Sex-specific association between microvascular health and coagulation parameters: the Netherlands epidemiology of obesity study

Yuan, L.S.; Han, J.H.; Velden, A.I.M.V.; Vink, H.; Mutsert, R. de; Rosendaal, F.R.; ... ; Berg, B.M. van den

Received: 22 November 2022 | Revised: 16 May 2023 | Accepted: 1 June 2023

https://doi.org/10.1016/j.jtha.2023.06.001

ORIGINAL ARTICLE

Sex-specific association between microvascular health and coagulation parameters: the Netherlands Epidemiology of Obesity study

Lushun Yuan[1] | Jihee Han[2] | Anouk I. M. van der Velden[1] | Hans Vink[3,4] | Renée de Mutsert[2] | Frits R. Rosendaal[2] | Astrid van Hylckama Vlieg[2] | Ruifang Li-Gao[2,5] | Ton J. Rabelink[1] | Bernard M. van den Berg[1]

[1]Einthoven Laboratory for Vascular and Regenerative Medicine, Department of Internal Medicine, Nephrology, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

[3]Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht, the Netherlands

[4]MicroVascular Health Solutions LLC, Alpine, Utah, USA

[5]Metabolon Inc, Morrisville, North Carolina, USA

Correspondence
Bernard M. van den Berg, Department of Internal Medicine C7-Q-36, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.
Email: b.m.van_den_berg@lumc.nl

Funding information
The Netherlands Epidemiology of Obesity study was supported by the participating departments; Division and the Board of Directors of the Leiden University Medical Centre; and Leiden University, Research Profile Area "Vascular and Regenerative Medicine." Coagulation factor analyses were funded by "Stichting De Merel." Stichting De Merel had no role in the study design; data collection and analysis; decision to publish; or preparation, review, or approval of the manuscript. This work was supported by the

Abstract

Background: Microvascular dysfunction is a growing determinant of sex differences in coronary heart disease (CHD). Dysregulation of the coagulation system is involved in CHD pathogenesis and can be induced by endothelial glycocalyx (EG) perturbation. However, little is known about the link between EG function and coagulation parameters in population-based studies on sex specificity.

Objectives: We sought to examine the sex differences in the relationship between EG function and coagulation parameters in a middle-aged Dutch population.

Methods: Using baseline measurements of 771 participants from the Netherlands Epidemiology of Obesity study (age, 56 years [IQR, 51-61 years]; 53% women; body mass index, 27.9 kg/m^2 [IQR, 25.1-30.9 kg/m^2]), associations between glycocalyx-related perfused boundary region (PBR) derived using sidestream dark-field imaging and coagulation parameters (factor [F]VIII/IX/XI; thrombin generation parameters; and fibrinogen) were investigated using linear regression analyses, adjusting for possible confounders (including C-reactive protein, leptin, and glycoprotein acetyls), followed by sex-stratified analyses.

Results: There was a sex difference in the associations between PBR and coagulation parameters. Particularly in women, 1-SD PBR (both total and feed vessel, indicating poorer glycocalyx status) was associated with higher FIX activity ([1.8%; 95% CI, 0.3%-3.3%] and [2.0%; 95% CI, 0.5%-3.4%], respectively) and plasma fibrinogen levels ([5.1 mg/dL; 95% CI, 0.4-9.9 mg/dL] and [5.8 mg/dL; 95% CI, 1.1-10.6 mg/dL], respectively). Furthermore, 1-SD PBR$_{capillary}$ was associated with higher FVIII activity (3.5%; 95% CI, 0.4%-6.5%) and plasma fibrinogen levels (5.3 mg/dL; 95% CI, 0.6-10.0 mg/dL).

Conclusion: We revealed a sex-specific association between microcirculatory health and procoagulant status, which suggests that microvascular health be considered during early development of CHD in women.

Manuscript handled by: Karen Vanhoorelbeke

Final decision: Karen Vanhoorelbeke, 01 June 2023

China Scholarship Council grant (201806270262 to L.Y.).

KEYWORDS
coagulation factors, endothelial glycocalyx (EG), fibrinogen, perfused boundary region (PBR), thrombin generation

1 | INTRODUCTION

In previous decades, men were thought to be more susceptible to coronary heart disease (CHD) than women [1]. However, the risk of CHD in women is frequently underestimated due to underrecognition of CHD and distinct clinical presentations, which results in a poor prognosis [2]. Nowadays, systemic nonobstructive microvascular dysfunction, which causes problems in small blood vessels feeding muscular tissue, which can result in CHD, is believed to be more common in women than in men. Therefore, this type of dysfunction is becoming a growing determinant of sex differences in patients with CHD along with normal or near-normal coronary angiographic assessment [3,4].

Previously, we examined endothelial surface perturbation using the noninvasive sidestream dark-field (SDF) imaging technique in a subpopulation of the Netherlands Epidemiology of Obesity (NEO) study [5–7]. Although SDF imaging provides high-contrast images of the sublingual microvasculature, allowing for determination of lateral red blood cell (RBC) movement within the vessels of interest, with newly developed software, automatic analysis of RBC velocity allows the inclusion of flow changes between feed vessels (10-25 μm) and capillaries (<10 μm) to be coupled to the perfused boundary region (PBR), vessel density, and capillary blood volume [8,9]. In various studies, it has already been shown that the detected changes in PBR, inversely related to EG thickness, are correlated with glycocalyx degradation products, including circulating syndecan-1, heparan sulfate (HS), hyaluronan, and soluble thrombomodulin, and endothelial activation markers such as E-selection, soluble angiopoietin-2, and soluble Tie-2 [10–13]. It was also found to be negatively correlated with coronary flow reserve, an assessment for evaluation of coronary microvascular dysfunction. Besides, according to the miCRovascular rarefaction in vascUlar Cognitive Impairment and heArt faiLure study protocol [14], the SDF imaging technique will be used to quantify the microvascular health of cerebral and cardiac microvasculature, suggesting that this technique is a valid surrogate for cardiac microvasculature health examination [15].

The endothelial glycocalyx (EG) is a negatively charged, gel-like surface matrix of proteoglycans and covalently bound glycosaminoglycans, glycoproteins, and glycolipids [16] and exerts anti-inflammatory and antithrombotic roles by covering various glycoprotein adhesion receptors for leukocytes [17] and platelets [18] or aiding in sequestration of antiadhesion factors [19]. EG perturbation acts as a representative of impaired microvascular function [6,9,20,21]. In particular, several EG-bound proteins, including specific sulfation patterns of HS proteoglycans, or HS-binding proteins, such as antithrombin III and tissue factor pathway inhibitor, could prevent the coagulation cascade *in vitro* and *in vivo* [22–24]. In disease cases such as nephrotic syndrome, sepsis, brain injury, and severe COVID-19, derangement of EG induced by microvascular dysfunction could accelerate hypercoagulation [8,25–28].

Essentials

- Little is known about the link between microvascular health and coagulation parameters in population-based studies on sex specificity.
- We studied sex differences in relation between endothelial glycocalyx function and coagulation parameters in a middle-aged Dutch population.
- Particularly in women, associations between endothelial glycocalyx health and coagulation parameters were observed.
- Microcirculatory changes, together with coagulation factors, provide possible cues to prevent the development of coronary heart disease in women.

Specifically, women were found to have already higher fibrinogen plasma levels than men of the same age and ethnic group [29]. Increased fibrinogen levels could push the balance from fibrinolysis to clotting [30] and have been reported to be a potential risk factor for CHD [31–34]. Furthermore, other coagulation factors were also associated with an increased risk of CHD [33,35]. However, little is known about the link between EG function and coagulation parameters in population-based studies and about whether a sex-specific determinant is present. Based on *in vitro* and *in vivo* evidence, we hypothesized that poorer EG function could lead to a procoagulant state in a population-based study, which might demonstrate sex specificity and be involved in the risk of CHD. The aim of our study was, therefore, to investigate the association between microvascular health, assessed based on new SDF imaging parameters, and levels of procoagulant factor activity (factor [F]VIII, FIX, and FXI), fibrinogen concentration, and thrombin generation parameters in the general Dutch population and further perform analyses stratified by sex, revealing sex differences in vascular vulnerability and its potential roles in the risk of CHD.

2 | METHODS

2.1 | Study population and study design

This study was conducted in a population-based prospective cohort, the NEO study [7]. All 6671 participants gave written informed consent, and the Medical Ethical Committee of the Leiden University Medical Center approved the study design. Initiated in 2008, the NEO

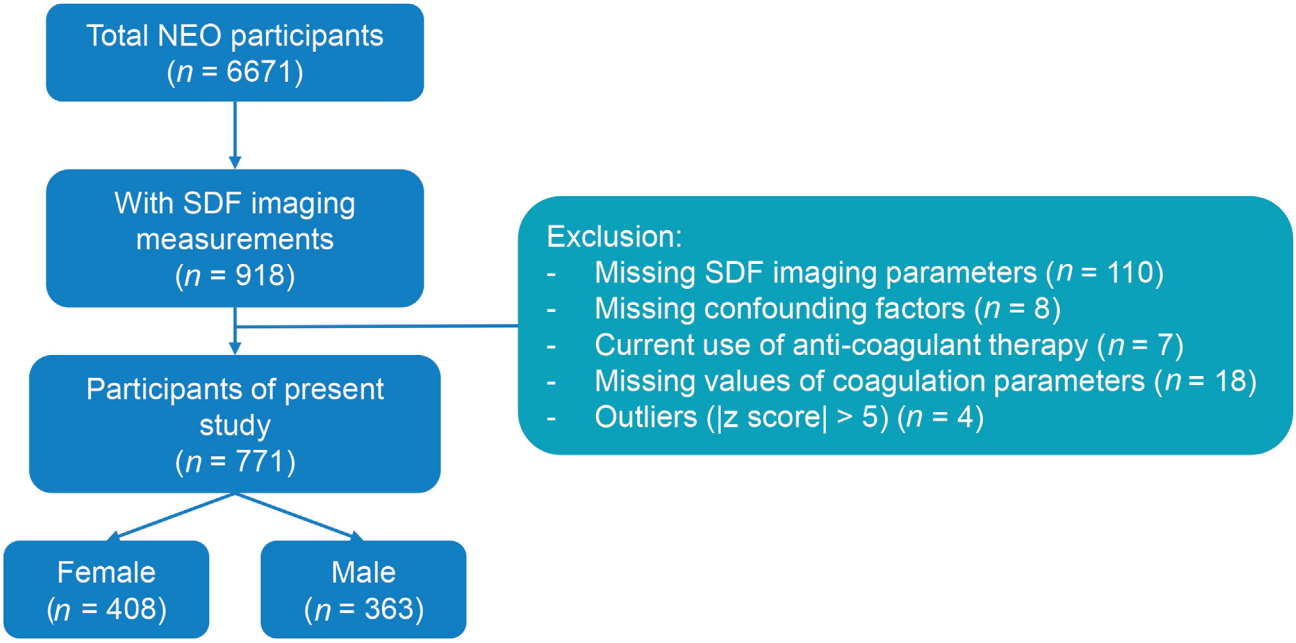

FIGURE 1 Study flow chart. NEO, Netherlands Epidemiology of Obesity; SDF, sidestream dark field.

study was designed to study pathways that lead to obesity-related diseases. Detailed information about the study design and data collection has been described elsewhere [7]. Briefly, men and women aged between 45 and 65 years with a self-reported body mass index (BMI) of ≥27 kg/m^2 living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from 1 municipality (Leiderdorp) were invited irrespective of their BMI, representing the BMI distribution of the Dutch general population. Prior to their visits, the participants completed questionnaires on demographic, lifestyle, and clinical information at home. At the baseline visit, fasting blood samples were drawn from the antecubital vein after the NEO participants had rested for 5 minutes. The present study is a cross-sectional analysis of a subpopulation of 918 NEO 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. Individuals were excluded from the analyses (Figure 1) if their SDF imaging parameters were missing (n = 110), their values of confounding factors were missing (n = 8), they were currently receiving anticoagulant therapy (using vitamin K antagonists or heparin; n = 7), their values of coagulation parameters were missing (n = 18), and they had outliers values (z score > 5) in outcomes (ie, coagulation factors and thrombin generation parameters; n = 4). Seven hundred seventy-one participants were included for all analyses using coagulation parameters as outcomes.

2.2 | SDF microcirculation imaging

Intravital microscopy was performed earlier on individuals in a supine position using an SDF camera (MicroVision Medical Inc) and images acquired using GlycoCheck software (Microvascular Health Solutions Inc) as described elsewhere [6,9]. The software automatically identifies all available measurable microvessels distributed at a 1-μm interval between 4 and 25 μm, and RBC velocity was included as a new parameter using the new software. After reanalyzing, the following validated parameters [8] were included in this study, which were divided into 2 categories: glycocalyx-related parameters—ie, total PBR (PBR_{Total}, 4-25 μm), PBR feed vessel ($PBR_{feed\ vessel}$, 10-19 μm), and PBR capillary ($PBR_{capillary}$, 4-9 μm); and microcirculatory perfusion–related parameters, ie, feed vessel RBC velocity ($RBCV_{feed\ vessel}$), capillary RBC velocity ($RBCV_{capillary}$), total valid vessel density (with measurable RBC velocity; D_{Total}), perfused feed vessel density ($D_{feed\ vessel}$), perfused capillary density ($D_{capillary}$), and capillary blood volume (CBV_{static}). Detailed information on the new software used in the NEO study has been described previously [8]. PBR_{Total}, $PBR_{feed\ vessel}$, and $PBR_{capillary}$ were expressed in micrometers. The levels of $RBCV_{feed\ vessel}$ and $RBCV_{capillary}$ were expressed in micrometer per second. The levels of D_{Total}, $D_{feed\ vessel}$, and $D_{capillary}$ were expressed in micrometer per squared millimeter. CBV_{static} was expressed in picoliter per squared millimeter.

2.3 | Coagulation factor activity

Blood samples for the measurement of coagulation factors' activity were collected, processed within 4 hours, and drawn into tubes containing 0.106M trisodium citrate (Sarstedt) without the measurement of residual platelets, as discussed earlier [7]. Fasting fibrinogen levels were measured according to the method of Clauss, as described earlier [36]. Fasting FVIII, FIX, and FXI activity was measured using a coagulometric clot detection method on an ACL TOP 700 analyzer (Werfen), and Werfen plasma was used as reference plasma to

calibrate factor measurements (Werfen). The levels of FVIII, FIX, and FXI activity were expressed in percentage. The levels of fibrinogen were expressed in milligram per deciliter.

2.4 | Thrombin generation

All blood samples were collected and processed within 4 hours. Tubes were centrifuged for 10 minutes at 2500 *g* at 18 °C, and plasma separated into aliquots was stored at −80 °C until further use, only after first-time thawing for 3 minutes at 37 °C (water bath), as discussed earlier [7]. Thrombin generation was measured using protocols described previously by Hemker et al. [37]: calibrated automated thrombogram (Thrombinoscope BV). Briefly, 20 μL of PPP-Reagent LOW (86194, TS31.00; Stago) and thrombin calibrator (86192, TS30.00; Stago) were dispensed into the wells of a round-bottom, 96-well plate (#3655; Thermo Scientific). A thermostable inhibitor of contact activation (PS-0177-oxoxox; Synapse Research Institute) was added to plasma samples from the participants in the NEO study and to normal pooled plasma (as an internal control for each plate). Then, 80 μL of mixed plasma was added to the plate, and the plate was placed in a fluorometer for incubation at 37 °C for 10 minutes. Thrombin formation was initiated by adding 20 μL of a fluorogenic substrate with calcium (FluCa-kit, 86197, TS 50.00; Stago). The final reaction volume was 120 μL. Thrombin formation was determined every 10 seconds for 50 minutes and corrected for the calibrator using the Thrombinoscope software. The thrombin generation parameters included lag time, time to peak (ttPeak), peak thrombin generation (Peak), endogenous thrombin potential (ETP), and thrombin generation velocity (VelIndex). The levels of lag time and ttPeak were expressed in minutes, the levels of Peak height were expressed in nanomolar, the levels of ETP were expressed in nanomolar × minutes, and the levels of VelIndex were expressed in nanomolar per minute.

2.5 | Serum inflammatory markers

The serum concentrations of C-reactive protein (CRP) were determined using a high-sensitivity CRP assay (TINA-Quant CRP HS system and Modular P800; Roche) [36]. Sserum leptin concentrations were measured using a human leptin competitive radioimmunoassay (HL-81HK; Merck Millipore). Leptin concentrations were measured using a gamma counter, as described elsewhere [38]. Glycoprotein acetyl (GlycA) concentrations were measured in plasma that had undergone 1 previous freeze-thaw cycle using high-throughput proton nuclear magnetic resonance spectroscopy (Nightingale Health Ltd) [39].

2.6 | Statistical analyses

Descriptive baseline characteristics of the study population were expressed as median with IQR for nonnormally distributed variables, mean with SD for normally distributed variables, or percentages for dichotomous variables.

First, the distributions of confounding factors and SDF imaging parameters were evaluated, and *z*-transformation (ie, with mean = 0 and SD = 1) was performed based on age, BMI, and SDF imaging parameters. After checking the distributions of outcome variables, thrombin generation velocity (VelIndex) showed a right-skewed distribution, and $\log_2$ transformation was performed using VelIndex. Afterward, the linear regression analysis was used to investigate the associations between SDF imaging parameters (exposure) and procoagulation factors (ie, FVIII, FIX, and FXI), fibrinogen, and thrombin generation parameters (outcomes). The analyses were adjusted for several potential confounding factors. First, crude analyses were performed (model 1). Second, we adjusted the models for the confounding factors age (unit, years; continuous variable) and sex (categories, women and men; dichotomous variable; model 2). Third, the models were adjusted for other confounding factors: BMI (unit, kg/m^2; continuous variable), current smoking status (categories, current users and noncurrent users; dichotomous variable), menopausal status (categories, premenopausal and postmenopausal; dichotomous variable), current use of an oral contraceptive pill (categories, current users and noncurrent users; dichotomous variable), and current use of hormone replacement therapy (categories, current users and noncurrent users; dichotomous variable; model 3). Fourth, we adjusted for serum CRP (unit, mg/L; continuous variable), serum leptin concentration (unit, μg/L; continuous variable), and GlycA (unit, mmol/L; continuous variable) in a separate model because these systemic inflammation markers might be potential confounders of the association between microvascular health and coagulation parameters (model 4). We calculated differences in the mean levels of fibrinogen, FVIII, FIX, FXI, and thrombin generation parameters, with 95% CIs, associated with SDF imaging parameters. Furthermore, the association between SDF imaging parameters and coagulation factor levels was examined using a sex-stratified analysis. The mean coagulation factor levels were calculated for sex-stratified SDF imaging parameters. As a sensitivity analysis, all analyses were performed separately in premenopausal and postmenopausal women.

To our knowledge, the present study is the first to investigate the association between the microvascular health index derived from SDF imaging and levels of coagulation factors as well as the parameters of thrombin generation at the population level; we had no prior information on effect sizes to perform a power calculation. Noteworthy, most previous studies with relatively small sample sizes have identified associations between the microvascular health index and disease outcomes, eg, COVID-19 in 38 participants [13], coronary artery disease in 115 participants [40], and sepsis in 51 participants [8]. To address the power that we could achieve using 771 individuals, we used the following setting: significant level = .05 and power = 80%, with the linear regression $PBR_{Total} \sim$ fibrinogen as an example; we may need 394, 54, and 24 samples for a small, medium, and large effect size, respectively [41]. Therefore, a sample size of 771 individuals may have the statistical power to capture even small effect sizes.

3 | RESULTS

3.1 | Participant characteristics

The Table shows the characteristics of individuals included in the present study. Of the 771 included participants, 53% were women, with a median age of 56 years (IQR, 51-61 years), and 12.5% were current smokers. The participants had a median BMI of 27.9 kg/m^2 (IQR, 25.1-30.9 kg/m^2). Among these women, 84% had a postmenopausal status, 7.6% used oral contraceptives, and 3.4 % used hormone replacement therapy. Apart from this, we observed a higher level of inflammatory markers and procoagulant status. Although the capillary glycocalyx is very important for proper endothelial function, PBR dimensions are measured in vessels with diameters ranging from 4 to 25 μm. Therefore, the contribution of the capillary glycocalyx to PBR_{Total} is very limited. PBR_{total} is comparable with $PBR_{feed\ vessel}$, and both are the main parameters presenting as possible disturbances of the endothelial surface layer, which, in women, are both significantly increased (median [women vs men]: PBR_{total}, 2.35 vs 2.3, respectively; $PBR_{feed\ vessel}$, 2.31 vs 2.24, respectively). The density of perfused capillaries is higher in women than in men, as measured by both a higher capillary density ($D_{capillary}$) and higher capillary blood volume (CBV_{static}), which is associated with a lower capillary RBC velocity ($RBCV_{capillary}$), suggesting a more advantageous capillary presence in women.

3.2 | Association between microvascular health and levels of coagulation parameters in the total population

Linear regression analyses were used to investigate the association between 1-SD difference in microvascular parameters and the coagulation factors. In model 3 of the total population, both glycocalyx-related parameters, PBR_{Total} and $PBR_{feed\ vessel}$, were associated with higher fibrinogen levels (Supplementary Table S1). Nevertheless, with additional adjustment for systemic inflammatory markers (model 4), only the association of $PBR_{feed\ vessel}$ persisted (Supplementary Table S1, Supplementary Figure S1), and we observed that 1 SD (ie, 0.3 μm) in $PBR_{feed\ vessel}$ was associated with a higher fibrinogen concentration of 3.2 mg/dL (95% CI, 0.02-6.4 mg/dL). Interestingly, the microcirculatory perfusion parameters, in particular all 3 vessel density parameters, were positively associated with ETP in all models, with very similar effect size estimations between model 3 and model 4 (Supplementary Table S2, Supplementary Figure S2). In model 4 of the total population, 1 SD in vessel density (the D_{Total}, $D_{feed\ vessel}$, and $D_{capillary}$ were 65.84, 42.17, and 41.39 μm/mm^2, respectively) corresponded to higher ETP levels ([31.9 nM × min; 95% CI, 9.6-54.2 nM × min]; [23.7 nM × min; 95% CI, 1.2-46.2 nM × min]; and [25.2 nM × min; 95% CI, 2.9-47.5 nM × min, respectively]).

3.3 | Effect modification by sex of the association between microvascular health and coagulation factors

After sex stratification, we observed differences between men and women between the glycocalyx-related parameters and coagulation factor levels (Figures 2 and 3). Consistent associations of PBR_{Total} and $PBR_{feed\ vessel}$ with FIX activity and plasma fibrinogen concentration were found specifically in women, which persisted after adjusting for inflammatory markers: 1 SD in PBR_{Total} or $PBR_{feed\ vessel}$ (0.25 μm and 0.3 μm, respectively) was associated with higher fibrinogen levels (5.1 mg/dL [95% CI, 0.4-9.9 mg/dL] and 5.8 mg/dL [95% CI, 1.1-10.6 mg/dL], respectively) and higher FIX activity (1.8% [95% CI, 0.3%-3.3%] and 2.0% [95% CI, 0.5%-3.4%], respectively) (Figure 2, Supplementary Table S3), whereas no associations were observed in men (Figure 3, Supplementary Table S4). For $PBR_{capillary}$, in addition to the association with fibrinogen concentration, $PBR_{capillary}$ was associated with FVIII activity, which persisted after further adjustment for systemic inflammatory markers (model 4): 1 SD in $PBR_{capillary}$ (0.11 μm) was associated with higher fibrinogen levels (5.3 mg/dL; 95% CI, 0.6-10.0 mg/dL) and FVIII activity (3.5%; 95% CI, 0.4%-6.5%) (Figure 2, Supplementary Table S3).

There were differences in the associations between microcirculatory perfusion–related parameters and coagulation factor levels stratified by sex. For women, associations between vessel density and ETP were observed in the crude as well as age- and sex-adjusted models (Supplementary Figure S3, Supplementary Table S5); however, only $D_{capillary}$ was positively associated with ETP after further adjusting for demographic and lifestyle factors (model 3) as well as systemic inflammatory markers (model 4), wherein 1 SD of $D_{capillary}$ (42.26 μm/mm^2) was associated with higher levels of ETP (32.0 nM × minutes; 95% CI, 0.1-63.8 nM × minutes). In men, only 1 consistent negative association was observed between $RBCV_{feed\ vessel}$ and the thrombin generation parameter ttPeak (Supplementary Figure S4, Supplementary Table S6), wherein 1 SD of $RBCV_{feed\ vessel}$ (61.52 μm/s) was associated with lower levels of ttPeak (−0.2; 95% CI, −0.4% to −0.02%). In addition, 1 SD of D_{Total} (68.08 μm/mm^2) was associated with higher ETP levels (31.9 nM × minutes; 95% CI, 1.3-62.6 nM × minutes).

Sensitivity analyses were performed to test robustness. The use of premenopausal women yielded even more notable results than the main analysis for total women that PBR difference was associated with higher fibrinogen concentrations, higher FXI and FVIII activity, and higher thrombin generation parameters (ETP, Peak, and VelIndex) (Supplementary Figure S5, Supplementary Table S7, Supplementary Figure S7, Supplementary Table S9). However, the results in postmenopausal women were less significant but comparable with those of the main analysis for all women (Supplementary Figure S6, Supplementary Table S8, Supplementary Figure S8, Supplementary Table S10).

TABLE Characteristics of the study population.

Personal characteristics	Total (100%)	Women (53%)	Men (47%)
Demographics			
Age, y	56 (51-61)	56 (50.75-61)	57 (51-61)
BMI, kg/m^2	27.93 (25.08-30.91)	27.75 (24.52-31.25)	27.99 (25.73-30.59)
Tobacco smoking (percentage of current user)	12.5	8.1	17.4
Menopause status (percentage of postmenopausal/ perimenopausal women)	NA	83.8	NA
Medication (current use)			
Oral contraceptive pill (percentage of women)	NA	7.6	NA
HRT (percentage of women)	NA	3.4	NA
Systemic inflammatory markers			
C-reactive protein, mg/L	1.37 (0.74-3.06)	1.61 (0.8-3.5)[a]	1.25 (0.64-2.26)
Leptin, µg/L	14.7 (8-27.15)	25.2 (16-37)[b]	8.4 (5.55-12.9)
Glycoprotein acetyls, mmol/L	1.23 (1.13-1.34)	1.21 (1.11-1.32)[c]	1.24 (1.14-1.35)
Coagulation parameters			
Fibrinogen, mg/dL	289.32 (257.77-330.88)	300.55 (265.66-346.02)[b]	278.98 (247.19-312.78)
Factor VIII, %	122.98 (101.39-147.01)	123.53 (102.05-148.34)	122.98 (101.39-145.04)
Factor IX, %	119.31 (108.41-134.28)	119.31 (107.27-136.11)	119.31 (110.74-131.41)
Factor XI, %	116.77 (103.75-129.16)	120.02 (107.58-134.01)[b]	111.56 (100.06-123.93)
Lag time, min	6.72 (6-7.75)	6.5 (5.75-7.58)[b]	7.08 (6.17-7.92)
ttPeak, min	14.75 (13.46-16.08)	14.36 (13.19-15.75)[b]	15.04 (13.83-16.33)
Peak, nM	83.27 (62.1-106.28)	84.77 (61.11-111.09)	81.69 (63.92-102.77)
ETP, nM/min	1149.22 (915.56-1396.66)	1175.06 (916.94-1439.8)[c]	1124.76 (914.49-1329.16)
VelIndex, nM/min	10.42 (7.47-14.63)	10.79 (7.36-15.32)	10.18 (7.76-13.8)
SDF imaging parameters			
PBR of total vessels, µm	2.33 (2.19-2.5)	2.35 (2.2-2.54)[d]	2.3 (2.17-2.44)
PBR of feed vessels, µm	2.28 (2.09-2.48)	2.31 (2.11-2.52)[d]	2.24 (2.06-2.44)
PBR of capillaries, µm	1.2 (1.13-1.27)	1.2 (1.14-1.27)	1.2 (1.13-1.26)
RBC velocity in feed vessels, µm/s	55.85 (43.93-68.91)	51.24 (39.7-64.4)[b]	60.26 (49.5-73.13)
RBC velocity in capillaries, µm/s	55.74 (41.94-70.55)	50.77 (37.78-66.86)[b]	60.45 (47.77-72.65)
Total vessel density, µm/mm^2	257.02 (209.81-309.06)	255.58 (206.18-309.23)	259.16 (213.37-306.45)
Feed vessel density, µm/mm^2	131.57 (107.1-160.52)	129.5 (103.51-158.26)[c]	134.83 (110.94-165.09)
Capillary density, µm/mm^2	100.71 (79.69-131.2)	104.25 (81.76-137.28)[c]	96.82 (74.81-121.83)
Capillary blood volume, pL/mm^2	2.52 (1.4-4.35)	2.81 (1.41-4.76)[c]	2.29 (1.38-3.92)

Data are shown as mean (± SD), median (25th percentile to 75th percentile), or percentage.

BMI, body mass index; ETP, endogenous thrombin potential; HRT, hormonal replacement therapy; NA, not applicable; PBR, perfused boundary region; peak, peak thrombin generation; RBC, red blood cell; ttPeak, time to peak.

[a] The nonpaired *t*-test or Wilcoxon test was performed between women and men; $P < .001$.

[b] The nonpaired *t*-test or Wilcoxon test was performed between women and men; $P < .0001$.

[c] The nonpaired *t*-test or Wilcoxon test was performed between women and men; $P < .05$.

[d] The nonpaired *t*-test or Wilcoxon test was performed between women and men; $P < .01$.

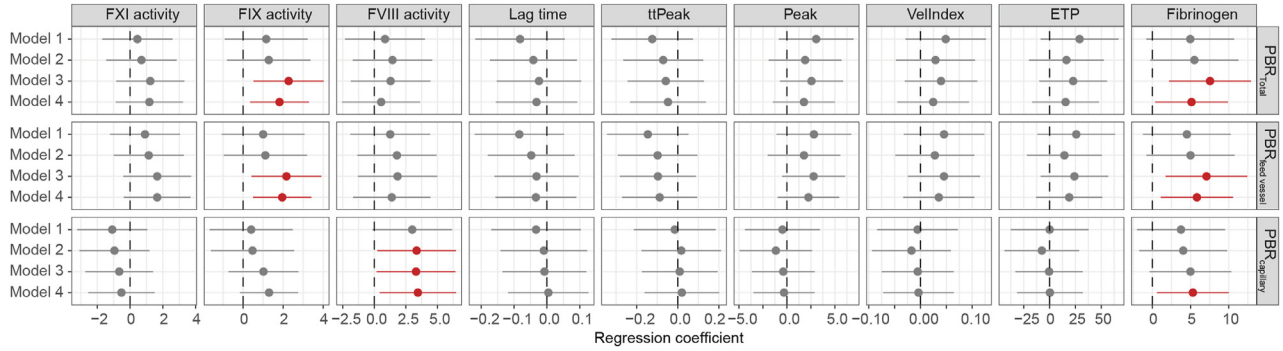

FIGURE 2 Association between glycocalyx-related sidestream dark-field imaging parameters and levels of coagulation parameters in women. After sex stratification, differences between the glycocalyx-related parameters and coagulation factor levels could be observed in women. Model 1: crude model. Model 2: model 1 + age. Model 3: model 2 + body mass index, current smoking status, menopausal status, current use of an oral contraceptive pill, and current use of hormone replacement therapy. Model 4 = model 3 + serum C-reactive protein, serum leptin concentration, and serum glycoprotein acetyl concentration. The effect size and 95% CI are depicted by a horizontal line with a dot. A nonsignificant association is represented in gray, and a substantial positive association is represented in red. Perfused boundary region $(PBR)_{Total}$: PBR of total vessels from 4 to 25 μm. $PBR_{feed\ vessel}$: PBR of feed vessels from 10 to 19 μm. $PBR_{capillary}$: PBR of capillaries from 4 to 9 μm. ETP, endogenous thrombin potential; FIX, factor IX; FVIII, factor VIII; FXI, factor XI; ttPeak, time to peak.

4 | DISCUSSION

In this population-based, cross-sectional study, the PBR in feed vessels was positively associated with plasma fibrinogen levels in the total population. Remarkably, we discovered a sex difference in the associations between PBR and coagulation parameters, in which higher PBR (both total and feed vessels, indicating perturbed glycocalyx) was associated with higher FIX activity and plasma fibrinogen levels in women. Furthermore, in women, higher $PBR_{capillary}$ (ie, poorer glycocalyx status in capillaries) was associated with higher FVIII activity and fibrinogen levels and higher $D_{capillary}$ was associated with higher levels of ETP, whereas none of these associations was present in men.

The SDF imaging technique could provide 2 types of parameters concerning EG and microcirculatory perfusion function. We proposed that changes in endothelial surface properties, ie, the EG layer (PBR), are likely a functional unit that could present as a marker of microvascular dysfunction. In the present study, we observed an association between early (preclinical) microvascular health changes and coagulation factor activation and discovered a striking sex difference in microvascular health, wherein women showed a perturbed EG concomitant with a more procoagulable endothelial surface. This association was not observed in men. These findings highlight the importance of sex differences in microcirculatory perturbation in CHD.

Previously, studies reported the association between CHD and the underlying systemic presence of a hypercoagulable state [42–44]. Our results showed that in women, 1 SD in PBR_{Total} and $PBR_{feed\ vessel}$ was associated with 5.1- and 5.8-mg/dL increases in fibrinogen concentration. Based on a large, individual-participant meta-analysis study that assessed the association between fibrinogen concentration

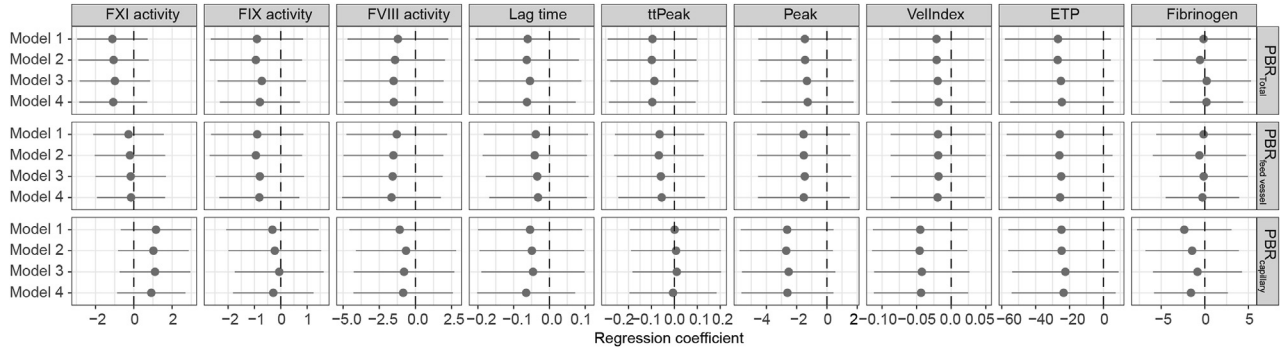

FIGURE 3 Association between glycocalyx-related sidestream dark-field imaging parameters and levels of coagulation parameters in men. After sex stratification, differences between the glycocalyx-related parameters and coagulation factor levels could be observed in men. Model 1: crude model. Model 2: model 1 + age. Model 3: model 2 + body mass index + current smoking status. Model 4: model 3 + serum C-reactive protein, serum leptin concentration, and serum glycoprotein acetyl concentration. The effect size and 95% CI are depicted by a horizontal line with a dot. A nonsignificant association is represented in gray, and a substantial positive association is represented in red. Perfused boundary region $(PBR)_{Total}$: PBR of total vessels from 4 to 25 μm; $PBR_{feed\ vessel}$: PBR of feed vessels from 10 to 19 μm; and $PBR_{capillary}$: PBR of capillaries from 4 to 9μm. ETP, endogenous thrombin potential; FIX, factor IX; FVIII, factor VIII; FXI, factor XI; ttPeak, time to peak.

and the risk of CHD [45], such increases in PBR would suggest a 12% to 14% increase in the risk of CHD in women, which could be clinically relevant. In line with our observations, Brands et al. [40] reported that reduced EG barrier properties were only found in women with CHD, whereas these were not present in men. In addition, in the REasons for Geographic and Racial Differences in Stroke Study, a large population-based observational study, higher levels of FVIII and FIX were associated with increased risk of CHD [35,46]. According to these associations, per-SD difference in PBR could correspond to a 2% to 5% increase in the risk of CHD in women. FVIII and von Willebrand factor (VWF) are 2 distinct but related glycoproteins that circulate in plasma as a tightly bound complex (FVIII/VWF) [47]. As one of the endothelial activation markers, VWF can bind to HS on sendothelial cell surface [26,48]. Based on the observed tight correlation between FVIII and VWF in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study [49], we deduced an association between EG health and VWF in the present study to further indicate the link between EG dysfunction and endothelial activation. Our current findings imply a role of microvascular health in the risk of CHD in women through the association between higher microvascular PBR (perturbed glycocalyx) and differences in both FIX and FVIII activity, together with the already implied high plasma fibrinogen levels (Figure 4) [19,22,23,26,47,50–55].

The present study also included various thrombin generation parameters that represent the global coagulation cascade in addition to coagulation factor activity. Interestingly, no associations were observed between the microvascular health parameters measured and the dynamic parameters of thrombin generation (such as lag time and ttPeak), except for an association between vessel density and ETP. Thrombin was found to be involved in both prothrombotic and inflammatory endothelial processes [56]. Although previous studies have reported an association between *in vivo* thrombin generation potential and the severity of coronary vessel disease [57], increased thrombin generation potential was a characteristic in patients with clinically stable CHD [58]. Although we found sex differences in the association between vessel density and ETP, when we compared the findings in the total population, men, and women, we found that the associations in men and women were close to significance; therefore, this observation might reflect a general feature of microvascular health and thrombin formation rather than a sex-specific feature. Furthermore, compared with previous studies using the thrombin generation assay, the participants in the present study were preclinical and did not have CHD yet, which might have limited the effect of thrombin and findings related to thrombin generation. In the context of the associations between EG (PBR) and coagulation factors, the associations between vessel densities and ETP were very marginal.

To the best of our knowledge, this is the first study to show an association between microvascular health derived from SDF imaging and the levels of coagulation factors and thrombin generation parameters in a large population-based study. In addition, adjustment for extensive potential confounding factors, including systemic inflammatory markers, strongly suggested a sex-specific role in monitoring microvascular health changes in women. Additionally, the present study used the new software, which included RBC velocity as a new parameter to better quantify the microcirculatory difference.

The limitations of the present study should also be addressed. First, it was an observational, cross-sectional study, and residual confounding may still have been present. Second, the present study population mainly comprised middle-aged European White participants (ie, aged 45-65 years). Therefore, it is not clear whether the results of the present study can be generalizable to other ethnicities or age groups. Third, the majority of the women were postmenopausal; although the sensitivity analysis revealed similar findings in premenopausal women, future studies including more premenopausal women need to be conducted. Fourth, a recent study reported the variability of microcirculatory measurements in healthy volunteers [59], showing that when 3 consecutive measurements are averaged, SDF imaging using the GlycoCheck software can be used with acceptable reliability and reproducibility for microcirculation measurements at the population level. In our current study, repeated measurement of SDF imaging was not performed for each participant, and limited information was extracted from the smallest capillaries (diameter, 4 μm). However, the measurement by itself is already an average of at least 10 recordings from 1 individual at the same time point rather than average of 3 consecutive measurements from the same individual, which could only lead to a random error and subsequent underestimation of the effect size. Fifth, due to the technical limitation of the *ex vivo* thrombin generation assay, we could not avoid batch effects, and this assay could not represent the real circumstances of the coagulation cascade in humans. Sixth, as a result of sample availability to measure the circulating endothelial glycocalyx (EG) disruption and endothelial cell (EC) activation markers, our study lacks possible mechanistic correlations between EG dysfunction, increased procoagulant state, and increased risk of CHD in women. Seventh, although we focused on the role of EG perturbation in coagulation in the present study, it is important to note that the formation of a procoagulant surface is a result of multiple mechanisms, such as, among others, gene transcription changes as well as protein expression and release. Finally, in model 4, we adjusted for systemic inflammatory markers (such as CRP, leptin, and GlycA) for the association between microvascular health and coagulation factor levels. It should be noted that these systemic inflammatory markers could act as mediators instead of confounders in the estimated associations. If so, adjustment for systemic inflammatory markers could lead to underestimation of the association between microvascular health and coagulation factor levels.

In conclusion, our data reveal a hitherto unreported sex-specific association between microcirculatory health and procoagulant status. The microcirculatory differences between men and women identified in our study implied that microvascular health changes might be the earliest detectable clue prior to the general higher procoagulant status in women ultimately developing CHD, which is also independent of increased systemic inflammatory state (Figure 4). Our study suggested the potential clinical utility of monitoring microcirculatory changes specifically in women to prevent the development of CHD.

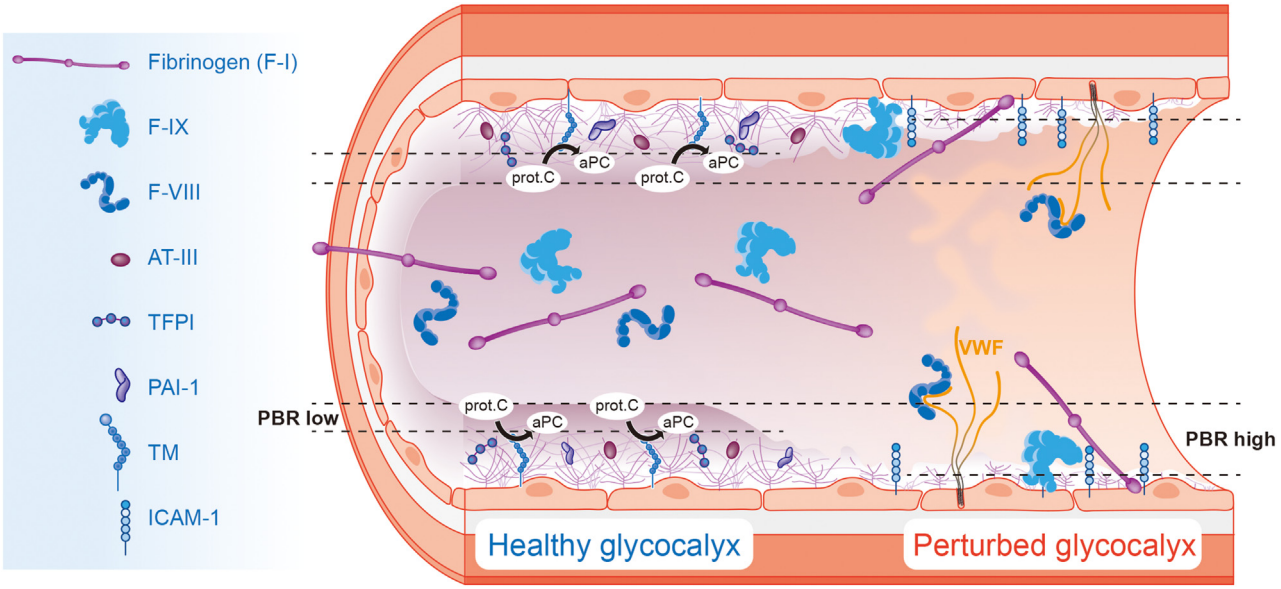

FIGURE 4 Proposed pathophysiologic concept of the association between microvascular health and endothelial response to coagulation. Here, a healthy glycocalyx (ie, perfused boundary region$_{low}$, marked by the arbitrary dotted lines) provides a robust anticoagulant barrier through binding of antithrombin III [22], presence of thrombomodulin [26], PAI-1 [50], and tissue factor pathway inhibitor [23] to heparan sulfate proteoglycans [19]. The thrombin-TM complex activates protein C to produce APC, which inactivates factor (F)VIIIa and FVa in the presence of protein S, thereby inhibiting further thrombin (F-II) formation [51]. The procoagulant endothelial surface is a result of multiple mechanisms involving effects such as gene transcription as well as protein expression and release. We hypothesized that in the event of a perturbed glycocalyx (ie, perfused boundary region$_{high}$, marked by arbitrary dotted lines further apart), a procoagulant surface appears, with increasing binding possibilities of fibrinogen (interacting with ICAM-1) [52,53], FXI [54,55], and FVIII (to surface-expressed von Willebrand factor) [47] and reduced presence of anticoagulant factors. Although FIX can bind directly to the cell surface or abluminal collagen, binding of fibrinogen and FVIII to the surface depends more on the activation state of endothelial cells. This increased interaction of coagulation FIX and FVIII activity and fibrinogen concentration, as observed in the present study, together with possible diminished surface anticoagulation pathways, would play a critical role in increased systemic microvascular dysfunction and development of coronary heart disease especially in women. aPC, activated protein C; AT-III, antithrombin III; ICAM-1, intercellular adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; PBR, perfused boundary region; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; VWF, von Willebrand factor.

ACKNOWLEDGMENTS

We express our gratitude toward all participants of the Netherlands Epidemiology of Obesity study. Furthermore, we thank P.R. van Beelen and all research nurses for data collection, P.J. Noordijk and her team for handling and storage of the blood samples, and I. de Jonge for data management.

AUTHOR CONTRIBUTIONS

R.d.M. and F.R.R. were involved in inclusion of participants for the Netherlands Epidemiology of Obesity (NEO) study, the study design of the NEO study, and acquisition of data of the NEO study. L.Y., J.H., R.L.-G., T.J.R., and B.M.v.d.B. conceived the study and design. L.Y., J.H., A.I.M.V.d.V., H.V., A.v.H.V., R.d.M., R.L.-G., T.J.R., and B.M.v.d.B. performed analyses and interpretation of data. L.Y., J.H., R.L.-G., T.J.R., and B.M.v.d.B. drafted the manuscript and design of figures. All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTERESTS

H.V. works for MicroVascular Health Solutions LLC. R.L.-G. works for Metabolon Inc. The remaining authors, including L.Y., J.H., A.I.M.v.d.V., R.d.M., F.R.R., A.v.H.V., T.J.R., and B.M.v.d.B., have no competing interests to disclose.

REFERENCES

[1] Bots SH, Peters SAE, Woodward M. Sex differences in coronary heart disease and stroke mortality: a global assessment of the effect of ageing between 1980 and 2010. *BMJ Glob Health*. 2017;2:e000298.

[2] Maas AH, Appelman YE. Gender differences in coronary heart disease. *Neth Heart J*. 2010;18:598–602.

[3] Koller A. Perspectives: microvascular endothelial dysfunction and gender. *Eur Heart J Suppl*. 2014;16:A16–9.

[4] Padro T, Manfrini O, Bugiardini R, Canty J, Cenko E, De Luca G, Duncker DJ, Eringa EC, Koller A, Tousoulis D, Trifunovic D, Vavlukis M, de Wit C, Badimon L. ESC Working Group on Coronary Pathophysiology and Microcirculation position paper on 'coronary microvascular dysfunction in cardiovascular disease'. *Cardiovasc Res*. 2020;116:741–55.

[5] Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, Cosentino F, Deanfield J, Gallino A, Ikonomidis I, Kremastinos D, Landmesser U, Protogerou A, Stefanadis C, Tousoulis D, Vassalli G, Vink H, Werner N, Wilkinson I, Vlachopoulos C. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *Eur J Cardiovasc Prev Rehabil*. 2011;18:775–89.

[6] Lee DH, Dane MJ, van den Berg BM, Boels MG, van Teeffelen JW, de Mutsert R, den Heijer M, Rosendaal FR, van der Vlag J, van Zonneveld AJ, Vink H, Rabelink TJ, NEO Study Group. Deeper penetration of erythrocytes into the endothelial glycocalyx is

associated with impaired microvascular perfusion. *PloS One*. 2014;9:e96477.

[7] de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, de Roos A, Cobbaert CM, Kloppenburg M, le Cessie S, Middeldorp S, Rosendaal FR. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol*. 2013;28:513–23.

[8] Rovas A, Sackarnd J, Rossaint J, Kampmeier S, Pavenstädt H, Vink H, Kümpers P. Identification of novel sublingual parameters to analyze and diagnose microvascular dysfunction in sepsis: the NOSTRADAMUS study. *Crit Care*. 2021;25:1–14.

[9] van der Velden AIM, van den Berg BM, de Mutsert R, van der Vlag J, Jukema JW, Rosendaal FR, Rabelink TJ, Vink H. Microvascular differences in individuals with obesity at risk of developing cardiovascular disease. *Obesity*. 2021;29:1439–44.

[10] Drost CC, Rovas A, Kusche-Vihrog K, Van Slyke P, Kim H, Hoang VC, Maynes JT, Wennmann DO, Pavenstädt H, Linke W, Lukasz A, Hesse B, Kümpers P. Tie2 activation promotes protection and reconstitution of the endothelial glycocalyx in human sepsis. *Thromb Haemost*. 2019;119:1827–38.

[11] Rovas A, Seidel LM, Vink H, Pohlkötter T, Pavenstädt H, Ertmer C, Hessler M, Kümpers P. Association of sublingual microcirculation parameters and endothelial glycocalyx dimensions in resuscitated sepsis. *Crit Care*. 2019;23:1–11.

[12] Bol ME, Huckriede JB, van de Pas KGH, Delhaas T, Lorusso R, Nicolaes GAF, Sels JEM, van de Poll MCG. Multimodal measurement of glycocalyx degradation during coronary artery bypass grafting. *Front Med*. 2022;9:1045728.

[13] Rovas A, Osiaevi I, Buscher K, Sackarnd J, Tepasse PR, Fobker M, Kühn J, Braune S, Göbel U, Thölking G, Gröschel A, Pavenstädt H, Vink H, Kümpers P. Microvascular dysfunction in COVID-19: the MYSTIC study. *Angiogenesis*. 2021;24:145–57.

[14] van Dinther M, Bennett J, Thornton GD, Voorter PHM, Ezponda Casajús A, Hughes A, Captur G, Holtackers RJ, Staals J, Backes WH, Bastarika G, Jones EAV, González A, van Oostenbrugge R, Treibel TA. Evaluation of miCRovascular rarefaction in vascUlar Cognitive Impairment and heArt faiLure (CRUCIAL): study protocol for an observational study. *Cerebrovasc Dis Extra*. 2023;13:18–32.

[15] Ikonomidis I, Pavlidis G, Lambadiari V, Rafouli-Stergiou P, Makavos G, Thymis J, Kostelli G, Varoudi M, Katogiannis K, Theodoropoulos K, Katsimbri P, Parissis J, Papadavid E. Endothelial glycocalyx and microvascular perfusion are associated with carotid intima-media thickness and impaired myocardial deformation in psoriatic disease. *J Hum Hypertens*. 2022;36:1113–20.

[16] Dane MJ, van den Berg BM, Lee DH, Boels MG, Tiemeier GL, Avramut MC, van Zonneveld AJ, van der Vlag J, Vink H, Rabelink TJ. A microscopic view on the renal endothelial glycocalyx. *Am J Physiol Renal Physiol*. 2015;308:F956–66.

[17] Schmidt EP, Yang Y, Janssen WJ, Gandjeva A, Perez MJ, Barthel L, Zemans RL, Bowman JC, Koyanagi DE, Yunt ZX, Smith LP, Cheng SS, Overdier KH, Thompson KR, Geraci MW, Douglas IS, Pearse DB, Tuder RM. The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. *Nat Med*. 2012;18:1217–23.

[18] Reitsma S, oude Egbrink MG, Viviane V, Megens RT, Engels W, Vink H, Slaaf DW, van Zandvoort MA. Endothelial glycocalyx thickness and platelet-vessel wall interactions during atherogenesis. *Thromb Haemost*. 2011;106:939–46.

[19] Boels MG, Lee DH, van den Berg BM, Dane MJ, van der Vlag J, Rabelink TJ. The endothelial glycocalyx as a potential modifier of the hemolytic uremic syndrome. *Eur J Intern Med*. 2013;24:503–9.

[20] de Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CD. Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation*. 2004;109:2529–35.

[21] Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. *J Am Soc Nephrol*. 2012;23:1900–8.

[22] Chopra P, Joshi A, Wu J, Lu W, Yadavalli T, Wolfert MA, Shukla D, Zaia J, Boons GJ. The 3-O-sulfation of heparan sulfate modulates protein binding and lyase degradation. *Proc Natl Acad Sci U S A*. 2021;118:e2012935118.

[23] Tinholt M, Stavik B, Louch W, Carlson CR, Sletten M, Ruf W, Skretting G, Sandset PM, Iversen N. Syndecan-3 and TFPI colocalize on the surface of endothelial-, smooth muscle-, and cancer cells. *PLoS One*. 2015;10:e0117404.

[24] Rezaie AR, Giri H. Anticoagulant and signaling functions of antithrombin. *J Thromb Haemost*. 2020;18:3142–53.

[25] Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care*. 2019;23:1–12.

[26] Yuan L, Cheng S, Sol WMPJ, van der Velden AIM, Vink H, Rabelink TJ, van den Berg BM. Heparan sulfate mimetic fucoidan restores the endothelial glycocalyx and protects against dysfunction induced by serum of COVID-19 patients in the intensive care unit. *ERJ Open Res*. 2022;8:00652–2021.

[27] Rabelink TJ, van den Berg BM, Garsen M, Wang G, Elkin M, van der Vlag J. Heparanase: roles in cell survival, extracellular matrix remodelling and the development of kidney disease. *Nat Rev Nephrol*. 2017;13:201–12.

[28] Haeren RH, Vink H, Staals J, van Zandvoort MA, Dings J, van Overbeeke JJ, Hoogland G, Rijkers K, Schijns OE. Protocol for intraoperative assessment of the human cerebrovascular glycocalyx. *BMJ Open*. 2017;7:e013954.

[29] Vorster HH. Fibrinogen and women's health. *Thromb Res*. 1999;95:137–54.

[30] Kim PY, Stewart RJ, Lipson SM, Nesheim ME. The relative kinetics of clotting and lysis provide a biochemical rationale for the correlation between elevated fibrinogen and cardiovascular disease. *J Thromb Haemost*. 2007;5:1250–6.

[31] Hsieh CT, Chien KL, Hsu HC, Lin HJ, Su TC, Chen MF, Lee YT. Associations between fibrinogen levels and the risk of cardiovascular disease and all-cause death: a cohort study from the Chin-Shan community in Taiwan. *BMJ Open*. 2022;12:e054638.

[32] Wilhelmsen L, Svärdsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med*. 1984;311:501–5.

[33] Rudnicka AR, Mt-Isa S, Meade TW. Associations of plasma fibrinogen and factor VII clotting activity with coronary heart disease and stroke: prospective cohort study from the screening phase of the Thrombosis Prevention Trial. *J Thromb Haemost*. 2006;4:2405–10.

[34] Lee AJ, Lowe GD, Smith WC, Tunstall-Pedoe H. Plasma fibrinogen in women: relationships with oral contraception, the menopause and hormone replacement therapy. *Br J Haematol*. 1993;83:616–21.

[35] Olson NC, Cushman M, Judd SE, Kissela BM, Safford MM, Howard G, Zakai NA. Associations of coagulation factors IX and XI levels with incident coronary heart disease and ischemic stroke: the REGARDS study. *J Thromb Haemost*. 2017;15:1086–94.

[36] Dekkers IA, de Mutsert R, de Vries APJ, Rosendaal FR, Cannegieter SC, Jukema JW, le Cessie S, Rabelink TJ, Lamb HJ, Lijfering WM. Determinants of impaired renal and vascular function are associated with elevated levels of procoagulant factors in the general population. *J Thromb Haemost*. 2018;16:519–28.

[37] Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, Lecompte T, Béguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33:4–15.

[38] Buis DTP, Christen T, Smit RAJ, de Mutsert R, Jukema JW, Cannegieter SC, Lijfering WM, Rosendaal FR. The association between leptin concentration and blood coagulation: results from the NEO study. *Thromb Res*. 2020;188:44–8.

[39] Huxley VH, Kemp SS. Sex-specific characteristics of the microcirculation. *Adv Exp Med Biol*. 2018;1065:307–28.
[40] Brands J, Hubel CA, Althouse A, Reis SE, Pacella JJ. Noninvasive sublingual microvascular imaging reveals sex-specific reduction in glycocalyx barrier properties in patients with coronary artery disease. *Physiol Rep*. 2020;8:e14351.
[41] Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. New York: Routledge; 1988.
[42] Song CJ, Nakagomi A, Chandar S, Cai H, Lim IG, McNeil HP, Freedman SB, Geczy CL. C-reactive protein contributes to the hypercoagulable state in coronary artery disease. *J Thromb Haemost*. 2006;4:98–106.
[43] Tantry US, Bliden KP, Suarez TA, Kreutz RP, Dichiara J, Gurbel PA. Hypercoagulability, platelet function, inflammation and coronary artery disease acuity: results of the Thrombotic RIsk Progression (TRIP) study. *Platelets*. 2010;21:360–7.
[44] Bratseth V, Pettersen AÅ, Opstad TB, Arnesen H, Seljeflot I. Markers of hypercoagulability in CAD patients. Effects of single aspirin and clopidogrel treatment. *Thromb J*. 2012;10:1–8.
[45] Fibrinogen Studies Collaboration. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA*. 2005;294:1799–809.
[46] Zakai NA, Judd SE, Kissela B, Howard G, Safford MM, Cushman M. Factor VIII, protein C and cardiovascular disease risk: the REasons for Geographic and Racial Differences in Stroke Study (REGARDS). *Thromb Haemost*. 2018;118:1305–15.
[47] Federici AB. The factor VIII/von Willebrand factor complex: basic and clinical issues. *Haematologica*. 2003;88:EREP02.
[48] Kalagara T, Moutsis T, Yang Y, Pappelbaum KI, Farken A, Cladder-Micus L, Vidal-Y-Sy S, John A, Bauer AT, Moerschbacher BM, Schneider SW, Gorzelanny C. The endothelial glycocalyx anchors von Willebrand factor fibers to the vascular endothelium. *Blood Adv*. 2018;2:2347–57.
[49] Rietveld IM, Lijfering WM, le Cessie S, Bos MHA, Rosendaal FR, Reitsma PH, Cannegieter SC. High levels of coagulation factors and venous thrombosis risk: strongest association for factor VIII and von Willebrand factor. *J Thromb Haemost*. 2019;17:99–109.
[50] Dólleman SC, Agten SM, Spronk HMH, Hackeng TM, Bos MHA, Versteeg HH, van Zonneveld AJ, de Boer HC. Thrombin in complex with dabigatran can still interact with PAR-1 via exosite-I and instigate loss of vascular integrity. *J Thromb Haemost*. 2022;20:996–1007.
[51] Ikezoe T. Thrombomodulin/activated protein C system in septic disseminated intravascular coagulation. *J Intensive Care*. 2015;3:1–8.
[52] Pluskota E, D'Souza SE. Fibrinogen interactions with ICAM-1 (CD54) regulate endothelial cell survival. *Eur J Biochem*. 2000;267:4693–704.
[53] Tsakadze NL, Zhao Z, D'Souza SE. Interactions of intercellular adhesion molecule-1 with fibrinogen. *Trends Cardiovasc Med*. 2002;12:101–8.
[54] Kossmann S, Lagrange J, Jäckel S, Jurk K, Ehlken M, Schönfelder T, Weihert Y, Knorr M, Brandt M, Xia N, Li H, Daiber A, Oelze M, Reinhardt C, Lackner K, Gruber A, Monia B, Karbach SH, Walter U, Ruggeri ZM, et al. Platelet-localized FXI promotes a vascular coagulation-inflammatory circuit in arterial hypertension. *Sci Transl Med*. 2017;9:eaah4923.
[55] Shariat-Madar Z, Mahdi F, Schmaier AH. Factor XI assembly and activation on human umbilical vein endothelial cells in culture. *Thromb Haemost*. 2001;85:544–51.
[56] Foley JH, Conway EM. Cross talk pathways between coagulation and inflammation. *Circ Res*. 2016;118:1392–408.
[57] Rho R, Tracy RP, Bovill EG, Ball SP, Becker RC. Plasma markers of procoagulant activity among individuals with coronary artery disease. *J Thromb Thrombolysis*. 1995;2:239–43.
[58] Tosi F, Micaglio R, Sandri M, Castagna A, Minguzzi D, Stefanoni F, Chiariello C, Franzese I, Luciani GB, Faggian G, Girelli D, Olivieri O, Martinelli N. Increased plasma thrombin potential is associated with stable coronary artery disease: an angiographically-controlled study. *Thromb Res*. 2017;155:16–22.
[59] Bol ME, Broddin BEK, Delhaas T, Sels JEM, van de Poll MCG. Variability of microcirculatory measurements in healthy volunteers. *Sci Rep*. 2022;12:19887.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.jtha.2023.06.001