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Age, waist circumference, and blood pressure are associated with skin microvascular flow motion: The Maastricht Study

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Objective: Skin microvascular flow motion (SMF) – blood flow fluctuation attributed to the rhythmic contraction and dilation of arterioles – is thought to be an important component of the microcirculation, by ensuring optimal delivery of nutrients and oxygen to tissue and regulating local hydraulic resistance. There is some evidence that SMF is altered in obesity, type 2 diabetes mellitus, and hypertension. Nevertheless, most studies of SMF have been conducted in highly selected patient groups, and evidence how SMF relates to other cardiovascular risk factors is scarce. Therefore, the aim of the present study was to examine in a population-based setting which cardiovascular risk factors are associated with SMF.

Methods: We measured SMF in 506 participants of the Maastricht Study without prior cardiovascular event. SMF was investigated using Fourier transform analysis of skin laser Doppler flowmetry at rest within five frequency intervals in the 0.01–1.6-Hz spectral range. The associations with SMF of the cardiovascular risk factors age, sex, waist circumference, total-to-high-density lipoprotein cholesterol, fasting plasma glucose, 24-h SBP, and cigarette smoking were analysed by use of multiple linear regression analysis.

Results: Per 1 SD higher age, waist circumference and 24-h SBP, SMF was 0.16 SD higher (95% confidence interval (CI) 0.07, 0.25; P < 0.001), −0.14 SD lower (95% CI −0.25, −0.04; P = 0.01), and 0.16 SD higher (95% CI 0.07, 0.26; P < 0.001), respectively, in fully adjusted analyses. We found no significant associations of sex, fasting plasma glucose levels, total-to-high-density lipoprotein cholesterol ratio, or pack years of smoking with SMF.

Conclusion: Age and 24-h SBP are directly, and waist circumference is inversely associated with SMF in the general population. The exact mechanisms underlying these findings remain elusive. We hypothesize that flow motion may be an important component of the microcirculation by ensuring optimal delivery of nutrients and oxygen to tissue and regulating local hydraulic resistance not only under physiological conditions but also under pathophysiological conditions when microcirculatory perfusion is reduced, such as occurs with ageing and higher blood pressure. In addition, obesity may result in an impaired flow motion with negative effects on the delivery of nutrients and oxygen to tissue and local hydraulic resistance.

Keywords: blood flow regulation, microcirculation, risk factors

Abbreviation: SMF, skin microvascular flow motion

INTRODUCTION

Microvascular vasomotion, that is rhythmic changes in (precapillary) arteriolar diameter, is thought to be an important component of microvascular function [1]. In fact, these rhythmic oscillations regulate microvascular flow distribution so that various tissue regions are intermittently perfused [1,2]. Indeed, theoretical and experimental studies demonstrate that an increase in vasomotion can increase total blood flow by 40–60% [3,4]. In addition, when active and passive vessels with the same average diameter are compared, active vessels have a lower vascular resistance (according to Poiseuille’s law) [5,6], that is, vasomotion decreases arterial pressure by 20% [7]. Thus, vasomotion may be an important component of the microcirculation by ensuring optimal delivery of nutrients and oxygen to tissue and regulating local hydraulic resistance (i.e. resistance of the microcirculatory bed to blood flow) [1,5,8]. The rhythmic changes in arteriolar diameter caused by vasomotion produce periodical fluctuations of flow known as microvascular flow motion [8]. Skin microvascular flow motion (SMF) can easily be assessed using
laser-Doppler flowmetry (LDF). In vivo measurements of SMF show a broad spectrum of oscillation frequencies, with high-frequency oscillations originating from the cardiac and respiratory cycles, and low-frequency oscillations originating from the endothelial, neurogenic, and myogenic cycles [8–10].

Central obesity and a sedentary lifestyle are important causes of type 2 diabetes mellitus (T2DM) and hypertension, respectively [11]. However, how obesity leads to T2DM and hypertension is incompletely understood. We and others have advanced the hypothesis that microvascular dysfunction may contribute both to the development of T2DM – by impairing the timely access of glucose and insulin to their target tissues – and to the development of hypertension – by increasing vascular resistance [12]. In addition, it has been suggested that obesity is a primary cause of microvascular dysfunction [13]. Therefore, microvascular dysfunction may be an intermediate step linking central obesity to T2DM and hypertension [14].

There is some evidence supporting the hypothesis that SMF, as an important component of microvascular function, is altered in obesity, T2DM, and hypertension [1,15–18]. Nevertheless, most studies on SMF have been conducted in small numbers of highly selected patients. Hence, whether the differences found in these studies can be translated to the general population is unknown. In addition, evidence on whether and to what extent SMF relates to other cardiovascular risk factors, such as smoking and cholesterol levels, is scarce [19].

Therefore, the aim of the present study was to examine in a population-based setting which cardiovascular risk factors are associated with SMF. In addition, we investigated whether any such associations are similar in a healthy subpopulation (free of obesity, hypertension, T2DM, and medication use).

Methods

Study population

In this study, we used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously [20]. In brief, the study focuses on the cause, 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 via e-mails. Recruitment was stratified according to known T2DM status for reasons of efficiency. The present study includes cross-sectional data from the first 866 participants, who completed the baseline survey between November 2010 and March 2012. 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 Netherlands Health Council under the Dutch ‘Law for Population Studies’ (Permit 131088–105234-PG). All participants gave written informed consent.

Skin microvascular flow motion measurements

All participants were asked to refrain from smoking and caffeine 3 h before the measurements. A light meal (breakfast and/or lunch), which was low in fat content, was allowed prior to the start of the measurements. LDF measurements were performed in a quiet, climate-controlled room (T = 24°C) with participants in the supine position, as previously described [1].

Cutaneous blood perfusion was measured by means of a laser-Doppler system (Periflux 5000; Perimed, Stockholm, Sweden), equipped with a thermostatic laser-Doppler probe (PF 457; Perimed) at the dorsal side of the wrist of the left hand. Since flow motion has predominantly been observed in participants with a skin temperature above 29.3°C [3], the laser-Doppler probe was set at 30°C. The LDF output was recorded for 25 min with a sample rate of 32 Hz, which gives a semi-quantitative assessment of skin microvascular blood perfusion expressed in arbitrary perfusion units (i.e. the product of the velocity and concentration of moving red blood cells [21]). LDF measurements from the skin reflect perfusion in predominantly arterioles and venules [22].

Fast-Fourier transform algorithm was performed by means of Perisoft dedicated software (PSW version 2.50; Perimed) to measure the power density of the LDF oscillation. The frequency spectrum between 0.01 and 1.6 Hz was divided into five SMF components: endothelial, 0.01–0.02 Hz; neurogenic, 0.02–0.06 Hz; myogenic, 0.06–0.15 Hz; respiratory, 0.15–0.40 Hz; and heart beat, 0.40–1.60 Hz [10]. In addition, total SMF energy was obtained by the sum of the power density values of the total frequency spectrum.

Definition of cardiovascular risk factors

Cardiovascular risk factors were adapted from the Framingham risk score [23], and included the following: age, sex, waist circumference, fasting plasma glucose, total-to-high-density lipoprotein (HDL) cholesterol ratio, 24-h SBP, and pack years of smoking.

Measurements of cardiovascular risk factors

Medical history, history of cardiovascular disease, medication use, and smoking behaviour were assessed by questionnaire [20]. Height, weight, waist circumference, glycated hemoglobin (HbA1c), glucose levels, total and HDL cholesterol levels, and triglycerides were determined as described elsewhere [20].

To determine glucose metabolism, all participants (except those who used insulin) underwent a standardized 2-h 75 g oral glucose tolerance test (OGTT) after an overnight fast. For safety reasons, participants with a fasting glucose level above 11.0 mmol/l, as determined by a finger prick, did not undergo the OGTT. For these individuals (n = 13), fasting glucose level and information about diabetes medication use were used to determine glucose metabolism status. Glucose metabolism was defined according to the WHO 2006 criteria into normal glucose metabolism, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and T2DM [24]. Additionally, individuals without type 1 diabetes mellitus (T1DM) and on diabetes medication were considered as having T2DM [20].

Ambulatory blood pressure (BP) was measured with ambulatory 24-h BP monitoring (WatchBP O3; Microlife)
Cardiovascular risk factors and skin microvascular flow motion

From the initial 866 participants included in this study, we excluded four participants with T1DM. In the remaining 862 participants, LDF data were available in 746 participants; the reason for the missing data was a defective LDF system (n = 78) and unsatisfactory LDF measurements (i.e. LDF recordings less than 10 min, n = 38). In addition, to avoid treatment-induced changes in microvascular function, we excluded participants with a prior cardiovascular event (n = 152). In the remaining 594 participants, all variables were available in the 506 participants. Data were missing on 24-h BP measurements (n = 47), pack-years of smoking (n = 36), cholesterol levels (n = 4), and waist circumference (n = 1) (Fig. 1). In addition, we selected a healthy subgroup of participants who were free of: impaired glucose metabolism (IGM) or T2DM; hypertension; glucose-lowering medication, antihypertensive medication, and lipid-modifying medication use; and BMI at least 30 kg/m². Complete data for this healthy subgroup were available in 193 participants (Fig. 1).

General characteristics
Baseline characteristics of the study population are shown in Table 1. The study population included 260 (51.4%) men, 73 (14.4%) current smokers, 120 participants (23.7%) with T2DM, and 189 participants (37.4%) with hypertension. The healthy subgroup included 80 (41.4%) men and 28 (14.5%) current smokers. In addition, Table 1 shows that, when compared with participants in the lowest tertile of SMF, those in the middle and highest tertiles had a higher age and higher BP values. The median value of total SMF energy was 14.5 (inter-quartile range 9.1–21.4) in the study population and 13.6 (7.8–22.1) in the healthy subgroup (Table 2).

 Associations of age with total skin microvascular flow motion energy and the skin microvascular flow motion components
Age was associated with a higher total SMF energy; per SD higher age (8.5 years) total SMF energy was 0.16 SD (95% CI 0.07, 0.25; P < 0.001) higher (Fig. 2). Age was not significantly associated with the energy contribution of the endothelial, neurogenic, and myogenic component, but was associated with a higher energy contribution of the respiratory and heart beat component [β 0.24 SD (0.15, 0.33); P < 0.001 and β 0.20 SD (0.11, 0.29); P < 0.001, respectively] (Fig. 3). We found similar results when the frequency components were divided into a low (0.01–0.15 Hz) and high (0.15–1.60 Hz) frequency component; age was not significantly associated with the energy contribution of the low-frequency component [β 0.02 SD (−0.08, 0.11); P = 0.73], but was associated with a higher energy contribution of the high-frequency component [β 0.21 SD (0.12, 0.30); P < 0.001]. These results did not materially change when analyses were restricted to the healthy subgroup (Figs. 4 and 5), although the association of a higher age with total SMF energy and the energy contribution of the heart beat component became somewhat weaker and borderline significant [β 0.13 SD (−0.02, 0.27); P = 0.09 and β 0.13 SD (−0.02, 0.28); P = 0.09, respectively].

 Associations of waist circumference with total skin microvascular flow motion energy and the skin microvascular flow motion components
Waist circumference was associated with a lower total SMF energy; per SD higher waist circumference (14.4 cm) total SMF energy was −0.14 SD (−0.25, −0.04; P = 0.01) lower (Fig. 2). In addition, waist circumference was associated with...
a lower energy contribution of all the five frequency components (Fig. 3). We found similar results with the low and high-frequency component; waist circumference was significantly associated with a lower energy contribution of the low as well as the high-frequency component. Restriction of these analyses to the healthy subpopulation showed similar results (Figs 4 and 5), although the association of a higher waist circumference with the energy contribution of the endothelial, neurogenic, and heart beat component became somewhat weaker and borderline significant \( \beta = 0.17 \text{ SD} (0.34, 0.01); P = 0.07, \beta = 0.16 \text{ SD} (0.35, 0.02); P = 0.09, \) and \( \beta = 0.15 \text{ SD} (0.33, 0.03); P = 0.10, \) respectively.

**Associations of 24-h SBP with total skin microvascular flow motion energy and the skin microvascular flow motion components**

Twenty-four-hour SBP was associated with a higher total SMF energy; per SD higher 24-h SBP (12 mmHg) total SMF energy was 0.16 SD (0.07, 0.26; \( P < 0.001 \)) higher (Fig. 2). In addition, 24-h SBP was associated with a higher energy contribution of all the five frequency components (Fig. 3). We found similar results with the low and high-frequency component; 24-h SBP was significantly associated with a higher energy contribution of the low as well as the high-frequency component. Restriction of these analyses to the healthy subpopulation showed similar results (Figs 4 and 5).

Analysis with 24-h DBP, mean arterial pressure, or pulse pressure, instead of 24-h SBP, gave similar results, both in the study population and the analyses restricted to the healthy subpopulation (data not shown).

**Associations of the other cardiovascular risk factors with total skin microvascular flow motion energy and the skin microvascular flow motion components**

We found no significant associations of sex, fasting plasma glucose levels, total-to-HDL cholesterol ratio, or pack-years of smoking with total SMF energy, both in the study population and the analyses restricted to the healthy subpopulation (data not shown).
Data are presented as medians [inter-quartile ranges], or numbers (percentages) of the total group and according to tertiles of total skin microvascular flow motion energy. HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP, mean arterial pressure; T2DM, type 2 diabetes mellitus. P value for linear trend or P value for linear-by-linear associations as appropriate.

*PSN measurements were based on data from only 391 of the 506 participants in the study population and 160 of the 193 participants in the healthy subpopulation.

**TABLE 1. Baseline characteristics of the study population and the healthy subpopulation in the total group and according to tertiles of total skin microvascular flow motion energy**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study population</th>
<th>Healthy subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 506)</td>
<td>Healthy subpopulation (n = 193)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.8 ± 8.5</td>
<td>58.2 ± 8.5</td>
</tr>
<tr>
<td>Sex men</td>
<td>260 (51.4)</td>
<td>80 (41.5)</td>
</tr>
<tr>
<td>BMI categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight (BMI &lt; 25 kg/m²)</td>
<td>171 (33.8)</td>
<td>80 (41.5)</td>
</tr>
<tr>
<td>Overweight (BMI ≥ 25 to &lt; 30 kg/m²)</td>
<td>243 (48.0)</td>
<td>85 (44.0)</td>
</tr>
<tr>
<td>Obese (BMI ≥ 30 kg/m²)</td>
<td>92 (18.2)</td>
<td>87 (51.5)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>100 ± 10.5</td>
<td>94.1 ± 7.4</td>
</tr>
<tr>
<td>Women</td>
<td>90.8 ± 13.5</td>
<td>89.4 ± 12.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.9 ± 0.8</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.9 ± 1.4</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.4 ± 1.1</td>
<td>5.7 ± 1.0</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.4 ± 1.0</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Total-to-HDL cholesterol ratio</td>
<td>4.2 ± 1.3</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.2 (0.8–1.7)</td>
<td>1.2 (0.8–1.7)</td>
</tr>
<tr>
<td>Total skin microvascular flow motion energy (A.U.)</td>
<td>14.5 [9.1–21.4]</td>
<td>13.6 [7.8–22.1]</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>173 (34.2)</td>
<td>79 (40.9)</td>
</tr>
<tr>
<td>Former</td>
<td>260 (51.4)</td>
<td>86 (44.6)</td>
</tr>
<tr>
<td>Current</td>
<td>73 (14.4)</td>
<td>28 (14.5)</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>14.0 (5.0–27.9)</td>
<td>10.5 (4.2–25.7)</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDM</td>
<td>297 (58.7)</td>
<td>193 (100)</td>
</tr>
<tr>
<td>IGM</td>
<td>89 (17.6)</td>
<td>86 (44.6)</td>
</tr>
<tr>
<td>T2DM</td>
<td>120 (23.7)</td>
<td>28 (14.5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>189 (37.4)</td>
<td>72 (36.7)</td>
</tr>
<tr>
<td>Peripheral sensory neuropathy</td>
<td>19 (4.9)</td>
<td>3 (1.8)</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD, medians [inter-quartile ranges], or numbers (percentages) of the total group and according to tertiles (T1–T3) of total skin microvascular flow motion energy. LDL, high-density lipoprotein; HDL, low-density lipoprotein; MAP, mean arterial pressure; T2DM, type 2 diabetes mellitus. P value for linear trend or P value for linear-by-linear associations as appropriate.

**TABLE 2. Flow motion in the study population and the healthy subpopulation**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study population (n = 506)</th>
<th>Healthy subpopulation (n = 193)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total skin microvascular flow motion energy (A.U.)</td>
<td>14.5 [9.1–21.4]</td>
<td>13.6 [7.8–22.1]</td>
</tr>
<tr>
<td>Energy contribution endothelial component (A.U.)</td>
<td>0.9 [0.5–1.4]</td>
<td>0.9 [0.5–1.4]</td>
</tr>
<tr>
<td>Energy contribution neurogenic component (A.U.)</td>
<td>1.4 [0.8–2.2]</td>
<td>1.3 [0.8–2.2]</td>
</tr>
<tr>
<td>Energy contribution myogenic component (A.U.)</td>
<td>1.4 [0.8–2.2]</td>
<td>1.4 [0.8–2.2]</td>
</tr>
<tr>
<td>Energy contribution respiratory component (A.U.)</td>
<td>2.1 [1.3–3.3]</td>
<td>1.9 [1.2–3.3]</td>
</tr>
<tr>
<td>Energy contribution heart beat component (A.U.)</td>
<td>8.2 [4.8–12.1]</td>
<td>7.3 [4.2–12.4]</td>
</tr>
</tbody>
</table>

Data are presented as medians [inter-quartile ranges]. A.U., arbitrary units.

**TABLE 2. Flow motion in the study population and the healthy subpopulation**

population (Fig. 2) and the healthy subpopulation (Fig. 4).

In addition, sex, fasting plasma glucose levels, total-to-HDL cholesterol ratio, and pack-years of smoking were not associated with a higher or lower energy contribution of the five SMF components (data not shown).

Analysis with HbA1c or diagnostic measures of T2DM [i.e. T2DM (yes/no), T2DM and insulin use (yes/no), T2DM and peripheral neuropathy (yes/no)] instead of fasting plasma glucose did not result in significant associations either (data not shown). Nevertheless, participants with a T2DM duration of at least 10 years had a lower energy contribution of the endothelial component as compared to participants with a T2DM for less than 10 years [sβ = −0.23 SD (−0.46, −0.01); P = 0.04].

**Additional analyses**

First, the associations of the cardiovascular risk factors with both total SMF energy and the energy contribution of the components did not differ by diabetes status (data not shown). Second, the five SMF components can also be...
expressed in relative energy contributions [30]. Analysis with the relative energy contribution instead of absolute energy contribution of the SMF components demonstrated that age was associated with a lower relative energy contribution of the endothelial, neurogenic, and myogenic components, but with a higher relative energy contribution of both the respiratory and the heart beat component. In addition, waist circumference and 24-h SBP were not associated with a higher or lower relative energy contribution of the five SMF components (data not shown). Third, additional adjustment for time of LDF measurements (to adjust for diurnal influences) and time from light meal till LDF measurements (to adjust for light meal influences) gave similar results (data not shown). Fourth, additional adjustment for glucose-lowering medication, antihypertensive medication, and lipid-modifying medication use in the study population gave similar results (data not shown).

DISCUSSION

The study represents a comprehensive analysis of the associations of cardiovascular risk factors with SMF in a population-based sample. The study had two main findings. First, age and 24-h SBP were directly, and waist circumference was inversely associated with SMF after adjustment for the other cardiovascular risk factors, diabetes status, glucose-lowering medication, antihypertensive medication, and lipid-modifying medication use. Second, these associations were similar both in the study population and in the healthy subpopulation. Importantly, the study population is likely to be representative of the source population in the study region (e.g. the prevalence of overweight, obesity, and hypertension was 48.0, 12.0, and 31.4%, respectively, in our dataset as compared to 48.0, 12.0, and 31.4% in the general Dutch population [31,32]). However, since the Maastricht Study focuses on the cause, pathophysiology, complications and comorbidities of T2DM, recruitment was stratified according to known T2DM status [20]. Nevertheless, restriction of these analyses to the healthy subpopulation (without IGM and T2DM) showed similar results, indicating that these associations were not determined by IGM or T2DM and these associations can be considered valid for the general population.

Our finding that age and 24-h SBP were directly and that waist circumference was inversely associated with total SMF energy is in line with previous studies [16,18,33,34].
Nevertheless, the age-related increases in the respiratory and heart beat component, the waist circumference-related decreases, and the BP-related increases in all the five frequency components demonstrated in our study somewhat contrast with findings of other studies which demonstrated age, obesity, and hypertension-related alterations in the low-frequency components [16,18,34]. The explanation for these discrepancies is not entirely clear, but may be related to the fact that we studied a larger group (i.e. we had more power to detect small differences) and previous studies investigated a non-random sample of the population which could have introduced selection bias.

There is convincing evidence that the regulation of whole body and tissue metabolism and BP by the microcirculation [14] may be mediated, at least partly, via effects on flow motion under both physiological and pathophysiological conditions [1,8]. Indeed, several studies demonstrated that insulin and meal ingestion stimulate microvascular flow motion, which is likely paralleled with an increased tissue perfusion and increased insulin-stimulated glucose uptake in skeletal muscle [1,35]. In addition, experimental data demonstrated that rabbits with microvascular flow motion had a 20% lower arterial pressure as compared to rabbits without flow motion [7]. Furthermore, tissue areas threatened by homeostatic and metabolic stress demonstrated enhanced flow motion [36]. In addition, patients with mild peripheral arterial occlusive disease are characterized by enhanced flow motion [37]. Interestingly, when these patients were divided into those exhibiting flow motion and those who did not, those with flow motion had significantly higher tissue oxygen levels than those patients without, despite similar blood flow [37].

Thus, considering the suggested role of flow motion, we may suppose that the positive association of age and 24-h SBP with total SMF energy could represent an adaptive response to ageing and increased BP, with beneficial effects on tissue perfusion and local hydraulic resistance. It should, however, be kept in mind that the positive associations of...
age and BP with SMF may reflect different aspects of microvascular function, which is reflected in the fact that we found positive associations of age with the high-frequency components and of BP with all frequency components. In addition, the negative association of waist circumference with total SMF energy could represent obesity-related disturbance of flow motion, with negative effects on the delivery of nutrients and oxygen to tissue and local hydraulic resistance, which is consistent with a role for microvascular dysfunction, specifically impaired SMF, in the development of obesity-related T2DM and hypertension [14].

Theoretical and experimental evidence for the concept that flow motion increases with age as an adaptive response is lacking. Hence, it should be realized that the positive association of age with SMF might be the consequence of a thinner epidermis with increased age, resulting in a greater measuring depth and thus increased perfusion signals. Nevertheless, studies investigating the effect of ageing on epidermal thickness demonstrated conflicting results [38,39], and therefore this concept remains controversial. In addition, increased perfusion signals due to a thinner epidermis would result in positive associations of all five frequency components. Here, we only demonstrated age-related increases of the respiratory and heart beat component, indicating that with ageing, there is a shift towards the contribution of the respiratory and heart beat component resulting in a, possibly adaptive, enhanced SMF.

With regard to BP, several experimental and mathematical models demonstrated that during BP elevations arterioles are constricted and an oscillating network can transiently dilate these arterioles, thereby increasing tissue perfusion and alter local hydraulic resistance [40,41]. Indeed, in the hamster microcirculation, vasoconstriction induced by N\(^{\text{-}}\)monomethyl-L-arginine caused a decrease in effective diameter and an increase in flow motion frequency [41]. These experimental studies may support our finding of a positive association of BP with total SMF energy, indicating that flow motion may prevail over autoregulation during BP elevations [41]. In relation to the possible mechanisms involved, we demonstrated BP-related increases in the energy contribution of all five frequency components. These findings suggest an improvement in the efficiency of all components of flow motion during BP elevation [18].

FIGURE 4 Associations between cardiovascular risk factors and total skin microvascular flow motion energy in the healthy subpopulation. Point estimates (standardized β) and 95% CIs represent the change in total skin microvascular flow motion energy (in SD) per SD increase (or men vs. women) in the cardiovascular risk factor resulting from a fully adjusted multivariate regression model. *P < 0.05; † P < 0.001. CI, confidence interval.
With regard to waist circumference, there is convincing evidence that obesity is a primary cause of microvascular dysfunction [13] and that microvascular dysfunction, in turn, may be an intermediate step linking obesity to T2DM and hypertension [14]. In relation to the possible mechanisms involved, there is an increase in several circulating adipose tissue-derived factors in obesity, whereas the anti-inflammatory adipokine, adiponectin, is decreased. These endocrine factors are likely candidates to influence microvascular function and thus flow motion [1,14]. Hence, the waist circumference-related decreases in the energy contribution of all five frequency components may reflect a decline in the efficiency of all the components of flow motion due to these endocrine factors. Interestingly, major weight loss in severely obese patients resulted in a full normalization of flow motion [33], suggesting a cause-effect relationship between obesity and impaired flow motion.

The lack of a significant association of fasting plasma glucose and other metabolic or diagnostic measures of T2DM with SMF was unexpected. Other studies suggested that humans with T1DM and T2DM are characterized by decreased flow motion patterns in the low-frequency oscillations [15,17]. The explanation for this discrepancy is not entirely clear, but may be related to the fact that the participants with T2DM in our study had a shorter diabetes duration [median of 7.0 (inter-quartile range 3.0–11.0) years] than in the other study (mean of 17.1 ± 2.3 years) [17]. Indeed, in the current study, participants with a T2DM duration of at least 10 years had a decreased energy contribution of the endothelial component as compared to participants with a T2DM for less than 10 years, suggesting that the altered patterns of flow motion as demonstrated in diabetes may be a complication of long-standing diabetes.

Sex, total-to-HDL ratio, and smoking are associated with macrovascular dysfunction [42]. However, we could not confirm these associations with SMF. Interestingly, several other studies did not find significant associations of sex, cholesterol, and smoking with measures of microvascular...
function either (i.e. skin capillary recruitment [43], and
generalized retinal arteriolar narrowing and venular dilata-
tion [44,45]), suggesting that these determinants are less
important for microvascular as compared to macrovascular
functioning.

The present study had some limitations. First, this study is
cross-sectional in nature and therefore it is not possible to
distinguish between cause and effect. Hence, further large-
scale (longitudinal) studies in this area are needed. Second,
observational studies like the Maastricht Study do not allow
invasive measurements (i.e. we studied skin and not muscle
microcirculation). Nevertheless, several studies have dem-
strated comparable metabolic [46] and vascular effects in
skin and muscle [47]. These studies strongly suggest that
vascular responses observed in skin are parallel to those in
muscle, and thus that measurement of the skin microvas-
cular is a valid tool for the assessment of microvascular
function [48]. Third, 86% of all participants complied with
the smoking, caffeine, and meal instructions before SMF
measurements. However, when analyses were restricted to
these participants, results did not change.

In conclusion, age and 24-h SBP are directly, and waist
circumference is inversely associated with SMF in the gen-
eral population. The exact mechanisms underlying these
findings remain elusive. We hypothesize that flow motion
may be an important component of the microcirculation by
ensuring optimal delivery of nutrients and oxygen to tissue
and regulate local hydraulic resistance not only under
physiological conditions but also under pathophysiologica-
conditions when microcirculatory perfusion is reduced,
such as that which occurs with ageing and higher BP. In
addition, obesity may result in an impaired flow motion with
negative effects on the delivery of nutrients and oxy-
gen to tissue and local hydraulic resistance.

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from their web-based electronic health record.

Conflicts of interest

There are no conflicts of interest.

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Predicting the 50-year risk of cardiovascular disease: the framingham
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Reviewers’ Summary Evaluations

Reviewer 1
This study provides data on skin microvascular flow motion recorded by Laser-Doppler-flowmetry (LDF) in a relatively large study population. Positive correlations for the power in the LDF signal vs. age and arterial pressure and a negative correlation for the power of the LDF signal vs. waist circumference are reported. The strengths of the study are the large sample investigated and the consistency of data obtained in the entire study population and in a healthy subgroup, excluding confounding treatment effects on the observed relationships between cardiovascular risk factors and skin microvascular flow motion. Mechanistic insights on microcirculatory function are limited.

Reviewer 2
This is a cross-sectional study, which investigated the association between cardiovascular risk factors and skin microvascular flow motion. The study being cross-sectional, caution is needed in consideration of any causal relationship. The clinical importance and significance of skin microvascular flow motion remains to be investigated in future large studies.