Prediabetes and Type 2 Diabetes Are Associated With Generalized Microvascular Dysfunction The Maastricht Study

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The Maastricht Study

BACKGROUND: Type 2 diabetes (T2DM) is associated with an increased risk of cardiovascular disease. This can be partly explained by large-artery dysfunction, which already occurs in prediabetes ("ticking clock hypothesis"). Whether a similar phenomenon also applies to microvascular dysfunction is not known. We therefore tested the hypothesis that microvascular dysfunction is already present in prediabetes and is more severe in T2DM. To do so, we investigated the associations of prediabetes, T2DM, and measures of hyperglycemia with microvascular function measured as flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia.

METHODS: In the Maastricht Study, a T2DM-enriched population-based cohort study (n=2213, 51% men, aged [mean±standard deviation] 59.7±8.2 years), we determined flicker light-induced retinal arteriolar %-dilation (Dynamic Vessel Analyzer), heat-induced skin %-hyperemia (laser-Doppler flowmetry), and glucose metabolism status (oral glucose tolerance test; normal glucose metabolism [n=1269], prediabetes [n=335], or T2DM [n=609]). Differences were assessed with multivariable regression analyses adjusted for age, sex, body mass index, smoking, physical activity, systolic blood pressure, lipid profile, retinopathy, estimated glomerular filtration rate, (micro)albuminuria, the use of lipid-modifying and blood pressure-lowering medication, and prior cardiovascular disease.

RESULTS: Retinal arteriolar %-dilation was (mean±standard deviation) 3.4±2.8 in normal glucose metabolism, 3.0±2.7 in prediabetes, and 2.3±2.6 in T2DM. Adjusted analyses showed a lower arteriolar %-dilation in prediabetes (B=–0.20, 95% confidence interval –0.56 to 0.15) with further deterioration in T2DM (B=–0.61 [–0.97 to –0.25]) versus normal glucose metabolism (*P* for trend=0.001). Skin %-hyperemia was (mean±standard deviation) 1235±810 in normal glucose metabolism, 1109±748 in prediabetes, and 937±683 in T2DM. Adjusted analyses showed a lower %-hyperemia in prediabetes (B=–46 [–163 to 72]) with further deterioration in T2DM (B=–184 [–297 to –71]) versus normal glucose metabolism (*P* for trend=0.001). In addition, higher glycohemoglobin A1c and fasting plasma glucose were associated with lower retinal arteriolar %-dilation and skin %-hyperemia in fully adjusted models (for glycohemoglobin A1c, standardized B=–0.10 [–0.15 to –0.05], *P*<0.001 and standardized B=–0.13 [–0.19 to –0.07], *P*<0.001, respectively; for fasting plasma glucose, standardized B=–0.09 [–0.15 to –0.04], *P*<0.001 and standardized B=–0.10 [–0.15 to –0.04], *P*=0.002, respectively).

CONCLUSION: Prediabetes, T2DM, and measures of hyperglycemia are independently associated with impaired microvascular function in the retina and skin. These findings support the concept that microvascular dysfunction precedes and thus may contribute to T2DM-associated cardiovascular disease and other complications, which may in part have a microvascular origin such as impaired cognition and heart failure.

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Clinical Perspective

What Is New?

- Macrovascular dysfunction in prediabetes may partly explain the increased risk of cardiovascular disease in prediabetes and type 2 diabetes (ticking clock hypothesis). We hypothesized that microvascular dysfunction also occurs in prediabetes, which may explain the increased risk of complications of microvascular origin in prediabetes and early type 2 diabetes.
- We demonstrated, in a population-based setting, impaired retinal and skin microvascular function in prediabetes with further deterioration in type 2 diabetes. Inverse linear associations were found between measures of glycemia (glycohemoglobin A1c, fasting and 2-hour postload glucose levels) and microvascular function. All associations were independent of cardiovascular risk factors.

What Are the Clinical Implications?

- These data extend the ticking clock hypothesis to microvascular dysfunction in prediabetes, which may explain the increased risk of complications that are (in part) of microvascular origin such as retinopathy and albuminuria, but also heart failure and cognitive decline.
- This suggests that both early hyperglycemia and microvascular dysfunction should be considered as potential targets of intervention for reducing the risk of microvascular complications in (pre)diabetes.

The current worldwide epidemic of type 2 diabetes (T2DM)[1] implies an epidemic of its complications, both macrovascular (myocardial infarction, stroke, and peripheral arterial disease)[1–3] and microvascular. The latter not only comprises retinopathy and nephropathy,[3] but also complications that are partly of microvascular origin, notably neuropathy, heart failure,[4] stroke, depression, and cognitive dysfunction.[5]

The pathogenesis of diabetic macrovascular complications is thought to involve large-artery endothelial dysfunction,[2] atherosclerosis,[6] and arterial stiffening.[7] Such macrovascular dysfunction also occurs, although in less severe forms, in prediabetes,[6–8] suggesting that the pathogenesis of T2DM-associated macrovascular disease starts before the diagnosis of T2DM (ticking clock hypothesis[9]), which also explains the increased risk of macrovascular disease in individuals with prediabetes.[10]

Whether individuals with prediabetes in addition have microvascular dysfunction has not been studied systematically.[8,11–13] However, some individuals have microvascular complications at the time of diagnosis of T2DM, and prediabetes has been associated not only with risk of retinopathy and nephropathy, but also with that of neuropathy, heart failure, stroke, and cognitive decline, which may, in part, have a microvascular origin.[5] Taken together, these data[5,9,10] raise the possibility that microvascular, similar to macrovascular, dysfunction also may occur before the diagnosis of T2DM.

In view of these considerations, we tested, in a population-based cohort study, the hypothesis that prediabetes, T2DM, and measures of hyperglycemia are associated with microvascular function in the retina and skin independently of potential confounders. We chose retina and skin because these are unique sites enabling direct and reproducible[14,15] assessment of microvascular function, as measured by flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia.[16,17]

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.[18] In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of T2DM 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 by mailings. Recruitment was stratified according to known T2DM status with an oversampling of individuals with T2DM 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 3 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. From the initial 3451 participants included, those with other types of diabetes than T2DM were excluded (n=41). Of the remaining 3410 participants, retinal arteriolar reactivity data were available in 2261 participants. The main reasons for missing data were logistic (n=882), contraindications (n=59), or insufficient measurement quality (n=208). Covariates were missing in 48 participants. The retinal arteriolar reactivity study population thus consisted of 2213 participants (Figure 1). Heat-induced skin hyperemia data were available in 1647 of the 3410 participants. The reason for missing data was logistic (n=1650), technical (n=24), or insufficient measurement quality (n=89). Covariates were missing in 52 participants. The heat-induced skin hyperemia study population thus consisted of 1595 participants (Figure 1).

Assessment of Glucose Metabolism Status

To assess glucose metabolism status, all participants (except those who used insulin) underwent a standardized 2-hour 75-g oral

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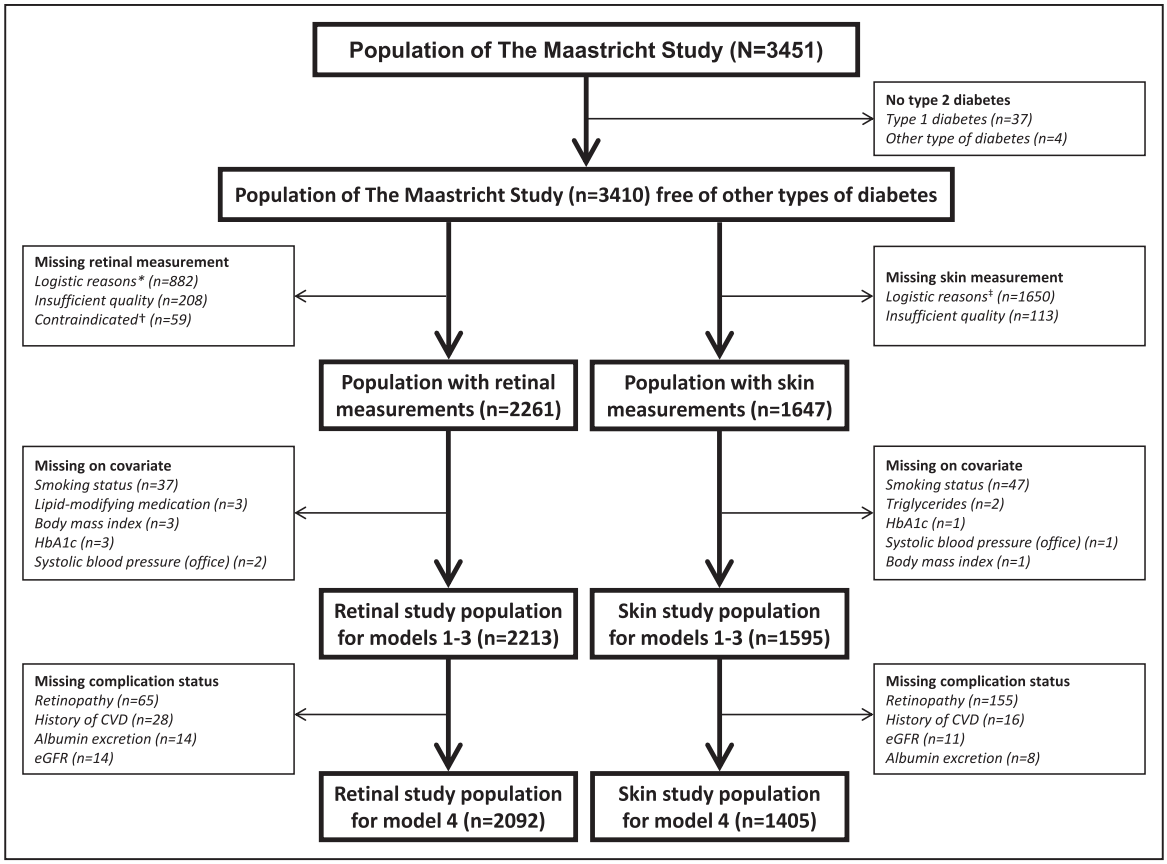

Figure 1. Retinal and skin study population selection.
CVD indicates cardiovascular disease; eGFR, estimated glomerular filtration rate; HbA1c, glycohemoglobin A1c. *Logistic reasons: no Dynamic Vessel Analyzer (DVA) equipment available (n=535), no trained researcher available (n=227), no eyedrops given for traffic safety reasons (n=120). †Contraindicated: history of epilepsy (n=14), allergy to eyedrops (n=31), glaucoma or lens implants (n=14). ‡Logistic reasons: no laser-Doppler equipment available (n=353), no trained researcher available (n=264), technical failure (n=1033).

glucose tolerance test 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 oral glucose tolerance test. 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 T2DM.[18]

Assessment of Microvascular Function

All participants were asked to refrain from smoking and drinking caffeine-containing beverages 3 hours before the measurement.[19] A light meal (breakfast or lunch), low in fat content, was allowed at least 90 minutes before the start of the measurements. For retinal measurements, pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine at least 15 minutes before the start of the examination. Skin blood flow measurements were performed in a climate-controlled room at 24°C.[20]

Retinal Arteriolar Dilation Response

The retinal arteriolar dilation response to flicker light, which is thought to be related to nutritive demands of activated retinal neurons,[21] was measured in a dimly lit room by use of the Dynamic Vessel Analyzer (IMEDOS, Jena, Germany). For safety reasons, participants with an intraocular pressure exceeding 30 mm Hg 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 diameters 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 12.5 Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The Dynamic Vessel Analyzer 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 reidentified.[21]

The integrated Dynamic Vessel Analyzer software (version 4.51; Imedos) automatically calculated baseline diameter and

percentage dilation. Baseline diameter was calculated as the average diameter size of the 20- to 50-second recording and was expressed in measurement units, where 1 measurement unit is equal to 1 μm of the Gullstrand eye.[22] 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 intervals of 0–10 seconds and 10–40 seconds during flicker stimulation) and averaged to assess average percentage dilation (Figure 2A). The software successfully assessed 2 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.

Skin Hyperemic Response

Skin blood flow was measured as described previously 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.[23] 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 (Appendix 1 in the online-only Data Supplement).[5] 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 hyperemic response was expressed as the percentage increase in average perfusion units during the 23-minute heating phase over the average baseline perfusion units (Figure 2B). The response is thought to be related to skin metabolic and thermoregulatory function.[24]

Validation of Measurements

Retinal and skin vasodilation measurements were performed by different observers after an intensive training period. Interobserver reliability (intraclass correlation coefficient) of the retinal arteriolar baseline diameter and percentage dilation response (n=9) between 2 randomly selected observers was 0.980 and 0.796, respectively. Retinal arteriolar dilation response curves were analyzed by 1 observer for measurement quality decisions, whereas heat-induced skin hyperemic response curves were analyzed by 2 observers (n=1760, intraclass correlation coefficient=0.839). Retinal and skin response curves with insufficient measurement quality, eg, insufficient measurement points or movement artifacts, were evaluated and discussed with a second observer and excluded on mutual agreement. To assess the intraclass correlation coefficient of retinal response curve quality decisions, 50 curves were evaluated by 2 observers (intraclass correlation coefficient=0.883).

Measurement of Covariates

History of cardiovascular disease, duration of diabetes, menopausal status, physical activity (hours/week), and smoking status (never, former, current) were assessed by questionnaire.[18] Use of lipid-modifying, antihypertensive, and glucose-lowering medication as well as postmenopausal hormone replacement therapy was assessed during a medication interview where

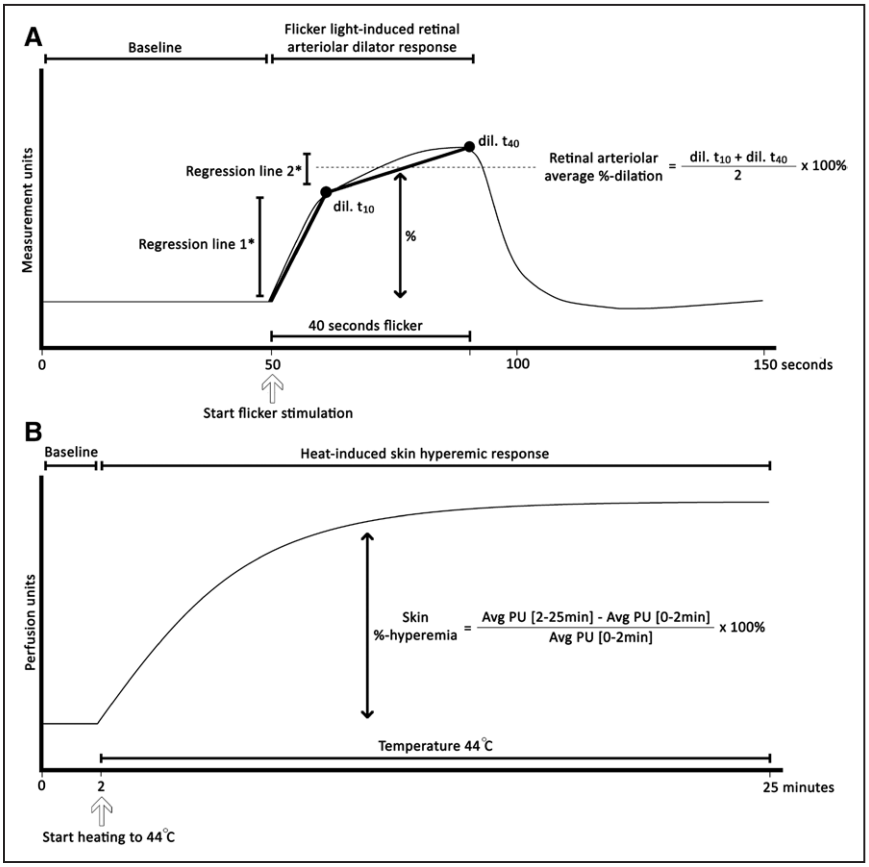

Figure 2. A, Schematic Dynamic Vessel Analyzer registration of a flicker light-induced retinal arteriolar dilation response. B, Schematic laser-Doppler registration of a heat-induced skin hyperemic response.

Avg indicates average; Dil, dilation; min, minutes; PU, perfusion units. *Regression lines were drawn at interval 0 to 10 seconds and 10 to 40 seconds during flicker stimulation.

generic name, dose, and frequency were registered.[18] We measured weight, height, body mass index, waist circumference, office and ambulatory 24-hour blood pressure, plasma glucose levels, serum creatinine, 24-hour urinary albumin excretion (twice), glycohemoglobin A1c (HbA1c), and plasma lipid profile as described elsewhere.[18] Estimated glomerular filtration rate (in mL/min/1.73 m^2) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation based on both serum creatinine and serum cystatin C.[25] The presence of retinopathy was assessed in both eyes by use of fundus photographs taken with an auto fundus camera (Model AFC-230; Nidek, Gamagori, Japan).[18]

Statistical Analysis

Statistical analyses were performed by use of the Statistical Package for Social Sciences (Version 22.0; IBM, Chicago, IL). Multiple linear regression analysis was used to determine the association of glucose metabolism (NGM, prediabetes, and T2DM), fasting plasma glucose, 2-hour postload oral glucose tolerance test plasma glucose levels, or HbA1c with retinal arteriolar dilation responses and heat-induced skin hyperemia. For linear trend analyses, the categorical variable glucose metabolism status (NGM=0, prediabetes=1, and T2DM=2) was used in the regression models. To assess regression coefficients per glucose metabolism group, pairwise analyses with dummy variables for prediabetes and T2DM were used. Model 1 was the crude model; model 2 was adjusted for age and sex; and model 3 was additionally adjusted for cardiovascular risk factors that have previously been associated with altered vessel responses and may therefore be potential confounders (body mass index, smoking status, systolic blood pressure, physical activity, use of antihypertensive and lipid-modifying drugs, fasting triglycerides and total-to-high-density lipoprotein cholesterol levels). In model 4, we additionally adjusted for a history of cardiovascular disease, retinopathy, estimated glomerular filtration rate, and urinary albumin excretion (the latter 2 as continuous variables). A P value <0.05 was considered statistically significant. Interaction terms (eg, prediabetes*sex or HbA1c*sex) were incorporated in the regression models to test for interaction among, on the one hand, prediabetes, T2DM, and measures of hyperglycemia and, on the other hand, sex, on retinal arteriolar dilation and heat-induced skin hyperemia. A $P_{interaction}$ <0.10 was considered statistically significant.

RESULTS

Characteristics of the Study Population

General characteristics of the population in which retinal reactivity data were available are shown in Table 1 stratified for glucose metabolism status. The study population consisted of 2213 individuals with a mean±standard deviation age of 59.7±8.2 years of whom 51.1% were men. Individuals with T2DM were, by design, oversampled (27.5%). The cardiometabolic risk profile deteriorated with glucose metabolism status. Individuals with T2DM were older, more often male and/or a current smoker, and had a higher body mass index, waist circumference, blood pressure, fasting plasma glucose and triglyceride levels, and less physical activity than individuals without T2DM (Table 1). The population in which heat-induced skin hyperemia data were available (n=1595) overlapped for 73% with the population in which retinal reactivity data were available and was comparable with regard to age, sex, and cardiometabolic risk profile (Tables I and II in the online-only Data Supplement). Individuals with missing data on retinal or skin reactivity measurements or covariates were to a great extent comparable with regard to age, sex, and cardiometabolic risk profile as compared with individuals included in the study populations (Tables I and II in the online-only Data Supplement).

Glucose Metabolism Status and Flicker Light-Induced Retinal Arteriolar Dilation

Arteriolar baseline diameter did not significantly differ among the 3 groups of glucose metabolism in crude or adjusted analyses, whereas the average %-dilation was lower in individuals with prediabetes and T2DM as compared with NGM (P for trend <0.001; Figure 3A and Table 2). Adjustment for age and sex attenuated the difference in %-dilation between individuals with T2DM or prediabetes and NGM, but the association remained statistically significant (P for trend <0.001). Additional adjustment for cardiovascular risk factors (model 3) and for history of cardiovascular disease, retinopathy, estimated glomerular filtration rate, and urinary albumin excretion (model 4) further attenuated the difference in %-dilation between individuals with T2DM or prediabetes and NGM, but the association again remained statistically significant with the unstandardized regression coefficient of prediabetes consistently approximately half to one fourth of the T2DM coefficient (Figure 3A and Table 2). Possible confounders, in the fully adjusted model, that were associated with the retinal dilator response were age (B=–0.04, 95% confidence interval –0.06 to –0.02), systolic blood pressure (B=0.01 [0.00–0.02]), triglycerides (B=–0.18 [–0.37 to 0.01]), and retinopathy (B=–1.34 [–2.41 to –0.26]; betas indicate the change in %-dilation per 1-U higher age, systolic blood pressure, and triglycerides or when having retinopathy).

Glucose Metabolism Status and Heat-Induced Skin Hyperemic Outcomes

Baseline skin blood flow did not significantly differ among the three groups of glucose metabolism in crude or adjusted analyses, whereas the hyperemic response was lower in individuals with prediabetes and T2DM as compared with NGM (P for trend <0.001; Figure 3B and Table 2). Adjustment for age and sex attenuated the difference in hyperemic response between individuals with T2DM or prediabetes and NGM, but the association remained statistically significant (P for trend <0.001).

Table 1. General Characteristics and Retinal and Skin Measures for the Retinal Study Population According to Glucose Metabolism Status

Characteristic	NGM (n=1269)	Prediabetes (n=335)	T2DM (n=609)
Age, y	57.8±8.2	61.3±7.5	62.8±7.6
Women	737 (58.1)	155 (46.3)	191 (31.4)
Postmenopausal	542 (75.2)	121 (81.2)	148 (87.6)
Hormone replacement therapy	13 (1.8)	2 (1.3)	3 (1.6)
Diabetes duration, y*	-	-	8.2±7.0
Body mass index, kg/m^2	25.4±3.5	27.5±4.3	29.8±4.9
Waist circumference, cm			
Men	96.1±9.2	101.9±10.8	107.4±12.7
Women	85.5±9.9	92.2±12.4	101.3±13.9
History of cardiovascular disease	141 (11.3)	40 (12.0)	158 (26.3)
Office SBP, mm Hg	130.9±17.3	137.3±17.2	142.1±17.6
Office DBP, mm Hg	75.4±9.9	78.1±9.7	77.4±9.6
Ambulatory 24-h SBP, mm Hg	117.3±11.4	119.9±11.2	122.5±11.7
Ambulatory 24-h DBP, mm Hg	73.6±7.2	74.4±7.2	72.9±7.0
Physical activity, h/wk	14.8±8.0	14.6±8.0	11.9±7.5
Smoking			
Never/former/current	508/614/147	95/199/41	177/346/86
Never/former/current, %	40.0/48.4/11.6	28.4/59.4/12.2	29.1/56.8/14.1
Fasting glucose, mmol/L	5.2±0.4	5.9±0.6	7.9±2.1
2-h postload glucose, mmol/L†	5.4±1.1	8.1±1.7	14.3±3.9
HbA1c, %	5.4±0.3	5.7±0.4	6.9±1.0
HbA1c, mmol/mol	35.7±3.7	38.5±4.5	51.7±11.4
Triglycerides, mmol/L	1.2±0.6	1.6±1.0	1.8±1.0
Total-to-HDL cholesterol ratio	3.5±1.1	3.8±1.2	3.7±1.1
Total cholesterol, mmol/L	5.6±1.0	5.5±1.2	4.4±1.0
HDL cholesterol, mmol/L	1.7±0.5	1.5±0.4	1.3±0.4
LDL cholesterol, mmol/L	3.3±0.9	3.2±1.0	2.4±0.9
Antihypertensive medication use	266 (21.0)	141 (42.1)	438 (71.9)
Lipid-modifying medication use	203 (16.0)	117 (34.9)	454 (74.5)
Diabetes medication use	0 (0)	0 (0)	476 (78.2)
Insulin	-	-	121 (19.9)
Metformin	-	-	423 (69.5)
Sulfonylureas	-	-	119 (19.5)
Thiazolidinediones	-	-	6 (1.0)
GLP-1 analogs	-	-	3 (0.5)
DPP-4 inhibitors	-	-	43 (7.1)
eGFR, mL/min/1.73 m^2	90.3±13.0	87.2±13.7	84.7±17.3
eGFR <60, mL/min/1.73 m^2	19 (1.5)	11 (3.3)	62 (10.3)
(Micro)albuminuria‡	55 (4.4)	18 (5.4)	106 (17.4)
Retinopathy	1 (0.1)	1 (0.3)	25 (4.2)
Baseline arteriolar diameter, MU	115.5±15.5	114.5±15.8	115.9±15.9

(*Continued*)

Table 1. Continued

Characteristic	NGM (n=1269)	Prediabetes (n=335)	T2DM (n=609)
Arteriolar average dilation, %			
Mean±SD	3.4±2.8	3.0±2.7	2.3±2.6
Median (interquartile range)	3.1 (1.2–5.3)	2.7 (0.8–4.9)	1.5 (0.4–3.8)
Baseline skin blood flow, PU§	11.1±6.8	11.5±6.8	10.9±5.6
Skin hyperemic response, %§			
Mean±SD	1234.9±810.4	1108.7±747.9	936.7±683.2
Median (interquartile range)	1097.4 (646.8–1630.7)	995.5 (567.0–1518.5)	821.3 (480.0–1207.8)

Data are reported as mean±SD or number (percentages) as appropriate. Data present the retinal study population for regression models 1–3.

DBP indicates diastolic blood pressure; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP, glucagon-like peptide; HbA1c, glycohemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MU, measuring units; NGM, normal glucose metabolism; PU, perfusion units; SBP, systolic blood pressure; SD, standard deviation; and T2DM, type 2 diabetes mellitus.

*Available in 420 T2DM individuals.

†Available in 471 T2DM individuals.

‡(Micro)albuminuria was defined as a urinary albumin excretion of >30 mg per 24 h.

§Heat-induced skin hyperemia measures were available in a different subset of n=1595 (Supplemental Table 1).

Additional adjustment for cardiovascular risk factors (model 3) and for history of cardiovascular disease, retinopathy, estimated glomerular filtration rate, and urinary albumin excretion (model 4) further attenuated the difference in hyperemic response between individuals with T2DM or prediabetes and NGM, but the association again remained statistically significant with the unstandardized regression coefficient of prediabetes consistently approximately half to one fourth of the T2DM coefficient (Figure 3B and Table 2). Possible confounders, in the fully adjusted model, that were significantly associated with the skin hyperemic response were age (B=–12, 95% confidence interval –19 to –6), female sex (B=311 [224–397]), body mass index (B=11 [1–21]), smoking status (B=–265 [–398 to –132]), systolic blood pressure (B=3 [0–5]), and triglycerides (B=–89 [–144 to –33]; betas indicate the change in %-hyperemia per unit higher age, body mass index, systolic blood pressure, and triglycerides or being female or a current [versus never] smoker).

Associations of Measures of Hyperglycemia With Retinal Arteriolar Dilation and Heat-Induced Skin Hyperemia

HbA1c, fasting plasma glucose, and 2-hour postload glucose levels were not associated with baseline retinal arteriolar diameter or baseline skin blood flow, either in crude or adjusted models (data not shown). In contrast, both HbA1c and fasting plasma glucose were associated with the average percentage dilation and hyperemic response, both in crude and adjusted models (Table 3 and Figure 4). The association of 2-hour postload glucose levels with retinal arteriolar percentage dilation was nonsignificant after adjustment for cardiovascular risk factors and remained significant for the skin hyperemic response (Table 3 and Figure 4).

Additional Analyses

With regard to the retinal arteriolar dilation analyses, the associations of prediabetes and T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with average percentage dilation remained unchanged after excluding participants of whom the average percentage dilation was based on one instead of two regression lines (n=102, data not shown). Second, no significant differences were found between average percentage dilation measured in the right versus the left eye (3.0±2.7% and 3.1±2.9%, respectively, *P*=0.153). Third, qualitatively similar associations of prediabetes, T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with the retinal arteriolar dilator response were found when using absolute retinal arteriolar diameter corrected for baseline arteriolar diameter (data not shown). Similar associations also remained when substituting office systolic blood pressure for 24-hour ambulatory systolic blood pressure (for retinal analyses, 24-hour ambulatory blood pressure was available in n=1962 individuals; Table III in the online-only Data Supplement) or adding physical activity (for retinal analyses, physical activity data were available in n=1974 individuals; Table IV in the online-only Data Supplement) or further specification of blood pressure-lowering medication into renin–angiotensin–aldosterone system inhibitors and other types of antihypertensives in the regression models (Table V in the online-only Data Supplement). Fourth,

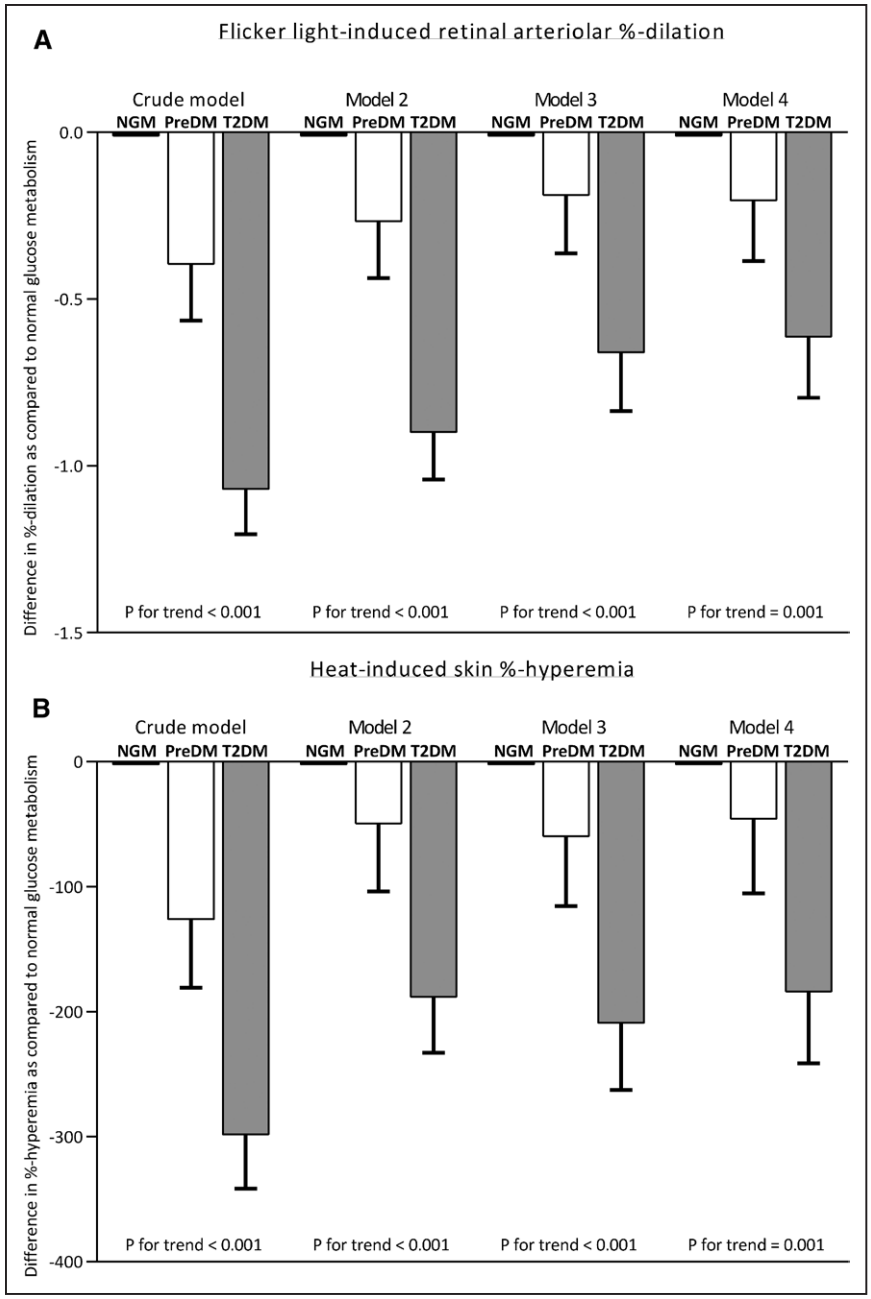

Figure 3. Multivariable adjusted differences in flicker light-induced retinal arteriolar %-dilation (A) and heat-induced skin %-hyperemia (B) between individuals with prediabetes (PreDM) and type 2 diabetes (T2DM) compared with normal glucose metabolism (NGM).

Bars represent the mean difference with standard error in retinal arteriolar %-dilation or heat-induced skin %-hyperemia for prediabetes and T2DM as compared with NGM. *P* values indicate trend analyses among NGM, prediabetes, and T2DM. NGM is the reference and is set to zero. Model 2: adjusted for age and sex. Model 3: additionally adjusted for body mass index, triglyceride levels, total-to-high-density lipoprotein cholesterol ratio, smoking status, systolic blood pressure, and use of antihypertensive and/or lipid-modifying drugs. Model 4: additionally adjusted for history of cardiovascular disease, retinopathy, estimated glomerular filtration rate (eGFR), and urinary albumin excretion.

analyses excluding T2DM participants with microvascular complications (retinopathy, estimated glomerular filtration rate <60 mL/min/1.73 m^2 and/or urinary albumin excretion >30 mg/24 hours; Table VI in the online-only Data Supplement) or using newer types of antidiabetic medication (such as glucagon-like peptide-1 analogs, thiazolidinediones, or dipeptidyl peptidase-4 inhibitors; Table VII in the online-only Data Supplement), gave similar results with regard to the associations of prediabetes and T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with average percentage dilation.

Fifth, with regard to heat-induced skin hyperemia analyses, qualitatively similar associations of prediabetes, T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with the heat-induced skin hyperemic response were found when using absolute skin blood flow corrected for baseline skin blood flow (data not shown). Similar associations also remained when substituting office systolic blood pressure for 24-hour ambulatory systolic blood pressure (for skin analyses, 24-hour ambulatory blood pressure was available in n=1402 individuals; Table III in the online-only Data Supplement) or adding physical activity (for skin analyses, physical activity data were available in n=1406 individuals; Table IV in the online-only Data Supplement) or further specification of blood pressure-lowering medication into renin–angiotensin–aldosterone system inhibitors and other types of antihypertensives in the regression models (Table V in

Table 2. Multivariable Adjusted Differences in Retinal Arteriolar Baseline Diameter, Flicker Light-Induced Retinal Arteriolar %-Dilation, Skin Baseline Blood Flow, and Heat-Induced Skin %-Hyperemia Among Individuals With Normal Glucose Metabolism (NGM), Prediabetes, and Type 2 Diabetes (T2DM)

Characteristic	Prediabetes*	T2DM*	*P* for Trend
Retinal arteriolar baseline diameter (MU)	B (95% CI)	B (95% CI)	
Crude	–1.02 (–2.91 to 0.86)	0.39 (–1.12 to 1.90)	0.749
Model 2	–0.69 (–2.60 to 1.22)	0.91 (–0.69 to 2.51)	0.334
Model 3	–0.62 (–2.58 to 1.35)	0.84 (–1.13 to 2.80)	0.490
Model 4	–0.05 (–2.06 to 1.96)	0.83 (–1.20 to 2.86)	0.457
Retinal arteriolar average dilation (%)	B (95% CI)	B (95% CI)	
Crude	–0.39 (–0.73 to –0.06)	–1.07 (–1.34 to –0.80)	<0.001
Model 2	–0.27 (–0.60 to 0.07)	–0.90 (–1.18 to –0.62)	<0.001
Model 3	–0.19 (–0.53 to 0.16)	–0.66 (–1.01 to –0.31)	<0.001
Model 4	–0.20 (–0.56 to 0.15)	–0.61 (–0.97 to –0.25)	0.001
Retinal arteriolar average dilation (SD)	stB (95% CI)	stB (95% CI)	
Crude	–0.05 (–0.09 to –0.01)	–0.17 (–0.21 to –0.13)	<0.001
Model 2	–0.03 (–0.08 to 0.01)	–0.14 (–0.19 to –0.10)	<0.001
Model 3	–0.02 (–0.07 to 0.02)	–0.11 (–0.16 to –0.05)	<0.001
Model 4	–0.03 (–0.07 to 0.02)	–0.10 (–0.16 to –0.04)	0.001
Skin baseline blood flow (PU)	B (95% CI)	B (95% CI)	
Crude	0.37 (–0.53 to 1.27)	–0.19 (–0.91 to 0.53)	0.689
Model 2	0.05 (–0.87 to 0.96)	–0.58 (–1.33 to 0.18)	0.151
Model 3	0.35 (–0.59 to 1.28)	0.00 (–0.90 to 0.91)	0.930
Model 4	0.15 (–0.86 to 1.17)	–0.25 (–1.23 to 0.72)	0.655
Heat-induced skin hyperemia (%)	B (95% CI)	B (95% CI)	
Crude	–126 (–233 to -19)	–298 (–384 to –213)	<0.001
Model 2	–49 (–156 to 57)	–188 (–276 to –100)	<0.001
Model 3	–60 (–169 to 50)	–209 (–314 to –104)	<0.001
Model 4	–46 (–163 to 72)	–184 (–297 to –71)	0.001
Heat-induced skin hyperemia (SD)	stB (95% CI)	stB (95% CI)	
Crude	–0.06 (–0.11 to –0.01)	–0.18 (–0.23 to –0.13)	<0.001
Model 2	–0.02 (–0.07 to 0.03)	–0.11 (–0.16 to –0.06)	<0.001
Model 3	–0.03 (–0.08 to 0.02)	–0.12 (–0.19 to –0.06)	<0.001
Model 4	–0.02 (–0.08 to 0.03)	–0.11 (–0.18 to –0.04)	0.001

Regression coefficients (B) indicate the mean difference (95% CI) in retinal and skin measures (in %) with NGM as a reference. Standardized regression coefficients (stB) and 95% CI represent the change in retinal and skin measures (in SD) as compared with NGM.

CI indicates confidence interval; MU, measuring units; PU, perfusion units; and SD, standard deviation.

Model 2: adjusted for age and sex.

Model 3: additionally adjusted for body mass index, triglyceride levels, total-to-high-density lipoprotein cholesterol ratio, smoking status, systolic blood pressure, and use of antihypertensive and/or lipid-modifying drugs.

Model 4: additionally adjusted for history of cardiovascular disease, retinopathy, estimated glomerular filtration rate, and urinary albumin excretion.

*The retinal reactivity population consisted of 335 individuals with prediabetes and 609 with T2DM; the heat-induced skin hyperemia population consisted of 254 individuals with prediabetes and 478 with T2DM.

the online-only Data Supplement). Sixth, analyses excluding T2DM participants with microvascular complications (Table VI in the online-only Data Supplement) or using newer types of antidiabetic medication (Table VII in the online-only Data Supplement) gave similar results with regard to the associations of prediabetes and T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with the heat-induced skin hyperemic response.

Table 3. Multivariable-Adjusted Associations of HbA1c, Fasting Glucose, and 2-Hour Postload Glucose Levels With the Flicker Light-Induced Retinal Arteriolar Dilator and the Heat-Induced Skin Hyperemic Response

	HbA1c		Fasting Glucose		2-H Postload Glucose	
Characteristic	**stB (95% CI)**	***P* Value**	**stB (95% CI)**	***P* Value**	**stB (95% CI)**	***P* Value**
Retinal arteriolar average dilation (%)						
Crude	–0.17 (–0.21 to –0.13)	<0.001	–0.16 (–0.20 to –0.12)	<0.001	–0.11 (–0.15 to –0.07)	<0.001
Model 2	–0.15 (–0.19 to –0.11)	<0.001	–0.14 (–0.18 to –0.10)	<0.001	–0.08 (–0.13 to –0.04)	<0.001
Model 3	–0.12 (–0.17 to –0.07)	<0.001	–0.11 (–0.16 to –0.06)	<0.001	–0.05 (–0.10 to 0.00)	0.054
Model 4	–0.10 (–0.15 to –0.05)	<0.001	–0.09 (–0.15 to –0.04)	<0.001	–0.04 (–0.10 to 0.01)	0.123
Heat-induced skin hyperemia (%)						
Crude	–0.17 (–0.21 to –0.12)	<0.001	–0.16 (–0.20 to –0.11)	<0.001	–0.14 (–0.19 to –0.09)	<0.001
Model 2	–0.12 (–0.17 to –0.07)	<0.001	–0.10 (–0.15 to –0.05)	<0.001	–0.08 (–0.13 to –0.03)	0.002
Model 3	–0.13 (–0.18 to –0.07)	<0.001	–0.10 (–0.15 to –0.04)	0.001	–0.10 (–0.16 to –0.04)	0.001
Model 4	–0.13 (–0.19 to –0.07)	<0.001	–0.10 (–0.15 to –0.04)	0.002	–0.09 (–0.15 to –0.02)	0.007

Point estimates (standardized betas) and 95% CIs represent the change in flicker-light induced retinal arteriolar %-dilation (in SD) and heat-induced skin %-hyperemia (in SD) per SD increase in HbA1c, fasting plasma glucose, or 2-h postload glucose level.

CI indicates confidence interval; HbA1c, glycohemoglobin A1c; SD, standard deviation; stB, standardized beta.

Model 2: adjusted for age and sex.

Model 3: additionally adjusted for body mass index, triglyceride levels, total-to-high-density lipoprotein cholesterol ratio, smoking status, systolic blood pressure, and use of antihypertensive and/or lipid-modifying drugs.

Model 4: additionally adjusted for history of cardiovascular disease, retinopathy, estimated glomerular filtration rate, and urinary albumin excretion.

Seventh, qualitatively similar associations of prediabetes, T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with the flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemic response were found when excluding outliers (defined as <3 standard deviations or >3 standard deviations; n=11 for retinal measures, n=25 for skin measures) (data not shown) or when selecting individuals who both had a retinal and skin measurement (n=1162) (data not shown). Finally, the associations among prediabetes, T2DM, and measures of hyperglycemia with flicker light-induced retinal arteriolar dilation and heat-induced skin hyperemia did not statistically significantly differ between men and women (*P* values for interaction >0.140). Moreover, additional adjustment for postmenopausal status and/or hormone replacement therapy in women did not influence the associations among prediabetes, T2DM, fasting glucose, 2-hour postload glucose, or HbA1c with flicker light-induced retinal arteriolar dilation or heat-induced skin hyperemia (Table VIII in the online-only Data Supplement).

DISCUSSION

This study demonstrates that both prediabetes and T2DM are associated with microvascular dysfunction in the retina and skin. In addition, measures of glycemia (HbA1c, fasting or 2-hour postload glucose levels) are linearly associated with microvascular dysfunction. These associations were independent of major cardiovascular risk factors and clinically defined diabetic retinopathy and nephropathy. Taken together, these data support the concept that generalized microvascular dysfunction occurs before the diagnosis of T2DM and may play a role in disorders that are (in part) of microvascular origin and that may occur early in the course of T2DM and indeed in prediabetes, notably retinopathy, nephropathy, neuropathy, heart failure, stroke, and cognitive decline.

This is the first large, population-based study to show that microvascular dysfunction is a feature of prediabetes and T2DM. Importantly, and in contrast to previous, smaller studies,[12,13,26–28] we showed that these findings were independent of a broad array of potential confounders, which, as compared with age- and sex-adjusted estimates, explained approximately 2% to 32% of the associations between (pre)diabetes and microvascular dysfunction (Table 2 and online-only Data Supplement Tables IV through VI). In addition, we showed that baseline values of retinal arteriolar diameters and skin perfusion were similar among the groups and thus did not explain group differences in microvascular responses. Retinal arteriolar diameters were obtained in a single, temporally located vessel; our data therefore do not necessarily contradict prior findings of wider central retinal arteriolar equivalent in prevalent diabetes,[16] because central retinal arteriolar equivalent represents averaged diameter of at least 6 arterioles radiating from the optic disc, whether temporally or nasally located.

We used linear trend analyses to test for a graded decline in microvascular function from NGM to prediabetes to T2DM, because a similar trend has been shown for macrovascular function.[6–8] Indeed, the decline in microvascular function in prediabetes was approximately

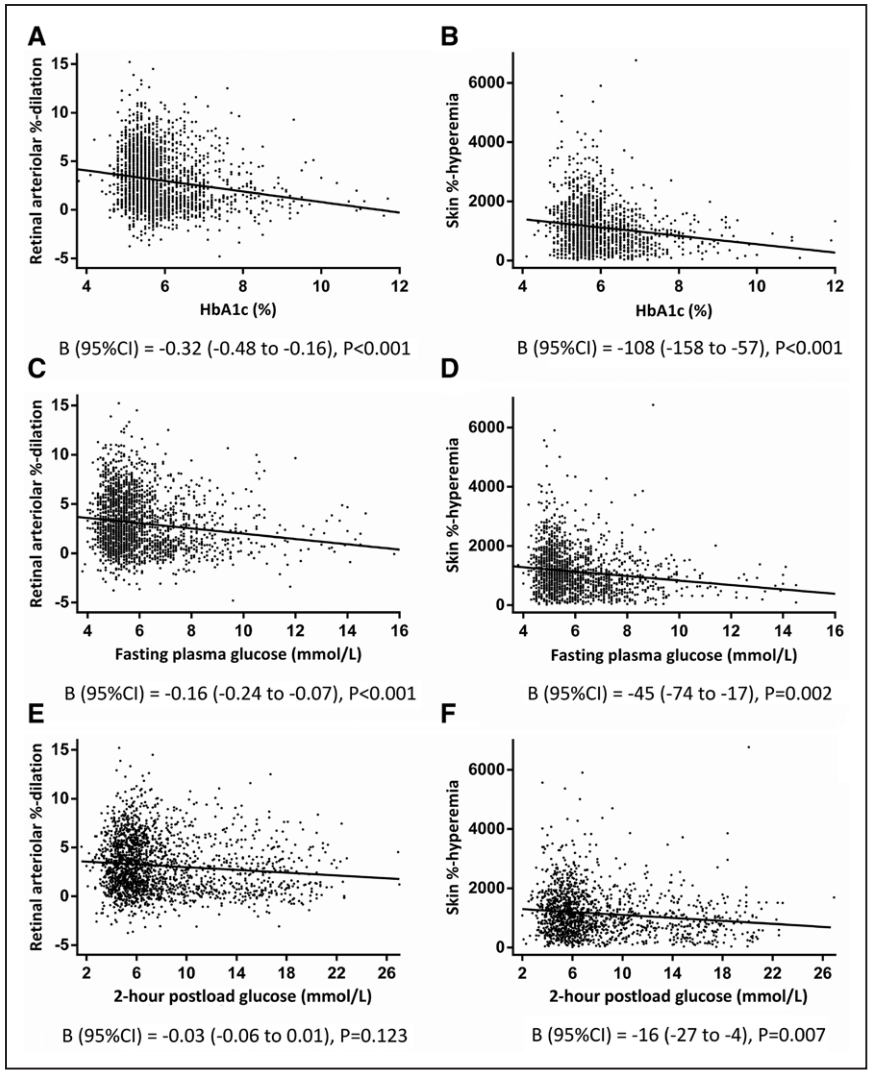

Figure 4. Associations of glycohemoglobin A1c (HbA1c) (top), fasting plasma glucose (middle), and 2-hour postload glucose (bottom) with the flicker light-induced retinal arteriolar dilator (left) and heat-induced skin hyperemic response (right).

A, Association between HbA1c and flicker light-induced retinal arteriolar %-dilation; **B,** Association between HbA1c and heat-induced skin %-hyperemia; **C,** Association between fasting plasma glucose and flicker light-induced retinal arteriolar %-dilation; **D,** Association between fasting plasma glucose and heat-induced skin %-hyperemia; **E,** Association between 2-hour postload glucose and flicker light-induced retinal arteriolar %-dilation; **F,** Association between 2-hour postload glucose and heat-induced skin %-hyperemia. Regression coefficients (B) indicate the adjusted mean difference and 95% confidence interval (95% CI) in retinal arteriolar %-dilation and skin %-hyperemia per 1% point increase in HbA1c or per mmol/L increase in fasting glucose or 2-hour postload glucose level. Note that the regression coefficients between retinal and skin responses differ as a result of differences in the scales of the response effect size. HbA1c indicates glycohemoglobin A1c.

half to one fourth of that in T2DM (Table 2). Our interpretation of a graded decline in microvascular function with worsening glucose tolerance is supported by the significant associations of higher levels of HbA1c and fasting plasma glucose with attenuated retinal and skin microvascular responses (Table 3 and Figure 4), which suggests that microvascular dysfunction is closely related to hyperglycemia. Thus, we attribute the fact that direct comparison of microvascular function between the NGM and prediabetes groups was not statistically significant in adjusted analyses to a type 2 statistical error, because power in such between-group comparisons is reduced compared with analyses of trends and of continuous variables such as HbA1c.

Impairments in both flicker light-induced retinal arteriolar dilation (which is nitric oxide-dependent[29,30]) and heat-induced skin hyperemia (which depends on nitric oxide and endothelium-derived hyperpolarizing factors[31,32]) are likely to reflect microvascular endothelial dysfunction,[29,32] possibly in conjunction with neuronal dysfunction.[13,17,31,33] The mechanism that may underlie the association between hyperglycemia and microvascular endothelial dysfunction may be bidirectional, ie, microvascular endothelial dysfunction may act as both a consequence and cause of hyperglycemia.[2] On the one hand, hyperglycemia can impair endothelial function through formation of advanced glycation endproducts, intraendothelial accumulation of glucose, and increased oxidative stress.[34] On the other hand, microvascular endothelial dysfunction can cause or aggravate hyperglycemia and lead to (pre)diabetes,[35,36] both by impairing the timely access of glucose and insulin to their target tissues[37] as well as by impairing insulin secretion.[38] Consequently, a vicious circle may exist between hyperglycemia and microvascular endothelial dysfunction.

Strengths of our study include its population-based design with oversampling of individuals with T2DM, which enables an accurate comparison of individuals without and with T2DM; the use of an oral glucose tolerance test and HbA1c levels to characterize glucose metabolism; the use of 2 independent methods to directly assess microvascular function instead of relying on indirect biomarkers[36,39] or estimates of microvascular structure[35]; the extensive assessment of potential confounders, including 24-hour

ambulatory blood pressure, 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. However, from the association between diabetes and microangiopathy it follows that there is a strong a priori likelihood that hyperglycemia can cause microvascular dysfunction. On the other hand, it appears likely that microvascular dysfunction can cause hyperglycemia,[37] and we in fact hypothesize that the association is bidirectional.[36,40] Second, we measured microvascular dysfunction in retina and skin and infer that findings can be generalized to other microvascular beds,[41] but this inference needs to be formally tested. Third, we studied white individuals aged 40 to 75 years who were relatively intensively treated with regard to vascular and metabolic risk factors; therefore, extrapolation to other groups will need further study. Fourth, our fully adjusted model may have been overadjusted,[42] because diabetic retinopathy and nephropathy have a microvascular origin. However, qualitatively similar results were obtained when we excluded T2DM participants with clinically apparent diabetic microangiopathy. Fifth, although we adjusted for many potential confounders, we cannot fully exclude residual confounding by variables not included in these analyses (eg, dietary habits). Sixth, a single oral glucose tolerance test may result in misclassification of long-term glucose tolerance status.[43] Therefore, what may be considered prediabetes may be unrecognized T2DM (which would overestimate microvascular dysfunction in prediabetes), or vice versa, and what may be considered NGM may be unrecognized prediabetes, or vice versa (all of which would underestimate microvascular dysfunction in prediabetes). When group size of the NGM, prediabetes, and T2DM groups and misclassification estimates[43] are taken into account, the net result of these opposing effects is likely to be underestimation of microvascular dysfunction in the prediabetes group.

In summary, we showed, in a population-based study, that prediabetes and T2DM, and continuous measures of hyperglycemia, are associated with impaired microvascular function in the retina and skin, independently of major cardiovascular risk factors and clinically defined diabetic retinopathy and nephropathy. These findings, therefore, support the concept that generalized microvascular dysfunction precedes the clinical diagnosis of T2DM and may contribute to the development of microvascular complications in T2DM and prediabetes such as retinopathy, nephropathy, neuropathy, heart failure, stroke, and cognitive decline. These findings suggest that both early hyperglycemia and microvascular dysfunction should be considered as potential targets of intervention.

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DISCLOSURES

None.

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FOOTNOTES

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