Universiteit
Leiden
The Netherlands

The glycocalyx: a diagnostic and therapeutic target in cardiometabolic diseases

Velden, A.I.M. van der

Citation
Velden, A. I. M. van der. (2024, September 3). *The glycocalyx: a diagnostic and therapeutic target in cardiometabolic diseases*. Retrieved from https://hdl.handle.net/1887/4039604

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4039604

Note: To cite this publication please use the final published version (if applicable).

CHAPTER 2

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

Anouk I.M. van der Velden
Bernard M. van den Berg
Renée de Mutsert
Johan van der Vlag
J. Wouter Jukema
Frits R. Rosendaal
Ton J. Rabelink
Hans Vink

Obesity (Silver Spring)
2021, 9:1439-1444

Abstract

Objective

To investigate microvascular differences in individuals with obesity at risk for developing cardiovascular diseases.

Methods

In this cross-sectional Netherlands Epidemiology of Obesity study, the sublingual microcirculation was assessed with a new developed GlycoCheck™ software, which integrates red blood cell (RBC) 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. Analysis of Variance was used to evaluate microvascular differences between the groups.

Results

A total of 813 participants were included. The high-risk group (n=168) was characterized by differences in the microvasculature compared to the low-risk group (n=392), with a 49% reduction in the number of smallest capillaries and a 9.1 μm/sec (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

We observed that with adding RBC velocity to the software, SDF imaging is able to detect microcirculatory differences in a cohort of individuals with obesity at risk for developing cardiovascular diseases.

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 resulted 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 sub-population of the Netherlands Epidemiology of Obesity (NEO) study [7], we present a newly developed software application which facilitates automatic analysis of red blood cell velocity, allowing to include flow changes between feed vessels and capillaries to be coupled to the perfused boundary region and perfused capillary density measurements. In the current study we re-analyzed our previous SDF measurements and divided the cohort into cardiovascular risk groups according to their Framingham Risk Score (FRS). 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.

As 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.

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 6671 individuals aged 45–65 years, with an oversampling of overweight individuals with a body mass index (BMI) of 27 kg/m^2 or higher. Detailed information about the NEO study design and data collection are 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.

In the present study, 918 participants in which SDF-imaging was performed between January and October 2012 as part of the baseline visit at the LUMC NEO study center were included.

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 a SDF camera (MicroVision Medical Inc., Wallingford, PA) and acquired using Glycocheck™ software (Microvascular Health Solutions Inc., Salt Lake City, UT, USA) as described elsewhere [5].

The new software includes red blood cell velocity as a new parameter. After re-analyzing the following parameters were obtained: perfused capillary density; capillary blood volume; RBC velocity; static and dynamic perfused boundary region. Detailed information about the new software used in the NEO study is described elsewhere [5, 9]

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 (<10%), intermediate (10% to 20%), or high (>20%) risk group. Data is presented as mean (standard deviation, SD), median (25^{th}-75^{th} percentile), or as percentage. Differences in microvascular parameters between the risk groups were analyzed by Analysis of Variance (ANOVA). Capillary blood volume and perfused capillary density were transformed into the natural logarithm. The percentage change of capillary density compared to the low risk group (reference) was calculated within the capillary diameters class. The above-mentioned analyses were performed with STATA Statistical Software (StataCorp, College Station, TX, version 14.1).

Results

Differences in microvascular parameters between Framingham risk groups

For stratifying the participants (n=918) by the Framingham risk score, participants with pre-existing cardiovascular disease (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 groups and in the total cohort are shown in table 1 and figure 1.

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

Table 1. Characteristics and SDF derived parameters of the study population stratified by Framingham risk group and total cohort.

	Low risk (N=392)	Intermediate risk (N=253)	High risk (N=168)	Total cohort (N=813)
Demographics				
Age (years)	54 (6)	57 (6)	60 (5)	56 (6)
Women (%)	81	37	12	53
Post-menopausal in women (% yes)	52	77	95	60
Ethnicity (% Caucasian)	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-to-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)
Hba1c (%)	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/1.73m^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/mm²)	40 (25-59)	36 (25-57)	33 (24-47)	37 (25-55)
RBC velocity feed vessels (µm/sec)	53 (18)	60 (19)	62 (17)	57 (18)
RBC velocity capillaries (µm/sec)	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)

Abbreviations: *BMI* body mass index, *M* men, *W* women, *HDL* High-density-lipoprotein, *HsCRP* high sensitivity c-reactive protein, *PBR* perfused boundary region, *RBC* red blood cell

Data are presented as mean (SD), median (25th -75th percentile) or percentage.

Framingham risk groups: low risk: <10%, intermediate risk: 10-20%, high risk: >20%

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

Missing: 12 Hba1c, 3 eGFR CKD-EPI, 3 Albumin/creatinine ratio

Abbreviations: *PBR* perfused boundary region, *RBC* red blood cell

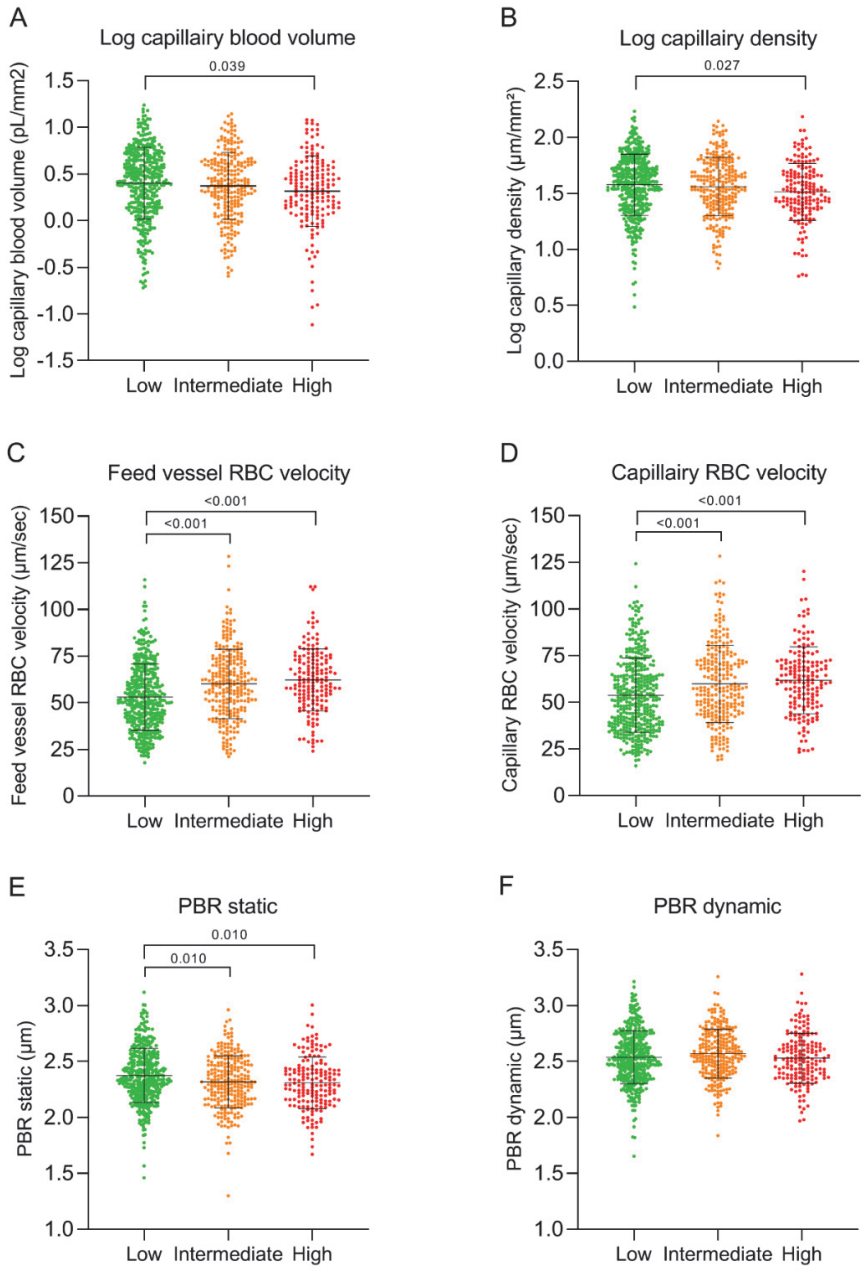

Figure 1. Sidestream dark field imaging derived parameters of the study population stratified by Framingham risk group. Log transformed capillary blood volume (A), capillary density (B), feed vessels red blood cell velocity (C), capillary red blood cell velocity (D), static perfused boundary region (PBR) (E) and dynamic perfused boundary region (F) in the low-, intermediate and high Framingham risk groups. Differences in microvascular parameters between the risk groups were analyzed by analysis of variance.

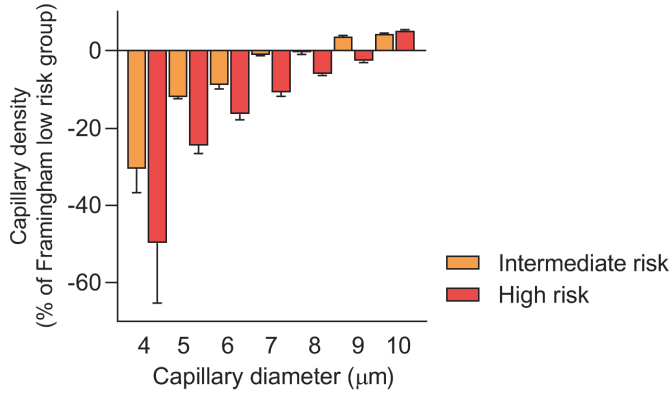

Figure 2. Capillary density of capillaries with different diameter in the intermediate and high-risk group compared to the low-risk group. Percentage difference in the number of perfused capillaries (capillary density) per capillary diameter group in the intermediate- and high Framingham risk groups compared to the low-risk group (reference).

Discussion

In the current study, we observed moderate microvascular differences detected with SDF-imaging in individuals with obesity at risk of developing cardiovascular disease. 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 group. RBC velocity at intermediate- and high-risk was higher in both feed vessels (>10 μm) and capillaries, possibly due to higher metabolic demand in tissues [10]. The loss of functional capillaries has been a consistent observation over the years in hypertensive or diabetic patients [11-13]. However, in our current study, we cannot distinguish between capillary rarefaction or reduced NO production due to endothelial dysfunction that could lead to impaired vasodilatation and perfusion.

While the estimated PBR (4-25 μm) seem to differ between 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 intra-variability across various studies possibly reflects the inter-individual 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 newly 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 cardiovascular disease in the future. A strength of the current study is the large number of participants in the cohort.

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

References

1. Perticone, F., et al., *Prognostic significance of endothelial dysfunction in hypertensive patients.* Circulation, 2001. **104**(2): p. 191-6.
2. Halcox, J.P., et al., *Prognostic value of coronary vascular endothelial dysfunction.* Circulation, 2002. **106**(6): p. 653-8.
3. van den Berg, B.M., et al., *Glomerular Function and Structural Integrity Depend on Hyaluronan Synthesis by Glomerular Endothelium.* J Am Soc Nephrol, 2019. **30**(10): p. 1886-1897.
4. Wang, G., et al., *Endothelial Glycocalyx Hyaluronan: Regulation and Role in Prevention of Diabetic Complications.* Am J Pathol, 2020. **190**(4): p. 781-790.
5. Lee, D.H., et al., *Deeper penetration of erythrocytes into the endothelial glycocalyx is associated with impaired microvascular perfusion.* PLoS One, 2014. **9**(5): p. e96477.
6. Eriksson, S., J. Nilsson, and C. Sturesson, *Non-invasive imaging of microcirculation: a technology review.* Med Devices (Auckl), 2014. **7**: p. 445-52.
7. de Mutsert, R., et al., *The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection.* Eur J Epidemiol, 2013. **28**(6): p. 513-23.
8. D'Agostino, R.B., Sr., et al., *General cardiovascular risk profile for use in primary care: the Framingham Heart Study.* Circulation, 2008. **117**(6): p. 743-53.
9. Rovas, A., et al., *Identification of novel sublingual parameters to analyze and diagnose microvascular dysfunction in sepsis: the NOSTRADAMUS study.* Crit Care, 2021. **25**(1): p. 112.
10. Segal, S.S., *Regulation of blood flow in the microcirculation.* Microcirculation, 2005. **12**(1): p. 33-45.
11. de Jongh, R.T., et al., *Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance.* Circulation, 2004. **109**(21): p. 2529-35.
12. Debbabi, H., et al., *Increased skin capillary density in treated essential hypertensive patients.* Am J Hypertens, 2006. **19**(5): p. 477-83.
13. Marin, P., et al., *Muscle fiber composition and capillary density in women and men with NIDDM.* Diabetes Care, 1994. **17**(5): p. 382-6.
14. Dane, M.J., et al., *Association of kidney function with changes in the endothelial surface layer.* Clin J Am Soc Nephrol, 2014. **9**(4): p. 698-704.
15. Ikonomidis, I., et al., *Association of impaired endothelial glycocalyx with arterial stiffness, coronary microcirculatory dysfunction, and abnormal myocardial deformation in untreated hypertensives.* J Clin Hypertens (Greenwich), 2018. **20**(4): p. 672-679.