.09)	-0.07 (-0.15; 0.00)	**0.00**

Betas and 95% confidence intervals represent the difference in the total MVD composite score or per measure of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption where a negative beta indicates less total MVD, less CSVD features, narrower retinal microvascular diameters, or less MVD estimated from the

flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score. Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For all analyses the value of one SD was numerically comparable to the value of one SD that was presented in the legend of Table 4.2. Variables in model 3B: age, sex, glucose metabolism status, educational level, waist circumference, smoking status, diet score; office systolic blood pressure, use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease and, only for heat-induced skin hyperaemia, baseline skin blood flow. Bold denotes P-value<0.05. Abbreviations: β, beta; CI: confidence interval; CSVD, cerebral small vessel disease; SD: standard deviation; eGFR, estimated glomerular filtration rate; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

Table S4.18 Associations of total alcohol consumption with CSVD features, retinal microvascular diameters, and measures of MVD, where educational level was replaced with income level (model 3C) or occupational status (model 3D); glucose metabolism status was replaced with fasting plasma glucose (model 3E), or 2-hour post load (model 3F) or HbA1c (model 3G); or office systolic blood pressure was replaced with office diastolic blood pressure (model 3H), 24-hour ambulatory systolic blood pressure (model 3I) or 24-hour ambulatory diastolic blood pressure (model 3J).

		Total alcohol consumption				
	Model	**Continuous β (95% CI)**	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Total MVD composite score, per SD	3C, n= 2382	**-0.05 (-0.07; -0.02)**	**0.14 (0.02; 0.25)**	**-0.14 (-0.25; -0.04)**	**-0.12 (-0.21; -0.03)**	**0.00**
	3D, n= 2594	**-0.05 (-0.07; -0.02)**	**0.12 (0.01; 0.22)**	-0.08 (-0.18; 0.01)	**-0.12 (-0.21; -0.04)**	**0.00**
	3E, n= 3119	**-0.05 (-0.07; -0.02)**	**0.13 (0.04; 0.23)**	**-0.10 (-0.19; -0.01)**	**-0.12 (-0.20; -0.04)**	**0.00**
	3F, n= 2880	**-0.03 (-0.06; -0.00)**	0.09 (-0.02; 0.20)	-0.09 (-0.18; 0.01)	-0.09 (-0.18; 0.01)	**0.00**
	3G, n= 3112	**-0.04 (-0.06; -0.01)**	**0.12 (0.02; 0.21)**	**-0.09 (-0.18; -0.00)**	**-0.11 (-0.19; -0.03)**	**0.00**
	3H, n= 3120	**-0.04 (-0.07; -0.02)**	**0.11 (0.00; 0.22)**	-0.10 (-0.20; 0.00)	**-0.11 (-0.20; 0.02)**	**0.00**
	3I, n= 2770	**-0.05 (-0.08; -0.02)**	**0.12 (0.01; 0.22)**	**-0.13 (-0.22; -0.04)**	**-0.14 (-0.22; -0.05)**	**0.00**
	3J, n= 2770	**-0.05 (-0.08; -0.02)**	**0.12 (0.01; 0.22)**	**-0.13 (-0.22; -0.04)**	**-0.14 (-0.22; -0.05)**	**0.00**
CSVD features composite score, per SD	3C, n= 1628	-0.00 (-0.04; 0.03)	0.00 (-0.15; 0.15)	-0.04 (-0.17; 0.09)	-0.02 (-0.13; 0.10)	0.72
	3D, n= 1770	0.01 (-0.03; 0.04)	-0.03 (-0.17; 0.11)	-0.04 (-0.16; 0.08)	-0.02 (-0.13; 0.08)	0.86
	3E, n= 2075	-0.00 (-0.04; 0.03)	-0.02 (-0.15; 0.11)	0.00 (-0.12; 0.11)	-0.03 (-0.13; 0.08)	0.78
	3F, n= 1961	0.01 (-0.03; 0.04)	-0.02 (-0.15; 0.12)	-0.00 (-0.12; 0.11)	-0.00 (-0.11; 0.10)	0.87
	3G, n= 2071	-0.00 (-0.03; 0.03)	-0.02 (-0.14; 0.11)	0.00 (-0.11; 0.12)	-0.02 (-0.12; 0.08)	0.86
	3H, n= 2075	-0.00 (-0.03; 0.03)	-0.02 (-0.15; 0.11)	-0.00 (-0.11; 0.11)	-0.02 (-0.12; 0.08)	0.83
	3I, n= 1852	-0.02 (-0.05; 0.02)	-0.02 (-0.16; 0.12)	-0.05 (-0.17; 0.07)	-0.06 (-0.17; 0.05)	0.35
	3J, n= 1852	-0.02 (-0.05; 0.02)	-0.02 (-0.15; 0.12)	-0.04 (-0.16; 0.08)	-0.06 (-0.17; 0.05)	0.33
Retinal microvascular diameters composite score, per SD	3C, n= 2092	-0.01 (-0.05; 0.02)	0.01 (-0.13; 0.15)	-0.11 (-0.23; 0.01)	-0.07 (-0.18; 0.04)	0.12
	3D, n= 2280	-0.00 (-0.04; 0.03)	0.00 (-0.13; 0.13)	-0.11 (-0.22; 0.01)	-0.06 (-0.16; 0.04)	0.18
	3E, n= 2720	-0.01 (-0.03; 0.02)	0.03 (-0.08; 0.15)	-0.09 (-0.20; 0.02)	-0.05 (-0.14; 0.05)	0.11
	3F, n= 2515	-0.00 (-0.03; 0.03)	0.03 (-0.10; 0.15)	-0.07 (-0.18; 0.04)	-0.03 (-0.13; 0.07)	0.27
	3G, n= 2715	-0.00 (-0.03; 0.03)	0.03 (-0.09; 0.15)	-0.09 (-0.20; 0.02)	-0.04 (-0.14; 0.05)	0.14
	3H, n= 2721	0.00 (-0.03; 0.03)	0.01 (-0.11; 0.12)	-0.09 (-0.19; 0.02)	-0.04 (-0.13; 0.06)	0.30
	3I, n= 2424	-0.01 (-0.04; 0.02)	0.01 (-0.11; 0.13)	-0.10 (-0.21; 0.02)	-0.04 (-0.14; 0.06)	0.30
	3J, n= 2424	-0.01 (-0.04; 0.03)	0.00 (-0.12; 0.13)	-0.11 (-0.21; 0.01)	-0.04 (-0.14; 0.06)	0.33
Flicker light-induced increase in retinal microvascular diameters composite score, per SD	3C, n= 1629	-0.01 (-0.05; 0.03)	-0.03 (-0.19; 0.12)	-0.10 (-0.24; 0.04)	-0.06 (-0.18; 0.07)	0.47
	3D, n= 1757	-0.00 (-0.04; 0.03)	-0.08 (-0.23; 0.08)	-0.05 (-0.19; 0.08)	-0.04 (-0.16; 0.08)	0.97
	3E, n= 2090	-0.01 (-0.04; 0.03)	-0.03 (-0.17; 0.10)	-0.09 (-0.21; 0.04)	-0.06 (-0.17; 0.05)	0.42
	3F, n= 1942	-0.01 (-0.05; 0.02)	-0.03 (-0.17; 0.12)	-0.08 (-0.20; 0.05)	-0.05 (-0.17; 0.06)	0.48
	3G, n= 2087	0.01 (-0.04; 0.03)	-0.04 (-0.17; 0.10)	-0.08 (-0.21; 0.04)	-0.05 (-0.16; 0.06)	0.53
	3H, n= 2090	-0.01 (-0.04; 0.03)	-0.03 (-0.16; 0.11)	-0.08 (-0.20; 0.04)	-0.05 (-0.16; 0.06)	0.45
	3I, n= 1862	-0.02 (-0.06; 0.02)	-0.05 (-0.19; 0.10)	-0.15 (-0.28; -0.02)	-0.11 (-0.23; 0.00)	0.10
	3J, n= 1862	-0.02 (-0.06; 0.02)	-0.05 (-0.19; 0.09)	**-0.15 (-0.28; -0.02)**	-0.11 (-0.22; 0.01)	0.13

Table S4.18 (continued)

	Model	Total alcohol consumption				
		Continuous β (95% CI)	**None vs. light β (95% CI)**	**Moderate vs. light β (95% CI)**	**High vs. light β (95% CI)**	**P for trend P-value**
Heat-induced skin hyperaemia, per SD	3C, n= 1168	-0.03 (-0.07; 0.01)	-0.02 (-0.20; 0.16)	-0.07 (-0.23; 0.09)	-0.11 (-0.25; 0.04)	0.17
	3D, n= 1271	-0.04 (-0.08; 0.01)	-0.08 (-0.25; 0.10)	-0.00 (-0.15; 0.15)	-0.11 (-0.25; 0.03)	0.34
	3E, n= 1517	-0.03 (-0.06; 0.01)	-0.06 (-0.22; 0.09)	-0.03 (-0.17; 0.11)	-0.11 (-0.23; 0.02)	0.28
	3F, n= 1395	-0.01 (-0.05; 0.03)	-0.04 (-0.20; 0.13)	0.01 (-0.14; 0.15)	-0.05 (-0.19; 0.08)	0.63
	3G, n= 1516	-0.02 (-0.06; 0.02)	-0.07 (-0.22; 0.09)	-0.01 (-0.15; 0.13)	-0.09 (-0.22; 0.03)	0.43
	3H, n= 1517	-0.02 (-0.06; 0.02)	-0.07 (-0.22; 0.09)	-0.01 (-0.15; 0.13)	-0.08 (-0.21; 0.04)	0.52
	3I, n= 1335	-0.03 (-0.07; 0.01)	-0.08 (-0.24; 0.09)	-0.02 (-0.17; 0.13)	**-0.15 (-0.29; -0.02)**	0.13
	3J, n= 1335	-0.03 (-0.07; 0.01)	-0.08 (-0.24; 0.09)	-0.01 (-0.16; 0.14)	**-0.14 (-0.28; -0.01)**	0.18
Logarithmically transformed UAE, per SD	3C, n= 2381	-0.02 (-0.05; 0.01)	0.03 (-0.09; 0.16)	-0.07 (-0.18; 0.04)	-0.05 (-0.16; 0.04)	0.11
	3D, n= 2598	-0.03 (-0.05; 0.00)	0.04 (-0.08; 0.15)	-0.08 (-0.18; 0.02)	-0.07 (-0.16; 0.02)	**0.04**
	3E, n= 3106	-0.02 (-0.05; 0.00)	0.04 (-0.06; 0.14)	-0.09 (-0.18; 0.01)	-0.07 (-0.15; 0.02)	**0.02**
	3F, n= 2868	-0.00 (-0.03; 0.03)	0.01 (-0.10; 0.12)	-0.04 (-0.15; 0.06)	-0.02 (-0.11; 0.07)	0.48
	3G, n= 3100	-0.02 (-0.04; 0.01)	0.03 (-0.07; 0.13)	-0.07 (-0.17; 0.02)	-0.05 (-0.13; 0.03)	0.08
	3H, n= 3107	-0.02 (-0.04; 0.01)	0.04 (-0.06; 0.15)	-0.07 (-0.17; 0.03)	-0.05 (-0.14; 0.03)	0.05
	3I, n= 2760	**-0.03 (-0.06; -0.00)**	0.05 (-0.06; 0.16)	-0.08 (-0.18; 0.02)	-0.07 (-0.16; 0.01)	**0.01**
	3J, n= 2760	-0.03 (-0.06; 0.00)	0.06 (-0.05; 0.17)	-0.06 (-0.16; 0.04)	-0.06 (-0.15; 0.03)	**0.03**
Plasma biomarkers of MVD composite score, per SD	3C, n= 2357	**-0.05 (-0.08; -0.02)**	**0.28 (0.16; 0.40)**	-0.03 (-0.14; 0.07)	-0.07 (-0.16; 0.02)	**0.00**
	3D, n= 2575	**-0.06 (-0.09; -0.03)**	**0.27 (0.16 0.38)**	0.02 (-0.08; 0.12)	-0.08 (-0.17; 0.01)	**0.00**
	3E, n= 3077	**-0.05 (-0.07; -0.03)**	**0.25 (0.15; 0.35)**	-0.00 (-0.09; 0.09)	**-0.08 (-0.16; -0.00)**	**0.00**
	3F, n= 2838	**-0.05 (-0.08; -0.02)**	**0.18 (0.07; 0.29)**	-0.03 (-0.12; 0.07)	**-0.10 (-0.18; -0.01)**	**0.00**
	3G, n= 3070	**-0.05 (-0.07; -0.02)**	**0.24 (0.14; 0.33)**	0.01 (-0.08; 0.10)	-0.07 (-0.15; 0.02)	**0.00**
	3H, n= 3078	**-0.05 (-0.08; -0.03)**	**0.24 (0.14; 0.34)**	0.00 (-0.09; 0.09)	-0.08 (-0.16; 0.00)	**0.00**
	3I, n= 2732	**-0.05 (-0.08; -0.03)**	**0.24 (0.13; 0.34)**	-0.03 (-0.13; 0.06)	-0.09 (-0.17; 0.00)	**0.00**
	3J, n= 2732	**-0.05 (-0.08; -0.02)**	**0.24 (0.13; 0.34)**	-0.03 (-0.13; 0.07)	-0.09 (-0.18; 0.00)	**0.00**

Betas and 95% confidence intervals represent the difference in the total MVD composite score or per measure of MVD (all per SD) per unit of total alcohol consumption or for none, moderate, or high versus light total alcohol consumption where a negative beta indicates less total MVD, less CSVD features, narrower retinal microvascular diameters, or less MVD estimated from the flicker light-induced increase in retinal microvascular diameters composite score, heat-induced skin hyperaemia, UAE, or the plasma biomarkers of MVD composite score. Total alcohol consumption was entered in the models as a continuous variable (per unit, i.e. 10 g/day), as dummies (none, moderate or high versus light alcohol consumption) or (for the P-for trend analyses) as a categorical variable (none, light, moderate, and high alcohol consumption). For all analyses the value of one SD was numerically comparable to the value of one SD that was presented in the legend of Table 4.2. Variables in model 3B: age, sex, glucose metabolism status (where applicable), educational level (where applicable), waist circumference, smoking status, diet score, office systolic blood pressure (where applicable), use of antihypertensive medication, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, prior cardiovascular disease and, only for heat-induced skin hyperaemia, for baseline skin blood flow. Bold denotes P-value<0.05. Abbreviations: β, beta; CI: confidence interval; CSVD, cerebral small vessel disease; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; SD: standard deviation; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor; MVD, microvascular dysfunction; HDL, high-density lipoprotein.

CHAPTER 5

Higher levels of daily physical activity are associated with better skin microvascular function in type 2 diabetes – The Maastricht Study

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Abstract

Objective

Physical activity may provide a means for the prevention of cardiovascular disease via improving microvascular function. Therefore, this study investigated whether physical activity was associated with skin and retinal microvascular function.

Methods

In The Maastricht Study, a population-based cohort study enriched with Type 2 diabetes (T2D; n=1,298, 47.3% women, aged 60.2±8.1 years, 29.5% T2D), we studied whether accelerometer-assessed physical activity and sedentary time were associated with skin and retinal microvascular function. Associations were studied by linear regression and adjusted for major cardiovascular risk factors. In addition, we investigated whether associations differed in strength between individuals with and without T2D.

Results

In individuals with T2D, total and higher-intensity physical activity were independently associated with greater heat-induced skin hyperaemia (regression coefficients per hour, [95%CI], 10 [1; 18] and 36 [14; 58] perfusion units, respectively). In individuals without T2D total and higher-intensity physical activity were not associated with heat-induced skin hyperaemia. No associations with retinal arteriolar %-dilation were identified.

Conclusion

Higher levels of total and higher-intensity physical activity were associated with greater skin microvascular vasodilation in individuals with, but not without, T2D.

Introduction

Microvascular dysfunction is an important underlying mechanism in common diseases, such as heart failure.[1] (lacunar) stroke[2], depression[3], cognitive decline[4], chronic kidney disease[5], and neuropathy[6], which occur in the general population and more frequently in individuals with type 2 diabetes (T2D).[7] It is therefore important to identify, in particular modifiable, factors that contribute to the development of microvascular dysfunction, as these can be targeted in preventive strategies.

Physical inactivity may be one such modifiable risk factor, as may sedentary behaviour, which refers to activities that do not increase energy expenditure substantially above the resting level, such as sitting, watching television and using the computer.[8]

Indeed, low levels of physical activity and high levels of sedentary time have been shown to be associated with *macrovascular* diseases[9] (e.g. stroke, myocardial infarction, and peripheral arterial disease) and may act through inducing large artery endothelial dysfunction.[10,11] Low levels of physical activity and high levels of sedentary time have also been shown to be associated with diseases that may be partly or wholly of microvascular origin.[12-18] Thus, we hypothesized that low levels of physical activity and high levels of sedentary time may affect microvascular (endothelial) function. In support, several small (mostly intervention) studies have shown beneficial effects of physical activity[19-22], and adverse effects of sedentary behavior[19,23-26], on microvascular function. However, it is unclear whether daily life habitual physical activity, as compared to exercise programs[20,22], also beneficially affects microvascular function. Earlier studies were conducted in small numbers of individuals[19-26], which limits translation to the general population, or were based on self-reported measures of physical activity and sedentary behavior[27] which are known to be less precise and valid than objective measures.[28]

Mechanistically, higher levels of physical activity and lower levels of sedentary time are thought to increase nitric oxide bioavailability[29,30], presumably in conjunction with beneficial effects on low-grade inflammation[31,32], both of which may improve microvascular (endothelial) function.[33] As hyperglycaemia has detrimental effects on nitric oxide bioavailability[34] and microvascular (endothelial) function[35], the associations of physical activity and sedentary behaviour with microvascular (endothelial) function may differ between (i.e. may be stronger in) individuals with as compared to those without T2D.

In view of these considerations, we investigated, in a population-based study with oversampling of individuals with T2D, whether objectively measured physical activity and sedentary behaviour were associated with skin microvascular and retinal arteriolar (endothelial) function. In addition, we examined whether these associations differed between individuals with and without T2D. We chose skin and retina for two reasons.

First, these sites are easily accessible, enabling direct and reproducible[36,37] assessment of microvascular (endothelial) function, as measured by heat-induced skin hyperaemia and flicker light-induced retinal arteriolar dilation. Second, skin and retina may differ in their associations with physical activity and sedentary behaviour, with skin potentially showing stronger associations. This is because skin is important in disseminating exercise-produced heat[38], whereas retinal blood flow, in contrast, has been shown to be relatively stable during exercise[39,40], presumably to maintain visual performance.[41]

Materials and methods

Study population and design

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously[42]. In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of T2D and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2D status, with an oversampling of individuals with T2D, for reasons of efficiency. The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Assessment of glucose metabolism status

To assess glucose metabolism status, all participants (except those who used insulin) underwent a standardized 2-h 75 gram oral glucose tolerance test (OGTT) after an overnight fast. For safety reasons, participants with a fasting glucose level above 11.0 mmol/L, as determined by a finger prick, did not undergo the OGTT. For these individuals fasting glucose level and information about diabetes medication use were used to assess glucose metabolism status. Glucose metabolism status was defined according to the World Health Organization 2006 criteria as normal glucose metabolism (NGM), impaired fasting glucose, impaired glucose tolerance (combined as

prediabetes) and T2D. Individuals without type 1 diabetes on glucose-lowering medication were classified as having T2D[42].

Assessment of microvascular function

All participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement. A light meal (breakfast and (or) lunch), low in fat content, was allowed if taken at least 90 minutes prior to the start of the measurements.

Skin blood flow measurements were performed in a climate-controlled room at 24°C with participants in a supine position. Skin blood flow was measured as described previously by means of a laser-Doppler system (Periflux 5000, Perimed, Järfälla, Sweden), equipped with a thermostatic laser-Doppler probe (PF457) at the dorsal side of the wrist of the left hand. The laser-Doppler output was recorded for 25 minutes with a sample rate of 32Hz, which gives semi-quantitative assessment of skin blood flow expressed in arbitrary perfusion units (PU). Skin blood flow was first recorded unheated for 2 minutes to serve as a baseline. After the 2 minutes of baseline, the temperature of the probe was rapidly and locally increased to 44°C, and was then kept constant until the end of the registration. In contrast to earlier work[43], heat-induced skin hyperaemia was expressed not as a percentage of baseline but as perfusion during the 23 minutes heating phase (in PU), because spurious associations between determinants and heat-induced skin hyperaemia expressed as percentage may occur when determinants are associated with baseline skin blood flow (as was the case here; see Supplemental results and Supplemental Table S5.3). To investigate associations of heat-induced skin hyperaemia independently of associations with baseline skin blood flow, we adjusted for baseline flow in the regression models (this will not lead to autocorrelation because there was only a weak association between baseline skin blood flow and heat-induced skin hyperaemia).[44,45]

For retinal measurements pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine at least 15 minutes prior to the start of the examination. Retinal arteriolar vasodilation to flicker light exposure was measured by the Dynamic Vessel Analyzer (Imedos, Jena, Germany). Briefly, a baseline recording of 50 seconds was followed by 40-second flicker light exposure followed by a 60-second recovery period. Baseline diameter was calculated as the average diameter size of the 20-50 seconds recording and was expressed in measurement units (MU). As determinants were not associated with retinal arteriolar baseline diameter (see Supplemental results and Supplemental Table S5.4), flicker light-induced retinal arteriolar dilation was expressed as a percentage over baseline, based on the average dilation achieved at time points 10 and 40 seconds during the flicker stimulation period. The measurement has extensively

been described previously[43] and more details are provided in the Supplemental material.

Assessment of physical activity and sedentary behaviour

Daily activity levels and patterns were measured using the activPAL3™ physical activity monitor (PAL technologies, Glasgow, UK) as has been described previously[46] and in the Supplemental Methods. Participants were asked to wear the accelerometer for 8 consecutive days, without removing the device at any time. The total amount of physical activity (stepping time) was based on the stepping posture and calculated as the mean time spent stepping during waking time per day, standing time was not included. The method used to determine waking time has been described elsewhere.[46] Higher-intensity physical activity was defined as hours with a step frequency $\geq$110 steps/min during waking time[47,48]; again standing time was not included. The total amount of sedentary time was based on the sedentary posture (sitting or lying) and calculated as the mean time spend in a sedentary position during waking time per day.[46]

Measurement of covariates

A history of cardiovascular disease (CVD), smoking status (never, former, current), alcohol consumption, level of education, occupational status, daily energy intake, mobility limitation, and duration of diabetes were assessed by questionnaire.[42] Mobility limitation was obtained from the EuroQol-5D questionnaire and was defined as having difficulty walking 500 meter or climbing the stairs in the previous week. Energy intake was obtained from a food frequency questionnaire and calculated as the mean energy intake (kcal) per day. Use of antihypertensive, lipid-modifying, and glucose-lowering medication was assessed during a medication interview where generic name, dose, and frequency were registered.[49] We measured height, weight, waist circumference, office and ambulatory 24-h blood pressure, plasma glucose levels, serum creatinine, 24-h urinary albumin excretion (twice), glycated haemoglobin A1c (HbA1c), and plasma lipid profile as described elsewhere.[42] Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Estimated glomerular filtration rate (eGFR; in mL/min/1.73m^2) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CDK-epi) equation based on both serum creatinine and serum cystatin C.[50] The presence of retinopathy was based on fundus photographs taken with an auto fundus camera (Model AFC-230, Nidek, Gamagori, Japan).

Statistical analyses

All analyses were performed with Statistical Package for Social Sciences version 23.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). Multivariable linear regression analyses were used to investigate the associations of total physical activity (h/day), higher-intensity physical activity (h/day), and total sedentary time (h/day) with baseline skin blood flow, baseline retinal arteriolar diameter, heat-induced skin hyperaemia, and flicker light-induced retinal arteriolar %-dilation. Model 1 was adjusted for age, sex, glucose metabolism status, baseline skin blood flow (only for analyses of heat-induced skin hyperaemia), and waking time (to exclude the possibility that estimated effects were influenced by differences in waking hours). Model 2 was additionally adjusted for potential confounders such as body mass index, educational level, mobility limitation, office systolic blood pressure, total-to-high-density lipoprotein (HDL) cholesterol ratio, triglycerides, smoking status, alcohol consumption, the use of antihypertensive- and/or lipid-modifying medication, and a history of cardiovascular disease. In model 3a higher-intensity physical activity was additionally adjusted for total sedentary time. In model 3b total sedentary time was additionally adjusted for higher-intensity physical activity. Data were expressed as regression coefficients and corresponding 95% confidence interval (95%CI).

The Maastricht Study by design oversampled individuals with T2D, and we therefore were able to investigate potential interactions between total physical activity, higher-intensity physical activity, and total sedentary time with T2D by adding interaction terms to the regression models. In stratified analyses in individuals without T2D, model 1 was additionally adjusted for prediabetes status. To assess whether associations differed by sex or age, interactions between total physical activity, higher-intensity physical activity, and total sedentary time with sex and age were investigated. In these analyses, interaction terms were the product of total physical activity, higher-intensity physical activity, or total sedentary time and T2D, sex, or age, respectively.

Collinearity diagnostics (i.e. tolerance <0.10 and/or variance inflation factor >10) were used to detect multicollinearity between covariates. A P-value <0.05 was considered statistically significant except for interaction analyses, where a $P_{interaction}$<0.10 was considered statistically significant.

Results

Characteristics of study population

From the initial 3,451 participants included, those with other types of diabetes than T2D were excluded (n=41). Of the remaining 3,410 participants, heat-induced skin

hyperaemia data were available in 1,647. The reasons for missing data were logistical (n=1,650, specified as no laser-Doppler equipment available [n=353], or no trained researcher available [n=264], or technical failure [n=1,033]) or insufficient measurement quality (n=113). Accelerometery variables were missing in 272 participants and data on potential confounders were missing in 77 participants. The heat-induced skin hyperaemia study population thus consisted of 1,298 participants. Retinal arteriolar reactivity data was available in 2,261 participants. The reasons for missing data were logistical (n=882, specified as no Dynamic Vessel Analyzer equipment available [n=535], or no trained researcher available [n=227], or no eye drops given for traffic safety reasons [n=120]), insufficient measurement quality (n=208) or contraindications (n=59). Accelerometery variables were missing in 330 participants, particularly due to device availability (n=233), and data on potential confounders were missing in 84 participants. The retinal arteriolar reactivity study population thus consisted of 1,847 participants (Figure 5.1 shows the flow chart).
General characteristics of skin reactivity study population are shown in Table 5.1, according to tertiles of total physical activity. This study population had a mean age ± standard deviation (SD) of 60.2±8.1 years, of whom 46.3% were women and 29.5% had T2D (oversampled by design). In addition, when compared to individuals in the lowest tertile of total physical activity, those in the middle and highest tertiles were on average younger, had lower glucose levels, and were less likely to have had a history of cardiovascular disease (Table 5.1). The skin study population overlapped for 73% with the retinal study population. Both populations were comparable with regard to age, sex, and cardio-metabolic risk profile (Table 5.1). Individuals excluded from the analyses due to missing data on skin or retinal reactivity measurements, accelerometery measurements or covariates were also highly comparable to individuals included in the study populations with regard to age, sex, and cardio-metabolic risk profile (Supplemental Tables S5.1 and S5.2).

Associations of total and higher-intensity physical activity with skin hyperaemia and retinal arteriolar dilation

In adjusted analyses, total physical activity and higher-intensity physical activity were not significantly associated with heat-induced skin hyperaemia (Table 5.2). However, the associations between total physical activity and higher-intensity physical activity with heat-induced skin hyperaemia differed between individuals without and with T2D ($P_{interaction}<0.10$; Table 5.2). In individuals without T2D, total and higher-intensity physical activity were not significantly associated with heat-induced skin hyperaemia.

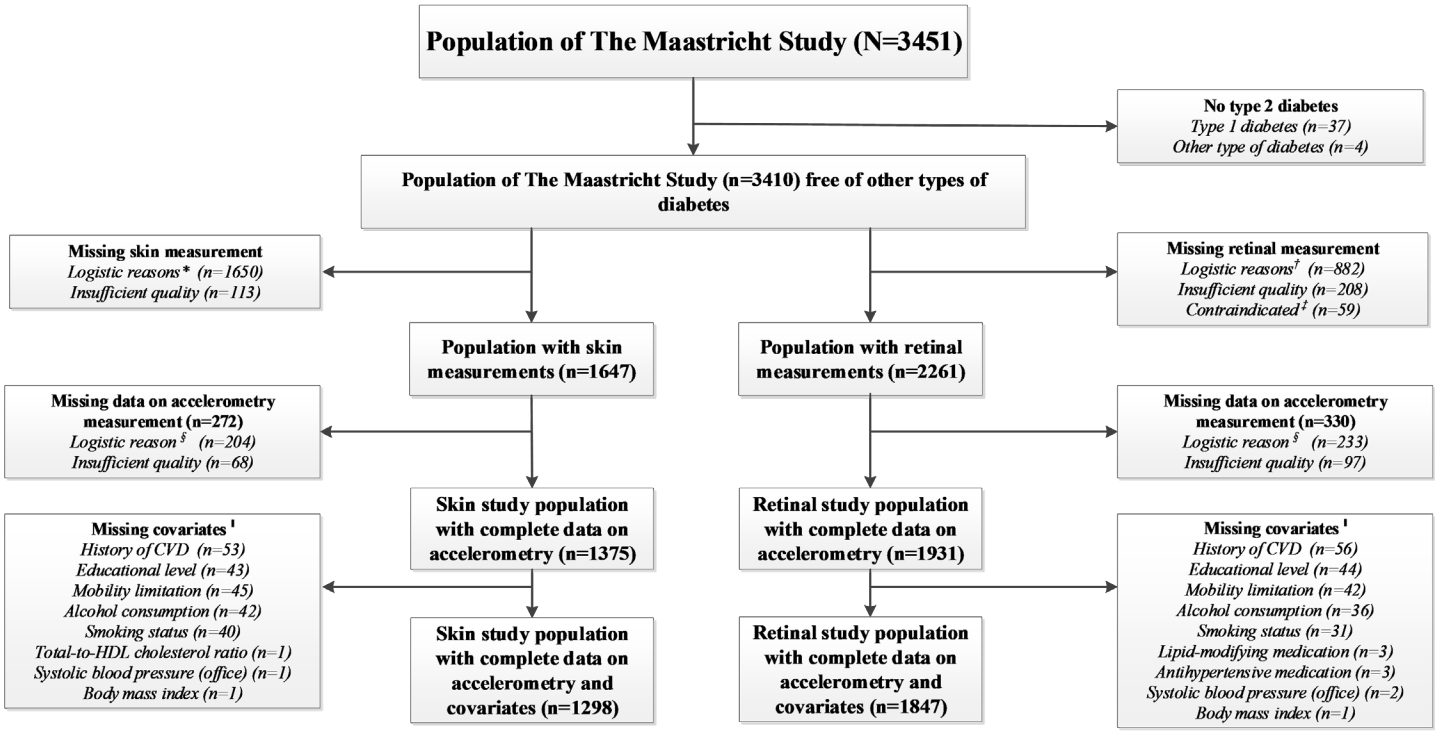

Figure 5.1 Depicts skin and retinal study population selection.
Symbols *=Logistical reasons: no laser-Doppler equipment available (n=353), no trained researcher available (n=264), technical failure (n=1033). †=Logistical reasons: no Dynamic Vessel Analyzer equipment available (n=535), no trained researcher available (n=227), no eye drops given for traffic safety reasons (n=120); ‡=Contraindicated: history of epilepsy (n=14), allergy to eye drops (n=31), glaucoma or lens implants (n=14); §=No accelerometer available. ‖=Missing values on covariates were not mutually exclusive.

Table 5.1 General characteristics of the skin and retina study populations according to sex-specific tertiles of total physical activity.

	Skin study population			Retinal study population		
Characteristic	**Tertile 1 of total physical activity (highest) n=432**	**Tertile 2 of total physical activity n=434**	**Tertile 3 of total physical activity (lowest) n=432**	**Tertile 1 of total physical activity (highest) n=616**	**Tertile 2 of total physical activity n=616**	**Tertile 3 of total physical activity (lowest) n=615**
Range of total physical activity (h/day) in men	2.21-4.69	1.52-2.21	0.22-1.52	2.25-4.67	1.56-2.25	0.19-1.56
Range of total physical activity (h/day) in women	2.27-5.38	1.73-2.27	0.30-1.73	2.33-5.38	1.77-2.32	0.30-1.77
Age (years)	59.0±7.5	60.4±8.0	61.1±8.5	58.8±7.7	59.5±8.2	60.5±8.4
Women	200 (46.3)	201 (46.3)	200 (46.3)	298 (48.4)	298 (48.4)	298 (48.5)
Educational level						
Low	131 (30.3)	128 (29.5)	173 (40.0)	185 (30.0)	177 (28.7)	230 (37.4)
Medium	113 (26.2)	130 (30.0)	116 (26.9)	163 (26.5)	190 (30.8)	182 (29.6)
High	188 (43.5)	176 (40.6)	143 (33.1)	268 (43.5)	249 (40.4)	203 (33.0)
Occupational status						
Unemployed	207 (55.1)	213 (56.6)	241 (65.1)	271 (50.5)	285 (53.4)	332 (61.7)
Employed	169 (44.9)	163 (43.4)	129 (34.9)	266 (49.5)	249 (46.6)	206 (38.3)
Glucose metabolism status						
Normal glucose metabolism	277 (64.1)	244 (56.2)	178 (41.2)	413 (67.0)	369 (59.9)	274 (44.6)
Prediabetes	73 (16.9)	83 (19.1)	60 (13.9)	93 (15.1)	106 (17.2)	87 (14.1)
Type 2 diabetes	82 (19.0)	107 (24.7)	194 (44.9)	110 (17.9)	141 (22.9)	254 (41.3)
Type 2 diabetes duration (years)	4.0 [2.5-7.0]	5.0 [2.0-11.0]	8.0 [3.0-12.5]	5.0 [3.0-9.5]	5.0 [2.0-10.0]	7.0 [3.0-13.0]
Body mass index (kg/m^2)	25.9±3.7	26.6±3.8	28.4±5.1	25.7±3.8	26.5±3.9	28.3±5.2
Waist circumference (cm)						
Men	97.0±10.0	100.4±10.0	106.5±13.3	96.4±10.0	100.3±9.9	106.7±13.6
Women	87.1±10.2	88.7±11.5	94.1±14.8	85.9±10.9	88.2±11.2	92.8±14.1
History of cardiovascular disease	59 (13.7)	72 (16.6)	96 (22.2)	69 (11.2)	94 (15.3)	123 (20.0)
Limited mobility	37 (8.6)	56 (12.9)	114 (26.4)	52 (8.4)	68 (11.0)	164 (26.7)
Office SBP (mmHg)	134.1±18.4	136.5±17.3	137.4±18.9	133.8±17.9	135.2±18.1	135.9±18.1
Office DBP (mmHg)	76.1±9.3	77.3±9.9	76.4±9.6	75.9±9.9	76.9±10.0	76.4±9.9
Ambulatory 24-h SBP (mmHg)	120.9±11.2	120.1±12.1	120.0±11.8	118.7±11.0	118.7±11.5	117.7±11.7
Ambulatory 24-h DBP (mmHg)	74.7±7.2	73.7±7.3	74.1±7.5	73.3±7.1	73.3±7.2	72.8±7.2
Smoking						
Never / former / current	169/227/36	140/259/35	120/228/84	265/304/47	211/345/60	173/326/116
% (never / former / current)	39.1/52.5/8.3	32.3/59.7/8.1	27.8/52.8/19.4	43.0/49.4/7.6	34.3/56.0/9.7	28.1/53.0/18.9

Table 5.1 (continued)

Characteristic	Skin study population			Retinal study population		
	Tertile 1 of total physical activity (highest) n=432	Tertile 2 of total physical activity n=434	Tertile 3 of total physical activity (lowest) n=432	Tertile 1 of total physical activity (highest) n=616	Tertile 2 of total physical activity n=616	Tertile 3 of total physical activity (lowest) n=615
Alcohol consumption (high)	142 (32.9)	113 (26.0)	95 (22.0)	182 (29.5)	145 (23.5)	135 (22.0)
Energy intake (kcal/day)	2221.3±586.1	2193.8±560.8	2101.3±586.6	2245.9±619.1	2155.3±566.6	2083.0±571.4
Fasting glucose (mmol/L)	5.8±1.2	6.0±1.5	6.5±2.0	5.8±1.4	5.9±1.4	6.5±2.0
2-h postload glucose (mmol/L)	7.3±4.0	7.9±4.2	9.2±4.7	7.1±3.7	7.6±4.0	8.9±4.6
HbA1c (%)	5.8±0.6	5.9±0.8	6.2±1.1	5.7±0.7	5.8±0.7	6.1±1.1
HbA1c (mmol/mol)	39.5±6.9	40.7±8.3	44.1±12.2	38.7±8.0	39.5±8.1	43.1±11.5
Total-to-HDL cholesterol ratio	3.5±1.0	3.7±1.2	3.8±1.2	3.3±1.0	3.6±1.2	3.8±1.2
Total cholesterol (mmol/L)	5.4±1.1	5.3±1.2	5.0±1.2	5.4±1.1	5.3±1.2	5.1±1.2
HDL cholesterol (mmol/L)	1.6±0.5	1.5±0.5	1.4±0.5	1.7±0.5	1.6±0.5	1.4±0.5
LDL cholesterol (mmol/L)	3.2±1.0	3.1±1.1	2.9±1.1	3.1±0.9	3.1±1.1	2.9±1.1
Triglycerides (mmol/L)	1.3±0.7	1.4±1.0	1.7±1.0	1.2±0.7	1.4±0.9	1.6±0.9
Antihypertensive medication use	137 (31.7)	176 (40.6)	239 (55.3)	175 (28.4)	233 (37.8)	307 (49.9)
Lipid-modifying medication use	132 (30.6)	161 (37.1)	219 (50.7)	172 (27.9)	205 (33.3)	275 (44.7)
Diabetes medication use						
Any type	54 (12.5)	87 (20.0)	163 (37.7)	79 (12.8)	102 (16.6)	210 (34.1)
Insulin	6 (1.4)	19 (4.4)	59 (13.7)	18 (2.9)	20 (3.2)	69 (11.1)
Oral glucose-lowering medication	52 (12.0)	84 (19.4)	149 (34.5)	72 (11.7)	97 (15.7)	196 (31.9)
eGFR (mL/min/1.73m^2)	91.2±13.4	88.1±14.1	85.2±15.8	91.1±13.0	89.0±14.4	85.0±15.4
eGFR<60 mL/min/1.73m^2	7 (1.6)	15 (3.5)	28 (6.6)	7 (1.1)	26 (4.2)	44 (7.2)
(Micro)albuminuria*	26 (6.1)	26 (6.0)	59 (13.8)	42 (6.9)	34 (5.6)	77 (12.4)
Retinopathy	3 (0.8)	4 (1.0)	10 (2.6)	5 (0.8)	6 (1.0)	14 (2.3)
Baseline skin blood flow before heating (PU)#	11.9±7.2	10.5±5.7	10.8±6.3	12.0±7.3	10.5±5.8	10.6±5.5
Skin hyperaemic response (%)#						
Mean ± SD	1077.2±731.2	1219.5±804.7	1083.5±763.6	1064.5±743.2	1204.7±771.1	1118.8±799.0
Median [interquartile range]	960.6 [570.8-1462.3]	1074.0 [636.8-1630.1]	910.5 [575.4-1407.2]	960.6 [557.8-1416.5]	1052.1 [642.7-1606.4]	979.0 [585.0-1464.3]
Skin hyperaemia during heating (PU)#						
Mean ± SD	114.8±58.0	116.3±59.0	106.3±54.8	114.2±58.7	115.0±57.5	108.8±57.8
Median [interquartile range]	107.6 [74.9-140.6]	103.3 [75.2-149.8]	97.0 [68.3-131.2]	108.1 [73.6-138.5]	102.3 [73.8-148.0]	98.7 [70.0-133.0]

Table 5.1 (continued)

	Skin study population			Retinal study population		
Characteristic	**Tertile 1 of total physical activity (highest) n=432**	**Tertile 2 of total physical activity n=434**	**Tertile 3 of total physical activity (lowest) n=432**	**Tertile 1 of total physical activity (highest) n=616**	**Tertile 2 of total physical activity n=616**	**Tertile 3 of total physical activity (lowest) n=615**
Baseline arteriolar diameter before flicker light exposure (MU)†	116.3±16.6	115.3±16.8	116.2±16.0	115.6±15.8	114.6±16.0	115.5±15.2
Arteriolar average dilation (%)†						
Mean ± SD	3.2±3.0	3.0±2.7	2.9±2.8	3.2±2.9	3.1±2.8	3.0±2.7
Median [interquartile range]	2.7 [1.0-5.2]	2.6 [0.9-4.7]	2.4 [0.8-4.4]	2.8 [1.0-5.1]	2.8 [0.9-5.1]	2.6 [0.9-4.8]
Arteriolar diameter during flicker light exposure (MU)†						
Mean ± SD	119.9±16.8	118.7±16.8	119.5±16.1	119.2±15.9	118.1±16.0	118.9±15.4
Median [interquartile range]	119.5 [107.9-129.4]	118.0 [105.9-128.3]	119.4 [107.9-129.4]	118.3 [107.9-129.2]	118.2 [107.3-127.9]	119.0 [108.1-128.8]
Accelerometry variables						
Valid worn days	6.2±1.2	6.2±1.1	6.2±1.1	6.3±1.3	6.3±1.1	6.3±1.2
Waking time (h/day)	16.0±0.8	15.7±0.9	15.4±1.0	15.9±0.8	15.8±0.9	15.5±1.0
Total physical activity (h/day)	2.7±0.4	1.9±0.2	1.3±0.3	2.8±0.4	2.0±0.2	1.3±0.3
Higher-intensity physical activity (h/day)	0.6±0.3	0.3±0.2	0.2±0.1	0.6±0.4	0.4±0.2	0.2±0.2
Sedentary time (h/day)	8.5±1.4	9.5±1.5	10.6±1.4	8.3±1.4	9.4±1.4	10.5±1.5

Data are reported as mean±SD, median [interquartile range], or number (percentages %) as appropriate. SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; MU, measurement units; PU, perfusion units; SD, standard deviation. *=(Micro)albuminuria was defined as a urinary albumin excretion of >30 mg per 24 hours. †=In the skin study population, flicker light-induced retinal arteriolar reactivity measures were available in n=952. #=In the retinal study population, heat-induced skin hyperaemia measures were available in n=952.

In contrast, in individuals with T2D, total and higher-intensity physical activity were directly associated with heat-induced skin hyperaemia (regression coefficients per hour increase were 10 PU (95%CI: 1; 18, P=0.026) and 36 PU (14; 58, P=0.002), respectively (Table 5.2).
In adjusted analyses, total physical activity and higher-intensity physical activity were not significantly associated with flicker light-induced retinal arteriolar %-dilation (Table 5.3). In addition, the associations between total physical activity and higher-intensity physical activity with retinal arteriolar %-dilation did not differ significantly between individuals without and with T2D ($P_{interaction}$>0.10; Table 5.3).

Table 5.2 Multivariable-adjusted associations of total physical activity, higher-intensity physical activity, and total sedentary time with heat-induced skin hyperaemia in the total skin study population and stratified according to type 2 diabetes status.

	Model 1	**Model 2**	**Model 3a**	**Model 3b**	**$P_{interaction}$**
Heat-induced skin hyperaemia (PU)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	
Total skin study population (n=1298)					
Total physical activity (h/day)	2 (-3; 7)	1 (-4; 6)	-	-	-
Higher-intensity physical activity (h/day)	6 (-5; 17)	3 (-9; 14)	4 (-8; 16)	-	-
Total sedentary time (h/day)	0 (-2; 2)	1 (-1; 3)	-	1 (-1; 3)	-
Without type 2 diabetes (n=915)					
Total physical activity (h/day)	-2 (-8; 4)	-3 (-9; 3)	-	-	-
Higher-intensity physical activity (h/day)	-3 (-16; 10)	-6 (-20; 7)	-3 (-17; 11)	-	-
Total sedentary time (h/day)	2 (-1; 4)	2 (-1; 5)	-	2 (-1; 5)	-
With type 2 diabetes (n=383)					
Total physical activity (h/day)	**11 (4; 19)**	**10 (1; 18)**	**-**	-	0.018
Higher-intensity physical activity (h/day)	**40 (20; 61)**	**38 (16; 60)**	**36 (14; 58)**	**-**	0.001
Total sedentary time (h/day)	**-3 (-6; -0)**	-2 (-6; 1)	-	-1 (-5; 2)	0.068

Regression results are presented as unstandardized coefficients (B) with 95% confidence intervals (95%CI). Boldface indicates statistical significance (P<0.05). $P_{interaction}$ (with type 2 diabetes) was based on model 2. PU, perfusion units. Model 1: adjusted for age, sex, glucose metabolism status (for 'total skin study population' only), waking time, and baseline skin blood flow. For analyses in individuals without type 2 diabetes, model 1 is also adjusted for prediabetes status. Model 2: additionally adjusted for educational level, body mass index, mobility limitation, office systolic blood pressure, total-to-HDL cholesterol ratio, triglycerides, antihypertensive and lipid-modifying medication, smoking status, alcohol consumption, and a history of cardiovascular disease. Model 3a: additionally adjusted for sedentary time. Model 3b: additionally adjusted for higher-intensity physical activity.

Associations of sedentary behaviour with skin hyperaemia and retinal arteriolar dilation

In adjusted analyses, total sedentary time was not associated with heat-induced skin hyperaemia (Table 5.2). Although associations between total sedentary time and heat-induced skin hyperaemia differed statistically between individuals without and with T2D ($P_{interaction}$<0.10), stratified analyses in individuals without and with T2D did not

show significant associations between total sedentary time and heat-induced skin hyperaemia (Table 5.2).
In adjusted analyses, total sedentary time was not significantly associated with flicker light-induced retinal arteriolar %-dilation (Table 5.3). In addition, the association between total sedentary time and retinal arteriolar %-dilation did not differ significantly between individuals without and with T2D ($P_{interaction}$>0.10; Table 5.3).

Table 5.3: Multivariable-adjusted associations of total physical activity, higher-intensity physical activity, and total sedentary time with retinal arteriolar %-dilation.

	Model 1	**Model 2**	**Model 3a**	**Model 3b**	**$P_{interaction}$**
Retinal arteriolar dilation (%)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	
Total physical activity (h/day)	-0.04 (-0.24; 0.15)	-0.09 (-0.30; 0.11)	-	-	0.814
Higher-intensity physical activity (h/day)	-0.16 (-0.60; 0.27)	-0.24 (-0.69; 0.22)	-0.24 (-0.71; 0.23)	-	0.226
Total sedentary time (h/day)	0.01 (-0.09; 0.08)	0.01 (-0.08; 0.10)	-	-0.00 (-0.09; 0.09)	0.806

Regression results are presented as unstandardized coefficients (B) with 95% confidence intervals (95%CI). Boldface indicates statistical significance (P<0.05). $P_{interaction}$ (with type 2 diabetes) was based on model 2. Model 1: adjusted for age, sex, glucose metabolism status, and waking time. Model 2: additionally adjusted for educational level, body mass index, mobility limitation, office systolic blood pressure, total-to-HDL cholesterol ratio, triglycerides, antihypertensive and lipid-modifying medication, smoking status, alcohol consumption, and a history of cardiovascular disease. Model 3a: additionally adjusted for sedentary time. Model 3b: additionally adjusted for higher-intensity physical activity.

Additional analyses

As associations of total and higher-intensity physical activity with heat-induced skin hyperaemia were stronger in individuals with as compared to those without T2D, we further explored interactions between continuous measures of glycaemia (i.e. HbA1c, fasting plasma glucose, and 2-h post load glucose) and total and higher-intensity physical activity. Statistically significant interactions were found between 2-h post load glucose and total physical activity, and between HbA1c, fasting plasma glucose, and 2-h post load glucose and higher-intensity physical activity (all $P_{interaction}$<0.10; data not shown). In individuals with prediabetes, regression coefficients of the associations of total and higher-intensity physical activity with skin hyperaemia, were numerically in between those of individuals without and with T2D (Supplemental Table S5.5).
Qualitatively similar associations of total physical activity, higher-intensity physical activity, and total sedentary time with skin hyperaemia and retinal arteriolar %-dilation were observed in a range of additional analyses. First, when heat-induced increase in skin blood flow (in PU) from skin baseline or flicker light-induced increase (in MU) in retinal arteriolar diameter from baseline were used as outcomes (data not shown). Second, we had similar findings when we replaced office systolic blood pressure with 24-h ambulatory systolic blood pressure (data not shown, for skin and retinal analyses,

24-h ambulatory blood pressure was available in n=1,151 individuals (n=812 without and n=339 with T2D) and n=1,632 (n=1,180 without and n=452 with T2D) respectively). Third, after additional adjustment for daily caloric intake (data not shown, for skin and retinal analyses, daily caloric intake was available in n=1,219 individuals (n=863 without and n=356 with T2D) and n=1,755 (n=1,276 without and n=479 with T2D) respectively) or occupational status (data not shown, for skin and retinal analyses, occupational status was available in n=1,122 individuals (n=807 without and n=315 with T2D) and n=1,609 (n=1,178 without and n=431 with T2D) respectively). Fourth, after additional adjustment for eGFR, urinary albumin excretion, and the presence of retinopathy (data not shown, for skin and retinal analyses, data on these additional covariates were available in n=1,163 individuals (n=809 without and n=354 with T2D) and n=1,774 (n=1,288 without and n=486 with T2D) respectively). Fifth, when antihypertensive medication was further specified into renin-angiotensin-aldosterone system (RAAS)-inhibiting (with or without other types of antihypertensives) and non-RAAS-inhibiting antihypertensives only (data not shown; RAAS-inhibiting antihypertensives included angiotensin-converting-enzyme, angiotensin receptor blockers and renin blockers). Sixth, after additional adjustment for time of the day or month of the year when the skin and retinal measurements were done (to adjust for possible diurnal or seasonal influences; data not shown). Seventh, associations of total physical activity, higher-intensity physical activity, and total sedentary time with skin hyperaemia and retinal arteriolar %-dilation did not differ between women and men or by age (all $P_{interaction}>0.10$). Last, collinearity diagnostics revealed no problematic multicollinearity in any of the analyses (i.e. all tolerance values ≥0.10 and variance inflation factors ≤10).

Discussion

In this population-based study, we tested the hypothesis that habitually low levels of physical activity and high levels of sedentary time (objectively quantified with an accelerometer) can affect microvascular endothelial function. There were three novel findings. First, higher levels of total and higher-intensity physical activity were independently associated with greater skin microvascular vasodilation in individuals with, but not in individuals without T2D. Second, and in contrast to our hypothesis, sedentary time was not associated with skin microvascular function. Third, physical activity and sedentary behaviour were not statistically significantly associated with retinal microvascular function. These findings suggest that increasing habitual daily physical activity should be investigated as a means to improving microvascular function in T2D, with the ultimate goal of reducing risk of heart failure[1], (lacunar)

stroke[2], depression[3], cognitive decline[4], retinopathy[6], chronic kidney disease[5], and neuropathy.[6]

Both heat-induced skin hyperaemia and flicker light-induced retinal arteriolar dilation are likely a reflection of microvascular *endothelial* function, as they both rely on nitric oxide bioavailability[51,52], possibly in conjunction [53]with vascular smooth muscle cell function[54,55], and/or neuronal function.[56,57] Mechanistically, higher levels of physical activity and lower levels of sedentary time are thought to increase nitric oxide bioavailability[29,30] On the one hand physical activity is thought to induce microvascular laminar shear stress and thereby stimulate the synthesis of nitric oxide by endothelial nitric oxide synthase (eNOS).[58] On the other hand, physical activity is thought to inhibit eNOS uncoupling via reduction of oxidative stress by improvement of insulin sensitivity.[58]

The associations of higher levels of total and higher-intensity physical activity with greater heat-induced skin hyperaemia in individuals with T2D are in agreement with findings from prior human and animal research.[53,59,60] As in individuals with T2D insulin sensitivity is thought is be worse, a likely explanation for stronger associations in T2D is that in T2D physical activity improves nitric oxide bioavailability via both stimulation of eNOS and inhibition of eNOS uncoupling.[58] Alternatively, as in individuals normal glucose metabolism insulin sensitivity is not or less strongly reduced, the effect of physical activity on nitric oxide bioavailability may be less strong because under normal glycaemic circumstances physical activity is thought to mostly improve nitric oxide bioavailability via stimulation of eNOS.[58]

The absence of an association between higher levels of habitual total and higher-intensity physical activity and greater skin microvascular function in individuals without T2D contrasts findings from earlier studies as previous studies showed beneficial effects of (chronic) intensive exercise on skin microvascular function in healthy individuals.[61] A possible explanation is that in individuals without T2D, physical activity-induced improvement in skin microvascular function is observed only at higher intensities and longer duration of physical activity than those in our study.[20,61]

The absence of an inverse association between higher levels of habitual total sedentary time and skin microvascular function contrasts with earlier studies with *uninterrupted* sedentary time protocols.[19,25,26] Mechanistically, sedentary behaviour is thought to reduce shear stress, leading to less nitric oxide synthesis and reduced endothelium-dependent vasodilation.[29] Insufficient duration of total sedentary time (mean $\pm$ SD: 9.5$\pm$1.7 h/day; range: 2.5 to 15.9 h/day) is unlikely to explain the absence of an inverse association between sedentary behaviour and microvascular function. A likelier explanation is that frequent transient interruptions of sedentary behaviour by standing

and/or walking can restore sedentary behaviour-induced microvascular dysfunction[19,25], presumably via changes in shear stress.[62] Indeed, a higher number of such interruptions, which in our study population occurred on average ~4 times per sedentary hour, have been shown to be associated with reduced cardiovascular mortality.[63]

Autoregulation of retinal blood flow can explain why physical activity and sedentary behaviour were not associated with retinal microvascular function.[39,40,64] During physical activity, constriction of small retinal arterioles increases retinal vascular resistance in proportion to the increase in ocular perfusion pressure[40] to stabilize retinal perfusion[39,40], presumably to maintain visual acuity[41]. Our finding of a numerically lower baseline retinal arteriolar diameter with increased levels of higher-intensity physical activity (Supplemental Table S5.4) is consistent with this interpretation. Whether chronic habitual sedentary behaviour alters ocular blood flow is currently unknown, but unlikely, as retinal autoregulation has been shown to preserve stable retinal perfusion during transient sedentary time at different postures (e.g. sitting and lying).[64]

Strengths of our study include its size and population-based design; the use of a waterproof-attached accelerometer, which has resulted in improved wear time compliance[65], and measures which are more precise and valid than when self-reported[28]; the extensive assessment of, and adjustment for, potential confounders; the use of two independent methods to directly assess microvascular function in different microvascular beds; and the broad array of additional analyses, which all gave consistent results.

Our study had some limitations. First, the data were cross-sectional. Therefore, we cannot exclude reverse causality, i.e. that microvascular dysfunction leads to suboptimal delivery of oxygen and nutrients to tissues (e.g. muscle) on demand, and therefore may hamper physical activity. Second, total and higher-intensity physical activity and total sedentary time were measured during one week, which may not truly reflect habitual behaviour. However, with an average wear time of 6.2 days in our study, compliance to the 8 days wear time protocol was good, and sufficient to reliably estimate habitual sedentary behaviour and close to optimal wear time for estimation of habitual physical activity.[66] Third, higher-intensity physical activity was based on step frequency, which is less precise than acceleration-based determination. However, we used a cut-off point of >110 steps/minute for higher-intensity physical activity, which roughly equals a Metabolic Equivalent of Task (MET)-score of $\geq$3.0 (which indicates moderate-to-vigorous intensity activity).[47] Last, although we adjusted for many potential confounders, we cannot fully exclude residual confounding by variables not included in these analyses (e.g. dietary habits).

This large population-based study, with postured-based accelerometery data, showed that higher levels of total and higher-intensity physical activity were independently associated with greater skin microvascular vasodilation in individuals with, but not in those without T2D. This is consistent with beneficial effects of physical activity on nitric oxide bioavailability, which are likely more prominent in individuals with hyperglycaemia. These findings suggest that increasing habitual daily physical activity should be investigated as a means to improving microvascular function in T2D, with the ultimate goal of reducing risk of heart failure[1], (lacunar) stroke[2], depression[3], cognitive decline[4], chronic kidney disease[5], and neuropathy.[6]

Perspectives

In this large population-based study, with postured-based accelerometery data, higher levels of total and higher-intensity physical activity were independently associated with greater skin microvascular vasodilation in individuals with T2D. However, physical activity was not associated with retinal microvascular dilatation response. Habitual daily physical activity should be investigated as a means to improving microvascular function in T2D, with the ultimate goal of reducing risk of heart failure, (lacunar) stroke, depression, cognitive decline, chronic kidney disease, and neuropathy.

References

1. Lee JF, Barrett-O'Keefe Z, Garten RS, Nelson AD, Ryan JJ, Nativi JN, Richardson RS and Wray DW. Evidence of microvascular dysfunction in heart failure with preserved ejection fraction. Heart. 2016;102:278-84.
2. Knottnerus IL, Ten Cate H, Lodder J, Kessels F and van Oostenbrugge RJ. Endothelial dysfunction in lacunar stroke: a systematic review. Cerebrovasc Dis. 2009;27:519-26.
3. Santos M, Xekardaki A, Kovari E, Gold G, Bouras C and Giannakopoulos P. Microvascular pathology in late-life depression. J Neurol Sci. 2012;322:46-9.
4. De Silva TM and Faraci FM. Microvascular Dysfunction and Cognitive Impairment. Cell Mol Neurobiol. 2016;36:241-58.
5. Zafrani L and Ince C. Microcirculation in Acute and Chronic Kidney Diseases. Am J Kidney Dis. 2015;66:1083-94.
6. Gupta A and Bhatnagar S. Vasoregression: A Shared Vascular Pathology Underlying Macrovascular And Microvascular Pathologies? OMICS. 2015;19:733-53.
7. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC and Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321:405-12.
8. Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, Chastin SFM, Altenburg TM, Chinapaw MJM and Participants STCP. Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. Int J Behav Nutr Phys Act. 2017;14:75.
9. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT and Lancet Physical Activity Series Working G. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380:219-29.
10. Chen SM, Tsai TH, Hang CL, Yip HK, Fang CY, Wu CJ and Guo GB. Endothelial dysfunction in young patients with acute ST-elevation myocardial infarction. Heart Vessels. 2011;26:2-9.
11. Witte DR, Westerink J, de Koning EJ, van der Graaf Y, Grobbee DE and Bots ML. Is the association between flow-mediated dilation and cardiovascular risk limited to low-risk populations? J Am Coll Cardiol. 2005;45:1987-93.
12. Young DR, Reynolds K, Sidell M, Brar S, Ghai NR, Sternfeld B, Jacobsen SJ, Slezak JM, Caan B and Quinn VP. Effects of physical activity and sedentary time on the risk of heart failure. Circ Heart Fail. 2014;7:21-7.
13. Willey JZ, Moon YP, Sacco RL, Greenlee H, Diaz KM, Wright CB, Elkind MS and Cheung YK. Physical inactivity is a strong risk factor for stroke in the oldest old: Findings from a multi-ethnic population (the Northern Manhattan Study). Int J Stroke. 2017;12:197-200.
14. Schuch F, Vancampfort D, Firth J, Rosenbaum S, Ward P, Reichert T, Bagatini NC, Bgeginski R and Stubbs B. Physical activity and sedentary behavior in people with major depressive disorder: A systematic review and meta-analysis. J Affect Disord. 2017;210:139-50.
15. Steinberg SI, Sammel MD, Harel BT, Schembri A, Policastro C, Bogner HR, Negash S and Arnold SE. Exercise, sedentary pastimes, and cognitive performance in healthy older adults. Am J Alzheimers Dis Other Demen. 2015;30:290-8.
16. Praidou A, Harris M, Niakas D and Labiris G. Physical activity and its correlation to diabetic retinopathy. J Diabetes Complications. 2017;31:456-61.
17. White SL, Dunstan DW, Polkinghorne KR, Atkins RC, Cass A and Chadban SJ. Physical inactivity and chronic kidney disease in Australian adults: the AusDiab study. Nutr Metab Cardiovasc Dis. 2011;21:104-12.
18. Singleton JR, Smith AG and Marcus RL. Exercise as Therapy for Diabetic and Prediabetic Neuropathy. Curr Diab Rep. 2015;15:120.
19. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ and Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol. 2015;100:829-38.
20. Tew GA, George KP, Cable NT and Hodges GJ. Endurance exercise training enhances cutaneous microvascular reactivity in post-menopausal women. Microvasc Res. 2012;83:223-8.

21. Roche DM, Rowland TW, Garrard M, Marwood S and Unnithan VB. Skin microvascular reactivity in trained adolescents. Eur J Appl Physiol. 2010;108:1201-8.
22. Wang JS. Effects of exercise training and detraining on cutaneous microvascular function in man: the regulatory role of endothelium-dependent dilation in skin vasculature. Eur J Appl Physiol. 2005;93:429-34.
23. Navasiolava NM, Dignat-George F, Sabatier F, Larina IM, Demiot C, Fortrat JO, Gauquelin-Koch G, Kozlovskaya IB and Custaud MA. Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. Am J Physiol Heart Circ Physiol. 2010;299:H248-56.
24. Vranish JR, Young BE, Kaur J, Patik JC, Padilla J and Fadel PJ. Influence of Sex on Microvascular and Macrovascular Responses to Prolonged Sitting. Am J Physiol Heart Circ Physiol. 2017:ajpheart 00823 2016.
25. Demiot C, Dignat-George F, Fortrat JO, Sabatier F, Gharib C, Larina I, Gauquelin-Koch G, Hughson R and Custaud MA. WISE 2005: chronic bed rest impairs microcirculatory endothelium in women. Am J Physiol Heart Circ Physiol. 2007;293:H3159-64.
26. Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, Gokce N, Ruderman NB, Keaney JF, Jr. and Vita JA. Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. Arterioscler Thromb Vasc Biol. 2007;27:2650-6.
27. Anuradha S, Dunstan DW, Healy GN, Shaw JE, Zimmet PZ, Wong TY and Owen N. Physical activity, television viewing time, and retinal vascular caliber. Med Sci Sports Exerc. 2011;43:280-6.
28. Downs A, Van Hoomissen J, Lafrenz A and Julka DL. Accelerometer-measured versus self-reported physical activity in college students: implications for research and practice. J Am Coll Health. 2014;62:204-12.
29. Thosar SS, Johnson BD, Johnston JD and Wallace JP. Sitting and endothelial dysfunction: the role of shear stress. Med Sci Monit. 2012;18:RA173-80.
30. Nyberg M, Blackwell JR, Damsgaard R, Jones AM, Hellsten Y and Mortensen SP. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. J Physiol. 2012;590:5361-70.
31. Lambert CP, Wright NR, Finck BN and Villareal DT. Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons. J Appl Physiol (1985). 2008;105:473-8.
32. Parsons TJ, Sartini C, Welsh P, Sattar N, Ash S, Lennon LT, Wannamethee SG, Lee IM, Whincup PH and Jefferis BJ. Physical Activity, Sedentary Behavior, and Inflammatory and Hemostatic Markers in Men. Med Sci Sports Exerc. 2017;49:459-65.
33. Matlung HL, Bakker EN and VanBavel E. Shear stress, reactive oxygen species, and arterial structure and function. Antioxid Redox Signal. 2009;11:1699-709.
34. Hamed S, Brenner B and Roguin A. Nitric oxide: a key factor behind the dysfunctionality of endothelial progenitor cells in diabetes mellitus type-2. Cardiovasc Res. 2011;91:9-15.
35. Sorensen BM, Houben A, Berendschot T, Schouten J, Kroon AA, van der Kallen CJH, Henry RMA, Koster A, Reesink KD, Dagnelie PC, Schaper NC, Schalkwijk CG, Schram MT and Stehouwer CDA. Hyperglycemia Is the Main Mediator of Prediabetes- and Type 2 Diabetes-Associated Impairment of Microvascular Function: The Maastricht Study. Diabetes Care. 2017;40:e103-5.
36. Nguyen TT, Kreis AJ, Kawasaki R, Wang JJ, Seifert BU, Vilser W, Nagel E and Wong TY. Reproducibility of the retinal vascular response to flicker light in Asians. Curr Eye Res. 2009;34: 1082-8.
37. Agarwal SC, Allen J, Murray A and Purcell IF. Comparative reproducibility of dermal microvascular blood flow changes in response to acetylcholine iontophoresis, hyperthermia and reactive hyperaemia. Physiol Meas. 2010;31:1-11.
38. Kenny GP and McGinn R. Restoration of thermoregulation after exercise. J Appl Physiol (1985). 2017;122:933-44.
39. Hayashi N, Ikemura T and Someya N. Effects of dynamic exercise and its intensity on ocular blood flow in humans. Eur J Appl Physiol. 2011;111:2601-6.
40. Risner D, Ehrlich R, Kheradiya NS, Siesky B, McCranor L and Harris A. Effects of exercise on intraocular pressure and ocular blood flow: a review. J Glaucoma. 2009;18:429-36.
41. Hayashi N, Someya N and Ikemura T. Effects of hyper- and hypocapnea on choroidal and retinal blood flows and the visual acuity. Faseb J. 2010;24.

42. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
43. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CD. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. Circulation. 2016;134:1339-52.
44. Vickers AJ. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Med Res Methodol. 2001;1:6.
45. Atkinson G and Batterham AM. The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. Vasc Med. 2013;18:354-65.
46. van der Berg JD, Willems PJ, van der Velde JH, Savelberg HH, Schaper NC, Schram MT, Sep SJ, Dagnelie PC, Bosma H, Stehouwer CD and Koster A. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. J Sports Sci. 2016;34:1867-73.
47. Tudor-Locke C, Craig CL, Brown WJ, Clemes SA, De Cocker K, Giles-Corti B, Hatano Y, Inoue S, Matsudo SM, Mutrie N, Oppert JM, Rowe DA, Schmidt MD, Schofield GM, Spence JC, Teixeira PJ, Tully MA and Blair SN. How many steps/day are enough? For adults. Int J Behav Nutr Phys Act. 2011;8:79.
48. Tudor-Locke C and Rowe DA. Using cadence to study free-living ambulatory behaviour. Sports Med. 2012;42:381-98.
49. Tremblay MS, Colley RC, Saunders TJ, Healy GN and Owen N. Physiological and health implications of a sedentary lifestyle. Appl Physiol Nutr Metab. 2010;35:725-40.
50. Martens RJ, Henry RM, Houben AJ, van der Kallen CJ, Kroon AA, Schalkwijk CG, Schram MT, Sep SJ, Schaper NC, Dagnelie PC, Muris DM, Gronenschild EH, van der Sande FM, Leunissen KM, Kooman JP and Stehouwer CD. Capillary Rarefaction Associates with Albuminuria: The Maastricht Study. J Am Soc Nephrol. 2016;27:3748-57.
51. Kellogg DL, Jr., Liu Y, Kosiba IF and O'Donnell D. Role of nitric oxide in the vascular effects of local warming of the skin in humans. J Appl Physiol (1985). 1999;86:1185-90.
52. Dorner GT, Garhofer G, Kiss B, Polska E, Polak K, Riva CE and Schmetterer L. Nitric oxide regulates retinal vascular tone in humans. Am J Physiol Heart Circ Physiol. 2003;285:H631-6.
53. Mitranun W, Deerochanawong C, Tanaka H and Suksom D. Continuous vs interval training on glycemic control and macro- and microvascular reactivity in type 2 diabetic patients. Scand J Med Sci Sports. 2014;24:e69-76.
54. Montero D, Pierce GL, Stehouwer CD, Padilla J and Thijssen DH. The impact of age on vascular smooth muscle function in humans. J Hypertens. 2015;33:445-53; discussion 453.
55. Lacolley P, Regnault V, Nicoletti A, Li Z and Michel JB. The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. Cardiovasc Res. 2012;95:194-204.
56. Minson CT, Berry LT and Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. J Appl Physiol (1985). 2001;91:1619-26.
57. Falsini B, Riva CE and Logean E. Flicker-evoked changes in human optic nerve blood flow: relationship with retinal neural activity. Invest Ophthalmol Vis Sci. 2002;43:2309-16.
58. Meza CA, La Favor JD, Kim DH and Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci. 2019;20.
59. Green DJ, Maiorana A, O'Driscoll G and Taylor R. Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol. 2004;561:1-25.
60. Machado MV, Martins RL, Borges J, Antunes BR, Estato V, Vieira AB and Tibirica E. Exercise Training Reverses Structural Microvascular Rarefaction and Improves Endothelium-Dependent Microvascular Reactivity in Rats with Diabetes. Metab Syndr Relat Disord. 2016;14:298-304.
61. Lanting SM, Johnson NA, Baker MK, Caterson ID and Chuter VH. The effect of exercise training on cutaneous microvascular reactivity: A systematic review and meta-analysis. J Sci Med Sport. 2017;20:170-7.
62. Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT and Green DJ. Impact of shear rate modulation on vascular function in humans. Hypertension. 2009;54:278-85.

63. Diaz KM, Howard VJ, Hutto B, Colabianchi N, Vena JE, Safford MM, Blair SN and Hooker SP. Patterns of Sedentary Behavior and Mortality in U.S. Middle-Aged and Older Adults: A National Cohort Study. Ann Intern Med. 2017.
64. Feke GT and Pasquale LR. Retinal blood flow response to posture change in glaucoma patients compared with healthy subjects. Ophthalmology. 2008;115:246-52.
65. Tudor-Locke C, Barreira TV, Schuna JM, Jr., Mire EF, Chaput JP, Fogelholm M, Hu G, Kuriyan R, Kurpad A, Lambert EV, Maher C, Maia J, Matsudo V, Olds T, Onywera V, Sarmiento OL, Standage M, Tremblay MS, Zhao P, Church TS, Katzmarzyk PT and Group IR. Improving wear time compliance with a 24-hour waist-worn accelerometer protocol in the International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE). Int J Behav Nutr Phys Act. 2015;12:11.
66. Barreira TV, Hamilton MT, Craft LL, Gapstur SM, Siddique J and Zderic TW. Intra-individual and inter-individual variability in daily sitting time and MVPA. J Sci Med Sport. 2016;19:476-81.

Supplemental materials

Supplemental methods

Assessment of retinal arteriolar microvascular function

Retinal arteriolar dilation response[1]

The retinal arteriolar dilation response to flicker light was measured in a dimly lit room by use of the Dynamic Vessel Analyzer (DVA; Imedos, Jena, Germany). For safety reasons, participants with an intraocular pressure exceeding 30 mmHg were excluded from retinal measurements. Per participant either the left or right eye was selected depending on the time of day the measurement was performed and without reference to participant characteristics.

During the measurement, the participant was instructed and encouraged to focus on the tip of a fixated needle inside the retinal camera (FF450; Carl Zeiss GmbH, Jena, Germany), while the fundus of the eye was examined under green measuring light (530-600 nm, illumination of fundus approximately 6500 lux). A straight arteriolar segment of approximately 1.5 mm in length located 0.5 to 2.0 disc diameter from the margin of the optic disc in the temporal section was examined. When the specific vessel profile was recognized, vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker light exposure period (flicker frequency 12.5Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The DVA automatically corrected for alterations in luminance caused by, for example, slight eye movements. During blinks and small eye movements, the registration stopped and restarted once the vessel segments were automatically re-identified.

Assessment of patterns of physical activity and sedentary behaviour

Daily activity levels and patterns were measured using the activPAL3^{TM} physical activity monitor (PAL technologies, Glasgow, UK) as has been described previously.[2] The activPAL3^{TM} is a small (53 x 35 x 7 mm), lightweight (15 gram) triaxial accelerometer that records movement in the vertical, anteroposterior and mediolateral axes, and also determines posture (sitting or lying, standing, and stepping) based on acceleration information. The device was attached directly to the skin on the front of the right thigh with transparent 3M Tegaderm^{TM} tape, after the device had been waterproofed using a nitrile sleeve. Participants were asked to wear the accelerometer for 8 consecutive days, without removing the device at any time. To avoid inaccurately identifying of non-wear time, participants were asked not to replace the device once removed. Data were uploaded using the active PAL software and processed using

customized software written in MATLAB R2013b (MathWorks, Natick, MA, USA). Data from the first day were excluded from the analysis because participants performed physical function tests at the research centre after the device was attached. In addition, data from the final wear day providing ≤14 waking hours of data were excluded from the analysis. Participants were included if they provided at least 1 valid day (≥10 hours of waking data).

Detailed assessment of covariates

Level of education was categorized into low (none, primary, or lower vocational education only), medium (intermediate general secondary, intermediate vocational or higher general secondary education) and high (higher vocational education or university level of education). Alcohol consumption was categorized into non-consumers, low consumers (≤7 glasses per week for women and ≤14 glasses per week for men), and high consumers (>7 glasses per week for women and >14 glasses per week for men). A history of cardiovascular disease (CVD) was assessed by web-based questionnaires and was defined as a history of myocardial infarction, stroke, or vascular surgery (including angioplasty) of coronary, carotid, abdominal aortic or peripheral arteries. Occupational status was assessed by questionnaire, and classified as self-employed, working for the government, salaried worker, disabled, rentier, retired, homemaker, unemployed or other. Those who selected the option disabled, rentier, retired, homemaker or unemployed were included in the non-employment group. Those in the class "other" were excluded, as their employment status could not be confirmed. Those who reported being self-employed, salaried worker, or working for the government were further classified as employed, and their occupational status was categorized according to the question "What category was your job?" as low (including options such as: without a profession, unschooled, schooled and lower employee), intermediate or high employee or self-employed.

Statistical analysis

Differences in general characteristics between individuals in the study populations and individuals excluded due to missing values were compared by Analyses of Variance (ANOVA) for continuous variables and χ^2-test for categorical variables.

Supplemental results

Associations of total and higher-intensity physical activity, and total sedentary time with baseline skin blood flow and baseline retinal arteriolar diameter

In adjusted analyses, total physical activity and higher-intensity physical activity were significantly associated with baseline skin blood flow (regression coefficients per hour increase were 0.89 PU (95%CI: 0.32; 1.46, p=0.002) and 2.14 PU (0.82; 3.46, p=0.002) respectively). These associations did not differ significantly between individuals without and with type 2 diabetes (T2D; $P_{interaction}$>0.10, Supplemental Table S5.3). Total sedentary time was associated with baseline skin blood flow (regression coefficient per hour increase was -0.24 PU [-0.48; -0.00, P=0.047]). In addition, this association was significantly different between individuals without and with T2D ($P_{interaction}$<0.10). In individuals without T2D total sedentary time was significantly associated with baseline skin blood flow (regression coefficient per hour increase was -0.45 PU [-0.74; -0.16, P=0.003]), whereas in individuals with T2D it was not (0.26 PU [-0.14; 0.66, P=0.198, Supplemental Table S5.3]).

In adjusted analyses, total physical activity, higher-intensity physical activity, and total sedentary time were not significantly associated with baseline retinal arteriolar diameter (Supplemental Table S5.4). In addition, the associations between total physical activity, higher-intensity physical activity, and total sedentary time with baseline retinal arteriolar diameter did not differ significantly between individuals without and with T2D ($P_{interaction}$>0.10, Supplemental Table S5.4).

Table S5.1 General characteristics of the skin hyperaemia study population and individuals excluded from analyses due to missing values.

Characteristic	Skin hyperaemia study population (n=1298)	Missings in skin hyperaemia study population*	Excluded due to missing values (n=2112)	Missings in population excluded due to missing values†	P-value
Age (years)	60.2±8.1	0	59.6±8.4	0	0.046
Women	601 (46.3)	0	1053 (49.9)	0	0.044
Educational level		0		75	0.750
Low	432 (33.3)		686 (33.7)		
Medium	359 (27.7)		581 (28.5)		
High	507 (39.1)		770 (37.8)		
Occupational status		176		354	0.766
Unemployed	661 (58.9)		1001 (56.9)		
Employed	461 (41.1)		757 (43.1)		
Glucose metabolism status		0		0	0.032
Normal glucose metabolism	699 (32.9)		1225 (58.0)		
Prediabetes	216 (16.6)		295 (14.0)		
Type 2 diabetes	383 (29.5)		592 (28.0)		
Diabetes duration (years)	6.0 [3.0-12.0]	109	7.0 [3.0-13.0]	202	0.102
Body mass index (kg/m^2)	27.0±4.4	0	27.2±4.7	3	0.202
Waist circumference (cm)		0		4	
Men	101.3±11.9		101.8±12.3		0.425
Women	89.9±12.7		90.1±13.2		0.765
History of cardiovascular disease	227 (17.5)	0	325 (16.2)	105	0.330
Limited mobility	207 (15.9)	0	365 (18.0)	78	0.184
Office SBP (mmHg)	136.0±18.3	0	134.4±18.1	2	0.014
Office DBP (mmHg)	76.6±9.6	0	76.0±10.0	2	0.054
Ambulatory 24-h SBP (mmHg)	120.3±11.7	147	118.4±11.9	252	<0.001
Ambulatory 24-h DBP (mmHg)	74.1±7.3	147	73.1±7.1	252	<0.001
Smoking		0		61	0.004
Never / former / current	429/714/155		731/1015/305		
% (never / former / current)	33.1/55.0/11.9		35.6/49.5/14.9		

Table S5.1 (continued)

Characteristic	Skin hyperaemia study population (n=1298)	Missings in skin hyperaemia study population*	Excluded due to missing values (n=2112)	Missings in population excluded due to missing values†	P-value
Alcohol consumption (high)	350 (27.0)	0	515 (25.2)	67	0.170
Energy intake (kcal/day)	2172.1±579.8	79	2178.9±618.4	143	0.757
Fasting glucose (mmol/L)	6.1±1.6	0	6.1±1.7	1	0.719
2-h postload glucose (mmol/L)	8.1±4.3	94	7.8±4.2	157	0.069
HbA1c (%)	5.9±0.9	1	5.9±0.9	12	0.140
HbA1c (mmol/mol)	41.4±9.6	1	40.9±10.2	12	0.138
Total-to-HDL cholesterol ratio	3.7±1.1	0	3.7±1.2	4	0.640
Total cholesterol (mmol/L)	5.2±1.2	0	5.2±1.1	4	0.578
HDL cholesterol (mmol/L)	1.5±0.5	0	1.5±0.5	4	0.488
LDL cholesterol (mmol/L)	3.1±1.1	0	3.1±1.0	4	0.998
Triglycerides (mmol/L)	1.5±0.9	0	1.4±0.8	4	0.120
Antihypertensive medication use	552 (42.5)	0	803 (38.1)	4	0.010
Lipid-modifying medication use	512 (39.4)	0	708 (33.6)	4	0.001
Diabetes medication use					
Any type	304 (23.4)	0	461 (21.9)	4	0.292
Insulin	84 (6.5)	0	132 (6.3)	4	0.807
Oral glucose-lowering medication	285 (22.0)	0	428 (20.3)	4	0.249
eGFR (mL/min/1.73m^2)	88.2±14.7	11	88.0±15.0	22	0.793
eGFR<60 mL/min/1.73m^2	50 (3.9)	11	93 (4.4)	22	0.429
(Micro)albuminuria‡	111 (8.6)	8	180 (8.7)	34	0.954
Retinopathy	17 (1.4)	118	23 (1.4)	469	0.928
Baseline skin blood flow before heating (PU)	11.1±6.5	0	11.1±6.1§	1763	0.972
Skin hyperaemic response (%)					
Mean ± SD	1126.9±769.4	0	1119.1±782.4§	1763	0.867
Median [interquartile range]	997.1 [587.0-1513.6]	0	993.8 [575.5-1463.6]§	1763	0.867
Skin hyperaemia during heating (PU)					
Mean ± SD	112.5±57.4	0	111.4±56.7§	1763	0.754
Median [interquartile range]	102.5 [72.8-139.8]	0	99.7 [73.5-134.8]§	1763	0.754

Table S5.1 (continued)

Characteristic	Skin hyperaemia study population (n=1298)	Missings in skin hyperaemia study population*	Excluded due to missing values (n=2112)	Missings in population excluded due to missing values†	P-value
Accelerometry variables					
Valid worn days	6.2±1.1	0	6.4±1.2‖	803	<0.001
Waking time (h/day)	15.7±0.9	0	15.7±0.9‖	803	0.463
Total physical activity (h/day)	2.0±0.7	0	2.0±0.7‖	803	0.147
Higher-intensity physical activity (h/day)	0.4±0.3	0	0.4±0.3‖	803	0.324
Sedentary time (h/day)	9.5±1.7	0	9.3±1.7‖	803	0.004

Data are reported as mean±SD, median [interquartile range], or number (percentages %) as appropriate. P-value indicates comparison between study population and individuals excluded due to missing values. SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; PU, perfusion units. *=Total number of missings for a specific variable in the skin hyperaemia study population, †=Total number of missings for a specific variable in the population which was excluded, ‡=(Micro)albuminuria was defined as a urinary albumin excretion of >30 mg per 24 hours, §=349 were excluded due to missing on accelerometry data or covariate. ‖=1309 were excluded due to missing on skin reactivity data or covariate.

Table S5.2 General characteristics of the retinal reactivity study population and individuals excluded from analyses due to missing values.

Characteristic	Retinal reactivity study population (n=1847)	Missings in retinal reactivity study population*	Excluded due to missing values (n=1563)	Missings in population excluded due to missing values†	P-value
Age (years)	59.6±8.2	0	60.1±8.4	0	0.089
Women	894 (48.4)	0	760 (48.6)	0	0.897
Educational level		0		75	0.130
Low	592 (32.1)		526 (35.3)		
Medium	535 (29.0)		405 (27.2)		
High	720 (39.0)		557 (37.4)		
Occupational status		238		292	<0.001
Unemployed	888 (55.2)		774 (60.9)		
Employed	721 (44.8)		497 (39.1)		
Glucose metabolism status		0		0	0.194
Normal glucose metabolism	1056 (57.2)		868 (55.5)		
Prediabetes	286 (15.5)		225 (14.4)		
Type 2 diabetes	505 (27.3)		470 (30.1)		
Diabetes duration (years)	6.0 [3.0-12.0]	167	7.0 [3.0-12.0]	144	0.072
Body mass index (kg/m^2)	26.9±4.5	0	27.4±4.7	3	0.001
Waist circumference (cm)		0		4	
Men	101.1±12.0		102.1±12.2		0.078
Women	89.0±12.5		91.4±13.5		<0.001
History of cardiovascular disease	286 (15.5)	0	266 (18.2)	105	0.035
Limited mobility	284 (15.4)	0	288 (19.4)	81	0.003
Office SBP (mmHg)	135.0±18.0	0	135.1±18.4	2	0.880
Office DBP (mmHg)	76.4±9.9	0	76.0±9.7	2	0.179
Ambulatory 24-h SBP (mmHg)	118.3±11.4	215	120.0±12.3	184	<0.001
Ambulatory 24-h DBP (mmHg)	73.1±7.2	215	73.9±7.2	184	0.002
Smoking		0		61	0.008
Never / former / current	649/975/223		511/754/237		
% (never / former / current)	35.1/52.8/12.1		34.0/50.2/15.8		
Alcohol consumption (high)	462 (25.0)	0	403 (26.9)	67	0.016
Energy intake (kcal/day)	2161.6±589.7	92	2194.3±620.5	130	0.129

Table S5.2 (continued)

Characteristic	Retinal reactivity study population (n=1847)	Missings in retinal reactivity study population*	Excluded due to missing values (n=1563)	Missings in population excluded due to missing values†	P-value
Fasting glucose (mmol/L)	6.0±1.6	0	6.1±1.7	1	0.113
2-h postload glucose (mmol/L)	7.8±4.2	122	8.0±4.3	129	0.425
HbA1c (%)	5.9±0.9	3	6.0±1.0	10	<0.001
HbA1c (mmol/mol)	40.4±9.5	3	41.8±10.4	10	<0.001
Total-to-HDL cholesterol ratio	3.6±1.1	0	3.8±1.2	4	<0.001
Total cholesterol (mmol/L)	5.2±1.2	0	5.2±1.2	4	0.327
HDL cholesterol (mmol/L)	1.6±0.5	0	1.5±0.4	4	<0.001
LDL cholesterol (mmol/L)	3.0±1.0	0	3.1±1.0	4	0.054
Triglycerides (mmol/L)	1.4±0.8	0	1.4±0.9	4	0.975
Antihypertensive medication use	715 (38.7)	0	640 (41.1)	4	0.164
Lipid-modifying medication use	652 (35.3)	0	568 (36.4)	4	0.492
Diabetes medication use					
Any type	391 (21.2)	0	374 (24.0)	4	0.049
Insulin	106 (5.7)	0	110 (7.1)	4	0.116
Oral glucose-lowering medication	365 (19.8)	0	348 (22.3)	4	0.067
eGFR (mL/min/1.73m^2)	88.4±14.5	12	87.8±15.3	21	0.262
eGFR<60 mL/min/1.73m^2	77 (4.2)	12	66 (4.3)	21	0.904
(Micro)albuminuria‡	152 (8.3)	13	139 (9.1)	29	0.426
Retinopathy	25 (1.4)	48	15 (1.5)	539	0.871
Baseline arteriolar diameter before flicker light exposure (MU)	115.2±15.6	0	116.5±15.6§	1149	0.118
Arteriolar average dilation (%)					
Mean ± SD	3.1±2.8	0	2.5±2.6§	1149	<0.001
Median [interquartile range]	2.7 [0.9-5.1]	0	1.8 [0.6-4.3]§	1149	<0.001
Arteriolar diameter during flicker light exposure (MU)					
Mean ± SD	118.7±15.8	0	119.4±15.7§	1149	0.409
Median [interquartile range]	118.6 [107.8-128.5]	0	118.7 [108.3-130.1]§	1149	0.409

Table S5.2 (continued)

Characteristic	Retinal reactivity study population (n=1847)	Missings in retinal reactivity study population*	Excluded due to missing values (n=1563)	Missings in population excluded due to missing values†	P-value
Accelerometry variables					
Valid worn days	6.3±1.2	0	6.2±1.1‖	803	0.083
Waking time (h/day)	15.7±0.9	0	15.7±0.9‖	803	0.519
Total physical activity (h/day)	2.0±0.7	0	1.9±0.7‖	803	0.014
Higher-intensity physical activity (h/day)	0.4±0.3	0	0.3±0.3‖	803	0.001
Sedentary time (h/day)	9.4±1.7	0	9.5±1.7‖	803	0.203

Data are reported as mean±SD, median [interquartile range], or number (percentages %) as appropriate. P-value indicates comparison between study population and individuals excluded due to missing values. SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; MU, measurement units. *=Total number of missings for a specific variable in the retinal reactivity study population, †=Total number of missings for a specific variable in the population which was excluded, ‡=(Micro)albuminuria was defined as a urinary albumin excretion of >30 mg per 24 hours, §=414 were excluded due to missing on accelerometry data or covariate. ‖=760 were excluded due to missing on retinal arteriolar reactivity data or covariate.

Table S5.3 Multivariable-adjusted associations of total physical activity, higher-intensity physical activity, and total sedentary time with baseline skin blood flow in the total skin study population and stratified according to type 2 diabetes status.

	Model 1	Model 2	Model 3a	Model 3b	$P_{interaction}$
Baseline skin blood flow (PU)	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	
Total skin study population (n=1298)					
Total physical activity (h/day)	**0.92 (0.37; 1.47)**	**0.89 (0.32; 1.46)**	-	-	-
Higher-intensity physical activity (h/day)	**2.20 (0.92; 3.48)**	**2.14 (0.82; 3.46)**	**1.91 (0.53; 3.29)**	-	-
Total sedentary time (h/day)	**-0.29 (-0.52; -0.06)**	**-0.24 (-0.48; -0.00)**	-	-0.14 (-0.39; 0.10)	-
Without type 2 diabetes (n=915)					
Total physical activity (h/day)	**1.07 (0.39; 1.76)**	**0.97 (0.28; 1.67)**	-	-	-
Higher-intensity physical activity (h/day)	**2.39 (0.89; 3.89)**	**2.08 (0.53; 3.61)**	1.47 (-0.15; 3.09)	-	-
Total sedentary time (h/day)	**-0.49 (-0.78; -0.21)**	**-0.45 (-0.74; -0.16)**	-	**-0.36 (-0.67; -0.05)**	-
With type 2 diabetes (n=383)					
Total physical activity (h/day)	0.45 (-0.47; 1.36)	0.75 (-0.27; 1.76)	-	-	0.164
Higher-intensity physical activity (h/day)	1.15 (-1.35; 3.66)	1.64 (-1.02; 4.31)	2.11 (-0.61; 4.84)	-	0.328
Total sedentary time (h/day)	0.21 (-0.16; 0.58)	0.26 (-0.14; 0.66)	-	0.33 (-0.08; 0.73)	0.049

Regression results are presented as unstandardized coefficients (B) with 95% confidence intervals (95%CI). Boldface indicates statistical significance (P<0.05). Pinteraction (with type 2 diabetes) was based on model 2. PU, perfusion units. Model 1: adjusted for age, sex, glucose metabolism status (for 'total skin study population' only), and waking time. For analyses in individuals without type 2 diabetes, model 1 is also adjusted for prediabetes status. Model 2: additionally adjusted for educational level, body mass index, mobility limitation, office systolic blood pressure, total-to-HDL cholesterol ratio, triglycerides, antihypertensive and lipid-modifying medication, smoking status, alcohol consumption, and a history of cardiovascular disease. Model 3a: additionally adjusted for sedentary time. Model 3b: additionally adjusted for higher-intensity physical activity

Table S5.4 Multivariable-adjusted associations of total physical activity, higher-intensity physical activity, and total sedentary time with baseline retinal arteriolar diameter.

	Model 1	Model 2	Model 3a	Model 3b	$P_{interaction}$
Baseline retinal arteriolar diameter (MU)	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	
Total physical activity (h/day)	-0.20 (-1.30; 0.91)	-0.01 (-1.21; 1.11)	-	-	0.935
Higher-intensity physical activity (h/day)	-1.09 (-3.56; 1.37)	-0.85 (-3.40; 1.71)	-0.75 (-3.39; 1.88)	-	0.897
Total sedentary time (h/day)	0.09 (-0.39; 0.56)	0.11 (-0.38; 0.60)	-	0.07 (-0.43; 0.58)	0.657

Regression results are presented as unstandardized coefficients (B) with 95% confidence intervals (95%CI). Boldface indicates statistical significance (P<0.05). $P_{interaction}$ (with type 2 diabetes) was based on model 2. MU, measurement units. Model 1: adjusted for age, sex, glucose metabolism status, and waking time. Model 2: additionally adjusted for educational level, body mass index, mobility limitation, office systolic blood pressure, total-to-HDL cholesterol ratio, triglycerides, antihypertensive and lipid-modifying medication, smoking status, alcohol consumption, and a history of cardiovascular disease. Model 3a: additionally adjusted for sedentary time. Model 3b: additionally adjusted for higher-intensity physical activity.

CHAPTER 6

Blood pressure variability and microvascular dysfunction: The Maastricht Study

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Abstract

Background

Microvascular dysfunction (MVD) contributes to stroke, dementia, depression, retinopathy, and chronic kidney disease. However, the determinants of MVD are incompletely understood. Greater blood pressure variability (BPV) may be one such determinant.

Methods and results

We used cross-sectional data of The Maastricht Study (n=2,773, age 59.9 years; 51.9% men) to investigate whether greater very short- to mid-term BPV is associated with various MVD measures. We standardized and averaged within-visit, 24-hour and 7-day BPV into a systolic and a diastolic BPV composite score. MVD measures included a composite score of MRI cerebral small vessel disease (CSVD) features (total brain parenchymal volume, white matter hyperintensity volume, lacunar infarcts, and cerebral microbleeds), a composite score of flicker light-induced retinal arteriolar and venular dilation response, albuminuria, heat-induced skin hyperaemia, and a composite score of plasma biomarkers of MVD (sICAM-1, sVCAM-1, sE-selectin and von Willebrand Factor). We used linear regression with adjustment for age, sex, glucose metabolism status, mean 24-hour systolic or diastolic blood pressure, cardiovascular risk factors, and antihypertensive medication. We found that higher systolic and diastolic BPV composite scores (per SD) were associated with higher albuminuria (higher ratio, 1.04 [95%CI 1.00-1.08] and 1.07 [1.03-1.11], respectively), but not with other measures of MVD tested.

Conclusions

Greater systolic and diastolic BPV was associated with higher albuminuria, but not with CSVD features, flicker light-induced retinal arteriolar and venular dilation response, heat-induced skin hyperemia, and plasma biomarkers of MVD. This suggests that the microvasculature of the kidneys is most vulnerable to the detrimental effects of greater BPV.

Introduction

Microvascular dysfunction (MVD) is an important contributor to various diseases that are (in part) of microvascular origin, including stroke,[1] dementia,[1] depression,[1] retinopathy,[2] and chronic kidney disease.[3] However, the determinants of MVD are incompletely understood. Greater blood pressure variability (BPV), i.e. greater fluctuations of blood pressure over time, may be one such determinant.

Greater BPV may lead to MVD both via increases in pulsatile pressure that can penetrate distally and damage the microcirculation,[4] and sudden falls in blood pressure leading to reduced microvascular perfusion.[5] The microvascular beds of organs with low vascular impedance (i.e. the microvasculature of the brain, eyes and kidneys) may be particularly vulnerable for these fluctuations in blood pressure.[4]

Microvascular function can be measured noninvasively in various organs. These measures include magnetic resonance imaging (MRI) features of cerebral small vessel disease (CSVD, i.e. lower total brain parenchyma volume, higher white matter hyperintensity volume, and presence of lacunar infarcts and cerebral microbleeds);[6] flicker light-induced retinal arteriolar and venular dilation response;[7] albuminuria ("urinary albumin excretion", UAE);[8] heat-induced skin hyperaemia;[7] and plasma biomarkers of MVD (i.e. soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin], and von Willebrand factor [vWF]).[9]

The associations between BPV and most of the above MVD measures remains incompletely understood. To date, only five studies have evaluated the association between BPV and CSVD features. These studies found an association between greater very short- to short-term systolic and diastolic BPV and cerebral atrophy,[10,11] higher white matter hyperintensity volume,[10-13] lacunar infarcts,[10,12] and enlarged perivascular spaces.[10,14] However, these studies were relatively small (n=<155),[11,13] were performed in selected populations (i.e. individuals with hypertension,[12] aged 70 years and older,[15] or admitted to the hospital[10,14]) or did not adjust for potentially important confounders (i.e. mean blood pressure[11, 13] or lifestyle factors[12]). For UAE, most previous studies,[16-27] but not all,[28-30] found an association with greater very short-to mid-term systolic or diastolic BPV. However, these studies did not adjust for potentially important confounders, including dietary habits and physical activity. For plasma biomarkers of MVD, only one study has been done, which included 190 individuals with newly diagnosed hypertension. This study found an association between greater short-term systolic BPV and higher sE-selectin.[31] Currently, no studies have investigated the association between BPV and flicker light-induced retinal arteriolar and venular dilation or heat-induced skin hyperaemia.

In view of the above, we investigated, in a large population-based cohort, whether very short- to mid-term BPV (i.e. within-visit, 24-hour, and 7-day BPV) is associated with a comprehensive set of MVD measures, including CSVD features, flicker light-induced retinal arteriolar and venular dilation response, UAE, heat-induced skin hyperaemia, and plasma biomarkers of MVD. We hypothesized that greater BPV would be more strongly associated with MVD in organs with a low vascular impedance, i.e. brain, eyes and kidneys, and would not be associated with MVD in organs with a high vascular impedance, e.g. skin.

Materials and methods

Study population and design

We used data from The Maastricht Study, an observational population-based cohort study. The rationale and methodology have been described previously.[32] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of diabetes mellitus type 2 (T2D) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns, the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2D status, with an oversampling of individuals with T2D for reasons of efficiency. The present report includes cross-sectional data from 3,451 participants who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare, and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent. Data are available from The Maastricht Study for any researcher who meets the criteria for access to confidential data, and the corresponding author may be contacted to request data.

Blood pressure measurements and determination of blood pressure variability

A detailed description of the office, 24-hour ambulatory, and 7-day home blood pressure measurements and determination of BPV has been reported previously.[33] Briefly, within-visit BPV was calculated as the standard deviation (SD) of three consecutive office blood pressure measurements assessed in the sitting position, with a 1-minute interval, after ten minutes of rest.[34] 24-hour BPV was calculated as the

average real variability of blood pressure readings taken every 15 minutes between 08:00 A.M. and 11:00 P.M., and every 30 minutes between 11:00 P.M. – 08.00 A.M.[34] 7-day BPV was calculated as the SD of home blood pressure measurements taken twice, with a 1-minute interval, each morning and evening, for 7 consecutive days.[34]

Microvascular dysfunction measures

For all MVD measures, participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement.[35] A light meal was allowed until ≥90 minutes prior to the examination. For retinal measurements, pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine ≥15 minutes before the start of the examination. Skin blood flow measurements were performed in a climate-controlled room at 24°C.[36] Here, we briefly describe the MVD measures used; a detailed description, including the reproducibility of the MVD measures, is provided in the Extended Methods (Supplementary Material).

Features of cerebral small vessel disease

Brain MRI measurements were implemented from December 2013 onwards and were available in 2,313 of the 3,451 participants (67%). Brain MRI was performed on a 3T MRI scanner (Siemens Magnetom Prisma-fit Syngo MR D13D, Erlangen, Germany). We evaluated four MRI CSVD features, i.e. total brain parenchyma volume, white matter hyperintensity volume, lacunar infarcts, and cerebral microbleeds. Briefly, the MRI protocol consisted of a 3D T1-weighted sequence, T2-weighted fluid-attenuated inversion recovery (FLAIR), and a gradient recalled echo (GRE) pulse sequence with susceptibility-weighted imaging (SWI).[37] T1-weighted images and FLAIR images were analysed by use of an automated method.[38,39] T1-weighted images were segmented into grey matter, white matter, and cerebrospinal fluid volumes.[38] Intracranial volume was calculated as the sum of grey matter, white matter (including white matter hyperintensity volume), and cerebrospinal fluid volumes. Total brain parenchyma volume was calculated as the sum of grey and white matter volumes. T1-weighted and FLAIR images were used to identify white matter hyperintensities.[39] White matter hyperintensity volume was summed to assess total white matter hyperintensity burden, and expressed relative to intracranial volume. Lacunar infarcts were defined as focal brain parenchyma defects of ≥3 mm and <15mm in size with a similar signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on FLAIR images.[6] Cerebral microbleeds were rated on three-dimensional T2* GRE imaging with SWI by use of the Microbleed Anatomical Rating Scale,[40] and were defined as focal lesions of ≥2 mm and ≤10mm in size with a hypointense signal.[6] The presence of lacunar infarcts and cerebral microbleeds was rated manually by three neuroradiologists.

Flicker light-induced retinal arteriolar and venular dilation response

We measured retinal arteriolar and venular dilation to flicker light exposure by the Dynamic Vessel Analyzer (Imedos, Jena, Germany), as previously described.[7,41] Briefly, a baseline recording of 50 seconds was followed by 40-second flicker light exposure followed by a 60-second recovery period. We calculated baseline diameters (in measurement units) as the average diameter during the 20-50 seconds recording. For both the arteriolar and venular dilation, percentage dilation over baseline was calculated using the average dilation achieved at time points 10 and 40 seconds during the flicker stimulation period.

Urinary albumin excretion

To assess UAE, participants were requested to collect two 24-hour urine samples. Urinary albumin concentration was measured with a standard immunoturbidimetric assay by an automatic analyser (due to a change of supplier, by the Beckman Synchron LX20 and the Roche Cobas 6000) and multiplied by collection volume to obtain 24-hour UAE. A urinary albumin concentration below the detection limit of the assay was set at 1.5 mg/L (2 mg/L for the Beckman Synchron LX20 and 3 mg/L for the Roche Cobas 6000) before multiplying by collection volume. Only urine collections with a collection time between 20 and 28 hours were considered valid. If needed, UAE was extrapolated to 24-hour excretion. For this study, UAE was preferably based on the average of two (available in 91.3% of participants) 24-hour urine collections.

Heat-induced skin hyperaemia

We measured heat-induced skin hyperaemia by laser Doppler flowmetry (Perimed, Järfälla, Sweden), as previously described.[7] Briefly, skin blood flow at the wrist, expressed in arbitrary perfusion units (PU), was recorded unheated for 2 minutes to serve as a baseline. After 2 minutes, the temperature of the laser Doppler probe was rapidly and locally increased to 44°C and was kept constant until the end of the registration. Skin hyperemia was expressed as the percentage increase in average PU during the 23 minutes heating phase over the 2 minutes average baseline PU.

Plasma biomarkers of microvascular dysfunction

We measured four plasma biomarkers of MVD: sICAM-1, sVCAM-1, sE-selectin and vWF.[42] sICAM-1, sVCAM-1 and sE-selectin were measured in EDTA plasma samples with commercially available 4-plex sandwich immunoassay kits with different standards and antibodies (Meso Scale Discovery, Rockville, Maryland, United States of America).

Covariates

We determined glucose metabolism status according to the World Health Organization 2006 criteria as normal glucose metabolism, prediabetes, or T2D.[43] Education level was classified into three groups: low (none, primary or lower vocational education only), intermediate (intermediate general secondary, intermediate vocational or higher general secondary education) and high (higher vocational education or university level of education). We determined alcohol consumption (none, low [women ≤7, men ≤14 units/week], high [women >7, men >14 units/week]), smoking status (never, former, current), medication use, body mass index, total/high density lipoprotein (HDL) cholesterol ratio, and prior cardiovascular disease as described previously.[7,8,32] We defined hypertension as use of antihypertensive medication, and/or systolic office blood pressure ≥140 mm Hg, and/or diastolic office blood pressure ≥90 mm Hg.[44] Estimated glomerular filtration rate (eGFR) was computed with the CKD-EPI (Chronic Kidney Disease Epidemiology collaboration) formula using serum creatinine and cystatin C.[45] Plasma biomarkers of low-grade inflammation (i.e. high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8, and tumour necrosis factor alpha) were determined as described previously.[32,46] Carotid-femoral pulse wave velocity, a measure of aortic stiffness,[44] was measured according to international guidelines[47] with the use of applanation tonometry (Sphygmocor, Atcor Medical, Sydney Australia) at the right common carotid and right common femoral arteries. As described previously, we used questionnaires to assess the Mediterranean diet score ("diet score"),[48] moderate-to-vigorous physical activity,[32] and socio-economic status (income level and occupation status).[49]

Statistical analysis

We inversed (i.e. multiplied by -1) total brain parenchyma volume, flicker light-induced retinal arteriolar and venular dilation response, and heat-induced skin hyperaemia so that higher values indicated worse microvascular function. White matter hyperintensity volume and UAE were log-transformed (base 2) to normalize their skewed distribution.

We summarized the three BPV measures (i.e. within-visit, 24-hour, and 7-day BPV) into a systolic and diastolic BPV composite score, as done previously.[50] We hypothesized that each BPV measure is associated with MVD according to similar underlying mechanisms, i.e. each greater BPV measure may be related to an increased pulsatile load, and these increased pulsatile loads may damage the microvascular beds of various organs in a similar way. Furthermore, a composite score reduces the influence of noise, or measurement error, of its components,[51] and it reduces the chance

of a type 1 error. The BPV composite scores were calculated when at least data on two of the three BPV measures were available. The scores were calculated by summation and subsequent standardization of the z-scores of the three systolic and diastolic BPV measures, respectively, so that a 1-unit increment is expressed as a 1-SD increment in the BPV composite score.

We also calculated separate composite scores for the CSVD features, for the flicker light-induced retinal arteriolar and venular dilation response and for the plasma biomarkers of MVD, respectively. The CSVD composite score was calculated as described previously;[52] one point per CSVD feature was assigned based on the following cut-offs: for lower total brain parenchyma volume quartile 1 vs. quartiles 2 to 4; for higher white matter hyperintensity volume quartile 4 vs. quartiles 1 to 3; and for lacunar infarcts and cerebral microbleeds presence vs. absence. The points for each feature were combined to compute the CSVD composite score (range 0-4). The composite scores for retinal arteriolar and venular dilation and plasma biomarkers of MVD were calculated by summation and averaging of the z-scores of the flicker light-induced retinal arteriolar and venular dilation responses and the four plasma biomarkers of MVD, respectively.

We used Poisson regression to investigate the association between the systolic and diastolic BPV composite scores and the CSVD composite score. We used linear regression to investigate the association between the systolic and diastolic BPV composite scores and the retinal arteriolar and venular dilation composite score, UAE, skin hyperaemia and the plasma biomarkers of MVD composite score. All analyses were adjusted for age and sex (model 1), and additionally for glucose metabolism status (model 2), mean 24-hour systolic or diastolic blood pressure (where appropriate) (model 3), and education level, body mass index, smoking status, alcohol consumption, total/HDL cholesterol ratio, lipid-modifying medication, and the individual classes of antihypertensive medication (i.e. beta blockers, diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers) (model 4). For analyses with the CSVD composite score, results were exponentiated to represent the risk ratio per point higher score. For analyses with log-transformed UAE as the outcome, regression coefficients were back-transformed and expressed as higher ratio per SD higher systolic and diastolic BPV.

We tested interaction terms of the BPV composite scores with age,[53] sex[54] ,glucose metabolism status[55] and hypertension status[12] to evaluate whether the associations between the BPV composite scores and the MVD measures differed according to these factors.

Several sensitivity and additional analyses were performed. First, we repeated the analysis with the individual BPV measures as the determinant, i.e. within-visit, 24-hour, and 7-day systolic and diastolic BPV. Second, we repeated the analysis using as

the outcome the individual CSVD features, the individual retinal arteriolar and venular dilation response and the individual plasma biomarkers of MVD, respectively. Third, we repeated the analysis with additional adjustment for eGFR, prior cardiovascular disease, plasma biomarkers of low-grade inflammation, and carotid-femoral pulse wave velocity. These covariates were entered into a separate model because of the risk of overadjustment bias: these factors may be confounders, but may also mediate any association between BPV and MVD. Fourth, we repeated the analysis additionally adjusting for the diet score, and moderate-to-vigorous physical activity, and for income level and occupational status (instead of educational level). Adjustment for these potential confounders was not included in the main analysis, because data were missing in a relatively large number of participants (n=1,133 missed data on one or more of these variables). Fifth, we used micro-albuminuria defined as ≥30 mg/24h vs. <30 mg/24h as the outcome instead of UAE per mg/24h.[56] Sixth, we studied the association between BPV and eGFR (continuously and categorically [≥60 vs. <60 ml/min/1.73m^2]).

All statistical analyses were performed with Statistical Package for Social Sciences (v22.0; IBM, Chicago, Illinois). A P value of <0.05 was considered statistically significant.[57]

Results

Study population

Figure 6.1 shows the derivation of the study population. In total, 2,773 participants had data available on the BPV composite scores, all potential confounders and at least one MVD measure, and were included in the analysis. CSVD features were available in 1,837 participants, retinal arteriolar and venular dilation response in 1,844, UAE in 2,748, skin hyperemia in 1,320, and plasma biomarkers of MVD in 2,726. These subpopulations were comparable with regard to age, sex, and cardiovascular risk profile (Supplemental Table S6.1). Participants excluded due to missing data had greater BPV and higher body mass index and more often had prior cardiovascular disease compared with those without missing data (Supplemental Table S6.1).

Table 6.1 and Supplemental Table S6.2 show the general characteristics for the total study population and according to tertiles of the systolic BPV composite score. Supplemental Table S6.3 shows the characteristics according to tertiles of the diastolic BPV composite score. The mean age was 59.9 years, 51.9% were men, and 26.9% had T2D. In general, participants with the highest compared with the lowest tertile of the systolic BPV composite score were older, less often male, had a worse cardiovascular risk profile and more often used lipid-modifying and antihypertensive medication.

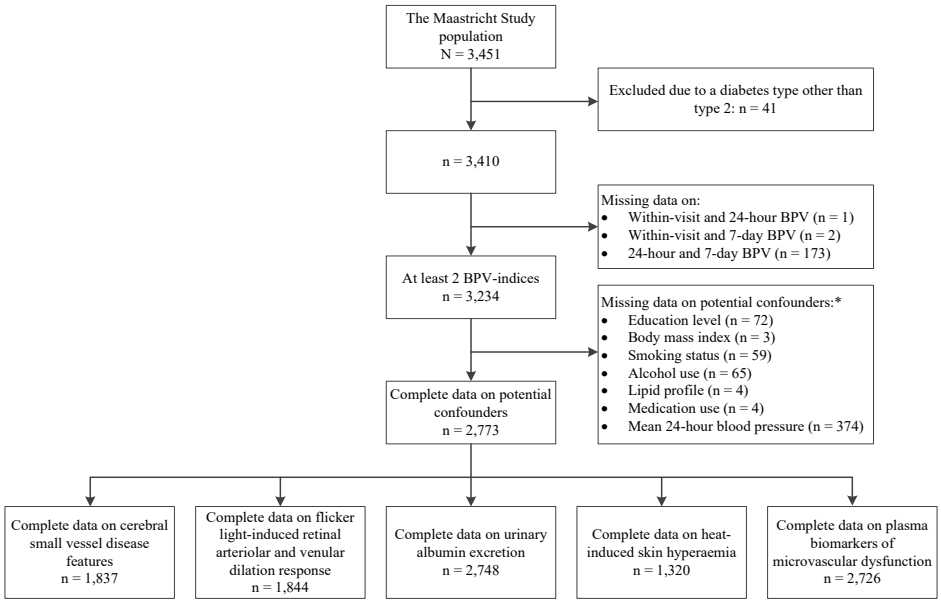

Figure 6.1 Flowchart delineating the derivation of the study population. *not mutually exclusive. Abbreviations: BPV, blood pressure variability.

Blood pressure variability and microvascular dysfunction

Higher systolic and diastolic BPV composite scores were associated with higher UAE (1.04 [95%CI 1.00-1.08] and 1.07 [1.03-1.11] higher ratio per 1 SD higher systolic and diastolic BPV composite score, respectively), after adjustment for all potential confounders (Table 6.2, model 4). Systolic and diastolic BPV composite scores were not associated with the other MVD measures: the CSVD composite score, the retinal arteriolar and venular dilation response composite score, skin hyperaemia, and the plasma biomarkers of MVD composite score, after full adjustment (Table 6.2, model 4).

We did not observe consistent interactions with age, sex, glucose metabolism status or hypertension status for the associations between systolic and diastolic BPV and any of the MVD measures (Supplemental Table S6.4).

Table 6.1 General study population characteristics.

Characteristic	Total study population (n=2,773)	Tertiles of systolic BPV composite score		
		Lowest tertile (n=921)	Middle tertile (n=934)	Highest tertile (n=918)
Demographics				
Age, years	59.9 ± 8.2	57.8 ± 8.6	60.3 ± 7.8	61.7 ± 7.6
Men	1,440 (51.9)	490 (53.2)	486 (52.0)	464 (50.5)
Lifestyle factors				
Smoking status:				
Never	980 (35.3)	366 (39.7)	297 (31.8)	317 (34.5)
Former	1,441 (52.0)	453 (49.2)	506 (54.2)	482 (52.5)
Current	352 (12.7)	102 (11.1)	131 (14.0)	119 (13.0)
Alcohol consumption				
None	509 (18.4)	148 (16.1)	179 (19.2)	182 (19.8)
Low (women ≤7, men ≤14 units/week)	1,550 (55.9)	562 (61.0)	516 (55.2)	472 (51.4)
High (women >7, men >14 units/week)	714 (25.7)	211 (22.9)	239 (25.6)	264 (28.8)
Body mass index, kg/m^2	27.0 ± 4.4	26.3 ± 4.3	27.0 ± 4.3	27.6 ± 4.5
Cardiovascular risk factors				
Total/HDL cholesterol ratio	3.7 ± 1.2	3.6 ± 1.2	3.6 ± 1.2	3.8 ± 1.2
Glucose metabolism status				
Normal glucose metabolism	1,575 (56.8)	612 (67.4)	522 (55.9)	432 (47.1)
Prediabetes	416 (15.0)	112 (12.2)	151 (16.2)	153 (16.7)
Type 2 diabetes	782 (28.2)	188 (20.4)	261 (27.9)	333 (36.3)
Use of lipid-modifying medication	1,004 (36.2)	278 (30.2)	330 (35.3)	396 (43.1)
Use of antihypertensive medication	1,101 (39.7)	284 (30.8)	371 (39.7)	446 (48.6)
Beta blockers	488 (17.6)	130 (14.1)	162 (17.3)	196 (21.4)
Diuretics	448 (16.2)	104 (11.3)	170 (18.2)	174 (19.0)
Calcium channel blockers	244 (8.8)	72 (7.8)	88 (9.4)	84 (9.2)
Angiotensin-converting enzyme inhibitors	342 (12.3)	77 (8.4)	107 (11.5)	158 (17.2)
Angiotensin II receptor blockers	491 (17.7)	122 (13.2)	174 (18.6)	195 (21.2)
Mean BP				
24-hour systolic BP, mmHg	120.1 ± 11.7	115.9 ± 9.7	120.0 ± 11.7	124.3 ± 12.7
24-hour diastolic BP, mmHg	74.4 ± 7.1	72.7 ± 6.3	74.4 ± 7.2	76.1 ± 7.4
BPV measures				
Within-visit systolic BPV, mmHg	4.69 ± 2.91	2.77 ± 1.46	4.43 ± 1.99	6.88 ± 3.30
Within-visit diastolic BPV, mmHg	2.51 ± 1.68	2.14 ± 1.31	2.41 ± 1.46	2.99 ± 2.07
24-hour systolic BPV, mmHg	10.03 ± 2.50	8.16 ± 1.32	9.88 ± 1.58	12.07 ± 2.61
24-hour diastolic BPV, mmHg	7.01 ± 1.86	6.24 ± 1.35	6.88 ± 1.65	7.91 ± 2.10
7-day systolic BPV, mmHg	9.25 ± 3.83	6.91 ± 1.70	8.84 ± 2.28	12.15 ± 4.80
7-day diastolic BPV, mmHg	5.76 ± 2.93	4.76 ± 1.65	5.37 ± 1.80	7.24 ± 4.14
Measures of MVD				
Cerebral small vessel disease composite score, per point	0.66 ± 0.84	0.52 ± 0.77	0.70 ± .086	0.78 ± 0.87
Flicker light-induced retinal arteriolar and venular dilation response composite score, SD	-0.01 ± 1.00	-0.05 ± 1.01	-0.04 ± 0.99	0.05 ± 0.99
Urinary albumin excretion, mg/24 hours	6.8 [4.1–11.8]	5.8 [3.7–9.9]	6.9 [4.0–12.5]	7.6 [4.7–13.6]
Heat-induced skin hyperaemia, %	1,124 ± 781	1,164 ± 841	1,130 ± 745	1,083 ± 756
Plasma biomarkers of MVD composite score, SD	-0.01 ± 0.99	-0.12 ± 0.99	-0.04 ± 0.98	0.12 ± 0.99

Data are presented as mean ± standard deviation, median [interquartile range] or n (%). Data were available for: within-visit blood pressure variability, n=2,768; 24-hour blood pressure variability, n=2,773 7-day blood pressure variability, n=1,950; cerebral small vessel disease composite score, n=1,837; flicker light-induced arteriolar and venular dilation, n=1,844; urinary albumin excretion, n=2,748; skin hyperaemia, n=1,320, and plasma biomarkers of microvascular dysfunction, n=2,685. Abbreviations: BP, blood pressure; BPV, blood pressure variability; HDL, high-density lipoprotein; MVD, microvascular dysfunction.

Table 6.2 Associations between systolic and diastolic blood pressure variability composite scores and microvascular dysfunction measures.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction measure	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease composite score, per point	1	**1.07**	**(1.01 – 1.13)**	**1.07**	**(1.00 – 1.13)**
	2	1.06	(1.00 – 1.12)	1.06	(0.99 – 1.12)
	3	1.03	(0.97 – 1.10)	1.03	(0.97 – 1.10)
	4	1.02	(0.96 – 1.09)	1.03	(0.97 – 1.10)
Flicker light-induced retinal arteriolar and venular dilation composite score, per SD	1	0.033	(-0.014 – 0.081)	0.021	(-0.030 – 0.071)
	2	0.021	(-0.027 – 0.069)	0.014	(-0.036 – 0.064)
	3	0.030	(-0.020 – 0.081)	0.027	(-0.023 – 0.078)
	4	0.031	(-0.019 – 0.082)	0.025	(-0.025 – 0.076)
Urinary albumin excretion, higher ratio	1	**1.14**	**(1.10 - 1.18)**	**1.13**	**(1.09 - 1.17)**
	2	**1.11**	**(1.07 - 1.15)**	**1.11**	**(1.09 - 1.15)**
	3	**1.05**	**(1.01 - 1.09)**	**1.09**	**(1.09 - 1.13)**
	4	**1.04**	**(1.00 - 1.08)**	**1.07**	**(1.03 - 1.11)**
Heat-induced skin hyperaemia, per SD	1	0.027	(-0.029 – 0.083)	0.007	(-0.050 – 0.064)
	2	0.006	(-0.051 – 0.063)	-0.009	(-0.066 – 0.048)
	3	-0.001	(-0.060 – 0.058)	-0.006	(-0.064 – 0.052)
	4	0.005	(-0.054 – 0.065)	-0.003	(-0.061 – 0.055)
Plasma biomarkers of microvascular dysfunction composite score, per SD	1	**0.089**	**(0.052 – 0.126)**	**0.082**	**(0.043 – 0.120)**
	2	**0.052**	**(0.015 – 0.088)**	**0.054**	**(0.017 – 0.092)**
	3	**0.057**	**(0.019 – 0.095)**	**0.056**	**(0.018 – 0.093)**
	4	0.035	(-0.001 – 0.072)	0.023	(-0.013 – 0.060)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light-induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat-induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: adjusted for age, sex; model 2: model 1 + glucose metabolism status; model 3: model 2 + mean 24-hour systolic or diastolic blood pressure (where appropriate); model 4: model 3 + educational level, body mass index, smoking status, alcohol consumption, total/high density lipoprotein cholesterol ratio, lipid-modifying medication, and the individual classes of antihypertensive medication (i.e. beta blockers, diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers). Bold denotes P value<.05.Abbreviations: CI: confidence interval; SD: standard deviation.

Sensitivity and additional analyses

For individual systolic and diastolic BPV measures we found the following results after full adjustment: systolic and diastolic within-visit BPV were both not associated with any measure of MVD; 24-hour systolic BPV was not associated with any measure of MVD; diastolic 24-hour, BPV was associated with a higher CSVD composite score, retinal arteriolar and venular dilation response composite score and UAE but not with any other measure of MVD; 7-day systolic BPV was associated with a higher CSVD composite score and a higher plasma biomarkers of MVD composite score, but not with any other measure of MVD; and 7-day diastolic BPV was associated with higher

UAE and a higher plasma biomarkers of MVD composite score, but not with any other measure of MVD (Supplemental Table S6.5). When we repeated the analysis using each individual MVD measure as the outcome, the systolic and diastolic BPV composite scores were associated with higher levels of sVCAM-1 and the systolic BPV composite score was associated with higher levels of vWF (Supplemental Table S6.6). Results were similar when we additionally adjusted for eGFR, prior cardiovascular disease, plasma biomarkers of low-grade inflammation, carotid-femoral pulse wave velocity, diet score, moderate-to-vigorous physical activity, income level or occupational status (Supplemental Tables S6.7-S6.14). Each SD higher BPV composite score was associated with higher odds of UAE ≥30 mg/24h; odds ratios were 1.19 (95%CI 1.02–1.38) for systolic BPV and 1.19 (95%CI 1.02–1.37) for diastolic BPV (Figure 6.2 and Supplemental Table S6.15). The systolic and diastolic BPV composite scores were not associated with eGFR (Supplemental Table S6.16).

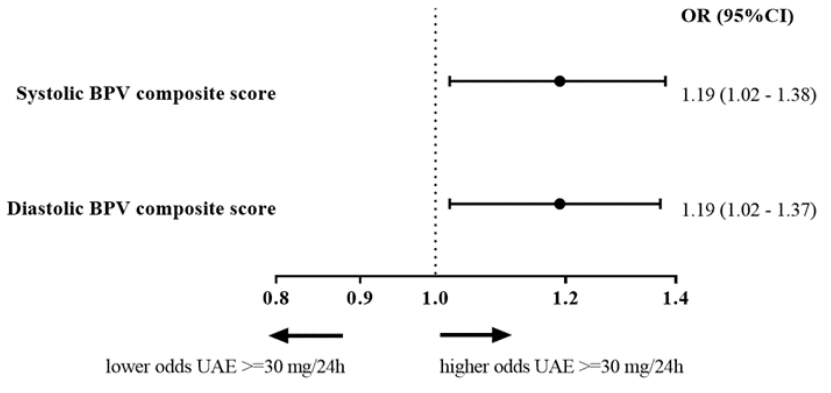

Figure 6.2 Associations of systolic and diastolic blood pressure variability composite scores with urinary albumin excretion dichotomized as ≥30 mg/24h vs. <30 mg/24h. Point estimates represent the odds ratio of urinary albumin excretion per standard deviation higher systolic or diastolic BPV composite score. Results are adjusted for age, sex, glucose metabolism status, mean 24-hour systolic or diastolic blood pressure (where appropriate), education level, body mass index, smoking status, alcohol consumption, total/high density lipoprotein cholesterol ratio, lipid-modifying medication, and the individual classes of antihypertensive medication. Abbreviations: BPV, blood pressure variability; CI, confidence interval; OR, odds ratio; UAE, urinary albumin excretion; 24h, 24 hours; mg, milligram.

Discussion

We found that greater very short- to mid-term systolic and diastolic BPV are associated with higher UAE, but not with other measures of MVD tested, i.e. the CSVD composite score, flicker light-induced retinal arteriolar and venular dilation response composite score, heat-induced skin hyperaemia, and the plasma biomarkers of MVD

composite score. The association with higher UAE was independent of age, sex, mean 24-hour systolic or diastolic blood pressure, education level, and lifestyle and cardiovascular risk factors. The strength of this association corresponds to a 1.2 higher odds of UAE ≥30 mg/24h as compared to a UAE of <30 mg/24h per SD higher systolic or diastolic BPV composite score.

Our study findings are in agreement with most,[16-27] but not all,[28-30] previous studies that investigated the association between BPV and UAE. Our study adds to the existing literature on UAE, because we were able to study this association in the context of various other MVD measures, and adjusted for potentially important confounders, including dietary habits and physical activity. Previous studies on BPV and UAE did not adjust for these potential confounders.

In disagreement with our hypothesis, we did not find an association between greater BPV and MVD measured in organs with low microvascular impedance other than the kidneys, i.e. the brain and eyes, and with plasma biomarkers of MVD, which at least partly reflect MVD in organs with low microvascular impedance. A possible explanation is that the kidney microvasculature has a lower impedance than the brain and eye microvasculature, e.g. blood flow to the kidneys relative to organ weight (360 ml/min/100 g kidney tissue) is higher than to the brain (50 ml/min/100 g brain tissue).[58] The kidney microvasculature may, therefore, in comparison be most vulnerable to the detrimental effects of BPV.

Although we found no significant associations between greater BPV and CSVD features, flicker light-induced retinal arteriolar and venular dilation response, and plasma biomarkers of MVD, these measures nevertheless reflect MVD in organs with low vascular impedance and may thus be vulnerable to an increased pulsatile load,[59] albeit to a lesser extent than UAE. Indeed, we found positive associations that were quantitatively similar for CSVD features, flicker light-induced retinal arteriolar and venular dilation response, and plasma biomarkers of MVD. Estimations of these associations may have been more inaccurate (i.e. larger confidence intervals), because of higher measurement errors in these MVD measures as compared with UAE: CSVD features, flicker light-induced retinal arteriolar and venular dilation response, and plasma biomarkers of MVD were measured only once, whereas UAE was based on two 24-hour urine samples. In addition, estimations of the associations may have been more inaccurate with CSVD features and retinal arteriolar and venular dilation response due to relatively less available data (n=1,837 and n=1,844, respectively) as compared with UAE (n=2,748).

As expected, we did not find an association between BPV and heat-induced skin hyperaemia. The skin has relatively high microvascular impedance,[4] and, therefore, most of the increased pulsatile energy related to greater BPV may be dissipated by arteries and large arterioles proximal to the skin capillaries.[60]

Strengths of this study include the large study population of community-dwelling participants, assessment of microvascular function in various vascular beds, and the extensive adjustment for potential confounders.

Our study has several limitations. First, our cross-sectional data preclude reaching causal conclusions about the study findings. Indeed, the reverse association may hold true as well, i.e. higher UAE (as a reflection of worse kidney function) may lead to greater BPV.[61] However, when we additionally adjusted our analyses for estimated glomerular filtration rate, results were similar. Second, as we performed a large number of tests in interaction analyses we cannot exclude that some findings may reflect the play of chance due to multiple testing. Future validation of these inconsistent (hypothesis-generating) observations is warranted. Third, the use of a BPV composite score assumes that all BPV measures share similar underlying mechanisms that lead to MVD, which may not necessarily be true. However, when we repeated the analyses with the individual BPV measures, results were qualitatively similar. This may suggest that BPV measures share similar underlying mechanisms that lead to MVD (even though individual measures of BPV are determined by different haemodynamic mechanisms). [62]Fourth, the association between greater BPV and UAE may be the result of residual confounding due to low-grade inflammation,[63,64] arterial stiffening,[4, 65] activation of the renin-angiotensin system,[66,67] unhealthy dietary habits,[68,69] physical inactivity[70,71] and lower socio-economic status.[72,73] However, when we adjusted for low-grade inflammation, carotid-femoral pulse wave velocity, the diet score, moderate-to-vigorous physical activity and factors related to socio-economic status (i.e. education, income level and occupational status), results did not materially change. In this study, no data were available on activation of the renin-angiotensin system, however, and this issue requires further study. Fifth, we may have underestimated the association between greater BPV and MVD, because individuals excluded for the present analysis due to missing data had greater BPV and a higher prevalence of prior cardiovascular disease than those included in the analysis. Finally, the study population consisted mainly of middle-aged individuals who were relatively well-educated and whose cardiovascular risk factors were relatively well-controlled. This may have led to an underestimation of the association between BPV and MVD.

Perspectives

In conclusion, this large, relatively healthy population-based study showed that greater very short- to mid-term systolic and diastolic BPV was associated with higher UAE, but not with other measures of MVD tested, i.e. CSVD features, flicker light-induced retinal arteriolar and venular dilation response, heat-induced skin hyperaemia, and

plasma biomarkers of MVD. This may suggest that the microvasculature of the kidneys is most vulnerable to the detrimental effects of greater BPV. Future longitudinal studies should confirm these associations and, if verified, intervention studies should assess whether lowering BPV may prevent albuminuria.

References

1. Rensma SP, van Sloten TT, Launer LJ, Stehouwer CDA. Cerebral small vessel disease and risk of incident stroke, dementia and depression, and all-cause mortality: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2018;90:164-173
2. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67:1729-1741
3. Bidani AK, Polichnowski AJ, Loutzenhiser R, Griffin KA. Renal microvascular dysfunction, hypertension and CKD progression. Curr Opin Nephrol Hypertens. 2013;22:1-9
4. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. J Appl Physiol (1985). 2008;105:1652-1660
5. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. Lancet. 2010;375:938-948
6. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. nEuroimaging STfRVco. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-838
7. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, et al. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. Circulation. 2016;134:1339-1352
8. Martens RJ, Henry RM, Houben AJ, van der Kallen CJ, Kroon AA, Schalkwijk CG, et al.. Capillary Rarefaction Associates with Albuminuria: The Maastricht Study. J Am Soc Nephrol. 2016;27: 3748757
9. van Sloten TT, Schram MT, Adriaanse MC, Dekker JM, Nijpels G, Teerlink T, et al. Endothelial dysfunction is associated with a greater depressive symptom score in a general elderly population: the Hoorn Study. Psychol Med. 2014;44:1403-1416
10. Yang S, Yuan J, Qin W, Yang L, Fan H, Li Y et al. Twenty-four-hour ambulatory blood pressure variability is associated with total magnetic resonance imaging burden of cerebral small-vessel disease. Clin Interv Aging. 2018;13:1419-1427
11. Goldstein IB, Bartzokis G, Guthrie D, Shapiro D. Ambulatory blood pressure and the brain: a 5-year follow-up. Neurology. 2005;64:1846-1852
12. Filomena J, Riba-Llena I, Vinyoles E, Tovar JL, Mundet X, Castane X, et al. Investigators I. Short-Term Blood Pressure Variability Relates to the Presence of Subclinical Brain Small Vessel Disease in Primary Hypertension. Hypertension. 2015;66:634-640
13. Gunstad J, Cohen RA, Tate DF, Paul RH, Poppas A, Hoth K et al. Blood pressure variability and white matter hyperintensities in older adults with cardiovascular disease. Blood Press. 2005;14:353-358
14. Yang S, Qin W, Yang L, Fan H, Li Y, Yin J et al. The relationship between ambulatory blood pressure variability and enlarged perivascular spaces: a cross-sectional study. BMJ Open. 2017;7:e015719
15. Yamaguchi Y, Wada M, Sato H, Nagasawa H, Koyama S, Takahashi Y et al. Impact of ambulatory blood pressure variability on cerebral small vessel disease progression and cognitive decline in community-based elderly Japanese. Am J Hypertens. 2014;27:1257-1267
16. Kawai T, Ohishi M, Kamide K, Onishi M, Takeya Y, Tatara Y et al. The impact of visit-to-visit variability in blood pressure on renal function. Hypertens Res. 2012;35:239-243
17. Kilpatrick ES, Rigby AS, Atkin SL. The role of blood pressure variability in the development of nephropathy in type 1 diabetes. Diabetes Care. 2010;33:2442-2447
18. Okada H, Fukui M, Tanaka M, Inada S, Mineoka Y, Nakanishi N et al. Visit-to-visit variability in systolic blood pressure is correlated with diabetic nephropathy and atherosclerosis in patients with type 2 diabetes. Atherosclerosis. 2012;220:155-159
19. Kristensen KS, Hoegholm A, Bang LE, Gustavsen PH, Poulsen CB. No impact of blood pressure variability on microalbuminuria and left ventricular geometry: analysis of daytime variation, diurnal variation and 'white coat' effect. Blood Press Monit. 2001;6:125-131
20. de la Sierra A, Pareja J, Yun S, Acosta E, Aiello F, Oliveras A et al. Central blood pressure variability is increased in hypertensive patients with target organ damage. J Clin Hypertens (Greenwich). 2018;20:266-272

21. Tatasciore A, Renda G, Zimarino M, Soccio M, Bilo G, Parati G et al. Awake systolic blood pressure variability correlates with target-organ damage in hypertensive subjects. Hypertension. 2007;50: 325-332
22. Yin LH, Yan WJ, Guo ZX, Zhou FZ, Zhang HY. Relation between blood pressure variability and early renal damage in hypertensive patients. Eur Rev Med Pharmacol Sci. 2017;21:2226-2231
23. Kagitani H, Hoshide S, Kario K. Optimal indicators of home BP variability in perimenopausal women and associations with albuminuria and reproducibility: The J-HOT home BP study. Am J Hypertens. 2015;28:586-594
24. Ushigome E, Fukui M, Hamaguchi M, Senmaru T, Sakabe K, Tanaka M et al. The coefficient variation of home blood pressure is a novel factor associated with macroalbuminuria in type 2 diabetes mellitus. Hypertens Res. 2011;34:1271-1275
25. Matsumoto S, Ushigome E, Matsushita K, Fukuda T, Mitsuhashi K, Majima S et al. Home Blood Pressure Variability From the Stored Memory Is Correlated With Albuminuria, but From the Logbook Is not. Am J Hypertens. 2017;30:993-998
26. Li CL, Liu R, Wang JR, Yang J. Relationship between blood pressure variability and target organ damage in elderly patients. Eur Rev Med Pharmacol Sci. 2017;21:5451-5455
27. Mule G, Calcaterra I, Costanzo M, Geraci G, Guarino L, Foraci AC et al. Relationship Between Short-Term Blood Pressure Variability and Subclinical Renal Damage in Essential Hypertensive Patients. J Clin Hypertens (Greenwich). 2015;17:473-480
28. Ceriello A, De Cosmo S, Rossi MC, Lucisano G, Genovese S, Pontremoli R et al. Group AM-AS. Variability in HbA1c, blood pressure, lipid parameters and serum uric acid, and risk of development of chronic kidney disease in type 2 diabetes. Diabetes Obes Metab. 2017;19:1570-1578
29. Wei FF, Li Y, Zhang L, Xu TY, Ding FH, Wang JG et al. Beat-to-beat, reading-to-reading, and day-to-day blood pressure variability in relation to organ damage in untreated Chinese. Hypertension. 2014;63:790-796
30. Madden JM, O'Flynn AM, Dolan E, Fitzgerald AP, Kearney PM. Short-term blood pressure variability over 24 h and target organ damage in middle-aged men and women. J Hum Hypertens. 2015;29: 719-725
31. Tatasciore A, Zimarino M, Renda G, Zurro M, Soccio M, Prontera C et al. Awake blood pressure variability, inflammatory markers and target organ damage in newly diagnosed hypertension. Hypertens Res. 2008;31:2137-2146
32. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-451
33. Zhou TL, Kroon AA, Reesink KD, Schram MT, Koster A, Schaper NC et al. Blood pressure variability in individuals with and without (pre)diabetes: The Maastricht Study. J Hypertens. 2018;36:259-267
34. Garhofer G, Resch H, Sacu S, Weigert G, Schmidl D, Lasta M et al. Effect of regular smoking on flicker induced retinal vasodilatation in healthy subjects. Microvasc Res. 2011;82:351-355
35. Pettersson A, Bostrom KB, Gustavsson P, Ekselius L. Which instruments to support diagnosis of depression have sufficient accuracy? A systematic review. Nord J Psychiatry. 2015;69:497-508
36. van Agtmaal MJM, Houben A, de Wit V, Henry RMA, Schaper NC, Dagnelie PC et al. Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. Diabetes Care. 2018;41: 2535-2543
37. Vrooman HA, Cocosco CA, van der Lijn F, Stokking R, Ikram MA, Vernooij MW et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. Neuroimage. 2007;37:71-81
38. de Boer R, Vrooman HA, van der Lijn F, Vernooij MW, Ikram MA, van der Lugt A et al. White matter lesion extension to automatic brain tissue segmentation on MRI. Neuroimage. 2009;45:1151-1161
39. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR et al. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. Neurology. 2009;73:1759-1766
40. Houben A, Martens RJH, Stehouwer CDA. Assessing Microvascular Function in Humans from a Chronic Disease Perspective. J Am Soc Nephrol. 2017;28:3461-3472
41. Muris DM, Houben AJ, Schram MT, Stehouwer CD. Microvascular dysfunction: an emerging pathway in the pathogenesis of obesity-related insulin resistance. Rev Endocr Metab Disord. 2013;14:29-38

42. Correction: Neighbourhood socioeconomic position and risks of major chronic diseases and all-cause mortality: a quasi-experimental study. BMJ Open. 2019;9:e018793corr018791
43. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T et al. Investigators C-E. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367: 20-29
44. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC et al. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-396
45. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T et al. Artery S, European Society of Hypertension Working Group on Vascular S, Function, European Network for Noninvasive Investigation of Large A. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30:445-448
46. Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocke MC, Peeters PH, et al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ. 2005;330:991
47. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N et al. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16
48. Zhou TL, Henry RMA, Stehouwer CDA, van Sloten TT, Reesink KD, Kroon AA. Blood Pressure Variability, Arterial Stiffness, and Arterial Remodeling. Hypertension. 2018;72:1002-1010
49. O'Brien PC. Procedures for comparing samples with multiple endpoints. Biometrics. 1984;40: 1079-1087
50. van Sloten TT, Sigurdsson S, van Buchem MA, Phillips CL, Jonsson PV, Ding J, et al. Cerebral Small Vessel Disease and Association With Higher Incidence of Depressive Symptoms in a General Elderly Population: The AGES-Reykjavik Study. The American journal of psychiatry. 2015;172:570-578
51. James MA, Tullett J, Hemsley AG, Shore AC. Effects of aging and hypertension on the microcirculation. Hypertension. 2006;47:968-974
52. Schiebinger L, Leopold SS, Miller VM. Editorial policies for sex and gender analysis. Lancet. 2016;388:2841-2842
53. Burke M, Pabbidi MR, Farley J, Roman RJ. Molecular mechanisms of renal blood flow autoregulation. Curr Vasc Pharmacol. 2014;12:845-858
54. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39:S1-266
55. Rothman KJ. No Adjustments Are Needed for Multiple Comparisons. Epidemiology. 1990;1:43-46
56. Hall J.E. Guyton and Hall textbook of medical physiology. Philadelphia, PA: Elsevier; 206.
57. van Sloten TT, Protogerou AD, Henry RM, Schram MT, Launer LJ, Stehouwer CD. Association between arterial stiffness, cerebral small vessel disease and cognitive impairment: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2015;53:121-130
58. van Sloten TT, Czernichow S, Houben AJ, Protogerou AD, Henry RM, Muris DM et al. Association Between Arterial Stiffness and Skin Microvascular Function: The SUVIMAX2 Study and The Maastricht Study. Am J Hypertens. 2015;28:868-876
59. Sarafidis PA, Ruilope LM, Loutradis C, Gorostidi M, de la Sierra A, de la Cruz JJ, et al. Blood pressure variability increases with advancing chronic kidney disease stage: a cross-sectional analysis of 16 546 hypertensive patients. J Hypertens. 2018;36:1076-1085
60. Pietri P, Vlachopoulos C, Tousoulis D. Inflammation and Arterial Hypertension: From Pathophysiological Links to Risk Prediction. Curr Med Chem. 2015;22:2754-2761
61. Virdis A, Masi S, Colucci R, Chiriaco M, Uliana M, Puxeddu I et al. Microvascular Endothelial Dysfunction in Patients with Obesity. Curr Hypertens Rep. 2019;21:32
62. Zhou TL, Henry RMA, Stehouwer CDA, van Sloten TT, Reesink KD, Kroon AA. Blood Pressure Variability, Arterial Stiffness, and Arterial Remodeling: The Maastricht Study. Hypertension. 2018;72:1002-1010
63. Ozkayar N, Dede F, Akyel F, Yildirim T, Ates I, Turhan T et al. Relationship between blood pressure variability and renal activity of the renin-angiotensin system. Journal of Human Hypertension. 2016;30:297-302

64. Min SH, Kong SH, Lee JE, Lee DH, Oh TJ, Kim KM et al. Association of angiotensin-II levels with albuminuria in subjects with normal glucose metabolism, prediabetes, and type 2 diabetes mellitus. J Diabetes Complicat. 2017;31:1499-1505
65. Maseli A, Aeschbacher S, Schoen T, Fischer A, Jung M, Risch M et al. Healthy Lifestyle and Blood Pressure Variability in Young Adults. Am J Hypertens. 2017;30:690-699
66. Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. Nutr Metab Cardiovasc Dis. 2014;24: 929-939
67. Simmons GH, Wong BJ, Holowatz LA, Kenney WL. Changes in the control of skin blood flow with exercise training: where do cutaneous vascular adaptations fit in? Exp Physiol. 2011;96:822-828
68. Pagonas N, Dimeo F, Bauer F, Seibert F, Kiziler F, Zidek W et al. The impact of aerobic exercise on blood pressure variability. J Hum Hypertens. 2014;28:367-371
69. Campbell TS, Seguin JR, Vitaro F, Tremblay RE, Ditto B. Childhood socioeconomic position and blood pressure dipping in early adulthood: a longitudinal study. Ann Behav Med. 2013;46:227-231
70. Kim D, Glazier RH, Zagorski B, Kawachi I, Oreopoulos P. Neighbourhood socioeconomic position and risks of major chronic diseases and all-cause mortality: a quasi-experimental study. BMJ Open. 2018;8:e018793.

Supplemental materials

Extended Methods

Brain magnetic resonance imaging

Brain magnetic resonance imaging (MRI) was performed on a 3T MRI scanner (Siemens Magnetom Prisma-fit Syngo MR D13D, Erlangen, Germany) by use of a 64-element head/neck coil for parallel imaging. The MRI protocol consisted of a 3D T_1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR/TI/TE 2300/900/2.98 ms, 176 slices, 256×240 matrix size, 1.00 mm cubic voxel size); a fluid-attenuated inversion recovery (FLAIR) sequence (TR/TI/TE 5000/1800/394 ms, 176 slices, 512×512 matrix size, 0.49×0.49×1.00 mm voxel size); a combined proton density (PD) and T_2-weighted turbo spin echo (TSE) pulse sequence (TR/TE1/TE2 3200/9.4/94 ms, 30 slices, 640×540 matrix size, 0.36×0.36×4.00 mm voxel size); and a susceptibility-weighted imaging (SWI) sequence (TR/TE 28/20 ms, 144 slices, 384×312 matrix size, 0.57×0.57×1.00 mm voxel size).

Contra-indications for MRI assessments were the presence of a cardiac pacemaker or implantable cardioverter-defibrillator, neurostimulator, non-detachable insulin pump, metallic vascular clips or stents in the head, cochlear implant, metal-containing intra-uterine device, metal splinters or shrapnel, dentures with magnetic clip, an inside bracket, pregnancy, epilepsy, and claustrophobia.

T_1-weighted images and FLAIR images were analysed by use of an ISO-13485:2012 certified, automated method (which included visual inspection).[1,2] T_1-weighted images were segmented into grey matter, white matter, and cerebrospinal fluid volumes (1 voxel = 1.00 mm^3 = 0.001 ml).[1] Intracranial volume was calculated as the sum of grey matter, white matter (including white matter hyperintensity volume), and cerebrospinal fluid volumes. Total brain parenchyma volume was calculated as the sum of grey and white matter volumes. White matter hyperintensity volume was summed to assess total white matter hyperintensity burden, and expressed relative to intracranial volume. Lacunar infarcts were defined as focal brain parenchyma defects of ≥3 mm and <15 mm in size with a similar signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on T_2 and FLAIR images.[3] Cerebral microbleeds were rated on T_2-weighted and SWI images by use of the Microbleed Anatomical Rating Scale,[4] and were defined as focal lesions of ≥2 mm and ≤10 mm in size with a hypointense signal.[3] The presence of lacunar infarcts and cerebral microbleeds was rated manually by three neuroradiologists. The two-way mixed effects, consistency, intraclass correlation coefficients for the three raters based on 50 randomly selected scans were 0.84 (95% confidence interval 0.74; 0.91) and 0.83 (0.72; 0.90) for the presence of lacunar infarcts and cerebral microbleeds, respectively.

Assessment of retinal microvascular dynamic function

The retinal arteriolar dilation response to flicker light, which is thought to be related to nutritive demands of activated retinal neurons,[5] was measured in a dimly lit room by use of the Dynamic Vessel Analyzer (DVA; IMEDOS, Jena, Germany). For safety reasons, participants with an intraocular pressure exceeding 30 mmHg were excluded from retinal measurements. Per participant, we randomly measured the left or right eye. During the measurement, the participant was instructed and encouraged to focus on the tip of a fixated needle inside the retinal camera (FF450; Carl Zeiss GmbH, Jena, Germany), while the fundus of the eye was examined under green measuring light (530-600 nm, illumination of fundus approximately 6500 lux). A straight arteriolar segment of approximately 1.5 mm in length located 0.5 to 2.0 disc diameter from the margin of the optic disc in the temporal section was examined. When the specific vessel profile was recognized, vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker-light exposure period (flicker frequency 12.5Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The DVA automatically corrected for alterations in luminance caused by, for example, slight eye movements. During blinks and small eye movements, the registration stopped and restarted once the vessel segments were automatically re-identified.[5]
The integrated DVA software (version 4.51, Imedos) automatically calculated baseline diameter and percentage dilation. Baseline diameter was calculated as the average diameter size of the 20-50 seconds recording and was expressed in measurement units (MU), where 1 MU is equal to 1μm of the Gullstrand eye.[6] Percentage dilation over baseline was based on the average dilation achieved at time-points 10 and 40 seconds during the flicker stimulation period. Two regression lines were drawn (at interval 0-10 seconds and 10-40 seconds during flicker stimulation) and averaged to assess average percentage dilation. The software successfully assessed two regression lines in 95.4% of the curves; only 102 dilation curves (4.6%) were based on one regression line. The purpose of taking the average dilation was to account for inter-individual variation in the curve shape during dilation.

Reproducibility and quality assessment

Retinal vascular %-dilation response measurements were performed by different observers, after an intensive training period. Inter‐observer reliability of the retinal baseline diameter and percentage dilation response between two randomly selected observers (N=9 participants) were, respectively, 0.980 and 0.796 for arteriolar vessels and 0.972 and 0.871 for venular vessels. Retinal arteriolar dilation response curves were analysed by one observer for measurement quality decisions. Retinal response

curves with insufficient measurement quality, e.g. insufficient measurement points or movement artifacts were evaluated and discussed with a second observer, and excluded on mutual agreement. To assess the inter‐observer reliability of retinal response curves quality decisions 50 curves were evaluated by two observers (Inter‐observer reliability =0.883).

Heat-induced skin hyperaemia

Skin blood flow was measured by means of a laser-Doppler system (Periflux 5000; Perimed, Järfalla, Sweden) equipped with a thermostatic laser-Doppler probe (PF457; Perimed) at the dorsal side of the wrist of the left hand. The laser-Doppler output was recorded for 25 minutes with a sample rate of 32 Hz, which gives semiquantitative assessment of skin blood flow expressed in arbitrary perfusion units. In 596 individuals, heat-induced skin hyperaemia measurements were recorded between 20-25 minutes. These data were extrapolated to 25 minutes using the last minute average as reference; with a weighted correction factor of 1.017. This correction factor is the ratio of the increase in average PU in the 20-25 minute interval as compared to the average PU in the 19-20 minute interval. Skin blood flow was first recorded unheated for 2 minutes to serve as a baseline. After the 2 minutes of baseline, the temperature of the probe was rapidly and locally increased to 44°C and was then kept constant until the end of the registration. The heat-induced skin hyperaemic response was expressed as the percentage increase in average perfusion units during the 23-minute heating phase over the average baseline perfusion units.

Plasma biomarkers of microvascular dysfunction

Plasma biomarkers microvascular dysfunction (MVD; sICAM-1, soluble vascular adhesion molecule-1 (sVCAM-1), soluble E-selectin (sE-selectin)) of the first 866 individuals of The Maastricht Study were measured in ethylenediaminetetraacetic acid (EDTA) plasma samples with commercially available 4-plex sandwich immunoassay kits (Meso Scale Discovery [MSD], Rockville, Maryland, United States of America) as described previously.[7]

From individual 867 onwards, plasma biomarkers were measured in EDTA plasma samples with renewed commercially available 4-plex sandwich immunoassay kits with different standards and antibodies (MSD, Rockville, Maryland, United States of America). For this technique in this study, the intra- and inter-assay coefficients of variation were 5.4 and 5.4 for CRP, 8.7 and 10.8 for SAA, 10.3 and 8.4 for sICAM-1, 13.2 and 11.9 for IL-6, 7.6 and 5.5 for IL-8, 4.3 and 6.2 for TNF-α, 5.0 and 4.7 for sVCAM-1, and 2.9 and 7.4 for sE-selectin, respectively.

Absolute values of plasma biomarkers differed between the individuals measured with the old and renewed 4-plex sandwich immunoassay kits. To realign the absolute values of individuals measured with the old 4-plex sandwich immunoassay to individuals measured with the renewed 4-plex sandwich immunoassay, realign formulas were calculated with Deeming regression analyses.[8] In order to do so, the first 419 out of 866 individuals, who were measured with the old 4-plex sandwich immunoassay, were measured with the renewed 4-plex sandwich immunoassay as well.

The biomarker of endothelial dysfunction Von Willebrand Factor (vWF) was quantified in citrate plasma using ELISA (Dako, Glostrup, Denmark). The intra- and inter-assay coefficients of variation were 3.0 and 4.3%, respectively.

References

1. Vrooman HA, Cocosco CA, van der Lijn F, et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. Neuroimage. 2007;37(1):71-81.
2. de Boer R, Vrooman HA, van der Lijn F, et al. White matter lesion extension to automatic brain tissue segmentation on MRI. Neuroimage. 2009;45(4):1151-1161.
3. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12(8):822-838.
4. Gregoire SM, Chaudhary UJ, Brown MM, et al. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. Neurology. 2009;73(21):1759-1766.
5. Nagel E, Vilser W. Flicker observation light induces diameter response in retinal arterioles: a clinical methodological study. Br J Ophthalmol. 2004;88(1):54-56.
6. Nagel E, Vilser W, Fink A, Riemer T. [Variance of retinal vessel diameter response to flicker light. A methodical clinical study]. Ophthalmologe. 2006;103(2):114-119.
7. van Dooren FE, Schram MT, Schalkwijk CG, et al. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56: 390-396.
8. van Bussel BC, Ferreira I, van de Waarenburg MP, et al. Multiple inflammatory biomarker detection in a prospective cohort study: a cross-validation between well-established single-biomarker techniques and an electrochemiluminescense-based multi-array platform. PLoS One. 2013;8(3):e58576.

Table S6.1 Characteristics of the study population and individuals excluded from the analyses due to missing values, according to measure of microvascular dysfunction.

	CSVD features			Flicker light-induced arteriolar and venular dilation			Urinary albumin excretion			Skin hyperemia			Plasma biomarkers of microvascular dysfunction		
	Complete (n=1,837)	No. of participants in complete / missing	Missing (n=1,614)	Complete (n=1,844)	No. of participants in complete / missing	Missing (n=1,607)	Complete (n=2,748)	No. of participants in complete / missing	Missing (n=703)	Complete (n=1,320)	No. of participants in complete / missing	Missing (n=2,131)	Complete (n=2,726)	No. of participants in complete / missing	Missing (n=725)
Demographics															
Age, years	59.3 ± 8.1	0/0	60.3 ± 8.4 *	59.8 ± 8.2	0/0	59.8 ± 8.3	60.0 ± 8.2	0/0	59.0 ± 8.7 *	60.4 ± 8.0	0/0	59.4 ± 8.4 *	59.9 ± 8.2	0/0	59.3 ± 8.6
Men	940 (51.2%)	0/0	835 (51.7%)	944 (51.2%)	0/0	831 (51.7%)	1,427 (51.9%)	0/0	348 (49.5%)	699 (53.0%)	0/0	1,076 (50.5%)	1,426 (52.3%)	0/0	349 (48.1%) *
Lifestyle factors															
Smoking behaviour		0/63	*		0/63	*		0/63	*		0/63	*		0/63	*
Never	697 (37.9%)		473 (30.5%)	660 (35.8%)		510 (33.0%)	974 (35.4%)		196 (30.6%)	439 (33.3%)		731 (35.3%)	964 (35.4%)		206 (31.1%)
Former	934 (53.4%)		815 (52.5%)	969 (52.5%)		780 (50.5%)	1,428 (52.0%)		321 (50.2%)	727 (55.1%)		1,022 (49.4%)	1,416 (51.9%)		333 (50.3%)
Current	206 (11.2%)		263 (17.0%)	215 (11.7%)		254 (16.5%)	346 (12.6%)		123 (19.2%)	154 (11.7%)		315 (15.2%)	346 (12.7%)		123 (18.6%)
Alcohol use		0/69	*		0/69			0/69			0/69			0/69	
None	314 (17.1%)		315 (20.4%)	338 (18.3%)		291 (18.9%)	499 (18.2%)		130 (20.5%)	235 (17.8%)		394 (19.1%)	497 (18.2%)		132 (20.1%)
Low	1,032 (56.2%)		843 (54.6%)	1,052 (57.0%)		823 (53.5%)	1,541 (56.1%)		334 (52.7%)	730 (55.3%)		1,145 (55.5%)	1,522 (55.8%)		353 (53.8%)
High	491 (26.7%)		387 (25.0%)	454 (24.6%)		454 (27.6%)	708 (25.8%)		170 (26.8%)	355 (26.9%)		523 (25.4%)	707 (25.9%)		171 (26.1%)
BMI, kg/m^2	26.5 ± 4.1	0/3	27.8 ± 5.0 *	26.9 ± 4.3	0/3	27.3 ± 4.8 *	27.0 ± 4.4	0/3	27.5 ± 5.2 *	26.9 ± 4.3	0/3	27.2 ± 4.7	27.0 ± 4.4	0/3	27.4 ± 5.1
MVPA, h/wk	4.8 [2.5 – 8.3]	186/271	4.5 [1.8 – 7.5] *	4.5 [2.3 – 8.0]	187/270	4.5 [2.3 – 7.8]	4.5 [2.3 – 8.0]	311/146	4.5 [1.8 – 7.5] *	4.5 [2.3 – 7.8]	140/317	4.5 [2.3 – 8.0]	4.5 [2.3 – 8.0]	315/142	4.5 [1.8 – 7.5]
CV risk factors															
History of CVD	216 (11.9%)	20/88	342 (22.4%) *	281 (15.4%)	20/88	277 (18.2%) *	453 (16.7%)	34/74	105 (16.7%)	235 (18.0%)	14/94	323 (15.9%)	448 (16.6%)	35/73	110 (16.9%)
Total-to-HDL ratio	3.7 ± 1.2	0/4	3.7 ± 1.2	3.6 ± 1.1	0/4	3.7 ± 1.2 *	3.7 ± 1.2	0/4	3.6 ± 1.2	3.7 ± 1.1	0/4	3.7 ± 1.2	3.7 ± 1.2	0/4	3.6 ± 1.2
eGFR	88.8 ± 14.2	15/18	87.4 ± 15.7 *	88.1 ± 14.7	13/20	88.1 ± 15.2	88.0 ± 14.6	9/24	88.6 ± 16.1	88.2 ± 14.6	11/22	88.1 ± 15.1	88.1 ± 14.7	6/27	88.4 ± 15.9
GMS		0/41	*		0/41			0/41			0/41	*		0/41	
NGM	1,139 (62.0%)		785 (49.9%)	1,052 (57.0%)		872 (55.7%)	1,561 (56.8%)		363 (54.8%)	706 (53.5%)		1,218 (58.3%)	1,547 (56.7%)		377 (55.1%)
Prediabetes	284 (15.5%)		227 (14.4%)	278 (15.1%)		233 (14.9%)	451 (15.1%)		96 (14.5%)	210 (15.9%)		301 (14.4%)	411 (15.1%)		100 (14.6%)
Type 2 diabetes	414 (22.5%)		561 (34.8%)	514 (27.9%)		461 (29.4%)	772 (28.1%)		203 (30.7%)	404 (30.6%)		571 (27.3%)	768 (28.2%)		207 (30.3%)
Use of lipid medication	561 (30.5%)	0/4	697 (43.3%) *	655 (35.5%)	0/4	603 (37.6%)	998 (36.3%)	0/4	260 (37.2%)	523 (39.6%)	0/4	735 (34.6%) *	998 (36.3%)	0/4	260 (37.2%)
Use of AHT	624 (34.0%)	0/4	754 (46.8%) *	707 (38.3%)	0/4	671 (41.9%) *	1093 (39.8%)	0/4	285 (40.8%)	559 (42.3%)	0/4	819 (38.5%) *	990 (36.3%)	0/4	268 (37.2%)
β-blockers	251 (13.7%)	0/4	369 (22.9%) *	308 (16.7%)	0/4	312 (19.5%) *	483 (17.6%)	0/4	137 (19.6%)	238 (18.0%)	0/4	382 (18.0%)	481 (17.6%)	0/4	139 (19.3%)
Diuretics	236 (12.8%)	0/4	329 (20.4%) *	291 (15.8%)	0/4	274 (17.1%)	446 (16.2%)	0/4	119 (17.0%)	226 (17.1%)	0/4	339 (15.9%)	444 (16.3%)	0/4	121 (16.8%)
CCB	117 (6.4%)	0/4	209 (13.0%) *	162 (8.8%)	0/4	164 (10.2%)	243 (8.8%)	0/4	83 (11.9%) *	134 (10.2%)	0/4	192 (9.0%)	241 (8.8%)	0/4	85 (11.8%) *
ACE-i	172 (9.4%)	0/4	254 (15.8%) *	218 (11.8%)	0/4	208 (13.0%)	338 (12.3%)	0/4	88 (12.6%)	173 (13.1%)	0/4	253 (11.9%)	337 (12.4%)	0/4	89 (12.3%)
ARBs	282 (15.4%)	0/4	339 (21.1%) *	314 (17.0%)	0/4	307 (19.2%)	489 (17.8%)	0/4	132 (18.9%)	253 (19.2%)	0/4	368 (17.3%)	486 (17.8%)	0/4	135 (18.7%)

Table S6.1 (continued)

	CSVD features			Flicker light-induced arteriolar and venular dilation			Urinary albumin excretion			Skin hyperemia			Plasma biomarkers of microvascular dysfunction		
	Complete (n=1,837)	No. of participants in complete / missing	Missing (n=1,614)	Complete (n=1,844)	No. of participants in complete / missing	Missing (n=1,607)	Complete (n=2,748)	No. of participants in complete / missing	Missing (n=703)	Complete (n=1,320)	No. of participants in complete / missing	Missing (n=2,131)	Complete (n=2,726)	No. of participants in complete / missing	Missing (n=725)
Plasma biomarkers of LGI		17/21			16/22			27/11			13/25			0/38	
CRP, µg/ml	1.14 [0.58–2.51]		1.39 [0.66–3.07]*	1.20 [0.62–2.66]		1.33 [0.61–2.94]	1.23 [0.61–2.76]		1.34 [0.64–2.99]	1.20 [0.62–2.72]		1.29 [0.61–2.86]	1.22 [0.61–2.78]		1.36 [0.64–2.92]
SAA, µg/ml	3.16 [1.97–5.32]		3.37 [2.12–5.65]	3.28 [1.04–5.37]		3.26 [2.04–5.61]	3.24 [1.04–5.39]		3.33 [2.05–5.79]	3.30 [1.10–5.45]		3.24 [2.00–5.46]	3.24 [1.03–5.40]		3.33 [2.09–5.75]
IL-6, pg/ml	0.54 [0.37–0.84]		0.65 [0.43–0.99]*	0.57 [0.39–0.88]		0.60 [0.40–0.94]	0.58 [0.39–0.89]		0.62 [0.40–0.96]	0.58 [0.40–0.88]		0.59 [0.39–0.92]	0.58 [0.39–0.89]		0.62 [0.41–0.95]
IL-8, pg/ml	4.01 [3.21–5.09]		4.32 [3.41–5.60]	4.06 [3.21–5.23]		4.24 [3.40–5.45]	4.13 [3.28–5.32]		4.16 [3.32–5.35]	4.14 [3.30–5.27]		4.13 [3.28–5.36]	4.13 [3.28–5.33]		4.18 [3.36–5.34]
TNF-α, pg/ml	2.15 [1.86–2.51]		2.23 [1.92–2.63]	2.21 [1.89–2.59]		2.18 [1.88–2.53]	2.19 [1.88–2.57]		2.21 [1.91–2.54]	2.19 [1.88–2.55]		2.19 [1.89–2.57]	2.19 [1.88–2.56]		2.21 [1.92–2.55]
Mean BP															
Office SBP, mmHg	133.8 ± 17.2	0/2	136.5 ± 19.2 *	134.9 ± 17.9	1/1	135.2 ± 18.5	135.0 ± 18.1	1/1	135.3 ± 18.5	135.9 ± 18.3	2/0	134.5 ± 18.2 *	135.0 ± 18.1	1/1	135.4 ± 18.5
Office DBP, mmHg	76.0 ± 9.7	0/2	76.3 ± 10.0	76.4 ± 9.9	1/1	75.9 ± 9.8	76.2 ± 9.9	1/1	75.8 ± 9.8	76.6 ± 9.6	2/0	75.9 ± 9.7	76.2 ± 9.9	1/1	75.8 ± 9.7
24-hour SBP, mmHg	119.7 ± 11.3	0/559	121.0 ± 12.8 *	120.1 ± 11.5	0/559	120.1 ± 12.5	120.1 ± 11.8	0/559	121.9 ± 13.7	120.9 ± 11.6	0/559	119.5 ± 12.0 *	120.1 ± 11.8	0/559	120.7 ± 13.5
24-hour DBP, mmHg	74.6 ± 7.0	0/559	76.9 ± 7.4 *	74.4 ± 7.1	0/559	74.2 ± 7.2	74.4 ± 7.1	0/559	73.9 ± 7.6	74.6 ± 6.9	0/559	74.2 ± 7.4	74.4 ± 7.1	0/559	73.4 ± 7.5
7-day SBP, mmHg	126.0 ± 12.8	526/478	129.5 ± 14.2 *	126.8 ± 13.2	580/424	128.4 ± 13.9 *	127.4 ± 13.5	791/213	128.3 ± 14.0 *	127.6 ± 13.1	333/1,004	127.6 ± 13.9	127.4 ± 13.5	792/212	128.2 ± 14.0
7-day DBP, mmHg	77.2 ± 8.2	526/478	77.4 ± 8.2	77.0 ± 8.1	580/424	77.6 ± 8.2	77.2 ± 8.1	791/213	77.5 ± 8.6	77.3 ± 7.8	333/1,004	77.3 ± 8.4	77.3 ± 8.2	792/212	77.3 ± 8.0
BPV															
WV-BPV, mmHg	4.6 ± 2.9	1/7	4.6 ± 2.9	4.6 ± 2.9	5/3	4.7 ± 2.9	4.7 ± 2.9	5/3	4.5 ± 2.9	4.8 ± 3.0	3/5	4.5 ± 2.8 *	4.7 ± 2.9	5/3	4.4 ± 2.8 *
WV-BPV, mmHg	2.5 ± 1.6	1/7	2.6 ± 1.8	2.5 ± 1.6	5/3	2.6 ± 1.8 *	2.5 ± 1.7	5/3	2.5 ± 1.9	2.6 ± 1.7	3/5	2.5 ± 1.7	2.5 ± 1.7	5/3	2.5 ± 1.9
24-h sBPV, mmHg	9.9 ± 2.4	0/559	10.4 ± 2.7 *	10.0 ± 2.5	0/559	10.1 ± 2.6	10.0 ± 2.5	0/559	10.2 ± 2.5	10.1 ± 2.4	0/559	10.0 ± 2.5	10.0 ± 2.5	0/559	10.4 ± 2.8
24-h sBPV, mmHg	6.9 ± 1.7	0/559	7.3 ± 2.0 *	6.9 ± 1.8	0/559	7.2 ± 2.0 *	7.0 ± 1.9	0/559	7.1 ± 1.9	7.0 ± 1.8	0/559	7.0 ± 1.9	7.0 ± 1.8	0/559	7.2 ± 2.1
7-d sBPV, mmHg	8.9 ± 3.6	539/486	9.9 ± 4.1 *	9.1 ± 3.8	597/428	9.6 ± 4.0 *	9.2 ± 3.8	597/428	9.8 ± 4.3 *	9.2 ± 3.6	539/486	9.5 ± 4.1	9.3 ± 3.8	812/213	9.6 ± 4.1 *
7-d dBPV, mmHg	5.5 ± 2.7	539/486	6.2 ± 3.5 *	5.7 ± 2.9	597/428	6.1 ± 3.2 *	5.7 ± 2.9	597/428	6.4 ± 3.8 *	5.7 ± 2.8	539/486	6.0 ± 3.2 *	5.8 ± 2.9	812/213	6.2 ± 3.7 *
Microvascular dysfunction															
CSVD features															
TBV, ml	1,133± 112	0/1,149	1,140 ± 111	-	-	-	-	-	-	-	-	-	-	-	-
WMH volume, ml	0.22 [0.07–0.73]	0/1,149	0.25 [0.07–0.81]	-	-	-	-	-	-	-	-	-	-	-	-
Presence of CMB	207 (11.3%)	0/1,195	64 (15.3%) *	-	-	-	-	-	-	-	-	-	-	-	-
Presence of lacunar infarcts	94 (5.1%)	0/1,152	30 (6.5%)	-	-	-	-	-	-	-	-	-	-	-	-

Table S6.1 (continued)

	CSVD features			Flicker light-induced arteriolar and venular dilation			Urinary albumin excretion			Skin hyperaemia			Plasma biomarkers of microvascular dysfunction		
	Complete (n=1,837)	No. of participants in complete / missing	Missing (n=1,614)	Complete (n=1,844)	No. of participants in complete / missing	Missing (n=1,607)	Complete (n=2,748)	No. of participants in complete / missing	Missing (n=703)	Complete (n=1,320)	No. of participants in complete / missing	Missing (n=2,131)	Complete (n=2,726)	No. of participants in complete / missing	Missing (n=725)
Flicker light-induced arteriolar and venular dilation															
Flicker light-induced arteriolar dilation, %	-	-	-	3.04 ± 2.81	0/1,161	2.84 ± 2.69	-	-	-	-	-	-	-	-	-
Flicker light-induced venular dilation, %	-	-	-	3.89 ± 2.20	0/1,120	3.72 ± 2.25	-	-	-	-	-	-	-	-	-
Urinary albumin excretion															
<15 mg/24h	-	-	-	-	-	-	2237 (81.4%)	0/42	516 (78.1%)	-	-	-	-	-	-
≥15-30 mg/24h	-	-	-	-	-	-	279 (10.2%)	0/42	78 (11.8%)	-	-	-	-	-	-
≥30 mg/24h	-	-	-	-	-	-	232 (8.4%)	0/42	67 (10.1%)	-	-	-	-	-	-
Skin hyperaemia, %	-	-	-	-	-	-	-	-	-	1,124 ± 781	0/1,775	1,107 ± 723	-	-	-
Plasma biomarkers of microvascular dysfunction															
sICAM-1, ng/ml	-	-	-	-	-	-	-	-	-	-	-	-	353 ± 99	0/251	361 ± 103
sVCAM-1, ng/ml	-	-	-	-	-	-	-	-	-	-	-	-	427 ± 101	0/251	433 ± 102
sE-selectin, ng/ml	-	-	-	-	-	-	-	-	-	-	-	-	119 ± 66.2	0/251	118 ±60.8
vWF, %	-	-	-	-	-	-	-	-	-	-	-	-	132 ± 48	0/253	135 ± 50

Data are presented as mean ± standard deviation, median [interquartile range] or n(%). *denotes a statistically significantly difference from the complete group, assessed by the student's t-test for normally distributed variables, Mann-Whitney U-test for skewed variables or Chi-square test for categorical variables. Abbreviations: BMI, body mass index; MVPA, MVPA, moderate-to-vigorous physical activity; CSVD, cerebral small vessel disease; CV, cardiovascular; CVD, cardiovascular disease; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; GMS, glucose metabolism status; NGM, normal glucose metabolism status; AHT, antihypertensive treatment; CCB, calcium channel blocker; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; LGI: low-grade inflammation; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BPV, blood pressure variability; WV, within-visit; TBV, total parenchymal brain volume; WMH, white matter hyperintensity; CMB, cerebral microbleeds; UAE, urinary albumin excretion; WMH, white matter hyperintensity; sICAM, soluble intercellular adhesuin molecule-1; sVCAM, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor.

Table S6.2 Study population characteristics of variables used in the additional analyses.

Characteristic	Total study population (n=2,773)	Tertiles of systolic BPV composite score		
		Lowest tertile (n=921)	Middle tertile (n=934)	Highest tertile (n=918)
Demographics				
Income level, euros	2,017 ± 814	2,050 ± 814	1,968 ± 795	2,032 ± 831
Occupation status				
Low	704 (30.4)	225 (27.9)	245 (32.2)	234 (31.3)
Middle	814 (35.2)	285 (35.4)	262 (34.4)	267 (35.7)
High	797 (34.4)	296 (36.7)	254 (33.4)	247 (33.0)
Lifestyle factors				
Greek Mediterranean diet score	4.4 ± 1.6	4.4 ± 1.7	4.4 ± 1.6	4.4 ± 1.6
Moderate-to-vigorous physical activity, h/wk	4.5 [2.3 – 8.0]	4.5 [2.3 – 7.5]	4.5 [2.3 – 8.3]	4.5 [2.3 – 7.7]
Cardiovascular risk factors				
History of cardiovascular disease	458 (16.5)	127 (14.0)	159 (17.2)	172 (18.7)
Estimated glomerular filtration rate, ml/min/1.73m^2	88.1 ± 14.7	90.3 ± 14.8	87.5 ± 14.2	86.3 ± 14.8
Estimated glomerular filtration rate <60 ml/min/1.73m^2	114 (4.1)	28 (3.0)	34 (3.7)	52 (5.7)
Markers of low-grade inflammation				
C-reactive protein (µg/ml)	1.23 [0.61 – 2.76]	1.10 [0.54 – 2.39]	1.20 [0.59 – 2.90]	1.44 [0.69 – 3.08]
Serum amyloid A (µg/ml)	3.24 [2.04 – 5.39]	3.08 [1.86 – 5.20]	3.18 [2.00 – 5.25]	3.51 [2.26 – 5.71]
Interleukin-6 (pg/ml)	0.58 [0.39 – 0.89]	0.52 [0.37 – 0.83]	0.58 [0.39 – 0.93]	0.65 [0.43 – 0.93]
Interleukin-8 (pg/ml)	4.12 [3.28 – 5.33]	3.92 [3.10 – 5.17]	4.17 [3.29 – 5.35]	4.27 [3.50 – 5.46]
Tumour necrosis factor alpha (pg/ml)	2.19 [1.88 – 2.57]	2.16 [1.83 – 2.51]	2.18 [1.88 – 2.54]	2.23 [1.92 – 2.66]
Carotid-femoral pulse wave velocity, m/s	9.0 ± 2.1	8.4 ± 1.9	9.0 ± 2.1	9.6 ± 2.2
Mean BP				
Office systolic BP, mmHg	135.0 ± 18.1	127.5 ± 15.3	135.1 ± 17.2	142.4 ± 18.7
Office diastolic BP, mmHg	76.2 ± 9.9	73.9 ± 9.0	76.4 ± 9.9	78.3 ± 10.1
7-day *s*ystolic BP, mmHg	127.5 ± 13.6	120.7 ± 10.4	126.9 ± 12.2	135.3 ± 13.9
7-day diastolic BP, mmHg	77.3 ± 8.2	75.1 ± 7.1	77.3 ± 7.9	79.6 ± 8.9
Microvascular dysfunction measures				
Cerebral small vessel disease features				
Total brain parenchyma volume, ml	1,133 ± 112.2	1,145 ± 116.6	1,131 ± 108.1	1,123 ± 110.2
White matter hyperintensity volume, ml	0.22 [0.07 – 0.73]	0.16 [0.05 – 0.48]	0.26 [0.08 – 0.81]	0.30 [0.10 – 1.01]
Presence of cerebral microbleeds	207 (11.3)	67 (10.1)	67 (10.9)	73 (13.1)
Presence of lacunar infarcts	94 (5.1)	22 (3.3)	38 (6.2)	34 (6.1)
Flicker light-induced arteriolar and venular dilation				
Flicker light-induced arteriolar dilation, %	3.04 ± 2.81	3.20 ± 2.82	3.13 ± 2.81	2.77 ± 2.79
Flicker light-induced venular dilation, %	3.89 ± 2.20	3.89 ± 2.21	3.94 ± 2.16	3.85 ± 2.22
Urinary albumin excretion ≥30 mg/24h				
Plasma biomarkers of microvascular dysfunction	232 (8.4)	48 (5.2)	87 (9.5)	97 (10.6)
sICAM-1, ng/ml	353 ± 99	348 ± 98	350 ± 94	363 ± 104
sVCAM-1, ng/ml	427 ± 101	422 ± 99	422 ± 98	438 ± 106
sE-selectin, ng/ml	119 ± 66	112 ± 68	121 ± 68	123 ± 62
vWF, %	132 ± 48	129 ± 47	131 ± 48	137 ± 50

Data are presented as mean ± standard deviation, median [interquartile range] or n (%). Data were available for: income level, n=2,137; diet score, n=2,632; occupation status, n=2,315; moderate-to-vigorous physical activity, n=2,464; carotid-femoral pulse wave velocity, n=2,354; history of cardiovascular disease, n=2,737; estimated glomerular filtration rate, n=2,749; markers of low-grade inflammation, n=2,745; within-visit blood pressure variability, n=2,768; 24-hour blood pressure variability, n=2,773; 7-day blood pressure variability, n=1,950; cerebral small vessel disease features, n=1,837; flicker light-induced arteriolar and venular dilation, n=1,844; urinary albumin excretion, n=2,748; plasma biomarkers of microvascular dysfunction, n=2,685. Abbreviations: BP, blood pressure; BPV, blood pressure variability; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor.

Table S6.3 Study population characteristics according to tertiles of diastolic blood pressure variability.

Characteristic	Study population (n=2,773)	Tertiles of diastolic BPV composite score		
		Lowest tertile (n=924)	Middle tertile (n=925)	Highest tertile (n=924)
Demographics				
Age, years	59.9 ± 8.2	59.0 ± 8.3	59.6 ± 7.9	61.2 ± 8.2
Men	1,440 (51.9)	463 (50.1)	484 (52.3)	493 (53.4)
Lifestyle factors				
Smoking status:				
Never	980 (35.3)	351 (38.0)	320 (34.6)	309 (33.4)
Former	1,441 (52.0)	465 (50.3)	484 (55.3)	484 (53.2)
Current	352 (12.7)	108 (11.7)	121 (13.1)	121 (13.1)
Alcohol consumption				
None	509 (18.4)	168 (18.2)	161 (17.4)	180 (19.5)
Low (women ≤7, men ≤14 units/week)	1,550 (55.9)	534 (57.8)	507 (54.8)	509 (55.1)
High (women >7, men >14 units/week)	714 (25.7)	222 (24.0)	257 (27.8)	235 (25.4)
Body mass index, kg/m^2	27.0 ± 4.4	26.2 ± 4.1	26.8 ± 4.2	28.0 ± 4.8
Cardiovascular risk factors				
History of cardiovascular disease	458 (16.5)	126 (13.6)	141 (15.5)	191 (21.0)
Total/high density lipoprotein cholesterol ratio	3.7 ± 1.2	3.6 ± 1.1	3.7 ± 1.2	3.8 ± 1.2
Estimate glomerular filtration rate, ml/min/1.73m^2	88.1 ± 14.7	88.9 ± 14.4	89.2 ± 14.2	85.9 ± 15.1
Glucose metabolism status				
Normal glucose metabolism	1,575 (56.8)	590 (63.9)	537 (58.1)	448 (48.5)
Prediabetes	416 (15.0)	119 (12.9)	152 (16.4)	145 (15.7)
Type 2 diabetes	782 (28.2)	215 (23.3)	236 (25.5)	331 (35.8)
Use of lipid-modifying medication	1,004 (36.2)	307 (33.2)	309 (33.4)	388 (42.0)
Use of antihypertensive medication	1,101 (39.7)	308 (33.3)	336 (36.3)	457 (49.5)
Beta blockers	488 (17.6)	134 (14.5)	146 (15.8)	208 (22.5)
Diuretics	448 (16.2)	120 (13.0)	137 (14.8)	191 (20.7)
Calcium channel blockers	244 (8.8)	80 (8.7)	75 (8.1)	89 (9.6)
Angiotensin-converting enzyme inhibitors	342 (12.3)	81 (8.8)	100 (10.8)	161 (17.4)
Angiotensin II receptor blockers	491 (17.7)	130 (14.1)	157 (17.0)	204 (22.1)
Markers of low-grade inflammation				
C-reactive protein (µg/ml)	1.23 [0.61–2.76]	1.19 [0.54–2.55]	1.12 [0.58–2.56]	1.43 [0.70–3.17]
Serum amyloid A (µg/ml)	3.24 [2.04–5.39]	3.09 [1.84–5.20]	3.20 [2.10–5.39]	3.49 [2.19–5.60]
Interleukin-6 (pg/ml)	0.58 [0.39–0.89]	0.54 [0.36–0.85]	0.56 [0.38–0.86]	0.65 [0.44–0.98]
Interleukin-8 (pg/ml)	4.12 [3.28–5.33]	4.02 [3.18–5.03]	4.08 [3.29–5.32]	4.23 [3.37–5.48]
Tumour necrosis factor alpha (pg/ml)	2.19 [1.88–2.57]	2.15 [1.83–2.53]	2.19 [1.87–2.54]	2.23 [1.91–2.64]
Mean BP				
Office systolic BP, mmHg	135.0 ± 18.1	130.7 ± 16.9	134.6 ± 17.9	139.7 ± 18.5
Office diastolic BP, mmHg	76.2 ± 9.9	74.5 ± 9.3	76.4 ± 9.6	77.8 ± 10.3
24-hour systolic BP, mmHg	120.1 ± 11.7	117.7 ± 10.7	120.1 ± 11.6	122.3 ± 12.5
24-hour diastolic BP, mmHg	74.4 ± 7.1	73.0 ± 6.7	74.7 ± 7.1	75.5 ± 7.3
7-day *s*ystolic BP, mmHg	127.5 ± 13.6	122.4 ± 11.5	127.3 ± 13.2	133.2 ± 13.9
7-day diastolic BP, mmHg	77.3 ± 8.2	75.2 ± 7.6	77.6 ± 7.6	79.2 ± 8.9

Table S6.3 (continued)

Characteristic	Study population (n=2,773)	Tertiles of diastolic BPV composite score: Lowest tertile (n=924)	Middle tertile (n=925)	Highest tertile (n=924)
BPV				
Within-visit systolic BPV, mmHg	4.69 ± 2.91	4.13 ± 2.52	4.67 ± 2.88	5.27 ± 3.19
Within-visit diastolic BPV, mmHg	2.51 ± 1.68	1.55 ± 0.83	2.31 ± 1.01	3.69 ± 2.10
24-hour systolic BPV, mmHg	10.03 ± 2.50	8.78 ± 1.85	10.00 ± 2.22	11.32 ± 2.67
24-hour diastolic BPV, mmHg	7.01 ± 1.86	5.63 ± 0.89	6.92 ± 1.18	8.48 ± 2.02
7-day systolic BPV, mmHg	9.25 ± 3.83	7.60 ± 2.30	8.89 ± 2.92	11.49 ± 4.89
7-day diastolic BPV, mmHg	5.76 ± 2.93	4.38 ± 1.15	5.42 ± 1.58	7.68 ± 4.19
Microvascular dysfunction measures				
Cerebral small vessel disease features				
Total brain parenchyma volume, ml	1,133 ± 112.2	1,140 ± 116.6	1,134 ± 112.3	1,125 ± 106.1
White matter hyperintensity volume, ml	0.22 [0.07 – 0.73]	0.21 [0.06 – 0.64]	0.20 [0.07 – 0.64]	0.31 [0.09 – 1.00]
Presence of cerebral microbleeds	207 (11.3)	65 (10.0)	72 (11.3)	70 (12.8)
Presence of lacunar infarcts	94 (5.1)	25 (3.8)	31 (4.9)	38 (6.9)
Flicker light-induced arteriolar and venular dilation				
Flicker light-induced arteriolar dilation, %	3.04 ± 2.81	3.15 ± 2.89	3.01 ± 2.67	2.95 ± 2.85
Flicker light-induced venular dilation, %	3.89 ± 2.20	3.90 ± 2.18	3.83 ± 2.13	3.95 ± 2.29
Urinary albumin excretion	6.8 [4.1–11.8]	6.1 [3.8–9.9]	6.5 [3.9–11.4]	7.9 [4.8–14.6]
≥30 mg/24h	232 (8.4)	51 (5.5)	72 (7.8)	109 (12.0)
Skin hyperaemia, %	1,124 ± 781	1,153 ± 791	1,103 ± 790	1,116 ± 763
Plasma biomarkers of microvascular dysfunction				
sICAM-1, ng/ml	353 ± 99	348 ± 92	349 ± 91	363 ± 112
sVCAM-1, ng/ml	427 ± 101	422 ± 92	425 ± 102	436 ± 109
sE-selectin, ng/ml	119 ± 66	115 ± 66	116 ± 61	124 ± 71
vWF, %	132 ± 48	130 ± 47	131 ± 48	136 ± 49

Data are presented as mean ± standard deviation, median [interquartile range] or n (%). Data available for: estimated glomerular filtration rate, n=2,749; history of cardiovascular disease, n=2,737; markers of low-grade inflammation, n=2,745; within-visit blood pressure variability, n=2,768; 7-day blood pressure variability, n=1,950; cerebral small vessel disease features, n=1,837; flicker light-induced arteriolar and venular dilation, n=1,844; skin hyperaemia, n=1,320; urinary albumin excretion, n=2,748; plasma biomarkers of microvascular dysfunction, n=2,685. Abbreviations: BP, blood pressure; BPV, blood pressure variability; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular adhesion molecule-1; sE-selectin, soluble E-selectin; vWF, von Willebrand factor.

Table S6.4 P-values of interactions with age, sex, glucose metabolism status, and hypertension status.

	Systolic blood pressure variability composite score		Diastolic blood pressure variability composite score	
Interaction with age (<60 vs. ≥60 years)				
Cerebral small vessel disease features	0.194		0.438	
Retinal arteriolar and venular dilation	0.916		0.461	
Urinary albumin excretion	0.039 *		0.171	
Skin hyperaemia	0.579		0.779	
Plasma biomarkers of MVD	0.389		0.046 *	
Interaction with sex				
Cerebral small vessel disease features	0.340		0.560	
Retinal arteriolar and venular dilation	0.458		0.788	
Urinary albumin excretion	<0.001 †		<0.001 †	
Skin hyperaemia	0.450		0.413	
Plasma biomarkers of MVD	0.150		0.013 †	
Interaction with glucose metabolism status	**Prediabetes**	**Type 2 diabetes**	**Prediabetes**	**Type 2 diabetes**
Cerebral small vessel disease features	0.458	0.005 ‡	0.751	0.042 ‡
Retinal arteriolar and venular dilation	0.959	0.132	0.473	0.066
Urinary albumin excretion	0.639	0.121	0.628	0.057
Skin hyperaemia	0.681	0.799	0.449	0.816
Plasma biomarkers of MVD	0.050	0.652	0.107	0.011 ‡
Interaction with hypertension status				
Cerebral small vessel disease features	0.247		0.966	
Retinal arteriolar and venular dilation	0.835		0.077	
Urinary albumin excretion	0.222		0.025 §	
Skin hyperaemia	0.388		0.827	
Plasma biomarkers of MVD	0.379		0.014 §	

Variables in models in addition to blood pressure variability and interaction term: age, sex, glucose metabolism status, mean 24-hour systolic or diastolic blood pressure (where appropriate), education level, body mass index, smoking status, alcohol consumption, total/high density lipoprotein cholesterol ratio, lipid-modifying medication, and the individual classes of antihypertensive medication (i.e. beta blockers, diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers). * For statistically significant interactions with age, associations appeared stronger in individuals ≥60 years. † For statistically significant interactions with sex, associations appeared stronger in men. ‡ For statistically significant interactions with glucose metabolism status, associations with cerebral small vessel disease features appeared stronger in individuals with normal glucose metabolism status, whereas associations with plasma biomarkers of MVD appeared stronger in individuals with type 2 diabetes. § For statistically significant interactions with hypertension status, associations with urinary albumin excretion and plasma biomarkers of MVD appeared stronger in individuals with hypertension. Abbreviations: MVD, microvascular dysfunction.

Table S6.5 Associations between within-visit, 24-hour and 7-day systolic and diastolic blood pressure variability and measures of microvascular dysfunction.

Microvascular dysfunction	Systolic blood pressure variability			Diastolic blood pressure variability		
	Within-visit	24-hour	7-day	Within-visit	24-hour	7-day
Model	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
Cerebral small vessel disease features, per point						
	n=1,836	n=1,839	n=1,298	n=1,836	n=1,839	n=1,298
1	1.037 (0.984 -1.092)	1.030 (0.972 - 1.092)	**1.095 (1.026 - 1.169)**	1.016 (0.958 - 1.078)	**1.078 (1.020 - 1.139)**	1.022 (0.946- 1.103)
2	1.032 (0.980 - 1.088)	1.017 (0.959 - 1.078)	**1.078 (1.009 - 1.153)**	1.012 (0.954 - 1.073)	**1.074 (1.016 - 1.135)**	1.008 (0.933- 1.089)
3	1.026 (0.973 - 1.082)	0.984 (0.925 - 1.048)	1.066 (0.995- 1.142)	1.002 (0.944 - 1.063)	1.055 (0.997 - 1.117)	0.997 (0.922- 1.079)
4	1.021 (0.969 -1.077)	0.983 (0.922 - 1.049)	1.057 (0.982 - 1.137)	0.997 (0.939 - 1.059)	**1.065 (1.005 - 1.128)**	0.990 (0.912- 1.075)
Flicker light-induced arteriolar and venular dilation, per SD						
	n=1,841	n=1,846	n=1,247	n=1,842	n=1,846	n=1,247
1	0.010 (-0.035- 0.055)	0.032 (-0.015 - 0.079)	0.025 (-0.034 - 0.083)	-0.021 (-0.070 - 0.029)	0.045 (-0.003 - 0.092)	0.000 (-0.059 - 0.059)
2	0.005 (-0.040 - 0.051)	0.022 (-0.025 - 0.069)	0.015 (-0.044 - 0.074)	-0.021 (-0.064 - 0.034)	0.039 (-0.009 - 0.086)	-0.005 (-0.065 - 0.054)
3	0.008 (-0.038 - 0.053)	0.034 (-0.016 - 0.085)	0.019 (-0.041 - 0.078)	-0.015 (-0.064 - 0.034)	**0.053 (0.005 - 0.101)**	0.000 (-0.059 - 0.059)
4	0.006 (-0.040- 0.051)	0.040 (-0.012 - 0.091)	0.022 (-0.039 - 0.083)	-0.018 (-0.067 - 0.031)	**0.055 (0.006 - 0.103)**	0.000 (-0.060 - 0.060)
Urinary albumin excretion, ratio increase						
	n=2,743	n=2,748	n=1,936	n=2,744	n=2,748	n=1,936
1	**1.04 (1.00 - 1.07)**	**1.13 (1.09 - 1.17)**	**1.11 (1.07 - 1.16)**	**1.04 (1.00 - 1.08)**	**1.11 (1.07 - 1.15)**	**1.09 (1.04 - 1.16)**
2	**1.03 (1.00 - 1.07)**	**1.10 (1.06 - 1.14)**	**1.08 (1.04 - 1.13)**	**1.04 (1.00 - 1.02)**	**1.09 (1.05 - 1.13)**	**1.07 (1.02 - 1.14)**
3	1.01 (0.98 - 1.04)	**1.04 (1.00 - 1.07)**	**1.05 (1.01 - 1.10)**	**1.03 (1.00 - 1.07)**	**1.07 (1.04 - 1.11)**	**1.06 (1.01 - 1.10)**
4	1.02 (0.99 - 1.05)	1.02 (0.98 - 1.05)	**1.04 (1.00 - 1.08)**	1.03 (0.99 - 1.06)	**1.05 (1.02 - 1.09)**	**1.05 (1.00 - 1.09)**
Skin hyperaemia, per SD						
	n=1,317	n=1,322	n=983	n=1,318	n=1,322	n=983
1	0.025 (-0.027- 0.076)	0.028 (-0.028- 0.084)	-0.021 (-0.086 - 0.044)	0.017 (-0.036 - 0.070)	0.000 (-0.054 - 0.055)	-0.018 (-0.084- 0.047)
2	0.016 (-0.036- 0.067)	0.015 (-0.041- 0.071)	-0.042 (-0.108 - 0.023)	0.012 (-0.040 - 0.065)	-0.009 (-0.063 - 0.044)	-0.035 (-0.100 - 0.031)
3	0.015 (-0.036- 0.067)	0.010 (-0.050- 0.070)	-0.052 (-0.119- 0.015)	0.013 (-0.040- 0.066)	-0.005 (-0.060- 0.050)	-0.035 (-0.101 - 0.032)
4	0.019 (-0.032- 0.071)	0.020 (-0.041-; 0.082)	-0.051 (-0.119- 0.016)	0.014 (-0.038- 0.067)	-0.001 (-0.057- 0.055)	-0.034 (-0.101 - 0.032)
Plasma biomarkers of microvascular dysfunction, per SD						
	n=2,721	n=2,728	n=1,914	n=2,722	n=2,728	n=1,914
1	0.007 (-0.030 - 0.043)	**0.080 (0.043 - 0.117)**	**0.121 (0.077 - 0.166)**	0.010 (-0.028 - 0.047)	**0.064 (0.027 - 0.100)**	**0.103 (0.057 - 0.149)**
2	-0.007 (-0.042 - 0.028)	**0.047 (0.011 - 0.083)**	**0.083 (0.040 - 0.126)**	0.004 (-0.032 - 0.039)	**0.041 (0.006 - 0.077)**	**0.074 (0.030 - 0.119)**
3	-0.007 (-0.042 - 0.028)	**0.054 (0.016 - 0.093)**	**0.087 (0.043 - 0.131)**	0.004 (-0.032 - 0.040)	**0.042 (0.006 - 0.078)**	**0.074 (0.030 - 0.119)**
4	0.003 (-0.030 - 0.037)	0.016 (-0.021 - 0.054)	**0.065 (0.022 - 0.107)**	0.005 (-0.030 - 0.039)	0.001 (-0.034 - 0.036)	**0.049 (0.005 - 0.092)**

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light-induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat-induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex; model 2: model 1 + glucose metabolism status; model 3: model 2 + mean 24-hour systolic or diastolic blood pressure (where appropriate); model 4: model 3 + education level, body mass index, smoking status, alcohol consumption, total/high density lipoprotein cholesterol ratio, lipid-modifying medication, and the individual classes of antihypertensive medication (i.e. beta blockers, diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers). Bold denotes P value<.05. Abbreviations: CI: confidence interval; SD: standard deviation.

Table S6.6 Associations between systolic and diastolic blood pressure variability and individual measures of microvascular dysfunction.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Cerebral small vessel disease features					
Total parenchymal brain volume, low vs. high	1	1.05	(0.93 – 1.19)	1.12	(0.98 – 1.28)
	2	1.02	(0.90 – 1.16)	1.11	(0.97 – 1.27)
	3	1.05	(0.92 – 1.20)	1.12	(0.98 – 1.29)
	4	1.01	(0.88 – 1.16)	1.09	(0.94 – 1.25)
White matter hyperintensity volume, high vs. low	1	**1.13**	**(1.01 – 1.28)**	1.06	(0.93 – 1.20)
	2	1.11	(0.98 – 1.25)	1.04	(0.92 – 1.19)
	3	1.01	(0.89 – 1.15)	0.97	(0.85 – 1.11)
	4	1.00	(0.87 – 1.14)	0.96	(0.84 – 1.11)
Cerebral microbleeds, presence vs. absence	1	1.12	(0.96 – 1.30)	1.09	(0.92 – 1.28)
	2	1.11	(0.96 – 1.29)	1.08	(0.92 – 1.27)
	3	1.08	(0.92 – 1.27)	1.07	(0.91 – 1.26)
	4	1.09	(0.92 – 1.28)	1.07	(0.91 – 1.27)
Lacunar infarcts, presence vs. absence	1	1.20	(0.98 – 1.47)	1.25	(1.01 – 1.55)
	2	1.17	(0.95 – 1.45)	1.23	(0.99 – 1.53)
	3	1.15	(0.93 – 1.44)	1.23	(0.99 – 1.52)
	4	1.16	(0.93 – 1.45)	1.22	(0.98 – 1.52)
Flicker light-induced retinal arteriolar and venular dilation	Model	β	(95% CI)	β	(95% CI)
Flicker light-induced arteriolar dilation, per SD	1	-0.028	(-0.076; 0.020)	-0.013	(-0.064; 0.037)
	2	-0.010	(-0.058; 0.038)	-0.004	(-0.054; 0.046)
	3	-0.021	(-0.071; 0.029)	-0.014	(-0.064; 0.037)
	4	-0.021	(-0.072; 0.030)	-0.014	(-0.066; 0.037)
Flicker light-induced venular dilation, per SD	1	-0.015	(-0.063; 0.032)	-0.018	(-0.067; 0.032)
	2	-0.013	(-0.061; 0.035)	-0.016	(-0.066; 0.033)
	3	-0.014	(-0.064; 0.036)	-0.027	(-0.077; 0.024)
	4	-0.016	(-0.066; 0.034)	-0.022	(-0.073; 0.028)
Plasma biomarkers of microvascular dysfunction	Model	β	(95% CI)	β	(95% CI)
Soluble intracellular adhesion molecule-1, per SD	1	**0.074**	**(0.035 – 0.112)**	**0.055**	**(0.016 – 0.094)**
	2	**0.041**	**(0.003 – 0.079)**	0.032	(-0.007 – 0.070)
	3	**0.041**	**(0.002 – 0.081)**	0.031	(-0.008 – 0.069)
	4	0.022	(-0.016 – 0.060)	0.005	(-0.033 – 0.042)
Soluble vascular adhesion molecule-1, per SD	1	**0.056**	**(0.018 – 0.094)**	**0.067**	**(0.028 – 0.105)**
	2	**0.038**	**(0.000 – 0.076)**	**0.054**	**(0.016 – 0.093)**
	3	**0.044**	**(0.005 – 0.084)**	**0.057**	**(0.018 – 0.096)**
	4	**0.042**	**(0.003 – 0.082)**	**0.045**	**(0.006 – 0.084)**
E-selectin, per SD	1	**0.053**	**(0.014 – 0.092)**	**0.047**	**(0.007 – 0.087)**
	2	0.009	(-0.028 – 0.047)	0.016	(-0.022 – 0.055)
	3	0.002	(-0.037 – 0.042)	0.009	(-0.030 – 0.048)
	4	-0.019	(-0.057 – 0.019)	-0.022	(-0.059 – 0.016)
Von Willebrand Factor, per SD	1	**0.058**	**(0.021 – 0.096)**	**0.054**	**(0.015 – 0.093)**
	2	**0.045**	**(0.007 – 0.083)**	**0.045**	**(0.006 – 0.083)**
	3	**0.062**	**(0.023 – 0.102)**	**0.054**	**(0.014 – 0.093)**
	4	**0.051**	**(0.012 – 0.091)**	0.036	(-0.003 – 0.075)

Regression coefficients (β) represent difference in markers of microvascular dysfunction for every 1 standard deviation increase in blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24-hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total-to-high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid-modifying medication. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation; OR, odds ratio.

Table S6.7 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for estimated glomerular filtration rate.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,822	1	1.072	(1.013 – 1.135)	1.062	(0.999 – 1.130)
	2	1.057	(0.998 – 1.119)	1.052	(0.989 – 1.120)
	3	1.035	(0.974 – 1.099)	1.032	(0.968 – 1.100)
	4	1.025	(0.964 – 1.090)	1.031	(0.966 – 1.100)
	5	1.026	(0.965 – 1.091)	1.032	(0.968 – 1.102)
Flicker light-induced arteriolar and venular dilation, per SD n=1,831	1	0.033	(-0.015 – 0.081)	0.019	(-0.031 – 0.070)
	2	0.020	(-0.028 – 0.069)	0.013	(-0.037 – 0.063)
	3	0.030	(-0.021 – 0.080)	0.026	(-0.024 – 0.077)
	4	0.031	(-0.020 – 0.082)	0.025	(-0.026 – 0.076)
	5	0.031	(-0.020 – 0.082)	0.025	(-0.026 – 0.076)
Urinary albumin excretion, ratio increase n=2,724	1	1.14	(1.10 - 1.18)	1.13	(1.09 - 1.17)
	2	1.10	(1.07 - 1.14)	1.10	(1.07 - 1.15)
	3	1.05	(1.01 - 1.09)	1.09	(1.05 - 1.13)
	4	1.04	(1.00 - 1.08)	1.07	(1.03 - 1.11)
	5	1.04	(1.00 - 1.08)	1.07	(1.03 - 1.11)
Skin hyperaemia, per SD n=1,309	1	0.028	(-0.028 – 0.085)	0.006	(-0.051 – 0.064)
	2	0.007	(-0.050 – 0.064)	-0.009	(-0.067 – 0.048)
	3	-0.000	(-0.060 – 0.059)	-0.007	(-0.065 – 0.051)
	4	0.007	(-0.053 – 0.066)	-0.003	(-0.061 – 0.055)
	5	0.007	(-0.053 – 0.066)	-0.003	(-0.061 – 0.055)
Plasma biomarkers of microvascular dysfunction, per SD n=2,720	1	0.090	(0.053 – 0.127)	0.083	(0.044 – 0.121)
	2	0.053	(0.016 – 0.089)	0.055	(0.018 – 0.092)
	3	0.057	(0.019 – 0.095)	0.056	(0.019 – 0.094)
	4	0.036	(-0.001 – 0.072)	0.024	(-0.013 – 0.060)
	5	0.037	(0.001 – 0.073)	0.025	(-0.010 – 0.061)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light-induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat-induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24-hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total-to-high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid-modifying medication. Model 5: model 4 + estimated glomerular filtration rate. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.8 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for prior cardiovascular disease.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,817	1	1.069	(1.010 – 1.132)	1.064	(1.001 – 1.132)
	2	1.054	(0.995 – 1.116)	1.055	(0.992 – 1.122)
	3	1.032	(0.971 – 1.096)	1.034	(0.97 – 1.102)
	4	1.022	(0.961– 1.087)	1.033	(0.969 – 1.102)
	5	1.019	(0.958– 1.084)	1.029	(0.964 – 1.097)
Flicker light–induced arteriolar and venular dilation, per SD n=1,824	1	0.032	(–0.015 – 0.080)	0.019	(–0.032 – 0.069)
	2	0.020	(–0.028 – 0.068)	0.012	(–0.038 – 0.062)
	3	0.031	(–0.019 – 0.081)	0.026	(–0.025 – 0.076)
	4	0.030	(–0.020 – 0.081)	0.023	(–0.027 – 0.074)
	5	0.029	(–0.021 – 0.080)	0.023	(–0.028 – 0.074)
Urinary albumin excretion, ratio increase n=2,714	1	1.14	(1.07 – 1.15)	1.13	(1.06 – 1.14)
	2	1.11	(1.01 – 1.09)	1.10	(1.05 – 1.12)
	3	1.05	(1.00 – 1.08)	1.08	(1.03 – 1.10)
	4	1.04	(1.00 – 1.08)	1.07	(1.03 – 1.10)
	5	1.04	(1.09 – 1.17)	1.07	(1.03 – 1.10)
Skin hyperaemia, per SD n=1,306	1	0.022	(–0.034 – 0.079)	0.008	(–0.050 – 0.065)
	2	0.002	(–0.055 – 0.059)	–0.008	(–0.065 – 0.050)
	3	–0.004	(–0.064 – 0.056)	–0.004	(–0.062 – 0.054)
	4	0.002	(–0.057 – 0.062)	0.000	(–0.059 – 0.058)
	5	0.002	(–0.057 – 0.062)	0.000	(–0.059 – 0.058)
Plasma biomarkers of microvascular dysfunction, per SD n=2,691	1	0.092	(0.055 – 0.129)	0.081	(0.042 – 0.120)
	2	0.056	(0.019 – 0.092)	0.054	(0.016 – 0.092)
	3	0.061	(0.023 – 0.099)	0.056	(0.017 – 0.093)
	4	0.040	(0.003 – 0.077)	0.023	(–0.014 – 0.059)
	5	0.038	(0.001 – 0.075)	0.020	(–0.017 – 0.056)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Model 5: model 4 + prior cardiovascular disease. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.9 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for plasma biomarkers of low–grade inflammation.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease					
features, per point	1	1.074	(1.015 – 1.137)	1.067	(1.004 – 1.135)
	2	1.059	(1.000 – 1.121)	1.058	(0.995 – 1.125)
n=1,820	3	1.035	(0.975 – 1.100)	1.036	(0.973 – 1.104)
	4	1.027	(0.965 – 1.092)	1.036	(0.971 – 1.105)
	5	1.026	(0.965 – 1.092)	1.036	(0.971 – 1.105)
Flicker light–induced arteriolar	1	0.035	(–0.013 – 0.083)	0.019	(–0.031 – 0.069)
and venular	2	0.023	(–0.026 – 0.071)	0.013	(–0.038 – 0.063)
dilation, per SD	3	0.032	(–0.018 – 0.083)	0.026	(–0.025 – 0.077)
n=1,828	4	0.034	(–0.017 – 0.085)	0.024	(–0.027 – 0.075)
	5	0.033	(–0.018 – 0.083)	0.025	(–0.026 – 0.076)
Urinary albumin excretion,	1	1.14	(1.10 – 1.18)	1.13	(1.09 – 1.17)
ratio increase	2	1.10	(1.07 – 1.14)	1.10	(1.07 – 1.15)
n=2,721	3	1.05	(1.01 – 1.09)	1.09	(1.05 – 1.13)
	4	1.04	(1.00 – 1.08)	1.07	(1.03 – 1.11)
	5	1.04	(1.00 – 1.08)	1.07	(1.03 – 1.11)
Skin hyperaemia, per SD	1	0.025	(–0.031 – 0.082)	0.005	(–0.052 – 0.063)
n=1,307	2	0.005	(–0.052 – 0.062)	–0.010	(–0.068 – 0.047)
	3	–0.002	(–0.061 – 0.058)	–0.007	(–0.066 – 0.051)
	4	0.005	(–0.054 – 0.065)	–0.004	(–0.062 – 0.054)
	5	0.002	(–0.054 – 0.065)	–0.004	(–0.062 – 0.054)
Plasma biomarkers of	1	0.089	(0.052 – 0.126)	0.082	(0.043 – 0.120)
microvascular	2	0.056	(0.015 – 0.088)	0.054	(0.017 – 0.092)
dysfunction, per SD	3	0.057	(0.019 – 0.095)	0.056	(0.018 – 0.093)
n=2,726	4	0.035	(–0.001 – 0.072)	0.023	(–0.013 – 0.060)
	5	0.033	(–0.002 – 0.069)	0.026	(–0.009 – 0.061)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Model 5: model 4 + composite score of plasma biomarkers of low–grade inflammation. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation; OR, odds ratio.

Table S6.10 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for carotid–femoral pulse wave velocity.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1552	1	1.080	(1.016 – 1.148)	1.066	(0.997 – 1.139)
	2	1.064	(1.001 – 1.132)	1.055	(0.987 – 1.127)
	3	1.044	(0.979 – 1.114)	1.030	(0.962 – 1.103)
	4	1.029	(0.963 – 1.099)	1.025	(0.956 – 1.100)
	5	1.027	(0.962 – 1.098)	1.025	(0.955 – 1.099)
Flicker light–induced retinal arteriolar and venular dilation, per SD n=1,519	1	0.013	(–0.039 – 0.065)	0.020	(–0.035 – 0.074)
	2	0.002	(–0.05 – 0.055)	0.013	(–0.041 – 0.068)
	3	0.015	(–0.040 – 0.070)	0.027	(–0.028 – 0.082)
	4	0.013	(–0.042 – 0.069)	0.023	(–0.032 – 0.079)
	5	0.013	(–0.042– 0.069)	0.024	(–0.031 – 0.079)
Urinary albumin excretion, ratio increase n=2,335	1	1.13	(1.09 – 1.17)	1.12	(1.08 – 1.16)
	2	1.10	(1.06 – 1.14)	1.10	(1.06 – 1.14)
	3	1.05	(1.01 – 1.09)	1.08	(1.04 – 1.13)
	4	1.04	(1.00 – 1.08)	1.07	(1.03 – 1.11)
	5	1.04	(1.00 – 1.08)	1.07	(1.03 – 1.11)
Skin hyperaemia, per SD n=1,142	1	0.047	(–0.014 – 0.109)	0.024	(–0.037 – 0.086)
	2	0.025	(–0.037 – 0.087)	0.007	(–0.054 – 0.069)
	3	0.019	(–0.046 – 0.084)	0.011	(–0.052 – 0.073)
	4	0.030	(–0.036 – 0.095)	0.017	(–0.046 – 0.080)
	5	0.030	(–0.035– 0.095)	0.017	(–0.046 – 0.080)
Plasma biomarkers of microvascular dysfunction, per SD n=2,313	1	0.072	(0.032 – 0.112)	0.083	(0.043 – 0.124)
	2	0.038	(–0.001 – 0.077)	0.059	(0.020 – 0.098)
	3	0.045	(0.005 – 0.086)	0.060	(0.021 – 0.100)
	4	0.029	(–0.010 – 0.068)	0.031	(–0.007 – 0.069)
	5	0.028	(–0.011 – 0.067)	0.031	(–0.007 – 0.069)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Model 5: model 4 + carotid–femoral pulse wave velocity. Bold denotes P value<.05.Abbreviations: BPV, blood pressure variability; CI, confidence interval; SD, standard deviation; OR, odds ratio.

Table S6.11 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for diet score.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,748	1	1.077	(1.017 – 1.142)	1.063	(0.998 – 1.132)
	2	1.063	(1.002 – 1.127)	1.053	(0.988 – 1.122)
	3	1.040	(0.978 – 1.106)	1.030	(0.965 – 1.099)
	4	1.032	(0.969 – 1.099)	1.032	(0.966 – 1.103)
	5	1.032	(0.969 – 1.099)	1.032	(0.966 – 1.103)
Flicker light–induced retinal arteriolar and venular dilation, per SD n=1,758	1	0.033	(–0.017 – 0.082)	0.014	(–0.038 – 0.065)
	2	0.021	(–0.029 – 0.070)	0.008	(–0.044 – 0.059)
	3	0.029	(–0.022 – 0.081)	0.021	(–0.031 – 0.073)
	4	0.032	(–0.021 – 0.084)	0.021	(–0.032 – 0.073)
	5	0.032	(–0.021 – 0.084)	0.021	(–0.032 – 0.073)
Urinary albumin excretion, ratio increase n=2,602	1	1.14	(1.09 – 1.18)	1.12	(1.08 – 1.17)
	2	1.10	(1.07 – 1.15)	1.10	(1.06 – 1.14)
	3	1.05	(1.01 – 1.09)	1.08	(1.04 – 1.12)
	4	1.04	(1.00 – 1.08)	1.06	(1.03 – 1.10)
	5	1.04	(1.00 – 1.08)	1.06	(1.03 – 1.10)
Skin hyperaemia, per SD n=1,249	1	0.040	(–0.018 – 0.098)	0.019	(–0.039 – 0.078)
	2	0.019	(–0.039 – 0.078)	0.004	(–0.054 – 0.063)
	3	0.014	(–0.047 – 0.075)	0.008	(–0.051 – 0.067)
	4	0.018	(–0.043 – 0.079)	0.009	(–0.050 – 0.068)
	5	0.018	(–0.043 – 0.08)	0.010	(–0.050 – 0.069)
Plasma biomarkers of microvascular dysfunction, per SD n=2,575	1	0.090	(0.051 – 0.128)	0.081	(0.041 – 0.121)
	2	0.052	(0.014 – 0.089)	0.055	(0.016 – 0.094)
	3	0.057	(0.018 – 0.097)	0.057	(0.018 – 0.096)
	4	0.036	(–0.002 – 0.074)	0.025	(–0.013 – 0.062)
	5	0.036	(–0.002 – 0.074)	0.025	(–0.013 – 0.063)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Model 5: model 4 + diet score. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.12 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for physical activity.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,651	1	1.086	(1.018 – 1.159)	1.062	(0.989 – 1.141)
	2	1.067	(0.998 – 1.140)	1.046	(0.974 – 1.124)
	3	1.043	(0.973 – 1.118)	1.027	(0.954 – 1.105)
	4	1.044	(0.971 – 1.122)	1.028	(0.952 – 1.110)
	5	1.086	(1.018 – 1.159)	1.062	(0.989 – 1.141)
Flicker light–induced retinal arteriolar and venular dilation, per SD n=1,657	1	0.029	(–0.021 – 0.079)	0.014	(–0.040 – 0.068)
	2	0.017	(–0.034 – 0.067)	0.006	(–0.048 – 0.060)
	3	0.025	(–0.028 – 0.077)	0.020	(–0.035 – 0.074)
	4	0.023	(–0.030 – 0.076)	0.017	(–0.038 – 0.072)
	5	0.023	(–0.030 – 0.076)	0.017	(–0.038 – 0.072)
Urinary albumin excretion, ratio increase n=2,437	1	1.14	(1.10 – 1.19)	1.13	(1.08 – 1.17)
	2	1.11	(1.07 – 1.16)	1.10	(1.06 – 1.15)
	3	1.05	(1.01 – 1.10)	1.08	(1.04 – 1.12)
	4	1.04	(1.00 – 1.08)	1.06	(1.02 – 1.11)
	5	1.04	(1.00 – 1.08)	1.06	(1.02 – 1.11)
Skin hyperaemia, per SD n=1,180	1	0.036	(–0.024 – 0.097)	0.014	(–0.048 – 0.076)
	2	0.017	(–0.044 – 0.078)	–0.001	(–0.063 – 0.061)
	3	0.011	(–0.053 – 0.075)	0.004	(–0.059 – 0.067)
	4	0.020	(–0.044 – 0.084)	0.006	(–0.057 – 0.070)
	5	0.019	(–0.045 – 0.083)	0.006	(–0.058 – 0.069)
Plasma biomarkers of microvascular dysfunction, per SD n=2,411	1	0.089	(0.050 – 0.128)	0.073	(0.032 – 0.115)
	2	0.055	(0.016 – 0.093)	0.048	(0.008 – 0.088)
	3	0.056	(0.016 – 0.096)	0.046	(0.006 – 0.087)
	4	0.036	(–0.002 – 0.075)	0.016	(–0.023 – 0.055)
	5	0.037	(–0.002 – 0.076)	0.017	(–0.022 – 0.057)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Model 5: model 4 + physical activity. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.13 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for income level

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,445	1	1.058	(0.994 – 1.126)	1.070	(1.001 – 1.144)
	2	1.040	(0.976– 1.108)	1.056	(0.987– 1.129)
	3	1.018	(0.952– 1.089)	1.035	(0.966 – 1.109)
	4	1.013	(0.945– 1.084)	1.035	(0.965 – 1.111)
Flicker light–induced retinal arteriolar and venular dilation, per SD n=1,439	1	0.017	(–0.037 – 0.071)	0.006	(–0.052 – 0.065)
	2	0.005	(–0.049 – 0.060)	–0.002	(–0.061 – 0.056)
	3	0.011	(–0.045 – 0.068)	0.010	(–0.05 – 0.069)
	4	0.017	(–0.040 – 0.075)	0.010	(–0.05 – 0.07)
Urinary albumin excretion, ratio increase n=2,121	1	1.14	(1.10 – 1.19)	1.13	(1.08 – 1.17)
	2	1.11	(1.06 – 1.15)	1.10	(1.05 – 1.14)
	3	1.05	(1.01 – 1.10)	1.08	(1.03 – 1.12)
	4	1.05	(1.01 – 1.09)	1.06	(1.02 – 1.11)
Skin hyperaemia, per SD n=1,017	1	0.031	(–0.030– 0.092)	0.027	(–0.037 – 0.091)
	2	0.014	(–0.048 – 0.077)	0.012	(–0.052 – 0.077)
	3	0.006	(–0.059 – 0.071)	0.015	(–0.050 – 0.080)
	4	0.011	(–0.054 – 0.077)	0.021	(–0.045 – 0.087)
Plasma biomarkers of microvascular dysfunction, per SD n=2,097	1	0.094	(0.052 – 0.137)	0.087	(0.042 – 0.131)
	2	0.058	(0.016 – 0.099)	0.057	(0.014 – 0.100)
	3	0.067	(0.023 – 0.110)	0.058	(0.014 – 0.102)
	4	0.048	(0.006 – 0.090)	0.025	(–0.018 – 0.067)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, income level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.14 Associations between systolic and diastolic blood pressure variability and measures of microvascular dysfunction, additionally adjusted for occupation status.

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Cerebral small vessel disease features, per point n=1,568	1	1.058	(0.994 – 1.126)	1.070	(1.001 – 1.144)
	2	1.040	(0.976 – 1.108)	1.056	(0.987 – 1.129)
	3	1.018	(0.952 – 1.089)	1.035	(0.966 – 1.109)
	4	1.013	(0.945 – 1.084)	1.035	(0.965 – 1.111)
Flicker light–induced retinal arteriolar and venular dilation, per SD n=1,552	1	0.038	(–0.016 – 0.091)	0.025	(–0.031– 0.080)
	2	0.024	(–0.029 – 0.078)	0.017	(–0.038 – 0.073)
	3	0.031	(–0.026 – 0.087)	0.030	(–0.026 – 0.086)
	4	0.033	(–0.024 – 0.090)	0.030	(–0.027 – 0.086)
Urinary albumin excretion, ratio increase n=2,302	1	1.15	(1.11 – 1.20)	1.14	(1.09 – 1.18)
	2	1.12	(1.07 – 1.16)	1.11	(1.07 – 1.16)
	3	1.06	(1.02 – 1.10)	1.09	(1.05 – 1.14)
	4	1.06	(1.01 – 1.10)	1.08	(1.04 – 1.12)
Skin hyperaemia, per SD n=1,113	1	0.068	(0.007 – 0.128)	0.048	(–0.013 – 0.110)
	2	0.046	(–0.016 – 0.107)	0.031	(–0.031 – 0.093)
	3	0.041	(–0.023 – 0.105)	0.032	(–0.031 – 0.094)
	4	0.044	(–0.021 – 0.108)	0.028	(–0.035 – 0.091)
Plasma biomarkers of microvascular dysfunction, per SD n=2,284	1	0.099	(0.058 – 0.141)	0.087	(0.043 – 0.130)
	2	0.058	(0.017 – 0.098)	0.057	(0.015 – 0.098)
	3	0.064	(0.022 – 0.107)	0.057	(0.015 – 0.099)
	4	0.042	(0.001– 0.083)	0.028	(–0.014 – 0.069)

Results (β [95% confidence interval]) are expressed as rate ratio for higher cerebral small vessel disease composite score, SD lower flicker light–induced retinal arteriolar and venular dilation composite score, higher ratio urinary albumin excretion, SD lower heat–induced skin hyperaemia, and SD higher plasma biomarkers of microvascular dysfunction composite score (all indicating worse microvascular function), and per SD higher systolic or diastolic blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, occupation status, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

Table S6.15 Associations between systolic and diastolic blood pressure variability and urinary albumin excretion as a categorical variable

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Microvascular dysfunction	**Model**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Urinary albumin excretion, ≥30 mg/24h vs. <30 mg/24h n=2,748	1	1.39	(1.22 – 1.59)	1.32	(1.16 – 1.50)
	2	1.30	(1.14 – 1.49)	1.25	(1.10 – 1.43)
	3	1.19	(1.03 – 1.38)	1.20	(1.05 – 1.38)
	4	1.19	(1.02 – 1.38)	1.19	(1.02 – 1.37)

Regression coefficients (β) represent odds ratio of ≥30 mg/24h urinary albumin excretion per 1 SD increase in blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Bold denotes P value<.05. Abbreviations: BPV, blood pressure variability; CI, confidence interval; OR, odds ratio.

Table S6.16 Associations between systolic and diastolic blood pressure variability and estimated glomerular filtration rate

		Systolic blood pressure variability composite score, per SD		Diastolic blood pressure variability composite score, per SD	
Estimated glomerular filtration rate	**Model**	**β**	**(95% CI)**	**β**	**(95% CI)**
Lower estimated glomerular filtration	1	0.007	(-0.026 - 0.041)	0.026	(-0.008 - 0.060)
rate, per SD	2	0.002	(-0.032 - 0.035)	0.022	(-0.012 - 0.057)
n=2,748	3	0.017	(-0.018 - 0.053)	0.026	(-0.008 - 0.061)
	4	-0.004	(-0.038 - 0.030)	-0.006	(-0.040 - 0.028)
	Model	**OR**	**(95%CI)**	**OR**	**(95%CI)**
Estimated glomerular filtration rate,	1	1.14	(0.95 - 1.36)	1.17	(0.98 - 1.40)
≥60 vs. <60 ml/min/1.73m^2, odds ratio	2	1.06	(0.87 - 1.27)	1.10	(0.92 - 1.32)
	3	1.10	(0.91 - 1.33)	1.14	(0.96 - 1.36)
n=2,748	4	1.04	(0.84 - 1.29)	1.02	(0.84 - 1.24)

Regression coefficients (β) represent standard deviation lower estimated glomerular filtration rate, and odds ratio for ≥60 vs. <60 ml/min/1.73m^2 estimated glomerular filtration rate, for every 1 SD increase in blood pressure variability composite score. Model 1: age, sex. Model 2: model 1 + glucose metabolism status. Model 3: model 2 + mean 24–hour systolic or diastolic blood pressure (where appropriate). Model 4: model 3 + body mass index, smoking status, alcohol use, education level, total–to–high density lipoprotein cholesterol ratio, (individual classes of) antihypertensive medication, lipid–modifying medication. Bold denotes P value<.05. Abbreviations: CI, confidence interval; SD, standard deviation.

CHAPTER 7

Carotid stiffness is associated with retinal microvascular dysfunction– The Maastricht Study

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Abstract

Objective

This study investigated whether arterial stiffening is a determinant of subtle retinal microvascular changes that precede diabetic retinopathy.

Research design and methods

This study used cross-sectional data from the Maastricht Study, a type 2 diabetes-enriched population-based cohort study. We used multivariable linear regression analysis to investigate, in individuals without and with type 2 diabetes, the associations of carotid distensibility coefficient and carotid-femoral pulse wave velocity with retinal microvascular diameters and flicker light-induced dilation and adjusted for cardiovascular and lifestyle risk factors.

Results

The retinal microvascular diameter study population consisted of N=2434 participants (51.4% men, mean ± SD age 59.8 ± 8.1 years and 28.1% type 2 diabetes). No measures of arterial stiffness were significantly associated with microvascular diameters. Greater carotid distensibility coefficient (i.e. lower carotid stiffness) was significantly associated with greater retinal arteriolar flicker light-induced dilation (per standard deviation, standardized beta [95%CI] 0.06 [0.00; 0.12]) and non-significantly, but directionally similarly, associated with greater retinal venular flicker light-induced dilation (0.04 [-0.02; 0.10]). Carotid-femoral pulse wave velocity (i.e. aortic stiffness) was not associated with retinal microvascular flicker light-induced dilation. The associations between carotid distensibility coefficient and retinal arteriolar and venular flicker light-induced dilation were two- to threefold stronger in individuals with type 2 diabetes than in those without.

Conclusion

In this population-based study greater carotid, but not aortic, stiffness was associated with worse retinal flicker light-induced dilation and this association was stronger in individuals with type 2 diabetes. Hence, carotid stiffness may be a determinant of retinal microvascular dysfunction.

Introduction

Clinical diabetic retinopathy is preceded by subtle retinal microvascular changes that include initial widening and later narrowing of retinal microvascular diameters, and impaired retinal microvascular flicker light-induced dilation.[1,2] These changes are thought to reflect impaired retinal autoregulation, which predisposes to enhanced retinal capillary pressure and consequent capillary dilation, leakage, rupture, and nonperfusion, which are hallmark features of non-proliferative diabetic retinopathy.[2] Widening and narrowing of retinal microvascular diameters are thought to reflect impairment of autoregulation and microvascular remodelling respectively.[2,3] In turn, lower retinal microvascular flicker light-induced dilation is thought to reflect dysfunction of the neurovascular coupling unit (i.e. dysfunction of both neuronal and endothelial cells).[4] Because hyperglycaemia is associated with neurodegeneration[5] and endothelial cell dysfunction[2,6,7] individuals with type 2 diabetes may be especially at risk for lower retinal microvascular flicker light-induced dilation. Indeed, we have observed lower retinal microvascular flicker light-induced dilation in individuals with prediabetes as well as in individuals with type 2 diabetes.[8]

Arterial stiffening may be a potentially reversible determinant of adverse changes in retinal vascular structure and function.[9] Mechanistically, arterial stiffening is thought to enhance propagation of arterial pressure and flow waves.[3] This increases transmission of detrimental pulsatile energy into the retinal microcirculation, which is especially vulnerable to arterial stiffening-induced hemodynamic stress because the retina is a high-flow, low-impedance microvascular system.[3,10]

Indeed, there is some evidence that arterial stiffening is associated with narrowing of retinal arterioles[11-15] and, possibly, widening of retinal venules,[13] although these studies did not adjust for potential confounders such as blood pressure,[14] diet,[11-16] and physical activity.[11-16] Current evidence is also limited because the associations between arterial stiffness and flicker light-induced retinal arteriolar and venular dilation have not yet been investigated.

In view of the above, we studied the associations between arterial stiffness and retinal arteriolar and venular diameters and flicker light-induced dilation in the population-based Maastricht Study. We hypothesized that greater arterial stiffness is associated with narrower retinal arterioles, possibly wider retinal venules, lower arteriolar-to-venular ratio and lower retinal arteriolar and venular flicker light-induced dilation, and that associations may be stronger in individuals with type 2 diabetes.

Methods

Study population and design

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously.[17] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Arterial stiffness

All measurements were done by trained vascular technicians unaware of the participants' clinical or diabetes mellitus status, in a dark, quiet, temperature-controlled room (21°C–23°C), as described previously.[18,19] Participants were asked to refrain from smoking and drinking coffee, tea or alcoholic beverages 3 hours before the study. Participants were allowed to have a light meal (breakfast or lunch). All measurements were performed in the supine position after 10 minutes of rest. Talking or sleeping was not allowed during the examination. During the vascular measurements (≈45 minutes), brachial systolic, diastolic, and mean arterial pressure (MAP) were determined every 5 minutes with an oscillometric device (Accutorr Plus, Datascope Inc, Montvale, NJ). The MAP and heart rate during these measurements were used in the statistical analysis. A 3-lead ECG was recorded continuously during the measurements to facilitate automatic signal processing.

Local carotid arterial elastic properties

Measurements of local carotid arterial properties were done at the left common carotid artery (10-mm proximal to the carotid bulb), with the use of an ultrasound scanner equipped with a 7.5-MHz linear probe (MyLab 70, Esaote Europe B.V., Maastricht,

The Netherlands). This setup enables the measurement of diameter, distension, and intima-media thickness (IMT) as described previously.[18,19] Briefly, during the ultrasound measurements a B-mode image on the basis of 19 M-lines was depicted on screen, and an online echo-tracking algorithm showed real-time anterior and posterior arterial wall displacements. The M-mode recordings were composed of 19 simultaneous recordings at a frame rate of 498 Hz. The distance between the M-line recording positions was 0.96 mm; thus, a total segment of 18.24 mm of each artery was covered by the scan plane. For offline processing, the radiofrequency signal was fed into a dedicated PC-based acquisition system (ART.LAB, Esaote Europe B.V. Maastricht, The Netherlands) with a sampling frequency of 50 MHz. Data processing was performed in MatLab (version 7.5, Mathworks, Natick, MA). The distension waveforms were obtained from the radio frequency data with the use of a wall track algorithm.[18] Carotid IMT was defined as the distance of the posterior wall from the leading edge interface between lumen and intima to the leading edge interface between media and adventitia.[19] The median diameter, distension, and IMT of 3 measurements were used in the analyses.
Local arterial elastic properties were quantified by calculating the following indices:[20]

- Distensibility coefficient (DC)
  $DC = (2\Delta D * D + \Delta D^2) / (PP * D^2)$ $(10^{-3}\ kPa^{-1})$
- Young's elastic modulus (YEM)
  $YEM = D / (IMT * DC)$ $(10^3\ kPa)$

where ΔD is, distension; IMT, intima-media thickness; and PP, brachial pulse pressure (calculated as systolic blood pressure (BP)minus diastolic BP).

Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity (cfPWV; in m/s) was determined according to international guidelines with the use of applanation tonometry (SphygmoCor, Atcor Medical, Sydney, Australia).[21] Pressure waveforms were determined at the right common carotid arteries and right common femoral arteries. Difference in the time of pulse arrival from the R-wave of the ECG between the 2 sites (transit time) was determined with the intersecting tangents algorithm. The pulse wave travel distance was calculated as 80% of the direct straight distance (measured with an infantometer) between the 2 arterial sites. The median of 3 consecutive cfPWV (defined as travelled distance/transit time) recordings was used in the analyses. Details are provided in the Supplementary Methods.

Retinal microvascular function measures

All participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement.[22,23] A light meal (breakfast and (or) lunch), low in fat content, was allowed if taken at least 90 minutes prior to the start of the measurements.
For retinal measurements pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine at least 15 minutes prior to the start of the examination.

Retinal microvascular diameters

All fundus photographs were taken with an auto-focus, auto-shot and auto-tracking fundus camera (Model AFC-230; Nidek, Gamagori, Japan) in an optic disc-centred field of view of 45° in a darkened room. Static retinal vessel analysis (one image of the left or right eye was randomly chosen per participant) was performed using the retinal health information and notification system (RHINO) software developed by the RetinaCheck group of the Technical University of Eindhoven (Eindhoven, the Netherlands).[24,25] Optic disc detection and arteriole/venule classification were corrected manually. Retinal vessel diameters were measured at 0.5–1.0 disc diameter away from the optic disc margin and were presented as central retinal arteriolar equivalent and central retinal venular equivalent (CRAE and CRVE, respectively) in measurement units (MU) and as arteriole-to-venule ratio (AVR). The scale factor is based on the optic disc diameter, which is assumed to be 1800 μm,[26] i.e. 1 MU = 1 pixel size× 1800 μm/pixel size of optic disc diameter. CRAE and CRVE represent the equivalent single-vessel parent diameter for the six largest arterioles and largest venules in the region of interest, respectively. The calculations were based on the improved Knudtson–Parr–Hubbard formula. [27]
Fundus photographs of insufficient quality, e.g. obstructed by lashes or defocused, were evaluated and discussed with a second observer and excluded on mutual agreement. We calculated the intraclass correlation coefficients for CRAE and CRVE to assess the agreement between analyses of the RHINO software with vs without manual identification of arterioles and venules using 2556 images. The intraclass correlation coefficient of CRAE was 0.910 and that of CRVE was 0.897.

Retinal microvascular flicker light-induced dilation

The retinal arteriolar and venular dilation response to flicker light, which is thought to be related to nutritive demands of activated retinal neurons[28], was measured in a dimly lit room by use of the Dynamic Vessel Analyzer (DVA; IMEDOS, Jena, Germany). Per participant, we randomly measured the left or right eye.

During the measurement, the participant was instructed and encouraged to focus on the tip of a fixated needle inside the retinal camera (FF450; Carl Zeiss GmbH, Jena, Germany), while the fundus of the eye was examined under green measuring light (530-600 nm, illumination of fundus approximately 6500 lux). A straight arteriolar or venular segment of approximately 1.5 mm in length located 0.5 to 2.0 disc diameter from the margin of the optic disc in the temporal section was examined. When the specific vessel profile was recognized, vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker-light exposure period (flicker frequency 12.5Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The DVA automatically corrected for alterations in luminance caused by, for example, slight eye movements. During blinks and small eye movements, the registration stopped and restarted once the vessel segments were automatically re-identified.[28]
The integrated DVA software (version 4.51, Imedos) automatically calculated baseline diameter and percentage dilation. Baseline diameter was calculated as the average diameter size of the 20-50 seconds recording and was expressed in measurement units (MU), where 1 MU is equal to 1µm of the Gullstrand eye.[29] Percentage dilation over baseline was based on the average dilation achieved at time-points 10 and 40 seconds during the flicker stimulation period. Two regression lines were drawn (at interval 0-10 seconds and 10-40 seconds during flicker stimulation) and averaged to assess average percentage dilation. The software successfully assessed two regression lines in 95.4% of the curves; only 102 dilation curves (4.6%) were based on one regression line. The purpose of taking the average dilation was to account for interindividual variation in the curve shape during dilation. More details are provided in the Supplementary Methods.

Covariates

We determined glucose metabolism status (normal glucose metabolism, prediabetes, type 1 diabetes or type 2 diabetes) based on a 75 gram oral glucose tolerance test and use of glucose lowering medication according to the World Health Organization 2006 criteria.[17] Education level ("educational status") was classified into three groups: low (none, primary or lower vocational education only), medium (intermediate general secondary, intermediate vocational or higher general secondary education) and high (higher vocational education or university level of education). Alcohol consumption was classified as non-consumer, low-consumer (≤7 alcoholic drinks/week for women; ≤14 alcoholic drinks/week for men) or high-consumer (>7 alcoholic drinks/week for women; >14 alcohol drinks/week for men). We determined age, sex, smoking status (never, former, current), medication use, waist circumference, body mass index (BMI),

total/high density lipoprotein (HDL) cholesterol ratio, triglycerides, fasting plasma glucose, 2-hour post load glucose, and glycated haemoglobin (HbA1c), office and ambulatory blood pressure, accelerometer-assessed physical activity, estimated glomerular filtration rate (eGFR), albuminuria, prior cardiovascular disease, plasma biomarkers of low-grade inflammation (i.e. high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8 and tumour necrosis factor alpha) and retinopathy as described previously.[5,17,30-32] An automated refractor (Tonoref II; Nidek, Gamagordi, Japan) was used for automated refraction and noncontact tonometry assessment in both eyes. We determined glaucoma as use of intraocular pressure-lowering medication or intraocular pressure greater than 21 mm Hg. We used a validated food frequency questionnaire to assess the Dutch Health Diet score ("diet score").[33,34] As described previously, we used questionnaires to assess income level and occupational status. [35]

Statistical analyses

All analyses were performed with Statistical Package for Social Sciences version 23.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). We used multivariable linear regression analysis to investigate the associations between (standardized) carotid DC, YEM and cfPWV (determinants) with (standardized) retinal microvascular diameters (i.e. CRAE, CRVE, AVR) and retinal arteriolar and venular flicker light-induced dilation (outcomes). We checked whether measures of arterial stiffness were associated with baseline diameter in analyses of retinal microvascular flicker light-induced percentage dilation because such associations may result in spurious estimates of associations, but this was not the case [data not shown].

Model 1 shows crude results. In model 2 we adjusted for age, sex, glucose metabolism status (entered as dummies [i.e. type 2 diabetes, or prediabetes, or other type of diabetes versus normal glucose metabolism status]). In model 3 we additionally adjusted for mean arterial pressure and heart rate. We chose these variables as they are key potential confounders (all) or were oversampled (type 2 diabetes). In model 4 we additionally adjusted for variables whose status as potential confounders has been less firmly established (use of antihypertensive medication [yes/no], waist circumference, total cholesterol / HDL cholesterol ratio, lipid-modifying medication, smoking status [current, ever, never], alcohol consumption status [none, low, high] and educational status [low, medium, high]).

Data were expressed as standardized regression coefficient and corresponding 95% confidence interval (95%CI). Collinearity diagnostics (i.e. tolerance <0.10 and/or variance inflation factor >10) were used to detect multicollinearity between covariates. For analyses P-value <0.05 was considered statistically significant.

To assess whether associations differed by type 2 diabetes status (i.e. between individuals with type 2 diabetes and individuals without diabetes) or sex we tested for interaction. We excluded participants with other types of diabetes from the interaction analyses because the number of participants with other types of diabetes was small. We hypothesized a priori that associations may be stronger individuals with type 2 diabetes as under hyperglycaemic circumstances the retinal microvasculature is likely more vulnerable. As most previous studies did not find interaction by sex we a priori did not expect interaction by sex to be likely.[11,14] $P_{interaction}$ <0.10 was considered statistically significant.

Additional analyses

First, we repeated the analysis additionally adjusting for the diet score, physical activity, and refractive error. Adjustment for these potential confounders was not included in the main analysis because data were missing in a relatively large number of participants (n=120-488 had missing data on one or more of these variables). Second, we additionally adjusted for kidney variables (eGFR and albuminuria), prior cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy and glaucoma. We adjusted for these covariates in a separate model because these variables may be confounders, but may also (partly) mediate the associations under study. Third, we substituted glucose metabolism status by fasting plasma glucose, 2-hour post load glucose or HbA1c; waist circumference by BMI; mean arterial pressure measured during the vascular ultrasound measurement by 24-hour mean arterial pressure; and educational status by occupational status and income level. Fourth, when type 2 diabetes status modified associations we repeated tests for interaction analyses with continuous measures of hyperglycaemia (i.e. fasting plasma glucose, 2-hour post load glucose and HbA1c).

Results

Selection and characteristics of the study population

Figure 7.1 shows an overview of the study population selection.

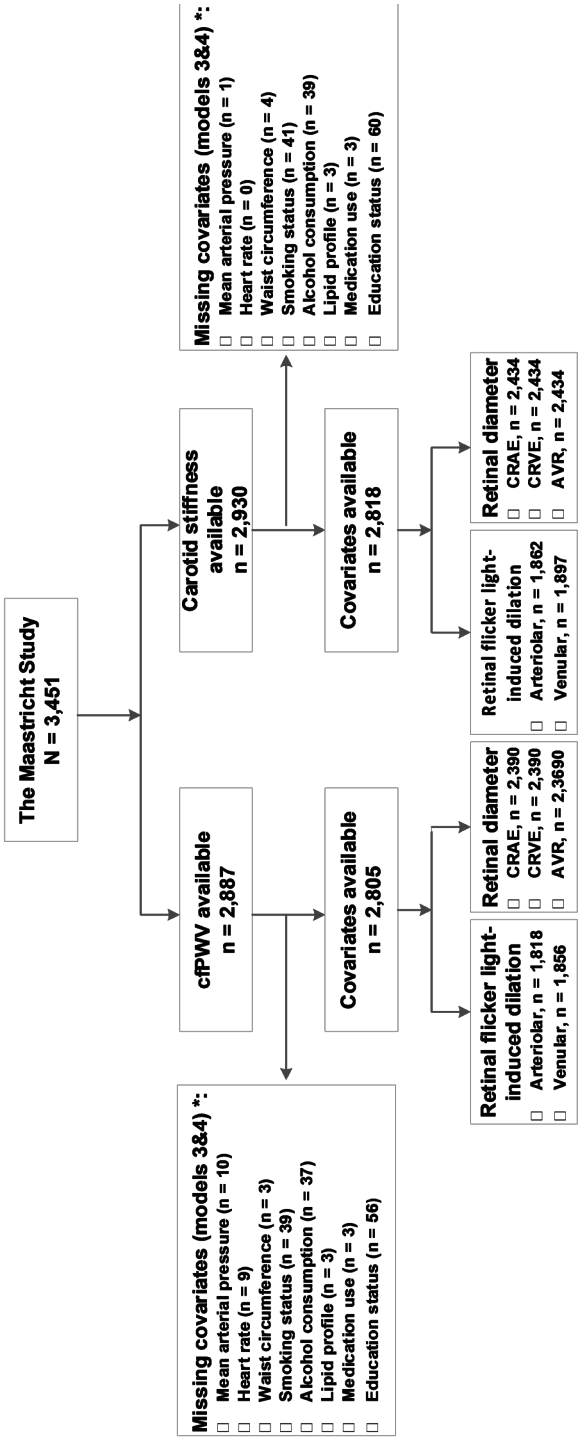


Figure 7.1 Retinal microvascular diameter and retinal microvascular flicker light-induced dilation study population selection.

Table 7.1 shows general characteristics according to retinal arteriolar diameter (CRAE) and Supplemental Table S7.1 shows general characteristics according to retinal venular flicker light-induced dilation. Overall, participants with a narrower arteriolar calibre and lower venular flicker light-induced dilation were more often men, had a higher age and had a more adverse cardiovascular risk profile. General characteristics of participants included in the study were highly comparable to those of participants with missing data (Supplemental Table S7.2).

Arterial stiffness and retinal microvascular diameters

After adjustment, neither carotid DC, nor carotid YEM, nor cfPWV were significantly associated with CRAE (per SD, standardized beta [95% CI], respectively -0.00 [-0.05, 0.05]; 0.02 [-0.02; 0.07]; 0.03 [-0.02; 0.08]), CRVE (-0.01 [-0.06; 0.04]; 0.02 [-0.02; 0.07]; 0.00 [-0.05; 0.05]) or AVR (0.01 [-0.04; 0.06]; 0.00 [-0.04; 0.05]; 0.04 [-0.01; 0.09]) (Table 7.2). Type 2 diabetes status and sex did not modify any of the above associations (Supplemental Table S7.3).

Arterial stiffness and flicker light-induced retinal arteriolar and venular dilation

After adjustment, greater carotid DC was significantly associated with greater retinal arteriolar and non-significantly, but directionally similarly, associated with greater retinal venular flicker light-induced dilation (respectively, 0.06 [0.00; 0.12]; 0.04 [-0.02; 0.10]; Table 7.2 and Figure 7.2). In contrast, carotid YEM and cfPWV were not significantly associated with retinal arteriolar flicker light-induced dilation (respectively, -0.01 [-0.06; 0.05] and -0.01 [-0.07; 0.05]) or with retinal venular flicker light-induced dilation (respectively, -0.02 [-0.07; 0.03] and -0.02 [-0.078 0.04]).
Type 2 diabetes modified the associations between carotid DC and arteriolar and venular flicker light-induced dilation ($P_{interaction}$=0.02 and $P_{interaction}$=0.01, respectively). Sex modified the associations between carotid DC and carotid YEM with venular flicker light-induced dilation ($P_{interaction}$=0.02 and $P_{interaction}$=0.02, respectively; $P_{interaction}$ values are shown in Supplemental Table S7.3). In participants with type 2 diabetes, but not in participants without diabetes, greater carotid DC was significantly associated with greater arteriolar and venular flicker light-induced dilation (respectively, 0.14 [0.04; 0.25] and 0.16 [0.06; 0.26] in participants with type 2 diabetes versus 0.03 [-0.04; 0.10] and -0.02 [-0.09; 0.05] in participants without diabetes; Supplemental Table S7.4 and Figure 7.2). In men, but not in women, carotid DC and YEM were significantly associated with retinal venular flicker light-induced dilation (respectively, 0.11 [0.03; 0.19] and -0.08 [-0.16; -0.01] in men versus -0.03[-0.11; 0.06] and 0.05 [-0.03; 0.12] in women; Supplemental Table S7.5). There was no three-way interaction by type 2 diabetes status and sex (Pinteraction>0.10; data not shown).

Table 7.1 General study population characteristics according to tertiles of arteriolar diameter.

Characteristic	Total study group (N = 2,434)	Tertiles of arteriolar diameter		
		Tertile 1 (low) (n = 811)	Tertile 2 (middle) (n =809)	Tertile 3 (high) (n = 814)
Demographics				
Age, years	59.8 ± 8.2	61.3 ± 7.9	59.6 ± 8.1	58.6 ± 8.3
Men	1252 (51.4%)	477 (58.8%)	414 (51.2%)	453 (55.7%)
Educational status				
Low	801 (32.9%)	269 (33.2%)	266 (32.9%)	266 (32.7%)
Medium	707 (29.0%)	230 (28.4%)	242 (29.9%)	235 (28.9%)
High	926 (38.0%)	312 (38.5%)	301 (37.2%)	313 (38.5%)
Occupational status *				
Low	654 (32.2%)	201 (29.6%)	212 (32.1%)	241 (35.0%)
Middle	701 (34.6%)	233 (34.3%)	242 (36.7%)	226 (32.8%)
High	673 (33.2%)	245 (36.1%)	216 (31.2%)	222 (32.2%)
Income per month, euros *	2016.8 ± 814.0	2084 ± 831	2007 ± 789	1959 ± 818
Lifestyle factors				
Smoking status				
Never	817 (33.6%)	255 (31.4%)	276 (34.1%)	286 (35.1%)
Former	1302 (53.5%)	467 (57.6%)	432 (53.4%)	403 (49.5%)
Current	315 (12.9%)	89 (11.0%)	101 (12.5%)	125 (15.4%)
Alcohol consumption				
None	440 (18.1%)	131 (16.2%)	135 (16.7%)	174 (21.4%)
Low (women ≤7, men ≤14)	1368 (56.2%)	441 (54.4%)	480 (59.3%)	447 (54.9%)
High (women >7, men >14)	626 (25.7%)	239 (29.5%)	194 (24.0%)	193 (23.7%)
Physical activity, hours/day *	2.0 ± 0.7	1.9 [1.5 – 2.5]	2.0 [1.5 – 2.4]	1.9 [1.5 – 2.4]
Dutch Healthy diet score, points	83.6 ± 14.6	82.8 ± 14.4	84.4 ± 14.4	83.7 ± 14.8
Cardiovascular risk factors				
Glucose metabolism status				
Normal glucose metabolism	1364 (56.0%)	429 (52.9%)	470 (58.1%)	465 (57.1%)
Prediabetes	357 (14.7%)	130 (16.0%)	119 (14.7%)	108 (13.3%)
Type 2 diabetes	684 (28.1%)	238 (29.3%)	212 (26.2%)	234 (28.7%)
Other types of diabetes	29 (1.2%)	14 (1.7%)	8 (1.0%)	7 (0.9%)

Table 7.1 (continued)

Characteristic	Total study group (N = 2,434)	Tertiles of arteriolar diameter		
		Tertile 1 (low) (n = 811)	Tertile 2 (middle) (n =809)	Tertile 3 (high) (n = 814)
Fasting plasma glucose, mmol/l*	5.6 [5.1 – 6.5]	5.6 [5.1 – 6.7]	5.5 [5.1 – 6.4]	5.5 [5.0 – 6.5]
2-hour post load plasma glucose, mmol/l*	6.2 [5.1 – 9.4]	6.5 [5.2 – 9.7]	6.2 [5.1 – 9.1]	6.1 [4.9 – 9.5]
HbA1c, %*	5.7 [5.4 – 6.2]	5.7 [5.4 – 6.3]	5.7 [5.4 – 6.2]	5.6 [5.4 – 6.2]
HbA1c, mmol/mol*	39 [36 – 44]	39 [36 – 45]	39 [36 – 44]	38 [36 – 44]
Glucose lowering medication	568 (23.3%)	201 (24.8%)	179 (22.1%)	188 (23.1%)
Waist circumference, cm	27.0 ± 4.5	27.0 ± 4.3	27.0 ± 4.3	27.1 ± 4.8
Body mass index, kg/m^2 *	27.0 ± 4.5	27.0 ± 4.3	27.0 ± 4.3	27.1 ± 4.8
History of cardiovascular disease*	405 (16.7%)	139 (17.3%)	124 (15.4%)	142 (17.6%)
eGFR, ml/min/1.73m^2*	88.3 ± 14.8	87.9 ± 14.9	88.7 ± 14.3	88.3 ± 15.2
Albuminuria, mg/24h*	6.6 [4.0 – 11.8]	7.2 [4.4 – 13.4]	6.3 [3.8 – 11.3]	6.2 [3.8 – 10.7]
Total-to-HDL cholesterol ratio	3.6 ± 1.2	3.6 ± 1.2	3.6 ± 1.2	3.6 ± 1.1
Triglycerides, mmol/l	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]
Use of lipid-modifying medication	910 (37.4%)	341 (42.0%)	279 (34.5%)	290 (35.6%)
Office systolic BP, mmHg *	134.1 ± 18.2	138.7 ± 18.3	134.2 ± 17.5	131.6 ± 18.0
Office diastolic BP, mmHg *	76.2 ± 10.0	78.1 ± 10.1	76.1 ± 9.7	74.2 ± 9.7
Mean arterial pressure, mmHg	96.5 ± 10.2	98.9 ±10.4	96.4 ± 9.9	94.3 ± 9.7
24-hour mean arterial pressure, mm Hg*	88.6 ± 8.0	90.5 ± 8.2	88.5 ± 7.8	86.7 ± 7.4
Heart rate, bpm	62.6 ± 9.4	62.6 ± 9.5	62.5 ± 9.1	62.7 ± 9.6
Use of antihypertensive medication	974 (40.0%)	354 (43.6%)	322 (39.8%)	298 (36.6%)
Inflammation markers*				
C-reactive protein, µg/ml	1.3 [0.6 – 2.7]	1.3 [0.6 – 2.6]	1.2 [0.6 – 2.7]	1.3 [0.6 – 2.9]
Serum amyloid A, µg/ml	3.3 [2.1 – 5.4]	3.2 [2.2 – 5.5]	3.3 [2.0 – 5.6]	3.4 [2.0 – 5.5]
Tumor necrosis factor α, pg/ml	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.5]	2.2 [1.9 – 2.6]
Interleukin-6, pg/ml	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]
Interleukin-8, pg/ml	4.1 [3.3 – 5.3]	4.2 [3.4 – 5.4]	4.1 [3.2 – 5.2]	4.0 [3.3 – 5.2]

Table 7.1 (continued)

Characteristic	Total study group (N=2,434)	Tertiles of arteriolar diameter		
		Tertile 1 (low) (n=811)	Tertile 2 (middle) (n=809)	Tertile 3 (high) (n=814)
Eye variables				
Retinopathy*	38 (1.7%)	13 (1.7%)	13 (1.7%)	12 (1.6%)
Glaucoma*	114 (5.2%)	46 (5.7%)	40 (4.9%)	28 (3.4%)
Refractive error*				
Right eye	0.1 [-1.5 – 1.1]	0.0 [-2.0 – 1.0]	0.1 [-1.50 – 1.1]	0.1 [-1.0 – 1.3]
Left eye	0.1 [-1.4 – 1.1]	-0.1 [-2.3 – 0.9]	0.13 [-1.4 – 1.13]	0.3 [-1.0 – 1.3]
Arterial stiffness				
cfPWV, m/s †	9.0 ± 2.1	9.3 ± 2.2	8.9 ± 2.0	8.8 ± 2.1
Carotid DC, 10^{-3}/kPa	14.5 ± 5.12	13.9 ± 5.1	14.3 ± 4.9	15.2 ± 5.4
Carotid YEM, 10^{3}/kPa*	0.73 ± 0.34	0.77 ± 0.35	0.75 ± 0.33	0.70 ± 0.33
Retinal microvascular function				
Arteriolar diameter (CRAE), µm	142.2 ± 20.4	120.2 ± 10.9	141.9 ± 5.2	164.6 ± 10.7
Venular diameter (CRVE), µm	214.4 ± 31.1	190.9 ± 24.5	213.1 ± 21.6	239.2 ± 25.7
Arteriole-to-venule ratio (AVR), no unit	0.67 ± 0.08	0.64 ± 0.08	0.67 ± 0.07	0.69 ± 0.07
Arteriolar flicker light-induced dilation, %‡	3.0 ± 2.8	3.2 ± 2.9	3.1 ± 2.8	2.8 ± 2.8
Venular flicker light-induced dilation, %‡	3.9 ± 2.2	3.9 ± 2.3	3.8 ± 2.1	4.0 ± 2.4

Data presented as mean ± standard deviation, median [interquartile range] or number (%). * data available for occupational status, n=2,028; income level, n=1,841; body mass index, n=2,433; physical activity, n=1,946; Dutch healthy diet score, n=2,314; fasting plasma glucose, n= 2,433; 2-hour post load plasma glucose, n=2,249; HbA1c, n=2,429; history of cardiovascular disease, n=2,418; eGFR, n=2,416; albuminuria, n=2,415; office systolic and diastolic blood pressure, n=2,432; 24-hour mean arterial pressure, n=2,169; inflammation markers, n=2,411; retinopathy, n=2,254; glaucoma, n=2,211; refractive error right eye, n=2,237, refractive error left eye, n=2,229; carotid YEM, n=2,432. † value shown for cfPWV study population with complete retinal arteriolar diameter data. ‡ value shown for carotid stiffness population with complete arteriolar or venular dilation data. Abbreviations: BP, blood pressure; carotid DC, carotid distensibility coefficient; carotid YEM, carotid Young elastic modulus; cfPWV, carotid -femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arterio-to-venule ratio; eGFR; estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high density lipoprotein.

Table 7.2 Associations of aortic and carotid stiffness with retinal microvascular diameter and flicker light-induced dilation.

		Retinal microvascular diameter			Retinal microvascular flicker light-induced dilation	
		CRAE	CRVE	AVR	Arteriolar dilation	Venular dilation
Arterial stiffness	**Model**	**Stβ (95%CI)**	**Stβ (95%CI)**	**Stβ (95%CI)**	**Stβ (95%CI)**	**Stβ (95%CI)**
Carotid DC, per SD	1	**0.11 (0.07; 0.15)**	0.03 (-0.01; 0.07)	**0.11 (0.07; 0.15)**	**0.09 (0.04 ;0.13)**	0.03 (-0.01; 0.08)
	2	**0.07 (0.03; 0.12)**	0.00 (-0.04; 0.05)	**0.09 (0.04; 0.13)**	0.03 (-0.02; 0.08)	0.01 (-0.04; 0.06)
	3	-0.00 (-0.05; 0.05)	-0.00 (-0.06; 0.04)	0.01 (-0.04; 0.06)	**0.06 (0.00; 0.12)**	0.04 (-0.01; 0.10)
	4	-0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.04)	0.01 (-0.04; 0.06)	**0.06 (0.00; 0.12)**	0.04 (-0.02; 0.10)
Carotid YEM, per SD	1	**-0.08 (-0.12; -0.04)**	-0.00 (-0.04; 0.04)	**-0.09 (-0.13; -0.05)**	-0.05 (-0.09; 0.00)	-0.02 (-0.07; 0.02)
	2	-0.04 (-0.08; 0.01)	0.02 (-0.03; 0.06)	**-0.06 (-0.11; -0.02)**	0.01 (-0.04; 0.05)	-0.00 (-0.05; 0.05)
	3	0.02 (-0.02; 0.07)	0.03 (-0.02; 0.07)	-0.00 (-0.05; 0.04)	-0.01 (-0.06; 0.04)	-0.03 (-0.08; 0.03)
	4	0.02 (-0.02; 0.07)	0.02 (-0.02; 0.07)	0.00 (-0.04; 0.05)	-0.01 (-0.06; 0.05)	-0.02 (-0.07; 0.03)
cfPWV, per SD	1	**-0.11 (-0.15; -0.07)**	-0.04 (-0.08; 0.01)	**-0.09 (-0.13; -0.05)**	**-0.08 (-0.13; -0.04)**	-0.04 (-0.09; 0.01)
	2	**-0.05 (-0.09; 0.00)**	-0.01 (-0.05; 0.04)	**-0.05 (-0.10; -0.01)**	0.00 (-0.05; 0.06)	0.00 (-0.05; 0.05)
	3	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.02 (-0.07; 0.04)	-0.03 (-0.08; 0.03)
	4	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.02 (-0.08; 0.04)

Standardized regression coefficient (stβ) represents arteriolar or venular dilation in SD per SD greater arterial stiffness. For the retinal microvascular diameter population 1 SD corresponds with 5.16 10^{-3}/kPa for carotid DC, 0.34 10^{3}/kPa for carotid YEM, 2.08 m/s for cfPWV, 20.39 µm for CRAE, 31.07 µm for CRVE and 0.08 (no unit) for AVR. For the retinal arteriolar flicker light-induced dilation population 1 SD corresponds with 5.14 10^{-3}/kPa for carotid DC, 0.37 10^{3}/kPa for carotid YEM, 2.07 m/s for cfPWV, 2.82% for flicker light-induced arteriolar dilation and 2.20% for flicker light-induced venular dilation. Bold denotes $p<.05$. Model 1: crude. Model 2: age, sex and glucose metabolism status; model 3: model 2 + mean arterial pressure and heart rate; model 4: model 2 + smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, educational status. Abbreviations: SD, standard deviation; CI, confidence interval; cfPWV, carotid-to-femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriole-to-venule-ratio; DC, distensibility coefficient; YEM, Young's elastic modulus.

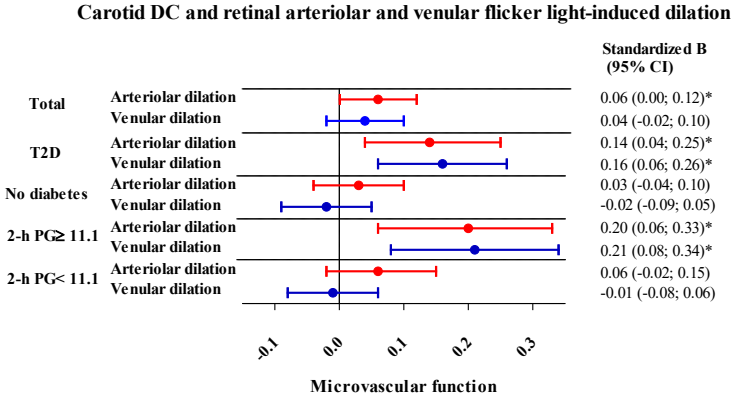


Figure 7.2 Associations between carotid DC (per SD) and retinal arteriolar and venular flicker light-induced dilation (per SD). Standardized regression coefficient (stβ) represents the difference in arteriolar or venular dilation in SD per SD greater carotid DC. In the total population 1 SD corresponds with 5.14 10-3/kPa for carotid DC, 2.82% for flicker light-induced arteriolar dilation and 2.20% for flicker light-induced venular dilation. In participants with type 2 diabetes 1 SD corresponds with 4.8 10-3/kPa for carotid DC, 2.7% for flicker light-induced arteriolar dilation, and 2.3% for flicker light-induced venular dilation in participants without diabetes. In participants without diabetes 1 SD corresponds with 5.2 10-3/kPa for carotid DC, 2.9% for flicker light-induced arteriolar dilation and 2.2% for flicker light-induced venular dilation. For participants with higher and lower levels of 2-h PG (≥11.1 mmol/l versus <11.1), values per SD were quantitatively similar to values per SD for participants with type 2 diabetes and without diabetes, respectively. Variables in model in addition to carotid DC: age, sex and glucose metabolism status, mean arterial pressure and heart rate, smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, educational status. In stratified analyses glucose metabolism status was not included in the model. *indicates statistically significant (P<0.05). Abbreviations: SD, standard deviation; CI, confidence interval; DC, distensibility coefficient; 2-h PG, 2-hour post load glucose; T2D, type 2 diabetes.

Additional analyses

Quantitatively similar results were observed in a range of sensitivity analyses. First, after additional adjustment for diet score, physical activity, and refractive error, associations did not materially change (Supplemental Table S7.6). Second, associations remained similar after additional adjustment for kidney variables, prior cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy and glaucoma (Supplemental Table S7.6). Third, associations were not materially altered when glucose metabolism status was substituted by fasting plasma glucose, 2-hour post load glucose or HbA1c; when waist circumference was substituted by BMI; when mean arterial pressure measured during the vascular ultrasound measurement was substituted by 24-hour mean arterial pressure; or when educational status was substituted by occupational status or income level (Supplemental Table S7.7). Fourth, when we replaced glucose metabolism status by continuous measures of hyperglycaemia, 2-hour post load glucose modified the association between carotid DC and retinal arteriolar

and venular flicker light-induced dilation ($P_{interaction}$=0.05 and $P_{interaction}$=0.03, respectively) and HbA1c modified the association between carotid DC and retinal venular flicker light-induced dilation ($P_{interaction}$=0.07; Supplemental Table S7.3). In stratified analyses the only significant associations were between carotid DC and arteriolar and venular flicker light-induced dilation in participants with WHO-defined[17] higher levels of 2-hour post load glucose (≥11.1 mmol/L; 0.20 [0.06; 0.33] and 0.21 [0.08; 0.34], respectively; Supplemental Table S7.5; Figure 7.2).

Discussion

The current population-based study has three main findings. First, carotid and aortic stiffness were not significantly associated with retinal arteriolar and venular diameters. Second, carotid but not aortic stiffness was significantly associated with worse retinal arteriolar flicker light-induced dilation in individuals with type 2 diabetes but not in individuals without diabetes. Third, carotid but not aortic stiffness was significantly associated with worse retinal venular flicker light-induced dilation in individuals with type 2 diabetes, but not in individuals without diabetes; and in men, but not in women.

In contrast to previous studies,[11-15] we observed no association between arterial stiffness and narrower retinal arteriolar diameters, possibly because inward remodelling (the proximate cause of arteriolar narrowing)[3] occurs in a more advanced disease stage than was present in the population that we studied. Additionally, and similar to most previous studies,[11,14,15] we observed no association of arterial stiffness with retinal venular diameters, possibly because hemodynamic stress in venules is insufficient to affect their structure.

Retinal arteriolar and venular flicker light-induced dilation (which represent measures of microvascular function and specifically neurovascular coupling) may be more easily affected by arterial stiffness than retinal arteriolar and venular diameters (which presumably mostly reflect microvascular structure).[36] Indeed, we observed that carotid stiffness was significantly associated with less retinal arteriolar and non-significantly with less venular flicker light-induced dilation. The observation that carotid stiffness was two- to threefold more strongly associated with lower flicker light-induced dilation under hyperglycaemic circumstances (i.e. in individuals with type 2 diabetes and with higher levels of 2-hour post load glucose) suggests that the retinal microvasculature may be more vulnerable to hemodynamic stress in the presence of hyperglycaemia. Associations were likely stronger because hyperglycaemia impairs both neuronal[1] and endothelial cell[2] function (i.e. neurovascular coupling) and intact neurovascular coupling is required for flicker light-induced dilation.[4,6,7]

Additionally, we consider the observation that the association between greater carotid stiffness and worse retinal venular dilation was stronger in men exploratory. Further investigation of this observation is warranted.

The lack of an association of both carotid YEM and aortic stiffness with retinal microvascular flicker light-induced dilation does not necessarily contradict the findings above. Carotid YEM reflects stiffness of material of the vessel wall[10], but not the arterial stiffening-induced hemodynamic stress which is thought to be detrimental to the retinal microvasculature.[11-15] Aortic stiffening, which reflects both muscular and elastic artery remodelling (in contrast to carotid stiffening which [mostly] reflects elastic remodelling), may not have yet occurred to such an extent that greater aortic pulsatile hemodynamic stress affects the retinal microvasculature.[37]

Strengths of this study are the large size of this population-based cohort study with oversampling of individuals with type 2 diabetes which enables accurate comparison of individuals with and without diabetes, the large number of potential confounders which were considered and the standardized assessment of all variables included in this study. Carotid and aortic stiffness as well as retinal microvascular diameters were measured with state-of-the-art methods.[21,23,25]

Limitations include the following. First, due to the cross-sectional nature of the study we cannot account for temporality and therefore causal inferences should be made with considerable caution. Although many data support the concept that arterial stiffening may lead to hemodynamic stress in the retinal microvasculature, in theory we cannot with the current data exclude that option that arterial stiffening and retinal microvascular dysfunction reflect separate entities of macrovascular and microvascular angiopathies in patients with diabetes.[3] Second, we may have underestimated the strength of the association between arterial stiffness and retinal microvascular flicker light-induced dilation response if such an association was stronger among participants that were excluded from the study population (who generally tend to be sicker). Additionally, the use of brachial rather than carotid pulse pressure to calculate carotid DC will again tend to underestimate associations through regression dilution.[10,38] Third, even though we took an extensive set of confounders into account, we cannot fully exclude residual confounding.[39] Fourth, we studied Caucasian individuals aged 40-75 years and therefore our results may be generalizable to such a population; whether these results also apply to other populations, e.g. older populations, requires further study.[40]

In summary, in this population-based study greater carotid, but not aortic, stiffness was associated with worse retinal flicker light-induced dilation and this association was stronger in individuals with type 2 diabetes. Hence, carotid stiffness may be a determinant of retinal microvascular dysfunction.

References

1. Simo R, Stitt AW and Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia. 2018;61:1902-12.
2. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. Diabetes. 2018;67:1729-41.
3. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. J Appl Physiol (1985). 2008;105:1652-60.
4. Lim M, Sasongko MB, Ikram MK, Lamoureux E, Wang JJ, Wong TY and Cheung CY. Systemic associations of dynamic retinal vessel analysis: a review of current literature. Microcirculation. 2013;20:257-68.
5. E.E.B. de Clerck JSAGS, T.T.J.M. Berendschot, H.J.M. Beckers, N.C. Schaper, M.T. Schram, C.D.A. Stehouwer, C.A.B. Webers. Loss of temporal peripapillary retinal nerve fibers in prediabetes or type 2 diabetes without diabetic retinopathy: The Maastricht Study. Investigative Ophthalmology and Visual Science. 2017;58:1017-27.
6. Metea MR and Newman EA. Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. J Neurosci. 2006;26:2862-70.
7. Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X and Nedergaard M. Astrocyte-mediated control of cerebral blood flow. Nat Neurosci. 2006;9:260-7.
8. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CD. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. Circulation. 2016;134:1339-52.
9. O'Rourke MF and Hashimoto J. Arterial stiffness: a modifiable cardiovascular risk factor? J Cardiopulm Rehabil Prev. 2008;28:225-37.
10. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H and European Network for Non-invasive Investigation of Large A. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27:2588-605.
11. Cheung N, Sharrett AR, Klein R, Criqui MH, Islam FM, Macura KJ, Cotch MF, Klein BE and Wong TY. Aortic distensibility and retinal arteriolar narrowing: the multi-ethnic study of atherosclerosis. Hypertension. 2007;50:617-22.
12. Lin F, Zhu, P., Huang, F., Li, Q., Yuan, Y., Gao, Z., et al. . Aortic stiffness is associated with the central retinal arteriolar equivalent and retinal vascular fractal dimension in a population along the southeastern coast of China. . Hypertension Research. 2015; 38:342.
13. Meyer ML, Klein BE, Klein R, Palta P, Sharrett AR, Heiss G, Nambi V, Wong TY and Tanaka H. Central arterial stiffness and retinal vessel calibers: the Atherosclerosis Risk in Communities Study-Neurocognitive Study. J Hypertens. 2020;38:266-73.
14. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, Strachan DP and Rudnicka AR. Associations of Retinal Microvascular Diameters and Tortuosity With Blood Pressure and Arterial Stiffness: United Kingdom Biobank. Hypertension. 2019;74:1383-90.
15. Wei F, Lutgarde T., Yu CC., Melgarejo JD. Zhang ZY., Maestre GE., Struijker-Boudier HAJ., Verhamme P., Staessen, J. . Retinal Microvasculature in relation to central hemodynamics in a Flemish population. Hypertension. 2019;74.
16. Liao D, Wong TY, Klein R, Jones D, Hubbard L and Sharrett AR. Relationship between carotid artery stiffness and retinal arteriolar narrowing in healthy middle-aged persons. Stroke. 2004;35:837-42.
17. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
18. Hermeling E RK, Kornmann LM, Reneman RS, Hoeks AP. The dicrotic notch as alternative time-reference point to measure local pulse wave velocity in the carotid artery by means of ultrasonography. J Hypertens. 2009;27:2028–35.
19. Willekes C, Hoeks AP, Bots ML, Brands PJ, Willigers JM and Reneman RS. Evaluation of off-line automated intima-media thickness detection of the common carotid artery based on M-line signal processing. Ultrasound Med Biol. 1999;25:57-64.
20. Reneman RS, Meinders JM and Hoeks AP. Non-invasive ultrasound in arterial wall dynamics in humans: what have we learned and what remains to be solved. Eur Heart J. 2005;26:960-6.
21. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T,

Artery S, European Society of Hypertension Working Group on Vascular S, Function and European Network for Noninvasive Investigation of Large A. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30:445-8.
22. Garhofer G, Resch H, Sacu S, Weigert G, Schmidl D, Lasta M and Schmetterer L. Effect of regular smoking on flicker induced retinal vasodilatation in healthy subjects. Microvasc Res. 2011;82:351-5.
23. Li W, Schram MT, Sorensen BM, Agtmaal MJM, Berendschot T, Webers CAB, Jansen JFA, Backes WH, Gronenschild E, Schalkwijk CG, Stehouwer CDA and Houben A. Microvascular Phenotyping in The Maastricht Study: Design, and Main findings, 2010-2018. Am J Epidemiol. 2020.
24. Bekkers E DR, Berendschot T, Romeny BT. . A Multi-Orientation Analysis Approach to Retinal Vessel Tracking. . J Math Imaging Vis 2014;49:583-610.
25. ter Haar Romeny BM BE, Zhang J et al. Braininspired algorithms for retinal image analysis. Mach Vis Appl. 2016;27:1117-35.
26. Jonas JB GG, Naumann GO. Optic disc, cup andneuroretinal rim size, configuration and correlations in normal eyes. Invest Ophthalmol Vis Sci. 1989;29:1151-8.
27. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R and Klein BE. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res. 2003;27:143-9.
28. Nagel E and Vilser W. Flicker observation light induces diameter response in retinal arterioles: a clinical methodological study. Br J Ophthalmol. 2004;88:54-6.
29. Nagel E, Vilser W, Fink A and Riemer T. [Variance of retinal vessel diameter response to flicker light. A methodical clinical study]. Ophthalmologe. 2006;103:114-9.
30. Martens RJH, Houben A, Kooman JP, Berendschot T, Dagnelie PC, van der Kallen CJH, Kroon AA, Leunissen KML, van der Sande FM, Schaper NC, Schouten J, Schram MT, Sep SJS, Sorensen BM, Henry RMA and Stehouwer CDA. Microvascular endothelial dysfunction is associated with albuminuria: the Maastricht Study. J Hypertens. 2018;36:1178-87.
31. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, Schaper NC, van der Kallen CJ, Koster A, Sep SJ, Denollet J, Verhey FR and Pouwer F. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-6.
32. Sorensen BM, van der Heide FCT, Houben A, Koster A, T TJMB, J SAGS, Kroon AA, van der Kallen CJH, Henry RMA, van Dongen M, S JPME, H HCMS, van der Berg JD, Schaper NC, Schram MT and Stehouwer CDA. Higher levels of daily physical activity are associated with better skin microvascular function in type 2 diabetes-The Maastricht Study. Microcirculation. 2020;27:e12611.
33. van Dongen MC, Wijckmans-Duysens NEG, den Biggelaar LJ, Ocke MC, Meijboom S, Brants HA, de Vries JH, Feskens EJ, Bueno-de-Mesquita HB, Geelen A, Stehouwer CD, Dagnelie PC and Eussen SJ. The Maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the Maastricht study. Nutrition. 2019;62:39-46.
34. Looman M, Feskens EJ, de Rijk M, Meijboom S, Biesbroek S, Temme EH, de Vries J and Geelen A. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20: 2289-99.
35. Qi Y, Koster A, van Boxtel M, Kohler S, Schram M, Schaper N, Stehouwer C and Bosma H. Adulthood Socioeconomic Position and Type 2 Diabetes Mellitus-A Comparison of Education, Occupation, Income, and Material Deprivation: The Maastricht Study. Int J Environ Res Public Health. 2019;16.
36. Newman A, Andrew N and Casson R. Review of the association between retinal microvascular characteristics and eye disease. Clin Exp Ophthalmol. 2018;46:531-52.
37. Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B and Laurent S. Association between local pulse pressure, mean blood pressure, and large-artery remodeling. Circulation. 1999;100:1387-93.
38. Hutcheon JA, Chiolero A and Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.
39. Liang W, Zhao Y and Lee AH. An investigation of the significance of residual confounding effect. Biomed Res Int. 2014;2014:658056.
40. Li X, Wong WL, Cheung CY, Cheng CY, Ikram MK, Li J, Chia KS and Wong TY. Racial differences in retinal vessel geometric characteristics: a multiethnic study in healthy Asians. Invest Ophthalmol Vis Sci. 2013;54:3650-6.

Supplemental methods

Clinometric properties

Arterial stiffness

Reproducibility was assessed in 12 individuals (six men; 60.8±6.8 years; six with type 2 diabetes) who were examined by two observers at two occasions spaced one week apart. The intra- and interobserver intraclass correlation coefficients were 0.87 and 0.69 for cfPWV; 0.85 and 0.73 for carotid DC; and 0.72 and 0.71 for carotid YEM.

Retinal microvascular flicker light-induced dilation

Retinal microvascular flicker light-induced dilation measurements were performed by different observers, after an intensive training period. Interobserver reliability of the retinal baseline diameter and percentage dilation response between two randomly selected observers (N=9 participants) were, respectively, 0.980 and 0.796 for arteriolar vessels and 0.972 and 0.871 for venular vessels. Retinal arteriolar and venular dilation response curves were analysed by one observer for measurement quality decisions. Retinal response curves with insufficient measurement quality, e.g. insufficient measurement points or movement artifacts were evaluated and discussed with a second observer, and excluded on mutual agreement. To assess the interobserver reliability of retinal response curves quality decisions 50 curves were evaluated by two observers (Inter-observer reliability =0.883).

Table S7.1 General study population characteristics according to tertiles of venular flicker light-induced dilation.

Characteristic	Total study group (N=1,897)	Tertiles of venular flicker light-induced dilation		
		Tertile 1 (low) (n=632)	Tertile 2 (middle) (n=630)	Tertile 3 (high) (n=635)
Demographics				
Age, years	59.9 ± 8.2	60.9 ± 8.1	59.4 ± 7.9	59.3 ± 8.4
Men	961 (50.7%)	354 (56.0%)	317 (50.3%)	290 (45.7%)
Educational status				
Low	611 (32.2%)	213 (33.7%)	199 (31.6%)	199 (31.3%)
Medium	555 (29.3%)	174 (27.5%)	195 (31.0%)	186 (29.3%)
High	731 (38.5%)	245 (38.8%)	236 (37.5%)	250 (39.4%)
Occupational status *				
Low	502 (31.8%)	155 (29.9%)	187 (35.2%)	160 (30.4%)
Middle	564 (35.8%)	192 (37.0%)	185 (34.8%)	187 (35.5%)
High	511 (32.4%)	172 (33.1%)	159 (29.9%)	180 (34.2%)
Income per month, euros *	2041 ± 808	2030 ± 779	2003 ± 787	2091 ± 856
Lifestyle factors				
Smoking status				
Never	648 (34.2%)	194 (30.7%)	207 (32.9%)	247 (38.9%)
Former	1015 (53.5%)	360 (57.0%)	348 (55.2%)	307 (48.3%)
Current	234 (12.3%)	78 (12.3%)	75 (11.9%)	81 (12.8%)
Alcohol consumption				
None	339 (17.9%)	111 (17.6%)	108 (17.1%)	120 (18.9%)
Low (women ≤7, men ≤14)	1077 (56.8%)	364 (57.6%)	363 (57.6%)	350 (55.1%)
High (women >7, men >14)	481 (25.4%)	157 (24.8%)	159 (25.2%)	165 (26.0%)
Physical activity, hours/day *	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.7
Dutch Healthy diet score, points*	83.9 ± 14.6	83.9 ± 14.5	82.7 ± 14.3	85.1 ± 14.8
Cardiovascular risk factors				
Glucose metabolism status				
Normal glucose metabolism	1072 (56.5%)	324 (51.3%)	371 (58.9%)	377 (59.4%)
Prediabetes	274 (14.4%)	92 (14.6%)	80 (12.7%)	102 (16.1%)
Type 2 diabetes	526 (27.7%)	201 (31.8%)	172 (27.3%)	153 (24.1%)
Other types of diabetes	25 (1.3%)	15 (2.4%)	7 (1.1%)	3 (0.5%)

Table S7.1 (continued)

Characteristic	Total study group (N=1,897)	Tertiles of venular flicker light-induced dilation		
		Tertile 1 (low) (n=632)	Tertile 2 (middle) (n=630)	Tertile 3 (high) (n=635)
Fasting plasma glucose, mmol/l	5.5 [5.0 – 6.5]	5.7 [5.1 – 8.8]	5.5 [5.0 – 6.5]	5.4 [5.0 – 6.2]
2-hour post load plasma glucose, mmol/l*	6.2 [5.1 – 9.4]	6.3 [5.1 – 9.9]	6.2 [5.1 – 9.4]	6.2 [5.1 – 8.8]
HbA1c, %*	5.7 [5.4 – 6.2]	5.7 [5.4 – 6.4]	5.7 [5.3 – 6.2]	5.6 [5.3 – 6.1]
HbA1c, mmol/mol*	39 [36 – 44]	39 [36 – 46]	39 [34 – 44]	38 [34 – 43]
Glucose-lowering medication	434 (22.9%)	182 (28.8%)	139 (22.1%)	113 (17.8%)
Waist circumference, cm	95.4 ± 13.6	96.5 ± 13.8	95.3 ± 13.4	94.3 ± 13.7
Body mass index, kg/m^2 *	26.9 ± 4.5	27.1 ± 4.4	27.1 ± 4.5	26.6 ± 4.5
History of cardiovascular disease*	294 (15.6%)	112 (17.9%)	112 (17.9%)	70 (11.0%)
eGFR, ml/min/1.73m^2*	88.2 ± 14.7	87.9 ± 15.5	88.1 ± 14.1	88.5 ± 14.5
Albuminuria, mg/24h*	6.5 [3.9 – 11.6]	6.8 [4.0 – 12.6]	6.6 [3.8 – 11.9]	6.3 [4.0 – 11.2]
Total-to-HDL cholesterol ratio	3.6 ± 1.2	3.6 ± 1.1	3.7 ± 1.1	3.6 ± 1.2
Triglycerides, mmol/l	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]	1.2 [0.9 – 1.7]
Use of lipid-modifying medication	702 (37.0%)	264 (41.8%)	245 (38.9%)	193 (30.4%)
Office systolic BP, mmHg *	135.1 ± 18.1	134.7 ± 18.9	135.8 ± 18.0	134.7 ± 17.5
Office diastolic BP, mmHg *	76.3 ± 10.0	74.9 ± 10.1	77.4 ± 9.7	76.7 ± 10.1
Mean arterial pressure, mmHg	96.7 ± 10.3	96.0 ±10.1	97.3 ± 10.5	96.7 ± 10.4
24-hour mean arterial pressure, mm Hg*	88.6 ± 7.9	88.2 ±7.8	89.2 ± 7.7	88.4 ± 8.3
Heart rate, bpm	62.5 ± 9.3	62.2 ± 9.2	62.4 ± 9.5	62.9 ± 9.2
Use of antihypertensive medication	733 (38.6%)	283 (44.8%)	243 (38.6%)	207 (32.6%)
Inflammation markers*				
C-reactive protein, µg/ml	1.3 [0.6 – 2.7]	1.2 [0.6 – 2.6]	1.2 [0.6 – 2.8]	1.3 [0.6 – 2.8]
Serum amyloid A, µg/ml	3.4 [2.1 – 5.5]	3.4 [2.0 – 5.5]	3.4 [2.2 – 5.5]	3.3 [2.1 – 5.6]
Tumor necrosis factor α, pg/ml	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.5]
Interleukin-6, pg/ml	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.8]
Interleukin-8, pg/ml	4.1 [3.3 – 5.2]	4.3 [3.4 – 5.4]	4.0 [3.2 – 5.2]	4.1 [3.2 – 5.1]
Eye variables				
Retinopathy*	31 (1.7%)	21 (3.4%)	4 (0.7%)	6 (1.0%)
Glaucoma*	81 (4.7%)	31 (5.3%)	28 (5.0%)	22 (3.8%)

Table S7.1 (continued)

Characteristic	Total study group (N=1,897)	Tertiles of venular flicker light-induced dilation		
		Tertile 1 (low) (n=632)	Tertile 2 (middle) (n=630)	Tertile 3 (high) (n=635)
Refractive error*				
Right eye	0.1 [-0.4 – 1.1]	0.3 [-1.1 – 1.3]	0.1 [-1.5 – 1.2]	-0.1 [-1.5 – 0.9]
Left eye	0.1 [-0.4 – 1.1]	0.3 [-1.0 – 1.1]	0.1 [-1.5 – 1.1]	0.00 [-1.6 – 0.9]
Arterial stiffness				
cfPWV, m/s †	9.0 ± 2.1	9.1 ± 2.3	8.9 ± 2.0	9.0 ± 2.0
Carotid DC, 10^{-3}/kPa	14.4 ± 5.1	14.3 ± 5.1	14.3 ± 5.0	14.6 ± 5.3
Carotid YEM, 10^{3}/kPa*	0.74 ± 0.37	0.75 ± 0.33	0.75 ± 0.44	0.73 ± 0.34
Retinal microvascular function				
Arteriolar flicker light-induced dilation, %	3.0 ± 2.8	2.1 ± 2.5	3.0 ± 2.7	4.0 ± 2.9
Venular flicker light-induced dilation, %	3.9 ± 2.2	1.7. ± 0.8	3.6 ± 0.5	6.3 ± 1.7

Data presented as mean ± standard deviation, median [interquartile range] or number (%). * data available for occupational status, n=1,577; income level, n=1,455; body mass index, n=1,896; physical activity, n=1,618; Dutch healthy diet score, n=1,805; 2-hour post load plasma glucose, n=1,753; HbA1c, n=1,894; history of cardiovascular disease, n=1,882; eGFR, n=1,885; albuminuria, n=1,884; office systolic and diastolic blood pressure, n=1,895; 24-hour mean arterial pressure, n=1,694; inflammation markers, n=1,882; retinopathy, n=1,842; glaucoma, n=1,722; refractive error right eye, n=1,745, refractive error left eye n=, n=1,737; carotid YEM, n=1,896. † value shown for cfPWV study population with complete retinal venular flicker light-induced dilation data. Abbreviations: BP, blood pressure; carotid DC, carotid distensibility coefficient; carotid YEM, carotid Young elastic modulus; cfPWV, carotid -femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arterio-to-venule ratio; eGFR; estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high density lipoprotein.

Table S7.2 Characteristics of in- and excluded participants of the retinal microvascular diameter and retinal venular flicker light-induced dilation study populations.

	Retinal microvascular diameter			Retinal venular flicker light-induced dilation		
Characteristic	**Characteristics of the included study population N=2,434**	**Missing data in-/excluded study population**	**Characteristics of the excluded study population N=1017**	**Characteristics of the included study population N=1897**	**Missing data in-/excluded study population**	**Characteristics of the excluded study population N=1554**
Demographics						
Age, years	59.8 ± 8.2	0/0	59.6 ± 8.5	59.9 ± 8.2	0/0	59.7 ± 8.4
Men	1252 (51.4%)	0/0	523 (51.4%)	961 (50.7%)	0/0	814 (52.4%)
Educational status						
Low	801 (32.9%)	0/74	334 (35.4%)	611 (32.2%)	0/74	524 (35.4%)
Medium	707 (29.0%)		247 (26.2%)	555 (29.3%)		399 (27.0%)
High	926 (38.0%)		362 (38.4%)	731 (38.5%)		557 (37.6%)
Occupational status						
Low	654 (32.2%)	406/221	239 (30.0%)	502 (31.8%)	320/307	391 (31.4%)
Middle	701 (34.6%)		294 (36.9%)	564 (35.8%)		431 (34.6%)
High	673 (33.2%)		263 (33.0%)	511 (32.4%)		425 (34.1%)
Income per month, euros	2016.8 ± 814	593/286	2016 ± 834	2041 ± 808	442/437	1985 ± 834
Lifestyle factors						
Smoking status						
Never	817 (33.6%)	0/50	355 (36.7%)	648 (34.2%)	0/50	524 (34.8%)
Former	1302 (53.5%)		457 (47.3%)	1015 (53.5%)		744 (49.5%)
Current	315 (12.9%)		155 (16.0%)	234 (12.3%)		236 (15.7%)
Alcohol consumption						
None	440 (18.1%)	0/48	196 (20.2%)	339 (17.9%)	0/48	297 (19.7%)
Low (women ≤7, men ≤14)	1368 (56.2%)		520 (53.7%)	1077 (56.8%)		811 (53.9%)
High (women >7, men >14)	626 (25.7%)		253 (26.1%)	481 (25.4%)		398 (26.4%)
Waist circumference, cm	95.7 ± 13.6	0/4	96.6 ± 14.2	95.4 ± 13.6	0/4	96.6 ± 14.0
Dutch Healthy diet score, points	83.6 ± 14.6	120/106	82.3 ± 15.0	83.9 ± 14.6	92/134	82.4 ± 14.9

Table S7.2 (continued)

Characteristic	Retinal microvascular diameter			Retinal venular flicker light-induced dilation		
	Characteristics of the included study population N=2,434	Missing data in-/excluded study population	Characteristics of the excluded study population N=1017	Characteristics of the included study population N=1897	Missing data in-/excluded study population	Characteristics of the excluded study population N=1554
Cardiovascular risk factors						
Glucose metabolism status						
Normal glucose metabolism	1364 (56.0%)	0/0	560 (57.3%)	1072 (56.5%)	0/0	852 (54.8%)
Prediabetes	357 (14.7%)		154 (14.6%)	274 (14.4%)		237 (15.3%)
Type 2 diabetes	684 (28.1%)		291 (28.1%)	526 (27.7%)		449 (28.9%)
Other types of diabetes	29 (1.2%)		12 (28.1%)	25 (1.3%)		16 (1.0%)
Fasting plasma glucose, mmol/l	5.6 [5.1 – 6.5]	1/0	5.6 [5.1 – 6.6]	5.5 [5.0 – 6.5]	0/1	5.6 [5.1 – 6.6]
2-hour post load plasma glucose, mmol/l	6.2 [5.1 – 9.4]	185/107	6.4 [5.1 – 8.9]	6.2 [5.1 – 9.4]	144/148	6.3 [5.0 – 9.3]
HbA1c, %	5.7 [5.4 – 6.2]	5/8	5.7 [5.4 – 6.3]	5.7 [5.4 – 6.2]	3/10	5.7 [5.4 –6.3]
HbA1c, mmol/mol	39 [36 – 44]	5/8	39 [36 – 45]	39 [36 – 44]	3/10	39 [36 – 45]
Glucose lowering medication	568 (23.3%)	0/0	239 (23.5%)	434 (22.9%)	0/0	373 (24.0%)
Waist circumference, cm	27.0 ± 4.5	1/2	27.3 ± 4.8	26.9 ± 4.5	1/2	27.3 ± 4.7
Body mass index, kg/m^2	2.0 ± 0.7	488/321	2.0 ± 0.7	2.0 ± 0.7	279/530	2.0 ± 0.7
History of cardiovascular disease	405 (16.7%)	16/51	163 (16.9%)	294 (15.6%)	15/52	274 (18.2%)
eGFR, ml/min/1.73m^2	88.3 ± 14.8	18/15	87.7 ± 15.3	88.2 ± 14.7	12/21	88.0 ± 15.3
Albuminuria, mg/24h	6.6 [4.0 – 11.8]	19/23	7.1 [4.3 – 12.6]	6.5 [3.9 – 11.6]	13/29	7.1 [4.3 – 12.5]
Total-to-HDL cholesterol ratio	3.6 ± 1.2	0/4	3.8 ± 1.2	3.6 ± 1.2	0/4	3.7 ± 1.2
Triglycerides, mmol/l	1.2 [0.9 – 1.7]	0/4	1.2 [0.9 – 1.8]	1.2 [0.9 – 1.7]	0/4	1.2 [0.9 – 1.7]
Use of lipid-modifying medication	910 (37.4%)	0/0	349 (34.3%)	702 (37.0%)	0/0	557 (35.8%)
Office systolic BP, mmHg	134.1 ± 18.2	2/0	135.6 ± 18.2	135.1 ± 18.1	2/0	135.1 ± 18.3
Office diastolic BP, mmHg	76.2 ± 10.0	2/0	76.2 ± 9.6	76.3 ± 10.0	2/0	75.9 ± 9.7
Mean arterial pressure, mmHg	96.5 ± 10.2	0/456	98.0 ± 10.8	96.7 ± 10.3	0/456	97.0 ± 10.3
24-hour mean arterial pressure, mm Hg*	88.6 ± 8.0	265/139	89.0 ± 8.0	88.6 ± 7.9	203/201	88.8 ± 8.1
Heart rate, bpm	62.6 ± 9.4	0/455	63.6 ± 9.3	62.5 ± 9.3	0/455	63.3 ± 9.5
Use of antihypertensive medication	974 (40.0%)	0/0	418 (41.1%)	733 (38.6%)	0/0	659 (42.4%)

Table S7.2 (continued)

Characteristic	Retinal microvascular diameter			Retinal venular flicker light-induced dilation		
	Characteristics of the included study population N=2,434	Missing data in-/excluded study population	Characteristics of the excluded study population N=1017	Characteristics of the included study population N=1897	Missing data in-/excluded study population	Characteristics of the excluded study population N=1554
Inflammation markers						
C-reactive protein, µg/ml	1.3 [0.6 – 2.7]	22/16	1.2 [0.6 – 3.0]	1.3 [0.6 – 2.7]	14/24	1.2 [0.6 – 2.9]
Serum amyloid A, µg/ml	3.3 [2.1 – 5.4]	22/16	3.2 [2.0 – 5.5]	3.4 [2.1 – 5.5]	14/24	3.2 [2.0 – 5.4]
Tumor necrosis factor α, pg/ml	2.2 [1.9 – 2.6]	22/16	2.2 [1.9 – 2.6]	2.2 [1.9 – 2.6]	14/24	2.2 [1.9 – 2.6]
Interleukin-6, pg/ml	0.6 [0.4 – 0.9]	23/16	0.6 [0.4 – 0.9]	0.6 [0.4 – 0.9]	15/24	0.6 [0.4 – 0.9]
Interleukin-8, pg/ml	4.1 [3.3 – 5.3]	22/16	4.2 [3.3 – 5.5]	4.1 [3.3 – 5.2]	14/24	4.2 [3.3 – 5.5]
Eye variables						
Retinopathy	38 (1.7%)	180/414	8 (1.3%)	31 (1.7%)	55/539	15 (1.5%)
Glaucoma	114 (5.2%)	223/216	44 (5.6%)	81 (4.7%)	175/274	77 (6.0%)
Refractive error						
Right eye	0.1 [-1.5 – 1.1]	197/216	0.0 [-1.5 – 1.1]	0.1 [-0.4 – 1.1]	152/261	0.1 [-1.6 – 1.1]
Left eye	0.1 [-1.4 – 1.1]	205/220	0.0 [-1.6 – 1.1]	0.1 [-0.4 – 1.1]	160/165	0.0 [-1.6 – 1.1]
Arterial stiffness						
cfPWV, m/s *	9.0 ± 2.1	0/564	9.2 ± 2.3	9.0 ± 2.1	0/564	9.1 ± 2.3
Carotid DC, 10^{-3}/kPa	14.5 ± 5.12	0/521	13.5 ± 4.9	14.4 ± 5.1	0/521	14.1 ± 5.1
Carotid YEM, 10^{3}/kPa	0.73 ± 0.34	2/521	0.83 ± 0.56	0.74 ± 0.37	1/522	0.78 ± 0.38
Retinal microvascular function						
Arteriolar diameter (CRAE), µm	142.2 ± 20.4	0/493	142.0 ± 20.8	NA	NA	NA
Venular diameter (CRVE), µm	214.4 ± 31.1	0/493	215.0 ±20.8	NA	NA	NA
Arteriole-to-venule ratio (AVR), no unit	0.67 ± 0.08	0/493	0.67 ± 0.08	NA	NA	NA
Arteriolar flicker light-induced dilation, % †	NA	NA	NA	3.0 ± 2.8	0/1161	2.9 ± 2.6
Venular flicker light-induced dilation, %	NA	NA	NA	3.9 ± 2.2	0/1120	3.8 ± 2.2

Data presented as mean ± standard deviation, median [interquartile range] or number (%). * value shown for cfPWV study population with complete retinal arteriolar diameter or retinal venular flicker light-induced dilation data. † value shown for carotid DC study population with complete retinal venular flicker light-induced dilation data. Abbreviations: BP, blood pressure; carotid DC, carotid distensibility coefficient; carotid YEM, carotid Young elastic modulus; cfPWV, carotid -femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arterio-to-venule ratio; eGFR; estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; NA, not applicable.

Table S7.3 P values of interaction terms with sex, type 2 diabetes status, fasting plasma glucose, 2-hour post load glucose and HbA1c.

	Retinal microvascular diameter			Retinal microvascular flicker light-induced dilation	
	CRAE	**CRVE**	**AVR**	**Arteriolar dilation**	**Venular dilation**
Arterial stiffness	**P-value**	**P-value**	**P-value**	**P-value**	**P-value**
Sex					
Carotid DC	0.64	0.17	0.25	0.15	0.02
Carotid YEM	0.28	0.20	0.83	0.12	0.02
cfPWV, per SD	0.24	0.15	0.60	0.78	0.23
Type 2 diabetes status					
Carotid DC	0.26	0.97	0.17	0.02	0.01
Carotid YEM	0.40	0.55	0.77	0.63	0.49
cfPWV, per SD	0.63	0.63	0.33	0.14	0.44
Continuous measures of hyperglycaemia: fasting plasma glucose/ 2-hour post load plasma glucose/ HbA1c					
Carotid DC	NA	NA	NA	0.83 /0.05/ 0.27	0.17/ 0.03/ 0.07
Carotid YEM	NA	NA	NA	NA	NA
cfPWV, per SD	NA	NA	NA	NA	NA

P-values indicate P-value of the interaction term (e.g. sex*[carotid DC]). The following variables in the model were included in the model (in addition to measure of arterial stiffness [either carotid DC or carotid YEM or cfPWV] and interaction term): age, sex, glucose metabolism status (entered as dummies [i.e. type 2 diabetes, prediabetes, other types of diabetes versus normal glucose metabolism status]), mean arterial pressure, heart rate, smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, educational status. For tests of interaction by type 2 diabetes the variable glucose metabolism status was replaced by type 2 diabetes status (versus without diabetes) and for tests of interaction by continuous measures of hyperglycaemia (only investigated if type 2 diabetes status modified the association) glucose metabolism status was not entered in the model. Bold denotes P value<0.10. Abbreviations: HbA1c, glycated haemoglobin; cfPWV, carotid-to-femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriole-to-venule-ratio; DC, distensibility coefficient; YEM, Young's elastic modulus; NA, not applicable.

Table S7.4 Associations of carotid distensibility coefficient with retinal microvascular flicker light-induced dilation stratified by type 2 diabetes status, level of 2-hour post load glucose (≥11.1 mmol/l versus <11.1 mmol/l) and level of HbA1c (≥6.5% versus <6.5%).

		Type 2 diabetes		Without diabetes	
		n=516	n=526	n=1321	n=1346
Arterial stiffness	Model	Arteriolar dilation Stβ (95%CI)	Venular dilation Stβ (95%CI)	Arteriolar dilation Stβ (95%CI)	Venular dilation Stβ (95%CI)
Carotid DC, per SD	1	0.15 (0.07; 0.24)	0.12 (0.03; 0.20)	0.04 (-0.01; 0.10)	-0.01 (-0.07; 0.04)
	2	0.13 (0.04; 0.22)	0.12 (0.03; 0.21)	-0.01 (-0.07; 0.05)	-0.04 (-0.10. 0.02)
	3	0.14 (0.04; 0.24)	0.16 (0.06; 0.26)	0.03 (-0.04; 0.09)	-0.01 (-0.08; 0.01)
	4	0.14 (0.04; 0.25)	0.16 (0.06; 0.26)	0.03 (-0.04; 0.10)	-0.02 (-0.09; 0.05)
		2-hour post load glucose (≥11.1 mmol/l)		2-hour post load glucose (<11.1 mmol/l)	
		n=327	n=334	n=1394	n=1419
Carotid DC, per SD	1	0.19 (0.08; 0.30)	0.12 (0.01; 0.23)	0.07 (0.01; 0.14)	-0.00 (-0.06; 0.05)
	2	0.17 (0.06; 0.29)	0.16 (0.05; 0.27)	0.01 (-0.07; 0.09)	-0.04 (-0.10; 0.02)
	3	0.19 (0.06; 0.32)	0.21 (0.08; 0.34)	0.06 (-0.03; 0.15)	-0.01 (-0.07; 0.06)
	4	0.20 (0.06; 0.33)	0.21 (0.08; 0.34)	0.06 (-0.02; 0.15)	-0.01 (-0.08; 0.06)
		HbA1c (≥6.5% mmol/l)		HbA1c (<6.5% mmol/l)	
			n=821		n=932
Carotid DC, per SD	1	NA	0.02 (-0.05; 0.09)	NA	0.02 (-0.04; 0.09)
	2	NA	0.01 (-0.06; 0.08)	NA	0.01 (-0.07; 0.08)
	3	NA	0.03 (-0.06; 0.11)	NA	0.05 (-0.03; 0.14)
	4	NA	0.02 (-0.07; 0.10)	NA	0.05 (-0.03; 0.13)

Standardized regression coefficient (stβ) represents arteriolar or venular dilation in SD per SD greater arterial stiffness in individuals with type 2 diabetes or without diabetes (other types of diabetes were excluded from analyses), in individuals with higher and lower levels of 2-hour post load glucose (≥ 11.1 mmol/l and < 11.1 mmol/l) and in individuals with higher and lower levels of HbA1c. For the retinal arteriolar flicker light-induced dilation population 1 SD corresponds with 4.8 10^{-3}/kPa for carotid DC participants with type 2 diabetes and 5.2 10^{-3}/kPa for carotid DC in participants without diabetes, 2.7% for flicker light-induced arteriolar dilation in participants with type 2 diabetes and 2.9% in participants without diabetes. For the venular arteriolar flicker light-induced dilation population 1 SD corresponds with 4.8 10^{-3}/kPa for carotid DC participants with type 2 diabetes and 5.2 10^{-3}/kPa for carotid DC in participants without diabetes, 2.3% for flicker light-induced venular dilation in participants with type 2 diabetes and 2.2% in participants without diabetes. For individuals with higher and lower levels of 2-hour post load glucose and Hba1c, values of arterial stiffness, retinal microvascular diameters or microvascular flicker light-induced dilation per SD were quantitively similar to values per SD for individuals with type 2 diabetes and without diabetes, respectively. Bold denotes p<.05. Model 1: crude. Model 2: age, sex and prediabetes (prediabetes was only entered in non-diabetes stratified analyses); model 3: model 2 + mean arterial pressure and heart rate; model 4: model 2 + smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, educational status. Abbreviations: HbA1c, glycated haemoglobin; SD, standard deviation; CI, confidence interval; cfPWV, carotid-to-femoral pulse wave velocity; DC, distensibility coefficient; NA, not applicable; YEM, Young's elastic modulus.

Table S7.5 Associations of carotid stiffness with retinal venular flicker light-induced dilation stratified by sex.

		Men, n=961	Women, n=936
Arterial stiffness	Model	Venular dilation Stβ (95%CI)	Venular dilation Stβ (95%CI)
Carotid DC, per SD	1	0.09 (0.02; 0.15)	-0.02 (-0.08; 0.05)
	2	0.05 (-0.02; 0.12)	-0.02 (-0.09; 0.06)
	3	0.11 (0.03; 0.19)	-0.01 (-0.10; 0.07)
	4	0.11 (0.03; 0.19)	-0.03 (-0.11; 0.06)
Carotid YEM, per SD	1	-0.07 (-0.13; -0.00)	0.04 (-0.03; 0.10)
	2	-0.04 (-0.10; 0.03)	0.04 (-0.03; 0.11)
	3	-0.09 (-0.16; -0.02)	0.04 (-0.03; 0.11)
	4	-0.08 (-0.16; -0.01)	0.05 (-0.03; 0.12)

Standardized regression coefficient (stβ) represents arteriolar or venular dilation in SD per SD greater arterial stiffness for men or women. For the retinal microvascular flicker light-induced dilation population 1 SD corresponds with 4.91 10^{-3}/kPa for carotid DC in men and 5.35 10^{-3}/kPa in women, 0.40 10^{3}/kPa for carotid YEM in men and 0.34 10^{3}/kPa in women, 3.72% for retinal flicker light-induced venular dilation in the men and 2.21% in women. Bold denotes p<.05. Model 1: crude. Model 2: age, glucose metabolism status; model 3: model 2 + mean arterial pressure and heart rate; model 4: model 2 + smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, educational status. Abbreviations: SD, standard deviation; CI, confidence interval; cfPWV, carotid-to-femoral pulse wave velocity; DC, distensibility coefficient; YEM, Young's elastic modulus.

Table S7.6 Associations of aortic and carotid stiffness with retinal microvascular diameter and retinal microvascular flicker light-induced dilation additionally adjusted for diet score, physical activity, refractive error, kidney variables, prior cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy, and glaucoma.

		Retinal microvascular diameter			Retinal microvascular flicker light-induced dilation	
		CRAE	CRVE	AVR	Arteriolar dilation	Venular dilation
Arterial stiffness	Model	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)
Carotid DC, per SD	4 + diet*	-0.01 (-0.06; 0.05)	0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.05)	0.05 (-0.01;0.11)	0.04 (-0.02; 0.09)
	4 + physical activity	-0.01 (-0.06; 0.05)	-0.02 (-0.07; 0.04)	0.01 (-0.05; 0.06)	0.07 (0.01; 0.13)	0.04 (-0.02; 0.10)
	4 + refractive error	-0.00 (-0.05; 0.05)	-0.01 (-0.07; 0.04)	0.02 (-0.04; 0.07)	0.05 (-0.01; 0.11)	0.05 (-0.01; 0.11)
	4 + kidney variables	-0.01 (-0.06; 0.04)	-0.01 (-0.06; 0.04)	0.00 (-0.05; 0.05)	0.06 (0.00; 0.12)	0.04 (-0.02; 0.10)
	4 + prior CVD	-0.01 (-0.06; 0.04)	-0.01 (-0.06; 0.04)	0.00 (-0.05; 0.05)	0.05 (-0.00; 0.11)	0.04 (-0.15; 0.10)
	4 + biomarkers of LGI	-0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.04)	0.01 (-0.04; 0.06)	0.06 (0.00; 0.12)	0.04 (-0.02; 0.10)
	4 + retinopathy	0.01 (-0.04; 0.06)	0.01 (-0.05; 0.06)	0.00 (-0.05; 0.06)	0.05 (-0.01; 0.11)	0.03 (-003; 0.09)
	4 + glaucoma	-0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.05)	0.01 (-0.04; 0.06)	0.04 (-0.02; 0.10)	0.04 (-0.02; 0.10)
Carotid YEM, per SD	4 + diet*	0.03 (-0.02; 0.07)	0.03 (-0.02; 0.07)	0.00 (-0.04; 0.05)	0.01 (-0.05; 0.06)	-0.01 (-0.07; 0.04)
	4 + physical activity	0.03 (-0.02; 0.08)	0.03 (-0.03; 0.08)	0.01 (-0.04; 0.06)	-0.02 (-0.07; 0.04)	-0.02 (-0.07; 0.04)
	4 + refractive error	0.02 (-0.02; 0.07)	0.03 (-0.02; 0.08)	-0.01 (-0.05; 0.04)	0.00 (-0.05; 0.06)	-0.03 (-0.08; 0.03)
	4 + kidney variables	0.03 (-0.02; 0.07)	0.03 (-0.02; 0.07)	0.00 (-0.04; 0.05)	-0.01 (-0.06; 0.05)	-0.02 (-0.07; 0.03)
	4 + prior CVD	0.02 (-0.02; 0.07)	0.02 (-0.02; 0.07)	0.01 (-0.04; 0.05)	-0.00 (-0.05; 0.05)	-0.02 (-0.07; 0.03)
	4 + biomarkers of LGI	0.02 (-0.02; 0.07)	0.02 (-0.02; 0.07)	0.00 (-0.04; 0.05)	-0.01 (-0.06; 0.05)	-0.02 (-0.07; 0.03)
	4 + retinopathy	0.01 (-0.03; 0.06)	0.01 (-0.04; 0.06)	0.01 (-0.04; 0.05)	-0.00 (-0.05; 0.05)	-0.02 (-0.07; 0.04)
	4 + glaucoma	0.03 (-0.02; 0.08)	0.03 (-0.01; 0.08)	-0.00 (-0.05; 0.05)	0.01 (-0.05; 0.06)	-0.02 (-0.08; 0.03)
cfPWV, per SD	4 + diet*	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.03 (-0.08; .03)
	4 + physical activity	0.02 (-0.03; 0.08)	-0.00 (-0.06; 0.05)	0.04 (-0.02; 0.09)	-0.02 (-0.08; 0.04)	-0.05 (-0.11; 0.02)
	4 + refractive error	0.02 (-0.03; 0.07)	-0.01 (-0.06; 0.04)	0.05 (-0.01 0.10)	-0.01 (-0.06; 0.06)	-0.01 (-0.07; 0.05)
	4 + kidney variables	0.03 (-0.02; 0.08)	-0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.02 (-0.08; 0.04)
	4 + prior CVD	0.03 (-0.02; 0.08)	-0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.02 (-0.07; 0.04)
	4 + biomarkers of LGI	0.04 (-0.01; 0.09)	0.00 (-0.05; 0.06)	0.04 (-0.01; 0.09)	-0.02 (-0.07; 0.04)	-0.02 (-0.08; 0.03)
	4 + retinopathy	0.04 (-0.01; 0.09)	0.00 (-0.05; 0.06)	0.04 (-0.01; 0.10)	-0.00 (-0.06; 0.06)	-0.02 (-0.08; 0.04)
	4 + glaucoma	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.05)	0.04 (-0.02; 0.09)	-0.01 (-0.07; 0.05)	-0.01 (-0.07; 0.05)

Standardized regression coefficient (stβ) represents retinal microvascular diameter or retinal microvascular dilation in SD per SD greater arterial stiffness where 1 SD (approximately) corresponds with 5.16 10^{-3}/kPa for carotid DC, 0.34 10^{3}/kPa for carotid YEM, 2.08 m/s for cfPWV, 20.39 µm for CRAE, 31.07 µm for CRVE and 0.08 (no unit) for AVR, 2.82% for flicker light-induced arteriolar dilation and 2.20% for flicker light-induced venular dilation (corresponding arterial stiffness and

retinal microvascular diameters and microvascular flicker light-induced dilation differ slightly per study population). For retinal microvascular diameters the size of the study population with full data on carotid DC and potential confounders was n=2316 for diet, n=1946 for physical activity, n=2247 for refractive error, n=2397 for kidney variables, n=2381 for prior CVD, n=2411 for biomarkers of LGI, n=2254 for retinopathy, and n=2211 for glaucoma. For retinal venular flicker light-induced dilation the size of the study population the size of the study population with full data on carotid DC and confounders was n=1807 for diet, n=1618 for physical activity 6, n=1753 for refractive error, n=1872 for kidney variables, n=1882 for prior CVD, n=1882 for biomarkers of LGI, n=1842 for retinopathy, and n=1722 for glaucoma. Bold denotes $p<.05$. Variables in model 4 are: age, sex, glucose metabolism status, mean arterial pressure, heart rate, smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, and educational status. *As alcohol consumption constitutes one of the items of the diet score we excluded alcohol consumption from model 5. Biomarkers of LGI are high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8 and tumor necrosis factor alpha. Abbreviations: SD, standard deviation; CI, confidence interval; cfPWV, carotid-to-femoral pulse wave velocity; CVD, cardiovascular disease; DC, distensibility coefficient; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriole-to-venule-ratio; eGFR, estimated glomerular filtration rate; LGI, low grade inflammation; YEM, Young's elastic modulus.

Table S7.7 Associations of aortic and carotid stiffness with retinal microvascular diameter and retinal microvascular flicker light-induced dilation, with glucose metabolism status substituted by fasting plasma glucose (model 4A), 2-hour post load plasma glucose (model 4B), or HbA1c (model 4C); with waist circumference substituted by body mass index (model 4D); with mean arterial pressure measured during the vascular ultrasound measurement substituted by 24-hour mean arterial pressure (model 4E); and with educational status substituted by occupational status (model 4F) or income level (model 4G).

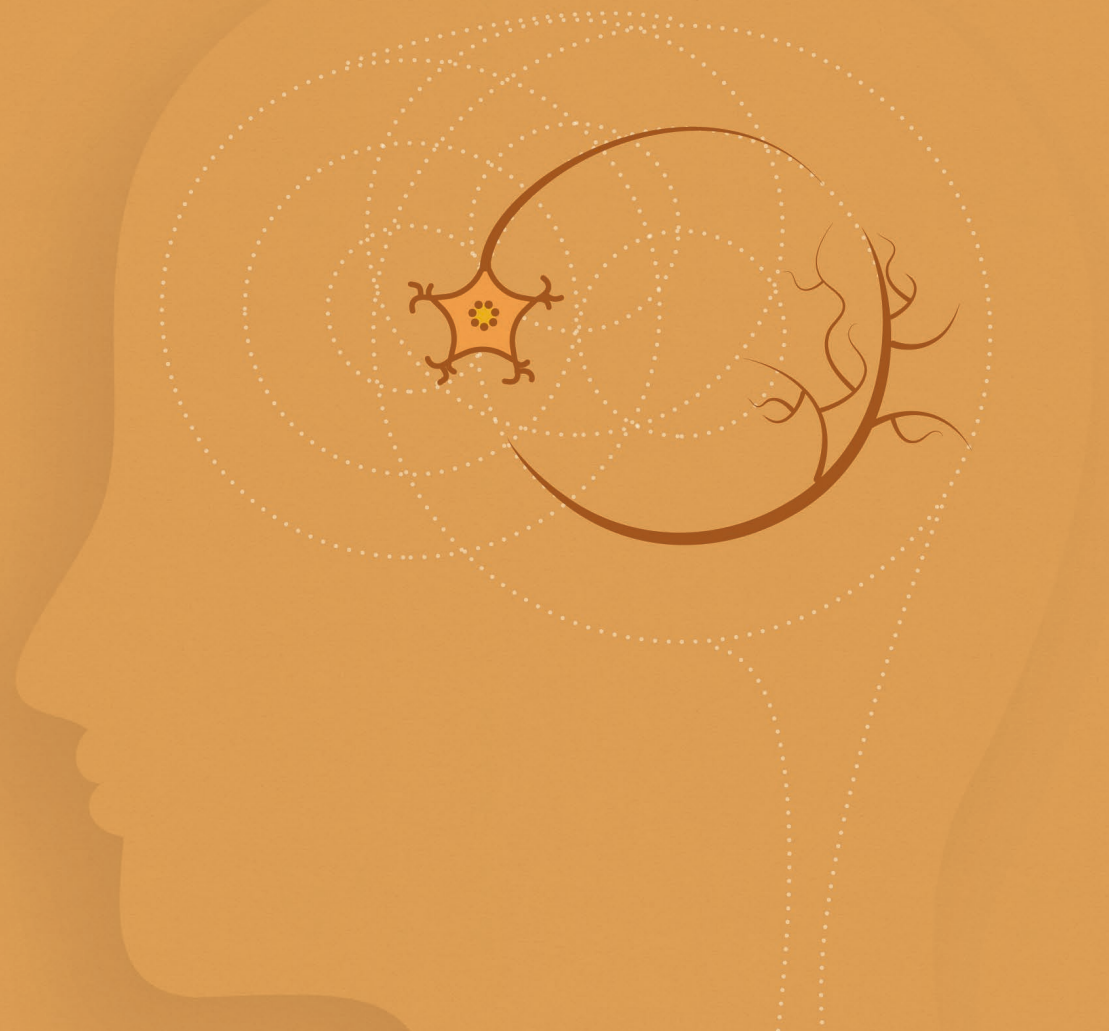
		Retinal microvascular diameter			Retinal microvascular flicker light-induced dilation	
		CRAE	CRVE	AVR	Arteriolar dilation	Venular dilation
Arterial stiffness	Model	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)	Stβ (95%CI)
Carotid DC, per SD	4A	-0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.04)	0.01 (-0.04; 0.06)	0.06 (0.00; 0.12)	0.04 (-0.02; 0.10)
	4B	0.01 (-0.05; 0.06)	-0.01 (-0.07; 0.04)	0.02 (-0.03; 0.08)	0.06 (0.00; 0.13)	0.04 (-0.02; 0.10)
	4C	-0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.05)	0.00 (-0.05; 0.05)	0.06 (-0.00; 0.11)	0.04 (-0.02; 0.10)
	4D	0.00 (-0.05; 0.05)	-0.00 (-0.05; 0.05)	0.00 (-0.05; 0.05)	0.06 (0.00; 0.12)	0.04 (-0.02; 0.10)
	4E	0.02 (-0.03; 0.07)	-0.00 (-0.05; 0.05)	0.03 (-0.02; 0.08)	0.04 (-0.02; 0.10)	0.04 (-0.02; 0.09)
	4F	0.03 (-0.03; 0.08)	0.02 (-0.04; 0.07)	0.01 (-00.04; 0.06)	0.06 (-0.01; 0.12)	0.04 (-0.03; 0.10)
	4G	-0.01 (-0.07; 0.04)	-0.01 (-0.07; 0.05)	-0.00 (-0.06; 0.06)	0.06 (-0.00; 0.13)	0.04 (-0.03; 0.10)
Carotid YEM, per SD	4A	0.02 (-0.02; 0.07)	0.02 (-0.03; 0.07)	0.00 (-0.04; 0.05)	-0.01 (-0.06; 0.04)	-0.02 (-0.07; 0.03)
	4B	0.02 (-0.03; 0.07)	0.03 (-0.02; 0.08)	-0.01 (-0.06; 0.03)	-0.01 (-0.06; 0.05)	-0.01 (-0.07; 0.04)
	4C	0.02 (-0.02; 0.07)	0.02 (-0.03; 0.07)	0.01 (-0.04; 0.05)	-0.01 (-0.06; 0.05)	-0.02 (-0.07; 0.03)
	4D	0.02 (-0.02; 0.06)	0.02 (-0.03; 0.06)	0.00 (-0.04; 0.05)	-0.00 (-0.06; 0.05)	-0.02 (-0.07; 0.03)
	4E	0.01 (-0.04; 0.05)	0.02 (-0.03; 0.06)	-0.01 (-0.05; 0.04)	0.01 (-0.05; 0.06)	-0.02 (-0.07; 0.04)
	4F	0.01 (-0.04; 0.05)	0.00 (-0.05; 0.05)	0.01 (-0.04; 0.06)	-0.02 (-0.07; 0.04)	-0.03 (-0.08; 0.03)
	4G	0.02 (-0.03; 0.07)	0.01 (-0.04; 0.07)	0.00 (-0.05; 0.05)	-0.01 (-0.07; 0.05)	-0.03 (-0.09; 0.03)
cfPWV, per SD	4A	0.04 (-0.02; 0.08)	0.01 (-0.04; 0.06)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.01 (-0.07; 0.04)
	4B	0.03 (-0.02; 0.08)	-0.01 (-0.06; 0.05)	0.05 (-0.00; 0.10)	-0.01 (-0.07; 0.05)	-0.00 (-0.06; 0.06)
	4C	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.05)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.01 (-0.07; 0.05)
	4D	0.03 (-0.02; 0.08)	0.00 (-0.05; 0.06)	0.04 (-0.01; 0.09)	-0.01 (-0.07; 0.05)	-0.02 (-0.08; 0.04)
	4E	0.01 (-0.04; 0.06)	-0.01 (-0.06; 0.04)	0.02 (-0.03; 0.07)	0.00 (-0.06; 0.06)	-0.01 (-0.07; 0.05)
	4F	0.04 (-0.01; 0.10)	0.01 (-0.04; 0.07)	0.04 (-0.02; 0.09)	-0.01 (-0.07; 0.05)	-0.04 (-0.10; 0.02)
	4G	0.04 (-0.01; 0.10)	0.02 (-0.03; 0.08)	0.03 (-0.03; 0.08)	-0.01 (-0.07; 0.06)	-0.01 (-0.08; 0.05)

Standardized regression coefficient (stβ) represents retinal microvascular diameter or retinal microvascular dilation in SD per SD greater arterial stiffness where 1 SD (approximately) corresponds with 5.16 10^{-3}/kPa for carotid DC, 0.34 10^{3}/kPa for carotid YEM, 2.08 m/s for cfPWV, 20.39 µm for CRAE, 31.07 µm for CRVE and 0.08 (no unit) for AVR, 2.82% for flicker light-induced arteriolar dilation and 2.20% for flicker light-induced venular dilation (corresponding arterial stiffness and retinal microvascular diameters and microvascular flicker light-induced dilation differ slightly per study population). For retinal microvascular diameters the size of the study population with full data on carotid DC and confounders was n=2433 in model 4A, n=2249 in model 4B, n=2429 in model 4C, n=2435 in model 4D, n=2170 in

model 4E, n=2041 in model 4F, and n=1851 in model 4G. For retinal venular flicker light-induced dilation the size of the study population the size of the study population with full data on carotid DC and confounders was n=1897 in model 4A, n=1753 in model 4B, n=1894 in model 4C, n=1896 in model 4D, n=1695 in model 4E, n=1587 in model 4F, and n=1463 in model 4G. Bold denotes p<.05. Variables in model 4 are: age, sex, glucose metabolism status, mean arterial pressure, heart rate, smoking status, alcohol consumption, waist circumference, total-to-high density lipoprotein cholesterol ratio, lipid-modifying and antihypertensive medication, and educational status. Abbreviations: HbA1c, glycated haemoglobin; SD, standard deviation; CI, confidence interval; cfPWV, carotid-to-femoral pulse wave velocity; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriole-to-venule-ratio; DC, distensibility coefficient; HbA1c, glycated haemoglobin; YEM, Young's elastic modulus.

Part III

Neurodegeneration and microvascular dysfunction in relation to depressive symptoms and cognitive performance



CHAPTER 8

Lower retinal nerve fibre layer thickness, an index of neurodegeneration, is associated with higher incidence of clinically relevant depressive symptoms and more depressive symptoms over time – The Maastricht Study

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Abstract

Importance
Whether neurodegeneration contributes to the early pathobiology of late-life depression remains incompletely understood.

Objective
The objective was to investigate whether lower retinal nerve fibre layer (RNFL) thickness, a marker of neurodegeneration, is associated with the incidence of clinically relevant depressive symptoms and depressive symptoms over time.

Design
An observational prospective cohort study from the Netherlands (The Maastricht Study) with baseline examination between 2010-20 and median follow-up of 5.0 years.

Setting
Population-based study.

Participants
Participants (mean age 60, 51% women, 20.5% type 2 diabetes) were recruited from the general population. Individuals with type 2 diabetes were oversampled by design. Up to n=4,934 participants were included in analyses.

Exposure
RNFL, an index of neurodegeneration, assessed with optical coherence tomography.

Main outcome
Depressive symptoms were assessed with the patient health questionnaire-9 (PHQ-9; continuous score 0-27) at baseline and, via annual assessments, over time. Presence of clinically relevant depressive symptoms was defined as PHQ-9 score ≥ 10.

Results
Lower RNFL thickness was associated with higher incidence of clinically relevant depressive symptoms (per standard deviation [SD], hazard ratio [95% CI], 1.11 [1.01;1.23]) and more depressive symptoms over time (per SD, rate ratio, 1.04 [1.01;1.06]), after adjustment for demographic, cardiovascular, and lifestyle factors.

Conclusions and relevance
The present population-based study found that lower RNFL thickness was associated with higher incidence of clinically relevant depressive symptoms and more depressive symptoms over time. Hence, neurodegeneration may contribute to the early pathobiology of late-life depression and early prevention of neurodegeneration may contribute to the prevention of late-life depression.

Introduction

Late-life depression is a debilitating disease that is in part caused by neurodegeneration.[1,2] Clinically, late life depression is associated with a high comorbidity of psychiatric and physical diseases including neurodegenerative diseases such as dementia.[3] Mechanistically, neurodegeneration in brain regions involved in reward, salience and cognitive function, is thought to lead to disturbances in mood, cognition, and motoric behaviour, all of which are clinical symptoms of late-life depression.[1]

Whether neurodegeneration contributes to the early pathobiology of late-life depression remains incompletely understood.[1] Cerebral neurodegeneration may be a gradual process that starts long before symptoms of late life depression are detectable.[4] Biologically, long-term exposure chronic exposure to stress, (micro)vascular dysfunction, amyloid accumulation, and exposure to adverse risk factors such as hyperglycaemia are all thought to contribute to deterioration of cerebral neurodegeneration.[1,4]

The retina may provide opportunity to study the early pathobiology of late life depression.[5] In the retina early neurodegeneration can be assessed as subtle neurodegenerative changes such as retinal nerve fibre layer (RNFL) thinning.[5] RNFL thinning is thought to reflect loss of retinal ganglion cell axons. Retinal neurodegeneration is a biologically plausible measure of cerebral neurodegeneration as the retina and brain have similar anatomical features and physiological properties.[5] Indeed, lower RNFL thickness has been associated with magnetic resonance imaging-assessed markers of cerebral neurodegeneration (i.e. lower grey and white matter volume),[6] cognitive decline,[7] and dementia.[8]

There are few data on the association between RNFL thickness and late-life depression. Some small studies have investigated the association between retinal neurodegeneration and depression, and found an association.[9-12] However, these studies have important limitations, i.e. they used cross-sectional data, used small, clinical study populations, and did not adjust for potential confounders.[9-12]

In view of the above, the objective was to investigate, using a large, well-characterized population-based cohort study, whether lower RNFL thickness was associated with incidence of clinically relevant depressive symptoms and depressive symptoms over time.

Materials and methods

Here key elements of the Material and Methods section are provided. More details are provided in the Supplemental Methods section.

Study population and design

We used data from The Maastricht Study, an observational, prospective population-based cohort study. The rationale and methodology have been described previously.[13] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes prospective data of n=7,689 individuals collected between November 2010 and December 2017. Prospective data were available for N=6,995, N=6,153, N=5,690, N=5,029, N=4,451, N=2,752, and N=1,469 participants at respectively one, two, three, four, five, six, and seven years of follow-up. The baseline examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Assessment of depressive symptoms and major depressive disorder

Depressive symptoms

Depressive symptoms were assessed with a validated Dutch version of the Patient Health Questionaire-9 (PHQ-9).[14] PHQ-9 data were collected both at baseline and during the seven years of annual follow-up. The PHQ-9 is a self-administered questionnaire based on the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) criteria for a major depressive disorder and consists of nine items.[15] Presence of clinically relevant depressive symptoms was defined as PHQ-9 score ≥10.[16] Incident clinically relevant depressive symptoms was defined as the absence of clinically relevant depressive symptoms at baseline (PHQ-9 score <10) and the presence of clinically relevant depressive symptoms (PHQ-9 score ≥10) on at least one follow-up assessment.

Assessment of the retinal nerve fibre layer thickness

We assessed RNFL thickness with optical coherence tomography (OCT; Spectralis unit; Heidelberg Engineering, Heidelberg, Germany). The RNFL thickness (μm) of both eyes was measured within a 3.45 mm diameter circular scan (12°, 768 voxels, 100 automatic real-time tracking) centred on the optic nerve head. All OCT scans were reviewed and their quality was scored. Intra- and interindividual reliability, expressed as intraclass correlation coefficients, were 0.97 and 0.96, respectively.[17]

Assessment of covariates

As previously described,[13] we used fasting plasma glucose and 2-hour post load glucose to assess glucose metabolism status according to the World Health Organization 2006 criteria as normal glucose metabolism, prediabetes, type 2 diabetes, type 1 diabetes, or other types of diabetes.[13] We assessed education status (low, middle, high), smoking status (never, current, former), alcohol consumption (none, low, high), and partner status (partner/no partner) with questionnaires;[18] assessed medication use via a medication interview; assessed waist circumference (cm) and office blood pressure (mmHg) as part of a physical examination; and determined total cholesterol/high density lipoprotein (HDL) ratio in fasting blood samples.[18,19]

Statistical analysis

We inversed (i.e. multiplied by -1) RNFL thickness, so that higher values indicate greater neurodegeneration (i.e. “thinner RNFL”). Next, if (for logistical reasons) the RNFL thickness was assessed at a later moment in time than at baseline (n=190 participants), we used the PHQ-9 score from the annual questionnaire closest in time to the assessment of the RNFL thickness as baseline PHQ-9 score (so that the time lag between measurements was minimized). Participants were eligible for inclusion in analyses if participants did not have clinically relevant depressive symptoms at baseline (i.e. PHQ-9 score <10) and if at least one PHQ-9 score assessment was available during annual follow up.

We used Cox proportional hazard regression analyses to study associations of standardized RNFL thickness with the incidence of clinically relevant depressive symptoms (i.e. present/absent) and we expressed results as hazard ratio with corresponding 95% confidence interval (CI). The date of censoring was defined as the first date at which the PHQ-9 score was ≥10 (event) or, otherwise, as the last date at which a PHQ-9 assessment was available (if no event had previously occurred). If any annual assessment of PHQ-9 score was missing before the date of censoring, the missing PHQ-9 scores were assumed to be <10 (i.e. ‘free of clinically relevant

depressive symptoms') to maximize the number of participants that could be used in the main analyses. Additionally, we made a Kaplan-Meier to visualize the association between RNFL thickness (entered as tertiles) and incidence of clinically relevant depressive symptoms.

We used generalized estimating equations analyses (exchangeable correlation structure; negative binomial mode) for analyses of standardized RNFL thickness with depressive symptoms over time (score 0-27). We used negative binomial regression as the PHQ-9 data is right-skewed (i.e. contains many null values). Results were expressed as rate ratio with corresponding 95% CI.

We checked whether we could assume that results were constant over time (i.e. proportional hazard assumption) by testing for interaction with time (i.e. entering an interaction term between RNFL thickness and time). If there was no interaction with time we considered results to be constant over time.

In model 1, we adjusted for age,[20,21] sex,[21,22] glucose metabolism status[23,24] (entered as dummy variables for prediabetes, type 2 diabetes, or other type of diabetes, with normal glucose metabolism status as reference), and education status (low [reference], middle, high).[25,26] We chose these variables as they are key potential confounders (all) or were oversampled by design (type 2 diabetes). In model 2, we additionally adjusted for variables of which their status as potential confounder has been less firmly established: waist circumference,[21,27] total cholesterol / HDL ratio,[28,29] use of lipid-modifying medication (yes/no)[28,29]. office systolic blood pressure[28,29], use of antihypertensive medication (yes/no)[28,30], smoking (current, ever, never [reference])[21,31], alcohol consumption (none [reference], low, high)[29,32], and partner status (partner/no partner).[31]

We used interaction analyses to test whether associations differed by sex (i.e. between men and women) or glucose metabolism status (i.e. between individuals with prediabetes, with type 2 diabetes, or with normal glucose metabolism). Because the number of participants with diabetes types other than type 2 was small, we excluded participants with other types of diabetes from tests of interaction with glucose metabolism status.

Additional analyses

To assess the robustness of our findings, we performed several additional analyses. First, we investigated the cross-sectional association between RNFL thickness and the presence of a major depressive disorder (assessed with the Mini-International Neuropsychiatric Interview, the gold standard for the assessment of a major depressive disorder).[33] Second, we studied the cross-sectional associations of RNFL thickness with depressive symptoms (continuous) and with the presence of clinically relevant

depressive symptoms (present/absent). Third, we performed additional prospective analyses in which we excluded participants with use of anti-depressive medication at baseline. Fourth, we repeated prospective analyses where we excluded participants with a major depressive disorder at baseline in addition to participants with clinically relevant depressive symptoms at baseline. Fifth, we studied prospective associations without exclusion of participants with clinically relevant depressive symptoms at baseline.[34] Sixth, we conducted prospective analyses in which we excluded participants without incident clinically relevant depressive symptoms when they had more than 2 missing assessments of PHQ-9 data during follow-up. Seventh, we studied associations after exclusion of participants with an age of onset of major depressive disorder ≤ 40 years. We excluded participants with an age of onset of major depressive disorder ≤ 40 years because the pathobiology of depression in these participants (i.e. early-life depression) may differ from the pathobiology of depression in participants with older age of onset of depression (i.e. late-life depression).[35] Eighth , we additionally adjusted for accelerometer-assessed physical activity[36], diet score[36], and spherical equivalent.[37]Adjustment for these potential confounders was not included in the main analyses because data were missing for a relatively large number of participants (up to N=617 participants had missing data on one or more of these variables). Nineth, we additionally adjusted for use of anti-depressive medication (assessed at baseline). As anti-depressive medication use may be a confounder and/or descendent of the outcome we adjusted for these covariates in additional analyses.[38] Tenth, we additionally adjusted for kidney variables (estimated glomerular filtration rate and albuminuria)[39], prior cardiovascular disease[40], retinopathy[41], glaucoma[42], and plasma biomarkers of low-grade inflammation[19] (all assessed at baseline) as they may be confounders and/or also (in part) be potential mediators. Eleventh, we performed additional analyses in which we excluded participants with baseline retinopathy or glaucoma. Last, we replaced glucose metabolism status with fasting plasma glucose, 2-hour post load plasma glucose, or haemoglobin A1c (HbA1c); waist circumference with body mass index; office systolic blood pressure with office diastolic blood pressure, or systolic or diastolic 24-hour ambulatory blood pressure; and education status with income level or occupation status.

All analyses were performed with Statistical Package for Social Sciences version 23.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). For all analyses (including interaction analyses) a P-value <0.05 was considered statistically significant in two-sided tests.

Results

Characteristics of the study population

Figure 8.1 shows a flow chart of the selection of participants for inclusion in analyses.

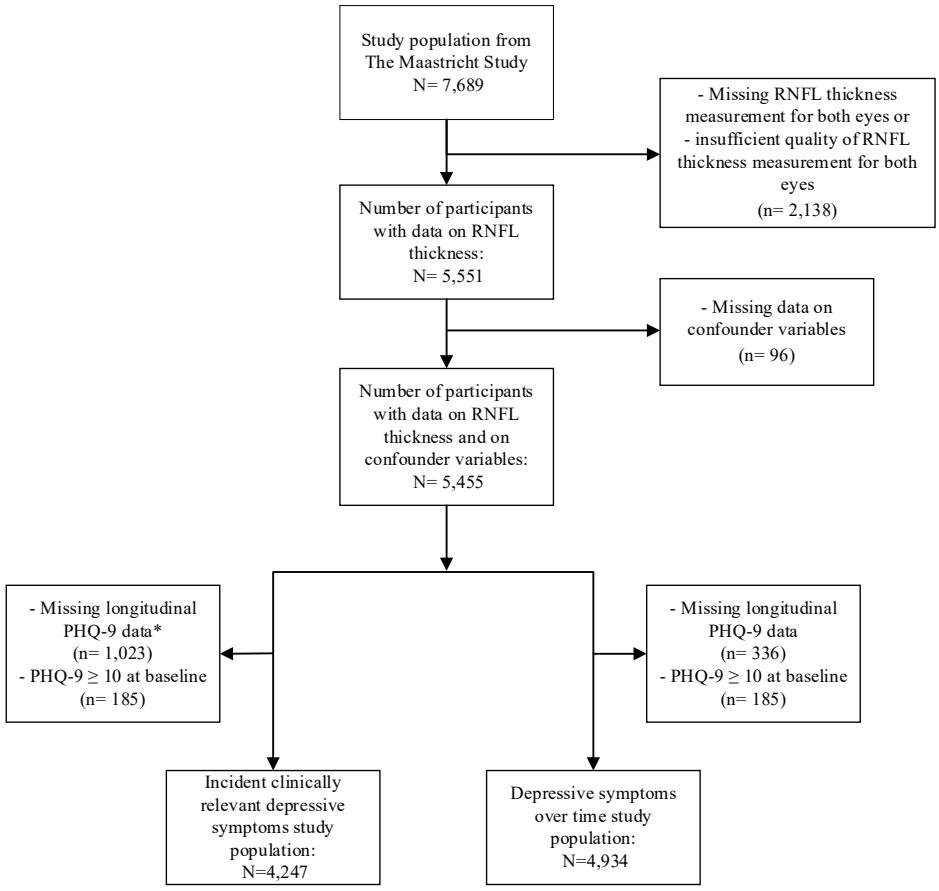


Figure 8.1 Flow chart for selection of participants for inclusion. * n=1023 were excluded as for these participants the assessment of clinically relevant depressive symptoms was missing at all annual follow up moments. Of the n=4,432 participants with data on incidence of clinically relevant depressive symptoms, n=3,910 participants had complete data at all annual assessments and respectively n=444, n=66, and n=12 participants had missing data at one, two, or three moments of annual follow up. Of the 4,934 participants with prospective data on depressive symptoms respectively n=4,822, n=4,280, n=3,911, n=3,467, n=2,854, n=1,636, n=710 participants had data on depressive symptoms after one, two, three, four, five, six, or seven years after baseline. Abbreviations: PHQ-9, 9-item Patient Health Questionnaire; RNFL, retinal nerve fibre layer.

Table 8.1 and Supplemental Table S8.1 show general characteristics of the participants. Overall, participants with a thinner RNFL at baseline were more often men and had a

more adverse cardiovascular risk profile. The median follow-up time was 5.0 years (interquartile range 3.0 - 6.0 years) and there were N=445 participants with incident clinically relevant depressive symptoms. General characteristics of the participants included in the analyses were highly comparable to those of participants with missing data (Supplemental Table S8.2).

Table 8.1 General characteristics of the prospective study population for incidence of clinically relevant depressive symptoms according to tertiles of RNFL thickness.

		Retinal nerve fiber layer thickness		
Characteristic	**Study population (N=4247)**	**Tertile 1 (low) (n=1415)**	**Tertile 2 (middle) (n=1416)**	**Tertile 3 (high) (n=1416)**
Covariates				
Age (years)	59.7 ± 8.4	60.3 ± 8.3	59.5 ± 8.4	59.3 ± 8.5
Women	2159 (50.8)	616 (43.5)	752 (53.1)	791 (55.9)
Glucose metabolism status				
Type 2 diabetes	870 (20.5)	352 (24.9)	257 (18.1)	261 (18.4)
Other type of diabetes	18 (0.4)	8 (0.6)	5 (0.4)	5 (0.4)
Prediabetes	649 (15.3)	218 (15.4)	219 (15.5)	212 (15.0)
Normal glucose metabolism	2710 (63.8)	837 (59.2)	935 (66.0)	938 (66.2)
Education status				
Low	1379 (32.5)	421 (29.8)	480 (33.9)	478 (33.8)
Middle	1187 (27.9)	371 (26.2)	390 (27.5)	426 (30.1)
High	1681 (39.6)	623 (44.0)	546 (38.6)	512 (36.2)
Waist circumference (cm)	94.0 ± 13.0	95.3 ± 13.1	93.2 ± 12.7	93.5 ± 13.2
Total-to-HDL cholesterol ratio	3.33 [2.8; 4.1]	3.36 [2.8; 4.1]	3.33 [2.8; 4.1]	3.3 [2.7; 4.1]
Use of lipid-modifying medication	1246 (29.3)	454 (32.1)	391 (27.6)	401 (28.3)
Office systolic blood pressure (mmHg)	133.2 ± 17.8	134.8 ± 17.3	132.8 ± 17.9	131.9 ± 18.0
Office diastolic blood pressure (mmHg) [a]	75.5 ± 9.7	76.5 ± 9.7	75.2 ± 9.7	74.8 ± 9.7
Use of antihypertensive medication	1482 (34.9)	570 (40.3)	463 (32.7)	449 (31.7)
Smoking status				
Non-smoker	1651 (38.9)	558 (39.4)	523 (36.9)	570 (40.3)
Former smoker	2130 (50.2)	721 (51.0)	739 (52.2)	670 (47.3)
Current smoker	466 (11.0)	136 (9.6)	154 (10.9)	176 (12.4)
Alcohol consumption				
None	682 (16.1)	204 (14.4)	234 (16.5)	244 (17.2)
Low	2507 (59.0)	836 (59.1)	814 (57.5)	857 (60.5)
High	1058 (24.9)	375 (26.5)	368 (26.0)	315 (22.2)
Partner status (with partner)	3510 (82.6)	1170 (82.7)	1155 (81.6)	1185 (83.7)
Retinal nerve fibre layer				
Peripapillary retinal nerve fiber layer thickness (µm)	94.8 ± 10.9	83.3 ± 6.3	95.2 ± 2.5	106.0 ± 6.6
Depressive symptoms				
Use of anti-depressive medication)	235 (5.5)	79 (5.6)	85 (6.0)	71 (5.0)
Major depressive disorder at baseline [b]	144 (3.0)	54 (3.4)	40 (2.5)	50 (3.1)

Table 8.1 (continued)

Characteristic	Study population (N=4247)	Retinal nerve fiber layer thickness Tertile 1 (low) (n=1415)	Tertile 2 (middle) (n=1416)	Tertile 3 (high) (n=1416)
Clinically relevant depressive symptoms at baseline [c]	236 (4.6)	69 (4.0)	86 (5.0)	81 (4.7)
Baseline depressive symptoms [c]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 1 year after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 2 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 3 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 4 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 5 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 6 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Depressive symptoms 7 years after baseline [d]	2 [0;4]	2 [0;4]	2 [0;4]	2 [0;4]
Incident clinically relevant depressive symptoms	445 (10.5)	162 (11.4)	150 (10.6)	133 (9.4)

Data are presented as n (%), mean ± standard deviation or median [interquartile range]. [a] data available for office diastolic blood pressure, n=4246. [b] Data are presented for the study population at baseline with complete data on the presence of a major depressive disorder, n=5071; [c] Data are presented for the study population at baseline with complete data on (clinically relevant) depressive symptoms, n=5170; [d] Data are presented for the prospective study population with complete data on depressive symptoms per moment of assessment, n=4934 (the number of participants with data available per year is reported in the legend of Figure 8.1). Abbreviations: HDL, high-density lipoprotein; RNFL, retinal nerve fibre layer thickness.

Analyses

After full adjustment (model 2), lower RNFL thickness was statistically significantly associated with a higher incidence of clinically relevant depressive symptoms and more depressive symptoms over time (per standard deviation, hazard ratio [95% CI], 1.11 [1.01; 1.23], and rate ratio [95%CI], 1.04 [1.01; 1.06], respectively; Figure 8,2 and Table 8.2). Figure 8.3 shows the Kaplan-Meier curve.

Time, sex and glucose metabolism status did not modify any of the above associations (P-values for interaction are presented in Supplemental Table S8.3).

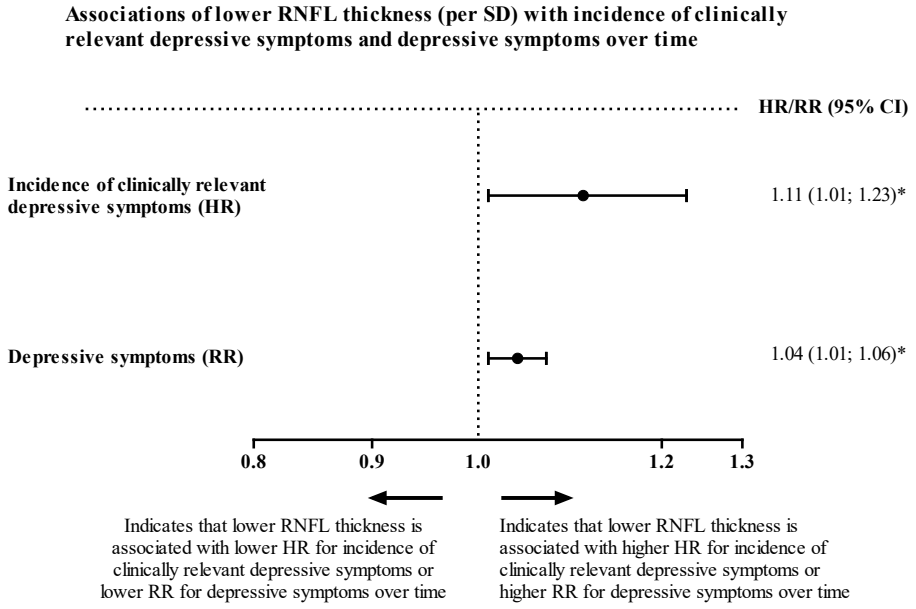


Figure 8.2 Associations of lower RNFL thickness (per SD) with incidence of clinically relevant depressive symptoms and depressive symptoms over time, where a higher hazard ratio or rate ratio indicates higher incidence of clinically relevant depressive symptoms or more depressive symptoms over time. One SD of RNFL thickness corresponds with 10.9 micrometres in the incidence of clinically relevant depressive symptoms study population and 11.0 micrometres in the depressive symptoms over time study population. Associations are adjusted for age, sex, glucose metabolism status, and education status, waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking, alcohol consumption, and partner status. * denotes P<0.05
Abbreviations RR, rate ratio; HR, hazard ratio; RNFL, retinal nerve fibre layer.

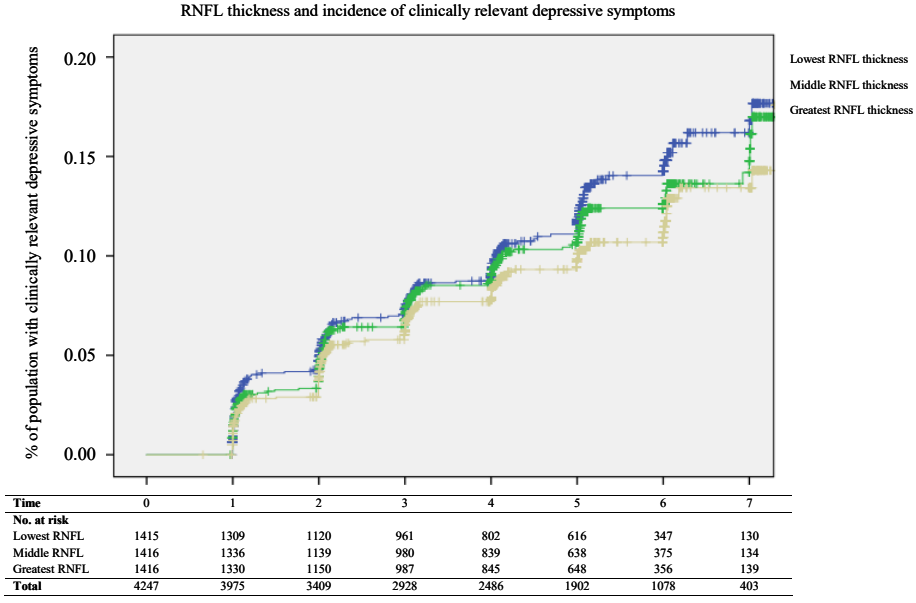
Additional analyses

We observed quantitively similar results in a range of additional analyses (Supplemental Results section). However, and only when education status was replaced with income level or occupation level, was the strength of the associations under study attenuated (Supplemental Table S8.7).

Table 8.2 Associations of lower RNFL thickness (per SD) with incidence of clinically relevant depressive symptoms and depressive symptoms over time.

	Crude	Model 1	Model 2
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Incidence of clinically relevant depressive symptoms	1.08 (0.99-1.19)	**1.11 (1.01-1.22)**	**1.11 (1.01-1.23)**
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Depressive symptoms over time	1.02 (1.00-1.05)	**1.04 (1.01-1.06)**	**1.04 (1.01-1.06)**

Regression coefficients represent the hazard ratio for the incidence of clinically relevant depressive symptoms or rate ratio for depressive symptoms over time per SD lower RNFL thickness, where a higher hazard ratio or rate ratio indicates higher incidence of clinically relevant depressive symptoms or more depressive symptoms over time. One SD of RNFL thickness corresponds with 10.9 micrometres in the incidence of clinically relevant depressive symptoms study population and 11.0 micrometres in the depressive symptoms over time study population. Model 1: age, sex, glucose metabolism status, and education status; Model 2: model 1 + waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking, alcohol consumption, and partner status. Bold indicates P<0.05. Abbreviations: CI, confidence interval; HR, hazard ratio; RR, rate ratio; RNFL, retinal nerve fibre layer thickness.



Time	0	1	2	3	4	5	6	7
No. at risk								
Lowest RNFL	1415	1309	1120	961	802	616	347	130
Middle RNFL	1416	1336	1139	980	839	638	375	134
Greatest RNFL	1416	1330	1150	987	845	648	356	139
Total	4247	3975	3409	2928	2486	1902	1078	403

Figure 8.3 Kaplan-Meier plot for hazard of incidence of clinically relevant depressive symptoms. Blue line indicates hazard of incidence of clinically relevant depressive symptoms for participants with RNFL thickness <90.75 micrometres (i.e. lowest RNFL thickness; indicating greatest extent of neurodegeneration). Green line indicates hazard of incidence of clinically relevant depressive symptoms for participants with RNFL thickness ≥90.76 to 99.59 micrometres. Light brown line indicates hazard of incidence of clinically relevant depressive symptoms for participants with RNFL thickness ≥99.60 micrometres (i.e. greatest RNFL thickness; indicating lowest extent of neurodegeneration). Of the participants with prospective data on depressive symptoms respectively n=4822, n=4280, n=3911, n=3467, n=2854, n=1636 and n=710 participants had data on depressive symptoms after one, two, three, four, five, six, or seven years after baseline. Abbreviations: RNFL, retinal nerve fibre layer.

Discussion

The present population-based found that lower RNFL thickness was statistically significantly associated with higher incidence of clinically relevant depressive symptoms and more depressive symptoms over time, after adjustment for demographic, cardiovascular, and lifestyle factors.

The present study is the first prospective study to investigate the association between RNFL thickness and depression as well as the first population-based study to investigate the association between RNFL thickness and depression.

Our results are consistent with the hypothesis that neurodegeneration contributes to the early pathobiology of late life depression. Lower RNFL thickness is thought to reflect more retinal neurodegeneration, whereas more symptoms of late-life depression are thought to reflect more cerebral neurodegeneration in brain areas involved in mood, cognition, and motor behaviour, such as the hippocampus and the frontal-subcortical brain areas.[1, 5]

Mechanistically, causes of neurodegeneration are thought to be chronic stress, (micro)vascular dysfunction, amyloid accumulation, and adverse exposure to risk factors such as hyperglycaemia. First, chronic exposure to stress is thought to lead to elevated levels of cortisol, which may increase levels of glucocorticoids and induce neurodegeneration.[1,4] Second, both (micro)vascular dysfunction and amyloid accumulation are thought to contribute to impaired functioning of hemodynamic autoregulation, which can predispose to ischemia and lead to higher levels of neuroinflammation, which is detrimental for neuronal cells.[1,2,8] Indeed, retinal as well as brain neuronal cells are highly susceptible to ischemia because they have a high energy demand and are fully dependent on the continuous supply of nutrients via the (micro)vasculature.[43] Third, amyloid accumulation and hyperglycaemia are thought to be neurotoxic and can lead to neurodegeneration.[44]

Associations were attenuated when education status was replaced with income level or occupation level, possibly because such adjustments may lead to collider bias. Neurodegeneration and depression may lead to lower income and occupation level, and therefore adjustment for these covariates may attenuate the strength of the associations under study.[45]

Our findings may be clinically relevant. First, RNFL thickness may be a feasible biomarker for identification of individuals at risk for late-life depression as its measurement is non-invasive and relatively inexpensive.[46] Indeed, RNFL thickness has been found to be a promising early biomarker for other neurodegenerative diseases such as multiple sclerosis.[47] Second, at an early stage there may be an opportunity to prevent depression by targeting early neurodegeneration via reducing exposure to

detrimental risk factors, such as hyperglycaemia, and lifestyle factors, such as an unhealthy diet.[48,49]

This study has the following strengths. First, the use of data from a large population-based cohort study reduces the chance that our results are affected by selection bias and enables us to draw conclusions that are valid in the general population.[50] Second, due to the prospective nature of the data we could account for temporality and, thus, can conclude that lower RNFL thickness precedes incident clinically relevant depressive symptoms.[51] Third, we adjusted for a large number of potential confounders, which reduces the chance that unmeasured confounding spuriously affects the strength of associations under study (i.e. confounding bias).[50] Fourth, all variables included in this study were assessed in a standardized manner with state-of-the-art methods (e.g. RNFL thickness), which reduces the chance that measurement error affects associations under study (i.e. information bias).[50]

This study also has limitations. First, the presence of a clinically relevant depressive disorder was prospectively assessed with the PHQ-9, which is a reasonably valid instrument for the diagnosis of the presence of a major depressive disorder (i.e. sensitivity 88% and specificity 85% at cut-off ≥10),[52] but not the current gold standard used in the clinic.[53] Second, we may have underestimated the strength of the association between RNFL thickness and late-life depression if participants with depressive symptoms or a major depressive disorder are more likely to have missing data.[50] For example, participants may be less likely to take part in a study or fill in a yearly questionnaire during a depressive episode. Third, we may have underestimated the strength of the association between RNFL thickness and late-life depression because we could not account for the use of anti-depressive medication that started after the baseline measurement. Fourth, even though we took an extensive set of confounders into account, we cannot fully exclude unmeasured confounding.[54] In particular, exposure to confounders may change over time and in this study confounders were only assessed at baseline. Fifth, some measurement error may have occurred as depressive symptoms were only measured at one time point per year (i.e. data are interval-censored) and the amount and intensity of depressive symptoms can fluctuate over time due to the nature of depression.[55] This may have led to an underestimation of the strength of prospective associations under study via regression dilution bias.[56] Last, we studied 40-to-75-year-old Caucasian individuals and therefore our results may be generalizable to such a population; whether these results also apply to other populations requires further study.

In summary, the present population-based study found that lower RNFL thickness was associated with a higher incidence of clinically relevant depressive symptoms and more depressive symptoms over time. Hence, neurodegeneration may contribute to the early pathobiology of late-life depression and early prevention of neurodegeneration may contribute to the prevention of late-life depression.

References

1. Alexopoulos GS. Mechanisms and treatment of late-life depression. Transl Psychiatry. 2019;9:188.
2. Hurley LL and Tizabi Y. Neuroinflammation, neurodegeneration, and depression. Neurotox Res. 2013;23:131-44.
3. Singh-Manoux A, Dugravot A, Fournier A, Abell J, Ebmeier K, Kivimaki M and Sabia S. Trajectories of Depressive Symptoms Before Diagnosis of Dementia: A 28-Year Follow-up Study. JAMA Psychiatry. 2017;74:712-8.
4. Leonard BE. Inflammation and depression: a causal or coincidental link to the pathophysiology? Acta Neuropsychiatr. 2018;30:1-16.
5. Cheung CY, Ikram MK, Chen C and Wong TY. Imaging retina to study dementia and stroke. Prog Retin Eye Res. 2017;57:89-107.
6. Mutlu U, Bonnemaijer PWM, Ikram MA, Colijn JM, Cremers LGM, Buitendijk GHS, Vingerling JR, Niessen WJ, Vernooij MW, Klaver CCW and Ikram MK. Retinal neurodegeneration and brain MRI markers: the Rotterdam Study. Neurobiol Aging. 2017;60:183-91.
7. Ko F, Muthy ZA, Gallacher J, Sudlow C, Rees G, Yang Q, Keane PA, Petzold A, Khaw PT, Reisman C, Strouthidis NG, Foster PJ, Patel PJ, Eye UKB and Vision C. Association of Retinal Nerve Fiber Layer Thinning With Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. JAMA Neurol. 2018;75:1198-205.
8. Gupta VB, Chitranshi N, den Haan J, Mirzaei M, You Y, Lim JK, Basavarajappa D, Godinez A, Di Angelantonio S, Sachdev P, Salekdeh GH, Bouwman F, Graham S and Gupta V. Retinal changes in Alzheimer's disease- integrated prospects of imaging, functional and molecular advances. Prog Retin Eye Res. 2020:100899.
9. Kalenderoglu A, Celik M, Sevgi-Karadag A and Egilmez OB. Optic coherence tomography shows inflammation and degeneration in major depressive disorder patients correlated with disease severity. J Affect Disord. 2016;204:159-65.
10. Schonfeldt-Lecuona C, Schmidt A, Kregel T, Kassubek J, Dreyhaupt J, Freudenmann RW, Connemann BJ, Pinkhardt EH and Gahr M. Retinal changes in patients with major depressive disorder - A controlled optical coherence tomography study. J Affect Disord. 2018;227:665-71.
11. Sonmez I, Kosger F and Aykan U. Retinal Nerve Fiber Layer Thickness Measurement by Spectral-Domain Optical Coherence Tomography in Patients with Major Depressive Disorder. Noro Psikiyatr Ars. 2017;54:62-6.
12. Yildiz M, Alim S, Batmaz S, Demir S, Songur E, Ortak H and Demirci K. Duration of the depressive episode is correlated with ganglion cell inner plexifrom layer and nasal retinal fiber layer thicknesses: Optical coherence tomography findings in major depression. Psychiatry Res Neuroimaging. 2016;251:60-6.
13. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
14. Kroenke K, Spitzer R and Williams J. The PHQ-9: Validity of a brief depression severity measure. Gen Intern Med. 2001;16:606-13.
15. Bell CC. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. . 1994;272(10):828.
16. Pettersson A, Bostrom KB, Gustavsson P and Ekselius L. Which instruments to support diagnosis of depression have sufficient accuracy? A systematic review. Nordic journal of psychiatry. 2015;69: 497-508.
17. Hong JT, Sung KR, Cho JW, Yun SC, Kang SY and Kook MS. Retinal nerve fiber layer measurement variability with spectral domain optical coherence tomography. Korean J Ophthalmol. 2012;26:32-8.
18. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
19. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, Schaper NC, van der Kallen CJ, Koster A and Sep SJ. Associations of low grade inflammation and endothelial dysfunction with depression–The Maastricht Study. Brain, behavior, and immunity. 2016;56:390-6.

20. Kessler RC, Birnbaum H, Bromet E, Hwang I, Sampson N and Shahly V. Age differences in major depression: results from the National Comorbidity Survey Replication (NCS-R). Psychol Med. 2010;40:225-37.
21. Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, Loeffler M, Colijn JM, Ikram MA, Vingerling JR, Williams KM, Hammond CJ, Creuzot-Garcher C, Bron AM, Silva R, Nunes S, Delcourt C, Cougnard-Gregoire A, Holz FG, Klaver CCW, Breteler MMB, Finger RP and European Eye Epidemiology C. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. Ophthalmology. 2018;125:1526-36.
22. Salk RH, Hyde JS and Abramson LY. Gender differences in depression in representative national samples: Meta-analyses of diagnoses and symptoms. Psychol Bull. 2017;143:783-822.
23. Chireh B, Li M and D'Arcy C. Diabetes increases the risk of depression: A systematic review, meta-analysis and estimates of population attributable fractions based on prospective studies. Prev Med Rep. 2019;14:100822.
24. De Clerck EEB, Schouten JSAG, Berendschot TTJM, Goezinne F, Dagnelie PC, Schaper NC, Schram MT, Stehouwer CDA and Webers CAB. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. Acta Ophthalmol. 2018;96:174-82.
25. Bjelland I, Krokstad S, Mykletun A, Dahl AA, Tell GS and Tambs K. Does a higher educational level protect against anxiety and depression? The HUNT study. Soc Sci Med. 2008;66:1334-45.
26. Khawaja AP, Chan MP, Broadway DC, Garway-Heath DF, Luben R, Yip JL, Hayat S, Khaw KT and Foster PJ. Laser scanning tomography in the EPIC-Norfolk Eye Study: principal components and associations. Invest Ophthalmol Vis Sci. 2013;54:6638-45.
27. Jeon SW, Lim SW, Shin DW, Ryu S, Chang Y, Kim SY, Oh KS, Shin YC and Kim YH. Metabolic syndrome and incident depressive symptoms in young and middle-aged adults: A cohort study. J Affect Disord. 2019;246:643-51.
28. Armstrong NM, Meoni LA, Carlson MC, Xue QL, Bandeen-Roche K, Gallo JJ and Gross AL. Cardiovascular risk factors and risk of incident depression throughout adulthood among men: The Johns Hopkins Precursors Study. J Affect Disorders. 2017;214:60-6.
29. Lamparter J, Schmidtmann I, Schuster AK, Siouli A, Wasielica-Poslednik J, Mirshahi A, Hohn R, Unterrainer J, Wild PS, Binder H, Lackner K, Beutel ME, Munzel T, Pfeiffer N and Hoffmann EM. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One. 2018;13:e0197682.
30. Sahin OZ, Sahin SB, Ayaz T, Karadag Z, Turkyilmaz K, Aktas E and Bostan M. The impact of hypertension on retinal nerve fiber layer thickness and its association with carotid intima media thickness. Blood Press. 2015;24:178-84.
31. Simayi A and Mohemaiti P. Risk and protective factors of co-morbid depression in patients with type 2 diabetes mellitus: a meta analysis. Endocr J. 2019;66:793-805.
32. Chang SC, Pan A, Kawachi I and Okereke OI. Risk factors for late-life depression: A prospective cohort study among older women. Prev Med. 2016;91:144-51.
33. DVSheehan Y, KHSheehan P, JJanavs E and THergueta R. GCDunbar (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry. 59:22-33.
34. Tizabi Y. Duality of Antidepressants and Neuroprotectants. Neurotoxicity Research. 2016;30:1-13.
35. Gollan J, Raffety B, Gortner E and Dobson K. Course profiles of early- and adult-onset depression. J Affect Disord. 2005;86:81-6.
36. Farioli Vecchioli S, Sacchetti S, Nicolis di Robilant V and Cutuli D. The Role of Physical Exercise and Omega-3 Fatty Acids in Depressive Illness in the Elderly. Curr Neuropharmacol. 2018;16:308-26.
37. Bikbov MM, Kazakbaeva GM, Gilmanshin TR, Zainullin RM, Arslangareeva, II, Salavatova VF, Bikbova GM, Panda-Jonas S, Nikitin NA, Zaynetdinov AF, Nuriev IF, Khikmatullin RI, Uzianbaeva YV, Yakupova DF, Aminev SK and Jonas JB. Axial length and its associations in a Russian population: The Ural Eye and Medical Study. PLoS One. 2019;14:e0211186.
38. Bubl E, Ebert D, Kern E, van Elst LT and Bach M. Effect of antidepressive therapy on retinal contrast processing in depressive disorder. Br J Psychiatry. 2012;201:151-8.

39. Hedayati SS, Jiang W, O'Connor CM, Kuchibhatla M, Krishnan KR, Cuffe MS, Blazing MA and Szczech LA. The association between depression and chronic kidney disease and mortality among patients hospitalized with congestive heart failure. Am J Kidney Dis. 2004;44:207-15.
40. Hare DL, Toukhsati SR, Johansson P and Jaarsma T. Depression and cardiovascular disease: a clinical review. Eur Heart J. 2014;35:1365-U13.
41. Chen X, Nie C, Gong Y, Zhang Y, Jin X, Wei S and Zhang M. Peripapillary retinal nerve fiber layer changes in preclinical diabetic retinopathy: a meta-analysis. PLoS One. 2015;10:e0125919.
42. Satyaprakash S VS, Arun Kumar S, Shivangi S, Kamaljeet S, Jagriti R. A Comparison of Retinal Nerve Fibre Layer Thickness by Spectral Domain Optical Coherence Tomography in Primary Open Angle Glaucoma, Normotensive Glaucoma and Glaucoma Suspect. 2018;3.
43. Butterfield DA and Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci. 2019;20:148-60.
44. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14:133-50.
45. Schisterman EF, Cole SR and Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20:488-95.
46. Srinivasan S, Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA and Efron N. Optical coherence tomography predicts 4-year incident diabetic neuropathy. Ophthalmic Physiol Opt. 2017;37:451-9.
47. Britze J and Frederiksen JL. Optical coherence tomography in multiple sclerosis. Eye (Lond). 2018;32:884-8.
48. Xu W, Tan L, Wang HF, Jiang T, Tan MS, Tan L, Zhao QF, Li JQ, Wang J and Yu JT. Meta-analysis of modifiable risk factors for Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2015;86:1299-306.
49. Yusufov M, Weyandt LL and Piryatinsky I. Alzheimer's disease and diet: a systematic review. Int J Neurosci. 2017;127:161-75.
50. Rothman KJ GS. Modern Epidemiology. Second Edition ed. Philadelphia: Lippincott Williams and Wilkins; 1998.
51. Rothman KJ and Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 2005;95 Suppl 1:S144-50.
52. Levis B, Benedetti A, Thombs BD and Collaboration DESD. Accuracy of Patient Health Questionnaire-9 (PHQ-9) for screening to detect major depression: individual participant data meta-analysis. BMJ. 2019;365:l1476.
53. Kroenke K, Spitzer RL and Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16:606-13.
54. Liang W, Zhao Y and Lee AH. An investigation of the significance of residual confounding effect. Biomed Res Int. 2014;2014:658056.
55. Almeida OP. Prevention of depression in older age. Maturitas. 2014;79:136-41.
56. Hutcheon JA, Chiolero A and Hanley JA. Random measurement error and regression dilution bias. BMJ. 2010;340:c2289.

Supplemental materials

Study population and design

We used data from The Maastricht Study, an observational, prospective population-based cohort study. The rationale and methodology have been described previously.[1] In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data of individuals collected between November 2010 and January 2019 (N=8,005 participants) and prospective data of individuals collected between November 2010 and December 2017 (N=7,689 participants). Prospective data were available for N=6,995, N=6,153, N=5,690, N=5,029, N=4,451, N=2,752, and N=1,469 participants at respectively one, two, three, four, five, six, and seven years of follow-up. The baseline examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Assessment of the retinal nerve fibre layer thickness

All participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement. A light meal (breakfast or lunch), low in fat content, was allowed if taken at least 90 minutes prior to the start of the measurements. Pupils were dilated with topical 0.5% tropicamide and 2.5% phenylephrine at least 15 minutes prior to the start of the examination.
We assessed RNFL thickness with optical coherence tomography (OCT; Spectralis unit and Eye Explorer version 5.7.5.0 software; Heidelberg Engineering, Heidelberg, Germany). The RNFL thickness (μm) of both eyes was measured within a 3.45 mm diameter circular scan (12°, 768 voxels, 100 automatic real-time tracking) centred on the optic nerve head by trained research assistants according to a standard operating procedure. The peripapillary OCT scans were reviewed and scored for the presence of measurement errors by experienced graders based on a predefined protocol. Graders were masked to clinical information of the participants. OCT images were excluded if one of the following criteria was present: scan errors (i.e. incomplete scan, poor

centring of the circular scan on the optic nerve head, RNFL layer incorrectly defined, or technical problem with the OCT device) and/or poor imaging quality (signal-to-noise ratio<15 dB). To reduce measurement error, the average RNFL thickness of both eyes was used in analyses (50.0% of participants). If data were only available for one eye the RNFL thickness was defined as the RNFL thickness of the eye for which data were available (50% of participants). The intra- and interrater reliability for the assessment of RNFL thickness are 0.966 and 0.963, respectively.[2]

Grading of OCT circle scans

OCT scans were considered of sufficient quality if all the following criteria were met: good centring of the circular scan on the optic nerve head (examples of good, poor and very poor centring are shown in Supplemental eFigure S1); complete (data of all 768 voxels was available); automatic quality ≥15 dB (an example of a scan with poor quality imaging is shown in Supplemental eFigure S8.2); no measurement error present (examples of all assessed measurement errors are shown in Supplemental eFigure S8.2). The percentage of agreement for selection of scans with sufficient quality ranged between 90% and 94% for four trained graders and was 70% for one grader (n=50 OCT scans per comparison).

Depressive symptoms

Presence and severity of clinically relevant depressive symptoms were assessed with a validated Dutch version of the Patient Health Questionaire-9 (PHQ-9) at baseline and during seven years of annual follow-up.[3] The PHQ-9 is a self-administered questionnaire based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for a major depressive disorder and consists of nine items.[4] We used PHQ-9 measurements with complete data, except that, in case when one or two items were missing, we recalculated the total score as 9 × (total points/9 minus number of missing items) and rounded to the nearest integer. Presence of clinically relevant depressive symptoms was defined as PHQ-9 score ≥10.[5] Incident clinically relevant depressive symptoms was defined as the absence of clinically relevant depressive symptoms at baseline (PHQ-9 score <10) and the presence of clinically relevant depressive symptoms (PHQ-9 score ≥10) at (at least) one follow-up assessment.

Major depressive disorder

The presence of a major depressive disorder in the preceding two weeks was assessed with the Mini-International Neuropsychiatric Interview (MINI), a short diagnostic structured interview, according to the DSM-IV criteria.[6] Major depressive disorder was

defined as the presence of at least one core symptom (i.e. depressed mood or loss of interest) and at least 4 other symptoms of depression (i.e. another core symptom, significant weight change or change in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, guilt or worthlessness, diminished ability to think or concentrate or indecisiveness, and suicidal thoughts or plans). Then, lifetime history of a major depressive disorder was assessed with the MINI and defined as the presence of symptoms of a major depressive disorder for at minimum two weeks during lifetime. Age of onset of lifetime major depressive disorder was defined as the youngest age at which a major depressive disorder occurred based on lifetime history. Presence of a major depressive disorder and lifetime history of a depressive disorder were both assessed at baseline.

Additional covariates

As previously described, we assessed history of cardiovascular disease by questionnaire, height (cm) and weight (kg) during a physical examination, and haemoglobin A1c (HbA1c) (mmol/mol), plasma biomarkers of low-grade inflammation (high sensitivity C-reactive protein (CRP) (μg/ml), serum amyloid A (SAA) (μg/ml), interleukin-6 (IL-6) (pg/ml), interleukin-8 (IL-8) (pg/ml) and tumour necrosis factor alpha (TNF-α) (pg/ml)) in fasting blood samples; 24-hour urinary albumin excretion (twice), defined as normal (<15 mg/24h), micro- (15-<30 mg/24h) and macroalbuminuria (≥30 mg/24h); estimated glomerular filtration rate (eGFR; in ml/min/1.73 m^2), calculated the estimated glomerular filtration rate (eGFR) based on serum creatinine only, since cystatin C was not presently available in all study participants; physical activity by accelerometer;[7] dietary intake with a validated food-frequency questionnaire (i.e. the Dutch Healthy Diet score);[8] spherical equivalent and intraocular pressure in both eyes with an automated refractor (Tonoref II; Nidek, Gamagori, Japan);[1,9] presence of retinopathy on fundus photos;[9] and 24-hour systolic and diastolic ambulatory blood pressure by use of an automated oscillometric upper arm blood pressure monitor (Omron 705IT, Omron, Japan).[1] The Dutch Healthy Diet score was recalculated so that the 'adjusted dietary intake score' reflects dietary intake without alcohol consumption (so that alcohol consumption and dietary intake could separately be included in statistical models). Body mass index (BMI) was calculated as kilograms per square meters (BMI=weight/length^2). Glaucoma was defined as use of intraocular pressure-lowering medication or an intraocular pressure higher than 21 mmHg in any eye (91.3% of all participants had data on intraocular pressure available for at least 1 eye). Spherical equivalent was defined as the mean spherical equivalent of both eyes (available for 91.1% of all participants) or as the spherical equivalent of the eye for which data was available. Use of anti-depressive medication was defined as use of one of the following types of medication: (selective or non-

selective) serotonin reuptake inhibitor, tricyclic antidepressant, tetracyclic antidepressant, monoamine oxidase inhibitor, melatonin receptor agonist, or atypical antidepressant.

Additional statistical analysis

We used negative binomial regression analyses to investigate the cross-sectional association between standardized RNFL thickness and depressive symptoms. We used negative binomial regression to account for the right-skewed PHQ-9 score which contained many null values. Results were expressed as rate ratio (RR) with corresponding 95% confidence interval (95% CI). Then, we used multivariable logistic regression analyses to cross-sectionally investigate the association between standardized RNFL thickness and the presence of clinically relevant depressive symptoms and the association between standardized RNFL thickness and the presence of a major depressive disorder. Results were expressed as odds ratio (OR) with corresponding 95% CI.
We used collinearity diagnostics (i.e. tolerance <0.10 and/or variance inflation factor >10) to detect multicollinearity between covariates (collinearity diagnostics were only available in cross-sectional analyses).

Supplemental results

Generally, we observed quantitively similar results in a range of additional analyses. First, in cross-sectional analyses, lower RNFL thickness was statistically significantly associated with more depressive symptoms but not with clinically relevant depressive symptoms, or the presence of a major depressive disorder (Supplemental Table S8.4). Second, associations did not materially change when we excluded participants with use of anti-depressive medication at baseline, when we excluded participants with a major depressive disorder at baseline in addition to participants with clinically relevant depressive symptoms at baseline, or when we studied associations without exclusion of participants with clinically relevant depressive symptoms at baseline (Supplemental Table S8.5). Third, we found numerically comparable associations when we excluded participants without incident clinically relevant depressive symptoms when they had more than 2 missing assessments of PHQ-9 data during follow-up and when we excluded participants with an age of onset of major depressive disorder ≤40 years (Supplemental Table S8.5). Fourth, associations remained similar when we additionally adjusted for physical activity, diet score, or spherical equivalent, use of anti-depressive medication, kidney variables, prior cardiovascular disease, retinopathy, glaucoma, or plasma biomarkers of low-grade inflammation (Supplemental Table S.6). Fifth, associations were similar when we excluded participants with retinopathy or glaucoma

(Supplemental Table S.6). Sixth, associations remained similar after substitution of glucose metabolism by fasting plasma glucose, 2-hour post load plasma glucose, or HbA1c; waist circumference by body mass index; and office systolic blood pressure by office diastolic blood pressure, or 24-hour ambulatory mean systolic or diastolic blood pressure (Supplemental Table S8.7). However, associations were less strong after substitution of education status by income level or occupation status (Supplemental Table S8.7).

References

1. Schram MT, Sep SJ, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, Henry RM and Stehouwer CD. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439-51.
2. Hong JT, Sung KR, Cho JW, Yun SC, Kang SY and Kook MS. Retinal nerve fiber layer measurement variability with spectral domain optical coherence tomography. Korean J Ophthalmol. 2012;26:32-8.
3. Kroenke K, Spitzer R and Williams J. The PHQ-9: Validity of a brief depression severity measure. Gen Intern Med. 2001;16:606-13.
4. Bell CC. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. . 1994;272(10):828.
5. Pettersson A, Bostrom KB, Gustavsson P and Ekselius L. Which instruments to support diagnosis of depression have sufficient accuracy? A systematic review. Nordic journal of psychiatry. 2015;69: 497-508.
6. DVSheehan Y, KHSheehan P, JJanavs E and THergueta R. GCDunbar (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry. 59:22-33.
7. Sorensen BM, van der Heide FCT, Houben A, Koster A, T TJMB, J SAGS, Kroon AA, van der Kallen CJH, Henry RMA, van Dongen M, S JPME, H HCMS, van der Berg JD, Schaper NC, Schram MT and Stehouwer CDA. Higher levels of daily physical activity are associated with better skin microvascular function in type 2 diabetes-The Maastricht Study. Microcirculation. 2020;27:e12611.
8. Looman M, Feskens EJM, de Rijk M, Meijboom S, Biesbroek S, Temme EHM, de Vries J and Geelen A. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr. 2017;20:2289-99.
9. De Clerck EEB, Schouten JSAG, Berendschot TTJM, Goezinne F, Dagnelie PC, Schaper NC, Schram MT, Stehouwer CDA and Webers CAB. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. Acta Ophthalmol. 2018;96:174-82.

Supplemental figures

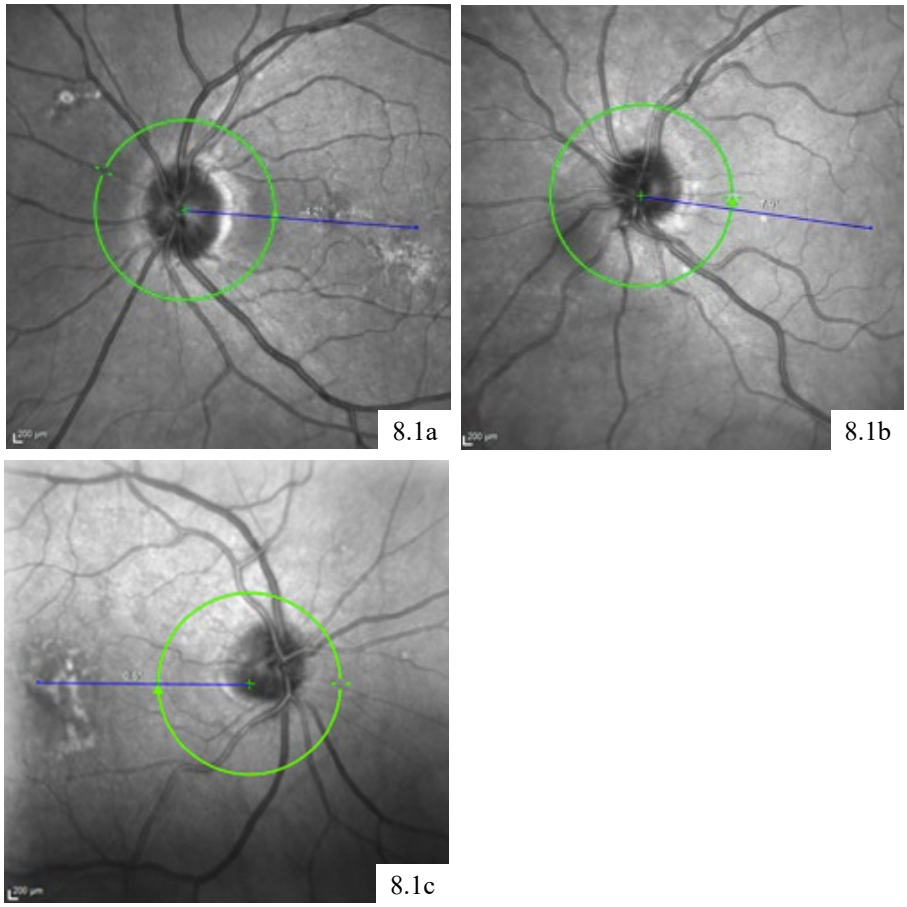
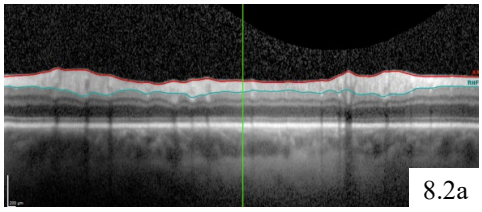


Figure S8.1 Examples of quality of centring of circular scans on the optic nerve head. Figure S8.1 shows examples of quality of centring of the circular scan on the optic nerve head: S8.1a shows good quality, S8.1b shows poor quality, and S8.1c shows very poor quality.



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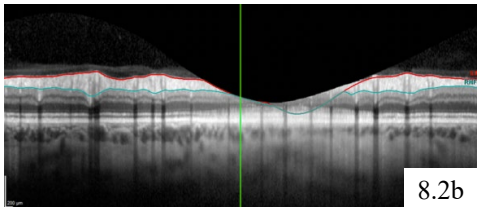

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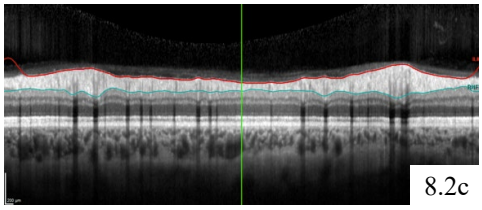

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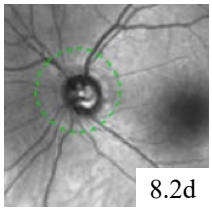

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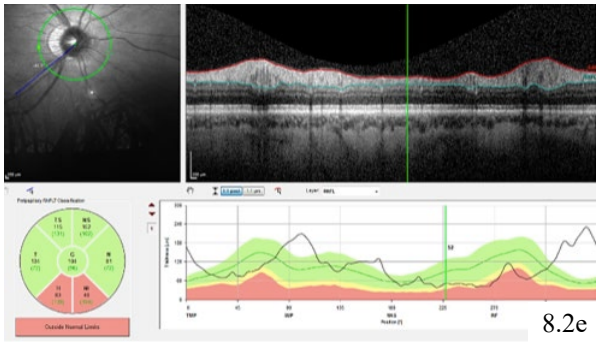

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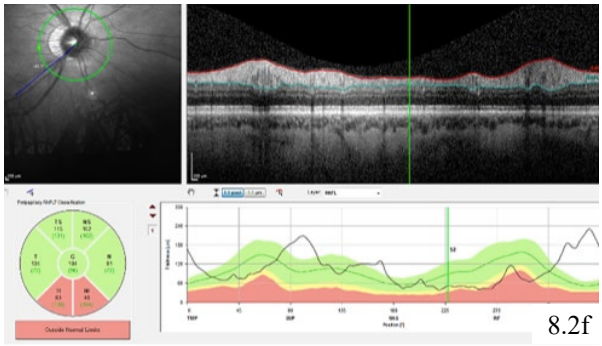

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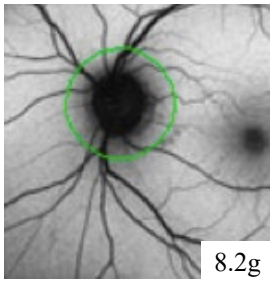

Figure S8.2 Examples of poor imaging quality and scan errors. S8.2a: Example of poor imaging quality (Signal-to-noise ratio<15 dB); S8.2b: OCT device too close to the eye; S8.2c: RNFL layer incorrectly defined; S8.2d: incorrect circle position (dashed line); S8.2e: participant does not look in the correct direction; S8.2f: technical problem with OCT device; S8.2g: autofluorescence on. Abbreviations: OCT, optical coherence tomography; RNFL, retinal nerve fibre layer thickness.

Supplemental tables

Table S8.1 Additional general characteristics of the prospective study population with complete data on the incidence of clinically relevant depressive symptoms, according to tertiles of RNFL thickness.

		Retinal nerve fibre layer thickness		
Characteristic	**Total study group (N=4247)**	**Tertile 1 (low) (n=1415)**	**Tertile 2 (middle) (n=1416)**	**Tertile 3 (high) (n=1416)**
Fasting plasma glucose (mmol/l) [a]	5.4 [5.0; 6.1]	5.4 [5.0; 6.3]	5.3 [4.9; 6.0]	5.3 [5.0; 6.0]
2-hour post load glucose (mmol/l) [a]	6.1 [4.9; 8.4]	6.3 [5.0; 9.0]	6.0 [4.9; 8.1]	6.0 [4.9; 8.0]
HbA1c (mmol/mol) [a]	37.0 [34.0; 41.0]	37.0 [34.0; 42.0]	37.0 [34.0; 40.5]	37.0 [34.0; 41.0]
Income level (euro) [a]	2078 ± 826	2092 ± 843	2089 ± 833	2045 ± 800
Occupation status [a]				
Low	381 (26.9)	129 (24.8)	120 (25.8)	132 (30.5)
Intermediate	542 (38.2)	185 (35.6)	188 (40.4)	169 (39.0)
High	495 (34.9)	206 (39.6)	157 (33.8)	132 (30.5)
Body mass index (kg/m2)	26.0 [23.7; 28.8]	26.2 [23.9; 28.9]	26.0 [23.5; 28.7]	26.0 [23.6; 28.8]
Systolic ambulatory blood pressure (mmHg) [a]	119.3 ± 11.4	120.2 ± 11.2	119.2 ± 11.4	118.5 ± 11.5
Diastolic ambulatory blood pressure (mmHg) [a]	73.8 ± 7.1	74.3 ± 6.9	73.8 ± 7.2	73.2 ± 7.1
Physical activity (hours/day)[a]	2.0 ± 0.7	2.00 ± 0.7	2.1 ± 0.7	2.0 ± 0.7
Dutch healthy diet score (points)*	84.5 ± 14.9	83.3 ± 14.7	84.9 ± 15.1	85.3 ± 14.7
Spherical equivalent (dioptre) [a]	0.1 [-1.4; 1.1]	-0.6 [-3.0; 0.5]	0.1 [-1.2; 1.1]	0.5 [-0.4; 1.5]
Kidney variables [a]				
eGFR (ml/min)	82.0± 13.5	81.4 ± 13.7	81.7± 13.4	82.8 ± 13.4
Albuminuria				
Normoalbuminuria	3932 (92.8)	1298 (92.0)	1316 (93.3)	1318 (93.2)
Microalbuminuria	282 (6.7)	103 (7.3)	86 (6.1)	92 (6.5)
Macroalbuminuria	22 (0.5)	10 (0.7)	8 (0.6)	4 (0.3)
Prior cardiovascular disease [a]	662 (15.6)	240 (17.0)	213 (15.1)	209 (14.8)
Retinopathy*	55 (1.3)	19 (1.4)	13 (0.9)	23 (1.7)
Glaucoma	149 (4.5)	93 (7.0)	36 (2.7)	50 (3.7)
Intraocular pressure (mmHg)	14.0 ± 3.0	14.4 ± 3.1	13.9 ± 2.9	13.8 ± 3.0
Plasma biomarkers of low-grade inflammation*				
C-reactive protein (µg/ml)	1.1 [0.6; 2.5]	1.2 [0.6; 2.7]	1.1 [0.6; 2.5]	1.1 [0.5; 2.4]
Serum amyloid A (µg/ml)	3.2 [2.0; 5.3]	3.3 [2.2; 5.6]	3.1 [2.0; 4.8]	3.2 [1.9; 5.4]
Interleukin-6 (pg/ml)	0.6 [0.4; 0.8]	0.6 [0.4; 1.0]	0.5 [0.4; 0.8]	0.5 [0.4; 0.8]
Interleukin-8 (pg/ml)	4.0 [3.2; 5.1]	4.2 [3.2; 5.3]	3.9 [3.2; 5.1]	3.9 [3.1; 4.8]
Tumour necrosis factor alpha (pg/ml)	2.2 [1.9; 2.5]	2.2 [1.9; 2.6]	2.1 [1.9; 2.5]	2.1 [1.8; 2.5]

Data are presented as n (%), mean ± standard deviation or median [interquartile range] [a] Number of participants with data available for fasting plasma glucose, n=4245; 2-hour post load glucose, n=4068; HbA1c, n=4243; income level, n=3420; occupation status, n=1418; office diastolic blood pressure, n=4246; systolic ambulatory blood pressure, n=3798; diastolic ambulatory blood pressure, n=3798; physical activity, n=3660; diet score, n=4016; spherical equivalent, n=4033; eGFR, n=4244; albuminuria, n=4236; prior cardiovascular disease, n=4233; retinopathy, n=4139; glaucoma, n=4001; intraocular pressure, n=4001; plasma biomarkers of low-grade inflammation, n=1840. Abbreviations: HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; RNFL, retinal nerve fibre layer thickness.

Table S8.2 General characteristics of the participants with complete data and missing data in the prospective study population with complete data on the incidence of clinically relevant depressive symptoms.

Characteristic, mean (SD)	Participants with complete data (n=4432)	N missing data in participants with complete/ missing data	Participants with missing data (n=3573)
Age (years)	59.6 ± 8.4	0/0	60.1 ± 9.0
Women	2269 (51.2)	0/0	1715 (48.0)
Glucose metabolism status			
Type 2 diabetes	925 (20.9)	0/0	995 (27.8)
Other type of diabetes	20 (0.5)		30 (0.8)
Prediabetes	676 (15.3)		514 (14.4)
Normal glucose metabolism	2811 (63.4)		2034 (56.9)
Fasting plasma glucose (mmol/l)	5.4 [5.0; 6.1]	2/2	5.5 [5.0; 6.5]
2-hour post load glucose (mmol/l)	6.1 [4.9; 8.4]	197/312	6.2 [5.0; 9.1]
HbA1c (mmol/mol)	37.0 [34.0; 41.0]	4/10	38.0 [35.0; 44.0]
Education status		0/116	
Low education	1457 (32.9)		1278 (37.0)
Medium education	1247 (28.1)		919 (26.6)
High education	1728 (39.0)		1260 (36.4)
Income level	2054 ± 827	873/1122	1942 ± 844
Occupation status			
Low	409 (27.9)	2964/2217	484 (35.7)
Intermediate	558 (38.0)		437 (32.2)
High	501 (34.1)		435 (32.1)
Waist circumference (cm)	94.1 ± 13.1	0/5	96.7 ± 14.2
Body mass index (kg/m2)	26.7 [24.2; 30.0]	0/3	26.7 [24.2; 30.0]
Total-to-HDL cholesterol ratio	3.3 [2.8; 4.1]	0/4	3.5 [2.8; 4.4]
Use of lipid-modifying medication	1314 (29.6)	0/6	1237 (34.7)
Office systolic blood pressure (mmHg)	133.1 ± 17.7	0/3	134.3 ± 18.2
Office diastolic blood pressure (mmHg)	75.5 ± 9.8	1/3	75.5 ± 9.8
Systolic ambulatory blood pressure (mmHg)	119.3 ± 7.1	476/609	119.9 ± 11.9
Diastolic ambulatory blood pressure (mmHg)	73.8 ± 7.1	476/609	74.0 ± 7.4
Use of antihypertensive medication	1566 (35.3)	0/6	1456 (40.8)
Smoking status		0/65	
Non-smoker	1714 (38.7)		1245 (35.5)
Former smoker	2211 (49.9)		1708 (48.7)
Current smoker	507 (11.4)		555 (15.8)
Alcohol consumption		0/66	
None	742 (16.7)		731 (20.8)
Low	2599 (58.6)		2029 (57.9)
High	1091 (24.6)		747 (21.3)
Partner status (with partner)	3635 (82.0)	0/58	2686 (76.4)
Physical activity (hours/day)	2.0 ± 0.7	617/657	1.9 ± 0.7
Dutch healthy diet score (points)	84.4 ± 14.9	253/431	83.0 ± 15.4
Spherical equivalent (dioptre)	0.1 [-1.4; 1.1]	218/416	0.1 [-1.6; 1.1]
Use of antidepressants	290 (6.5)	0/6	287 (8.0)
Kidney variables			
eGFR (ml/min)	82.0 ± 13.6	3/5	82.5 ± 15.0

Table S8.2 (continued)

Characteristic, mean (SD)	**Participants with complete data (n=4432)**	**N missing data in participants with complete/ missing data**	**Participants with missing data (n=3573)**
Albuminuria		13/74	
Normoalbuminuria	4097 (92.7)		3157 (90.2)
Microalbuminuria	299 (6.8)		309 (8.8)
Macroalbuminuria	23 (0.5)		33 (0.9)
Prior cardiovascular disease	699 (15.8)	15/86	638 (18.3)
Retinopathy	57 (1.3)	111/420	68 (2.2)
Glaucoma	187 (4.5)	251/444	148 (4.7)
Intraocular pressure (mmHg)	14.03 ± 3.04	251/444	13.9 ± 3.1
Plasma biomarkers of low-grade inflammation			
C-reactive protein (µg/ml)	1.1 [0.6; 2.6]	2521/1718	1.1 [0.7; 3.0]
Serum amyloid A (µg/ml)	3.2 [2.0; 5.3]	2521/1718	3.4 [2.1; 5.5]
Interleukin-6 (pg/ml)	0.6 [0.4; 0.9]	2521/1718	0.6 [0.4; 1.0]
Interleukin-8 (pg/ml)	4.0 [3.2; 5.2]	2521/1719	4.3 [3.4; 5.6]
Tumour necrosis factor alpha (pg/ml)	2.2 [1.9; 2.5]	2521/1718	2.2 [1.9; 2.6]
Peripapillary retinal nerve fibre layer thickness (µm)	94.8 ± 10.9 [a]	0/2454	94.7 ± 10.8 [a]
Depressive symptoms 1 year after baseline	2 [0; 4]	132/1920	2 [0; 4]
Depressive symptoms 2 years after baseline	2 [0; 4]	550/2195	2 [0; 4]
Depressive symptoms 3 years after baseline	2 [0; 4]	883/2317	2 [0; 4]
Depressive symptoms 4 years after baseline	2 [0; 4]	1253/2514	2 [0; 4]
Depressive symptoms 5 years after baseline	2 [0; 4]	1869/2607	2 [0; 4]
Depressive symptoms 6 years after baseline	2 [0; 4]	2950/3048	2 [0; 4]
Depressive symptoms 7 years after baseline	2 [0; 4]	3822/3320	2 [0; 4]
Incident clinically relevant depressive symptoms [b]	567 (12.8)	0†/1716	250 (13.5)
Median follow-up time (years)	5.0 [3.0-6.0]	0/2306	4.9 [2.1-6.0]

Data are presented as n (%), mean ± standard deviation or median [interquartile range]. Bold indicates P<0.05. Please note that the study population with complete data available (N=4432) differs in size from the prospective study population (N=4247) because for the main analyses participants with clinically relevant depressive symptoms (N=185) were excluded in the main analyses. [a] OCT scans of insufficient quality were not included in the calculation of the mean retinal nerve fibre layer thickness. [b] n=3910 participants had complete data on incidence of clinically relevant depressive symptoms at all annual assessments and respectively n=444, n=66, and n=12 participants had missing data for one, two, or three assessments of clinically relevant depressive symptoms over time. Abbreviations: HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; OCT, optical coherence tomography.

Table S8.3 P-values of interaction terms of RNFL thickness with sex, glucose metabolism status, or time.

	Sex	Prediabetes	Type 2 diabetes	Time
Prospective				
Incident clinically relevant depressive symptoms	0.60	0.48	0.94	0.06
Depressive symptoms	0.25	0.96	0.29	0.47

P-values for the interaction terms of sex, glucose metabolism status (i.e. prediabetes versus normal glucose metabolism status or type 2 diabetes versus normal glucose metabolism status), and time with RNFL thickness (e.g. sex*RNFL thickness). Variables in the model in addition to RNFL thickness and interaction term(s) with sex, glucose metabolism status and time are: age, sex, glucose metabolism status, education level, waist circumference, total cholesterol/HDL ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking status, alcohol consumption and partner status. Bold indicates P<0.05. Abbreviations: NA, not applicable; RNFL, retinal nerve fibre layer.

Table S8.4 Cross-sectional associations of lower RNFL thickness (per SD) with (clinically relevant) depressive symptoms or the presence of a major depressive disorder.

	Crude	Model 1	Model 2
Cross-sectional	RR (95% CI)	RR (95% CI)	RR (95% CI)
Depressive symptoms	1.01 (0.98-1.05)	1.04 (1.01-1.08)	1.04 (1.00-1.07)
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
Clinically relevant depressive symptoms	0.94 (0.82-1.06)	0.96 (0.84-1.10)	0.96 (0.84-1.10)
Major depressive disorder	1.06 (0.90-1.24)	1.07 (0.91-1.26)	1.08 (0.92-1.27)

Regression coefficients represent the rate ratio or odds ratio (where applicable) for the presence of (clinically relevant) depressive symptoms or the presence of a major depressive disorder (cross-sectional analyses), per SD lower RNFL thickness, where a higher odds ratio or rate ratio indicates higher odds of the presence of clinically relevant depressive symptoms or the presence of a major depressive disorder, or higher rate ratio for more depressive symptoms. One SD of RNFL thickness corresponds with 10.86 micrometres in the cross-sectional (clinically relevant) depressive symptoms study population and 10.81 micrometres in the major depressive disorder study population. Model 1: age, sex, glucose metabolism status, and education status; Model 2: model 1 + waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking, alcohol consumption, and partner status. Bold indicates P<0.05. Abbreviations: CI, confidence interval; OR, odds ratio; RR, rate ratio; RNFL, retinal nerve fibre layer.

Table S8.5 Associations of lower RNFL thickness (per SD) with the incidence of clinically relevant depressive symptoms and depressive symptoms over time, additional analyses.

	Crude	Model 1	Model 2
	HR/RR (95% CI)	HR/RR (95% CI)	HR/RR (95% CI)
After exclusion of participants with use of anti-depressive medication at baseline			
Incidence of clinically relevant depressive symptoms, HR (95%CI), n=4012	1.07 (0.97-1.18)	1.09 (0.98-1.21)	1.10 (0.99-1.22)
Depressive symptoms, RR (95%CI), n=4463	1.02 (0.99-1.04)	1.03 (1.01-1.06)	1.03 (1.01-1.06)
After exclusion of participants with clinically relevant depressive symptoms and/or a major depressive disorder at baseline			
Incidence of clinically relevant depressive symptoms, HR (95%CI), n=3913	**1.12 (1.01-1.23)**	**1.15 (1.04-1.27)**	**1.15 (1.04-1.27)**
Depressive symptoms, RR (95%CI), n=4526	**1.02 (1.00-1.05)**	**1.04 (1.02-1.07)**	**1.04 (1.02-1.07)**
Without exclusion of participants with clinically relevant depressive symptoms at baseline			
Incidence of clinically relevant depressive symptoms, HR (95%CI), n=4432	1.05 (0.96-1.14)	1.08 (0.99-1.17)	1.08 (1.00-1.18)
Depressive symptoms, RR (95%CI), n=5170	1.01 (0.99-1.04)	1.03 (1.01-1.06)	1.03 (1.01-1.06)
After exclusion of participants without incident clinically relevant depressive symptoms when they had at least 2 missing assessments of PHQ-9 data over 7 follow-up measurements			
Incidence of clinically relevant depressive symptoms, HR (95%CI), n=4231	1.08 (0.99-1.19)	**1.11 (1.01-1.22)**	**1.12 (1.01-1.23)**
Depressive symptoms, RR (95%CI), n=4160	1.00 (1.00-1.05)	1.02 (0.99-1.04)	1.02 (0.99-1.04)
After exclusion of participants with an age of major depressive disorder onset of ≤ 40 years			
Incidence of clinically relevant depressive symptoms, HR (95%CI), n=3755	1.08 (0.97-1.20)	1.09 (0.98-1.22)	1.10 (0.99-1.23)
Depressive symptoms, RR (95%CI), n=4350	1.02 (0.99-1.18)	**1.04 (1.01-1.07)**	**1.04 (1.01-1.07)**

Regression coefficients represent the hazard ratio for the incidence of clinically relevant depressive symptoms per SD lower RNFL thickness, where a higher hazard ratio or rate ratio indicates higher incidence of clinically relevant depressive symptoms or more depressive symptoms over time. In all models 1 SD corresponds with 10.9µm. Model 1: age, sex, glucose metabolism status, and education status; Model 2: model 1 + waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking, alcohol consumption, and partner status. Bold indicates P<0.05. Abbreviations: RR, rate ratio; HR, hazard ratio; CI, confidence interval; RNFL, retinal nerve fibre layer; PHQ-9, patient health questionnaire-9; NA, not applicable.

Table S8.6 Associations of lower RNFL thickness (per SD) with the incidence of clinically relevant depressive symptoms and depressive symptoms over time, additionally adjusted for physical activity (model 3), diet score (model 4), spherical equivalent (model 5), use of anti-depressive medication (model 6), kidney variables (model 7), prior cardiovascular disease (model 8), retinopathy (model 9), glaucoma (model 10), and plasma biomarkers of low-grade inflammation (model 11), or after exclusion of individuals with retinopathy (model 12), or glaucoma (model 13).

	Incidence of clinically relevant depressive symptoms HR (95%CI)	Depressive symptoms RR (95%CI)
Model 3	**1.12 (1.01-1.24)**	**1.04 (1.01-1.07)**
Model 4 [a]	1.10 (1.00-1.22)	**1.04 (1.01-1.06)**
Model 5	**1.15 (1.03-1.27)**	**1.04 (1.02-1.07)**
Model 6	1.10 (1.00-1.21)	**1.03 (1.01-1.06)**
Model 7	**1.11 (1.01-1.22)**	**1.04 (1.01-1.06)**
Model 8	**1.12 (1.01-1.23)**	**1.04 (1.01-1.06)**
Model 9	**1.12 (1.01-1.23)**	**1.04 (1.01-1.06)**
Model 10	**1.15 (1.04-1.28)**	**1.05 (1.02-1.07)**
Model 11	1.13 (0.97-1.30)	**1.04 (1.01-1.08)**
Model 12	**1.13 (1.02-1.24)**	**1.04 (1.01-1.07)**
Model 13	**1.16 (1.05-1.28)**	**1.05 (1.02-1.08)**

Regression coefficients represent hazard ratio for incidence of depressive symptoms or rate ratio for depressive symptoms over time per SD lower RNFL thickness, where a higher hazard ratio or rate ratio indicates higher incidence of clinically relevant depressive symptoms or more depressive symptoms over time. One SD of RNFL thickness corresponds with 10.9 micrometres in the incidence of clinically relevant depressive symptoms study population in model 3 and 11.0 micrometres in the depressive symptoms over time study population. In other models the value per SD was numerically comparable. Variables entered in all models : age, sex, and glucose metabolism status, education status, waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure, use of antihypertensive medication, smoking, alcohol consumption, and partner status. [a] Model 4 was adjusted for the 'adjusted dietary intake score'(i.e. dietary intake score without alcohol consumption as part of the dietary intake score). Number of participants with respectively complete data for incidence of depressive symptoms or depressive symptoms over time : model 3, n=3658 and n=4242; model 4, n=4012 and n=4620; model 5, n=4029 and n=4683; model 6, n=4247 and n=4934; model 7, n=4229 and n=4913; model 8, n=4229 and n=4916; model 9, n=4136 and n=4809; model 10, n=3997 and n=4641; model 11, n=1836 and n=2151; model 12, n=4970 and n=4742; model l3, n=3822 and n=4429. Bold indicates P<0.05. Abbreviations: RR, rate ratio; HR, hazard ratio; CI, confidence interval; RNFL, retinal nerve fibre layer.

Table S8.7 Associations of lower RNFL thickness (per SD) with the incidence of clinically relevant depressive symptoms and depressive symptoms over time, replacement of glucose metabolism status with fasting plasma glucose (model 3A), 2-hour post load glucose (model 3B) or HbA1c (model 3C), waist circumference with BMI (model 3D), office systolic blood pressure with office diastolic blood pressure (model 3E), 24-hour ambulatory systolic blood pressure (model 3F) and 24-hour ambulatory diastolic blood pressure (model 3G), and education status with income level (model 3H) or occupation status (model 3I).

	Incident clinically relevant depressive symptoms, HR (95%CI)	Depressive symptoms RR (95%CI)
Model 3A	**1.11 (1.01-1.23)**	**1.04 (1.01-1.06)**
Model 3B	1.10 (1.00-1.22)	**1.04 (1.01-1.06)**
Model 3C	**1.11 (1.01-1.23)**	**1.04 (1.01-1.06)**
Model 3D	**1.11 (1.01-1.23)**	**1.04 (1.01-1.06)**
Model 3E	**1.11 (1.01-1.22)**	**1.04 (1.01-1.06)**
Model 3F	**1.13 (1.02-1.25)**	**1.03 (1.01-1.06)**
Model 3G	**1.13 (1.02-1.25)**	**1.03 (1.01-1.06)**
Model 3H	1.04 (0.93-1.16)	1.04 (1.00-1.08)
Model 3I	1.05 (0.88-1.26)	1.01 (0.97-1.06)

Regression coefficients represent hazard ratio for incidence of depressive symptoms or rate ratio for depressive symptoms over time per SD lower RNFL thickness, where a higher hazard ratio or rate ratio indicates higher incidence of clinically relevant depressive symptoms or more depressive symptoms over time. One SD of RNFL thickness corresponds with 10.9 micrometres in the incidence of clinically relevant depressive symptoms study population in model 3A and 11.0 micrometres in the depressive symptoms over time study population, and. In other models the value per SD was numerically comparable. Variables in models 3A-4I: age, sex, and glucose metabolism status (where applicable), education status (where applicable), waist circumference, total cholesterol / HDL cholesterol ratio, use of lipid-modifying medication, office systolic blood pressure (where applicable), use of antihypertensive medication, smoking, alcohol consumption, and partner status. Number of participants with respectively complete data for incidence of depressive symptoms or depressive symptoms over time: model 3A, n=4241 and n=4932; model 3B, n=4065 and n=4711; model 3C, n=4239 and n=4929; model 3D, n=4243 and n=4934; model 3E, n=4242 and n=4933; model 3F, n=3795 and n=4380; model 3G, n=3795 and n=4380; model 3H, n=3420 and n=1654,; model 3I, n=1177 and n=1362. Bold indicates P<0.05. Abbreviations: RR, rate ratio; HR, hazard ratio; CI, confidence interval; RNFL, retinal nerve fibre layer.

CHAPTER 9

Neurodegeneration, microvascular dysfunction, and their interaction: associations with cognitive performance – The Maastricht Study

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CHAPTER 10

Summary and general discussion

Summary and General discussion

This chapter consists of five subsections. First, we present main findings of this thesis. Second, we compare our findings with existing literature. Third, we discuss the (biological) interpretation of our findings in detail (we start with a brief overview of the biology that we presume to underlie neuronal and microvascular changes; then we discuss the presumed biology that underlies associations of cardiovascular and lifestyle factors with neurodegeneration and microvascular dysfunction [chapters 2-7]; and, last, we discuss the biology which underlies the association of neurodegeneration with depressive symptoms; and the interaction between neurodegeneration and microvascular dysfunction in the associations of these factors with cognitive performance [chapters 8-9]). Fourth, we discuss the advantages and limitations of the methodology used in this thesis. Last, we discuss the (potential) implications of findings in this thesis for the clinic and for future research.

1. Main findings

This thesis, which used population-based data from up to n=5,666 participants from the Maastricht Study, has four main findings. First, we found that most cardiovascular risk factors, i.e. hyperglycaemia, daily glucose variability, dyslipidaemia, hypertension, blood pressure variability, arterial stiffening, and obesity, and adverse lifestyle factors, i.e. smoking, physical inactivity, lower cardiorespiratory fitness, an unhealthy diet, and [excessive] alcohol consumption, were cross-sectionally associated with neurodegeneration, estimated from lower retinal nerve fibre layer (RNFL) thickness, and/or microvascular dysfunction, estimated from a range of indices of microvascular function (which reflect MVD in various organs; chapters 2-7; Table 10.1 shows main findings and is [additionally] supplemented with previous findings from The Maastricht Study [in italics]). Overall, type 2 diabetes was the strongest determinant of neurodegeneration and microvascular dysfunction. In addition, we had similar findings in analyses with retinal sensitivity, an index of retinal function, as outcome (chapter 3; Table 10.1). Second, in individuals with, versus without, prediabetes and/or type 2 diabetes, we found stronger associations of hypertension with RNFL thickness and retinal sensitivity (chapter 3); of dyslipidaemia with RNFL thickness (chapter 3); and of arterial stiffening (chapter 7), alcohol consumption (chapter 4), and physical activity (chapter 5) with microvascular dysfunction, estimated from a range of indices of microvascular dysfunction (in various organs). Third, in prospective analyses, we found that greater neurodegeneration, estimated from lower RNFL thickness was associated with greater incidence of clinically relevant depressive symptoms (chapter 8). Fourth, in cross-sectional analyses, we found that in neurodegeneration,

estimated from RNFL thickness, was associated with lower cognitive performance in individuals with, but not in individuals without, microvascular dysfunction, where microvascular dysfunction was estimated from retinal venular diameter; and that microvascular dysfunction, estimated from retinal venular diameter, was associated with lower cognitive performance in individuals with, but not in individuals without, neurodegeneration, where neurodegeneration was estimated from RNFL thickness (chapter 9). In addition, both these associations were stronger in individuals with, versus without, hypertension.

2. Findings of this thesis in relation to the existing literature

Findings of this thesis are generally in line with findings from previous population-based studies (a detailed comparison with the existing literature can be found in chapters 2-9).

Findings of the present thesis add to the existing literature in three ways (a detailed literature overview per association can be found in chapters 2-9). First, the present thesis reports an array of associations that have not yet been studied in population-based studies (n>1,000). We list a few examples: no previous population-based studies have yet investigated associations of cardiovascular and lifestyle factors with retinal sensitivity; no previous population-based studies have studied associations of certain cardiovascular risk factors and lifestyle factors such as daily glucose variability and physical activity with RNFL thickness;[1,2] no previous population-based studies have yet reported the association of RNFL thickness with incident clinically relevant depressive symptoms;[3] and no previous population-based studied have investigated whether neurodegeneration and microvascular dysfunction, assessed in the retina, modify each other's associations with cognitive performance.[4] Second, the present thesis reported many associations in a methodologically superior manner to previous studies.[1,2] We adjusted for certain confounders which most previous population-based studies did not take in to account (e.g. physical activity and diet) and we used certain measurement tools which are considered to more precise and valid than measurement tools used in most previous studies (e.g. accelerometer instead of questionnaire-assessed physical activity and 24-hour ambulatory instead of office blood pressure).[5,6] Third, the present thesis comprehensively investigated whether associations differ in strength by sex, glucose metabolism status, and in certain cases, by other cardiovascular risk factors (e.g. by history of cardiovascular disease; in analyses with alcohol consumption as main determinant). These analyses add to the current literature because such analyses that have not, or not so extensively, been investigated by previous population-based studies.[1,2]

Table 10.1 Associations of cardiovascular and lifestyle factors with retinal sensitivity, RNFL thickness, flicker light-induced increase in retinal arteriolar and venular diameters (composite score), and retinal arteriolar and venular diameters (composite score), adjusted for demographic, cardiovascular, and life style factors (model 3), where results in italics indicate previous work from The Maastricht Study (i.e. work that is not part of the present thesis).

		Retinal Sensitivity, per SD		RNFL thickness, per SD		Flicker light-induced increase in retinal microvascular diameter (composite score), per SD		Retinal microvascular diameter (composite score), per SD	
		Number of participants in analyses	stβ (95% CI)	Number of participants in analyses	stβ (95% CI)	Number of participants in analyses	stβ (95% CI)	Number of participants in analyses	stβ (95% CI)
Traditional cardiovascular risk factors									
24-hour ambulatory systolic blood pressure, per SD	3	N=5,074	-0.01 (-0.04; 0.02)	N=4,746	-0.01 (-0.04; 0.02)	*N=1,991*	*0.03 (-0.02; 0.08)**	NA	NA
24-hour ambulatory diastolic blood pressure, per SD	3	N=5,074	0.03 (-0.00; 0.05)	N=4,746	**-0.03 (-0.06; -0.00)**	NA	NA	NA	NA
Mean arterial pressure, per SD	3	N=5,074	0.01 (-0.02; 0.04)	N=4,746	-0.03 (-0.06; 0.01)	NA	NA	NA	NA
Total cholesterol, per SD	3	N=5,664	**0.05 (0.02; 0.08)**	N=5,255	0.03 (-0.00; 0.06)	NA	NA	NA	NA
Waist circumference, per SD	3	N=5,666	-0.01 (-0.05; 0.02)	N=5,255	0.03 (-0.00;0.07)	*N=1,991*	*0.03 (-0.03; 0.08)**	NA	NA
HbA1c, per SD	3	N=5,662	**-0.05 (-0.08;-0.02)**	N=5,249	**-0.05 (-0.08; -0.02)**	*N=2,213*	***-0.12 (-0.17; -0.07*)***	*N=2,876*	*-0.02 (-0.05; 0.01)**
Prediabetes versus NGM	3	NA	NA	N=5,455	-0.05 (-0.13; 0.03)	*N=2,213*	*-0.02 (-0.07; 0.02*)*	*N=2,881*	*-0.04 (-0.15; 0.07)**
Type 2 diabetes versus NGM	3	NA	NA	N=5,455	**-0.16 (-0.25; -0.08)**	*N=2,213*	***-0.11 (-0.16; -0.05)****	*N=2,881*	***-0.14 (-0.24; -0.03)****
Novel cardiovascular risk factors									
Daily glucose variability (assessed as incremental glucose peak), per SD	3	NA	NA	2,407	**-0.06 (-0.12; -0.01)**	NA	NA	NA	NA
Systolic blood pressure variability, per SD	3	NA	NA	NA	NA	N=1,844	-0.03 (-0.08; 0.02)	NA	NA
Diastolic blood pressure variability, per SD	3	NA	NA	NA	NA	N=1,844	-0.03 (-0.08; 0.02)	NA	NA
Lower carotid distensibility coefficient, per SD	3	NA	NA	NA	NA	N=1, 882	**-0.06 (-0.12; -0.00)***	N=2,434	-0.00 (-0.05; 0.05)*
Carotid Young's elastic modulus, per SD	3	NA	NA	NA	NA	N=1, 882	-0.01 (-0.06; 0.05)*	N=2,434	0.02 (-0.02; 0.07)*
Carotid-to-femoral pulse wave velocity, per SD	3	NA	NA	NA	NA	N=1,818	-0.01 (-0.07; 0.05)*	N=2,390	0.03 (-0.02; 0.08)*

Table 10.1 (continued)

Lifestyle factors									
Lower diet score, per SD	3	N=5,369	**-0.06 (-0.09;-0.03)**	N=4,981	**-0.03 (-0.06;-0.00)**	NA	NA	NA	NA
Alcohol consumption		N=5,377		N=4,989		N=2,090			
- None versus light	3		-0.03 (-0.11; 0.05)		0.01 (-0.08; 0.09)		0.02 (-0.11; 0.16)		-0.02 (-0.13; 0.10)
- Moderate versus light	3		0.06 (-0.01; 0.13)		0.04 (-0.04; 0.11)		0.09 (-0.04; 0.21)		0.08 (-0.02; -0.19)
- High versus light	3		0.04 (-0.03; 0.10)		**-0.08 (-0.16; -0.01)**		0.06 (0.06; 0.16)		0.04 (-0.06; 0.14)
Smoking		N=5,666		N=5,255		*N=1,991*			
-Former versus never	3		**0.05 (0.00; 0.11)**		-0.07 (-0.07; 0.05)		NA	NA	NA
-Current versus never	3		**-0.14 (-0.22 ;-0.06)**		0.09 (-0.00;0.18)		*-0.09 (-0.24; 0.05)**	NA	NA
Lower cardiorespiratory fitness, per SD	3	N=4,899	**-0.05 (-0.08; -0.01)**	N=4,542	-0.03 (-0.07; 0.01)	NA	NA	NA	NA
Lower total physical activity, per SD	3	N=5,027	0.01 (-0.02; 0.04)	N=4,510	-0.01 (-0.04; 0.02)	N=1,847	0.09 (-0.11; 0.30)*	NA	NA

Standardized regression coefficients (stβ) represent the difference in retinal sensitivity, RNFL thickness, flicker light-induced increase in retinal microvascular diameters composite score, and retinal microvascular diameters (in SD) for one SD greater 24-hour ambulatory systolic or diastolic blood pressure; greater mean arterial pressure; greater total cholesterol; greater waist circumference; greater HbA1c; greater incremental glucose peak (i.e. daily glucose variability); greater systolic or diastolic blood pressure variability; lower carotid distensibility coefficient; greater carotid Young's elastic modulus; greater carotid-to-femoral pulse wave velocity; lower diet score (without alcohol consumption); lower cardiorespiratory fitness; or lower total physical activity; or for prediabetes or type 2 diabetes versus NGM; former or current versus never smoking; or none, moderate or high versus light total alcohol consumption. A negative beta indicates lower retinal sensitivity, lower RNFL thickness, lower flicker light-induced increase in retinal arteriolar and venular diameters; and wider arteriolar (CRAE) and/or venular (CRVE) diameter (wider retinal diameter is presumed to reflect worse microvascular function). The composite score for flicker light-induced increase in retinal microvascular diameters was calculated as the average of the z-scores for flicker light-induced increase in retinal arteriolar and venular diameter and the composite score for retinal microvascular diameters was calculated as the average of the z-scores for CRAE and CRVE. Carotid distensibility coefficient, diet score, cardiorespiratory fitness, and total physical activity were inverted (i.e. expressed per SD lower instead of per SD higher) so that higher levels per SD reflect exposure to higher levels of adverse cardiovascular risk factors (i.e. greater carotid stiffness, less healthy diet, worse cardiorespiratory fitness, or lower total physical activity). One SD corresponds with 11.6 and 7.2 mm Hg for systolic and diastolic 24-hour ambulatory blood pressure, respectively; 8.0 mm Hg for mean arterial pressure; 1.1 mmol/L for total cholesterol; 13.4 cm for waist circumference; 0.9% for HbA1c; 2.9 mmol/L incremental glucose peak (i.e. daily glucose variability); 5.14 10-3/kPa for carotid distensibility coefficient, 0.37 103/kPa for carotid Young's elastic modulus; 2.07 m/s for carotid-to-femoral pulse wave velocity; 14.6 points for the diet score (without alcohol consumption); 0.6 W/kg for cardiorespiratory fitness; 40.9 minutes/day for physical activity, 1.6 dB for retinal sensitivity; 10.8 micrometres for RNFL thickness; 3.6 for flicker light-induced increase in retinal arteriolar diameter; 4.1 for flicker light-induced increase in retinal venular diameter; 20.2 µm for CRAE; 31.4 µm for CRVE. All values have been calculated in chapters 2-7. The values per SD for systolic or diastolic blood pressure variability are not reported. * indicates that results are shown for flicker light-induced increase in retinal arteriolar diameter, instead of for the composite score based on flicker light-induced increase in retinal arteriolar and venular diameters or for CRAE, instead of for the composite score based on CRAE and CRVE. Additionally, and only for associations of 24-hour ambulatory blood pressure, waist circumference, HbA1c, prediabetes, type 2 diabetes, carotid distensibility coefficient, carotid Young's elastic modulus, carotid-to-femoral pulse wave velocity, total physical activity and current smoking with flicker light-induced increase in retinal arteriolar diameters, flicker light-induced increase in retinal arteriolar diameter was calculated as percentage instead of in absolute increase versus baseline. Bold denotes P<0.05. Results in italics indicate that results of previous work within The Maastricht Study (not part of this thesis) is shown. These results have previously been reported.[1-3] Variables in model 3: age, sex, glucose metabolism status (where applicable), education level, office systolic blood pressure (where applicable), use of antihypertensive medication, waist circumference (where applicable), total cholesterol/ HDL ratio (where applicable), lipid-modifying medication, smoking (where applicable), and alcohol consumption (where applicable). Additionally, for daily glucose variability, we entered HbA1c in model 3 to account for mean glycaemic level; for systolic blood pressure variability we entered mean systolic blood pressure in model 3; and for diastolic blood pressure variability we entered mean diastolic blood pressure in model 3. For reasons of parsimony, results are not shown for the associations in which fasting plasma glucose, 2-hour post load glucose, advanced glycation endproducts assessed with skin autofluorescence, continuous glucose monitoring assessed daily glucose variability, sedentary time and higher intensity physical activity were main determinants. Abbreviations: RNFL, retinal nerve fibre layer; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; SD, standard deviation; CI, confidence interval; HbA1c, glycated haemoglobin A1c; HDL, high density lipoprotein; NGM, normal glucose metabolism; NA not assessed.

3. Biological interpretation of findings

3.1. Interpretation of measurements used in this thesis

In the present thesis we used a range of measures to assess neurodegeneration and microvascular dysfunction. First, we assessed RNFL thickness. Biologically, lower RNFL thickness is thought to reflect a lower number of retinal ganglion cell axons and lower numbers of glial cells, including astrocytes (i.e. indicating neurodegeneration).[7] Second, we assessed retinal microvascular diameters. Biologically, widening and narrowing of retinal arteriolar diameters are thought to reflect early-stage microvascular dysfunction (i.e. impairment of autoregulation) and late-stage microvascular dysfunction (i.e. microvascular remodelling), respectively; and widening of retinal venular diameters is thought to reflect worse microvascular function (in contrast to the pathobiology of the retinal arteriolar microvasculature, the pathobiology of venular deterioration is not biphasic [i.e. venules are thought to widen but not narrow]).[8] Third, we assessed flicker light-induced increase in retinal diameters. Biologically, lower flicker light-induced increase in retinal arteriolar and venular diameters are thought to reflect worse function of the neurovascular coupling unit (i.e. function of neuronal and microvascular structures and their interaction).[9] Fourth, we assessed retinal sensitivity. Biologically, lower retinal sensitivity is thought to reflect worse retinal function, where retinal function relies on the function of retinal neurons.[10] Fifth, we assessed features of cerebral small vessel disease, i.e. white matter hyperintensity volume, cerebral microbleeds, lacunar infarcts, and total brain volume. Biologically, the presence of more features of cerebral small vessel disease reflects greater cerebral microvascular dysfunction.[8,11] Sixth, we assessed skin hyperaemia, albuminuria, and plasma biomarkers of microvascular dysfunction (i.e. intercellular adhesion molecule-1 [sICAM-1], soluble vascular adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin], and Von Willebrand Factor [vWF]). Biologically, lower skin hyperaemia, higher albuminuria, and higher levels of plasma biomarkers of microvascular dysfunction reflect greater microvascular dysfunction.[8,11,12] Seventh, we assessed depressive symptoms and cognitive performance. Biologically, more depressive symptoms and lower cognitive performance are thought to reflect worse brain function, presumably as a consequence of cerebral neurodegeneration and dysfunction of neural networks in brain regions which control these brain functions.[8,13]

3.2. Neuronal and microvascular changes as part of the pathobiology of major clinical disease

The neurovascular coupling unit is an important regulator of autoregulatory function in the brain and retina and impairment of autoregulatory function is thought to be an

important contributor to the early pathobiology of disease of neuronal and microvascular origin.[8,14-16] Pathomechanistically, impaired functioning of the autoregulation is thought to predispose to an enhanced capillary pressure and, consequently, capillary dilation; rupture; leakage; and non-perfusion, ultimately resulting in ischaemia, which can lead to brain and/or retinal injury.[8]

As postulated in "the vascular hypothesis", initial damage to the microvasculature is thought to cause secondary damage to neuronal structures in the brain and retina.[17,18] Biologically, in the arterioles and venules, damage to the endothelial cells impairs haemodynamic autoregulation. Mechanistically, one the hand, dysfunction of arterioles and venules can lead to suboptimal distribution of blood flow to the capillaries and therefore can predispose to ischaemia.[8] Then, on the other hand, dysfunction of arterioles and venules hampers regulation of the hydrostatic pressure, predisposing the capillaries to a detrimentally high intracapillary pressure, which can result in damage to the blood-brain and blood-retinal barrier, and can lead to neurodegeneration.[8,18] Biologically, damage to the blood-brain or blood-retinal barrier enables toxic blood-derived molecules, cells and microbial agents to enter the brain or retina, respectively, and results in impaired interstitial fluid composition, and activation of inflammatory and immune-mediated pathways that can induce neurodegeneration.[8,18]

Mechanistically, oxidative stress is thought to be an important cause of microvascular dysfunction and neurodegeneration.[18-20] Pathomechanistically, oxidative stress can impair endothelial cell nitric oxide (NO) bioavailability, which is detrimental for the microvasculature; and can activate pathways that can lead to neurodegeneration.[18-20] Then, biologically, with regard to the microvasculature, oxidative stress can induce endothelial NO synthase (eNOS) uncoupling which impairs endothelial cell NO bioavailability and can cause endothelial cell dysfunction (at a cellular level, eNOS uncoupling comprises that NO synthesis is reduced and that NO is scavenged by oxidative stress).[20] Next, with regard to neuronal structures, oxidative stress can induce glutamate excitotoxicity which can cause neuronal dysfunction and neurodegeneration (at a cellular level, excess levels of glutamate can cause an enhanced inflow of calcium into neurons, which can activate cellular processes that lead to deterioration of mitochondrial structure and function, and can consequently result in impaired intracellular energy availability; structural neurodegenerative changes [e.g. cytoskeletal disruption and severe myelin damage]; and activation of pathways [e.g. the nuclear factor kappa-beta pathway] that lead to neuronal apoptosis).[18,19] Additionally, also ischaemia may, independently of glutamate toxicity-mediated pathways, activate pathways that lead to mitochondrial damage, and via mitochondrial damage lead to neuronal apoptosis.[21]

3.3. Biological interpretation of associations of cardiovascular risk and adverse life style factors as determinants of neurodegeneration and microvascular dysfunction

3.3.1. Hyperglycaemia and daily glucose variability (chapters 2 and 3)

We had three findings. First, we found that greater glycaemia, estimated from fasting plasma glucose, 2-hour post load glucose, haemoglobin A1c, advanced glycation end products (AGEs; assessed as skin autofluorescence with the AGE reader), and/or duration of diabetes, was associated was lower RNFL thickness and lower retinal sensitivity. Second, we found that prediabetes and type 2 diabetes (versus normal glucose metabolism as reference) were associated with lower RNFL thickness. Third, we found that greater daily glucose variability was associated with lower RNFL thickness.

These findings are consistent with the concept that hyperglycaemia is detrimental for the microvasculature as well as for neurons and extend previous work from the Maastricht Study.[8,9,22,23] Mechanistically, hyperglycaemia is thought to induce intracellular oxidative stress in endothelial cells and neurons via the activation of four intracellular pathways (i.e. polyol pathway, hexosamine pathway, protein kinase C activation, and the receptor for AGEs [RAGE] activation).[8,24] Additionally, a vicious circle likely exists between hyperglycaemia and microvascular dysfunction, where hyperglycaemia can induce microvascular dysfunction and microvascular dysfunction can lead to an impaired disposal of glucose, which can further aggravate microvascular dysfunction.[8]

Daily glucose variability, which reflects peak high and/or peak low levels of glycaemia, may be detrimental for microvascular and neuronal structures via two mechanisms. First, peak high glucose levels may induce greater levels of oxidative stress than average high glucose levels.[25,26] Second, hypoglycaemic nadirs (in individuals with diabetes treated with agents that can induce hypoglycaemia) may impair glucose availability, an important source of energy supply for neurons, and predispose to ischaemia.[25,26]

The finding that RNFL thickness was increasingly lower in individuals with prediabetes and type 2 diabetes is in agreement with “ticking clock hypothesis”.[8,27-30] Hyperglycaemia-induced neurodegeneration and microvascular dysfunction is likely a continuous, gradual process that starts in prediabetes, progresses with the onset of type 2 diabetes, and continues during type 2 diabetes.[8,27-30]

3.3.2. Dyslipidaemia (chapter 3)

We found that greater lipid levels ("dyslipidaemia"), estimated from total cholesterol, were associated with *greater* RNFL thickness and *greater* retinal sensitivity, the former in individuals with, but not in individuals without, type 2 diabetes. These findings do not support the concept that dyslipidaemia may be a risk factor for the retinal neurodegeneration and dysfunction.

Mechanistically, greater total cholesterol, which reflects higher levels of circulating cholesterol, may be beneficial for the retina as cholesterol can increase synaptogenesis in the retina.[31] Then, as under hyperglycaemic circumstances neuronal and microvascular structures (i.e. neurovascular coupling unit) are functionally impaired, the ability to ensure a continuous supply of cholesterol to the retina is likely reduced, resulting in lower levels of retinal cholesterol, and subsequently less synaptogenesis, in individuals with, versus without, type 2 diabetes.[8,31]

3.3.3. Obesity (chapter 3)

We found that greater waist circumference was neither associated with RNFL thickness nor with retinal sensitivity. These findings do not support the concept that obesity may be a risk factor for the retinal neurodegeneration and dysfunction.

Mechanistically, higher levels of visceral (white) adipose tissue, which greater waist circumference represents, likely leads to higher levels of adipokines in the circulation, which may have bidirectional effects on retinal neurons.[32] For example, directionally opposing effects have been reported for tumour necrosis factor-alpha [TNF-alpha]), a relatively well-studied adipokine.[32] On the one hand TNF-alpha has been found to have neuroprotective effects (by reducing levels of oxidative stress in neurons).[32] On the other hand TNF-alpha has been found to have effects which can predispose to neurodegeneration (TNF-alpha can increase levels of oxidative stress in the retinal microvasculature, which can lead to blood-retinal barrier dysfunction).[32]

3.3.4. Hypertension (chapter 3), blood pressure variability (chapter 6), and arterial stiffness (chapter 7)

We had three findings. First, we found that antihypertensive medication use, an index of history of hypertension, was associated with lower RNFL thickness, but not retinal sensitivity; that greater 24-hour ambulatory diastolic blood pressure was associated with lower RNFL thickness, but not retinal sensitivity; and that greater 24-hour ambulatory systolic blood pressure was more strongly associated with lower RNFL thickness and retinal sensitivity in individuals with, versus without, type 2 diabetes. Second, we found that greater blood pressure variability was associated with greater microvascular dysfunction, estimated from albuminuria, plasma biomarkers of

microvascular dysfunction, features of cerebral small vessel disease, and flicker light-induced increase in retinal diameters, but, in contrast, blood pressure variability was not associated with microvascular dysfunction estimated from heat-induced skin hyperaemia. Third, we found that greater carotid stiffness was associated with lower flicker light-induced increase in retinal arteriolar and venular diameters, associations that were stronger in individuals with, versus without type 2 diabetes. Then, we found that aortic stiffness was not associated with flicker light-induced increase in retinal diameters; and that carotid and aortic stiffness were not associated with retinal arteriolar and venular diameters.

These findings are consistent with the concept that hypertension, blood pressure variability, and arterial stiffening are detrimental for the microvasculature, and via microvascular dysfunction may lead to neuronal injury and neurodegeneration. Below we discuss the pathobiology of hypertension, blood pressure variability, and arterial stiffness.

3.3.4.1 Hypertension

Hypertension, assessed as elevated blood pressure in the brachial artery, is thought to induce endothelial cell dysfunction via impairing NO bioavailability.[33-35] Pathobiologically, an elevated blood pressure can expose the microvasculature to oscillatory disturbed shear stress, which can induce eNOS uncoupling and lead to microvascular dysfunction (in contrast to physiological circumstances, where normal blood flow induces unilamellar shear stress that leads to the activation of cell-membrane located mechanosensors and subsequent NO synthesis).[33-35] Then, as hyperglycaemia is detrimental for neuronal and microvascular structures, which regulate capillary pressure, individuals with, versus without, type 2 diabetes may be more susceptible to hypertension.[8]

Then, a vicious circle between microvascular dysfunction and hypertension likely exists.[8] First, hypertension can cause loss of capillaries (i.e. capillary rarefaction), which can increase peripheral resistance and lead to an increase blood pressure.[8] Second, the microvasculature is thought to remodel inwardly in response to hypertension (in order to raise peripheral resistance and prevent a detrimentally high capillary pressure) and such inward remodelling can also increase peripheral resistance and lead to an increased blood pressure.[8]

3.3.4.2 Blood pressure variability

Greater blood pressure variability is thought to reflect dysfunction of mechanisms that serve to stabilize the blood pressure (e.g. dysfunction of the parasympathetic, and sympathetic autonomic nerve system and the baroreceptor reflex) and can be detrimental for the microvasculature via two mechanisms. First, greater blood pressure can induce a pulsatile pressure that can penetrate distally and damage the

microcirculation.[36] Second, greater blood pressure can lead to sudden falls in blood pressure, which can reduce microvascular perfusion.[37] Pathobiologically, both mechanisms can lead to oscillatory disturbed shear stress and thereby induce endothelial cell dysfunction (as described above for hypertension).[33,38] In addition, sudden falls in blood pressure may impair the continuous flow of blood and nutrients to the capillaries and therefore predispose to ischaemia, which can activate pathways that can induce neuronal apoptosis.[38]

A likely explanation why blood pressure variability, in contrast to other findings, was not associated with heat-induced skin hyperaemia, is that the skin, in contrast to other organ systems under study, has a relatively high microvascular impedance.[36] Therefore, most of the increased pulsatile energy related to greater blood pressure variability may have dissipated by arteries and large arterioles proximal to the skin capillaries.[39]

3.3.4.3. Arterial stiffness

Arterial stiffening is thought to reflect impaired functioning of the cushioning capacity of large arteries due to large artery remodelling.[40] Mechanistically, impaired cushioning capacity is thought to lead to an improper propagation of flow waves which can cause both a detrimental elevation of the systolic blood pressure as well as a detrimental reduction in diastolic blood pressure.[40] Biologically, an elevated systolic blood pressure can result in the transmission of detrimentally high pulsatile energy into the microcirculation and a relatively too low diastolic blood pressure can lead to a reduced (i.e. too low) flow of blood in the capillaries during the diastole.[40] In addition, both mechanisms can lead to oscillatory disturbed shear stress and thereby induce endothelial cell dysfunction (as described above for hypertension).[33,40] Additionally, a relatively too low diastolic blood pressure may impair the continuous flow of blood and nutrients to the capillaries and therefore predispose to ischaemia, which can activate pathways that lead to neurodegeneration.[8,18]

The retinal microvasculature may be more vulnerable to haemodynamic stress in the presence of hyperglycaemia.[8,18] Associations of carotid stiffness with flicker light-induced increase in retinal arteriolar and venular diameters were likely stronger because hyperglycaemia impairs both neuronal[41] and endothelial cell[8] function (i.e. neurovascular coupling) and intact neurovascular coupling is required for optimal function of the neurovascular coupling unit.[15]

We observed no association of arterial stiffness with (narrower) retinal arteriolar diameters, possibly because inward remodelling (the proximate cause of arteriolar narrowing)[36] occurs in a more advanced disease stage than was present in the population that we studied. Additionally, and similar to most previous studies,[42-44] we observed no association of arterial stiffness with retinal venular diameters, possibly because arterial stiffness-induced haemodynamic stress in venules is insufficient to affect their structure.

Aortic stiffening, which reflects both muscular and elastic arterial remodelling (in contrast to carotid stiffening which [mostly] reflects elastic remodelling), may not have yet occurred to such an extent that greater aortic stiffening-induced pulsatile haemodynamic affects the retinal microvasculature.[45] Therefore, we may not have found associations between aortic stiffness and flicker light-induced increase in retinal microvascular diameters and aortic stiffness and retinal microvascular diameters.

3.3.5. Smoking (chapter 3)

We found that current smoking was associated was greater RNFL thickness and lower retinal function; and that the former association was strongly attenuated after adjustment for spherical equivalent.

These observations are in part consistent with the concept that smoking may be detrimental for microvascular and neuronal structures. Mechanistically, smoking, which represents greater exposure to nicotine and free oxygen radicals (i.e. the bioactive components of cigarette smoke) is thought to be detrimental for the retinal microvasculature and neuronal cells via increasing levels of oxidative stress.[19,46]

Possibly, smoking may not be a (strong) determinant of RNFL thickness, as found in the UK biobank (n~32,000),[1] and RNFL thickness layer may in smokers, spuriously, seem thicker, due to effects of smoking on the cornea.[47] Mechanistically, smoking-associated corneal changes can cause hyperopia and hyperopia-associated over-convergence of light may lead to the assessment of peripapillary RNFL thickness at a location that is relatively too close to the optic nerve head (where there are relatively more axons and the RNFL thickness is thus greater).[48,49]

3.3.6 Lower physical activity (chapters 2 and 5) and cardiorespiratory fitness (chapter 2)

We had three findings. First, we found that lower cardiorespiratory fitness, but not lower total physical activity, was associated with lower RNFL thickness and lower retinal function. Second, we found that lower total physical activity and lower high-intensity physical activity, but not sedentary time, were associated with lower heat-induced skin hyperaemia; and that the associations of greater total physical activity and high-intensity physical activity with greater heat-induced skin hyperaemia were stronger in individuals with, versus without type 2 diabetes. Third, we found that total physical activity, high-intensity physical activity, and sedentary time were not associated with flicker light-induced increase in retinal arteriolar diameter.

These findings in part support the concept that physical activity may have certain beneficial effects on the microvasculature as well as on neurons. Hence physical inactivity may be a risk factor for the development of neurodegeneration and microvascular dysfunction.

The mechanisms which underlie how physical activity can be neuroprotective and increase microvascular function are distinct.[20,50-52] With regard to neurons, mechanistically, in response to physical activity muscles can release levels of certain bioactive factors, including brain-derived nerve factor (BDNF) which can pass the blood-brain barrier and can induce neuroprotective pathways.[50] For example, BDNF is thought to activate a number of pathways, that are involved in regulation of neuronal cell survival, proliferation, and axon and dendrite growth.[53] In addition, physical activity can induce epigenetic modifications (e.g. DNA methylation), which can alter neuronal cell gene expression and change neuronal cell function.[50] For example, via epigenetic effects physical activity is thought to regulate the elimination of toxic proteins that can aggregate in neuronal tissue.[50] Next, with regard to the microvasculature, mechanistically, physical activity can increase blood flow and thereby induce an increase in microvascular laminar shear stress, which can stimulate the synthesis of NO by eNOS, increasing NO bioavailability.[20]

In contrast to lower cardiorespiratory fitness, which represents physiological adaptation to long-term exposure to physical activity, lower total physical activity, which represents lower exposure to physical activity, was not associated with lower retinal sensitivity or lower RNFL thickness. Possibly we had null findings for total physical activity because other factors than the amount of physical activity determine the extent of the neuroprotective physiological response to physical activity.[54] Indeed, besides the amount of physical activity, also genetic factors and the type, frequency, and intensity of activity have been found to determine the physiological response to physical activity.[50,54]

Total and higher-intensity physical activity were more strongly associated with greater heat-induced skin hyperaemia in individuals with, versus without, type 2 diabetes, possibly because physical activity can besides stimulation of NO synthesis also reduce endothelial cell insulin resistance and thereby inhibit eNOS uncoupling.[20,51]

Greater sedentary time was not associated with lower heat-induced skin hyperaemia, likely because frequent transient interruptions of sedentary behaviour by standing and/or walking can restore sedentary behaviour‐induced microvascular dysfunction, presumably via changes in shear stress.[54] Indeed, a higher number of such interruptions, which in our study population occurred on average ~ 4 times per sedentary hour, have been shown to be associated with reduced cardiovascular mortality.[50,54]

Greater total physical activity and greater sedentary time were not associated with flicker light-induced increase in retinal arteriolar diameter, possibly due to autoregulation in the retina.[55] During physical activity, constriction of small retinal arterioles increases retinal vascular resistance in proportion to the increase in ocular perfusion pressure to stabilize retinal perfusion, presumably to maintain visual acuity.[55]

Therefore the extent of physical activity-induced shear stress that reaches the retina may be too low to be able to lead to an increase in microvascular function.

3.3.7. An unhealthy diet (chapter 3) and alcohol consumption (chapters 3 and 4)

We had three main findings. First, we found that lower adherence to a healthy diet (where alcohol consumption was left out of this adherence to a healthy diet score) was associated with lower RNFL thickness and lower retinal sensitivity. Second, we found that high versus light alcohol consumption was associated with lower RNFL thickness, but was not associated with retinal sensitivity. Third, we found a J-shaped association between alcohol consumption and microvascular dysfunction, estimated in various organ systems; where moderate versus light alcohol consumption was associated with less microvascular dysfunction and higher-than-moderate, versus light, alcohol consumption was associated with more microvascular dysfunction. In addition, the location of the mathematical minimum of the J-curve (i.e. the turning point of the J-curve [which indicates from which quantity of alcohol consumption more alcohol consumption is associated with more instead of less microvascular dysfunction]) differed by history of cardiovascular disease and sex; and the depth of the minimum of the J-curve (which indicates the strength of the effects of alcohol consumption on the microvasculature) differed by history of cardiovascular disease. In addition, the location of the minimum of the J-curve and the depth of the J-curve also differed by glucose metabolism status, and other cardiovascular risk factors (including hypertension, dyslipidaemia status, and smoking status).

These findings are in line with the concept that an unhealthy diet and high alcohol consumption are detrimental for the microvasculature as well as for neurons.

Mechanistically, an unhealthy diet may be detrimental because it lacks a range of potentially beneficial nutrients and because it lacks certain essential nutrients that cannot be synthesized within the human body.[56-59] We discuss two examples. First, a healthy diet contains vitamins C, vitamin E, and β-carotene, and certain polyphenols (e.g. resveratrol).[57-59] These nutrients are thought to have antioxidant effects (i.e. thus can reduce levels of oxidative stress).[57-59] Second, an unhealthy diet may lack certain long-chain polyunsaturated fatty acids (e.g. omega 3 and 6 polyunsaturated fatty acids [n-3 and n-6 PUFAs]) which are fats that cannot be synthetized in the central nervous system but are essential for the synthesis and maintenance of cell membranes.[56,60]

Mechanistically, alcohol consumption, which reflects intake of ethanol (the main bioactive component in alcoholic beverages), can be detrimental for neurons and the microvasculature as at higher concentrations of alcohol consumption (i.e. greater than moderate alcohol consumption) levels of ethanol can be so high that they cause oxidative stress in neurons and endothelial cells.[61] However, the exact concentration at which alcohol consumption becomes detrimental likely depends on the level of

background oxidative stress in an individual (higher levels of alcohol consumption may be required before ethanol-induced oxidative stress exceeds [background] cardiovascular risk factor-induced oxidative stress); and on the sex of an individual (the distribution volume and rate of breakdown of ethanol differ between men and women).[61] In addition, the effects of alcohol consumption on the microvasculature are likely stronger in individuals with, versus without a history of cardiovascular disease (i.e. with presumed higher, versus lower, levels of oxidative stress) because polyphenols in alcoholic beverages can scavenge oxidative stress (thereby directly reducing levels of oxidative stress) as well as can intervene in an oxidative stress-induced vicious circle that leads to higher levels of oxidative stress.[62,63]

3.4. Retinal neurodegeneration in relation to depressive symptoms (chapter 8) and the interaction between retinal neurodegeneration and microvascular dysfunction in relation to cognitive performance (chapter 9)

We had two main findings. First, we found that lower RNFL thickness, was associated with greater incidence of clinically relevant depressive symptoms. Second, we found that low versus high RNFL thickness, was associated with lower cognitive performance in individuals with, but not in individuals without microvascular dysfunction, where microvascular dysfunction was estimated from retinal venular diameter; and we found that wide versus narrow retinal venular diameter was associated with lower cognitive performance in individuals with, but not in individuals without, neurodegeneration, where neurodegeneration was estimated from RNFL thickness. In addition, the latter associations were stronger in individuals with, versus without hypertension.

3.4.1. Neurodegeneration and microvascular dysfunction in the retina and brain in relation to each other

Both findings are in support of the concept that the pathophysiology of neurodegeneration and microvascular dysfunction in the retina and brain may be similar.

A similar pathobiology for neurodegeneration and microvascular dysfunction in the retina and brain is biologically plausible. First, retinal and brain neuronal and microvascular structures are similar as they have a shared embryology.[64] Second, the retina and brain have a similar anatomy and physiology.[64] For example, in the retina and in the brain the blood-retina barrier and the blood-brain barrier, respectively, are anatomically and physiologically similar.[64] Third, pathobiological processes that have been found lead to neurodegeneration and microvascular dysfunction in the brain have

been found to occur in the retina. For example, accumulation of amyloid beta has been found in the brain as well as in the retina of individuals with dementia.[64]
Certain differences may exist between the retinal and brain pathobiology. Impairment of autoregulation may induce detectable neuronal and microvascular degeneration in the retina before such degeneration has occurred in the brain.[26] Biologically, axons of neuronal cells located in the retina are energetically inefficient because they lack myelin.[26] Therefore, retinal neuronal cells may be more susceptible to any early impairment of autoregulation than brain neuronal cells.[26]

3.4.2. Retinal neurodegeneration, microvascular dysfunction and their interaction in relation to cognitive performance

Low versus high RNFL thickness, which reflects more loss of retinal ganglion cell axons and astrocytes,[8] and wide versus narrow retinal venular diameter, which reflects more endothelial dysfunction,[8] were non-linearly associated with lower global cognitive performance, which reflects lower function of neural networks in brain regions which control cognitive function, in, respectively, individuals with a wide retinal venular diameter (i.e. “with presumed microvascular dysfunction”) and a low RNFL thickness (i.e. “with presumed neurodegeneration”).[65] Mechanistically, loss of neurons and astrocytes as well as endothelial cell dysfunction likely leads to lower function of the neurovascular coupling unit, which can lead to impairment of autoregulatory function and dysfunction of the blood-brain barrier, both processes which can predispose to cerebral neurodegeneration. Pathobiologically, these processes can result in the impaired availability of nutrients (i.e. ischaemia); the leakage of toxic compounds from the blood (e.g. amyloid beta); and the impaired clearance of waste products (e.g. amyloid beta); and all these processes can, in turn, activate pathways that lead to cerebral neurodegeneration, dysfunction of neural networks, and lower global cognitive performance.[18]
Low versus high RNFL thickness and wide versus narrow retinal venular diameter were not associated with lower global cognitive performance in, respectively, individuals with a narrow, retinal venular diameter (i.e. “without presumed microvascular dysfunction”) and a high RNFL thickness (i.e. “without presumed neurodegeneration”). Mechanistically, in individuals with microvascular dysfunction or, but not and, neurodegeneration, the ability to control autoregulation and the permeability of the blood brain barrier may not be so strongly impaired that ischaemia, the leakage of toxins, and the clearance of waste products occurs to such an extent that cerebral neurodegeneration and dysfunction of neural networks occurs.[15,18,66] Possibly, this may be because within the neurovascular coupling unit neuronal and microvascular structures may be able to compensate for each other.[15,18,66] Mechanistically, via feed-forward and feed-back interactions between neuronal and microvascular structures

autoregulation may to a certain extent be maintained.[15,18,66] In addition, as the permeability of the blood-brain barrier is regulated by both neurons and endothelial cells via tight junctions, in individuals with either neurodegeneration or microvascular dysfunction tight junctions may remain sufficiently intact to (up to a certain extent) adequately regulate the permeability of the blood-brain-barrier.[15,18]
Hypertension modified the above associations, where RNFL thickness and retinal venular diameter were more strongly associated with lower global cognitive performance in individuals with, versus without, respectively, hypertension as well as a wide retinal venular diameter (i.e. "with presumed microvascular dysfunction") and hypertension as well as a low RNFL thickness (i.e. "with presumed neuro-degeneration"). In individuals with, versus without, hypertension associations were likely stronger because in the presence of hypertension levels of haemodynamic stress are higher and normal autoregulatory function is likely required to under such circumstances achieve a normal capillary pressure.[8,18,66,67] Mechanistically, in individuals with, versus without, both a worse autoregulatory function and greater haemodynamic stress, capillary pressure is more likely to be detrimentally high, resulting in more loss of retinal capillaries (i.e. rarefaction), greater levels of ischaemia, and a lower ability to clear waste products; all of which are processes that may lead to a greater loss of cerebral neuronal structures and a stronger reduction in global cognitive performance in such individuals.[18,66,67]

4. Methodological considerations

This thesis used prospective and cross-sectional data from an observational population-based cohort study. Such an approach has a certain strengths and limitations. Below we discuss strengths and limitations with regard to selection bias, confounding bias, information bias, other sources of bias, and causality.

4.1. Selection bias

An important strength of this thesis is the use of data from a large number of individuals (up to n~5,600) from the general population. The large number of individuals and the sampling of individuals from the general population both reduce the chance of selection bias. In addition, the oversampling of individuals with type 2 diabetes reduces the chance of selection bias in this clinically-relevant subgroup of individuals.
Limitations of population-based studies in general are that relatively more healthy individuals may be more likely to take part in population-based studies; and that

relatively sicker individuals that take part in population-based studies may be more likely to have missing data. In this thesis we did not find strong evidence in support of these potential limitations. For example, the proportion of individuals with a high education level (~35% in The Maastricht Study) is similar to the proportion of individuals with a high education level according to the Dutch national registry (~ 30%; "Central Bureau voor de Statistiek").[68] Then, we found that general characteristics of individuals with missing data (i.e. individuals excluded from analyses) did not clinically-relevantly differ from general characteristics of individuals with complete data (i.e. individuals included in analyses). However, even though these differences were not clinically relevant, individuals who were excluded from analyses generally were generally older and had somewhat more adverse general characteristics (e.g. were more likely to have type 2 diabetes).

To conclude, in our opinion findings from the population-based Maastricht Study provide a representative cross-section of the general population and therefore findings can be considered valid in the general population.

4.2. Confounding bias

An important strength of this thesis is that a large number of important confounders were included in analyses. The use of a large range of important confounders reduces the chance that the strength of associations in this thesis were spuriously estimated due to residual confounding.[69] Indeed, we adjusted for key cardiovascular as well as lifestyle factors.[69] Additionally, we also accounted for important covariates which may be confounders and/or mediators and/or descending/ascending proxies (e.g. history of cardiovascular disease, kidney variables, ocular variables, and plasma biomarkers of low-grade inflammation).[69,70]

Another strength of this thesis is that we investigated whether glucose metabolism status or sex modified associations under study.

A limitation of this thesis is that we did not account for genetic and environmental confounders. Therefore, certain residual confounding due to unmeasured factors may affect the results of this study.[69] For example, air quality may be an environmental confounder.[71] Biochemically, the amount of pollution may differ between certain neighbourhoods in Maastricht, e.g. in neighbourhoods with more traffic the air may contain relatively more toxic compounds than in neighbourhoods with less traffic.[72] Additionally, certain interactions between genetic and/or environmental factors and certain cardiovascular and/or lifestyle factors may exist. For example, physical activity may more strongly prevent neurodegeneration in individuals with, versus without, certain apolipoprotein E (APOE) mutations.[73]

Although we cannot fully exclude that some residual confounding due to genetic and environmental factors may have occurred, the influence of genetic and environmental confounders is likely minimalized by the design of The Maastricht Study. First, participants were included from a relatively small region (i.e. Maastricht and surrounding villages), therefore a large number of environmental factors may be generally similar for most participants of the Maastricht Study.[69] Second, the population may be relatively stable over time, therefore the heterogeneity in genetics may be relatively smaller than if such a study would be performed in a different city (e.g. New York).[69] Indeed, the migration of individuals to Maastricht is relatively low (~0.04%).[74]

To conclude, in our opinion confounding was well controlled for in the present thesis and therefore findings of this thesis likely provide numerically accurate estimates of the associations under study in the general population.

4.3. Information bias

An important strength of this thesis is that covariates used in analyses in this thesis were measured with state-of-the-art measurement techniques with high reliability (Table 10.2 shows interobserver reliability for the most-used covariates in this thesis).[69] The use of such state-of-the-art measurement techniques reduces the chance that we spuriously estimated the strength of associations under study due to measurement error.[69] In addition, the assessment of certain factors with objective measurement techniques instead of relatively more subjective measurement techniques reduces the chance of measurement error (e.g. the assessment of physical activity with an accelerometer instead of with a questionnaire).[69]

The use of certain measurement techniques, such as questionnaire, may have limitations.[69] For example, average daily alcohol consumption was measured with a questionnaire and as a questionnaire is less objective measure than an objective biometric measure (e.g. a blood test) the use of a questionnaire may to a certain extent result in some measurement error.[69] Indeed, individuals with high alcohol consumption may underreport their alcohol consumption.[75]

To conclude, overall, the impact of any measurement error on our findings is likely small, therefore, in our opinion, findings of this thesis can be considered to be numerically accurate estimates of the strengths of associations under study in the general population.

Table 10.2 Inter-observer reliability of the most used measures in the present thesis.

Variables	Inter-observer reliability, expressed as ICC	References
Retinal nerve fibre layer thickness	0.96	[76]
Flicker light induced increase in retinal arteriolar diameter	0.80	[9]
Flicker light induced increase in retinal venular diameter	0.87	[77]
Central retinal arteriolar equivalent (retinal arteriolar diameter)	0.88	[78]
Central retinal venular equivalent (retinal venular diameter)	0.90	[78]
Retinal sensitivity	0.95	[79]
Fasting plasma glucose	0.98*	[80]
Office systolic blood pressure	0.90*	[81]
Total cholesterol	0.93*	[82]
Waist circumference	0.85	[83]

Inter-observer reliability, expressed as ICC, is shown for the measures that are most used in the main analyses. If data were available within The Maastricht Study, the inter-observer reliability calculated in The Maastricht Study are shown. If these data were not available within The Maastricht Study, the inter-observer reliability from other studies was shown. No inter-observer reliability data were available for age, sex, education status or medication use. Data on the percentage of agreement for quality decisions and data on inter-observer reliability for other measures are reported in the (Supplemental) methods sections in chapters 2-9. * for certain covariates we could not identify inter-observer reliability expressed as ICC in literature. For these covariates the number presented was calculated as 100% minus the coefficient of variation (so that for all measures higher numbers correspond with better reliability). Abbreviation: ICC, intraclass correlation coefficient.

4.4. Other sources of bias

We made certain methodological choices in our analyses, choices which have certain advantages and in some cases can have some potential disadvantages. Here we discuss a number of these choices, i.e. the use of composite scores in analyses; analyses of change variables; and the handling of missing data.

4.4.1. Use of composite scores

We used composite scores in several chapters, including for cognitive performance, blood pressure variability, and microvascular function.

The use of a composite score has certain advantages. One important advantage is that a composite measure provides a simple, interpretable overall measure.[84] A second advantage is that a composite score can reduce the 'noise' due to measurement error. However, it is important to note that this noise may be not only be fully caused by measurement error, but may (in part) be due to biological heterogeneity.[84] For example, the pathophysiology which leads to lower memory function, lower executive function, and lower information processing speed may in part be similar but in part be distinct (regions that regulate these brain functions differ and between these regions anatomical and physiological differences may exist).[85]

Next, the use of a composite score may also have certain disadvantages, including that the use of a composite score may result in the loss of information and a lower statistical

efficacy.[84] For example, when continuous variables are combined into a categorical variable as a composite score, the statistical efficacy of analyses may be reduced.[69] Certain examples of composite scores that are not statistically optimal can be found in this thesis. A first example, to calculate the adherence to the Dutch healthy diet composite score scores for 15 individual dietary components are combined and to calculate this sum score certain continuous variables are (partially) categorized: e.g. alcohol consumption up to moderate alcohol consumption is scored as 1 to 10 points but all alcohol consumption greater than moderate, independent of the precise quantity, is scored as 0 points (i.e. no distinction is made between high and very high alcohol consumption). A second example, we used a categorical composite score for cerebral small vessel disease to study the association of blood pressure variability with cerebral small vessel disease, where certain continuous covariates were categorized (chapter 6). In retrospect, for optimal statistical efficiency, we should have composed a continuous composite score for cerebral small vessel disease, as we did when we investigated alcohol consumption as a determinant of cerebral small vessel disease (chapter 4).
Then, another disadvantage of the use of a composite score may be that the use of a composite score can result in a somewhat spurious estimate of the strength of associations. We present one example from this thesis. We investigated whether adherence to a healthy diet (based on a composite score where individual dietary components were combined) was associated with RNFL thickness and retinal sensitivity; and may have underestimated the overall strength of the effects of diet because we combined components in a composite score (i.e. we may have found stronger associations if we would have calculated the total effect of dietary intake on outcomes by summing the betas for individual components). We checked whether this reasoning was correct by comparing the strength of the association of alcohol consumption with RNFL thickness with the strength of the associations of dietary intake, estimated from either all 15 components or 14/15 components (i.e. without alcohol) of a Dutch healthy diet. Indeed, we found that the beta for alcohol consumption entered separately in to the model was larger than the difference in beta between composite scores for the Dutch healthy diet with and without alcohol consumption (after adjustment for cardiovascular and lifestyle factors; data not shown).

4.4.2. Analyses with change variables

The present thesis used two so-called ‘change variables’, i.e. flicker light-induced increase in retinal microvascular diameters and heat-induced skin hyperaemia. For these measures the size of the change in diameter or perfusion, induced by exposure to flicker light or heat (where appropriate), is the index of interest. The ‘change’ is calculated based on the baseline value (the diameter or perfusion before exposure to flicker-light or heat) and the post-value (the average diameter or perfusion during the

exposure to the flicker-light or heat). In the present thesis we expressed change as the absolute increase in diameter or absolute increase in perfusion (i.e. post-value minus baseline value); as the percentage increase (i.e. [post-value minus baseline value]/baseline value); and as the post-value (with adjustment for the baseline value).
Methodologically, certain definitions of 'change variables' can have certain methodological advantages and disadvantages.[86,87]
First, from a methodological perspective, expressing change as a percentage is, of the above reported options, likely the least advantageous method. Mathematically, percentage change is a ratio and as certain covariates may be differently associated with the numerator and denumerator, in certain cases analyses with a change variable expressed as percentage may result in a spurious estimate of the strength of an association.[87] Indeed, we observed this in the association between physical activity and heat-induced skin hyperaemia (Chapter 5). In this chapter we handled this situation by using the post-value and adjusting for the baseline value. However, this phenomenon does not have to occur. Indeed. in other chapters where we expressed change as a percentage we did not observe spurious estimates (Chapters 6 and 7).
Second, the statistical power in analyses can differ depending on the definition of a change variable, where this difference in power between different definitions of change variables depends on the strength of the correlation between the baseline and post-value score.[87] When there is a high correlation (ρ=0.80), the statistical power for change expressed as "post-value minus the baseline value"; and "post-value with adjustment for baseline" are similar (statistical power of ~98%).[87] However, when there is a low correlation (ρ=0.20), the "post-value with adjustment for the baseline value"-method is a statistically more powerful technique than the "post-value minus baseline value" method (statistical power of ~70% instead of ~50%, respectively).[87] We calculated the correlations for the measures used in this thesis and found that the correlation between baseline arteriolar or venular diameter and flicker light-induced increase in retinal arteriolar or venular diameter was high (ρ=0.98 and ρ=0.97, respectively); and, opposingly, the correlation between heat-induced skin hyperaemia and baseline perfusion was low ρ=0.22). Therefore there is no single statistically most efficient, thus methodologically most advantageous, approach for flicker light-induced increase in retinal microvascular diameters, but there is for heat-induced skin hyperaemia. For analyses with heat-induced skin hyperaemia, analysing associations with the "post-value" as outcome and adjusting these associations for the baseline value is the most advantageous. Therefore we used the latter approach for analyses with heat-induced skin hyperaemia in chapters 4 and 5.

4.4.3. Missing data

In the present thesis we used complete-case analysis in all analyses because for nearly all associations under study, less than 5% of individuals with data available on the independent and the dependent variable had missing data on covariates. We did not impute missing data because imputation would for these analyses not result in a relevant increase in statistical power or better representation of the general population.[88]

Only for few associations under study in this thesis slightly more than 5% of individuals that complete data on the determinant and outcome had missing data on covariates (i.e. for the associations of alcohol consumption and physical activity with heat-induced skin hyperaemia [chapters 4 and 5, respectively] and for the associations of RNFL thickness and CRVE with cognitive performance [chapter 9]). To check whether imputation would lead to different results we, as an example, analysed the association between alcohol consumption and heat-induced skin-hyperaemia after missing data was imputed (settings multiple imputation; predicted mean matching, 100 iterations; 6 datasets because 6% of participants had missing data). After adjustment for potential confounders (same confounders as used in the main analyses), we found numerically similar results (data not shown).

4.4.4. Conclusion with regard to other sources of bias

To conclude, overall, the impact of 'other sources of bias' on our findings is likely small. Therefore, in our opinion, this thesis provides numerically accurate estimates of the associations under study in the general population.

4.5. Causality

In order to be able to conclude that a causal relationship exists between two covariates certain criteria need to be satisfied. The main criteria are 1) that temporality should be accounted for and 2) that modification of a first covariate should lead to a change in a second covariate.[89] Additionally, other criteria have been proposed, however, although these additional criteria may seem to be plausible, these criteria have been refuted as universally valid causal criteria.[89]

In the present thesis we could account for the first criterion, i.e. temporality, in the association between RNFL thickness and incident depression due to the availability prospective data (Chapter 8).[69] However, we could not account for temporality in the other analyses (Chapters 2-7 and Chapter 9).

In the present thesis we could not account for the second criterion as we used data from an observational study.[89] In order to be able to conclude the second criterion is accounted for intervention studies (i.e. trials) are required. Methodologically, as

intervention studies provide greater control over 'residual error' than observational studies, causality is generally assumed to be inferable from interventional research but not from observational research.[89]

In conclusion, causality cannot be concluded. However, the level of evidence of this thesis may be near to the level of evidence that is required to infer causality.[89] It can be argued that the extent of control over 'residual error' not only depends on the design but is also affected by the control over sources of potential bias.[89] Hence, as the impact of potential bias due to selection bias, information bias, confounding bias and other sources of bias on the findings of the present thesis is likely small, the level of evidence of population-based findings in this thesis may not be importantly distinct from the level of evidence of a small randomized controlled trial.[89] Indeed, residual error (e.g. due to measurement error) is more likely to occur in a randomized controlled trial with a smaller, versus a larger, study population.[89]

5. Potential clinical implications of findings in this thesis

Findings of this thesis are mostly in agreement with guidelines for current clinical practice and, in addition, may have certain novel implications for clinical practice.

5.1. Findings of this thesis which are in line with recommendations from existing guidelines

In agreement with guidelines for current clinical practice, we found that the potentially modifiable adverse factors hyperglycaemia, hypertension, smoking, lower physical activity, lower cardiorespiratory fitness, high alcohol consumption, and an unhealthy diet were associated with neurodegeneration and microvascular dysfunction.[90,91] Therefore, early monitoring and treatment of hyperglycaemia and hypertension and modification of adverse lifestyle factors, i.e. smoking, low physical activity, low cardiorespiratory fitness, high alcohol consumption, and an unhealthy diet, may contribute to the prevention of neuronal and microvascular degeneration.[90,91]

Importantly, physicians may choose to prioritize early prevention of hyperglycaemia over modification of other cardiovascular risk factors or lifestyle factors as type 2 diabetes was the strongest determinant of neurodegeneration and microvascular dysfunction, an observation that was consistent for RNFL thickness, flicker light-induced increase in retinal arteriolar diameter,[9] and retinal arteriolar diameter.[23] In addition, also in support of this concept, prevention of hyperglycaemia is likely important because certain risk factors may be more detrimental in the co-presence of hyperglycaemia. Indeed, we found that certain cardiovascular risk factors were more

strongly or only associated with neuronal and microvascular changes in individuals with, but not without, type 2 diabetes. Then, as hyperglycaemia-mediated neurodegeneration and microvascular dysfunction likely is a gradual process, there may already be a window of opportunity to prevent neuronal and microvascular degeneration in prediabetes.[8,92]

We did not find evidence that associations under study, except for alcohol consumption, differed by sex, therefore, other than for alcohol consumption, no sex-specific strategies may be required for the prevention of neurodegeneration and microvascular dysfunction. For alcohol consumption, as alcohol consumption is already detrimental at lower levels for women than for men, lower levels of alcohol consumption in women versus men should be recommended.[93]

5.2. Findings of this thesis which are not in line with recommendations from existing guidelines

In disagreement with guidelines for current clinical practice, we found that dyslipidaemia was associated with greater RNFL thickness and retinal sensitivity; and that waist circumference was not associated with these outcomes.[90,91] In our opinion this does not mean that dyslipidaemia or obesity can be advocated as both factors are risk factors for cardiovascular disease.[90,91]

In addition, we found that the quantity of alcohol consumption up to which alcohol consumption was associated with less microvascular dysfunction was at higher levels of alcohol consumption than advocated in clinical guidelines. However, alcohol consumption cannot be advocated because greater consumption of alcohol is associated with increased risk of all-cause mortality.[94]

5.3. Novel findings of this thesis which are not yet part of existing guidelines

This thesis has three findings which may have novel implications for clinical practice.

First, we found that certain risk factors, of which regulation is currently not part of standard-care, were determinants of neuronal and microvascular changes.[90,91] We found that greater daily glucose variability was associated with more neurodegeneration; and that greater blood pressure variability and arterial stiffening were associated with more microvascular dysfunction. Therefore, early monitoring of daily glucose variability, blood pressure variability, and arterial stiffness and the deployment of interventional strategies to prevent daily glucose variability, blood pressure variability, and arterial stiffness may contribute to the prevention of neurodegeneration and microvascular dysfunction.[95-97] Specifically, daily glucose variability may be reduced via dietary interventions and treatment with certain drugs (e.g. glucagon-like peptide-1

analogues);[95] blood pressure variability may be reduced via treatment with certain antihypertensive medication (e.g. calcium channel blockers);[97] and arterial stiffness may be lowered via reducing overweight and increasing physical activity.[96]
Second, we found that, in the co-presence of hyperglycaemia, hypertension and arterial stiffness were, respectively, more strongly associated with neurodegeneration and microvascular dysfunction. Therefore, clinicians may consider to more strictly regulate blood pressure and aim to reduce arterial stiffness in individuals with prediabetes or type 2 diabetes. In addition, physical activity was more strongly associated with lower microvascular dysfunction in individuals with prediabetes and type 2 diabetes. Therefore, clinicians may expect stronger effects of reducing physical inactivity on the microvasculature in individuals with prediabetes and type 2 diabetes.
Third, we found that retinal neurodegeneration and microvascular dysfunction were associated with, and/or precede, clinical symptoms of major clinical brain disease. Therefore, retinal imaging of neuronal and microvascular structures may provide a means to identify individuals at risk for major clinical brain disease and a means to monitor early-stage (i.e. subclinical) disease progression.[64] Importantly, the combination of the monitoring of exposure to risk factors with monitoring actual neurodegeneration and microvascular dysfunction may enable better personalized prevention of major clinical disease than currently possible. For example, more strict control of cardiovascular and lifestyle factors may be necessary in individuals in which neurodegeneration and microvascular dysfunction occur at an accelerated rate.

6. Implications of findings in this thesis for future research

Future research is warranted to be able to translate findings of this thesis into clinical practice. We discuss six matters that require further study
First, from a methodological perspective, re-studying associations in prospective cohort studies and randomized controlled trials is warranted to provide evidence of a sufficient level of evidence to be able to implement findings in clinical practice.[69,98] To start, findings from cross-sectional analyses require confirmation in prospective observational cohort studies. Then, if findings are confirmed in prospective cohort studies, further validation of findings in randomized controlled trials is necessary to provide evidence of a high enough level of evidence so that implementation in guidelines for clinical practice can be considered.[69] In practice, conducting prospective cohort studies and randomized controlled trials may be feasible at the same time. Indeed, the collection of prospective data on neuronal and microvascular changes is ongoing in The Maastricht Study; and neuronal and microvascular measures could be added as secondary endpoints in planned randomized controlled trials that investigate

the effects of lifestyle and/or cardiovascular interventions on (sub)clinical endpoints (e.g. the FINGER trial in the Netherlands in which the effects of lifestyle interventions on cognitive performance are investigated).[99]

Second, certain associations under study could be investigated in a more detailed manner. To start, the potential beneficial effects of diet on neurodegeneration and microvascular dysfunction may be greater than findings of this thesis show.[84] We investigated dietary intake as adherence to a healthy diet (based on a composite score where individual dietary components were combined), however, possibly, if all components of a healthy diet would be individually investigated, certain dietary component may prove to be more strongly associated with outcomes under study than other dietary components.

Third, a better understanding of how (patho)biological changes in the retina are associated with (patho)biological changes in the brain is required.[64] We report a few examples of associations that require further study. The association between biomarkers of synaptopathy (e.g. amyloid beta) and retinal neurodegeneration requires study (currently there are no population-based studies available on this association); the association between retinal neurodegeneration and diffusion tensor imaging-assessed brain connectivity requires study (currently there are few population-based data available on this association);[100] the association between retinal neurodegeneration and incident mild cognitive impairment and dementia requires further study as few prospective data, with small numbers of incident cases, are currently available;[101] further in detail investigation retinal biomarkers required, as most studies that investigated associations between retinal neurodegeneration and brain function or structure used RNFL thickness or ganglion cell layer thickness, but did not study associations with other (individual) retinal layers;[101] and research on the association between the interaction of neurodegeneration and microvascular dysfunction in the associations of retinal indices of neurodegeneration and microvascular dysfunction with brain structure (e.g. magnetic resonance imaging-assessed hippocampus volume and diffusion tensor-imaging assessed brain connectivity) is required as few population-based data are currently available.[102]

In addition, if results of the analyses above are promising it may be relevant investigate how well combinations of certain neuronal and microvascular measures may function as clinical tools in a clinical setting. For example, the diagnostic value of retinal measures could be investigated by comparing the diagnostic discriminatory value of retinal measurements with magnetic resonance imaging in a memory clinic setting. For such a comparison individual retinal layers could be studied and multiple indices of microvascular changes could be evaluated, e.g. fundoscopy-assessed retinal diameters, fractal dimension, bifurcation angles, and optical coherence tomography angiography-assessed capillary density.[64]Additionally, in a later stage, the performance of neuronal

and microvascular changes as clinical tools may be further improved via using artificial intelligence-based deep learning algorithms.[103] For example, such algorithms may be able to detect novel indices of retinal neurodegeneration and microvascular dysfunction besides currently known retinal indices. In addition, as artificial intelligence-based algorithms will likely be able to at a high speed analyse images, the use of retinal indices of neurodegeneration and microvascular dysfunction may be feasible in the clinic in the future.[103]

Fourth, normative data in the general population are required on the 1) normal and pathological rate of deterioration of retinal neuronal and microvascular degeneration and 2) on whether and within which timeframe modification of cardiovascular risk factors and adverse lifestyle factors can lead to detectable changes in retinal neurodegeneration and microvascular dysfunction. Currently, few data are available on the normal and pathological rate of deterioration of neuronal and microvascular structures, most of the currently existing data is derived from small selected populations, e.g. in patients with multiple sclerosis or with type 2 diabetes.[104,105] Then, presently, few data from trials, and mostly with only small numbers of participants, on the effects of modification of lifestyle factors and cardiovascular risk factors on retinal neuronal and (most) microvascular changes are available (e.g. studies on the effects of physical activity on flicker light-induced increase in retinal microvascular diameters are scarce).[106]

Fifth, sources of unmeasured residual confounding could be further investigated to provide quantitative insight in how strong the effects of residual unmeasured bias may be.[69,107] To start, certain well-established genetic factors could be investigated (e.g. certain APOE mutations).[108] Next, important environmental factors could be investigated (e.g. common air pollutants).[109]

Sixth, whether associations under study in this thesis differ by ethnicity requires further study.[110,111] Certain differences may exist, although such differences may be small.[110, 111] Indeed, in ethnicity-stratified analyses small differences in the strength of associations between measures of glycaemia and retinal microvascular diameters have been reported.[111]

7. General conclusion

The present thesis has two main findings. First, potentially modifiable cardiovascular and lifestyle factors were associated with neurodegeneration and microvascular dysfunction. In addition, a number of cardiovascular and lifestyle factors were more strongly associated with neurodegeneration and microvascular dysfunction in individuals with, versus without, type 2 diabetes or prediabetes. Second

neurodegeneration, microvascular dysfunction, and their interaction are associated with, and/or precede, symptoms of major clinical brain disease.

Hence, there may be a new horizon for the early prevention of major clinical disease of neuronal and microvascular origin, including dementia, late-life depression, and diabetic retinopathy, via early modification of adverse cardiovascular and lifestyle factors. In addition, (retinal) indices of neurodegeneration and microvascular dysfunction may provide a means for the early identification of individuals at risk for major clinical disease as well as for the monitoring of disease progression in an early stage (i.e. in the absence of symptoms of major clinical disease). Future research is warranted to translate findings of this thesis into clinical practice.

8. References

1. Khawaja AP, Chua S, Hysi PG, Georgoulas S, Currant H, Fitzgerald TW, Birney E, Ko F, Yang Q, Reisman C, Garway-Heath DF, Hammond CJ, Khaw PT, Foster PJ, Patel PJ, Strouthidis N, Eye UKB and Vision C. Comparison of Associations with Different Macular Inner Retinal Thickness Parameters in a Large Cohort: The UK Biobank. *Ophthalmology*. 2020;127:62-71.
2. Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, Loeffler M, Colijn JM, Ikram MA, Vingerling JR, Williams KM, Hammond CJ, Creuzot-Garcher C, Bron AM, Silva R, Nunes S, Delcourt C, Cougnard-Gregoire A, Holz FG, Klaver CCW, Breteler MMB, Finger RP and European Eye Epidemiology C. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. *Ophthalmology*. 2018;125:1526-1536.
3. Garcia-Portilla MP, Garcia-Alvarez L, de la Fuente-Tomas L, Velasco-Iglesias A, Saiz PA, Gonzalez-Blanco L, Bobes Bascaran MT, Baamonde B, Alcalde I, Merayo-Lloves J and Bobes J. Could structural changes in the retinal layers be a new biomarker of mental disorders? A systematic review and thematic synthesis. *Rev Psiquiatr Salud Ment (Engl Ed)*. 2019;12:116-129.
4. Ravi Teja KV, Tos Berendschot T, Steinbusch H, Carroll Webers AB, Praveen Murthy R and Mathuranath PS. Cerebral and Retinal Neurovascular Changes: A Biomarker for Alzheimer's Disease. *J Gerontol Geriatr Res*. 2017;6.
5. Skender S, Ose J, Chang-Claude J, Paskow M, Bruhmann B, Siegel EM, Steindorf K and Ulrich CM. Accelerometry and physical activity questionnaires - a systematic review. *BMC Public Health*. 2016;16:515.
6. Myers MG and Kaczorowski J. Office blood pressure is lower than awake ambulatory blood pressure at lower targets for treatment. *J Clin Hypertens (Greenwich)*. 2017;19:1210-1213.
7. Drexler W and Fujimoto JG. State-of-the-art retinal optical coherence tomography. *Prog Retin Eye Res*. 2008;27:45-88.
8. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. *Diabetes*. 2018;67:1729-1741.
9. Sorensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CD. Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction: The Maastricht Study. *Circulation*. 2016;134:1339-1352.
10. Kaczorowski K, Mulak M, Szumny D and Misiuk-Hojlo M. Heidelberg Edge Perimeter: The New Method of Perimetry. *Adv Clin Exp Med*. 2015;24:1105-12.
11. Li W, Schram MT, Sorensen BM, van Agtmaal MJM, Berendschot T, Webers CAB, Jansen JFA, Backes WH, Gronenschild E, Schalkwijk CG, Stehouwer CDA and Houben A. Microvascular Phenotyping in the Maastricht Study: Design and Main Findings, 2010-2018. *Am J Epidemiol*. 2020;189:873-884.
12. Prasad RM and Tikaria R. Microalbuminuria *StatPearls* Treasure Island (FL); 2021.
13. Alexopoulos GS. Mechanisms and treatment of late-life depression. *Transl Psychiatry*. 2019;9:188.
14. Silverman A and Petersen NH. Physiology, Cerebral Autoregulation *StatPearls* Treasure Island (FL); 2021.
15. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. *Neuron*. 2017;96:17-42.
16. Guerra G, Lucariello A, Perna A, Botta L, De Luca A and Moccia F. The Role of Endothelial Ca(2+) Signaling in Neurovascular Coupling: A View from the Lumen. *Int J Mol Sci*. 2018;19.
17. Soto-Rojas LO, Pacheco-Herrero M, Martinez-Gomez PA, Campa-Cordoba BB, Apatiga-Perez R, Villegas-Rojas MM, Harrington CR, de la Cruz F, Garces-Ramirez L and Luna-Munoz J. The Neurovascular Unit Dysfunction in Alzheimer's Disease. *Int J Mol Sci*. 2021;22.
18. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol*. 2018;14:133-150.
19. Rosso M and Chitnis T. Association Between Cigarette Smoking and Multiple Sclerosis: A Review. *JAMA Neurol*. 2020;77:245-253.
20. Meza CA, La Favor JD, Kim DH and Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? *Int J Mol Sci*. 2019;20.
21. Hardingham GE, Fukunaga Y and Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci*. 2002;5:405-14.

22. Sorensen BM, Houben A, Berendschot T, Schouten J, Kroon AA, van der Kallen CJH, Henry RMA, Koster A, Dagnelie PC, Schaper NC, Schram MT and Stehouwer CDA. Cardiovascular risk factors as determinants of retinal and skin microvascular function: The Maastricht Study. *PLoS One*. 2017;12:e0187324.
23. Li W, Schram MT, Berendschot T, Webers CAB, Kroon AA, van der Kallen CJH, Henry RMA, Schaper NC, Huang F, Dashtbozorg B, Tan T, Zhang J, Abbasi-Sureshjani S, Ter Haar Romeny BM, Stehouwer CDA and Houben A. Type 2 diabetes and HbA1c are independently associated with wider retinal arterioles: the Maastricht study. *Diabetologia*. 2020;63:1408-1417.
24. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54:1615-25.
25. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, Garg S, Heinemann L, Hirsch I, Amiel SA, Beck R, Bosi E, Buckingham B, Cobelli C, Dassau E, Doyle FJ, 3rd, Heller S, Hovorka R, Jia W, Jones T, Kordonouri O, Kovatchev B, Kowalski A, Laffel L, Maahs D, Murphy HR, Norgaard K, Parkin CG, Renard E, Saboo B, Scharf M, Tamborlane WV, Weinzimer SA and Phillip M. International Consensus on Use of Continuous Glucose Monitoring. *Diabetes Care*. 2017;40:1631-1640.
26. Yu DY, Cringle SJ, Balaratnasingam C, Morgan WH, Yu PK and Su EN. Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. *Prog Retin Eye Res*. 2013;36:217-46.
27. Coopmans C, Zhou TL, Henry RMA, Heijman J, Schaper NC, Koster A, Schram MT, van der Kallen CJH, Wesselius A, den Engelsman RJA, Crijns H and Stehouwer CDA. Both Prediabetes and Type 2 Diabetes Are Associated With Lower Heart Rate Variability: The Maastricht Study. *Diabetes Care*. 2020;43:1126-1133.
28. Vergoossen LW, Schram MT, de Jong JJ, Stehouwer CD, Schaper NC, Henry RM, van der Kallen CJ, Dagnelie PC, van Boxtel MP, Eussen SJ, Backes WH and Jansen JF. White Matter Connectivity Abnormalities in Prediabetes and Type 2 Diabetes: The Maastricht Study. *Diabetes Care*. 2020;43:201-208.
29. van der Velde J, Koster A, Strotmeyer ES, Mess WH, Hilkman D, Reulen JPH, Stehouwer CDA, Henry RMA, Schram MT, van der Kallen CJH, Schalkwijk CG, Savelberg H and Schaper NC. Cardiometabolic risk factors as determinants of peripheral nerve function: the Maastricht Study. *Diabetologia*. 2020;63:1648-1658.
30. van Agtmaal MJM, Houben A, de Wit V, Henry RMA, Schaper NC, Dagnelie PC, van der Kallen CJ, Koster A, Sep SJ, Kroon AA, Jansen JFA, Hofman PA, Backes WH, Schram MT and Stehouwer CDA. Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. *Diabetes Care*. 2018;41:2535-2543.
31. Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A and Pfrieger FW. CNS synaptogenesis promoted by glia-derived cholesterol. *Science*. 2001;294:1354-7.
32. Parimisetty A, Dorsemans AC, Awada R, Ravanan P, Diotel N and Lefebvre d'Hellencourt C. Secret talk between adipose tissue and central nervous system via secreted factors-an emerging frontier in the neurodegenerative research. *J Neuroinflammation*. 2016;13:67.
33. Bleakley C, Hamilton PK, Pumb R, Harbinson M and McVeigh GE. Endothelial Function in Hypertension: Victim or Culprit? *J Clin Hypertens (Greenwich)*. 2015;17:651-4.
34. Climie RE, van Sloten TT, Bruno RM, Taddei S, Empana JP, Stehouwer CDA, Sharman JE, Boutouyrie P and Laurent S. Macrovasculature and Microvasculature at the Crossroads Between Type 2 Diabetes Mellitus and Hypertension. *Hypertension*. 2019;73:1138-1149.
35. McSweeney SR, Warabi E and Siow RC. Nrf2 as an Endothelial Mechanosensitive Transcription Factor: Going With the Flow. *Hypertension*. 2016;67:20-9.
36. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J Appl Physiol (1985)*. 2008;105:1652-60.
37. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet*. 2010;375:938-48.
38. Sheng Y and Zhu L. The crosstalk between autonomic nervous system and blood vessels. *Int J Physiol Pathophysiol Pharmacol*. 2018;10:17-28.
39. van Sloten TT, Czernichow S, Houben AJ, Protogerou AD, Henry RM, Muris DM, Schram MT, Sep SJ, Dagnelie PC, van der Kallen CJ, Schaper NC, Blacher J, Hercberg S, Levy BI and Stehouwer CD. Association Between Arterial Stiffness and Skin Microvascular Function: The SUVIMAX2 Study and The Maastricht Study. *Am J Hypertens*. 2015;28:868-76.
40. Stehouwer CD, Henry RM and Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia*. 2008;51:527-39.

41. Simo R, Stitt AW and Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61:1902-1912.
42. Cheung N, Sharrett AR, Klein R, Criqui MH, Islam FM, Macura KJ, Cotch MF, Klein BE and Wong TY. Aortic distensibility and retinal arteriolar narrowing: the multi-ethnic study of atherosclerosis. *Hypertension*. 2007;50:617-22.
43. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, Strachan DP and Rudnicka AR. Associations of Retinal Microvascular Diameters and Tortuosity With Blood Pressure and Arterial Stiffness: United Kingdom Biobank. *Hypertension*. 2019;74:1383-1390.
44. Wei F, Lutgarde T., Yu CC., Melgarejo JD. Zhang ZY., Maestre GE., Struijker-Boudier HAJ., Verhamme P., Staessen, J. . Retinal Microvasculature in relation to central hemodynamics in a Flemish population. *Hypertension*. 2019;74.
45. Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B and Laurent S. Association between local pulse pressure, mean blood pressure, and large-artery remodeling. *Circulation*. 1999;100:1387-93.
46. Ambrose JA and Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43:1731-7.
47. Lee CS, Owen JP, Yanagihara RT, Lorch A, Pershing S, Hyman L, Miller JW, Haller JA, Chiang MF, Lum F and Lee AY. Smoking Is Associated with Higher Intraocular Pressure Regardless of Glaucoma: A Retrospective Study of 12.5 Million Patients Using the Intelligent Research in Sight (IRIS(R)) Registry. *Ophthalmol Glaucoma*. 2020;3:253-261.
48. Sowmya V, Venkataramanan VR and Prasad V. Effect of Refractive Status and Axial Length on Peripapillary Retinal Nerve Fibre Layer Thickness: An Analysis Using 3D OCT. *J Clin Diagn Res*. 2015;9:NC01-4.
49. Ma X, Chen Y, Liu X and Ning H. Effect of Refractive Correction Error on Retinal Nerve Fiber Layer Thickness: A Spectralis Optical Coherence Tomography Study. *Med Sci Monit*. 2016;22:5181-5189.
50. Di Liegro CM, Schiera G, Proia P and Di Liegro I. Physical Activity and Brain Health. *Genes (Basel)*. 2019;10.
51. Iaccarino G, Franco D, Sorriento D, Strisciuglio T, Barbato E and Morisco C. Modulation of Insulin Sensitivity by Exercise Training: Implications for Cardiovascular Prevention. *J Cardiovasc Transl Res*. 2021;14:256-270.
52. Lanting SM, Johnson NA, Baker MK, Caterson ID and Chuter VH. The effect of exercise training on cutaneous microvascular reactivity: A systematic review and meta-analysis. *J Sci Med Sport*. 2017;20:170-177.
53. Bathina S and Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci*. 2015;11:1164-78.
54. Raichlen DA, Klimentidis YC, Bharadwaj PK and Alexander GE. Differential associations of engagement in physical activity and estimated cardiorespiratory fitness with brain volume in middle-aged to older adults. *Brain Imaging Behav*. 2020;14:1994-2003.
55. Risner D, Ehrlich R, Kheradiya NS, Siesky B, McCranor L and Harris A. Effects of exercise on intraocular pressure and ocular blood flow: a review. *J Glaucoma*. 2009;18:429-36.
56. Burckhardt M, Herke M, Wustmann T, Watzke S, Langer G and Fink A. Omega-3 fatty acids for the treatment of dementia. *Cochrane Database Syst Rev*. 2016;4:CD009002.
57. Man AWC, Li H and Xia N. Impact of Lifestyles (Diet and Exercise) on Vascular Health: Oxidative Stress and Endothelial Function. *Oxid Med Cell Longev*. 2020;2020:1496462.
58. Mielech A, Puscion-Jakubik A, Markiewicz-Zukowska R and Socha K. Vitamins in Alzheimer's Disease-Review of the Latest Reports. *Nutrients*. 2020;12.
59. Prinelli F, Fratiglioni L, Kalpouzos G, Musicco M, Adorni F, Johansson I, Marseglia A and Xu W. Specific nutrient patterns are associated with higher structural brain integrity in dementia-free older adults. *Neuroimage*. 2019;199:281-288.
60. Joyal JS, Gantner ML and Smith LEH. Retinal energy demands control vascular supply of the retina in development and disease: The role of neuronal lipid and glucose metabolism. *Prog Retin Eye Res*. 2018;64:131-156.
61. Phillips SA, Osborn K, Hwang CL, Sabbahi A and Piano MR. Ethanol Induced Oxidative Stress in the Vasculature: Friend or Foe. *Curr Hypertens Rev*. 2020;16:181-191.
62. Louis XL, Raj P, Chan L, Zieroth S, Netticadan T and Wigle JT. Are the cardioprotective effects of the phytoestrogen resveratrol sex-dependent? (1). *Can J Physiol Pharmacol*. 2019;97:503-514.
63. Arnal JF, Fontaine C, Billon-Gales A, Favre J, Laurell H, Lenfant F and Gourdy P. Estrogen receptors and endothelium. *Arterioscler Thromb Vasc Biol*. 2010;30:1506-12.
64. Kashani AH, Asanad S, Chan JW, Singer MB, Zhang J, Sharifi M, Khansari MM, Abdolahi F, Shi Y, Biffi A, Chui H and Ringman JM. Past, present and future role of retinal imaging in neurodegenerative disease. *Prog Retin Eye Res*. 2021:100938.

65. Pulvermuller F, Tomasello R, Henningsen-Schomers MR and Wennekers T. Biological constraints on neural network models of cognitive function. *Nat Rev Neurosci*. 2021.
66. Iadecola C and Gottesman RF. Neurovascular and Cognitive Dysfunction in Hypertension. *Circ Res*. 2019;124:1025-1044.
67. Csiszar A, Tarantini S, Fulop GA, Kiss T, Valcarcel-Ares MN, Galvan V, Ungvari Z and Yabluchanskiy A. Hypertension impairs neurovascular coupling and promotes microvascular injury: role in exacerbation of Alzheimer's disease. *Geroscience*. 2017;39:359-372.
68. Centraal Bureau voor de Statistiek (CBS), 2018 Trends in Nederland, cijfers- onderwijs, access date 5-21-21,https://longreads.cbs.nl/trends18/maatschappij/cijfers/onderwijs/#:~:text=Cijfers%20%2D%20Onderwijs&text=In%20Nederland%20heeft%20bijna%2040,heen%20in%20omvang%20vrij%20stabiel.
69. Rothman KJ GS, Lash TL. *Modern epidemiology.* Third edition ed: Lippincott Williams & Wilkin; 2008.
70. Schisterman EF, Cole SR and Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology*. 2009;20:488-95.
71. Peters R, Ee N, Peters J, Booth A, Mudway I and Anstey KJ. Air Pollution and Dementia: A Systematic Review. *J Alzheimers Dis*. 2019;70:S145-S163.
72. Deng Q, Wei Y, Chen L, Liang W, Du J, Tan Y and Zhao Y. Relationship between Air Pollution and Regional Longevity in Guangxi, China. *Int J Environ Res Public Health*. 2019;16.
73. de Frutos-Lucas J, Frost N, Erickson KI, Serrano JM, Maestu F, Laws SM and Brown BM. Does APOE genotype moderate the relationship between physical activity, brain health and dementia risk? A systematic review. *Ageing Res Rev*. 2020;64:101173.
74. Gemeente Maastricht, 2021, Vestiging en vertrek, access date 21-4-21, https://maastricht.incijfers.nl/dashboard/vestiging-en-vertrek.
75. Gmel GR, J. . Measuring Alcohol Consumption. *Contemporary Drug Problems*. 2004;31 (3):467-540.
76. Hong JT, Sung KR, Cho JW, Yun SC, Kang SY and Kook MS. Retinal nerve fiber layer measurement variability with spectral domain optical coherence tomography. *Korean J Ophthalmol*. 2012;26:32-8.
77. van der Heide FCT, Zhou TL, Henry RMA, Houben A, Kroon AA, Dagnelie PC, van Dongen M, Eussen S, Berendschot T, Schouten J, Webers CAB, Schram MT, van Greevenbroek MMJ, Wesselius A, Schalkwijk CG, Koster A, Savelberg H, Schaper NC, Reesink KD and Stehouwer CDA. Carotid stiffness is associated with retinal microvascular dysfunction-The Maastricht study. *Microcirculation*. 2021:e12702.
78. Sherry LM, Wang JJ, Rochtchina E, Wong T, Klein R, Hubbard L and Mitchell P. Reliability of computer-assisted retinal vessel measurementin a population. *Clin Exp Ophthalmol*. 2002;30:179-82.
79. Cui QN, Gogt P, Lam JM, Siraj S, Hark LA, Myers JS, Katz LJ and Waisbourd M. Validation and reproducibility of the Heidelberg Edge Perimeter in the detection of glaucomatous visual field defects. *Int J Ophthalmol*. 2019;12:577-581.
80. Bur A, Herkner H, Woisetschlager C, Vlcek M, Derhaschnig U and Hirschl MM. Is fasting blood glucose a reliable parameter for screening for diabetes in hypertension? *Am J Hypertens*. 2003;16:297-301.
81. Coleman A, Freeman P, Steel S and Shennan A. Validation of the Omron 705IT (HEM-759-E) oscillometric blood pressure monitoring device according to the British Hypertension Society protocol. *Blood Press Monit*. 2006;11:27-32.
82. Glasziou PP, Irwig L, Heritier S, Simes RJ, Tonkin A and Investigators LS. Monitoring cholesterol levels: measurement error or true change? *Ann Intern Med*. 2008;148:656-61.
83. Nadas J, Putz Z, Kolev G, Nagy S and Jermendy G. Intraobserver and interobserver variability of measuring waist circumference. *Med Sci Monit*. 2008;14:CR15-18.
84. Barclay M, Dixon-Woods M and Lyratzopoulos G. The problem with composite indicators. *BMJ Qual Saf*. 2019;28:338-344.
85. Riordan H. Constructing Composites to Optimise Cognitive Outcomes. *Journal for Clinical Studies* 2017;9:40-45.
86. Van Breukelen GJ. ANCOVA versus change from baseline: more power in randomized studies, more bias in nonrandomized studies [corrected]. *J Clin Epidemiol*. 2006;59:920-5.
87. Vickers AJ. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Med Res Methodol*. 2001;1:6.
88. Jakobsen JC, Gluud C, Wetterslev J and Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials - a practical guide with flowcharts. *BMC Med Res Methodol*. 2017;17:162.
89. Rothman KJ and Greenland S. Causation and causal inference in epidemiology. *Am J Public Health*. 2005;95 Suppl 1:S144-50.

90. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Juni P, Lettino M, Marx N, Mellbin LG, Ostgren CJ, Rocca B, Roffi M, Sattar N, Seferovic PM, Sousa-Uva M, Valensi P, Wheeler DC and Group ESCSD. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J*. 2020;41:255-323.
91. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, Brayne C, Burns A, Cohen-Mansfield J, Cooper C, Costafreda SG, Dias A, Fox N, Gitlin LN, Howard R, Kales HC, Kivimaki M, Larson EB, Ogunniyi A, Orgeta V, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbaek G, Teri L and Mukadam N. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*. 2020;396:413-446.
92. Haffner SM, Stern MP, Hazuda HP, Mitchell BD and Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA*. 1990;263:2893-8.
93. Erol A and Karpyak VM. Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations. *Drug Alcohol Depend*. 2015;156:1-13.
94. Collaborators GBDA. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2018;392:1015-1035.
95. Frontoni S, Di Bartolo P, Avogaro A, Bosi E, Paolisso G and Ceriello A. Glucose variability: An emerging target for the treatment of diabetes mellitus. *Diabetes Res Clin Pract*. 2013;102:86-95.
96. Zieman SJ, Melenovsky V and Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2005;25:932-43.
97. Höcht C. Blood Pressure Variability: Prognostic Value and Therapeutic Implications. *International Scholarly Research Notices*. 2013.
98. Grimshaw JM, Eccles MP, Lavis JN, Hill SJ and Squires JE. Knowledge translation of research findings. *Implement Sci*. 2012;7:50.
99. Deckers K, Kohler S, Ngandu T, Antikainen R, Laatikainen T, Soininen H, Strandberg T, Verhey F, Kivipelto M and Solomon A. Quantifying dementia prevention potential in the FINGER randomized controlled trial using the LIBRA prevention index. *Alzheimers Dement*. 2021.
100. Mutlu U, Bonnemaijer PWM, Ikram MA, Colijn JM, Cremers LGM, Buitendijk GHS, Vingerling JR, Niessen WJ, Vernooij MW, Klaver CCW and Ikram MK. Retinal neurodegeneration and brain MRI markers: the Rotterdam Study. *Neurobiol Aging*. 2017;60:183-191.
101. Chan VTT, Sun Z, Tang S, Chen LJ, Wong A, Tham CC, Wong TY, Chen C, Ikram MK, Whitson HE, Lad EM, Mok VCT and Cheung CY. Spectral-Domain OCT Measurements in Alzheimer's Disease: A Systematic Review and Meta-analysis. *Ophthalmology*. 2019;126:497-510.
102. Chua SYL, Lascaratos G, Atan D, Zhang B, Reisman C, Khaw PT, Smith SM, Matthews PM, Petzold A, Strouthidis NG, Foster PJ, Khawaja AP, Patel PJ and Uk Biobank Eye VC. Relationships between retinal layer thickness and brain volumes in the UK Biobank cohort. *Eur J Neurol*. 2021;28:1490-1498.
103. Ting DSW, Peng L, Varadarajan AV, Keane PA, Burlina PM, Chiang MF, Schmetterer L, Pasquale LR, Bressler NM, Webster DR, Abramoff M and Wong TY. Deep learning in ophthalmology: The technical and clinical considerations. *Prog Retin Eye Res*. 2019;72:100759.
104. Aschauer J, Pollreisz A, Karst S, Hulsmann M, Hajdu D, Datlinger F, Egner B, Kriechbaum K, Pablik E and Schmidt-Erfurth UM. Longitudinal analysis of microvascular perfusion and neurodegenerative changes in early type 2 diabetic retinal disease. *Br J Ophthalmol*. 2020.
105. Schurz N, Sariaslani L, Altmann P, Leutmezer F, Mitsch C, Pemp B, Rommer P, Zrzavy T, Berger T and Bsteh G. Evaluation of Retinal Layer Thickness Parameters as Biomarkers in a Real-World Multiple Sclerosis Cohort. *Eye Brain*. 2021;13:59-69.
106. Streese L, Guerini C, Buhlmayer L, Lona G, Hauser C, Bade S, Deiseroth A and Hanssen H. Physical activity and exercise improve retinal microvascular health as a biomarker of cardiovascular risk: A systematic review. *Atherosclerosis*. 2020;315:33-42.
107. Currant H, Hysi P, Fitzgerald TW, Gharahkhani P, Bonnemaijer PWM, Senabouth A, Hewitt AW, Eye UKB, Vision C, International Glaucoma Genetics C, Atan D, Aung T, Charng J, Choquet H, Craig J, Khaw PT, Klaver CCW, Kubo M, Ong JS, Pasquale LR, Reisman CA, Daniszewski M, Powell JE, Pebay A, Simcoe MJ, Thiadens A, van Duijn CM, Yazar S, Jorgenson E, MacGregor S, Hammond CJ, Mackey DA, Wiggs JL, Foster PJ, Patel PJ, Birney E and Khawaja AP. Genetic variation affects morphological retinal phenotypes extracted from UK Biobank optical coherence tomography images. *PLoS Genet*. 2021;17:e1009497.
108. Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: A review. *Atherosclerosis*. 2016;255:145-155.

109. Bellou V, Belbasis L, Tzoulaki I, Middleton LT, Ioannidis JPA and Evangelou E. Systematic evaluation of the associations between environmental risk factors and dementia: An umbrella review of systematic reviews and meta-analyses. *Alzheimers Dement*. 2017;13:406-418.
110. Ho H, Tham YC, Chee ML, Shi Y, Tan NYQ, Wong KH, Majithia S, Cheung CY, Aung T, Wong TY and Cheng CY. Retinal Nerve Fiber Layer Thickness in a Multiethnic Normal Asian Population: The Singapore Epidemiology of Eye Diseases Study. *Ophthalmology*. 2019;126:702-711.
111. Jeganathan VS, Sabanayagam C, Tai ES, Lee J, Lamoureux E, Sun C, Kawasaki R and Wong TY. Retinal vascular caliber and diabetes in a multiethnic Asian population. *Microcirculation*. 2009;16:534-43.

Chapter 11.1 Dutch summary

Inleiding

Dementie, depressie, en diabetische retinopathie zijn veelvoorkomende ziekten waarvoor preventie in een zo vroeg mogelijk stadium belangrijk. Deze ziekten zijn relevant omdat ze de kwaliteit van leven van patiënten en hun familie aanzienlijk negatief kunnen aantasten; de ermee gepaard gaande kosten voor de samenleving hoog zijn; en er verwacht wordt dat het aantal mensen dat aan deze ziekten zal lijden in de komende 25 jaar aanzienlijk gaat toenemen. Gemiddeld genomen ontwikkelt 1 op de 3 vrouwen en 1 op de 7 mannen een vorm van dementie tijdens hun leven (anno 2021 zijn er 290.000 patiënten met dementie in Nederland); maakt 1 op de 9 mensen een depressie door tijdens hun leven; en ontwikkelt 1 op de 2 mensen met suikerziekte tekenen van diabetische retinopathie (op dit moment hebben ongeveer 1 miljoen mensen suikerziekte in Nederland en het gaat dus om ongeveer ~500.000 mensen).
Mogelijk kan het ontstaan van dementie, depressie, en diabetische retinopathie (gedeeltelijk) voorkomen en/of vertraagd worden door vroege tekenen van schade die voorafgaan aan deze ziekten te voorkomen. Er wordt gedacht dat, voordat symptomen van deze ziekten detecteerbaar zijn, vroege zenuwschade en kleine bloedvatschade ontstaan die over de tijd erger worden en op den duur kunnen leiden tot de beschadiging van organen, en uiteindelijk symptomen van dementie, depressie, en diabetische retinopathie. Vanuit een biologisch perspectief is het plausibel dat zenuwschade en kleine bloedvatschade relevant zijn omdat zenuwen en bloedvaten samen de aanvoer van voedingsstoffen en de afvoer van afvalstoffen reguleren (zodat metabolisme adequaat plaats kan vinden). Er wordt namelijk gedacht dat de aanvoer van te weinig voedingsstoffen en de te slechte afvoer van afvalstoffen beiden kunnen leiden tot beschadigingen van de zenuwen in de hersenen en het netvlies. Verder ontstaan beschadigingen misschien sneller bij patiënten met suikerziekte type 2 of een voorstadium van suikerziekte type 2 (wat bij tot wel 30% van de mensen in de algemene bevolking voorkomt) en zijn patiënten met suikerziekte type 2 daarom (deels) misschien meer kwetsbaar voor het ontstaan van dementie, depressie, en diabetische retinopathie.
De huidige wetenschappelijke literatuur heeft belangrijke beperkingen op het gebied van kennis over vroege zenuwschade en kleine bloedvatschade. Er zijn specifiek twee thema's waarover meer kennis nodig is. Ten eerste is het nodig om beter te begrijpen hoe potentieel modificeerbare cardiovasculaire risicofactoren en leefstijlfactoren verband houden met vroege zenuwschade en kleine bloedvatschade. Mogelijk kunnen door het tijdig aanpassen van cardiovasculaire risicofactoren en ongezonde leefstijlfactoren vroege zenuwschade en kleine bloedvatschade voorkomen worden. Ten tweede is er meer kennis nodig over hoe vroege zenuwschade en kleine bloedvatschade verband houden met symptomen van hersenziekten. Misschien kunnen

mensen met een verhoogd risico op het ontstaan van deze ziekten opgespoord worden aan de hand van vroege zenuwschade en kleine bloedvatschade; en misschien kan middels vroege zenuwschade en kleine bloedvatschade het vroege preklinische ziektestadium van belangrijke hersenziekten gemonitord worden. Tot slot, er is relatief weinig kennis over of mensen met suikerziekte type 2 (of een voorstadium van suikerziekte type 2) misschien kwetsbaarder zijn voor vroege zenuwschade en kleine bloedvatschade door cardiovasculaire risicofactoren en ongezonde leefstijlfactoren.
Door de recente ontwikkeling van nieuwe technieken kunnen tekenen van vroege zenuwschade en kleine bloedvatschade op een niet-invasieve, dus patiëntvriendelijke, manier gemeten worden. Daardoor is het nu mogelijk om onderzoek te doen naar vroege zenuwschade en kleine bloedvatschade. Met deze nieuwe technieken kunnen vroege zenuwschade en kleine bloedvatschade heel precies gemeten worden in verschillende orgaansystemen, waaronder in de hersenen, het netvlies, de nier, de huid, en in het bloed. Van deze organen is het netvlies in het bijzonder relevant omdat in het netvlies zowel zenuwschade als kleine bloedvatschade kunnen worden gemeten. Verder zijn voordelen van het meten van zenuwschade en kleine bloedvatschade in het netvlies dat de metingen relatief sneller en goedkoper zijn dan de metingen in de meeste andere orgaansystemen.
Gezien bovenstaande heeft dit proefschrift twee hoofddoelstellingen. De eerste hoofddoelstelling is het onderzoeken of potentieel modificeerbare cardiovasculaire risicofactoren en ongezonde leefstijlfactoren verband houden met vroege zenuwschade en kleine bloedvatschade. De tweede hoofddoelstelling is om te onderzoeken of vroege zenuwschade en kleine bloedvatschade verband houden met symptomen van hersenziekten (bijvoorbeeld depressieve klachten of een verminderd denkvermogen). Verder zijn nevendoelstellingen het onderzoeken of mensen met, ten opzichte van zonder, suikerziekte type 2 of een voorstadium van suikerziekte type 2 misschien kwetsbaarder zijn voor vroege zenuwschade en kleine bloedvatschade door cardiovasculaire risicofactoren en ongezonde leefstijlfactoren; en het onderzoeken of mannen en vrouwen in gelijke mate kwetsbaar zijn voor vroege zenuwschade en kleine bloedvatschade door potentieel modificeerbare cardiovasculaire risicofactoren en ongezonde leefstijlfactoren.

Methodologie

De Maastricht Studie

Om de doelstellingen van dit proefschrift te onderzoeken zijn er in dit proefschrift gegevens gebruikt van De Maastricht Studie. De Maastricht Studie is een

observationele cohort studie waarbinnen gegevens over de gezondheid van de algemene bevolking worden verzameld. Belangrijke doelstellingen van De Maastricht Studie zijn om meer kennis te verkrijgen over het ontstaan van suikerziekte type 2, het ziekteproces van suikerziekte type 2, en het ontstaan van complicaties en andere ziektes die gerelateerd zijn aan suikerziekte type 2. Inclusiecriteria voor deelname aan De Maastricht Studie zijn een leeftijd tussen 40 en 75 jaar en woonachtig zijn in Maastricht of omstreken. Verder zijn relatief meer patiënten met suikerziekte type 2 opgenomen in de studie dan in de algemene bevolking voorkomen zodat suikerziekte type 2 goed bestudeerd kan worden (bijna 30% van de deelnemers in De Maastricht Studie heeft suikerziekte type 2 terwijl in de algemene bevolking suikerziekte voorkomt bij ongeveer 5% van de mensen). In dit proefschrift zijn gegevens gebruikt van tot wel n=8.005 deelnemers. Deze gegevens zijn verzameld tussen november 2010 en januari 2019. Er is in het grootste deel van het proefschrift gebruik gemaakt van zogenaamde ‘cross-sectionele’ gegevens (gegevens die op een bepaald moment in de tijd zijn verzameld) en in een hoofdstuk is gebruik gemaakt van zogenaamde ‘longitudinale’ gegevens (gegevens die op meer dan een moment zijn verzameld; longitudinale gegevens waren alleen beschikbaar voor depressieve symptomen).

Het meten van zenuwschade en kleine bloedvatschade

In dit proefschrift zijn gegevens van metingen van zenuwschade en kleine bloedvatschade in het netvlies gebruikt en zijn gegevens van metingen van kleine bloedvatschade in de hersenen, nieren, huid, en in het bloed gebruikt.

Metingen van schade aan zenuwen en kleine bloedvaten in het netvlies

In het netvlies hebben we nabij de blinde vlek de dikte van de ‘retinal nerve fibre layer’ gemeten (de zogenaamde “zenuwvezellaag”; een van de lagen van het netvlies); de globale gevoeligheid van het netvlies voor het waarnemen van lichtprikkels gemeten; de dikte van bloedvaten ‘in rust’ gemeten; en de verwijdingsrespons van kleine bloedvaten als reactie op flikkerlicht blootstelling gemeten. Er wordt aangenomen dat een dunnere zenuwvezellaag duidt op minder zenuwbundels, dus meer zenuwschade in het netvlies; dat een verminderde gevoeligheid voor het waarnemen van lichtprikkels duidt op een minder goede functie van het netvlies als gevolg van meer beschadiging van de zenuwen van het netvlies; dat een wijdere of smallere breedte van een netvlies slagader of een wijdere ader duidt op meer kleine bloedvatschade; en dat een verminderde verwijdingsreactie als reactie op flikkerlicht blootstelling wijst op een verminderde gecombineerde functie van zenuwen en kleine bloedvaten in het netvlies.

Metingen van kleine bloedvatschade in andere orgaansystemen dan het netvlies

We hebben kleine bloedvatschade gemeten in de hersenen, nieren, huid, en in het bloed.
In de hersenen hebben we middels zogenaamde 'magnetic resonance imaging (MRI)' vier verschillende markers van schade in de hersenen gemeten (witte stof afwijkingen, kleine bloedingen, kleine infarcten, en totaal hersenvolume). Er wordt aangenomen dat meer witte stof afwijkingen, meer kleine bloedingen, meer kleine infarcten, en minder totaal hersenvolume duiden op meer kleine vaatschade. Verder, hebben we in de huid de toename in bloeddoorstroming gemeten als reactie op een warmte prikkel. Aangenomen wordt dat des te minder de bloeddoorstroming toeneemt in de huid als reactie op een gestandaardiseerde warmteprikkel, des te meer kleine bloedvatschade aanwezig is in de kleine bloedvaten van de huid. Daarnaast hebben we in de urine (die gedurende 24 uur verzameld was) de hoeveelheid albumine bepaald (de urine was tweemaal gemeten). Er wordt gedacht dat meer albumine in de urine duidt op meer kleine bloedvatschade in de nieren. Tot slot, hebben we in het bloed vier markers van kleine bloedvatschade gemeten. Aangenomen wordt dat hogere spiegels van deze markers duiden op meer kleine bloedvatschade in het gehele lichaam (niet beperkt tot een bepaald orgaan).

Cardiovasculaire risicofactoren en leefstijlfactoren

In dit proefschrift hebben we de volgende risicofactoren onderzocht: de suikerspiegel (gemeten in het bloed); de dagelijkse suikerspiegelschommelingen (gemeten in het bloed); de cholesterol spiegel (gemeten in het bloed); de bloeddruk; korte termijn bloeddrukschommelingen; de stijfheid van grote vaten; buikomtrek (als een maat voor overgewicht); roken; fysieke activiteit; fysieke fitheid, en dieet (waarbij alcohol inname los van andere bestanddelen van een [ongezond] dieet is bestudeerd).

Symptomen van brein disfunctie

In dit proefschrift hebben we depressieve symptomen en denkfunctie gemeten. Er wordt aangenomen dat zowel meer depressieve symptomen als een verminderde denkfunctie op breinschade en breindisfunctie duiden.

Statistiek

We hebben de verbanden tussen variabelen onderzocht met zogenaamde 'regressieanalyse'. Hierbij wordt middels een wiskundige berekening een lijn geschat die zo goed mogelijk het verband tussen twee variabelen weergeeft. De hellingshoek van deze lijn wordt de 'bèta' genoemd en geeft de sterkte van het verband tussen twee variabelen aan (des te steiler de lijn is, des te sterker is het verband tussen twee

variabelen). Bij het berekenen van de bèta's in dit proefschrift hebben we rekening gehouden met een groot aantal mogelijk verstorende variabelen (zogenaamde "confounder" variabelen) omdat deze verstorende variabelen kunnen leiden tot een foutieve schatting van de bèta. We hebben onder andere rekening gehouden met leeftijd, geslacht, opleidingsniveau, suikerziekte, roken, alcohol inname, cholesterol, bloeddruk, en buikomtrek (details zijn te vinden in de hoofdstukken 2-9). Tot slot hebben we onderzocht of de sterkte van de bèta verschilde tussen mannen en vrouwen; of tussen mensen met of zonder suikerziekte type 2 of een voorstadium van suikerziekte type 2.

Resultaten

Dit proefschrift heeft vijf hoofdbevindingen.

Ten eerste (hoofstukken 2-3), hebben we gevonden dat verhoogde suikerspiegels verband houden met een dunnere zenuwvezellaag in het netvlies (hoofdstuk 2) en een verminderde gevoeligheid van het netvlies voor lichtprikkels (hoofdstuk 3); dat een verhoogde mate van dagelijkse suikerschommelingen verband houdt met een dunnere zenuwvezellaag in het netvlies (hoofdstuk 2); dat roken ten opzichte van niet roken verband houdt met een verminderde gevoeligheid van het netvlies voor lichtprikkels, maar niet met de dikte van de zenuwvezellaag (hoofdstuk 3), dat een ongezonder dieet verband houdt met een dunnere zenuwvezellaag en een verminderde gevoeligheid van het netvlies voor lichtprikkels (hoofdstuk 3); dat een verhoogde bloeddruk verband houdt met een dunnere zenuwvezellaag, maar niet met een verminderde gevoeligheid van het netvlies voor lichtprikkels (hoofdstuk 3); dat een verminderde fysieke fitheid verband houdt met een verminderde gevoeligheid van het netvlies voor lichtprikkels, maar niet met de dikte van de zenuwvezellaag (hoofdstuk 3); dat veel ten opzichte van weinig alcohol inname verband houdt met een dunnere zenuwvezellaag in het netvlies, maar niet met de gevoeligheid van het netvlies voor lichtprikkels (hoofdstuk 3); dat een verhoogde cholesterolspiegel verband houdt met een grotere gevoeligheid van het netvlies voor lichtprikkels en bij mensen met, maar niet zonder, suikerziekte type 2 met een dikkere zenuwvezellaag (hoofdstuk 3); en dat er geen verband was tussen buikomvang of fysieke activiteit met de dikte van de zenuwvezellaag of de gevoeligheid van het netvlies voor lichtprikkels (hoofdstuk 3). Tot slot, het sterkst gevonden verband in dit proefschrift was het verband tussen suikerziekte type 2 (met ten opzichte van zonder) en de dikte van de zenuwvezellaag (hoofdstuk 2).
Ten tweede (hoofdstukken 3-7), hebben we gevonden dat matig ten opzichte van weinig alcoholinname verband houdt met minder kleine bloedvatschade en dat veel ten opzichte van weinig alcoholinname verband houdt met meer kleine bloedvatschade

(waarbij kleine bloedvatschade gemeten was in de hersenen, in het netvlies [als een wijdere slagader en ader en als een verminderde verwijdingsreactie na blootstelling aan flikkerlicht], in de nieren, in de huid, en in het bloed; hoofdstuk 4). Verder vonden we dat een hogere mate van schommelingen van de bloeddruk verband houdt met meer kleine bloedvatschade in de nieren, en in mindere mate met kleine bloedvatschade in de hersenen, het netvlies (gemeten als een verminderde verwijdingsreactie na blootstelling aan flikkerlicht), in de huid, en in het bloed (hoofdstuk 6). Daarnaast vonden we dat een hogere mate van vaatstijfheid verband houdt met meer kleine bloedvatschade (gemeten als een verminderde verwijdingsreactie na blootstelling aan flikkerlicht; hoofdstuk 7). We vonden dit verband echter niet wanneer we het verband tussen vaatstijfheid en de dikte van slagaders en aders in het netvlies onderzochten (hoofdstuk 7). Verder vonden we dat minder fysieke activiteit geen verband hield met kleine bloedvatschade gemeten in het netvlies (gemeten als de verwijdingsreactie na blootstelling aan flikkerlicht) of in de huid (hoofdstuk 5).
Ten derde (hoofdstukken 3,4,5, en 7), hebben we gevonden dat bij deelnemers met, ten opzichte van zonder, suikerziekte type 2 het verband tussen een hogere bloeddruk en zenuwschade sterker is (hierbij was zenuwschade in het netvlies gemeten als een dunnere zenuwvezellaag en een verminderde gevoeligheid voor lichtprikkels; hoofdstuk 3); dat bij deelnemers met, ten opzichte van zonder, suikerziekte type 2 matig ten opzichte van weinig alcoholinname sterker verband houdt met minder kleine bloedvatschade (gemeten in de hersenen, in het netvlies [als een wijdere slagader en ader en als een verminderde verwijdingsreactie na blootstelling aan flikkerlicht], in de nieren, in de huid, en in het bloed; hoofdstuk 4). Tevens zagen we ook dat het verband tussen alcoholinname en kleine bloedvatschade bij deelnemers met, ten opzichte van zonder, andere cardiovasculaire risicofactoren dan type 2 diabetes ook verschillend was (het verband van matig ten opzichte van lichte alcoholinname met minder kleine bloedvatschade was ook sterker bij mensen met, ten opzichte van zonder, een hoge bloeddruk; bij mensen die wel, ten opzichte van niet, roken; en bij mensen met een hogere ten opzichte van een lagere cholesterolspiegel; hoofdstuk 4). Verder vonden we dat minder fysieke activiteit bij deelnemers met, maar niet zonder, suikerziekte type 2, verband houdt met minder kleine bloedvatschade (gemeten in de huid; hoofdstuk 5); en dat het verband tussen een hogere mate van vaatstijfheid met kleine bloedvatschade (in het netvlies gemeten als een verminderde verwijdingsreactie na blootstelling aan flikkerlicht) sterker was bij deelnemers met ten opzichte van zonder suikerziekte type 2 (hoofdstuk 7). Tot slot vonden we dat het omslagpunt vanaf wanneer meer alcoholinname verband hield met meer kleine bloedvatschade verschilde tussen mannen en vrouwen, waarbij alcoholinname bij vrouwen al bij een lagere hoeveelheid alcoholinname dan bij mannen verband hield met meer kleine bloedvatschade (hoofdstuk 4).

Ten vierde (hoofdstuk 8), hebben we gevonden dat een dunnere zenuwvezellaag verband houdt met een grotere kans op het ontstaan van klinisch-relevante depressieve symptomen over de tijd (gemeten gedurende een periode van 7 jaar).
Ten vijfde (hoofdstuk 9), hebben we gevonden dat het verband tussen een dunnere zenuwvezellaag in het netvlies (zenuwschade) en een verminderde denkfunctie sterker is onder deelnemers die ook kleine bloedvatschade hebben (gemeten als een wijdere netvlies ader). Tevens, hebben we ook gevonden dat het verband tussen kleine bloedvatschade (gemeten als een wijdere netvliesader) en een verminderde denkfunctie sterker is onder deelnemers die ook een dunnere zenuwvezellaag (zenuwschade) hebben.

Discussie

Resultaten van dit proefschrift in vergelijking met de bestaande literatuur

De bevindingen in dit proefschrift komen in het algemeen overeen met de bevindingen van de huidige literatuur op het gebied van bevolkingsstudies. Verder zijn er tal van resultaten van dit proefschrift die een belangrijke aanvulling vormen op de reeds bestaande literatuur. Ten eerste worden in dit proefschrift enkele verbanden onderzocht die nog niet eerder zijn gepubliceerd. Een eerste voorbeeld hiervan is dat het verband tussen cardiovasculaire- en leefstijlfactoren en de gevoeligheid van het netvlies voor licht-prikkels nog niet onderzocht is in grote bevolkingsstudies (eerdere studies hebben minder dan n=1.000 deelnemers gebruikt en zijn derhalve niet als bevolkingsstudies te beschouwen). Een tweede voorbeeld is dat de verbanden tussen de mate van suikerspiegelschommelingen, fysieke activiteit, en fysieke fitheid met de dikte van de zenuwvezellaag nog niet onderzocht zijn in een bevolkingsstudie. Een derde voorbeeld is dat het verband tussen de dikte van de zenuwvezellaag en het ontstaan van klinische relevante symptomen van depressie nog niet eerder onderzocht is in een bevolkingsonderzoek. Een vierde voorbeeld is dat nog niet eerder onderzocht is op bevolkingsniveau of mensen met zowel meer zenuwschade (gemeten in het netvlies als een dunnere zenuwvezellaag) als meer kleine bloedvatschade (gemeten in het netvlies als een wijdere ader) een aanzienlijk verminderde denkfunctie hebben dan mensen die een of geen van deze factoren hebben. Ten tweede vormt dit proefschrift een aanvulling op de bestaande literatuur omdat in dit proefschrift enkele verbanden op een methodologisch gezien betere manier onderzocht zijn dan tot nu toe was gedaan door andere bevolkingsstudies. Een eerste voorbeeld hiervan is dat we in dit proefschrift rekening hebben gehouden met fysieke activiteit en dieet als verstorende variabelen (“confounders”). Veel eerdere studies hebben geen rekening gehouden met deze

verstorende variabelen. Dit is relevant omdat verstorende variabelen (confounders) tot een verkeerde schatting van de sterkte van een verband kunnen leiden (bijvoorbeeld een overschatting). Een tweede voorbeeld hiervan is dat we in dit proefschrift bepaalde variabelen op een preciezere manier hebben gemeten dan eerdere studies. Wij hebben bijvoorbeeld de bloeddruk gemeten gedurende een periode van 24 uur en de meeste eerdere studies hebben bloeddruk gemeten gedurende een veel kortere periode. Dit is relevant omdat een meting over een kortere tijd meer gevoelig is voor meetfouten dan een meting over een langere periode; en meetfouten tot een verkeerde schatting van de sterkte van een verband kunnen leiden. Ten derde vormt dit proefschrift een aanvulling op de bestaande literatuur omdat we in dit proefschrift onderzocht hebben of de verbanden die we bestudeerden in sterkte verschilden tussen mannen en vrouwen of tussen deelnemers met, ten opzichte van zonder, suikerziekte type 2 (of een voorstadium hiervan). Eerdere studies hebben dit niet of in een (veel) beperktere mate onderzocht. Dit is relevant omdat de schatting van de sterkte van verbanden in de algemene bevolking misschien niet in alle gevallen geldig (valide) is in bepaalde subgroepen van de bevolking, bijvoorbeeld onder mensen met suikerziekte type 2.

Biologische interpretatie van de bevindingen van dit proefschrift

Zenuwen en kleine bloedvaten zijn belangrijke regelaars van de aanvoer van voedingsstoffen en de afvoer van afvalstoffen. Schade aan de zenuwen en kleine bloedvaten kan ertoe leiden dat haarvaten beschadigd worden, waardoor deze haarvaten gaan verwijden, lekken, scheuren, en de bloedstroom verstoord wordt, waardoor een tekort aan voedingsstoffen kan ontstaan (bijvoorbeeld zuurstoftekort), wat kan leiden tot weefselschade.
Er wordt gedacht dat schade aan de kleine bloedvaten voorafgaat aan schade aan zenuwen in de hersenen en het netvlies. Enerzijds leidt schade in de kleine slagaders en aders van ongeveer 150 micrometer dikte tot een minder goede verdeling van bloed over de haarvaten, wat kan predisponeren tot een tekort aan voedingsstoffen (sommige haarvaten zijn in rusttoestand niet doorbloed en moeten worden opengezet bij een hogere behoefte aan voedingsstoffen). Anderzijds predisponeert schade in de kleine slagaders en aders tot een verhoogde druk in de haarvaten, wat kan leiden tot mechanische schade in de haarvaten (de haarvaten zijn heel kwetsbaar en bij een hoge druk kunnen ze beschadigd worden). Schade van de haarvaten kan ertoe leiden dat de scheidingsbarrière tussen het bloed en de zenuwen onvoldoende functioneert (de zogenaamde bloed-brein barrière in de hersenen; en de bloed-retina barrière in het netvlies). Hierdoor kan de concentratie van voedingsstoffen in de nabijheid van zenuwcellen verstoord raken en kunnen giftige en ontsteking-stimulerende cellen in

contact kunnen komen met zenuwcellen waardoor schade aan de zenuwcellen kan ontstaan.
Schade in de vaten en zenuwen ontstaat op een cellulair niveau in belangrijke mate door oxidatieve stress, wat door cardiovasculaire risicofactoren en ongezonde leefstijlfactoren veroorzaakt kan worden. In de kleine bloedvaten kan oxidatieve stress endotheel cellen, die aan de binnenzijde van de vaten zitten, beschadigen. Endotheel cellen zijn voor hun functie voor een groot deel afhankelijk van de beschikbaarheid van stikstofoxide en oxidatieve stress kan de beschikbaarheid van stikstofoxide in de endotheelcellen verlagen (enerzijds door de aanmaak van stikstofoxide te verminderen en anderzijds door beschikbare 'vrije' stikstofoxide weg te vangen). Verder kan in zenuwen oxidatieve stress leiden tot geprogrammeerde celdood via verschillende stappen. Oxidatieve stress kan zogenaamde "glutamaat excitotoxiciteit" veroorzaken wat, achtereenvolgens, kan leiden tot mitochondriële disfunctie; een tekort aan intracellulaire energie beschikbaarheid; structurele degeneratie van zenuwcellen (bijvoorbeeld cytoskelet beschadigingen en myelineschade), en uiteindelijk activatie van cellulaire "pathways" (routes) die tot geprogrammeerde celdood kunnen leiden (bijvoorbeeld de zogenaamde "nuclear factor kappa-beta pathway"). Tot slot, een tekort aan voedingsstoffen (bijvoorbeeld hypoxie) kan ook -onafhankelijk van glutamaat excitotoxiciteit- leiden tot activatie van cellulaire "pathways" die tot geprogrammeerde cel dood van zenuwcellen kunnen leiden.

Potentiële klinische implicaties van de bevindingen van dit proefschrift

De bevindingen van dit proefschrift komen grotendeels overeen met de aanbevelingen voor de klinische praktijk die opgenomen zijn in bestaande richtlijnen; en er zijn enkele bevindingen die nog niet in richtlijnen opgenomen zijn en derhalve mogelijk een aanvulling kunnen zijn op bestaande richtlijnen.
In overstemming met klinische richtlijnen vonden wij dat hogere suikerspiegels; hogere bloeddruk; roken; minder fysieke activiteit; en een ongezond dieet verband hielden met zenuwschade en kleine bloedvatschade. De implicatie van deze bevindingen is dat het vroegtijdig voorkomen van risicofactoren voor zenuwschade en kleine bloedvatschade mogelijk bij zou kunnen dragen aan het voorkomen van vroege zenuw- en kleine bloedvatschade. Verder vonden wij, behalve voor alcoholinname, geen aanwijzingen dat er tussen mannen en vrouwen verschillen bestaan in hoe deze risicofactoren verband houden met vroege zenuw- of kleine bloedvatschade. Dit betekent dat er nu geen aanwijzingen zijn dat er voor de vroege preventie van zenuwschade en kleine bloedvatschade geslachts-specifieke interventies nodig zijn (er is voor alcoholinname geen geslacht specifieke richtlijn nodig want de huidige richtlijnen geven aan dat alcoholinname niet aanbevolen wordt).

Enkele bevindingen komen niet overeen met aanbevelingen die opgenomen zijn in huidige richtlijnen. Ten eerste, wij vonden dat een verhoogde cholesterolspiegel verband hield met minder zenuwschade. Echter, omdat een verhoogd cholesterol een risicofactor is voor het ontstaan van diabetes en hart- en vaatziekte kan een verhoogde cholesterolspiegel niet aanbevolen worden. Ten tweede, wij vonden dat de inname van hogere hoeveelheden alcohol verband hield met minder kleine bloedvatschade bij mensen met, ten opzichte van zonder, suikerziekte type 2. Echter alcoholinname kan niet worden aanbevolen, omdat alcoholinname verband houdt met een grotere kans op vroegtijdig sterven en meer verloren productieve levensjaren door lichamelijke beperkingen.

Dit proefschrift heeft drie nieuwe bevindingen die een aanvulling zouden kunnen vormen op aanbevelingen uit bestaande richtlijnen. Ten eerste vonden wij dat een verhoogde mate van dagelijkse suikerspiegelschommelingen; een verhoogde mate van bloeddrukschommelingen; en meer vaatstijfheid verband hielden met vroege zenuw- of kleine bloedvatschade. Het zou daarom een aanvulling op bestaande richtlijnen kunnen zijn om vroegtijdig dagelijkse suikerspiegelschommelingen; bloeddrukschommelingen; en vaatstijfheid te monitoren en te behandelen. Dagelijkse suikerspiegelschommelingen en bloeddrukschommelingen zouden bijvoorbeeld via dieet interventies en bepaalde medicijnen verminderd kunnen worden; en vaatstijfheid zou misschien via gewichtsverlies en fysieke activiteit verminderd kunnen worden. Ten tweede vonden we dat bij mensen met, ten opzichte van zonder, suikerziekte type 2 een hogere bloeddruk en meer vaatstijfheid sterker verband hielden met, respectievelijk, zenuwschade en kleine bloedvatschade. Een implicatie van de eerstgenoemde bevinding zou kunnen zijn dat het streng reguleren van de bloeddruk in het bijzonder belangrijk is bij patiënten met suikerziekte type 2. Verder vonden we dat het verband tussen fysieke activiteit en minder bloedvatschade (gemeten in de huid) alleen aanwezig was bij patiënten met suikerziekte type 2. Een implicatie van deze bevinding is dat er misschien een sterker effect van bewegen op kleine bloedvatschade verwacht zou mogen worden bij patiënten met, ten opzichte van zonder, suikerziekte type 2. Ten derde vonden we dat zenuwschade en kleine bloedvatschade in het netvlies verband houden met, en/of voorafgaan aan, symptomen van breinziekten (klinisch relevante depressieve symptomen en een verminderde denkfunctie). Implicaties van deze bevindingen zijn dat het netvlies misschien in de toekomst gebruikt kan worden om mensen met een verhoogd risico op het ontstaan van breinziekten vroegtijdig op te sporen; en dat het misschien mogelijk is om vroegtijdige ziekteprogressie (voordat klachten optreden) al te monitoren via zenuwschade en kleine bloedvatschade in het netvlies. Dit zou kunnen bijdragen aan het beter voorkomen van vroege zenuwschade

en kleine bloedvatschade en (mede) daardoor mogelijk ook het voorkomen van dementie, depressie, en diabetische retinopathie.

Implicaties van de bevindingen van dit proefschrift voor toekomstig onderzoek

De bevindingen van dit proefschrift zijn veelbelovend en meer onderzoek kan mogelijk bijdragen aan het vertalen van deze onderzoeksbevindingen naar de klinische praktijk. We bespreken enkele belangrijke onderwerpen die meer onderzoek vereisen.
Ten eerste is het methodologisch gezien van belang om de bevindingen van dit proefschrift te bevestigen in longitudinale bevolkingsonderzoeken en om te onderzoeken in gerandomiseerd interventie onderzoek of het vroegtijdig modificeren van cardiovasculaire- en leefstijl-factoren ook daadwerkelijk bijdraagt aan minder zenuwschade, minder kleine bloedvatschade, en minder ziekte. Ten tweede zouden bepaalde bevindingen van dit proefschrift nog in meer detail onderzocht kunnen worden. Mogelijk zou het gedetailleerd bestuderen van individuele voedingsgroepen van een dieet meer inzicht kunnen geven in de mate waarin bepaalde voedingsstoffen invloed hebben op zenuwschade en kleine bloedvatschade. Verder zou onderzocht kunnen worden hoe groot de invloeden zijn van erfelijke aanleg, omgevingsfactoren (bijvoorbeeld luchtkwaliteit), en etnische achtergrond op de verbanden die in dit proefschrift onderzocht zijn. Ten derde is er meer onderzoek nodig naar hoe veranderingen in het netvlies precies verband houden met veranderingen in de hersenen. In dit proefschrift hebben we slechts een klein gedeelte van dit onderwerp onderzocht.

Conclusie

Dit proefschrift heeft twee hoofdbevindingen. Ten eerste, potentieel modificeerbare cardiovasculaire risicofactoren en leefstijlfactoren, waarvan suiker ziekte type 2 het sterkste voorbeeld is, houden verband met zenuwschade en kleine bloedvatschade. Ten tweede, zenuwschade en kleine bloedvatschade houden verband met, en/of gaan vooraf aan, symptomen van belangrijke breinziekten.
Een eerste belangrijke implicatie van deze bevindingen is dat het vroegtijdig behandelen van cardiovasculaire risicofactoren en ongezonde leefstijlfactoren (in het bijzonder verhoogde suikerspiegels zoals bij suikerziekte type 2) misschien bij kan dragen aan het vroegtijdig voorkomen van zenuwschade en kleine bloedvatschade en (hierdoor) ook aan het voorkomen van dementie, depressie, en diabetische retinopathie.
Een tweede belangrijke implicatie van deze bevindingen is dat vroege zenuwschade en

kleine bloedvatschade (gemeten in het netvlies) misschien gebruikt kunnen worden om mensen met een verhoogd risico op dementie, depressie, en diabetische retinopathie vroegtijdig op te sporen en/of om vroegtijdige ziekteprogressie (voordat symptomen van deze ziekten detecteerbaar zijn) te monitoren.
Tot slot, gezien de veelbelovende resultaten van dit proefschrift is het zinvol om in de toekomst meer onderzoek te verrichten om de bevindingen uit dit proefschrift te vertalen naar de klinische praktijk.

Chapter 11.2 Impact paragraph

Impact paragraph

In the impact paragraph we discuss the novelty of the findings of this thesis as well as the potential clinical, societal, and scientific impact of the findings of this thesis. The impact paragraph is composed as follows. First, we provide background information on the topic; second, we provide a brief summary of the main findings of this thesis; third, we discuss the novelty of the findings of this thesis; and fourth we, consecutively, discuss the potential clinical, societal, and scientific impact of the findings of this thesis.

Background

There is an imperative for the early prevention of major clinical disease of a neuronal and microvascular origin such as dementia,[1] late-life depression,[2] and diabetic retinopathy.[3] Importantly, these diseases can have debilitating effects on the quality of life of patients and their family; are associated with a high societal burden in terms of health costs; and an epidemic increase in the number of patients with these diseases is expected in the next 25 years.[1-4] Prevention of the above major clinical diseases may be possible via prevention of neurodegeneration and microvascular dysfunction, which precede symptoms of these major clinical diseases. However, the existing literature on the causes and consequences of neurodegeneration and microvascular dysfunction has important knowledge gaps.[5,6]

In view of the above, we in this thesis investigated 1) whether potentially modifiable cardiovascular and lifestyle factors are determinants of neurodegeneration and microvascular dysfunction; and 2) how neurodegeneration and microvascular dysfunction are associated with (early-stage) symptoms of major clinical disease, including more (clinically relevant) depressive symptoms and lower cognitive performance. In this thesis neurodegeneration was assessed in the retina; and microvascular dysfunction was estimated from indices of microvascular dysfunction assessed in the brain, retina, skin, kidney, and blood.

Main findings

Main findings of the present thesis are that potentially modifiable cardiovascular and lifestyle factors may be determinants of neurodegeneration and microvascular dysfunction; and that neurodegeneration and microvascular dysfunction are associated with, and/or precede, symptoms of major clinical disease (i.e. [clinically relevant] depressive symptoms and lower cognitive performance). Importantly, of the potentially modifiable cardiovascular and lifestyle factors under study, type 2 diabetes was the strongest determinant; and in individuals with, versus without type 2 diabetes certain

determinants under study (e.g. hypertension and arterial stiffness) were more strongly associated with neurodegeneration or microvascular dysfunction.

Novelty of concepts under study in this thesis

The concept of preventing major clinical disease of a neuronal and microvascular origin via early-stage modification of cardiovascular risk factors and adverse lifestyle factors is not novel. Alternatively, the following concepts, i.e. potential implications of the findings of this thesis, are relatively novel: 1) (retinal) indices of neurodegeneration and microvascular dysfunction may be biomarkers for the early-stage identification of individuals at risk for major clinical disease; and 2) (retinal) indices of neurodegeneration and microvascular dysfunction may be biomarkers for the monitoring of early-stage (subclinical) disease progression.

Potential clinical impact

We address two main clinical implications of our findings. First, findings of this thesis support the concept that there may be an opportunity to prevent and/or slow the onset of major clinical disease of neuronal and microvascular origin via the early-stage prevention of exposure to cardiovascular risk factors and adverse lifestyle factors. Possibly, implementation of therapeutic strategies that aim to prevent exposure to these factors may result in a reduction in neurodegeneration and microvascular dysfunction, and, consequently, to a reduction in the number of patients that develop major clinical disease. Second, findings of this thesis are in support of the concept that (the investigated) indices of neurodegeneration and microvascular dysfunction may be biomarkers for the early-stage identification of individuals at risk for major clinical disease; and that (the investigated) indices of neurodegeneration and microvascular dysfunction may be biomarkers for the early-stage monitoring of (subclinical) disease. Hence, potentially in a near future, there may be an opportunity to prevent major clinical disease in a more personalized and targeted (thus better) manner than currently possible.

Potential societal impact

If findings of this thesis are translated in to clinical practice the societal impact may be large.

Better prevention of major clinical disease may increase the health of a large number of individuals in the (near and late) future as a large number of individuals are at risk for dementia, late-life depression, and diabetic retinopathy. Epidemiologically, up to 1/3 and 1/7 women and men, respectively, are expected to develop dementia in their

lifetime[7] (in the Netherlands in 2021 currently N=290,000 individuals suffer from dementia); up to 1/9 individuals is at risk for a lifetime episode of depressive symptoms;[8] and up to 1/2 individuals with diabetes, currently approximately 1 million individuals in The Netherlands, is expected to develop some sign of diabetic retinopathy during their life time (i.e. ~500,000 individuals).[3] Additionally, as individuals with type 2 diabetes have an increased risk of developing dementia and late-life depression and the number of individuals with type 2 diabetes is expected to strongly increase in the upcoming 25 years, specific targeted prevention in individuals with type 2 diabetes may (considerably) contribute to the prevention of major clinical disease.[1-4]

Any reduction in the speed of the onset of major clinical disease, and/or any prevention of dementia, late-life depression, and diabetic retinopathy may have an important impact on the burden of major clinical diseases, both at the level of an individual (i.e. impacting the quality of life of patients and their family and friends) and at the level of the society. An example of the impact on the personal and societal burden is that prevention and/or slowing of the onset of diabetic retinopathy may prevent and/or reduce the number of life years with visual impairment and blindness (which can, at the level of an individual, improve quality of life; and, at a societal level, can reduce the loss of productive years of professional labour).[3] A second example of the impact on the societal burden is that prevention of major clinical disease may relevantly reduce the financial burden of these diseases on the national health budget (as health costs due to major clinical disease can be very high; e.g. as nearly 10% of the annual healthcare budget of the Netherlands is spent on healthcare for individuals with dementia, reducing the number of individuals that develops dementia may importantly reduce the financial burden of dementia on the healthcare budget of The Netherlands).[9]

Scientific impact

Findings of this thesis may have certain implications for future clinical research.

Here we discuss three main implications (a more detailed overview of implications for future research is presented in the general summary and discussion section [Chapter 10]).

First, in order to enable moving from a research setting to clinical practice, future researchers should aim to further confirm findings of this thesis in cohort studies with longitudinal data available; and (in a later stage) quantify the impact of early modification of cardiovascular risk factors and lifestyle factors on neurodegeneration and microvascular dysfunction in randomized controlled trials. We discuss two examples of such trials that could be conducted. A first example is that future researchers may investigate in clinical trials whether early monitoring and treatment of

daily glycaemic variability, blood pressure variability, and arterial stiffness can result in a clinically relevant reduction of neurodegeneration and microvascular dysfunction (on top of therapies which are currently used in clinical practice). A second example is that future researchers may aim to develop and evaluate type 2 diabetes-specific strategies to prevent neurodegeneration and microvascular dysfunction.[10,11]

Second, future researchers should further investigate how retinal and brain neuronal and microvascular (patho)biology relate to each other and perform research which aims to move retinal biomarkers from a preclinical research setting to a clinical setting. To start, future aetiological research may provide (cellular) population-based insight in the early pathobiology of brain disease (e.g. by using novel imaging techniques such as adaptive optics to image neurodegeneration at a cellular level in the retina).[12] Then, research with a clinical focus may seek to quantify the diagnostic potential of retinal indices of neurodegeneration and microvascular dysfunction (e.g. by investigating the clinical diagnostic value of these tools in a memory clinic setting). Next, in order to enable monitoring of neurodegeneration and microvascular dysfunction in the clinic, researchers should perform research that aims to obtain reference values for change in retinal neuronal and microvascular structures over time.[13]

Third, this thesis demonstrates that combining multiple (medical) disciplines, which are traditionally separated from each other, is a promising strategy for future research. In this thesis the disciplines ophthalmology, neurology/psychiatry, and internal medicine were combined. Therefore, future researchers may consider to pursue collaborations in multidisciplinary research teams.

References

1. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol*. 2018;14:133-50.
2. Alexopoulos GS. Mechanisms and treatment of late-life depression. *Transl Psychiatry*. 2019;9:188.
3. Wong TY and Sabanayagam C. The War on Diabetic Retinopathy: Where Are We Now? *Asia Pac J Ophthalmol (Phila)*. 2019;8:448-56.
4. Collaborators GBDN. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18:459-80.
5. Perneczky R. Dementia prevention and reserve against neurodegenerative disease. *Dialogues Clin Neurosci*. 2019;21:53-60.
6. Stehouwer CDA. Microvascular Dysfunction and Hyperglycemia: A Vicious Cycle With Widespread Consequences. *Diabetes*. 2018;67:1729-41.
7. van Bussel EF, Richard E, Arts DL, Nooyens AC, Coloma PM, de Waal MW, van den Akker M, Biermans MC, Nielen MM, van Boven K, Smeets H, Matthews FE, Brayne C, Busschers WB, van Gool WA and Moll van Charante EP. Dementia incidence trend over 1992-2014 in the Netherlands: Analysis of primary care data. *PLoS Med*. 2017;14:e1002235.
8. Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW and Ho RC. Prevalence of Depression in the Community from 30 Countries between 1994 and 2014. *Sci Rep*. 2018;8:2861.
9. Alzheimer Nederland; Fact sheet cijfers en feiten over dementie - https://www.alzheimer-nederland.nl/factsheet-cijfers-en-feiten-over-dementie; access date 03.01.2021. 2021.
10. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Juni P, Lettino M, Marx N, Mellbin LG, Ostgren CJ, Rocca B, Roffi M, Sattar N, Seferovic PM, Sousa-Uva M, Valensi P, Wheeler DC and Group ESCSD. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J*. 2020;41:255-323.
11. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, Brayne C, Burns A, Cohen-Mansfield J, Cooper C, Costafreda SG, Dias A, Fox N, Gitlin LN, Howard R, Kales HC, Kivimaki M, Larson EB, Ogunniyi A, Orgeta V, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbaek G, Teri L and Mukadam N. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*. 2020;396:413-46.
12. Burns SA, Elsner AE, Sapoznik KA, Warner RL and Gast TJ. Adaptive optics imaging of the human retina. *Prog Retin Eye Res*. 2019;68:1-30.
13. Kashani AH, Asanad S, Chan JW, Singer MB, Zhang J, Sharifi M, Khansari MM, Abdolahi F, Shi Y, Biffi A, Chui H and Ringman JM. Past, present and future role of retinal imaging in neurodegenerative disease. *Prog Retin Eye Res*. 2021:100938.

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Chapter 11.4 Curriculum vitae

Curriculum vitae

Frank Cornelis Theodorus van der Heide was born on the 11th of June 1991 in Veldhoven, The Netherlands. He lived in Veldhoven (The Netherlands; 1991-1996), Sawley (United Kingdom; 1996-2002), Neer (The Netherlands; 2002-2009), and Maastricht (The Netherlands; 2009- present). In 2009 he graduated from high school (Gymnasium; St Ursula, Horn). Then, between 2009 and 2017, he studied medicine at Maastricht University (bachelor and master) as well as classical piano at The Maastricht Music Academy (conservatorium Maastricht; bachelor and master). Next, between 2017 and 2020, he studied a master in epidemiology at the Vrije Universiteit in Amsterdam; and, between 2017 and 2021, he completed a PhD thesis on the causes and consequences of neurodegeneration and microvascular dysfunction at Maastricht University (The Maastricht Study). The PhD was supervised by prof. dr. C. Stehouwer (main supervisor [promotor]; internal medicine specialist; principal investigator of The Maastricht Study); dr. R. Henry (copromotor; internal medicine specialist); and dr. J. Schouten (copromotor; ophthalmologist). Presently (2021) Frank van der Heide is working as a post-doctoral researcher on ocular biomarkers in relation to cerebral neurodegenerative disease under supervision of prof. dr. C. Stehouwer.