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BRIEF CUTTING EDGE REPORT

Epidemiology/Genetics

Microvascular differences in individuals with obesity at risk of developing cardiovascular disease

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Abstract

Objective: This study aimed to investigate microvascular differences in individuals with obesity at risk for developing cardiovascular disease.

Methods: In this cross-sectional Netherlands Epidemiology of Obesity study, participant sublingual microcirculation was assessed with a newly developed GlycoCheck software (Microvascular Health Solutions Inc., Salt Lake City, Utah), which integrates red blood cell velocity within the smallest capillaries (4-7 μm) and feed vessels (>10 μm). Framingham Risk Score was used to calculate 10-year cardiovascular risk, divided into low-, intermediate-, and high-risk groups. ANOVA was used to evaluate microvascular differences among the groups.

Results: A total of 813 participants were included. The high-risk group (n = 168) was characterized by differences in the microvasculature compared with the low-risk group (n = 392): the high-risk group had a 49% reduction in the number of smallest capillaries and a 9.1-μm/s (95% CI: 5.2-12.9) higher red blood cell velocity in the feed vessels. No differences in velocity-corrected perfused boundary regions were found.

Conclusions: It was observed that, with adding red blood cell velocity to the software, sidestream dark field imaging is able to detect microcirculatory differences in a cohort of individuals with obesity at risk for developing cardiovascular disease.

INTRODUCTION

Obesity is a well-established risk factor for developing cardiovascular disease (CVD), the leading cause of mortality worldwide. One of the earliest changes in CVD pathogenesis is microvascular endothelial dysfunction (1,2). Recently, we showed that, early in diabetes, the endothelial glycocalyx is perturbed, which results in reduced tissue perfusion and decreased perfused capillary density (3,4). Detecting early microvascular changes, long before the onset of clinical symptoms of CVD, and monitoring the response of therapeutic interventions may improve cardiovascular outcome. However, techniques to easily assess the dynamic microcirculation in humans are limited (5,6).

Subsequent to our previous sidestream dark field (SDF) imaging analysis in a subpopulation of the Netherlands Epidemiology of Obesity (NEO) study (7), we present a newly developed software application that facilitates automatic analysis of red blood cell (RBC) velocity, allowing us to include flow changes between feed vessels and capillaries to be coupled to perfused boundary region (PBR) and perfused capillary density measurements. In the current study, we reanalyzed our previous SDF measurements and divided the cohort into cardiovascular risk groups according to Framingham Risk Score. The Framingham Risk Score is a sex-specific algorithm that is widely used to assess the risk of cardiovascular events (coronary, cerebrovascular, and peripheral artery disease and heart failure) within 10 years.

Because microvascular dysfunction is one of the first signs of CVD, we aimed to investigate whether individuals with obesity and a high risk for developing CVD could be characterized by microvascular changes measured with SDF imaging.

Study Importance

What is already known?

► Microvascular changes due to endothelial dysfunction is an early step in the pathogenesis of cardiovascular disease. Sidestream dark field imaging is a noninvasive technique to detect these microvascular changes.

What does this study add?

► By analyzing densities of blood perfused microvessels in a diameter-dependent manner, it is demonstrated that significant reductions in the number of the smallest capillaries can be identified in individuals with obesity exposed to increased cardiovascular risk according to the Framingham Risk Score. This new analysis could be used for early detection of microvascular changes in individuals with obesity that might aid in monitoring such individuals, e.g., using various interventions.

How might your results change the direction of research or the focus of clinical practice?

► By adding red blood cell velocity calculations to the new sidestream dark field imaging software, a better estimate of perfused boundary regions can be implemented that can be used to detect early signs of microvascular damage. Automated imaging can be used to detect early microvascular changes and monitor clinical interventions aimed at restoring microvascular health.

METHODS

Study design and population

The population-based prospective-cohort NEO study, designed to investigate pathways leading to obesity-related diseases, started in 2008 and included 6,671 individuals aged 45 to 65 years, with an oversampling of individuals with overweight (BMI of 27 kg/m^2 or higher). Detailed information about the NEO study design and data collection is described elsewhere (7). The Medical Ethical Committee of the Leiden University Medical Center approved the design of the study. All participants gave their written informed consent.

The present study included 918 participants in whom SDF imaging was performed between January and October 2012 as part of the baseline visit at the Leiden University Medical Center NEO study center.

Framingham Risk Score

The Framingham Risk Score was used to calculate the risk of general CVD by using the risk factors gender, age, total and high-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, current smoking, and diabetes status. The Framingham Risk Score was reported as absolute risk percentage classified as low (<10%), intermediate (10%-20%), or high (>20%) 10-year predicted risk of CVD (8).

SDF microcirculation imaging

Intravital microscopy was performed earlier using an SDF camera (MicroVision Medical Inc., Wallingford, Pennsylvania) and acquired using Glycocheck software (Microvascular Health Solutions Inc., Salt Lake City, Utah) as described elsewhere (5).

The new software includes RBC velocity as a new parameter. After reanalyzing, the following parameters were obtained: perfused capillary density, absolute and static capillary blood volume, RBC velocity, and static and dynamic PBR. Detailed information about the new software used in the NEO study is described elsewhere (5,9)

TABLE 1 Characteristics and SDF-derived parameters of the study population stratified by Framingham Risk Score group and total cohort

	Low risk ($n = 392$)	Intermediate risk ($n = 253$)	High risk ($n = 168$)	Total cohort ($n = 813$)
Demographics				
Age (y)	54 (6)	57 (6)	60 (5)	56 (6)
Women (%)	81	37	12	53
Postmenopausal in women (% yes)	52	77	95	60
Ethnicity (% White)	94	94	98	95
Tobacco smoking (% current)	5	12	32	12
Prevalent diabetes[a] (%)	1	6	23	7
Treatment for hypertension (% yes)	17	30	50	28
Anthropometrics				
Systolic blood pressure (mmHg)	123 (13)	136 (14)	143 (16)	131 (16)
Diastolic blood pressure (mmHg)	80 (8)	87 (10)	88 (9)	84 (10)
BMI (kg/m^2), M/W	27.0 (3.6)/27.8 (5.1)	28.2 (3.5)/29.0 (4.1)	29.1 (4.1)/31.8 (6.3)	28.3 (3.8)/28.3 (5.2)
Waist circumference (cm), M/W	96.9 (11.5)/90.5 (13.1)	99.9 (9.2)/94.7 (12.2)	103.5 (12.2)/100.8 (13.8)	100.7 (11.2)/91.9 (13.2)
Waist-hip ratio, M/W	0.92 (0.07)/0.84 (0.07)	0.95 (0.06)/0.88 (0.07)	0.97 (0.07)/0.90 (0.07)	0.95 (0.07)/0.85 (0.07)
Total body fat (%), M/W	24 (7)/39 (7)	26 (5)/41 (6)	29 (6)/42 (6)	27 (6)/40 (7)
Laboratory markers				
Fasting glucose (mmol/L)	5.2 (4.9-5.6)	5.5 (5.2-5.9)	5.7 (5.3-6.6)	5.4 (5.0-5.9)
Fasting insulin (IU/L)	8.5 (5.8-12.3)	9.9 (6.3-14.4)	12.4 (9.0-18.2)	9.6 (6.2-14.3)
HbA_{1c} (%)	5.31 (0.33)	5.42 (0.50)	5.69 (0.87)	5.42 (0.55)
Total cholesterol (mmol/L)	5.64 (1.00)	5.92 (1.12)	6.00 (1.07)	5.80 (1.06)
Triglycerides (mmol/L)	0.84 (0.63-1.21)	1.20 (0.82-1.65)	1.36 (0.98-1.98)	1.05 (0.73-1.48)
HDL cholesterol (mmol/L)	1.67 (0.44)	1.42 (0.38)	1.25 (0.31)	1.51 (0.43)
hsCRP (mg/L)	1.32 (0.7-3.10)	1.31 (0.73-2.92)	1.66 (0.88-3.55)	1.37 (0.73-3.06)
eGFR CKD-EPI (mL/min/1.73 m^2)	87 (12)	86 (12)	84 (12)	86 (12)
Albumin/creatinine ratio (mg/mmol)	0.43 (0.26-0.69)	0.41 (0.26-0.60)	0.43 (0.30-0.71)	0.42 (0.27-0.68)
Microvascular parameters				
Capillary blood volume (pL/mm^2)	2.74 (1.41-4.87)	2.41 (1.43-4.24)	2.21 (1.36-3.52)	2.52 (1.40-4.31)
Capillary density (µm/mm^2)	40 (25-59)	36 (25-57)	33 (24-47)	37 (25-55)
RBC velocity in feed vessels (µm/s)	53 (18)	60 (19)	62 (17)	57 (18)
RBC velocity in capillaries (µm/s)	54 (20)	60 (21)	62 (18)	57 (20)
PBR_{static} (µm)	2.37 (0.24)	2.32 (0.23)	2.31 (0.23)	2.34 (0.24)
$PBR_{dynamic}$ (µm)	2.54 (0.24)	2.57 (0.22)	2.53 (0.22)	2.55 (0.23)

Data are presented as mean (SD), median (25th-75th percentile), or percentage. Framingham Risk Score groups: low risk: <10%; intermediate risk: 10%-20%; high risk: >20%. Missing: 12 HbA_{1c}, 3 eGFR CKD-EPI, and 3 albumin/creatinine ratio.

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration formula; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density-lipoprotein; hsCRP, high-sensitivity C-reactive protein; M, men; PBR, perfused boundary region; pL, picoliter; RBC, red blood cell; SDF, sidestream dark field; W, women.

[a]Self-reported diabetes mellitus I or II, medication use, or fasting plasma glucose >7.0 mmol/L.

Statistical analysis

The Framingham Risk Score was calculated by using the Stata module of A. Linden, installed with the syntax "ssc install framingham." The resulting participants were divided into absolute risk percentage groups classified as low risk (<10%), intermediate risk (10%-20%), or high (>20%) risk. Data are presented as mean (SD), median (25th-75th percentile), or percentage. Differences in microvascular parameters between the risk groups were analyzed by ANOVA. Capillary blood volume and perfused capillary density we transformed into the natural logarithm. The percentage change of capillary density compared with the low-risk group (reference) was calculated within the capillary

diameters class. The previously mentioned analyses were performed with Stata software (version 14.1; StataCorp, College Station, Texas).

RESULTS

For stratifying the participants ($n = 918$) by Framingham Risk Score, participants with preexisting CVD ($n = 60$) were excluded for this analysis, as were participants with missing data on diabetes status ($n = 5$), systolic blood pressure ($n = 2$), $PBR_{4\text{-}25\mu m}$ ($n = 30$), and RBC velocity measurements ($n = 8$). This resulted in a total of 813 participants (382 men and 431 women) included in the present analysis. For each participant, the Framingham Risk Score was calculated, and individuals were divided into risk groups reported as low, intermediate, or high 10-year predicted risk of CVD. Study characteristics and microvascular parameters derived from SDF imaging stratified by Framingham Risk Score groups and in the total cohort are shown in Table 1 and Figure 1. For the statistical analysis,

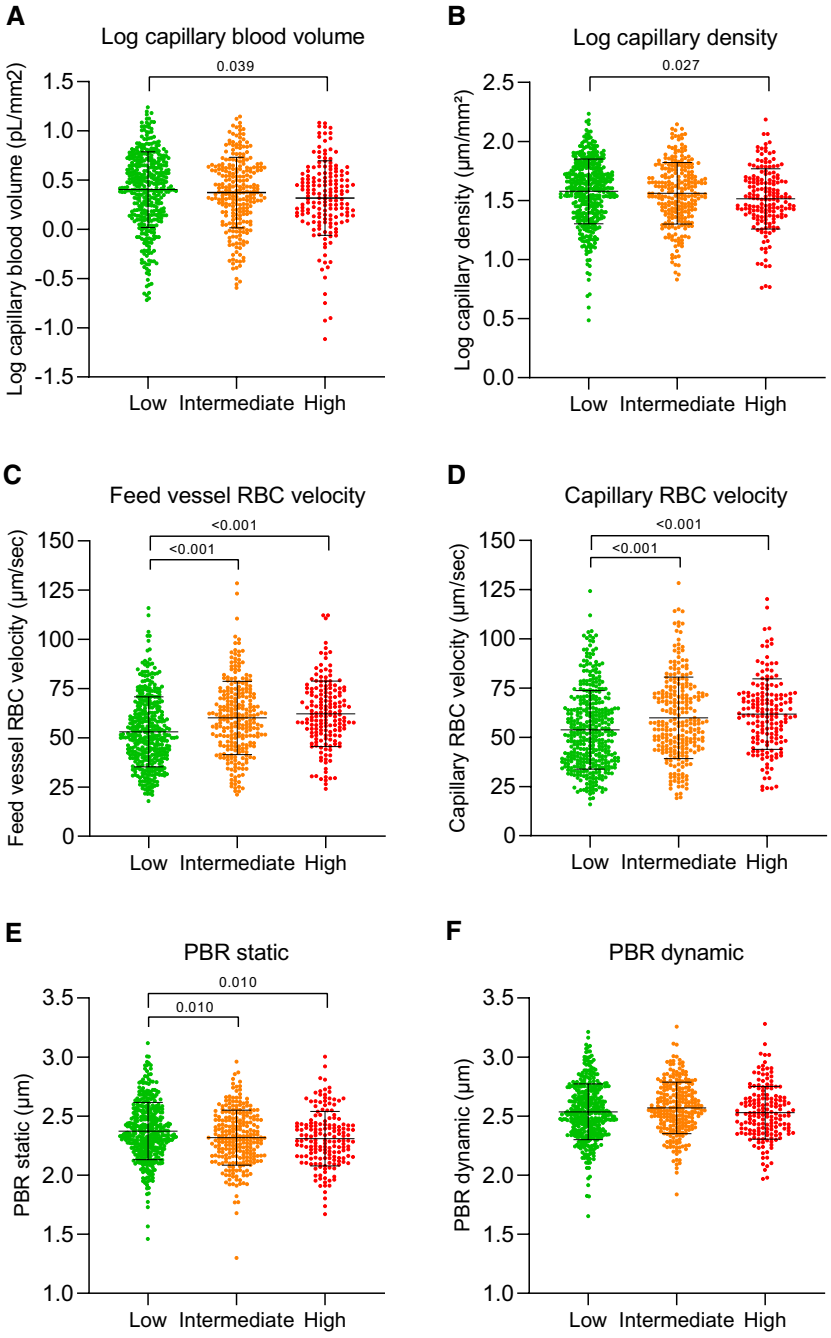

FIGURE 1 Sidestream dark field imaging–derived parameters of the study population stratified by Framingham Risk Score group. Log-transformed (A) capillary blood volume (B) and capillary density, (C) feed vessel red blood cell (RBC) velocity, (D) capillary RBC velocity, (E) static perfused boundary region (PBR), and (F) dynamic PBR in the low, intermediate, and high Framingham Risk Score groups. Differences in microvascular parameters among the risk groups were analyzed by ANOVA [Color figure can be viewed at wileyonlinelibrary.com]

capillary blood volume and capillary density were log transformed. After log transformation, capillary blood volume was 0.085 (95% CI: 0.003-0.166; Figure 1A) lower in the high-risk group compared with the low-risk group, and the capillary density was 0.063 (95% CI: 0.006-0.121; Figure 1B) lower in the high-risk group compared with the low-risk group. This reduced number of perfused capillary density in the high-risk group was accompanied by increased RBC velocity in the feed vessels and capillaries. Compared with the low-risk group, RBC velocity in the feed vessels was higher in the intermediate-risk group (difference 7.0 μm/s [95% CI: 3.7 to 10.4], Figure 1C) and the high-risk group (difference 9.1 μm/s [95% CI: 5.2 to 12.9], Figure 1C). This higher RBC velocity was also observed within capillaries, with an increase of 6.1 μm/s (95% CI: 2.4 to 9.8) in the intermediate-risk group and an increase of 8.1 μm/s (95% CI: 3.8 to 6.6) in the high-risk group, compared with the low-risk group (Figure 1D). The PBR static was lower in the intermediate-risk group compared with the low-risk group (difference −0.06 μm [95% CI: −0.10 to −0.01], Figure 1E) and in the high-risk group compared with the low-risk group (difference of −0.06 μm [95% CI: −0.12 to −0.01], Figure 1E). However, velocity-corrected PBR (PBR dynamic), based on per-group analysis (9), did not differ across the Framingham Risk Score groups (Figure 1F).

An in-depth analysis of perfused capillary density loss is shown in Figure 2. Capillaries were categorized according to their diameter, and percentage change in capillary density in intermediate- and high-risk groups compared with the low-risk group was calculated. The number of capillaries with a diameter of 4 μm was 49% lower in the high-risk group and 29% lower in the intermediate-risk group. Similarly, densities of 5-μm capillaries were 23% and 10% lower in the high- and the intermediate-risk groups, respectively.

DISCUSSION

In the current study, we observed moderate microvascular differences detected with SDF imaging in individuals with obesity at risk of developing CVD. The number of the smallest functional perfused capillaries (4-6 μm) in individuals with a high risk for developing CVD was reduced, coinciding with moderately lower perfused capillary density and capillary blood volume in the intermediate- and high-risk groups. RBC velocity at intermediate and high risk was higher in both feed vessels (>10 μm) and capillaries, possibly because of higher metabolic demand in tissues (10). The loss of functional capillaries in hypertensive or diabetic patients has been a consistent observation over the years (11-13). However, in our current study, we cannot distinguish between capillary rarefaction or reduced nitric oxide production owing to endothelial dysfunction that could lead to impaired vasodilatation and perfusion.

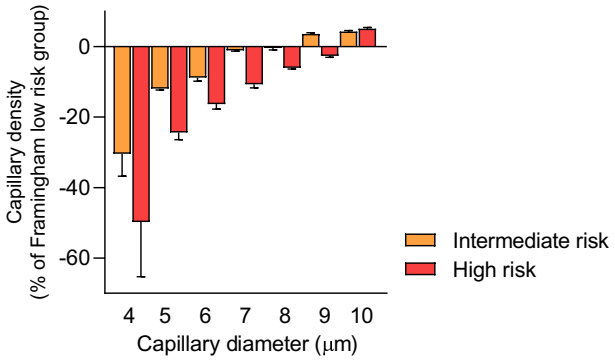

FIGURE 2 Percentage difference in the number of perfused capillaries (capillary density) per capillary diameter group in the intermediate- and high-risk Framingham groups compared with the low-risk group (reference) [Color figure can be viewed at wileyonlinelibrary.com]

Although the estimated PBR (4-25 μm) seemed to differ among the risk groups, the difference was abolished when PBR was corrected for RBC velocity. In previous studies, PBR was shown to discriminate between specific patient groups and controls (14,15). Interestingly, there is an inconsistency in the range of the measured PBR values in healthy individuals across these studies and our current study. This intravariability across various studies possibly reflects the interindividual variability due to the different flow stages within one person at the time of the SDF measurement, especially in healthy persons. By correcting the PBR for these velocity changes, the new PBR (dynamic) will represent a better estimate of changes in the endothelial glycocalyx layer, as also observed between sepsis patients and healthy controls (9).

A limitation of the current study is that only one SDF measurement per individual was performed. To capture different flow states of the feed vessels and capillaries, new recording and analysis strategies have to be developed to calculate microvascular changes on a per-patient basis. Another limitation is the cross-sectional design of the study. In the current study, minor differences between the cardiovascular risk groups could be detected, with a considerable overlap between the groups. It would be interesting to investigate whether high-risk individuals with microvascular changes develop CVD in the future. A strength of the current study is the large number of participants in the cohort.

In conclusion, we observed that, with adding RBC velocity to the software tool, SDF imaging was able to detect differences within the microvasculature in a cohort of individuals with obesity stratified by CVD risk profile.O

CONFLICT OF INTEREST

HV is Chief Science Officer at GlycoCheck BV (Maastricht, The Netherlands). The other authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

JvdV and TJR generated funding. AIMvdV, RdM, and BMvdB were involved in the design of the study. AIMvdV and HV processed data. AIMvdV analyzed the data. All authors were involved in data interpretation and writing of the paper and had final approval of the submitted and published versions.

CLINICAL TRIAL REGISTRATION

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