Association Between Arterial Stiffness and Skin Microvascular Function: The SUVIMAX2 Study and The Maastricht Study

ORIGINAL ARTICLE

Association Between Arterial Stiffness and Skin Microvascular Function: The SUVIMAX2 Study and The Maastricht Study

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BACKGROUND
It has been hypothesized that arterial stiffness leads to generalized microvascular dysfunction and that individuals with type 2 diabetes mellitus (T2DM) are particularly prone to the detrimental effects of arterial stiffness. However, evidence for an association between stiffness and markers of generalized microvascular dysfunction is lacking. We therefore investigated the association between arterial stiffness and skin microvascular function in individuals without and with T2DM.

METHODS
Cross-sectional data were used of The Supplementation en Vitamines et Mineraux Antioxydants 2 (SUVIMAX2) Study (n = 284/62.2 years/48.6% women/0% T2DM (by design)) and The Maastricht Study (n = 737/59.7 years/45.2% women/28.8% T2DM (by design)). Arterial stiffness was determined by carotid-femoral pulse wave velocity (cfPWV). Skin capillaroscopy was used to determine capillary density at baseline, and during reactive hyperemia and venous congestion. Laser Doppler flowmetry was used to assess acetylcholine- and local heating-induced vasoreactivity, and skin flowmotion.

RESULTS
In The SUVIMAX2 Study, cfPWV (per +1 SD) was not associated with baseline capillary density (regression coefficient: −0.48 (95% confidence interval: 2.37; 1.41)) or capillary recruitment during venous congestion (0.54% (−0.74; 1.81%)). In addition, cfPWV was not associated with acetylcholine (−0.02% (−0.14; 0.10%)) or local heating-induced vasoreactivity (0.03% (−0.07; 0.12%)). In The Maastricht Study, in individuals without T2DM, cfPWV was not associated with baseline capillary density (−1.20 (−3.17; 0.77)), and capillary recruitment during reactive hyperemia (1.22% (−0.41; 2.84%)) or venous congestion (1.50% (−0.25; 3.25%)). In addition, cfPWV was not associated with flowmotion (−0.01 (−0.07; 0.06)). Results were adjusted for age and sex. Additional adjustments for confounders did not materially change these results. Results were qualitatively similar in individuals with T2DM.

CONCLUSIONS
Arterial stiffness is not associated with skin microvascular function, irrespective of the presence of T2DM.

Keywords: blood pressure; epidemiology; hypertension; microcirculation; type 2 diabetes mellitus; vascular stiffness.

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Stiffening of large arteries impairs their cushioning function and increases pressure and flow pulsatility, which transmits distally and can damage the microcirculation.[1,2] Indeed, previous studies have shown an association between greater arterial stiffness and markers of microvascular dysfunction in the brain (cerebral small vessel lesions),[3] eye (retinal

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arteriolar narrowing),[4] and kidney (microalbuminuria).[5] These organs are, however, especially vulnerable to the detrimental effects of increased pressure and flow pulsatility, as their microvasculature is characterized by low impedance, allowing the pulsatile load to penetrate deeply into their microvascular beds.[1,2] Nevertheless, it has been hypothesized that increased arterial stiffness leads to generalized microvascular dysfunction, i.e., dysfunction not limited to microvascular beds characterized by low impedance.[6] Such a phenomenon, if it exists, may explain the association between arterial stiffness and different diseases, including peripheral neuropathy,[7] type 2 diabetes mellitus (T2DM),[8] and osteoporosis.[9] Microvascular dysfunction is a common element in the pathophysiology of these diseases.[6] However, evidence for an association between arterial stiffness and markers of generalized microvascular dysfunction is lacking.

The skin is a unique site which enables direct and non-invasive assessment of microvascular function both at rest and during provocative stimuli. Importantly, the skin microcirculation is considered a representative vascular bed to examine generalized microvascular phenomena.[10,11] Skin microvascular dysfunction is associated with cardiovascular disease (CVD) risk factors, including, the metabolic syndrome,[12] T2DM,[13] obesity,[14–16] and hypertension.[17] In addition, microvascular responses observed in skin parallel those in other tissues, including muscle.[18,19] To date, only one small study (n = 76)[20] has evaluated the association between arterial stiffness and skin microvascular function and did not find a significant association.

In view of the above, the aim of the present study was to evaluate the association between arterial stiffness, as determined by carotid-femoral pulse wave velocity (cfPWV), and skin microvascular function. Skin microvascular function was determined by baseline capillary density, capillary recruitment during reactive hyperemia after arterial occlusion and during venous congestion, endothelium-dependent and -independent skin vasoreactivity, and skin flowmotion. The associations were evaluated in 2 large studies: The Supplementation en Vitamines et Mineraux Antioxydants 2 (SUVIMAX2) Study and The Maastricht Study. In addition, it has been hypothesized that individuals with T2DM are particularly prone to the detrimental effects of increased pressure and flow pulsatility on the microcirculation, because T2DM may be associated with low microvascular impedance.[1,21] We therefore additionally investigated whether any association between stiffness and microvascular function was stronger in individuals with T2DM as compared to those without T2DM.

METHODS

Study design

The present study used cross-sectional data of The SUVIMAX2 Study and The Maastricht Study.

The SUVIMAX Study (n = 12,749) was a prevention trial designed to investigate the effect of antioxidant supplementation on CVD and cancer, and was conducted in France between 1994 and 2002.[22] In 2006–2007, 7,200 participants of the SUVIMAX trial participated in The SUVIMAX2 Study, an observational prospective cohort study on diet and aging. Of these, all individuals (n = 291) living in Paris without T2DM, hypertension, and prior CVD, underwent measurements on arterial stiffness and skin microvascular function.[14]

The Maastricht Study is an ongoing observational prospective population-based cohort study that aims to include 10,000 participants. The present study includes cross-sectional data from the first 866 participants, who completed the baseline survey between November 2010 and March 2012. The rationale and methodology have been described previously.[23] 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 40–75 years and living in the southern part of the Netherlands. The study area is defined by postal codes. The study area encloses 82,462 inhabitants aged 40–75 years, including an estimated 7,000 individuals with T2DM. Participants were recruited from the general population through mass media campaigns and from the municipal registries. In addition, individuals with T2DM were recruited through the regional Diabetes Patient Registry *via* mailings. Recruitment was stratified according to known T2DM status for reasons of efficiency. All examinations of each participant were performed within a time window of 3 months.

Both studies were approved by ethics committees: review committees of Paris-Cochin hospital (n°2364) and the Comité National Informatique et des Libertés (n°907094) for The SUVIMAX2 Study; and Maastricht University Medical centre (n°NL31329.068.10) and the Netherlands Health Council under the Dutch "Law for Population Studies" (n°131088-105234-PG) for The Maastricht Study. All participants gave written informed consent.

In both The SUVIMAX2 Study and The Maastricht Study, arterial stiffness was determined by cfPWV. In The SUVIMAX2 Study, microvascular function was assessed using skin capillaroscopy and endothelium-dependent and -independent skin vasoreactivity. In The Maastricht Study, microvascular function was assessed using skin capillaroscopy and skin flowmotion.

Vascular measurements in The SUVIMAX2 Study

All measurements were done by trained technicians unaware of the participants' clinical status in dark, quiet, temperature-controlled (21–24 °C) rooms as described previously.[14,24,25] Participants were asked to refrain from smoking and eating ≤12 h prior to the measurements. Measurements were done after 5 min of rest and talking or sleeping was not allowed during the examination.

CfPWV

CfPWV was determined with applanation tonometry (SphygmoCor; Atcor Medical, Sydney, Australia).[24] Pressure waveforms were determined at the right common carotid and right common femoral arteries. Difference in the time of pulse arrival from the R-wave of the electrocardiogram between the 2 sites (transit time) was determined with the maximum upstroke algorithm. The pulse wave travel

distance was calculated as the total direct straight distance (measured with an infantometer) between the 2 arterial sites. The mean of 3 consecutive cfPWV (defined as travel distance/transit time) recordings was used in the analysis.

Skin capillaroscopy

Capillaries were visualized in the dorsal skin of the middle phalanx of the dominant hand using a digital video microscope (micro-ScopernanMS-500C; Moritex, Tokyo, Japan; system magnification ×200).[14,25] A region of interest of 3 mm^2 skin area on the middle third of the phalanx was defined. Four microscopic fields of 1 mm^2 were randomly chosen in this area. Capillary density (mean of 4 fields) was measured under 2 conditions. First, baseline capillary density was assessed. Second, capillary recruitment during venous congestion was determined, which is a measure of structural capillary reserve capacity.[17] For venous congestion, a miniature cuff was applied on the base of the investigated finger and inflated to 50 mm Hg for 2 minutes. The number of perfused capillaries was counted manually using freeze-framed reproductions of the videotape by one investigator who was blinded to participants' clinical status. The intra- and interobserver coefficient of variations were 4.3% and 5.9%, respectively, as described previously.[20]

Endothelium-dependent and -independent skin vasoreactivity

Endothelium-dependent and -independent skin vasoreactivity were determined by acetylcholine iontophoresis and local skin heating, respectively.[14] Skin blood perfusion was measured using a laser Doppler system (Periflux 5000; Perimed, Stockholm, Sweden), equipped with a thermostatic laser probe (PF 457, Perimed) at the dorsal side of the wrist. Skin temperature was monitored continuously and maintained at 33 °C. Baseline skin blood perfusion was defined as the mean value during a time period of 4 min. Acetylcholine chloride (2% solution, 800 µl) was delivered on the dorsal side of the wrist using an anodal current (3 doses of 10 mA for 10 seconds with a 2-minute interval) and maximal increase in blood perfusion was measured. Next, the laser probe was heated to 44 °C for 5 minutes and maximal increase in blood perfusion was measured.

Vascular measurements in The Maastricht Study

All measurements were done by trained technicians unaware of the participants' clinical status, in dark, quiet, temperature-controlled (21–24 °C) rooms as described previously.[10,23] Participants were asked to refrain from smoking and drinking coffee, tea, or alcoholic beverages ≤3 hours prior to the study. Participants were allowed to have a light meal (breakfast and/or lunch). All measurements were performed after 10 minutes of rest and talking or sleeping was not allowed during the examination.

CfPWV

CfPWV was determined according to recent guidelines[26] with applanation tonometry (SphygmoCor; Atcor Medical). Pressure waveforms were determined at the right common carotid and right common femoral arteries. Transit time was determined with the intersecting tangents algorithm. The pulse wave travel distance was calculated as 80% of the direct straight distance (measured with an infantometer) between the 2 arterial sites. The median of 3 consecutive cfPWV recordings was used in the analysis. Reproducibility was assessed in 12 individuals (6 men; 60.8 ± 6.8 years; 6 T2DM) who were examined by 2 observers at 2 occasions spaced 1 week apart. The intra- and interobserver coefficient of variations were 13.5% and 16.2%, respectively.

Skin capillaroscopy

Capillaries were visualized in the dorsal skin of the distal phalanges of the third and fourth finger of the right hand using a digital video microscope (Capiscope; KK Technology, Honiton, UK; system magnification ×100).[10] Capillaries were visualized 4.5 mm proximal to the terminal row of capillaries in the middle of the nailfold. The investigator selected a region of interest of 1 mm^2 skin area. Capillary density (mean of 2 fields) was measured under 3 conditions. First, baseline capillary density was measured. Second, capillary recruitment during reactive hyperemia was assessed after 4 minutes of arterial occlusion. Capillary recruitment during reactive hyperemia reflects functional and/or structural capillary reserve capacity.[17] For the assessment of reactive hyperemia, a miniature cuff was applied on the base of the investigated finger and inflated to suprasystolic pressure (260 mm Hg) for 4 minutes. Third, venous congestion was applied, with the cuff inflated to 60 mm Hg for 2 minutes. The number of continuously perfused capillaries was counted using a semi-automatic procedure (CapiAna) and running movie files by 2 investigators who were blinded to participants' clinical status.[10] The intra- and interobserver coefficient of variations of CapiAna were 2.5% and 5.6%, respectively, as described previously.[10]

Skin flowmotion

Skin flowmotion, i.e., blood flow fluctuation attributed to the rhythmic contraction of arterioles, is an important component of the microcirculation.[16] It is involved in optimal delivery of nutrients to tissue and regulation of local vascular resistance.[16,27] To assess flowmotion, skin blood perfusion was measured using a laser Doppler system (Periflux 5000; Perimed), equipped with a thermostatic laser probe (PF 457; Perimed) at the dorsal side of the wrist. Skin temperature was monitored continuously and maintained at 30 °C. The Doppler flowmetry output was recorded for 25 minutes (sample rate 32Hz). Fast-Fourier Transform algorithm was performed using Perisoft dedicated software (PSW version 2.50) to measure the power density of the flowmetry oscillation. The frequency spectrum between 0.01 and 1.6 Hz was divided into 5 flowmotion components[28]: (i) endothelial, 0.01–0.02 Hz; (ii) neurogenic, 0.02–0.06 Hz; (iii) myogenic, 0.06–0.15 Hz; (iv) respiratory, 0.15–0.40 Hz; and (v) heartbeat, 0.40–1.60 Hz. In addition, total flowmotion energy was obtained by the sum of the power density values of the total frequency spectrum.

Potential confounders

CVD risk factors were determined as described previously in The SUVIMAX2 Study[14,24,25] and The Maastricht Study.[23]

Statistical analysis

All analyses were performed with PASW Statistics (Version 21). Separate analyses were done for The SUVIMAX2 Study and The Maastricht Study. In addition, because The Maastricht Study population is enriched with individuals with T2DM, results are presented separately for individuals without and with T2DM. Microvascular recruitment during reactive hyperemia after arterial occlusion and during venous congestion was expressed as percentage change in capillary density from baseline. Additional analyses were done using absolute recruitment during reactive hyperemia after arterial occlusion and capillary density during venous congestion as the outcome (both in capillaries/mm^2) instead of percentage recruitment. Acetylcholine- and local heating-induced skin vasoreactivity were expressed as percentage increase in perfusion from baseline. In addition, acetylcholine- and local heating-induced skin vasoreactivity and skin flowmotion energy were logarithmically transformed to normalize their skewed distribution. Linear regression analysis was used to evaluate the association between cfPWV and measures of skin microvascular function. Adjustments were made for the following potential confounders: age and sex (model 1); additionally for mean arterial pressure and heart rate (model 2); and CVD risk factors: waist-to-hip ratio, fasting plasma glucose, total/high-density lipoprotein cholesterol ratio, triglycerides, smoking habits (current, ever, and never smoker), use of lipid-lowering medication and (for The Maastricht Study) prior CVD and use of antihypertensive medication (model 3). Analyses with flowmotion as the outcome were additionally adjusted for skin temperature in all models. We used interaction terms to explore whether any association differed according to T2DM, fasting glucose, age, presence of hypertension, use of antihypertensive medication, and/or study population (The SUVIMAX2 Study vs. The Maastricht Study).[1] In addition, analyses were repeated with tertiles of cfPWV. Finally, analyses were repeated with brachial pulse pressure (systolic minus diastolic pressure), a surrogate measure of arterial stiffness, as the determinant instead of cfPWV.

RESULTS

Analytic sample

Of the 291 participants of The SUVIMAX2 Study, data were missing on cfPWV (n = 2), capillaroscopy (n = 2), skin vasoreactivity (n = 3), or potential confounders (n = 3). Therefore, 284 participants were eligible for analysis with capillary density and 283 for analysis with skin vasoreactivity, respectively. Of the 866 participants of The Maastricht Study, 4 participants with type 1 diabetes mellitus were excluded. In the remaining 862 participants, data were missing on cfPWV (n = 46; due to logistical reasons (n = 33) or insufficient quality (n = 13)), capillaroscopy (n = 38; due to logistical reasons (n = 31) or insufficient quality (n = 7)), skin flowmotion (n = 128; due to logistical reasons (n = 97) or insufficient quality (n = 31)), or potential confounders (n = 46). Therefore, 732 participants were eligible for analysis with capillary density (524 without T2DM and 208 with T2DM) and 648 for analysis with skin flowmotion (473 without T2DM and 175 with T2DM), respectively.

Clinical characteristics

Table 1 shows the clinical characteristics of The SUVIMAX2 Study and Table 2 of The Maastricht Study. Individuals of The SUVIMAX2 Study were, as compared to those of The Maastricht Study without T2DM, older and had a better CVD risk factor pattern (Tables 1 and 2). In addition, in The SUVIMAX2 Study, cfPWV and baseline capillary density were higher than in The Maastricht Study, but capillary recruitment during venous congestion was lower (Tables 1 and 2). These differences were most likely due to use of different measurement techniques (see below). Within The Maastricht Study, individuals with T2DM were, as compared to those without T2DM, older, less often female, and had a worse CVD risk factor pattern; and had a higher cfPWV and lower capillary recruitment during reactive hyperemia after arterial occlusion and during venous congestion (Table 2). The median duration of T2DM was 7 years (interquartile range: 3–11). Individuals with T2DM were treated with diet only (22%), oral glucose-lowering medication only (59%), or insulin (with or without oral glucose-lowering medication; 19%).

Association between cfPWV and baseline capillary density and capillary recruitment

In The SUVIMAX2 Study, cfPWV was not associated with baseline capillary density and capillary recruitment during venous congestion, after adjustment for age and sex (Table 3, model 1). Further adjustments for mean arterial pressure and heart rate (model 2) and CVD risk factors (model 3) did not materially change these results. Similarly, in The Maastricht Study, both in individuals without and with T2DM, cfPWV was not associated with baseline capillary density and capillary recruitment during reactive hyperemia after arterial occlusion or during venous congestion (Table 3, models 1–3).

Association between cfPWV and skin vasoreactivity

In The SUVIMAX2 Study, cfPWV was not associated with acetylcholine- or local heating-induced skin vasoreactivity (Table 4, models 1–3).

Association between cfPWV and skin flowmotion

In The Maastricht Study, both in individuals without and with T2DM, cfPWV was not associated with total skin

Table 1. Study population characteristics according to median cfPWV values for The SUVIMAX2 Study

	The SUVIMAX2 Study		
	According to median cfPWV value (10.5 m/s)		
	Total (*n* = 284)	Low cfPWV (*n* = 137)	High cfPWV (*n* = 147)
Clinical characteristics			
Age, years	62.2 ± 5.9	61.5 ± 5.6	62.9 ± 6.0
Women, % (*n*)	48.6 (138)	58.4 (80)	39.5 (58)
Smoking status, % (*n*)			
Never	50.4 (143)	52.6 (72)	48.3 (71)
Former	40.8 (116)	35.0 (48)	46.3 (68)
Current	8.8 (25)	12.4 (17)	5.4 (8)
Prior cardiovascular disease, % (*n*)	0 (0)[a]	0 (0)[a]	0 (0)[a]
Body mass index, kg/m^2	25.1 ± 3.4	24.9 ± 3.4	25.3 ± 3.4
Systolic blood pressure, mm Hg	119 ± 13	114 ± 11	123 ± 12
Diastolic blood pressure, mm Hg	77 ± 9	74 ± 8	79 ± 9
Hypertension, % (*n*)	0 (0)[a]	0 (0)[a]	0 (0)[a]
Fasting glucose, mmol/l	5.4 ± 0.6	5.3 ± 0.6	5.4 ± 0.5
Total cholesterol, mmol/l	6.0 ± 1.0	5.9 ± 1.0	6.0 ± 1.0
Lipid-lowering medication, % (*n*)	8.5 (24)	6.6 (9)	10.2 (15)
Antihypertensive medication, % (*n*)	0 (0)[a]	0 (0)[a]	0 (0)[a]
Vascular measures			
cfPWV, m/s	10.9 ± 2.1	9.4 ± 0.8	12.4 ± 1.9
Capillary density, capillaries/mm^2			
Baseline	90 ± 16	90 ± 14	90 ± 17
Venous congestion	96 ± 17	96 ± 15	96 ± 18
Capillary recruitment, % (*n*)			
Venous congestion	7 ± 11	7 ± 11	7 ± 11
Skin vasoreactivity, %			
Acetylcholine-induced	349 (172–572)	364 (181–573)	338 (159–576)
Local heating-induced	568 (379–875)	595 (393–914)	538 (362–856)
Total skin flowmotion energy, AU	n/a	n/a	n/a

Data are presented as percentage (number) of participants, mean ± SD or median (interquartile range).
Abbreviations: AU, arbitrary units; cfPWV, carotid-femoral pulse wave velocity; n/a, not available.
[a]By design.

flowmotion energy (Table 4, models 1–3), or with any of the individual skin flowmotion components (data not shown).

Additional analyses

There was no interaction with T2DM, fasting glucose, age, presence of hypertension, or use of antihypertensive medication for any of the skin microvascular function measures in either The SUVIMAX2 Study or The Maastricht Study (P for interaction >0.09, after adjustment for all potential confounders). In addition, there was no interaction with the study populations of The SUVIMAX2 Study vs. The Maastricht Study for baseline capillary density and capillary recruitment during venous congestion (P for interaction >0.67, after adjustment for all potential confounders). When we used absolute recruitment during hyperemia after arterial occlusion and capillary density during venous congestion, instead of percentage recruitment, results were qualitatively similar (data not shown). In addition, when the analyses were repeated with tertiles of cfPWV, instead of per +1 SD, results were qualitatively similar (data not shown). Results were qualitatively similar when we used brachial pulse pressure as the determinant instead of cfPWV (data not shown). Finally, when results of both studies were pooled, results were qualitatively similar (data not shown).

DISCUSSION

The main finding of the present study is that, in middle-aged individuals with or without T2DM, arterial stiffness

Table 2. Study population characteristics according to median cfPWV values for The Maastricht Study

	The Maastricht Study					
	Without type 2 diabetes			With type 2 diabetes		
	According to median cfPWV value (8.2 m/s)			According to median cfPWV value (9.5 m/s)		
	Total (*n* = 524)	Low cfPWV (*n* = 260)	High cfPWV (*n* = 264)	Total (*n* = 208)	Low cfPWV (*n* = 103)	High cfPWV (*n* = 105)
Clinical characteristics						
Age, years	58.1 ± 8.5	54.4 ± 8.1	61.8 ± 7.2	63.3 ± 7.2	61.1 ± 7.7	65.5 ± 6.0
Women, % (*n*)	51.5 (270)	58.1 (151)	45.1 (119)	29.3 (61)	30.1 (31)	28.6 (30)
Smoking status, % (n)						
Never	34.3 (180)	36.5 (95)	32.2 (85)	23.1 (48)	24.3 (25)	21.9 (23)
Former	50.0 (262)	47.7 (124)	52.3 (138)	62.5 (130)	621.1 (64)	62.9 (66)
Current	15.8 (83)	16.9 (44)	14.8 (39)	14.9 (31)	14.6 (15)	15.2 (16)
Prior cardiovascular disease, % (*n*)	12.8 (67)	9.6 (25)	15.9 (42)	29.3 (61)	28.2 (29)	30.5 (32)
Body mass index, kg/m^2	26.2 ± 3.9	25.6 ± 3.6	26.8 ± 4.1	29.7 ± 4.7	30.0 ± 5.1	29.5 ± 4.3
Systolic blood pressure, mm Hg	133 ± 18	127 ± 15	140 ± 17	148 ± 19	142 ± 18	154 ± 18
Diastolic blood pressure, mm Hg	76 ± 11	74 ± 10	78 ± 71	79 ± 10	79 ± 12	79 ± 9
Hypertension, % (*n*)	46.0 (241)	29.2 (76)	62.5 (165)	86.5 (180)	81.6 (84)	91.4 (96)
Fasting glucose, mmol/l	5.4 ± 0.6	5.3 ± 0.5	5.5 ± 0.6	7.9 ± 1.8	7.8 ± 1.6	8.0 ± 2.1
Total cholesterol, mmol/l	5.5 ± 1.1	5.5 ± 1.1	5.6 ± 1.1	4.5 ± 1.1	4.4 ± 0.9	4.6 ± 2.1
Lipid-lowering medication, % (*n*)	19.8 (104)	15.0 (39)	24.6 (65)	75.5 (157)	77.7 (80)	73.3 (77)
Antihypertensive medication, % (*n*)	26.7 (140)	17.7 (46)	35.6 (94)	70.7 (147)	70.9 (73)	70.5 (74)
RAAS inhibitors	18.9 (99)	11.5 (30)	26.1 (69)	57.2 (119)	57.3 (59)	57.1 (60)
Diuretics	10.5 (55)	6.9 (18)	14.0 (37)	26.0 (54)	25.2 (26)	26.7 (28)
Calcium channel blockers	4.8 (25)	2.7 (7)	6.8 (18)	17.3 (36)	12.6 (13)	21.9 (23)
Beta-blockers	11.5 (60)	9.2 (24)	13.6 (36)	35.1 (73)	32.0 (33)	38.1 (40)
Vascular measures						
cfPWV, m/s	8.5 ± 1.8	7.2 ± 0.7	9.9 ± 1.5	9.9 ± 2.4	8.0 ± 1.0	11.7 ± 2.0
Capillary density, capillaries/mm^2						
Baseline	72 ± 17	72 ± 17	72 ± 18	77 ± 18	76 ± 18	78 ± 18
Hyperemia	104 ± 17	104 ± 17	103 ± 18	102 ± 19	100 ± 20	103 ± 17
Venous congestion	104 ± 18	105 ± 17	104 ± 18	102 ± 19	101 ± 21	103 ± 17
Capillary recruitment, % (*n*)						
Reactive Hyperemia	46 ± 30	49 ± 30	50 ± 30	35 ± 25	34 ± 25	35 ± 25
Venous congestion	50 ± 32	50 ± 33	51 ± 32	35 ± 26	35 ± 26	36 ± 26
Total skin flowmotion energy, AU	14 (9–21)	14 (8–20)	15 (10–21)	15 (10–23)	14 (10–21)	18 (10–23)

Data are presented as percentage (number) of participants, mean ± SD or median (interquartile range). Abbreviations: AU, arbitrary units; cfPWV, carotid-femoral pulse wave velocity; n/a, not available; RAAS, renin-angiotensin system.

was not associated with skin microvascular dysfunction, as determined by baseline capillary density; capillary recruitment during reactive hyperemia after arterial occlusion and during venous congestion; endothelium-dependent and -independent skin vasoreactivity; and skin flowmotion.

Strengths of the present study include its extensive measurement of skin microvascular function and assessment of arterial stiffness by cfPWV, which is considered the "gold standard" measurement.[26] In addition, the associations were investigated in 2 large studies performed by independent investigators. It is therefore unlikely that the negative findings are due to technical measurement issues or insufficient statistical power, although we did not do a formal power calculation. Furthermore, The Maastricht Study is a population-based study with oversampling of

Table 3. Association between carotid-femoral pulse wave velocity and skin capillary density and recruitment

	Baseline capillary density (capillaries/mm^2)	Recruitment during reactive hyperemia after arterial occlusion (%)	Recruitment during venous congestion (%)
	Regression coefficient (95% confidence interval) for +1 SD cfPWV		
The SUVIMAX2 Study			
1	−0.48 (−2.37; 1.41)	n/a	0.54 (−0.74; 1.81)
2	−0.56 (−2.57; 1.44)	n/a	0.59 (−0.76; 1.94)
3	−0.27 (−2.32; 1.77)	n/a	0.62 (−0.77; 2.01)
The Maastricht Study			
Individuals without type 2 diabetes mellitus			
1	−1.63 (−3.31; 0.06)	1.22 (−0.41; 2.84)	1.50 (−0.25; 3.25)
2	−1.56 (−3.51; 0.39)	0.78 (−1.10; 2.66)	1.01 (−1.01; 3.03)
3	−1.20 (−3.17; 0.77)	0.41 (−1.49; 2.31)	0.62 (−1.42; 2.67)
Individuals with type 2 diabetes mellitus			
1	0.79 (−1.84; 3.42)	0.003 (−1.53; 1.53)	0.14 (−1.45; 1.73)
2	0.06 (−2.82; 2.94)	0.32 (−1.35; 1.99)	0.39 (−1.34; 2.13)
3	0.12 (−2.74; 3.00)	0.38 (−1.29; 2.06)	0.42 (−1.33; 2.16)

Model 1: adjusted for age and sex; model 2: additionally adjusted for heart rate and mean arterial pressure; model 3: additionally adjusted for waist-to-hip ratio, smoking habits, fasting glucose, total/high-density lipoprotein cholesterol ratio, triglycerides, use of lipid-lowering medication and (for The Maastricht Study) prior cardiovascular disease and use of antihypertensive medication.

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; n/a, not available.

Table 4. Association between carotid-femoral pulse wave velocity on the one hand and acetylcholine- and local heating-induced skin vasoreactivity and skin flowmotion on the other

	Acetylcholine-induced skin vasoreactivity (%)[a]	Local heating-induced skin vasoreactivity (%)[a]	Total skin flowmotion energy (AU)[a]
	Regression coefficient (95% confidence interval) for +1 SD cfPWV		
The SUVIMAX2 Study			
1	−0.02 (−0.14; 0.10)	0.03 (−0.07; 0.12)	n/a
2	−0.07 (−0.19; 0.06)	−0.01 (−0.11; 0.09)	n/a
3	−0.09 (−0.21; 0.04)	−0.02 (−0.12; 0.08)	n/a
The Maastricht Study			
Individuals without type 2 diabetes mellitus			
1	n/a	n/a	−0.01 (−0.07; 0.06)
2	n/a	n/a	−0.02 (−0.10; 0.06)
3	n/a	n/a	−0.01 (−0.09; 0.07)
Individuals with type 2 diabetes mellitus			
1	n/a	n/a	−0.05 (−0.15; 0.04)
2	n/a	n/a	−0.05 (−0.15; 0.06)
3	n/a	n/a	−0.04 (−0.14; 0.07)

[a]Data were logarithmically transformed. Model 1: adjusted for age, sex, and skin temperature; model 2: additionally adjusted for heart rate and mean arterial pressure; model 3: additionally adjusted for waist-to-hip ratio, smoking habits, fasting glucose, total/high-density lipoprotein cholesterol ratio, triglycerides, use of lipid-lowering medication and (for The Maastricht Study) prior cardiovascular disease and use of antihypertensive medication.

Abbreviations: AU, arbitrary units; cfPWV, carotid-femoral pulse wave velocity; n/a, not available.

individuals with T2DM. This allowed us to investigate any potential contrast between individuals with and without T2DM in the association between cfPWV and microvascular function.

Limitations of the present study include its cross-sectional design; we therefore cannot exclude the possibility of an association between greater arterial stiffness and decrement of skin microvascular function over time. In addition, not

all measures of skin microvascular function were available in each study. Finally, the intra- and interobserver reproducibility was not tested of the laser Doppler flowmetry measurements in The SUVIMAX2 Study and The Maastricht Study, and of the cfPWV measurement in The SUVIMAX2 Study. However, previous studies[29–32] have shown that laser Doppler flowmetry[29–31] and cfPWV measurements[33] have a relatively high reproducibility.

The lack of an association between arterial stiffness and skin microvascular function suggests that arterial stiffness does not lead to generalized microvascular dysfunction. There is substantial evidence that the skin microcirculation is representative of the microcirculation in general.[11,13–15,18] Possibly, the microcirculation of most organs is able to protect itself against the detrimental effects of increased arterial stiffness and pressure and flow pulsatility. This may be due to the fact that most organs have relatively high microvascular impedance. Therefore, most of the increased pulsatile energy is dissipated by arteries and large arterioles proximal to the capillaries.[1] In addition, a vascular remodeling response may be induced by the increased pulsatile load, which raises vascular resistance and, thereby, limits penetration of the pulsatile load into its microvascular bed. Other data[6,34,35] suggest that a remodeling response does occur in relatively large arterioles. For example, previous studies[35–37] have shown an association between greater arterial stiffness and a higher wall-to-lumen ratio of large arterioles in the subcutis[35,36] and retina.[37] The present study suggests that such remodeling, however, does not occur in small arterioles, because arterial stiffness was not associated with impaired acetylcholine-induced vasoreactivity. Remodeling of small arterioles is usually accompanied by impairment of this response.[38] In addition, the present study suggests that the previously observed remodeling response in large arterioles is effective, because arterial stiffness was not associated with capillary rarefaction. This indicates that capillaries are "protected" against the potential detrimental effects of increased pressure and flow pulsatility.

The present results do, however, not exclude the possibility that arterial stiffness is associated with microvascular dysfunction in the brain, eye, and kidney. The microvascular beds of these organs are characterized by low impedance, and, therefore, these organs are especially vulnerable for the detrimental effects of an increased pulsatile load.[1,2] Indeed, previous studies have shown an association between arterial stiffness and markers of microvascular dysfunction in these organs.[3–5]

In The SUVIMAX2 Study and The Maastricht Study, slightly different measurement techniques were used to assess cfPWV and capillary density, which can explain the difference in absolute values of these measures between the studies. Median cfPWV was higher in The SUVIMAX2 Study than in The Maastricht Study, most likely due to a difference in the calculation of pulse wave travel distance. In The SUVIMAX2 Study, the total carotid to femoral distance was used as travel distance, whereas in The Maastricht Study 80% of the total carotid to femoral distance was used. In addition, in The SUVIMAX2 Study, baseline capillary density was higher and capillary recruitment during venous congestion was lower than in The Maastricht Study. This may be due to use of a higher microscopic magnification in The SUVIMAX2 Study (×200 vs. ×100), differences in the method used to count capillaries (using primarily freeze-framed reproductions in The SUVIMAX2 Study vs. running movie files in The Maastricht Study) and lower cuff inflation during venous congestion (50 mm Hg in The SUVIMAX2 Study vs. 60 mm Hg in The Maastricht Study). In addition, the differences in absolute values of the vascular measures may be due to the differences in clinical characteristics between The SUVIMAX2 Study and The Maastricht Study. The net effect of such differences in characteristics is, however, difficult to quantify, because the different characteristics (both measured and unmeasured) may have influenced absolute values in various manners. Furthermore, it is difficult to disentangle the effects of the differences in clinical characteristics from the effects of the differences in measurement techniques between the 2 studies.

In conclusion, the present study shows that greater arterial stiffness is not associated with skin microvascular dysfunction in middle-aged individuals either with or without T2DM.

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DISCLOSURE

The authors declared no conflict of interest.

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