Characteristics and Determinants of the Sublingual Microcirculation in Populations of Different Ethnicity

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Abstract—No previous population study assessed sublingual capillary density (CD) or perfused boundary region (PBR). Lower PBR indicates greater glycocalyx width. In 252 Han and 220 She Chinese and 254 Flemish people (mean age, 51.1 years; 54.7% women), representing random population samples, we measured total and perfused CD and PBR in the sublingual capillary bed, using oblique profiled epi-illumination, and cardiovascular risk factors. In multivariable analyses, we modeled ethnicity as random effect. Significance level was $\alpha \leq 0.05$. Compared with Chinese, Flemish had lower total (577 versus 546 n°/mm^2) and perfused (338 versus 320 n°/mm^2) CD, but similar perfused-to-total CD ratio (mean, 0.59). Perfused-to-total CD ratio increased with age (effect size per 1–SD increase, +0.015 per year), body mass index (+0.008 per kg/m^2), total cholesterol (+0.012 per mmol/L), and Framingham risk score (+0.018 per point) with no ethnic differences in these associations. For age and Framingham risk score, associations with perfused-to-total CD ratio were driven by positive relationships with perfused CD, whereas associations with total CD were nonsignificant. Chinese when compared with Flemish had higher hematocrit (43.0 versus 41.1%), PBR (2010 versus 1876 nm), and pulse rate (72.6 versus 63.3 bpm). PBR standardized for hematocrit, perfused CD, and pulse rate decreased with body mass index (–26.7 nm/kg/m^2), mean arterial pressure (–30.6 nm/mm Hg), and diastolic pressure (–28.5 nm/mm Hg) with no ethnic differences in these associations. In conclusion, a higher cardiovascular risk profile is associated with functional recruitment of capillaries with preserved glycocalyx that protects the endothelial lining. **(*Hypertension*. 2015;65:993-1001. DOI: 10.1161/HYPERTENSIONAHA.114.05119.)** ● **Online Data Supplement**

Key Words: capillaries ■ glycocalyx ■ microcirculation ■ population ■ risk factors

Hypertension is consistently associated with capillary rarefaction in animal models and human patients, but little is known about the association of capillary density with blood pressure or other cardiovascular risk factors in the general population. The endothelial lining of capillaries is protected by the glycocalyx.[1] As a reservoir of enzymatic systems, the glycocalyx, a gel-like layer, plays an important role in maintaining the balance between vasoconstriction and vasodilatation, pro- and anticoagulant activity, pro- and anti-inflammatory stimuli, and pro- and antioxidative factors.[1] The endothelial glycocalyx regulates nitric oxide synthase activity and serves as a physical barrier for macromolecules, including plasma proteins and lipoproteins.[2] In addition, the glycocalyx attenuates platelet and leukocyte adhesion.[2] The volume of the glycocalyx depends on the balance between biosynthesis and the enzymatic or shear-dependent shedding of its components.[2]

The development of capillaroscopy made it possible to visualize the sublingual capillaries[3,4] by orthogonal polarization spectral or sidestream dark field imaging and to estimate the dimensions of the glycocalyx by measuring the sublingual perfused boundary region. Most studies published to date had a small sample size or a case–control design, including selected volunteers[5] or patients with diabetes mellitus,[6,7] renal dysfunction,[8–10] cardiovascular disease,[11,12] sepsis or critically ill patients.[13,14] To our knowledge, no previous population study focused on the sublingual microcirculation to assess capillary density and glycocalyx width. Our current study aimed to characterize these microcirculatory traits in relation

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to anthropometric characteristics and cardiovascular risk factors. To assess consistency of these novel phenotypes, which is a requirement for their future use in epidemiological studies, we recruited 3 ethnic groups with different lifestyle: Han Chinese, She Chinese, and white Flemish.

Methods

Study Population

The Chinese and Flemish population studies complied with the Helsinki Declaration for investigation of human subjects. They received ethical approval from the competent Institutional Review Boards of the Shanghai Jiaotong University School of Medicine and the Faculty of Medicine of the University of Leuven. All participants provided written informed consent. The Chinese investigators applied the same epidemiological methods and phenotyping protocols as used in the Flemish studies.

Chinese Population Study

Recruitment for the Chinese population study started in 2003 and continued until 2013. The field workers visited all homes in 24 villages randomly selected from the Jingning County, a mountainous rural area ≈500 km south of Shanghai and ≈800 m above the sea level. We invited all inhabitants aged ≥12 years to take part. The initial participation rate was 76.5%. Approximately 50% of the participants are She Chinese, and the remainder belongs to the Han ethnicity. In 2013, we invited 931 subjects for a baseline examination, including sublingual capillaroscopy and 663 (71.2%) gave informed consent. We excluded 191 participants from analysis because sublingual capillaroscopy was not performed (n=16) or imaging was unsuccessful (n=126), because measurements deviated >3 SDs from the mean (n=18), because of missing hematocrit (n=30), or because ethnicity was not Han or She (n=1). Thus, the number of Chinese participants statistically analyzed totaled 472, including 252 Han and 220 She people.

Flemish Population Study

Recruitment for the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) started in 1985 and continued until 2004. The altitude of the Flemish catchment area is ≈60 m above sea level. The initial participation rate was 78.0%. The participants were repeatedly followed up. From April 2013 to June 2014, we mailed an invitation letter to 556 former participants for a follow-up examination. However, 69 were unavailable because they had been institutionalized or were too ill (n=17) or because they had moved out of the area (n=52). Of the remaining 487 former participants, 320 renewed informed consent. The participation rate was therefore 65.7%. We excluded 66 participants from analysis because sublingual capillaroscopy was not performed (n=33), because imaging of the sublingual capillaries was of insufficient quality (n=21), because measurements deviated >3 SDs from the mean (n=6), or because of missing hematocrit (n=6). Thus, the number of Flemish participants statistically analyzed totaled 254.

Assessment of the Endothelial Perfused Boundary Region

In the Chinese and Flemish population studies, the sublingual capillaries were visualized at both sides of the frenulum of the tongue using the Handheld Video Capillary Microscope (KK Research Technology Ltd., Honiton Town, Devon, United Kingdom), interfaced with a laptop computer running the GlycoCheck software (GlycoCheck BV, Maastricht, The Netherlands). The microscope implements oblique profiled epi-illumination. The probe has an array of 520 nm light-emitting diodes positioned around the tip of the lens. Their output is slightly shaded over the field of view (profiled), so that the illumination onto the surface (epi-illumination) is from an angle outside the aperture of the objective (oblique).

The microscope, covered by a disposable transparent plastic cap, enables visualizing perfused capillaries. The GlycoCheck software starts recording videos automatically, when image quality, as gauged by reflected light intensity, focus (2.8), and movement of the probe is within acceptable limits. The software stops recording after acquisition of images in 3000 capillary segments 10 μm apart and determines the red blood cell column width at each segment with a red blood cell filling of ≥50%. The distribution of the red blood cell column width at each capillary segment is used to calculate the perfused boundary region, which is the distance between the median and the outer edge of the red blood cell perfused lumen (5th–95th percentile interval of the recorded red blood cell column width values; Figure S1 in the online-only Data Supplement). Data acquisition is stratified by the width of the red blood cell column width in 1-μm steps ranging from 1 to 50 μm. The median perfused boundary region values were determined for each width class before calculating the average over the 5- to 25-μm range. The perfused boundary region reflects the thickness of the endothelial glycocalyx, based on the idea that loss of its integrity allows deeper penetration of the red blood cells into the gel-like layer covering the endothelial lining. Higher perfused boundary region, therefore, indicates thinner glycocalyx. The software also returns total and perfused capillary density in segments per millimeter square.

To remove observer-induced variability, the GlycoCheck software fully controls image acquisition and the automated exportation of results. This approach does not require offline analysis and post-processing of the videos by readers. For total density of the sublingual capillaries, the coefficient of variation determined by manually counting small-sized (1–10 μm), medium-sized (11–20 μm), and large-sized sublingual capillaries (21–50 μm) ranged from 4.1% to 6.2% for intraobserver variability and from 12.5% to 21.4% for interobserver variability.[15] For the perfused boundary region, the coefficient of variation ranged from 5.6%[6] to 11.9%.[12]

Other Measurements

Participants were asked to refrain from smoking, heavy exercise, and drinking alcohol or caffeinated beverages for ≥6 hours before the examination. After they had rested in the sitting position for ≥5 to 10 minutes, trained observers performed 5 consecutive blood pressure readings to the nearest 2 mm Hg by auscultation of the Korotkoff sounds, using a standard mercury sphygmomanometer, according to European guidelines,[16] and next counted pulse rate >1 minute. The 5 blood pressure readings were averaged for analysis. Pulse pressure was systolic minus diastolic blood pressure. Mean arterial pressure was diastolic pressure plus one third of pulse pressure. Hypertension was a blood pressure of ≥140 mm Hg systolic or 90 mm Hg diastolic or use of antihypertensive drugs.

The observers measured each participant's anthropometric characteristics and administered a standardized questionnaire to collect information about medical history, smoking and drinking habits, and intake of medications. Body mass index was weight in kilograms divided by the square of height in meters. With the participants fasting for ≥6 hours, venous blood samples were drawn for measurement of hematocrit, plasma glucose and serum cholesterol, γ-glutamyltransferase, and creatinine. Diabetes mellitus was the use of antidiabetic drugs or a fasting or random plasma glucose of 7.0 or ≥11.1 mmol/L.[17] We calculated the Framingham risk score as described by Wilson et al.[18]

Statistical Analysis

For database management and statistical analysis, we used the SAS system, version 9.3 (SAS Institute Inc, Cary, NC). Means were compared using the *t* test or ANOVA and proportions by Fisher exact test. Statistical significance was a 2-sided *P* value of <0.05 with Bonferroni correction for multiple testing where appropriate.

We searched for independent determinants of capillary density and the perfused boundary region, using a stepwise regression procedure with *P* values for covariables to enter and stay in the models set at 0.15. We applied the deviation from mean coding, which expresses the ethnic differences relative to the average in the whole study population. The other covariables considered for entry into the regression models were female sex (0, 1), age (continuous), systolic, diastolic,

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mean and pulse pressure (continuous), smoking (0, 1), total cholesterol (continuous), plasma glucose (continuous), γ-glutamyltransferase (continuous), antihypertensive drug treatment (0, 1), history of cardiovascular disease (0, 1), and the Framingham risk score.[18] To avoid leading zeros in the regression estimates, we expressed the perfused boundary region in nanometer instead of micrometer, where appropriate.

In analyses stratified by ethnicity, we assessed the associations of capillary density and the perfused boundary region with significant covariables within each ethnicity. We introduced the appropriate interaction terms in the regression models to search for ethnic differences in the associations. Finally, we used a mixed model with ethnicity modeled as a random effect to obtain pooled estimates of the relation of capillary density and perfused boundary region with covariables across ethnicities.

Results

Characteristics of Participants

Table 1 lists the characteristics of participants by ethnicity. When compared with Han, diastolic blood pressure (77.5 versus 81.3 and 82.5 mm Hg) and mean arterial pressure (93.1 versus 97.2 and 98.0 mm Hg) were higher in She and Flemish ($P \leq 0.005$). When compared with Han and She ($P \leq 0.0001$), Flemish had higher body mass index (23.2 and 23.5 versus 26.5 kg/m^2), included more drinkers (34.1 and 31.4 versus 76.4%), but had lower total cholesterol (5.40 and 5.57 versus 4.82 mmol/L; $P \leq 0.0001$), and plasma glucose (4.99 and 4.79 versus 4.57 mmol/L; $P \leq 0.008$). When compared with Han ($P \leq 0.021$), Flemish had higher systolic blood pressure (124.3 versus 129.0 mm Hg), lower γ-glutamyltransferase (20.4 versus17.8 μmol/L), and lower prevalence of diabetes mellitus (6.4 versus 0.4%). When compared with She ($P \leq 0.002$), Flemish had higher serum creatinine (76.0 versus 70.9 μmol/L), and included fewer women (46.9 versus 62.7%).

Sublingual Capillary Density

The total and perfused capillary density averaged 580 and 342 n°/mm^2 in Han, 573 and 334 n°/mm^2 in She, and 546 and 320 n°/mm^2 in Flemish. The perfused-to-total capillary density ratio did not differ across ethnicities ($P \geq 0.70$), averaging 0.59 in all participants combined.

In all participants (Table 2), perfused and total capillary densities were higher than average in Han ($P \leq 0.035$) and lower in Flemish ($P \leq 0.011$). Perfused capillary density increased with age ($P=0.002$) and the Framingham risk score ($P=0.009$). There were no ethnic differences in the perfused-to-total capillary density ratio ($P \geq 0.26$). The ratio increased with age, body mass index, total cholesterol, and the Framingham risk score, but was lower in smokers than in nonsmokers ($P \leq 0.039$ for all; Table 2). The explained variance ranged from 1.4% for total capillary density in model 2 to 4.8% for perfused-to-total capillary density ratio in model 1.

With adjustment for smoking applied, the within-ethnicity associations of the perfused-to-total capillary density ratio (Figure 1) were significant for age in Flemish ($P<0.001$), for body mass index in Han ($P=0.006$), for total cholesterol in She ($P=0.019$), and for the Framingham risk score in all 3 ethnicities ($P \leq 0.036$). However, the ethnic differences in these associations were all nonsignificant ($P \geq 0.10$). In all participants combined (Figure 1), with adjustment for smoking applied, the perfused-to-total capillary density ratio increased with age (effect size per 1-SD increase, 0.015 per year; 95% confidence interval [CI], 0.008–0.023; $P<0.0001$), body mass index (0.008/kg/m^2; 95% CI, 0.001–0.016; $P=0.027$), total cholesterol (0.012/mmol/L; 95% CI, 0.005–0.020; $P=0.001$), and the Framingham risk score (0.018 per point; 95% CI, 0.011–0.025; $P<0.0001$). Figure 2 depicts the regression lines relating in all participants combined the perfused-to-total capillary density ratio with the aforementioned covariables with adjustments applied for ethnicity and shows that these associations were mainly driven by positive associations with the perfused capillary density, in particular, for age and Framingham risk score. All associations with total capillary density were nonsignificant ($P \geq 0.30$).

Determinants of the Perfused Boundary Region

The sublingual perfused boundary region averaged 2019 nm in Han, 2000 nm in She, and 1876 nm in Flemish. Figure S2 shows the frequency distributions of the perfused boundary region, which deviated from normality only in Flemish. In each ethnicity (Figure S3), the perfused boundary region independently decreased with hematocrit ($P<0.0001$) and the perfused capillary density ($P<0.0001$). When compared with Han and She (Table 1), Flemish participants had a not only lower hematocrit (43.3 and 42.7 versus 41.1%; $P<0.001$) and perfused capillary density (342 and 334 versus 320 n°/mm^2; $P<0.001$) but also lower pulse rate (72.1 and 73.2 versus 63.3 bpm; $P<0.0001$). In view of these ethnic differences, we standardized the perfused boundary region to the mean values of hematocrit, perfused capillary density, and pulse rate within each ethnicity. The standardized perfused boundary region averaged 2024 nm in Han, 1990 nm in She, and 1875 nm in Flemish (Table 1; Figure S2).

Table S1 lists the characteristics of the participants by fourths of the ethnicity-specific distributions of the standardized perfused boundary region. Body mass index, diastolic, and mean arterial pressure decreased with higher category of the standardized perfused boundary region ($P \leq 0.031$). The perfused boundary region and the total capillary density increased, whereas the perfused-to-total capillary density ratio decreased with higher category of the standardized perfused boundary region ($P<0.0001$). Because of the standardization, there were no differences in hematocrit ($P=0.72$), perfused capillary density ($P=0.86$), and pulse rate ($P=0.73$) across the categories in Table S1.

Table 3 shows the results of the stepwise regression analysis. Mean arterial pressure and pulse pressure were considered as covariables in model 1, and the Framingham risk score in model 2. The other covariables considered for entry in the regression models are listed in the Methods section of this article and in the footnote to Table 3. Compared with the average in the whole study population, Flemish people had smaller standardized perfused boundary region (≈75 nm; $P<0.0001$), whereas Han (≈50 nm) had a slightly but significantly ($P \leq 0.0002$) higher value than average. With adjustment for ethnicity and per 1-SD increment in the explanatory variables, the standardized perfused boundary region decreased by –18.4 nm ($P=0.074$) and –23.3 nm ($P=0.016$) in relation to body mass index and mean arterial blood pressure in model 1. When we offered systolic and diastolic blood pressure as covariables to

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Table 1. Characteristics of Participants by Ethnicity

Characteristics	Han (n=252)	She (n=220)	Flemish (n=254)
No. of participants (%)			
Women	140 (55.6)	138 (62.7)*	119 (46.9)
Smokers	55 (21.8)	38 (17.3)	36 (14.2)
Drinking alcohol	86 (34.1)	69 (31.4)*	194 (76.4)†
Hypertension	74 (29.4)	79 (35.9)	96 (37.8)
Antihypertensive treatment	31 (12.3)	38 (17.3)	43 (16.9)
Previous cardiovascular disease	8 (3.2)	4 (1.8)	14 (5.5)
Diabetes mellitus	16 (6.4)	4 (1.8)	1 (0.4)†
Mean (SD) of characteristics			
Age, y	51.8 (13.6)	51.7 (12.4)	49.8 (14.8)
Body mass index, kg/m^2	23.2 (3.2)	23.5 (2.9)*	26.5 (4.5)†
Systolic blood pressure, mm Hg	124.3 (20.8)	128.9 (22.7)	129.0 (15.9)†
Diastolic blood pressure, mm Hg	77.5 (11.2)‡	81.3 (12.0)	82.5 (9.8)†
Mean arterial pressure, mm Hg	93.1 (13.6)‡	97.2 (14.6)	98.0 (10.7)†
Pulse pressure, mm Hg	46.8 (14.1)	47.6 (15.7)	46.5 (12.5)
Pulse rate, bpm	72.1 (9.3)	73.2 (10.5)*	63.3 (8.2)†
Hematocrit, %	43.3 (4.1)	42.7 (3.5)*	41.1 (3.4)†
Total cholesterol, mmol/L	5.40 (1.03)	5.57 (0.99)*	4.82 (0.88)†
Blood glucose, mmol/L	4.99 (1.15)	4.79 (0.99)*	4.57 (0.50)†
γ-glutamyltransferase, units/L	20.4 (14.1–26.9)	20.0 (12.9–28.2)	17.8 (12.0–25.7)†
PBR, nm	2019 (284)	2000 (270)*	1876 (254)†
Standardized PBR, nm	2024 (261)	1990 (242)*	1875 (239)†
Total capillary density, n°/mm^2	580 (134)	573 (134)*	546 (99)†
Perfused capillary density, n°/mm^2	342 (88)	334 (86)	320 (69)†
Perfused-to-total capillary density ratio	0.59 (0.11)	0.59 (0.10)	0.59 (0.09)

Blood pressure was the average of 5 consecutive readings. Hypertension was defined an office blood pressure of ≥140 mm Hg systolic, ≥90 mm Hg diastolic, or use of antihypertensive drugs. For γ-glutamyltransferase and the Framingham risk score, reported values are geometric mean (interquartile range) and median (interquartile range), respectively. PBR indicates perfused boundary region.

Significance of the difference between ethnicities (P≤0.05 adjusted for multiple comparisons by Bonferroni method): *She vs Flemish, †Han vs Flemish, and ‡Han vs She.

model 1 instead of mean arterial pressure and pulse pressure, the association size for diastolic blood pressure was (–25.4 nm/mm Hg; 95% CI, –44.5 to –6.29; P=0.009). The associations of the standardized perfused boundary region with systolic blood pressure in model 1 and with the Framingham risk score in model 2 were not significant (P≥0.25).

Within ethnicity (Figure 3), the inverse associations of the standardized perfused boundary region with mean arterial pressure in She and Flemish participants were significant (P≤0.020). However, the ethnic differences in these associations were nonsignificant (P≥0.39). In all participants combined (Figure 3; Figure S4), the standardized perfused boundary region decreased with body mass index (–26.7 nm/kg/m^2; 95% CI, –7.3 to –46.0; P=0.007), mean arterial pressure (–28.5 nm/mm Hg; 95% CI, –10.3 to –46.7; P=0.002) and diastolic blood pressure (–30.6 nm/mm Hg; 95% CI, –12.4 to –48.9; P=0.001).

Sensitivity Analysis

Excluding 112 participants on antihypertensive drug treatment produced results for the perfused-to-total capillary density ratio (Figure S5) and the standardized perfused boundary region (Figure S6) that were not materially different from those in the whole study population (Figures 1 and 3, respectively). As in the main analysis (Figures 1 and 3), none of the comparisons between ethnicities reached significance (Figures S5 and S6; P≥0.11) except for the comparison between Han and She in the association of the perfused-to-total capillary density with body mass index (Figure S6; P=0.026).

Discussion

To our knowledge, our current study is the first to investigate the characteristics of the sublingual capillary bed in people randomly recruited from different populations. The key findings can be summarized as follows: (1) higher age, body mass index, total cholesterol, and Framingham risk score were associated with capillary recruitment, as exemplified by the perfused capillary density and the perfused-to-total capillary density ratio; (2) in perfused capillaries, the glycocalyx width increased with higher body mass index, mean arterial pressure, and diastolic blood pressure; (3) there were no ethnic differences in these associations. Although resulting from

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Table 2. Correlates of the Sublingual Capillary Density in 726 Participants

Correlates	Model 1	Model 2
Perfused capillary density		
Han (vs average)	10.0 (1.79, 18.3)*	9.29 (1.02, 17.6)*
She (vs average)	1.58 (–6.94, 10.1)	1.49 (–7.05, 10.0)
Flemish (vs average)	–11.6 (–19.9, –3.38)†	–10.8 (–19.1, –2.49)*
Age (+13.7 y)	9.31 (3.42, 15.2)†	…
Framingham risk score (+6.53 points)	…	7.93 (2.00,13.9)†
Total capillary density		
Han (vs average)	15.2 (2.50, 27.8)*	13.5 (0.97, 26.0)*
She (vs average)	6.65 (–6.28, 19.6)	6.59 (–6.35, 19.5)
Flemish (vs average)	–21.8 (–34.4, –9.16)‡	–20.1 (–32.5, –7.58)†
Perfused-to-total capillary density ratio		
Han (vs average)	0.0060 (–0.0044, 0.0164)	0.0050 (–0.0055, 0.0154)
She (vs average)	–0.0051 (–0.0159, 0.0057)	–0.0027 (–0.0134, 0.0080)
Flemish (vs average)	–0.0009 (–0.0123, 0.0104)	–0.0023 (–0.0134,0.0089)
Age (+13.7 y)	0.0092 (0.0007, 0.0177)*	…
Body mass index (+3.90 kg/m^2)	0.0085 (0.0007, 0.0164)*	0.0066 (–0.0015, 0.0146)
Total cholesterol (+1.02 mmol/L)	0.0102 (0.0024, 0.0179)*	…
Smoking (0, 1)	–0.0202 (–0.0393, –0.0011)*	…
Framingham risk score (+6.53 points)	…	0.0145 (0.0069, 0.0220)‡

Correlates of the sublingual capillary density were identified by a stepwise regression procedure with *P* values for covariables to enter and stay in the models set at 0.15. Ethnicity was forced in the models and was coded using the deviation from mean coding, which expresses the ethnic differences relative to the average in the whole study population. The covariables considered for entry in model 1 were female sex (0, 1), age (continuous), body mass index (continuous), smoking (0, 1), total cholesterol (continuous), plasma glucose (continuous), γ-glutamyltransferase (continuous), antihypertensive drug treatment (0, 1), and history of cardiovascular disease (0, 1). Mean arterial pressure and pulse pressure, or systolic and diastolic blood pressures were also offered to model 1, but did not enter. Covariables in model 2 were body mass index, γ-glutamyltransferase, Framingham risk score, antihypertensive drug treatment, and history of cardiovascular disease. For continuous variables, the association sizes are expressed for a 1-SD increase. Bracketed values denote the 95% confidence interval.

Significance of the independent associations: *$P \leq 0.05$, †$P \leq 0.01$, ‡$P \leq 0.001$.

cross-sectional observations, our current findings suggest that a higher cardiovascular risk profile is associated with functional recruitment of capillaries with preserved glycocalyx that protects the endothelial lining.

In our current study, perfused capillary density refers to sublingual capillary segments with a red blood cell filling of ≥50% and total capillary density denotes perfused segments with any degree of red blood cell filling. Imaging by oblique profiled epi-illumination does not allow visualizing capillaries without any red blood cell filling. This explains why the perfused-to-total capillary density ratio in our hands on average was 0.59 and why this ratio is a measure

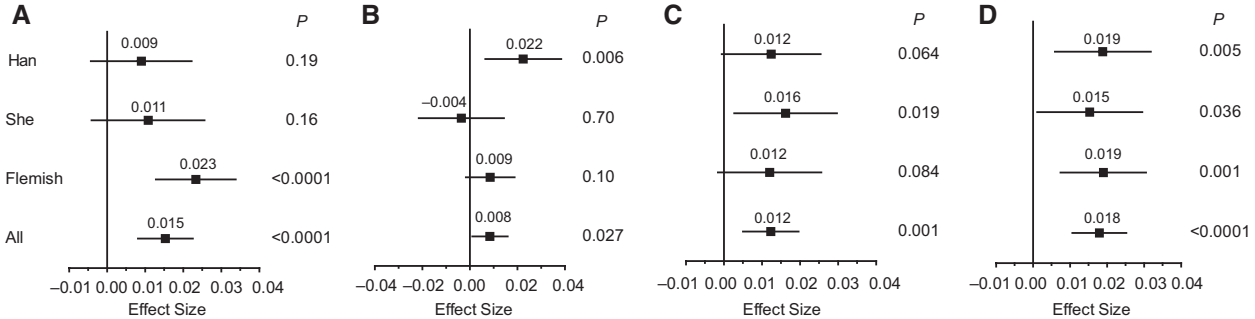

Figure 1. Association of the perfused-to-total capillary density ratio (adjusted for smoking) with age (**A**), body mass index (**B**), total cholesterol (**C**), and Framingham risk score (**D**) in 726 participants (252 Han, 220 She, and 254 Flemish). Estimates, presented with 95% confidence interval, are for a 1-SD increase in continuous explanatory variables (Table 1). *P* values are for the significance of the associations. None of the between-ethnicity comparisons of the association sizes reached significance (adjusted for multiple testing using Bonferroni method).

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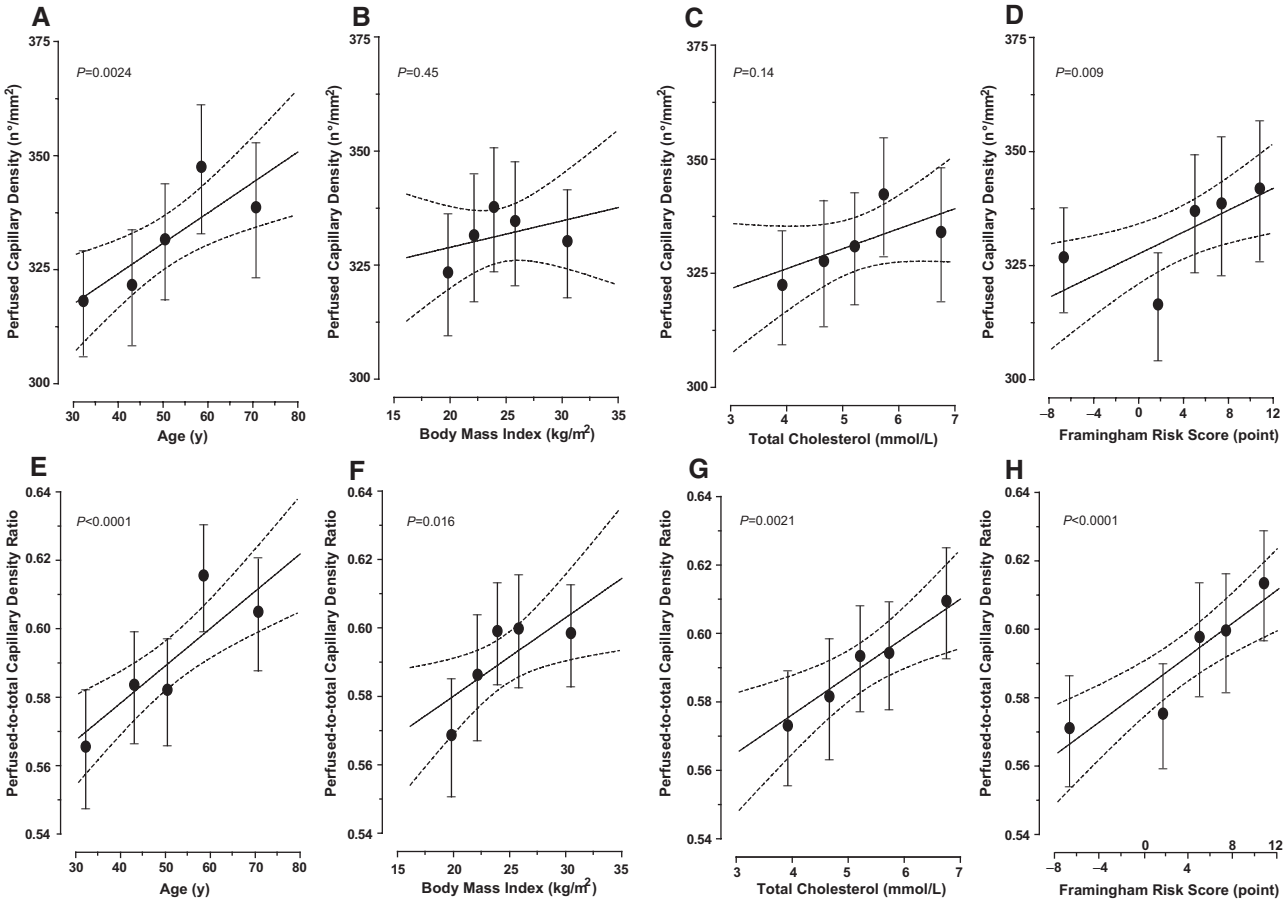

Figure 2. Association of perfused (**A**–**D**) capillary density and the perfused-to-total capillary density ratio (**E**–**H**) with age (**A** and **E**), body mass index (**B** and **F**), mean arterial pressure (**C** and **G**), and Framingham risk score (**D** and **H**). The independent variables were standardized for ethnicity. The regression lines with 95% confidence interval were computed using the data of individual people. *P* values denote the significance of the associations. The dots plotted over the regression line represent the means of the explanatory variables by fifths of their distribution. Vertical bars represent the 95% confidence interval of these means. The figure confirms the adequacy of the linear model.

of functional rather than structural capillary recruitment. We noticed that with an increasing cardiovascular risk profile, as reflected by higher age, body mass index, total cholesterol, and Framingham risk score, the perfused capillary density and the perfused-to-total capillary density ratio increased. This might indicate recruitment of capillaries with preserved functionality. Hypertension is consistently associated with capillary rarefaction in patients[19,20] and animal models alike.[21] However, as reviewed elsewhere,[22] in most vascular beds, not all microvessels are perfused at any one time. The fraction of

Table 3. Correlates of the Standardized Perfused Boundary Region in 726 Participants

Correlates	Model 1	Model 2
Han (vs average)	50.4 (24.5, 76.3)*	53.6 (27.8, 79.5)*
She (vs average)	24.3 (–2.18, 50.8)	20.8 (–5.61, 47.3)
Flemish (vs average)	–74.7 (–101.8, –47.6)*	–74.5 (–101.6, –47.3)*
Body mass index, +3.90 kg/m²	–18.4 (–38.6, 1.78)	–25.3 (–44.8, –5.85)§
Mean arterial pressure, +13.1 mm Hg	–23.3 (–42.2, –4.42)§	…

Correlates of the standardized perfused boundary region were identified by a stepwise regression procedure with *P*–values for covariables to enter and stay in the models set at 0.15. Ethnicity was modeled using the deviation from mean coding, which expresses the ethnic differences relative to the average in the whole study population. The covariables considered for entry in Model 1 were female sex (0, 1), age (continuous), body mass index (continuous), smoking (0, 1), mean arterial pressure (continuous), pulse pressure (continuous), total cholesterol (continuous), plasma glucose (continuous), γ–glutamyltransferase (continuous), antihypertensive drug treatment (0, 1), and history of cardiovascular disease (0, 1). Covariables considered for Model 2 were body mass index, γ-glutamyltransferase, the Framingham risk score, antihypertensive drug treatment, and history of cardiovascular disease. The Framingham risk score did not enter Model 2 (*P*=0.20). For continuous variables, the association sizes are expressed for a 1–SD increase. Bracketed values denote the 95% confidence interval.

Significance of the independent associations: *$P \leq 0.0001$, §$P \leq 0.05$.

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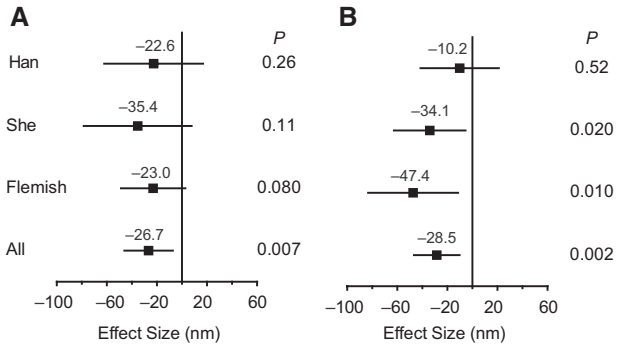

Figure 3. Association of the standardized perfused boundary region with body mass index (**A**) and mean arterial pressure (**B**) in 726 participants (252 Han, 220 She, and 254 Flemish). Estimates, presented with 95% confidence interval, are for a 1-SD increase in continuous explanatory variables (Table 1). *P* values are for the significance of the associations. None of the between-ethnicity comparisons of the association sizes reached significance (adjusted for multiple testing using Bonferroni method).

nonperfused vessels constitutes a reserve that may be called on when the vascular bed is under stress. Our current observations are novel in that they extend findings in hypertensive patients and animal models to ethnically diverse population samples with different lifestyle. To our knowledge, only 1 previous study in genetically engineered mice showed that the endothelial glycocalyx increased with age, but did not report on capillary density or capillary recruitment.[23] In 46 healthy volunteers, Ijzerman et al[24] demonstrated that a higher Framingham risk score was associated with impaired endothelium-dependent vasodilatation and reduced recruitment of skin capillaries. In the study of Ijzerman et al,[24] capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nail fold skin. The percentage capillary recruitment was assessed by dividing the increase of perfused capillary density after 4 minutes of arterial occlusion by the baseline value.[24]

Imaging as implemented in our current or previous studies[6–13,25] does not capture the endothelial lining of the capillaries. The perfused boundary region is, therefore, an indirect estimate of glycocalyx width. However, Nieuwdorp et al[26] elegantly validated the method in type 1 diabetic patients with or without microalbuminuria and matched healthy controls. These researchers determined the systemic glycocalyx volume by comparing the intravascular distribution volume of a glycocalyx permeable tracer (dextran 40) with that of a glycocalyx impermeable tracer (labeled erythrocytes). Glycocalyx volume decreased in a stepwise fashion from controls over diabetic patients without microalbuminuria to diabetic patients with microalbuminuria (1.5 versus 0.8, versus 0.2 l).[26] Moreover, compared with normal controls, type 1 diabetic patients had a thinner glycocalyx in sublingual capillaries, but higher levels of circulating hyaluronidase and hyaluronan, a principal glycocalyx constituent.[26]

The current literature on the perfused boundary region,[6–13,25] from which the glycocalyx width is inferred by sidestream darkfield imaging, is limited to case–control studies with sample size ranging from 16[8] to 150.[12] All studies focused on the sublingual capillaries with the exception of 1 study of the peritubular microcirculation studied at the time of renal transplantation.[13] Cases were patients with diabetes mellitus,[6,7] cardiovascular disease[11,12] stroke,[25] end-stage renal disease,[8–10] or sepsis.[13] When compared with controls, the glycocalyx width estimated from the perfused boundary region was smaller in cases with type 2 diabetes mellitus[6,7] or end-stage renal disease.[8–10] Among patients with cardiovascular disease and matched controls, the results were contradictory, cases having a decreased[11] or similar[12] perfused boundary region. The sublingual perfused boundary region did not differ between cases and controls in studies of lacunar stroke[25] or sepsis.[13] An issue to be considered in this regard is the high biological variability in microcirculatory phenotypes. Capillaries in which there is no flow at a given time may be well perfused a few minutes later, and perfused vessels may later have no flow, depending on systemic conditions or local tissue needs of oxygenation or transport of energy and metabolites.

Our current study moves beyond the state of the art because it includes randomly selected people from 2 populations, representing 3 ethnicities. Moreover, in contrast to previous studies in selected patients,[7,8,12] we examined the correlates of the perfused boundary region in detail. With ethnicity modeled as a random effect, the perfused boundary region decreased with body mass index, diastolic blood pressure, and mean arterial pressure. Furthermore, we did not observe ethnic differences in these associations, confirming the consistency of our findings across populations. Appendix I in the online-only Data Supplement further clarifies why we standardized the perfused boundary region to ethnicity-specific mean values of hematocrit, perfused capillary density, and pulse rate.

On the contrary, our current study should be interpreted within the context of some potential limitations. First, we assessed capillary properties only in the sublingual bed. To what extent our current findings can be extrapolated to other organs remains uncertain. However, changes in the coronary or renal microcirculation parallel those in retinal microvessels.[27,28] Second, to address any difference in methodology between the Chinese and Flemish studies, in our pooled analyses, we modeled ethnicity as a random effect. The CIs of the effect sizes relating perfused-to-total capillary density ratio or perfused boundary region to the explanatory variables were widely overlapping. However, formal power calculations to detect ethnic differences in these associations at a α-level of 0.05 showed that the β values were all <0.57. Therefore, we cannot exclude that for these analyses our study was underpowered. Third, the cross-sectional nature of our observations precludes making any extrapolation to the timing of events or causal relations. Fourth, the technology applied in our current study does not provide any information on the structural characteristics of the microcirculation because it does not visualize nonperfused capillaries. Finally, imposed ethical restraints in population-based research did not allow us to study recruitment of capillaries, using pharmacological interventions, such as the administration of nitrates or local superfusion of acetylcholine.

Perspectives

Our study was the first to investigate the sublingual microcirculation in general populations, including Chinese and

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Flemish people. It suggests that higher cardiovascular risk profile is associated with functional recruitment of capillaries with preserved glycocalyx that protects the endothelial lining. Given these observations, longitudinal studies should clarify whether microvascular alterations are early markers of disease, secondary to the primary pathological process itself, eg, hypertension, diabetes mellitus, or chronic kidney disease, or whether pathological changes in the microcirculation might be the primary instigator of disease with subsequently occurring organ dysfunction, such as for instance diastolic left ventricular dysfunction.[29] In addition, given the current literature, future research should elucidate the relation of left ventricular function[29] or renal function with peripherally measured microvascular phenotypes.

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Disclosures

None.

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Novelty and Significance

What Is New?

- To our knowledge, no previous population study assessed in the sublingual microcirculation capillary density and the perfused boundary region, which is inversely correlated with glycocalyx width. There is, therefore, no information on the distribution of this novel phenotypes in diverse ethnic groups.

What Is Relevant?

- Higher age, body mass index, total cholesterol, and Framingham risk score are associated with functional capillary recruitment, as exemplified by the perfused-to-total capillary density ratio.
- In perfused capillaries, the glycocalyx width increases with higher body mass index, mean arterial pressure, and diastolic blood pressure.
- There are no ethnic differences in these associations.

Summary

Cardiovascular risk factors are associated with functional recruitment of capillaries with preserved glycocalyx that protects the endothelial lining.

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Data Supplement

Characteristics and Determinants of the Sublingual Microcirculation in Populations of Different Ethnicity

Short title: The Sublingual Microcirculation in Populations

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Standardization

To eliminate bias, we standardized the perfused boundary region to ethnicity-specific mean values of hematocrit, perfused capillary density and pulse rate. In line with differences in altitude (800 *vs.* 60 m), Chinese had a higher hematocrit, a measurement that is intrinsically related to red blood cell count and therefore to red blood cell capillary filling. Pulse rate was higher in Chinese than Flemish and in women than in men. These observations remained consistent after excluding hypertensive patients on β-blockers, which were less prescribed in Chinese tha Flemish (0.4% *vs.* 9.4%). The ethnic and sex differences in pulse rate are in keeping with those reported in previous publications with larger sample size (1688 Chinese[1] and 797 Flemish[2]). They are probably largely attributable to the shorter stature of Chinese and women, compared with Flemish and men, respectively. Indeed, arterial wave reflection sites are numerous and diffuse, but have been approximately localized, for the lower part of the body, to a region extending from the renal arteries to the aortic bifurcation.[3] Because people of short stature necessarily have correspondingly shorter arterial trees, their reflecting sites are closer to the heart.[4] To optimize central hemodynamics, a short body height is associated with a faster heart rate.[5]

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Table S1. Characteristics of Participants by Fourths of the Ethnicity-Specific Distributions of the Standardized Perfused Boundary Region in 726 subjects (Starts)

Characteristic	Low	Medium–Low	Medium–High	High	*P*
N° of participants with characteristic (%)					
Ethnicity					
Han	63 (34.6)	63 (34.8)	63 (34.6)	63 (34.8)	>0.99
She	55 (30.2)	55 (30.4)	55 (30.2)	55 (30.4)	>0.99
Flemish	64 (35.2)	63 (34.8)	64 (35.2)	63 (34.8)	>0.99
Women	92 (50.6)	103 (56.9)	102 (56.0)	100 (55.3)	0.62
Smokers	32 (17.6)	28 (15.5)	39 (21.4)	30 (16.6)	0.48
Drinking alcohol	87 (47.8)	85 (47.0)	88 (48.4)	89 (49.2)	0.98
Hypertension	66 (36.3)	56 (30.9)	66 (36.3)	61 (33.7)	0.67
Antihypertensive treatment	30 (16.5)	22 (12.1)	31 (17.0)	29 (16.0)	0.55
Mean (SD) of characteristics					
Age, years	50.3 (13.0)	51.9 (13.3)	52.4 (13.8)	49.6 (14.6)	0.16
Body mass index, kg/m^2	25.4 (4.1)	23.9 (3.3)	24.3 (4.3)	24.3 (3.8)	0.003
Systolic pressure, mmHg	129.2 (22.0)	126.1 (18.9)	128.5 (19.1)	125.6 (19.8)	0.22
Diastolic pressure, mmHg	82.5 (11.9)	79.4 (10.1)	80.9 (11.5)	78.8 (10.9)	0.009
Mean arterial pressure, mmHg	98.1 (14.4)	95.0 (12.0)	96.8 (13.0)	94.4 (12.9)	0.031
Pulse pressure, mmHg	46.7 (14.8)	46.6 (13.9)	47.6 (13.6)	46.8 (14.1)	0.90
Pulse rate, beats per minute	68.9 (9.8)	70.0 (10.5)	69.6 (10.3)	69.1 (10.7)	0.73

Table S1. Characteristics of Participants by Fourths of the Ethnicity-Specific Distributions of the Standardized Perfused Boundary Region in 726 subjects (Continues)

Characteristic	Low	Medium–Low	Medium–High	High	*P*
Red blood cell count,	4.75 (0.53)	4.73 (0.45)	4.66 (0.40)	4.76 (0.46)	0.17
Hematocrit, %	42.2 (4.0)	42.4 (3.7)	42.2 (3.4)	42.5 (4.0)	0.72
Total cholesterol, mmol/L	5.24 (1.01)	5.37 (1.09)	5.23 (0.96)	5.16 (1.01)	0.29
Plasma glucose, mmol/L	4.83 (0.93)	4.74 (1.07)	4.82 (0.85)	4.74 (0.87)	0.71
γ-glutamyltransferase, units/L	20.0 (12.9–28.8)	19.1 (12.9–29.5)	18.6 (12.9–24.0)	18.6 (12.9–25.7)	0.65
Framingham risk score	5 (1, 8)	0 (4, 8)	5 (1, 8)	4 (–1, 7)	0.27
Perfused boundary region, nm	1661 (159)	1871 (128)	2035 (125)	2286 (199)	<0.0001
Total capillary density, n°/mm^2	543 (108)	541 (125)	580 (129)	601 (119)	<0.0001
Perfused capillary density, n°/mm^2	335 (78)	328 (84)	333 (84)	330 (81)	0.86
Perfused-to-total capillary density ratio	0.62 (0.10)	0.61 (0.09)	0.58 (0.10)	0.55 (0.10)	<0.0001

Blood pressure was the average of five consecutive readings at a single contact. Hypertension was a blood pressure of ≥140 mmHg systolic, or ≥90 mm Hg diastolic, or use on antihypertensive drugs. For γ–glutamyltransferase and the Framingham risk score, reported values are geometric mean (interquartile range) and median (interquartile range), respectively. *P*–values denote the significance of linear trend across fourths of the ethnic-specific distribution of the standardized perfused boundary region.

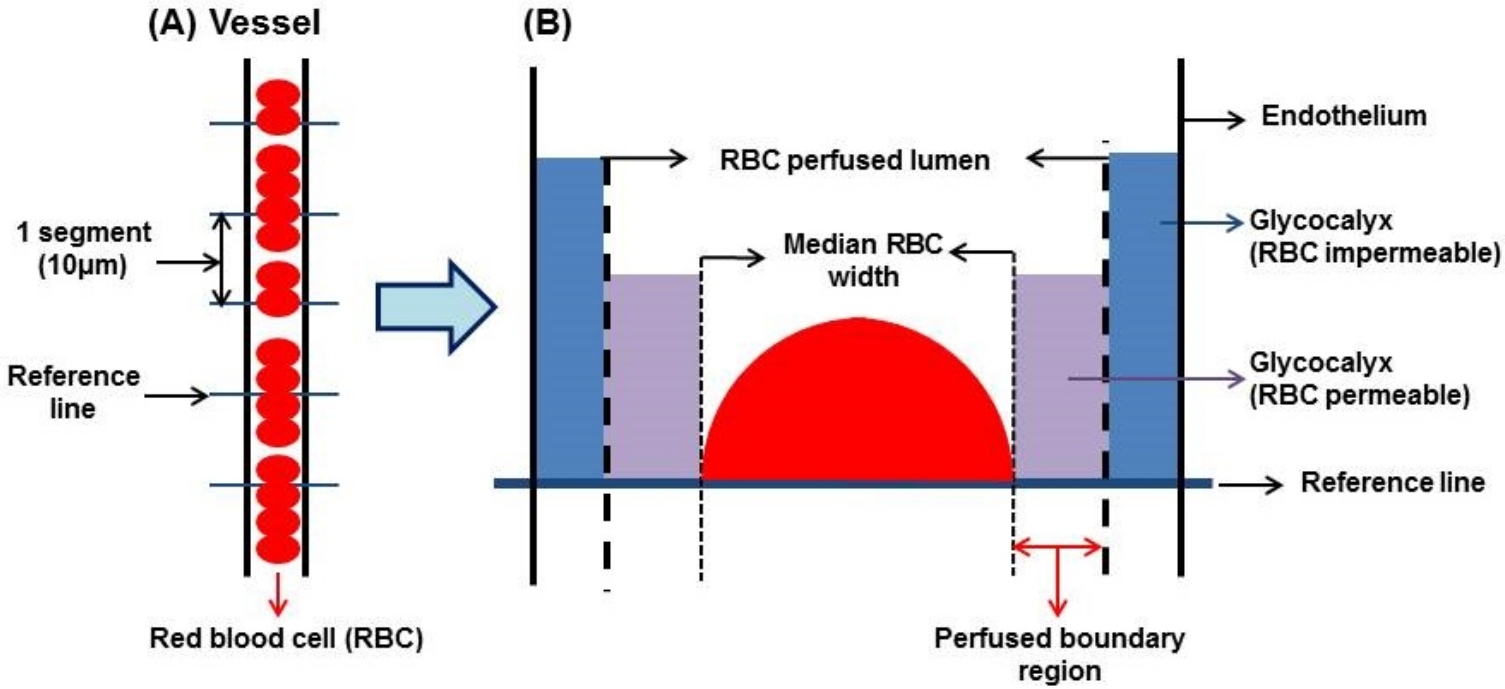

Figure S1. The GlycoCheck software acquires images in 3000 capillary segments 10 µm apart (A) and determines the red blood cell column width at each segment with a red blood cell filling of at least 50% (B). The distribution of the red blood cell column width at each capillary segment is used to calculate the perfused boundary region, which is the distance between the median and the outer edge of the red blood cell perfused lumen (5th to 95th percentile interval of the recorded red blood cell column width values).

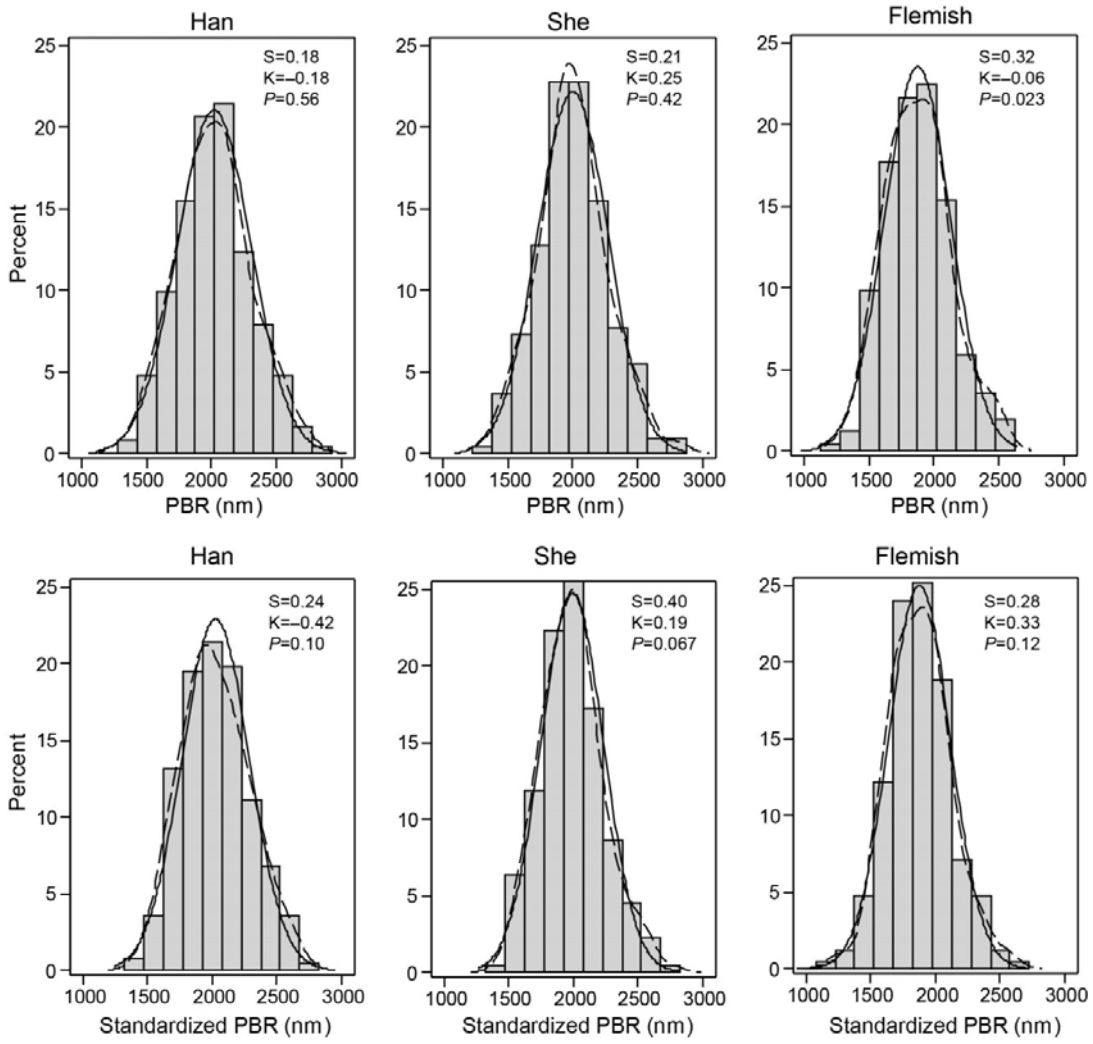

Figure S2. Frequency distributions of original and standardized perfused boundary region in Han, She, and Flemish. PBR indicates the perfused boundary region. S and K are the coefficients of skewness and kurtosis. The *P*–value is for departure of the actually observed distribution (kernel distribution; dotted line) from normality (full line).

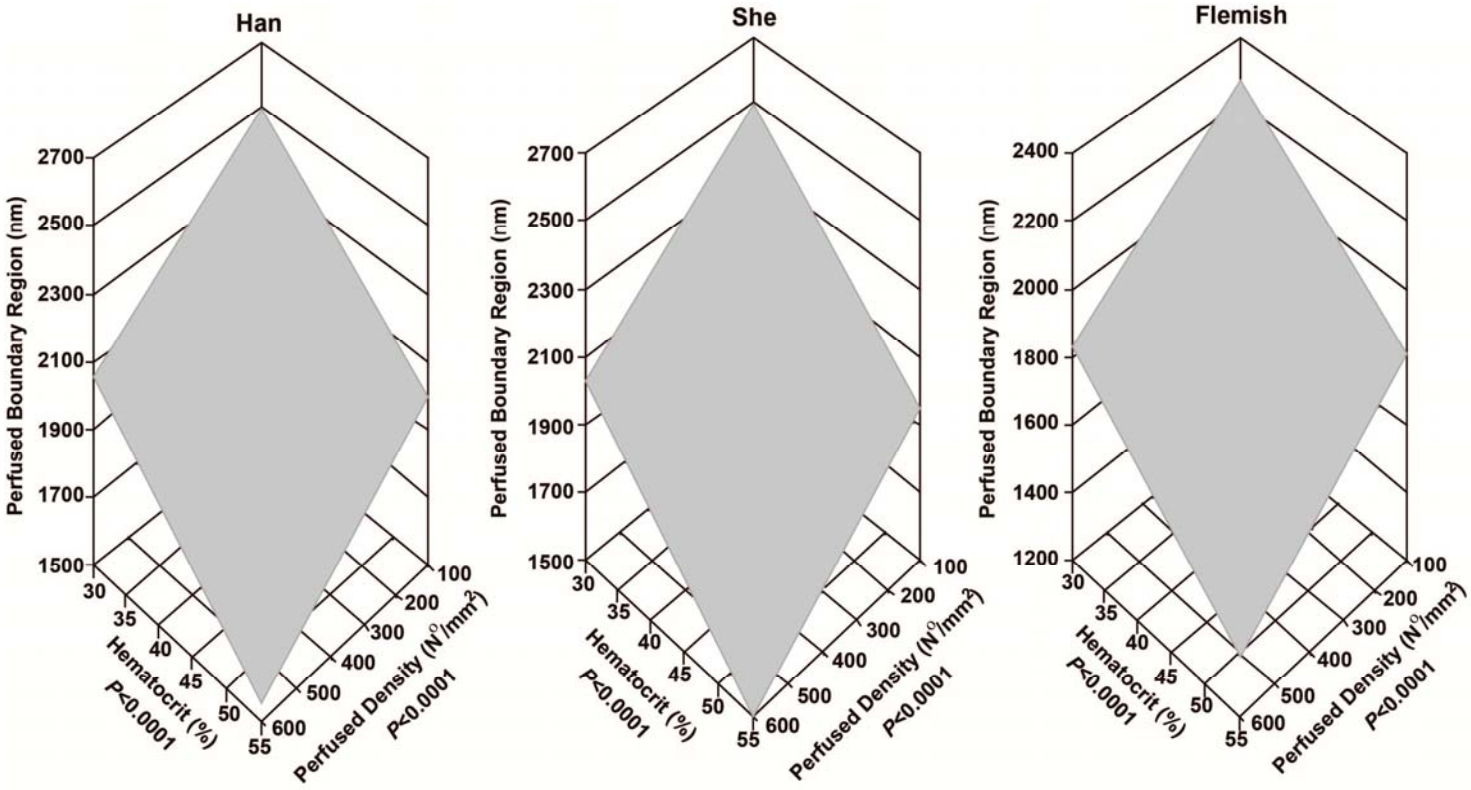

Figure S3. Association of the perfused boundary region with hematocrit and perfused capillary density by ethnicity. The scales of hematocrit and the perfused boundary region increase from back to front.

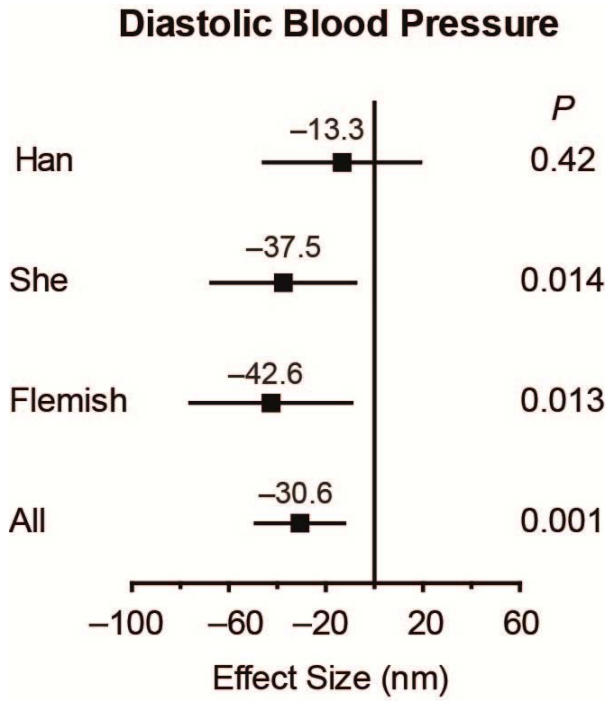

Figure S4. Association of the standardized perfused boundary region with diastolic blood pressure in 726 participants (252 Han, 220 She and 254 Flemish). Estimates, presented with 95% confidence interval, are for a 1–SD increase in continuous explanatory variables (see Table 1). *P*–values on the figure panel are for the significance of the associations. None of the comparison between ethnicities in the association sizes, adjusted for multiple testing using Bonferroni method reached significance.

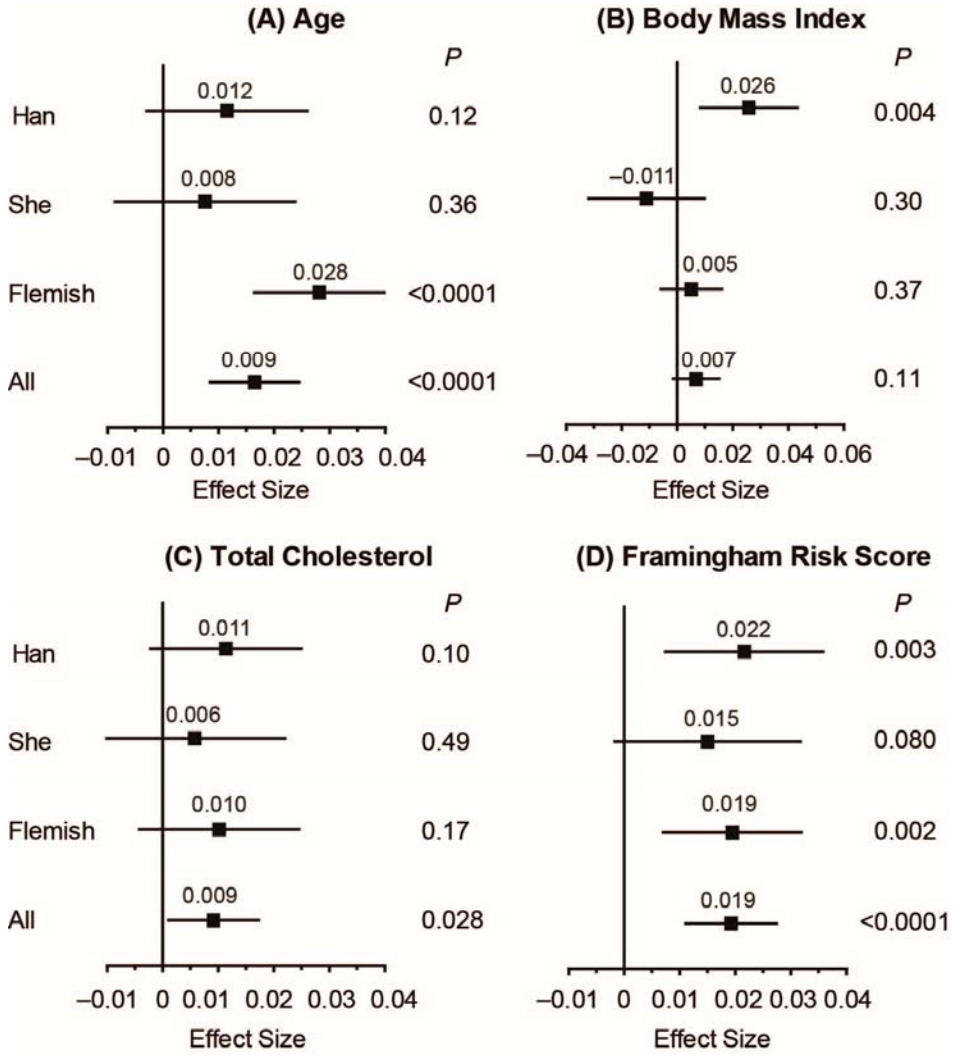

Figure S5. Association of the perfused-to-total capillary density ratio with age (A), body mass index (B), total cholesterol (C) and Framingham risk score (D) in 614 participants without antihypertensive drug treatment (221 Han, 182 She and 211 Flemish). Estimates, presented with 95% confidence interval, are for a 1–SD increase in continuous explanatory variables (see Table 1). *P*–values on the figure panel are for the significance of the associations. None of the comparison between ethnicities in the association sizes, adjusted for multiple testing using Bonferroni method reached significance ($P \geq 0.11$) with the exception of the comparison between Han and She in the association of the perfused-to-total capillary density with body mass index ($P = 0.026$).

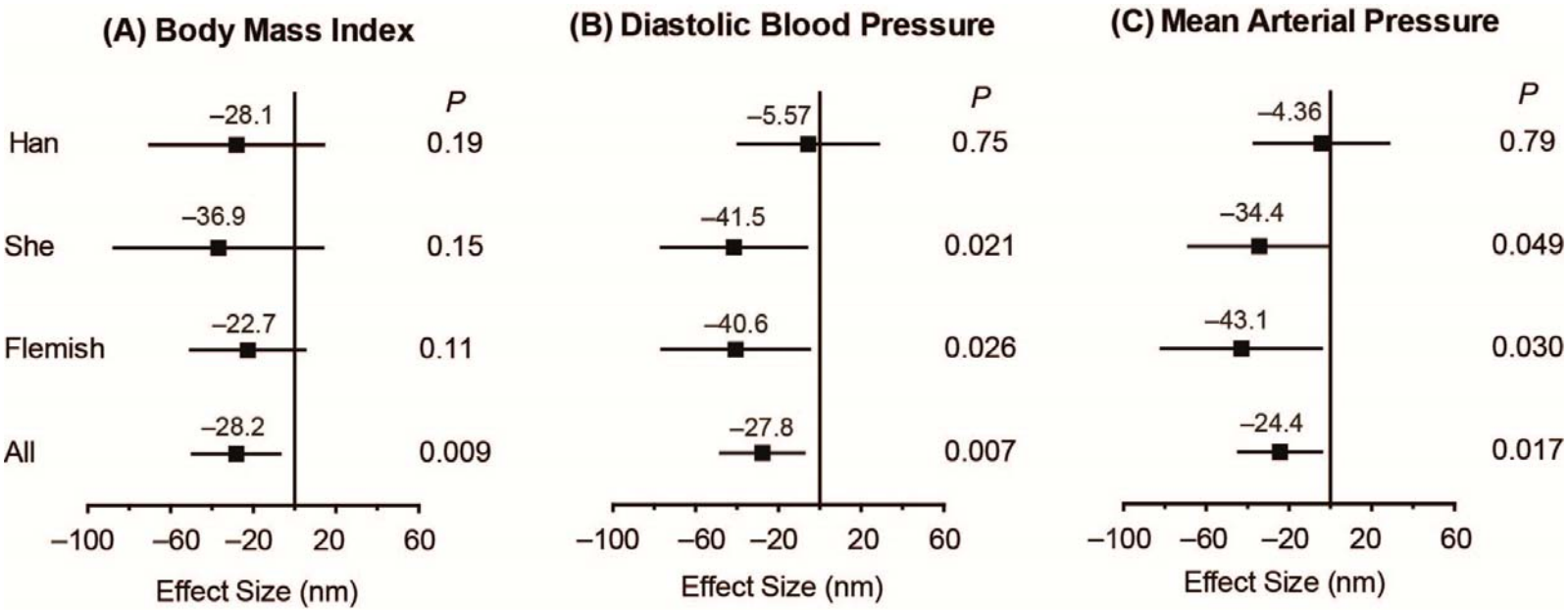

Figure S6. Association of the standardized perfused boundary region with body mass index (A), diastolic blood pressure (B) and mean arterial pressure (C) in 614 participants without antihypertensive drug treatment (221 Han, 182 She and 211 Flemish). Estimates, presented with 95% confidence interval, are for a 1–SD increase in continuous explanatory variables (see Table 1). *P*–values on the figure panel are for the significance of the associations. None of the comparison between ethnicities in the association sizes, adjusted for multiple testing using Bonferroni method reached significance.