Microvascular dysfunction in healthy insulin-sensitive overweight individuals

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Background **Obesity is associated with increased cardiovascular morbidity. The skin is a unique site allowing simple, noninvasive assessment of capillary density and endothelial function. In the present study, we measured skin capillary density and endothelial function in a group of normotensive overweight/obese nondiabetic individuals and healthy lean controls.**

Methods and results **We examined 120 relatively insulin-sensitive overweight individuals (BMI $27.9 \pm 2.7\ kg/m^2$, mean ± SD) with normal blood pressure and fasting plasma glucose and 130 lean (BMI $22.4 \pm 1.7\ kg/m^2$) controls. We used video microscopy to measure skin capillary density in the resting state and during venous occlusion. Laser Doppler flowmetry, combined with iontophoresis of acetylcholine (endothelial-dependent vasodilation) and following skin heating (endothelial-independent dilation), was performed. Resting capillary density was negatively correlated with BMI ($r = -0.130$, $P < 0.05$). Resting capillary density (mean ± SE) was lower, however nonsignificantly, in overweight as compared with the lean individuals (88.6 ± 1.5 vs. 91.8 ± 1.4, $P = 0.117$). Capillary recruitment, defined as the percentage increase in capillary density during venous congestion, was higher in overweight ($9.5 \pm 1.0\%$) than in controls ($5.4 \pm 0.9\%$, $P = 0.003$), which remained significant after adjustment for age, sex, mean arterial pressure and fasting glucose. As a consequence, capillary density during venous occlusion was similar between the groups. Endothelial-dependent and independent cutaneous vasodilation was also similar between groups. No correlations were found between capillary density and plasma markers of adiposity, inflammation or endothelial dysfunction.**

Conclusion **BMI was inversely correlated with resting capillary density. This suggests a lower baseline tissue perfusion associated with higher vasomotor tone. Despite this, capillary recruitment was higher in overweight as compared with lean individuals, resulting in similar capillary density during venous congestion. Our results suggest that skin microcirculation abnormalities, in the absence of endothelial dysfunction, may be one of the earliest detectable alterations in vascular function in overweight individuals.** ***J Hypertens*** **28:325–332 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: body fat, capillary recruitment, microcirculation, skin capillary density

Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IMT, intima–media thickness; LDF, laser Doppler flowmetry; LDL, low-density lipoprotein; MAP, mean arterial pressure; MCP-1, monocyte chemotactic protein-1; sE-selectin, soluble endothelium selectin; sICAM-1, soluble intercellular adhesion molecule-1; Svcam, soluble vascular cell adhesion molecule-1

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Introduction

Overweight and obesity are associated with increased cardiovascular disease (CVD) morbidity and mortality [1–3]. Whether obesity *per se* is directly linked to CVD remains under debate, given the frequent association of obesity with CVD risk factors, including arterial hypertension and type 2 diabetes. Multiple potential pathways linking adiposity and CVD have been identified, including biomarkers or intermediate clinical markers such as intima–media thickness (IMT) or arterial stiffness [4,5]. Previous studies [6–9] have demonstrated deleterious associations between body fat composition and distribution and the structure and function of large arteries in young and middle-aged adults. The metabolic syndrome, a cluster of bioclinical abnormalities with insulin resistance as a major characteristic, has also been positively associated with IMT and pulse wave velocity [10–12]. Dysfunction of the vascular endothelium (assessed by biomarkers, blood flow or vascular reactivity) has been described as an early abnormality in the development of CVD, in part through oxidative stress [13].

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Adipose tissue is no longer solely recognized as a passive fat storehouse and its endocrine synthesis capacities have been extensively investigated [14]. Leptin, adiponectin [15] and other adipokines (including cytokines and chemokines) produced by the adipocytes have been the most commonly studied molecules. They may play a role in the pathogenesis of CVD through a chronic subinflammatory state in obese and insulin-resistant patients. Yudkin *et al*. [16] have recently proposed that perivascular fat depots, in both large and small vessels, might contribute to insulin resistance and CVD through local cytokine production and its adverse impact on blood flow regulation. Insulin effects on nitric oxide-mediated vasodilatation may also be involved; however, this hypothesis must be validated in larger human studies.

Because of the various local interactions between adipose tissue secretions and small vessels, significant interest has been developed in the study of structure and function of the microcirculation. Impaired tissue perfusion via the microcirculatory network is a factor common to chronic conditions and may underlie much of the tissue and organ dysfunction related to associated ischemic complications. The cutaneous circulation is a valuable model of generalized microcirculation and may be considered as representative of the microcirculation of most target organs of CVDs. Skin capillary rarefaction has been demonstrated in overweight hypertensive patients [17–20], nondiabetic nonhypertensive obese women [21] and nondiabetic men and women [22,23].

The purpose of our current study was to compare skin capillary density (at baseline and after venous occlusion) and endothelium-dependent and independent vasodilation in healthy normal-weight individuals (BMI $< 25\,kg/m^2$) and normoglycemic normotensive predominantly overweight individuals (BMI $\geq 25\,kg/m^2$). We also investigated the associations between circulating adiposity and inflammation markers and skin capillary density and endothelial function in these populations.

Methods

Individuals and study design

The SU.VI.MAX study [24] (1994–2002) was a prevention trial designed to test the efficacy of an antioxidant supplementation on ischemic heart disease and cancer. Since 2006, 7200 individuals have agreed to be followed up in SU.VI.MAX-2, an observational prospective cohort study, with the aim of exploring the associations between diet and aging in France. A specific subprotocol was designed to assess microcirculation parameters in participants of the SU.VI.MAX-2 cohort living in the Paris area.

A total of 291 individuals were examined; however, 41 participants were excluded because of incomplete data. The final sample included 250 individuals (age range 48–74 years) without a history of type 2 diabetes (defined as fasting glucose $> 1.25\,g/l$ and/or taking diabetes medication), hypertension (defined as BP $\geq 140/90\,mmHg$ and/or taking antihypertensive medication) and cancer or CVD events since 1994.

Individuals were studied at the Department of Physiology and Noninvasive Investigations (Hôpital Lariboisière, Paris) between November 2006 and July 2007. SU.VI.MAX-2 Study was approved by the local Ethical Committee (CCP no. 2364, August 2006) and the 'Commission Nationale de l'Informatique et des Libertés' (no. 907094, July 2007). Signed informed consent was obtained from each participant.

Microvascular assessment

Intravital video capillaroscopy allows visualization of the skin in real time. In combination with video and computer technology, capillaroscopy generates high-contrast numeric images of dermal capillaries. Capillaroscopy was carried out using a standardized validated technique detailed elsewhere [19,20,25]. Briefly, individuals were studied between 0800 and 1200 h after an overnight fast. They were asked not to smoke from the previous evening. The capillaroscopy studies were performed in a temperature-controlled quiet room (21–24°C) after a 10-min rest in the semisupine position. Patients were seated with the forearm and hand supported at heart level.

Video microscopy was performed with an epiilluminated optic fiber microscope containing a 100-W mercury vapour lamp light source and a M200 objective (Moritex, micro-ScopernanMS-500C, Tokyo, Japan); final 200× magnification was used. Microscopic images were transferred for storage and analysis to a personal computer with a video image converter (Microvision, Evry, France). The skin of the dorsum of the middle phalanx of the nondominant hand was examined. This location has been chosen by most authors because it is expected that there is minimal effect of fat or muscle accumulation in this site. Indeed, the site-to-site variability impacts significantly on reproducibility of the measure [25]. The skin of the dorsum of the middle phalanx of the nondominant hand is a unique site where the capillary network is uniform, with capillary loops perpendicular to the skin surface and relatively homogeneous. An approximately 3×3-mm skin area on the middle third of the phalanx was defined. Four microscopic fields ($1\,mm^2$ each) were randomly chosen in this area for recording and measurements.

Mean capillary density was defined as the number of capillaries per unit area of skin and was calculated as the mean of four measurements performed in each individual. For each individual, images were acquired at baseline, to quantify the total number of continuously erythrocyte-perfused capillaries per dermal surface unit (resting or functional capillary density), and during

venous congestion (by applying a cuff to the wrist and maintaining a 50-mmHg inflating pressure for 2 min), in order to obtain the maximal response of all existing capillaries and to assess structural (anatomical) capillary density. Indeed, it has been shown that this procedure maximizes visible capillary number more than reactive hyperemia [26]. A previous study [19] has shown that intraobserver and interobserver repeatability was 4.3 and 5.9%, respectively. Capillary recruitment (%) was defined as follows: (capillary density during venous occlusion − resting capillary density)/resting capillary density $\times 100$.

Endothelial function: laser Doppler flowmetry and iontophoresis of acetylcholine

Endothelial-dependent and independent vasodilation of the forearm skin microcirculation was evaluated by iontophoresis and skin heating in combination with laser Doppler flowmetry (LDF). A laser beam penetrates the skin and a fraction of the light is backscattered by moving blood objects and undergoes a frequency shift according to the Doppler principle, generating a signal proportional to tissue perfusion. Forearm skin blood perfusion was measured by means of a LDF apparatus (Periflux PF5000; Perimed, Stockholm, Sweden) with the following characteristics: 780-nm wavelength, 10 Hz to 19 kHz bandwidth, 0.1 s time constant and 32-Hz sampling frequency. Calibration was performed using colloidal latex particles whose Brownian motion provides the standard value. The LDF outputs were recorded continuously with an interfaced computer (DELL E521; DELL, Round Rock, Texas, USA) equipped with Perisoft dedicated software allowing measurement of LDF output (mV). After recording video capillaroscopy images, the LDF probe was applied to the anterior part of the forearm with a plastic holder. The skin temperature was monitored throughout and maintained at 33°C by the same LDF heating probe.

Baseline skin blood perfusion was defined as the mean value recorded during a 4-min time period. In order to investigate the endothelium-dependent vasodilation, iontophoresis of graduated doses of acetylcholine was undertaken. Iontophoresis is a noninvasive standard method of drug application that allows the local transfer of electrically charged substances across the skin by using a small electric current. The electrical potential difference actively causes ions in solution to migrate according to their electrical charge. A battery-powered iontophoresis controller (Perijont 328; Perimed) was used to provide a direct current for iontophoresis. Acetylcholine was delivered with an anodal current. The battery-powered iontophoresis controller was connected to the LDF apparatus and provided the chosen current to an electrode chamber (PF 383; Perimed, Jarfallan, Sweden) allowing the passage of the laser light to the skin.

Acetylcholine chloride 2% solution (800 μl) was used to fill the chamber. The positive lead of the current source was connected to the chamber, and the negative lead was attached to a conductive hydrogel pad on the wrist, which served as the reference electrode. We used a delivery current of 10 mA and administered three successive doses of acetylcholine for 10 s with an interval of 2 min between each dose in order to achieve a plateau of the response following each dose delivery. The laser probe was heated to 44°C for 5 min and we recorded the maximal response to local skin heating, that is, the endothelium-independent maximal vasodilation. In order to eliminate baseline variability, the maximum skin perfusion value following iontophoresis was expressed as maximum percentage change from baseline. The following parameters (arbitrary unit) were determined: basal perfusion index (skin temperature at 33°C, arbitrary unit), perfusion index after the third dose of acetylcholine iontophoresis, that is, the maximal endothelial response and the maximum perfusion index after heat hyperemia (skin temperature at 44°C).

Hemodynamic anthropometric and body composition measurements

Brachial blood pressure (BP) and heart rate (HR) were measured in the sitting position with a semiautomatic oscillometric device (Dinamap PRO 400V2; General Electric, Fairfield, Connecticut, USA) with appropriate cuff size. After 10 min of rest, two measurements in each arm were obtained at 5-min intervals. SBP and DBP were calculated as the mean of the left and right second measurements. Mean arterial pressure (MAP) was calculated as: (DBP) + (SBP − DBP)/3. Peripheral pulse pressure (PP) was defined as the difference between the values of brachial systolic and diastolic pressures. Central aortic BP was derived from the radial artery pressure wave using the SphygmoCor system (PWV Medical, Sydney, Australia) [27,28]. This device applies a generalized transfer function to determine the aortic BP curve from the radial pressure wave and has been validated in detail elsewhere [29].

Body weight and body composition were measured using the Tanita DC-320 (Tanita Corp., Tokyo, Japan) bioelectrical impedance device based on four separate footpad electrodes mounted on the system's base [30]. All measurements were carried out at 50 kHz with a 0.8 mA, with individuals in indoor clothing and no shoes. Percentage body fat (%body fat = fat mass/body weight) and visceral fat level (arbitrary unit), an indirect surrogate of visceral adipose tissue (VAT) are presented. Waist circumference was measured as the circumference midway between lower ribs and iliac crests [10].

Biochemical measurements

Serum total cholesterol, high-density lipoprotein-cholesterol and triglyceride concentrations were measured using standard methods. Low-density lipoprotein

Table 1 Characteristics of the population

	BMI $< 25\,kg/m^2$ $n = 130$	BMI $\geq 25\,kg/m^2$ $n = 120$	*P*
Age (years)	62.0 ± 5.9	61.9 ± 5.6	0.954
Women (%)	54.6	49.2	0.389
Smokers (%)	13.1	5.8	0.140
Weight (kg)	62.18 ± 7.64	76.44 ± 10.16	<0.0001
BMI (kg/m^2)	22.44 ± 1.66	27.86 ± 2.73	<0.0001
Waist circumference (cm)	84.57 ± 6.72	96.86 ± 8.04	<0.0001
Total body fat (%)	24.22 ± 6.37	31.36 ± 7.44	<0.0001
Visceral fat level (arbitrary unit)	7.95 ± 2.50	11.39 ± 2.92	<0.0001
Peripheral (brachial) BP			
SBP (mmHg)	115 ± 11.4	118 ± 9.5	0.011
DBP (mmHg)	73.6 ± 7.4	77.1 ± 6.3	0.001
Mean BP (mmHg)	87.4 ± 8.4	90.8 ± 6.5	0.001
PP (mmHg)	41.3 ± 6.7	41.3 ± 7.8	0.976
Central BP			
SBP (mmHg)	112 ± 11.5	114 ± 10.1	0.121
DBP (mmHg)	76.3 ± 7.7	79.9 ± 6.9	0.001
Mean BP (mmHg)	91.6 ± 8.8	94.9 ± 7.3	0.001
PP (mmHg)	35.8 ± 7.3	34.3 ± 8.5	0.153
Heart rate (beats/min)	61.9 ± 9.4	64.4 ± 10.9	0.05
Total cholesterol (g/l)	2.27 ± 0.43	2.34 ± 0.34	0.176
LDL-cholesterol (g/l)	1.45 ± 0.37	1.52 ± 0.27	0.085
HDL-cholesterol (g/l)	0.65 ± 0.14	0.61 ± 0.15	0.019
Triglycerides (g/l)[a]	0.80 [0.75–0.85]	0.97 [0.91–1.04]	<0.0001
Lipid-lowering treatment (%)	7.7	7.5	0.954
Fasting glucose (mmol/l)	5.23 ± 0.51	5.45 ± 0.48	0.001
Fasting insulin (μu/ml)[a]	3.73 [3.43–4.04]	5.73 [5.26–6.24]	<0.0001
HOMA-IR[a]	0.86 [0.79–0.94]	1.38 [1.26–1.52]	<0.0001
Leptin (ng/ml)[a]	4.85 [4.15–5.67]	10.57 [8.98–12.44]	<0.0001
Total adiponectin (μg/ml)[a]	13.88 [12.79–15.05]	10.88 [10.00–11.85]	<0.0001
HMW-adiponectin (μg/ml)[a]	11.43 [10.34–12.63]	8.5 [7.66–9.43]	<0.0001
CRP (mg/l)[a,b]	1.21 [1.01–1.44]	1.76 [1.47–2.10]	0.004
sICAM-1 (ng/ml)	239.5 ± 59.49	268.4 ± 64.49	0.001
sVCAM-1 (ng/ml)	748.4 ± 215.4	738.2 ± 184.1	0.687
sE-selectin (ng/ml)	25.71 ± 12.00	25.04 ± 9.61	0.627
MCP-1 (pg/ml)	395.5 ± 110.4	419.7 ± 120.8	0.099

Data are mean ± SD for continuous variables and percentage for categorical variables. CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein-1; PP, pulse pressure; sE-selectin, soluble endothelium selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, vascular cell adhesion molecule-1. [a]Log-transformed variables, geometric means [95% CI]. [b]$n = 243$.

(LDL)-cholesterol was calculated by the Friedwald formula. Fasting plasma glucose was assayed enzymatically (hexokinase) using a multiparametric analyzer (C8000 Architect Abbott analyzer, Rungis, France). Fasting plasma insulin was measured by microparticle enzyme immunoassay (Axsym Abbott analyzer, Rungis, France). Insulin resistance was estimated by the calculation of the homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting plasma insulin × fasting plasma glucose)/22.5. Leptin, soluble endothelium selectin (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and chemokine (C–C motif) ligand 2/monocyte chemotactic protein-1 (MCP-1) were determined by ELISA (respective Quantikine immunoassays; R&D, Oxford, UK). Total adiponectin was assayed by radioimmunoassay (Linco Research, St. Charles, Missouri, USA) and high-molecular-weight (HMW) adiponectin was determined using a recently developed ELISA system (Fujirebio Inc., Tokyo, Japan) [31]. Hs CRP was assessed by immunonephelometry on IMMAGE analyser (Beckman-Coulter, Villepinte, France)

Statistical analyses

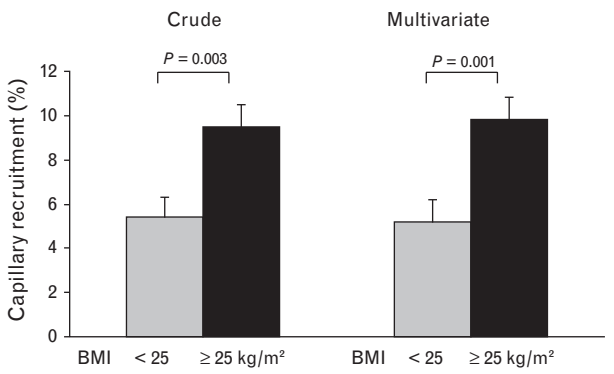
Descriptive data (Table 1) are expressed as mean ± SD for continuous variables and percentage for categorical variables. Capillary densities and recruitment and endothelial function parameters are given as mean ± SE (Table 2 and Fig. 1). Spearman's correlations were used for the crude associations between variables. Comparisons between the two groups were performed using unpaired *t*-tests, analysis of variance (ANOVA) or χ^2 tests wherever appropriate. Mean capillary density during venous occlusion was compared with baseline values

Table 2 Mean capillary density and recruitment and endothelial function parameters

	BMI $< 25\,kg/m^2$ $n = 130$	BMI $\geq 25\,kg/m^2$ $n = 120$	*P*
Mean capillary density ($capillary/mm^2$)			
Resting	91.8 ± 1.4	88.6 ± 1.5	0.117
During venous occlusion[a]	96.2 ± 1.4	96.5 ± 1.5	0.901
Capillary recruitment (%)	5.4 ± 0.9	9.5 ± 1.0	0.003
Acetylcholine-mediated vasodilation (%)[b]	416 ± 32	478 ± 34	0.193
Heating-mediated vasodilation (%)[b]	625 ± 36	713 ± 38	0.100

Data are mean ± SE. [a]Significantly different from resting values in each group. [b]$n = 248$.

Fig. 1



Crude and multivariate-adjusted capillary recruitment (mean ± SE). Multivariate models are adjusted for age, sex, mean arterial pressure, total cholesterol, triglycerides and fasting blood glucose.

using paired *t*-tests. Crude and adjusted (age, sex, fasting blood glucose and mean BP) mean capillary density was compared between the two groups with unpaired *t*-tests or ANOVA. Multivariate-independent associations between adiposity measures (BMI, waist circumference, %body fat and VAT), biological markers [fasting plasma glucose and insulin, HOMA-IR, leptin, adiponectin, sICAM-1, sVCAM-1, sE-selectin, MCP-1 and high-sensitivity C-reactive protein (CRP)] with both capillary and endothelial markers were assessed using linear regression models. Age, sex, fasting blood glucose, total cholesterol, triglycerides and mean BP multivariate models are presented. Correlation coefficient (β) was presented for each independent model. Statistical significance was judged at α value less than 0.05. All statistical analyses were performed using SAS version 8.2 (SAS, Cary, North Carolina, USA).

Results

Cohort characteristics

Table 1 presents the characteristics of the lean and overweight/obese populations. Only 17% of the latter group was obese (BMI $\geq 30\,kg/m^2$). There was no difference between the groups in age, sex and smoking status. Most of the peripheral and central BP measures and HR were significantly higher in overweight/obese individuals. As expected, all body fat measures, fasting insulin, HOMA-IR and leptin were significantly higher in overweight/obese individuals, whereas total and HMW adiponectin were lower. Of the endothelial adhesion markers, only sICAM-1 and MCP-1 were higher in overweight/obese as compared with lean individuals ($P < 0.0001$ and $P = 0.099$, respectively). Because of the inclusion of predominantly healthy normoglycemic individuals only, HOMA-IR values were low, even in the overweight and obese group.

Determinants of capillary recruitment measures

Age was positively related to resting capillary density ($r = 0.131$, $P < 0.05$), but was unrelated to capillary density during venous occlusion and capillary recruitment. SBP, DBP and mean BP were unrelated to these latter markers; however, peripheral PP was negatively related to capillary recruitment ($r = -0.173$, $P < 0.05$).

There was no difference in mean resting capillary density between men and women (mean ± SD 89.0 ± 14.5 and 91.5 ± 17.1 capillaries/mm^2, respectively, $P = 0.208$). Mean resting capillary density was similar ($P = 0.320$) in smokers (mean ± SD 87.0 ± 18.0 capillaries/mm^2), nonsmokers (mean ± SD 90.5 ± 15.7 capillaries/mm^2) and previous smokers (mean ± SD 90.8 ± 15.9 capillaries/mm^2). Similar results were found during venous occlusion ($P = 0.663$).

Microvascular and endothelial data in lean and overweight/obese individuals

There was a tendency to lower resting capillary density in overweight/obese as compared with lean individuals (Table 2). Capillary recruitment was higher in overweight (mean ± SE, $9.5 \pm 1.0\%$) than in lean (mean ± SE, $5.4 \pm 0.9\%$) individuals ($P = 0.003$, Table 2). Further adjustment for age, sex, mean BP, total cholesterol, triglycerides and fasting blood glucose did not modify the associations (mean ± SE, 9.8 ± 1.0 vs. $5.2 \pm 1.0\%$, $P = 0.001$, Fig. 1). Because of the greater degree of recruitment in overweight/obese individuals, there was no difference in capillary density during venous congestion, suggesting no reduction in the anatomical number of capillaries in insulin-sensitive overweight/obese individuals (Table 2).

Endothelial-dependent (416 ± 32 vs. $478 \pm 34\%$) and independent (625 ± 36 vs. $713 \pm 38\%$) cutaneous vasodilation was similar in both groups ($P = 0.193$ and $P = 0.100$, respectively, Table 2).

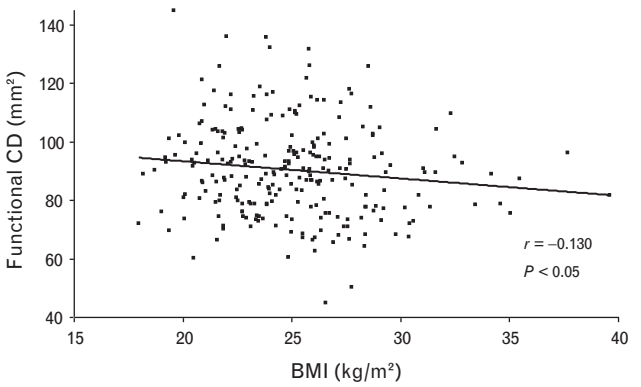
Correlation analyses

In crude analyses, resting capillary density was significantly inversely associated with BMI ($r = -0.130$, $P < 0.05$, Fig. 2). In multivariate analysis, the inverse relationship between resting capillary density and BMI became nonsignificant (Table 3). Consistent with the results in Table 2, capillary recruitment was significantly positively related to BMI ($r = 0.129$, $P < 0.05$) in crude analyses. Relationships remained significant after adjustment for age, sex, mean arterial BP and fasting glucose (Table 3). Adjustment for PP instead of MAP did not modify the associations (data not shown).

Inverse age and sex-adjusted correlations were found between fasting triglycerides or plasma glucose with both capillary density at baseline and during venous occlusion (Table 4). LDL-cholesterol was inversely associated with capillary density during venous occlusion only.

Except for LDL-cholesterol with capillary density during venous occlusion that became nonsignificant ($P = 0.10$),

Fig. 2



Spearman's correlation of functional (baseline) mean capillary density (CD) with BMI (kg/m^2).

the above associations with triglycerides and plasma glucose remained significant in a multivariate model, including age, sex, triglycerides, LDL-cholesterol and MAP (data not shown). No associations were observed with leptin, adiponectin, inflammation or endothelial vascular markers with capillary markers.

Discussion

To our knowledge, this is the largest study examining the relationships between adiposity, metabolic and cardiovascular risk markers and skin capillary density in healthy normotensive, normoglycemic insulin-sensitive predominantly overweight individuals. The main finding of this study was that, despite nonsignificant lower resting capillary density in overweight/obese individuals, capillary recruitment was significantly higher in the latter group, resulting in no difference in capillary density during venous occlusion. The difference in capillary recruitment between the groups persisted after adjustment for age, sex, MAP and fasting glucose. In line with the higher HR in the overweight group, these findings suggest lower baseline tissue perfusion, associated with higher vasomotor tone, in overweight individuals. Because endothelial-dependent and independent vasodilation were similar in the two groups, our results suggest that changes in the cutaneous microcirculation, may be the earliest alterations in vascular function associated with overweight/obesity, independent of known cardiovascular risk factors such as raised BP, insulin resistance or type-2 diabetes.

Table 3 Multivariate linear regression models with capillary variables as dependent variable

	Mean capillary density (capillaries/mm^2)		
	Baseline	During venous occlusion	Capillary recruitment (%)
BMI (kg/m^2)	−0.401	0.003	0.462*
Waist circumference (cm)	−0.089	0.008	0.100
Total body fat (%)	−0.182	0.146	0.374**
Visceral fat level (arbitrary unit)	−0.663	0.082	0.841**

Models are adjusted for age, sex, mean blood pressure, triglycerides, total cholesterol and fasting blood glucose. Partial correlation coefficients (β) are presented. * $P < 0.05$. ** $P < 0.01$.

Table 4 Age and sex-adjusted linear regression models with capillary variables as dependent variable ($n = 250$)

	Mean capillary density (capillaries/mm^2)		
	Baseline	During venous occlusion	Capillary recruitment (%)
Total cholesterol (g/l)	−3.384	−4.752	−1.452
LDL-cholesterol (g/l)	−4.624	−6.498*	−2.046
HDL-cholesterol (g/l)	9.776	9.840	−0.200
Triglycerides (g/l)	−6.034*	−6.030*	0.361
Fasting glucose (mmol/l)	−4.061*	−4.590*	−0.465
Fasting insulin (μu/ml)	−0.450	−0.228	0.257
HOMA-IR	−1.895	−1.139	0.894
Leptin (ng/ml)	−0.192	−0.143	0.051
Total adiponectin (μg/ml)	0.112	−0.031	−0.166
HMW-adiponectin (μg/ml)	0.163	0.050	−0.121
CRP (mg/l)†	−0.409	−0.192	0.298
sICAM-1 (ng/ml)	−0.007	0.005	0.018
sVCAM-1 (ng/ml)	0.003	0.002	−0.001
sE-selectin (ng/ml)	0.086	0.114	0.035
MCP-1 (pg/ml)	−0.008	−0.007	0.002

Partial correlation coefficients (β) are presented. CRP, c-reactive protein; HDL, high-density lipoprotein; HMW, high-molecular-weight; HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein-1; sE-selectin, soluble endothelium selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, vascular cell adhesion molecule-1. * $P < 0.05$. † $n = 243$.

Few studies have examined the relationship between adiposity *per se* and microvascular function. In a small study [21] in lean and obese women, no difference in resting capillary density was reported. In contrast to a previous study [22], we observed a positive association between total and central adiposity markers and capillary recruitment during venous congestion. Another group [32] reported no association between BMI and capillary density before and after venous occlusion and capillary recruitment.

Our study did not show differences in structural (anatomical) capillary rarefaction or endothelial-dependent and independent vasodilation between overweight/obese and lean individuals. Nevertheless, there is evidence that obesity is associated with impaired endothelial-dependent vasodilation in children [33] and adults [34]. In these studies, fasting insulin levels were higher than those in individuals included in our study. In the present study, overweight/obese individuals were relatively insulin-sensitive as evidenced by a low–normal HOMA-IR, a consequence of our stringent exclusion criteria. Indeed, mean HOMA-IR values close to 6 have been observed in type 2 diabetic individuals in previous studies [35,36]. Furthermore, sICAM-1, sVCAM-1, sE-selectin and CRP levels in our study were in the 'low–normal' range as compared with a group of healthy obese men [37]. As sICAM-1 and E-selectin have been associated with

insulin resistance in other populations, they are considered good correlates of impaired vascular reactivity [38,39]. Therefore, our study suggests that in the absence of insulin resistance, overweight/obese individuals do not exhibit structural (anatomical) capillary rarefaction or endothelial-dependent vasodilation in comparison with lean individuals. Interestingly, it should be noted that different obesity phenotypes may exist and this could be taken into consideration for the interpretation of our results. Indeed, in a recent study [40] in 314 individuals, it was shown that a subgroup of metabolically benign obesity could be identified. In this 'obese insulin-sensitive' population, insulin level was similar to the normal weight population and the IMT was low, even if the mean BMI was at least 30 kg/m^2.

It has been postulated that diminished microvascular (capillary) recruitment in muscle may precede and contribute to insulin resistance [23,34]. In line with this hypothesis, previous studies [21,41] have shown that recruitment of the microvasculature by insulin temporally precedes insulin's effect on skeletal muscle glucose uptake. Furthermore, it has been demonstrated that insulin's recruitment of the microvasculature is impaired in insulin-resistant Zucker rats [42]. Even though no studies have yet directly compared skin and muscle capillary recruitment as markers of microvascular function in humans, skin capillary measurement can be performed noninvasively in large populations and appears to be a good surrogate for muscle capillary recruitment. In previous studies, Serne *et al.* [20,23] demonstrated that skin capillary recruitment was strongly and positively related to insulin sensitivity measured by hyperinsulinemic euglycemic clamps in normotensive, but not hypertensive, normoglycemic individuals. In another study [21] by the same group, despite an overall relationship between capillary recruitment and insulin sensitivity in healthy, lean individuals, the association appeared to be abolished in 12 obese women. Therefore, the lack of insulin resistance in overweight/obese group in our study may explain the absence of defective skin capillary recruitment in this group during venous congestion and the absence of an association between HOMA-IR and capillary recruitment.

Our study has several strengths, including the large sample size and inclusion of well phenotyped men and women. Our exclusion of individuals with metabolic disease allowed us to examine the effect of obesity *per se* on microvascular structure and function. Potential limitations include the lack of glucose tolerance test to identify glucose-intolerant and diabetic individuals. Because of the cross-sectional design, we are unable to address cause and effect between adiposity, insulin sensitivity and microvascular dysfunction. In the resting state, only a proportion of the total number of muscle capillaries is perfused, with additional capillaries recruited as required, for example, during exercise. Procedures such as venous congestion and postocclusive hyperemia are used to recruit nonperfused capillaries [26]. Previous studies have demonstrated lower capillary density in muscle [43] and skin [21] in obese individuals, suggesting that cutaneous measurements of the microcirculation are acceptable surrogates of integrated microvascular function in muscle in the resting state [44]. However, it is unclear whether this is also the case during venous congestion or postocclusive hyperemia. Further studies correlating changes in the cutaneous microcirculation under basal and maximal conditions with changes in other vascular beds (coronary, muscle and renal) are required. In addition, the unsolved issue of distrectuality in capillaries function in individuals with abdominal adiposity or high visceral fat and the possible differences in structural characteristics of the capillary districts remains unsolved.

In conclusion, we have demonstrated that despite a crude inverse correlation between BMI and resting capillary density, insulin-sensitive overweight/obese individuals exhibit preserved (enhanced) capillary recruitment during venous congestion, contributing to the lack of anatomical defect. Our results suggest alterations of the microcirculation in overweight individuals, independently of BP and type-2 diabetes and in the absence of endothelial dysfunction, may be considered as the earliest marker of cardiovascular risk. Because a large body of evidence has shown that hypertension and diabetes result in impaired microvascular function [18,20,45,46], future studies are required to determine the effect of clustering of these abnormalities in the metabolic syndrome on the structure and function of the cutaneous microcirculation.

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