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Determinants of microvascular function in individuals with and without type 2 diabetes

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Chapter 6

Summary and general discussion

Summary and general discussion

The incidence of type 2 diabetes (T2D), with or without an adverse cardiovascular risk profile has increased dramatically since decades[1,2]. Importantly, cardiovascular risk not only affects individuals with diabetes, but also individuals in the general population, without diabetes[3,4]. An adverse cardiovascular risk profile and/or having T2D are associated with an increased risk of *macrovascular* diseases (e.g. myocardial infarction, stroke, and peripheral arterial disease) and diseases which are (partly) of *microvascular* origin (e.g. heart failure, (lacunar) stroke, depression, cognitive decline, retinopathy, chronic kidney disease, and neuropathy). Evidence indicates that endothelial dysfunction is an important underlying mechanism of both macrovascular[5-8] and microvascular diseases[9-14]. Endothelial cells line the interior surface of all blood vessels, both in the macrocirculation and microcirculation, and are important in blood vessel physiology. This dissertation focused on determinants of microvascular (endothelial) dysfunction in individuals with and without T2D in order to understand its pathophysiology better.

Macrovascular (endothelial) dysfunction occurs in prediabetes with further deterioration in T2D[15-17]. This suggests that the pathogenesis of T2D-associated macrovascular disease starts before diabetes occurs (ticking clock hypothesis[18]). However, whether *microvascular* endothelial dysfunction already occurs in prediabetes has not been investigated extensively[17,19]. Key metabolic features of T2D are insulin resistance and β-cell dysfunction leading to hyperglycemia and abnormal insulin signaling. In addition, T2D typically develops with an adverse cardiovascular risk profile consisting of low-grade inflammation[20], dyslipidemia[21], hypertension[22], and arterial stiffness[16]. We investigated to what extent prediabetes- and T2D-associated microvascular dysfunction were potentially attributable to these metabolic and vascular risk factors.

In the general population, aging, male sex, dyslipidemia, obesity, hyperglycemia (including prediabetes and T2D), hypertension, current smoking, low levels of physical activity, and high levels of sedentary time are major determinants of *macrovascular* diseases. These risk factors may act via inducing *large-artery* endothelial dysfunction[8,23], atherosclerosis[24,25], and/or arterial stiffening[26-28]. However, because of endothelial cell heterogeneity (i.e. endothelial cells differ remarkably in function and structure depending on their localization), this does not necessarily imply that *microvascular* endothelial function is affected similarly[29]. As many of the risk factors of *macrovascular* diseases are also associated with *microvascular* diseases[30-33], we hypothesized that they are also determinants of microvascular endothelial function.

Against this background, we investigated in a population-based cohort study, whether (1) prediabetes and T2D are associated with microvascular endothelial

dysfunction, and whether (pre)diabetes-associated microvascular dysfunction is potentially attributable to metabolic and/or vascular risk factors. (2) We further investigated associations between (modifiable) cardiovascular risk factors and microvascular endothelial function in the general population. An epidemiological approach, like we used in The Maastricht Study, has multiple advantages over small-scale (intervention) studies; first, it allows assessment of various outcomes and determinants. Second, it enables investigation of relatively unbiased associations (e.g. without (or with proper adjustment for) confounding and without selection bias). Third, it allows translation of results to the source population, and possibly also the general population[34]. In this final chapter the key findings of the dissertation are summarized and discussed in the light of the current literature and methodological considerations.

Summary of the main findings

Microvascular (endothelial) dysfunction has been shown to be associated with, and may therefore be an important underlying mechanism of common diseases, such as heart failure[9], (lacunar) stroke[10], depression[11], cognitive decline[14], retinopathy[12], chronic kidney disease[13], and neuropathy[12]. These diseases occur in the general population and more frequently in individuals with T2D[35]. In this dissertation we focused on microvascular (endothelial) (dys)function, more specifically on the stimulus-induced vasodilation capacity of the retinal arteriolar microcirculation and skin microvasculature, measured by flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia, respectively. Impairments in stimulus-induced retinal arteriolar dilation and skin hyperemia can be seen as a reflection of microvascular endothelial dysfunction[36,37], as impairments in both responses depend on decreased bioavailability of (endothelium-derived) vasodilators such as nitric oxide and endothelium-dependent hyperpolarizing factors[36-38]. Both responses are (partly) blunted by inhibition of N^G-monomethyl-L-arginine (L-NMMA); a non-selective inhibitor of nitric oxide synthase[36-39]. Nitric oxide is an important endothelium-derived cellular signaling molecule, which relaxes vascular smooth muscle cells and thereby acts as a potent vasodilator. However, this implies that impairments in retinal and skin microvascular vasodilation responses possibly also depend on vascular smooth muscle cell dysfunction[40]. In addition, these responses may also be caused by neuronal dysfunction, as intact retinal and skin nerve signaling is necessary to sense and conduct the flicker and heat stimulus, respectively[37,41].

All findings described in this dissertation are based on data from the first 3451 individuals of The Maastricht Study[42]. The Maastricht Study is an ongoing prospective

population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of T2D[42].

In chapter 2 we tested and confirmed the hypothesis that microvascular endothelial dysfunction is already present in prediabetes with further deterioration in T2D. The regression coefficient of prediabetes was consistently ~1/2 to 1/4 of the T2D coefficient. The interpretation of a graded decline in microvascular function with worsening glucose tolerance is supported by the significant linear associations of higher levels of glycated hemoglobin A1c (HbA1c) and fasting plasma glucose with attenuated retinal and skin microvascular responses. Importantly, all associations were independent of major cardiovascular risk factors.

In chapter 3 we investigated, with mediation analyses, whether and to what extent prediabetes- and T2D-associated retinal arteriolar and skin microvascular dysfunction are potentially attributable to key metabolic and vascular features associated with T2D, such as hyperglycemia, insulin resistance, elevated blood pressure, arterial stiffness, dyslipidemia, and low-grade inflammation. We showed that hyperglycemia itself, rather than the cardiovascular risk context associated with (pre)diabetes, is the main contributor to both prediabetes- and T2D-associated retinal and skin microvascular dysfunction. In contrast, in this relatively well-treated population, insulin resistance, blood pressure, arterial stiffness, lipid profile, and low-grade inflammation did not significantly contribute to (pre)diabetes-associated microvascular dysfunction.

From chapter 2 and 3 it follows that microvascular endothelial dysfunction is a feature of both prediabetes and T2D and is mainly attributable to hyperglycemia itself, rather than the cardiovascular risk context in which (pre)diabetes typically develops. This implies an early detrimental effect of hyperglycemia on the retinal and skin microvascular responses. Our findings therefore suggest early and intensive glycemic control in (pre)diabetes as a promising therapeutic target for the prevention of (pre)diabetes-associated microvascular dysfunction.

In chapter 4 we investigated whether determinants of *macrovascular* dysfunction (aging, male sex, hypertension, dyslipidemia, hyperglycemia, higher waist circumference, and current smoking) were also determinants of *microvascular* dysfunction. We demonstrated that aging and higher levels of glycemia were inversely associated with retinal and skin microvascular vasodilation. Male sex and current smoking were associated with impaired heat-induced skin hyperemia. 24-h systolic blood pressure, waist circumference, and total-to-HDL cholesterol ratio were not significantly associated with these microvascular functions. Hence, it follows that the pattern of associations between cardiovascular risk factors and retinal and skin *microvascular* function partly resembles the one between cardiovascular risk factors and *macrovascular* function. Thus, impairment of microvascular function may constitute a pathway through which an

adverse cardiovascular risk factor pattern may increase risk of diseases of (partly) microvascular origin.

In chapter 5 we examined associations of the modifiable lifestyle risk factors, little habitual physical activity and sedentary behavior, with skin and retinal microvascular function. In addition, we investigated whether these associations were stronger in individuals with T2D as compared to those without. There were three novel findings. First, higher levels of habitual total and higher-intensity physical activity were independently associated with greater skin microvascular vasodilation in individuals with, but not in those without T2D. Second, habitual sedentary time was not associated with skin microvascular function. Third, habitual physical activity and sedentary behavior were not associated with retinal microvascular function. These findings suggest that increasing habitual daily physical activity levels should be investigated as a means to improve microvascular function, at least in individuals with T2D.

Concluding remarks

In conclusion, using an epidemiological approach, we showed that prediabetes, T2D, and multiple cardiovascular risk factors (i.e. aging, male sex, hyperglycemia, current smoking, and low levels of physical activity) are associated with retinal and/or skin microvascular endothelial dysfunction. Importantly, all associations were independent of major cardiovascular risk factors and were based on data derived from a population-based study, which expanded the evidence of the associations as found in small studies with selected patient groups[43-48] to the general population. As all our conclusions were based on cross-sectional data, longitudinal studies are needed to further investigate temporality. Longitudinal studies should elucidate whether adverse cardiometabolic risk factors increase the risk of microvascular diseases via impairments in microvascular endothelial function. From a clinical point of view, it is of great importance to target individual risk factors, or clusters of risk factors, in order to reduce microvascular endothelial dysfunction. The ultimate goal is to reduce and/or prevent common diseases of microvascular origin such as heart failure, (lacunar) stroke, depression, cognitive decline, retinopathy, chronic kidney disease, and neuropathy.

Main findings and their interpretation

Hyperglycemia is the main contributor to prediabetes- and type 2 diabetes-associated retinal and skin microvascular endothelial dysfunction

From chapter 2 it follows that microvascular endothelial dysfunction is a feature of both prediabetes and T2D[49,50], which was supported by linear associations of higher levels of HbA1c and fasting plasma glucose with attenuated retinal and skin microvascular responses. These findings are in line with earlier insufficiently-adjusted studies, in which small numbers of highly selected individuals were studied[19,51,52]. In contrast to those studies, we expanded the validity of the findings to a population-based level. Importantly, all associations were independent of a broad array of major cardiovascular risk factors.

We earlier showed significant interaction between on the one hand prediabetes and T2D and on the other *large-artery* endothelial dysfunction with regard to the risk of macrovascular diseases[53]. This implies that individuals with prediabetes and T2D, as compared to those without, are more vulnerable to the detrimental effects of large-artery endothelial dysfunction in the development of cardiovascular disease[54]. Interactions may also be present between on the one hand prediabetes and T2D and on the other *microvascular* endothelial dysfunction with regard to the risk of microvascular diseases. Microvascular endothelial dysfunction has been associated with, and thus may contribute to the development of these common diseases of microvascular origin[9-14]. Indeed it has been shown that both microvascular endothelial dysfunction and microvascular diseases occur more frequently in individuals with prediabetes and T2D than in those without[31,35,49]. Thus, our findings support the concept that microvascular endothelial dysfunction precedes the clinical diagnosis of T2D and may contribute to the development of microvascular diseases in prediabetes and T2D[35].

In chapter 3 we demonstrated that prediabetes- and T2D-associated retinal arteriolar and skin microvascular dysfunction are mainly attributable to hyperglycemia. Hyperglycemia may impair microvascular function, via the formation of advanced glycation end products and/or reactive oxygen species, both of which can lead to impaired nitric oxide bioavailability, by quenching endothelium-derived nitric oxide and directly inhibit nitric oxide synthase activity[55-60]. However, a vicious circle may exist between hyperglycemia and microvascular endothelial function[61], as microvascular endothelial function can cause hyperglycemia by impairing insulin secretion[62] and/or by impairing the timely access of glucose and insulin to their target tissue[63].

In contrast, indices of insulin resistance, blood pressure, arterial stiffness, lipid profile, and low-grade inflammation did not significantly contribute to (pre)diabetes-

associated microvascular dysfunction. These findings were (partly) unexpected, and for reasons explained below, need to be interpreted with caution. There is evidence that each of these comorbid vascular risk factors is also linked to microvascular dysfunction, through adverse effects on the insulin signaling pathway, impairment of nitric oxide bioavailability, abnormal regulation of vasomotor tone, and/or increased detrimental pulsatile flow[45,61,64-66].

First, the homeostatic model assessment (HOMA2)-index we used to assess insulin resistance may differ pathophysiologically from the hyperinsulinemic-euglycemic clamp, which is the gold standard[67]. Clamp-derived insulin resistance mainly measures insulin-mediated glucose disposal in peripheral tissue such as muscle[68], whereas HOMA2 better reflects hepatic insulin resistance[69]. The HOMA2-index we used was likely to be less accurate than for instance the index of hyperglycemia (based on the oral glucose tolerance test (OGTT)) and blood pressure (based on ambulatory 24-h blood pressure measures). Second, the frequent use of antihypertensive and lipid-modifying medication in individuals with prediabetes and T2D, likely caused the small differences in actual blood pressure and lipid profile between individuals without and with T2D. These small differences may have limited the possibility to assess a mediating effect of actual blood pressure and/or lipid profile on (pre)diabetes-associated microvascular endothelial dysfunction. Indeed, the use of antihypertensive medication partly contributed to microvascular endothelial dysfunction in (pre)diabetes, suggesting that previous exposure to elevated blood pressure, rather than actual blood pressure may be important. Third, the absence of a significant contribution of arterial stiffness on, especially, (pre)diabetes-associated retinal microvascular dysfunction was somewhat unexpected, as the retina, as low-impedance tissue, is known to be sensitive to the higher flow pulsatility associated with arterial stiffnening[70]. Possibly, the difference in arterial stiffness between individuals without and with (pre)diabetes was too small in this relatively well-treated population[71]. Last, inflammatory markers drawn from venous plasma, as compared to local measurement, may have underestimated the contribution of the low-grade inflammation index on (pre)diabetes-associated microvascular endothelial dysfunction[72].

From **chapter 2** and **3** it follows that hyperglycemia itself, rather than the cardiovascular risk context in which diabetes typically develops, is the main contributor to the differences in retinal and skin microvascular function between individuals with prediabetes or relatively well-controlled T2D and individuals with normal glucose metabolism (NGM). These findings suggest early and intensive glycemic control in (pre)diabetes as a promising therapeutic target for the prevention of (pre)diabetes-associated microvascular endothelial dysfunction, with the final goal to reduce and/or prevent microvascular diseases.

(Modifiable) cardiovascular risk factors as determinants of retinal arteriolar and skin microvascular endothelial function

Microvascular diseases not only occur in individuals with T2D, but are also common in the general population. It is therefore important to unravel determinants of microvascular endothelial dysfunction in the general population. As a consequence of endothelial cell heterogeneity (i.e. diversity between endothelial cells depending on their localization[29]) determinants of *macrovascular* endothelial dysfunction in the general population, such as aging, male sex, hypertension, dyslipidemia, hyperglycemia, higher waist circumference, and current smoking do not necessarily also have to be determinants of *microvascular* endothelial dysfunction. In chapter 4 we investigated whether determinants of *macrovascular* endothelial dysfunction were also determinants of *microvascular* endothelial dysfunction. We demonstrated that aging and higher levels of glycemia were inversely associated with retinal and skin microvascular vasodilation. Male sex and current smoking were associated with impaired heat-induced skin hyperemia. 24-h systolic blood pressure, waist circumference, and total-to-HDL cholesterol ratio were not significantly associated with these microvascular functions.

As The Maastricht Study by design oversampled individuals with T2D, and it is known that individuals with T2D have an adverse cardiovascular risk factor profile, we investigated, with interaction analyses, whether associations between cardiovascular risk factors and microvascular endothelial function were driven by the oversampling of individuals with T2D. We found non-significant interactions between any cardiovascular risk factor and T2D with regard to retinal and skin microvascular function. This implies that the associations observed in this T2D-enriched population can be considered valid for a non-oversampled population, i.e. the general population[73]. For our findings this means that aging and higher levels of glycemia, along its entire range, are associated with impaired retinal and skin microvascular function, and that these associations were not driven by the T2D oversampling. This again supports a detrimental effect of early hyperglycemia on microvascular function, which is in line with the conclusions in chapters 2 and 3.

Current smoking was associated with heat-induced skin hyperemia, but not with retinal arteriolar microvascular function, which is in line with earlier studies[46,74]. Mechanistically, smoking may induce microvascular dysfunction via increased formation of reactive oxygen species and/or inhibition of nitric oxide synthase activity[75]. The absence of an association between smoking and retinal arteriolar microvascular dysfunction was likely to be explained by detrimental effects of smoking on the 'smaller' microvasculature (such as those involved in heat-induced skin hyperemia) and not on the relatively large retinal arterioles we measured[74]. This was supported by the observation from others, that smoking was indeed associated with impaired vasodilation

of relatively large retinal venules[74]. This suggests that dysfunction of small retinal arterioles and capillaries, leads to impaired downstream blood flow and thereby further hampers the relatively passive venous vasodilation[74].

The absence of associations of 24-h systolic blood pressure and total-to-HDL cholesterol ratio with retinal and skin microvascular function was unexpected, as earlier small studies have shown microvascular dysfunction to be associated with elevated blood pressure[19] and hypercholesterolemia[76]. A potential explanation is that in this relatively healthy and well-treated population, the blood pressure and total-to-HDL cholesterol ratio range was insufficiently broad for such associations to appear. In The Maastricht Study, approximately 40% and 37% of the individuals used antihypertensive and lipid-modifying medication, respectively. In particular the use of angiotensin-converting-enzyme (ACE)-inhibitors may also directly have improved microvascular endothelial function[77].

The absence of an inverse association of higher waist circumference and/or body mass index with retinal and skin microvascular function was unexpected[43,63]. Apparently, waist circumference and body mass index do not affect microvascular function as assessed here. However, earlier reports have shown that microvascular vasomotion[78], post-occlusive reactive hyperemia[79], and insulin-mediated vasodilation[63] are indeed impaired in obese individuals. Mechanistically, higher waist circumference and/or body mass index may induce microvascular dysfunction systemically, via an increase in circulating adipose tissue-derived factors, such as tumor necrosis factor-α and free fatty acids, and/or a decrease in the anti-inflammatory adipokine adiponectin[63], which may consequently impair insulin-mediated vasodilation[63]. In addition, adipokines released by local fat deposits next to the microvasculature (i.e. perivascular adipose tissue), may locally and directly inhibit vasodilatory pathways[80].

From **chapter 4** it follows that the pattern of associations between cardiovascular risk factors and retinal and skin *microvascular* function partly resembles the one between cardiovascular risk factors and *macrovascular* function. Thus, impairment of microvascular function may constitute a pathway through which an adverse cardiovascular risk factor pattern may increase risk of diseases of (partly) microvascular origin.

In **chapter 5** we examined associations of the modifiable cardiovascular risk factors; levels of habitual physical activity and levels of sedentary time with skin and retinal microvascular function. We hypothesized that these associations were stronger in individuals with T2D as compared to those without, as hyperglycemia is a potent inducer of impaired nitric oxide bioavailability[81] and microvascular endothelial dysfunction[49].

There were three novel findings. First, higher levels of habitual total and higher-intensity physical activity were independently associated with greater skin microvascular

vasodilation in individuals with, but not in those without T2D. These findings are in line with earlier smaller studies[82,83], and are likely to be explained by beneficial effects of physical activity on (hyperglycemia-induced) impaired nitric oxide bioavailability[84]. In individuals without T2D, physical activity-induced improvement in skin microvascular function may only be observed at higher intensities and longer duration of physical activity than those in our study[85,86], which explains the absence of an association in these individuals. Second, habitual sedentary time was not associated with skin microvascular function. This may be explained by frequent transient interruptions of habitual sedentary behavior by walking and/or standing, in such a way that detrimental effects did not appear[87,88]. Such interruptions have also been shown to be beneficial for cardiovascular risk[89]. Third, habitual physical activity and sedentary behavior were not associated with retinal microvascular function. This may be explained by autoregulation of retinal blood flow, which during physical activity preserves stable retinal perfusion[90,91], and consequently does not increase shear stress, in order to maintain visual acuity. In conclusion, results from chapter 5 suggest that increasing habitual daily physical activity should be investigated as a means to improve microvascular function at least in individuals with T2D.

Methodological considerations and challenges

The studies presented in this dissertation were based on data of The Maastricht Study, an ongoing prospective population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of T2D. The results need to be interpreted in light of its methodological limitations. Internal and external validity as well as causal inference will be discussed.

Internal validity

Internal validity refers to the approximate truth about inferences regarding the source population[34]. In cohort studies, confounding, overadjustment bias, information bias, and selection bias may be potential threats to the internal validity.

Confounding and overadjustment bias

Confounding occurs when a third variable affects the association between the variable of interest and outcome[92]. To minimize the possibility of confounding, an extensive phenotyping approach of individuals of The Maastricht Study was used, which enabled us to correct for various potential confounders in the regression models. However, in

observational studies, it is impossible to fully exclude the possibility of residual confounding, for instance caused by errors in the measurement of confounders and/or unmeasured confounders[34]. For example, the set of confounders we used may not truly reflect cumulative lifetime exposure. In addition, data on dietary habits, which may affect microvascular endothelial function[93,94], were not yet available. To test the robustness of our findings, we conducted several sensitivity analyses, with extra confounders and/or substitution of confounders in the regression models. All these sensitivity analyses gave qualitatively similar results and only marginally changed regression coefficients when compared to the main regression model. This makes it unlikely that residual confounding has influenced the associations reported.

As a consequence of the adjustment for multiple confounders, overadjustment bias[95] may have occurred. Overadjustment bias leads to underestimation of associations and may occur when analyses are adjusted for a proposed confounder that could also act as 1) an intermediate (between determinant and outcome) and/or 2) a proximal causal factor (a factor that lies in the causal pathway prior to the determinant), and/or 3) a proxy of the outcome[95]. To minimize the effects of overadjustment bias, we constructed different regression models. These were based on covariates grouped according to their putative roles, and we examined changes in regression coefficients after sequentially adding these groups of variables to the regression models. The regression models in which we adjusted for (diabetic) retinopathy, nephropathy, and history of cardiovascular disease are likely overadjusted as those diseases have a (micro)vascular origin. Results from these models should therefore be interpreted conservatively.

Measurement and information bias

Errors in the measurement of determinants and outcomes (measurement bias) may result in biased associations between determinants and outcomes (information bias)[34]. In this section we will discuss the validity of the primary outcome variables (flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia) and (modifiable) cardiovascular risk factors as determinants of retinal and skin microvascular endothelial function.

Flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia were used as direct reproducible[96,97] estimates of microvascular endothelial function. Measurement bias may have occurred in two ways. First, impairments in both these responses reflect decreased bioavailability of nitric oxide and are therefore likely a reflection of microvascular endothelial dysfunction[36,37]. However, both responses may also depend on intact neuronal[37,41] (e.g. for the retina; neurovascular coupling, for the skin; axonal reflexes) and vascular smooth muscle cell functioning[40]. It is therefore important to acknowledge that stimulus-induced retinal arteriolar and skin vasodilation

may not purely be a reflection of microvascular *endothelial* function. Second, as a consequence of the deep phenotyping approach of The Maastricht Study, we were not fully able to measure microvascular function under strict standardized conditions (e.g. in a fasting state, after abstention of exercise for a specific period of time, after an extensive acclimatization period prior to the measurement, and/or with measurements performed only at a fixed time of the day (to avoid diurnal influences)). However, to minimize information bias as a consequence of dietary influences on the microcirculation[93,94], participants were asked to refrain from caffeine consumption and smoking at least 3 hours before the measurement and were only allowed to have a light meal (breakfast and/or lunch), low in fat content, at least 90 minutes before the measurement. In addition, skin measurements were performed in a climate-controlled room (24˚C) after ~10 minutes acclimatization. To reduce diurnal influences on microvascular function, participants of The Maastricht Study were randomly assigned to morning, afternoon, or evening measurement time slots. In addition, analyses (in chapter 5) were adjusted for the part of the day in which the measurement was performed. This did not change the associations reported.

Another type of measurement bias may occur when data are expressed incorrectly. In this dissertation, flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia, were generally expressed as percentage increase from baseline retinal arteriolar diameter and skin blood flow, respectively. The widespread use of ratio variables (percentages) in medical research is generally accepted and implemented mainly because of two reasons; first, there is a need for standardization in clinical and epidemiological studies; second, a ratio variable incorporates two variables (baseline value and post-stimulus value) in a single measure that can be used in multivariable linear regression models[98]. However, some studies argue that ratio variables are statistically inefficient as data expressed in percentages do not accurately scale across the range of baseline values[98-100]. As we acknowledge this possible drawback, we additionally analyzed our data when we used flicker light-induced increase (in measurement units) in retinal arteriolar diameter from baseline diameter, or heat-induced increase in skin blood flow (in perfusion units) from skin baseline blood flow, as outcomes, rather than their percentages. All these additional analyses did not materially change our results and conclusions, which highlights the robustness of our findings.

In chapters 2 and 3 we used the OGTT to classify prediabetes and T2D in order to investigate whether microvascular dysfunction is a feature of both prediabetes and T2D. However, the OGTT may misclassify. Therefore it is important to speculate on whether misclassification could have biased our results; specifically, whether misclassification of (untreated) individuals with T2D as having prediabetes could have caused an *overestimation* of microvascular dysfunction in prediabetes. Whether misclassification

has occurred or not, the finding of microvascular dysfunction in prediabetes with deterioration in T2D was supported by significant inverse linear associations between measures of hyperglycemia and retinal and skin microvascular function. In addition, for reasons described below, we consider it likely that our results actually *underestimate* rather than *overestimate* microvascular dysfunction in prediabetes.

An OGTT does not have perfect reproducibility and therefore misclassification (here defined as a second OGTT that does not yield the same classification as the first) occurs, and has been quantified in individuals with NGM, prediabetes, and T2D[101]. However, and crucially, misclassification will occur in all directions, of prediabetes as T2D *and* vice versa; of prediabetes as NGM *and* vice versa; etcetera. It is important to note that misclassification of T2D as prediabetes will cause *over*estimation of microvascular dysfunction in prediabetes. However, misclassification of 1) prediabetes as NGM, of 2) NGM as prediabetes, and 3) of prediabetes as T2D will all cause *under*estimation of microvascular dysfunction in prediabetes. Direct misclassification of NGM as T2D or of T2D as NGM could be neglected[101]. Thus, it follows that the net result of these two opposing types of misclassification is likely to be an underestimation of microvascular dysfunction in prediabetes. The more so because in our study (in contrast to[101]) the NGM group is much larger than the prediabetes group; the quantitatively largest misclassification will therefore be of NGM as prediabetes, i.e. a type of misclassification that results in underestimation.

Importantly, the term 'misclassification' may be considered questionable, because the OGTT is the current gold standard and so its results are 'true' by definition. In the example[101], the second OGTT is considered the gold standard. A better approach would have been to use the mean of both OGTTs as the gold standard, but these calculations were not available to us. Given abovementioned considerations, we conclude that misclassification is likely to have occurred, but will likely have resulted in underestimation (bias towards the null) of microvascular dysfunction in prediabetes and therefore did not affect the conclusions of the studies reported in chapters 2 and 3 of this dissertation.

In chapter 4 we investigated whether major cardiovascular risk factors were determinants of retinal arteriolar and skin microvascular function. Cardiovascular risk factors and microvascular function were measured according to well-accepted methods with state of the art technologies[42]. In order to investigate the robustness of the associations (and minimize information bias) of blood pressure, obesity, hyperglycemia, and smoking with microvascular function, we substituted determinants in the analyses (e.g. waist circumference for body mass index, fasting plasma glucose for HbA1c or 2-h postload glucose, 24-h systolic blood pressure for 24-h diastolic blood pressure, 24-h mean arterial pressure, or 24-h pulse pressure, and smoking status for pack-years

of smoking). In particular 24-h ambulatory blood pressure is a very accurate measure of blood pressure, which is not prone to the 'white coat' effect that may occur when measured in the office. In addition, earlier studies have shown that cardiovascular risk was better predicted by 24-h ambulatory blood pressure than office blood pressure[102].

In **chapter 5** we used the activePAL3^{TM} accelerometer to objectively measure daily levels of habitual physical activity and sedentary time, which in contrast to self-reported questionnaires are more precise[103] and not affected by report bias[104]. One important drawback of the accelerometer is that information about the context (e.g. leisure or work-related, individually or in groups) and type (e.g. desk work, driving, or watching television) of physical activity and sedentary behavior is unknown. In addition to the length of time the accelerometer was worn (in this study 24 hours a day), the total number of days it was worn (in this study 8 consecutive days) is also important to outbalance irregularities in physical activity and sedentary behavior levels as a consequence of awareness due to wearing the device. Participants of The Maastricht Study were highly compliant wearing the accelerometer; namely 6.3 valid days, which is longer than required for adequate assessment of sedentary behavior and close to that required for optimal estimation of physical activity[105].

Selection of the study population

Associations in a study may differ from those observed in the source population when participants are selectively included (selection bias)[34]. Selection bias can be caused by factors that influence study participation (e.g. self-selection bias) and/or procedures used to selectively include participants (e.g. complete-case analysis)[34]. In The Maastricht Study, self-selection bias may likelier have resulted in *underestimations*, rather than *overestimations* of the associations (**chapters 2, 3, 4,** and **5**) and mediation effects reported (**chapter 3**). As participants in The Maastricht Study were asked if they were able and/or willing to participate in 3 half-day visit rounds, relatively healthy and/or well-treated individuals may consequently have been attracted (i.e. individuals with an unhealthy vascular risk profile and/or longstanding diabetes, were less likely to participate as a consequence of the disease and/or (vascular) complications). Indeed, self-selection bias has earlier been shown to occur in population-based studies, in which participants were healthier than non-responders[106,107].

A second type of selection bias may have occurred as we used a complete-case analysis approach in which participants with missing data were excluded. In our studies, data were missing in approximately 35-65% of the participants (mainly due to logistical reasons (e.g. device unavailability or no trained researcher available to perform the measurement)). If missing values caused by this complete-case analysis approach are completely at random, results remain unbiased[108]. In the studies described in this

dissertation, individuals excluded from the analyses due to missing data on retinal or skin reactivity measurements and/or covariates were highly comparable to individuals included in the study populations with regard to age, sex, and cardiometabolic risk profile. We therefore assume it unlikely that selection bias has affected the associations reported.

Temporality of the associations

The present dissertation enhanced the understanding of (pre)diabetes-associated microvascular endothelial dysfunction and highlights which cardiovascular risk factors are determinants of, and thus may contribute to, microvascular dysfunction in the general population. However, we should emphasize that data were cross-sectional. In chapter 3, we conducted mediation analyses to investigate whether metabolic and vascular risk factors contribute to (pre)diabetes-associated microvascular dysfunction, in order to carefully investigate causality. However, cross-sectional studies do not formally allow drawing conclusions of direct causality of associations, as associations may be prone to the possibility of reverse causality[109]. Implications about causality should therefore be provided with caution. In chapters 4 and 5, reverse causality obviously is not an issue for associations of age and sex with microvascular dysfunction. However, reverse causality may be especially relevant for hyperglycemia[61] (i.e. microvascular dysfunction may lead to impaired timely access of glucose and insulin to their target tissues[63] as well as impaired insulin secretion[62]), hypertension (i.e. microvascular dysfunction may increase peripheral resistance)[110], dyslipidemia (i.e. microvascular dysfunction may impair action of endothelial-bound lipoprotein lipase, and thereby hamper triglyceride utilization, which may lead to increased plasma triglycerides and reduced HDL-cholesterol)[111], and physical activity (i.e. microvascular dysfunction may lead to suboptimal delivery of oxygen and nutrients to tissues (e.g. muscle) on demand[61], and therefore may hamper physical activity). Despite the limitations of cross-sectional data, we believe this dissertation is an important first step in understanding the complexity of the pathophysiology of microvascular dysfunction on a population-based level. Longitudinal studies are needed to further clarify the temporality of the associations reported.

External validity

External validity is the extent in which results of a study can be generalized to other situations and/or to other people (e.g. populations)[95]. The design of The Maastricht Study at least allows generalizability of our findings to middle-aged and older individuals

of Caucasian origin. However, associations may differ in populations with a different distribution of determinants. Chapter 4 showed hyperglycemia, aging, current smoking, and male sex as determinants of microvascular function on a population-based level. Therefore, our results may not be representative for populations-based studies, in which by design, predominantly males, current smokers, and/or only elderly people were included. In addition, our findings may not necessarily be translatable to other racial or ethnic groups.

Conclusions and future directions

The overarching aim of this dissertation was to provide more insight into the pathophysiology of microvascular endothelial dysfunction, an important underlying mechanism in common diseases such as heart failure, (lacunar) stroke, cognitive decline, depression, chronic kidney disease, retinopathy, and neuropathy. These diseases occur in the general population and more frequently in individuals with T2D and put an enormous burden on patients, their families, and social health care systems. We therefore explored determinants of microvascular endothelial dysfunction in individuals with and without T2D.

In conclusion, using an epidemiological approach, we showed that prediabetes and T2D are associated with retinal and skin microvascular endothelial dysfunction. Indeed continuous levels of glycemia below the clinical threshold of T2D were already associated with microvascular dysfunction. Hyperglycemia itself, rather than the cardiovascular risk context associated with (pre)diabetes, is the main contributor to (pre)diabetes-associated impaired retinal and skin microvascular function. In individuals with T2D, higher levels of physical activity were associated with greater skin microvascular endothelial function. These findings suggest that early and intensive treatment of hyperglycemia as well as increasing physical activity should be investigated as a means to improve microvascular function in T2D. These findings may open the discussion on the implementation of screening for prediabetes and/or early treatment of individuals with prediabetes with glucose-lowering medication[112]. However, future research should first focus on how to implement and improve screening programs for chronic (mild) hyperglycemia. In addition, the benefits of screening and early treatment on outcomes, such as reductions in micro- and macrovascular complications, transition from prediabetes to T2D, side-effects, cost-efficiency, and quality of life should be further investigated[113].

In addition, in the general population, aging and hyperglycemia were associated with retinal and skin microvascular endothelial dysfunction, and male sex and smoking were

associated with skin microvascular endothelial dysfunction. This implicates that the pattern of associations between cardiovascular risk factors and retinal and skin *microvascular* function partly resembles the one between cardiovascular risk factors and *macrovascular* function. Thus, impairment of microvascular function may constitute a pathway through which an adverse cardiovascular risk factor pattern may increase risk of diseases of (partly) microvascular origin. Importantly, all associations were independent of major cardiovascular risk factors and were based on data derived from a population-based study, which expanded the evidence of the associations as found in small studies with selected patient groups to the general population.

As all conclusions in this dissertation were based on cross-sectional data, longitudinal studies are needed to further investigate temporality. The Maastricht Study represents a valuable cohort for the investigation of longitudinal associations between determinants and microvascular endothelial function. Annual follow-up on mortality, morbidity, and disease development is already in progress, and follow-up measurements of determinants and microvascular function will be planned as soon as budget becomes available. A longitudinal design should elucidate whether adverse cardiometabolic risk factors increase the risk of microvascular diseases via impairments in microvascular endothelial function. From a clinical point of view, physicians should be aware of individual risk factors or clustering of risk factors of microvascular endothelial dysfunction in their patients. The effect of strategies which target these risk factors should be investigated with regard to the efficacy of improving microvascular endothelial function. The ultimate goal is to reduce and/or prevent common diseases of microvascular origin such as heart failure, (lacunar) stroke, depression, cognitive decline, retinopathy, chronic kidney disease, and neuropathy.

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Nederlandstalige samenvatting

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Type 2 diabetes (T2D) is een chronische stofwisselingsziekte gekenmerkt door verhoogde glucosewaarden in het bloed. Deze aandoening komt voor bij ruim 1,2 miljoen Nederlanders. Daarnaast heeft ook een groot aantal mensen diabetes, of een voorstadium van diabetes dat prediabetes wordt genoemd, zonder het te weten. Van prediabetes is sprake wanneer de glucosewaarden licht verhoogd zijn maar nog niet zo hoog dat het T2D genoemd wordt. Verhoogde glucosewaarden kunnen leiden tot chronische schade aan zenuwen (neuropathie), ogen (retinopathie) en nieren (nefropathie), de zogenoemde *microvasculaire* ziekten. Andere veelvoorkomende ziekten die een deels *microvasculaire* oorzaak hebben zijn hartfalen, (lacunaire) beroerte, depressie en cognitieve achteruitgang. Verhoogde glucosewaarden kunnen ook leiden tot ziekten aan de grote vaten, de zogenoemde *macrovasculaire* ziekten, zoals een hartinfarct, perifeer arterieel vaatlijden en een beroerte. Naast de verhoogde bloedglucosewaarden gaat T2D, maar ook prediabetes, meestal gepaard met een ongunstig cardiovasculair risicoprofiel bestaande uit bijvoorbeeld hypertensie, overgewicht, laaggradige inflammatie en dislipidemie. Deze risicofactoren verhogen de kans op het krijgen van *macrovasculaire* en *microvasculaire* ziekten. Een ongunstig cardiovasculair risicoprofiel kan ook zonder de aanwezigheid van (pre)diabetes leiden tot deze ziekten.

De microcirculatie bestaat uit alle vaten kleiner dan 200-150 µm in diameter en omvat de arteriolen, capillairen en venulen. De microcirculatie speelt een belangrijke rol bij het transport en de uitwisseling van zuurstof, voedingsstoffen en hormonen naar de weefsels. Daarnaast is de microcirculatie belangrijk bij de regulering van de perifere vaatweerstand waardoor grote fluctuaties in hydrostatische druk op het niveau van de capillairen worden voorkomen zodat de lokale bloeddruk optimaal is voor uitwisseling. Belangrijk voor deze functies is een goede balans tussen microvasculaire vaatverwijding en vaatvernauwing. De binnenbekleding van de bloedvatwand, bestaande uit endotheelcellen, speelt hierbij een belangrijke rol. Verschillende uit endotheelcellen afkomstige stoffen (bijvoorbeeld stikstofmonoxide en endotheel afkomstig hyperpolariserende factor (EDHF)) zijn vaatverwijders. Endotheline-1, ook afkomstig uit het endotheel, zorgt juist voor vaatvernauwing. Endotheelcellen komen voor in alle bloedvaten, zowel in de macro- als in de microcirculatie. In dit proefschrift onderzochten wij microvasculaire endotheelfunctie, gemeten in het oog en in de huid als respectievelijk flikker licht geïnduceerde retinale arteriolaire vaatverwijding en hitte geïnduceerde huidhyperemie. Beide vaatverwijdingsresponsen zijn afhankelijk van de biobeschikbaarheid van stikstofmonoxide. Een verminderde stimulus geïnduceerde microvasculaire vaatverwijdingsrespons van retina en/of huid werd gedefinieerd

als microvasculaire *endotheeldisfunctie*, mogelijk in combinatie met gladde spierceldisfunctie en/of neuronale disfunctie.

Microvasculaire endotheeldisfunctie is een belangrijk onderliggend mechanisme van veelvoorkomende ziekten zoals hartfalen, (lacunaire) beroerte, depressie, cognitieve achteruitgang, retinopathie, chronisch nierfalen en neuropathie. Deze ziekten komen voor in de algemene populatie en vaker bij mensen met (pre)diabetes, en vormen een enorme belasting voor de patiënten, hun families en het zorgsysteem. Een mogelijke aanpak om deze ziekten te voorkomen is het tegengaan van microvasculaire endotheeldisfunctie. Eerst dient bekend te zijn welke (cardiovasculaire) risicofactoren daarmee geassocieerd zijn en daar dus mogelijk aan kunnen bijdragen. In dit proefschrift ligt de focus op het ontrafelen van determinanten van microvasculaire endotheelfunctie bij mensen met en zonder T2D.

Van *macrovasculaire* endotheeldisfunctie is al langer bekend dat het voorkomt bij mensen met prediabetes en T2D. De aanwezigheid van macrovasculaire disfunctie bij prediabetes kan verklaren waarom mensen met prediabetes, of mensen die nog niet zo lang gediagnosticeerd zijn met T2D, al wel *macrovasculaire* ziekten kunnen hebben (de zogenoemde "tikkende klok" hypothese). Het is echter onbekend of eenzelfde hypothese ook geldt voor *microvasculaire* endotheeldisfunctie, wat kan verklaren waarom mensen met prediabetes of mensen die pas net zijn gediagnosticeerd met T2D al wel *microvasculaire* ziekten kunnen hebben. Belangrijke metabole kenmerken van T2D zijn insulineresistentie en hyperglykemie. Daarbij gaat T2D vaak gepaard met een ongunstig cardiometabool risicoprofiel bestaande uit hypertensie, arteriële vaatstijfheid, dislipidemie en laaggradige inflammatie. We onderzochten of deze metabole en vasculaire risicofactoren bijdragen aan met (pre)diabetes geassocieerde microvasculaire disfunctie.

In de algemene populatie zijn veroudering, het mannelijk geslacht, dislipidemie, hyperglykemie (zoals bij prediabetes en T2D), hoge bloeddruk, verhoogde middelomtrek, roken, weinig fysieke activiteit en veel zitten belangrijke determinanten van *macrovasculaire* ziekten. Dit kan mogelijk verklaard worden door endotheel-disfunctie in de *grote vaten*, atherosclerose en/of arteriële vaatstijfheid. Als gevolg van endotheelcel heterogeniteit (het gegeven dat endotheelcellen verschillen in functie en structuur, afhankelijk van hun lokalisatie), betekent dit niet dat *microvasculaire* endotheelfunctie dezelfde determinanten heeft. Veel van de risicofactoren voor *macrovasculaire* ziekten zijn echter ook geassocieerd met *microvasculaire* ziekten. Onze hypothese is dat deze risicofactoren ook determinanten zijn van microvasculaire endotheelfunctie.

In het licht van bovenstaande had dit proefschrift 3 doelstellingen. Ten eerste, meer inzicht krijgen in de aanwezigheid van microvasculaire endotheeldisfunctie bij mensen met prediabetes en T2D (hoofdstuk 2). Vervolgens werd nagegaan of, en welke,

metabole en vasculaire risicofactoren bijdragen aan met (pre)diabetes geassocieerde microvasculaire endotheeldisfunctie (hoofdstuk 3). Ten tweede, onderzoeken of, en welke, cardiovasculaire risicofactoren determinanten zijn van microvasculaire endotheeldisfunctie (hoofdstuk 4). Ten derde, het bestuderen van dagelijkse fysieke activiteit en zitgedrag als mogelijke beïnvloedbare risicofactoren voor microvasculaire endotheeldisfunctie (hoofdstuk 5).

Alle doelstellingen in dit proefschrift werden onderzocht met gegevens van de eerste 3451 deelnemers van de De Maastricht Studie. Dit is een grootschalig lopend bevolkingsonderzoek naar T2D, de complicaties hiervan en andere vaak met T2D geassocieerde chronische aandoeningen. Het onderzoek wordt uitgevoerd bij personen van 40-75 jaar die woonachtig zijn in de regio Maastricht-Heuvelland. Een epidemiologische benadering, met een grootschalig bevolkingsonderzoek, heeft verschillende voordelen ten opzichte van kleinschalige (interventie) studies. 1) Het geeft de mogelijkheid om verschillende determinanten en uitkomsten te bepalen en te onderzoeken. 2) De kans op bias in de associaties is kleiner (als gevolg van de correctie voor verschillende veronderstelde confounders). 3) De bevindingen kunnen vertaald worden naar tenminste de bronpopulatie en mogelijk ook de algemene bevolking.

In hoofdstuk 2 onderzochten en bevestigden wij de hypothese dat microvasculaire endotheeldisfunctie (van de retinale arteriole en de huid) al aanwezig is bij mensen met prediabetes en verslechtert bij T2D. De regressiecoëfficiënt van prediabetes was consistent ~1/2 tot 1/4 ten opzichte van de regressiecoëfficiënt van mensen met T2D. De interpretatie van deze stapsgewijze afname in microvasculaire endotheelfunctie met verslechtering van glucosetolerantie werd ondersteund door de significante lineaire associaties tussen hogere waarden van hemoglobine A1c en nuchtere bloedglucose met verslechterde microvasculaire functie van retina en huid.

In hoofdstuk 3 onderzochten wij door middel van mediatieanalyse of, en welke, vaak met (pre)diabetes geassocieerde, metabole en vasculaire risicofactoren (zoals hyperglykemie, insulineresistentie, verhoogde bloeddruk, arteriële vaatstijfheid, dislipidemie en laaggradige inflammatie) bijdragen aan met (pre)diabetes geassocieerde microvasculaire endotheeldisfunctie. De resultaten lieten zien dat hyperglykemie zelf het meeste bijdroeg aan zowel met prediabetes als T2D geassocieerde microvasculaire endotheeldisfunctie van retina en huid. In tegenstelling droegen andere ongunstige cardio-vasculaire risicofactoren zoals insulineresistentie, verhoogde bloeddruk, arteriële vaatstijfheid, dislipidemie en laaggradige inflammatie niet bij aan deze disfunctie.

In hoofdstuk 4 onderzochten wij of cardiovasculaire risicofactoren als determinanten van *macrovasculaire* endotheeldisfunctie, namelijk veroudering, het mannelijk geslacht, dislipidemie, hyperglykemie (zoals bij prediabetes en T2D), hoge bloeddruk, verhoogde middelomtrek en roken ook determinanten zijn van *microvasculaire* endotheeldisfunctie.

Veroudering en hyperglykemie waren geassocieerd met zowel microvasculaire disfunctie van retina als huid. Bovendien hadden mannen en huidige rokers een lagere microvasculaire functie in de huid dan vrouwen en niet-rokers. De 24-uurs systolische bloeddruk, middelomtrek en het cholesterolprofiel waren niet significant geassocieerd met deze microvasculaire functiematen. Dit onderzoek toont aan dat cardiovasculaire risicofactoren voor *microvasculaire* endotheeldisfunctie gedeeltelijk overeenkomen met de determinanten van *macrovasculaire* endotheeldisfunctie.

In hoofdstuk 5 onderzochten wij de associaties tussen beïnvloedbare risicofactoren, zoals weinig fysieke activiteit en veel zitgedrag, en microvasculaire endotheeldisfunctie van retina en huid. Bovendien onderzochten wij of deze associaties sterker waren bij mensen met T2D vergeleken met mensen zonder T2D. Er waren 3 nieuwe bevindingen. Ten eerste toonden wij aan dat bij mensen met T2D zowel meer totale beweging als meer zware inspanning geassocieerd waren met betere microvasculaire vaatverwijding in de huid. Deze associatie was niet aanwezig in mensen zonder T2D. Ten tweede bleek, bij zowel mensen met als zonder T2D, zitgedrag niet geassocieerd te zijn met microvasculaire endotheeldisfunctie in de huid. Ten derde, en zoals verwacht, waren er geen associaties tussen fysieke activiteit en zitgedrag enerzijds en retinale arteriolaire microvasculaire functie anderzijds. Autoregulatie van de retinadoorbloeding kan hiervoor een verklaring zijn.

Conclusies

Het overkoepelende doel van dit proefschrift was om meer inzicht te krijgen in de determinanten van microvasculaire endotheeldisfunctie bij mensen met en zonder T2D.

Uit hoofdstuk 2 en 3 volgt dat microvasculaire endotheeldisfunctie zowel voorkomt bij mensen met prediabetes en T2D. (Vroege) hyperglykemie draagt het meest bij aan de met (pre)diabetes geassocieerde microvasculaire disfunctie, in tegenstelling tot andere cardiovasculaire risicofactoren die niet significant bijdroegen. De resultaten uit hoofdstuk 5 toonden aan dat bij mensen met T2D zowel meer totale beweging als meer zware inspanning geassocieerd waren met grotere microvasculaire hyperemie van de huid. Samengenomen suggereren deze resultaten dat bij mensen met T2D een vroege en intensieve hyperglykemische controle evenals meer bewegen aanknopingspunten zijn voor therapeutische strategieën om microvasculaire endotheeldisfunctie te verminderen dan wel tegen te gaan.

In hoofdstuk 4 toonde wij op populatieniveau aan dat verschillende cardiovasculaire risicofactoren zoals veroudering, mannelijk geslacht, hyperglykemie (zoals bij prediabetes en T2D) en roken geassocieerd waren met microvasculaire endotheeldisfunctie van de retinale arteriole en/of huid. Dit onderzoek verruimt het bewijs van cardiovasculaire risicofactoren als determinanten van microvasculaire

endotheeldisfunctie, zoals gevonden in kleine studies met sterk geselecteerde patiënten groepen, naar de doorsnee bevolking. Microvasculaire disfunctie zou een 'pathway' kunnen vormen waardoor een ongunstig cardiovasculair risicoprofiel de kans op ziekten met een microvasculaire oorzaak verhoogt.

Alle associaties waren gebaseerd op cross-sectionele gegevens en waren gecorrigeerd voor, en dus onafhankelijk van, belangrijke cardiovasculaire risicofactoren (zoals leeftijd, geslacht, bloeddruk, body mass index, het cholesterolgehalte en de voorgeschiedenis van hart- en vaatziekten). Door deze correcties is getracht de kans op rest confounding in de gevonden associaties zo klein mogelijk te houden. Uit cross-sectionele gegevens kunnen geen directe uitspraken over oorzaak-gevolg gedaan worden, hiervoor zijn longitudinale onderzoeken nodig. Deze longitudinale onderzoeken zouden ten doel kunnen hebben om te ontrafelen of een ongunstig cardiovasculair profiel, bij mensen met dan wel zonder T2D, een verhoogd risico geeft op microvasculaire ziekten via microvasculaire endotheeldisfunctie. Vanuit een klinisch oogpunt is het doel om individuele en/of clusters van risicofactoren aan te grijpen om zodoende microvasculaire endotheeldisfunctie te voorkomen. Het uiteindelijke doel is de vermindering en/of het voorkomen van veelvoorkomende ziekten met een microvasculaire origine zoals hartfalen, (lacunaire) beroerte, depressie, cognitieve achteruitgang, retinopathie, chronisch nierfalen en neuropathie.