

Vascular health assessment with flow-mediated dilatation and retinal image analysis: a pilot study in an adult population from Cape Town

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Abstract

Aim: Flow-mediated dilatation (FMD) and retinal vascular analysis (RVA) may assist in predicting cardiovascular disease (CVD) but are poorly characterised in South Africa. We recorded baseline FMD and retinal vascular widths in healthy participants, and investigated associations with cardiovascular risk factors.

Methods: Endothelial function (measured with FMD), microvascular structure (evaluated via fundus image analysis) and major CVD risk factors were assessed in 66 participants from Cape Town.

Results: Median FMD% was 9.6%, with higher values in females. Mean retinal arteriolar and venular widths were ~156 and ~250 μm , respectively. FMD was not associated with CVD risk factors. Hypertension was associated with narrower retinal arterioles and venules.

Conclusions: We report novel baseline FMD data in healthy South African adults from the Western Cape, and show that retinal microvascular calibres are associated with blood pressure. Our baseline FMD and RVA data could serve as a reference for future studies in South Africa.

Keywords: vascular health, endothelial function, flow-mediated dilatation, retinal imaging, cardiovascular risk.

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Cardiovascular disease (CVD) accounts for an estimated 17.7 million deaths globally, of which more than 70% occur in low- and middle-income countries such as South Africa.¹ The prevalence of CVD varies among different regions and populations, and while a steady decline has been noted in many high-income countries, this has not been the case in large parts of sub-Saharan Africa.²

In the Western Cape Province of South Africa, where Cape Town is located, cardiovascular and cardiometabolic diseases account for a large proportion of the primary causes of death, including diabetes mellitus, ischaemic heart disease and cerebrovascular disease.³ According to the South African Medical Research Council (SAMRC) Western Cape Mortality Profile 2013, cardiovascular diseases accounted for 24.3% of all deaths across all age groups in the province (compared to 23% of deaths attributed to infectious diseases).⁴ Previous studies have reported a high prevalence of cardiovascular risk factors in Cape Town populations, including type 2 diabetes mellitus, metabolic syndrome, hypertension and smoking.^{5,6}

Many traditional cardiovascular risk factors have been implicated in the development of endothelial dysfunction (ED).^{7,8} ED, characterised by a loss of vascular homeostasis and reduced nitric oxide (NO) bioavailability, is an early precursor of atherosclerosis and a marker and predictor of CVD.^{9–14} Flow-mediated dilatation (FMD) has become the gold-standard non-invasive assessment tool for endothelial function measurement in clinical research.¹⁵ In previous studies, mainly in populations from developed countries, FMD has been shown to be associated with cardiovascular risk factors^{11,16,17} and future CVD events.^{11,12,14} Yet, despite its potential as a clinical tool that can serve as a surrogate marker of CVD risk, we are not aware of studies utilising FMD in adult South African populations.

Retinal microvascular calibres, measured by non-invasive fundus photography and computerised analysis, are associated with long-term cardiovascular mortality and impaired endothelial function.^{18,19} The Multi-Ethnic Study of Atherosclerosis (MESA) reported that smaller retinal arteriolar calibre (central retinal arteriolar equivalent; CRAE) was associated with hypertension, whereas larger arteriolar calibre was associated with diabetes, current cigarette smoking and higher levels of plasma fibrinogen. Larger retinal venular calibre (central retinal venular equivalent; CRVE) has been shown to be associated with diabetes, current cigarette smoking, obesity, dyslipidaemia and systemic markers of inflammation and endothelial dysfunction.²⁰

There is a lack of retinal imaging studies in the South African context, particularly in populations of the Western Cape. One Cape Town-based study reported on retinal microvascular calibres in a cohort of HIV-infected participants,²¹ however, little is known about the relationship between retinal microvascular

and geometric characteristics and cardiovascular risk in South African populations.

The non-invasive assessment of vascular health with FMD and retinal imaging are deemed useful as a marker of cardiovascular risk and disease, yet there is a paucity of studies utilising these technologies in the South African research context. The growing interest in vascular and endothelial measurements as future diagnostic and screening tools in the clinical setting necessitates that baseline values are established for a variety of populations, including those living in low- to middle-income countries such as South Africa. In South Africa, current evidence suggests that the Western Cape Province has a particularly high prevalence of cardiovascular risk factors.

In view of the above, the present proof-of-concept study was undertaken to record baseline FMD and retinal microvascular and geometric data in a cohort of apparently healthy participants from Cape Town. The study additionally aimed to determine whether a relationship exists between FMD, retinal parameters and traditional cardiovascular risk factors in the study participants.

Methods

For this cross-sectional study, 66 HIV-free and otherwise apparently healthy participants were recruited from the Uitsig community health clinic near Cape Town between September 2014 and July 2015, as a pilot to a larger parent study.²² Participants were eligible for inclusion if they were adults (18 years or older), willing to give written consent for participation in the study and undergo HIV testing. Screening for HIV infection was performed with a rapid HIV test (SD Bioline HIV 1/2 3.0 immunochromatographic test kit; Standard Diagnostics, Republic of Korea). Participants were excluded if they were pregnant or tested positive for HIV infection.

The study received ethics approval from the Health Research Ethics Committee (HREC) of Stellenbosch University (HREC reference number: N13/05/064) and was conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council of South Africa Ethical Guidelines for Research. All participants supplied written, informed consent for participation in the study, and additionally provided written consent for HIV testing for which appropriate pre- and post-test counselling was provided by a qualified research nurse.

Information regarding participants' medical history, including smoking status, was gathered via a structured interview and health questionnaire. Additionally, participants were weighed and anthropometric measurements recorded to determine body mass index (BMI), waist circumference and waist-to-hip ratio (WHR) according to international guidelines.²³ A return visit was scheduled where participants were required to fast from 22h00 the previous night, and to refrain from smoking, drinking coffee or doing exercise for four to six hours prior to the assessments in order to comply with standard subject preparation recommendations for FMD measurements.²⁴

Blood samples were collected and immediately transported to the closest laboratory of the National Health Laboratory Services [a South African National Standard (SANS) accredited laboratory service provider] where the following fasting

biochemical measurements were performed: total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, glucose and glycated haemoglobin (HbA_{1c}). Appropriate assays were used for quantifying the concentrations of the above markers with a Roche/Hitachi cobas c 311, cobas c 501/502 analyser.

Subsequently, before endothelial function assessments were performed, systolic and diastolic blood pressures were measured in the left arm at three different occasions, two to five minutes apart, with an Omron M6 automatic digital blood pressure monitor (Omron Healthcare, Kyoto, Japan).

The FMD protocol is designed to expose the brachial artery to post-occlusion hyperaemia and the subsequent shear stress-induced release of NO and other pro-vasodilatory factors, resulting in vasodilation that can be imaged and quantified as an index of vasomotor function.²⁵ In this study, FMD assessment was performed using a MyLab™ Five mobile ultrasound system (Esaote, Italy). The FMD protocol was similar to previously published recommendations.^{25,26}

Participants were asked to lie supine on an examination bed, with their right arm abducted and supinated. A blood pressure cuff (deflated) was placed around the proximal part of the forearm. Subsequently, the ultrasound probe was positioned proximal to the cubital fossa (mid to distal humerus), just below the biceps brachii muscle belly, until the brachial artery was located visually on the ultrasound image. The ultrasound probe was secured in this position using a probe holder. Next, cross-sectional still images were captured with the ultrasound probe at three different locations along the designated section of the artery to obtain baseline brachial artery diameter measurements.

Following this, the blood pressure cuff was inflated to 200 mmHg (or 50 mmHg supra-systolic in the case of individuals with a systolic blood pressure greater than 150 mmHg) and blood flow to the forearm was occluded for five minutes. After the blood pressure cuff was deflated, additional cross-sectional ultrasound stills were taken along the same section of artery for a duration of two minutes.

For still analysis, the MyLab™ Five mobile ultrasound system built-in manual measurement tool was used to measure the brachial artery diameter in millimetres, consistently at the end of diastole, in all the stills. The three baseline measurements were used to calculate a mean baseline brachial artery diameter, and the maximum post-occlusion measurement, usually at approximately 60 seconds after blood pressure cuff release, was used to calculate the FMD percentage according to the following formula:

$$\text{FMD \%} = \frac{\text{maximum post-occlusion (diameter (mm))} - \text{mean baseline diameter (mm)}}{\text{mean baseline diameter (mm)}} \times 100$$

In order to ensure reliable data collection, the ultrasound operators were subjected to stringent training by experts and multiple practice sessions on student volunteers and colleagues. To keep inter-operator variability to the minimum, only three trained and experienced operators were employed in this study, and only one person independently performed the image analysis and data acquisition.

Retinal images were captured using a Canon CR-2 non-mydratic digital retinal camera (Canon Europa NV, the Netherlands) based on protocols previously published.²⁷ Optic disc-centred fundus images of both eyes were obtained. For the static retinal microvascular assessments, images were analysed using MONA REVA™ software version 2.0.2 (Vito, Belgium).

Vessel widths were calculated by measuring the six largest arterioles and six largest venules coursing through a zone between 0.5 and one disc diameter from the optic disc margin. Estimates are summarised as CRAE and CRVE, representing the average diameter (μm) of the arterioles and venules, respectively.²⁸ Standard procedure was to calculate the CRAE and CRVE values from the optic disc-centred image of the right eye. The left eye was used only when gradable right eye images were not obtained.

The retinal arteriolar-venular ratio (AVR) was calculated separately, which serves as a dimensionless measurement independent of the optical properties of the eye and camera. Additionally, fundus images were analysed by an independent retinal grading expert blinded to the participants' demographic, medical and other study results.

The presence or absence of the following parameters, all associated with possible underlying cardiometabolic disorders, were assessed qualitatively in a binary fashion: retinal tortuosity, cotton wool spots, retinal haemorrhage, telangiectasia and micro-aneurysms. The presence of any cotton wool spots, retinal haemorrhage, telangiectatic vessels or micro-aneurysms were counted as positive for each parameter, whereas retinal tortuosity was determined according to a pictorial grading scale, as previously published.²⁹

Statistical analysis

All data were statistically analysed with Statistica™ version 13.3 (TIBCO Software Inc, CA, USA). Continuous variables with normal distributions are expressed as mean (standard deviation) or mean [95% confidence interval (CI)], and non-parametric data as median (interquartile range). Independent *t*-tests and Mann-Whitney *U*-tests (with continuity correction) were used to compare parametric and non-parametric continuous variables, respectively, among male and female groups, and the Fisher exact (two-tailed) test to compare categorical variables. Correlations between continuous cardiometabolic variables and vascular variables (FMD%, CRAE, CRVE and AVR) were evaluated by Pearson's correlation coefficient (non-parametric data were normalised by logarithmic transformation where indicated). The relationship between categorical cardiovascular risk factor variables (expressed as 'yes' or 'no') and vascular variables was evaluated by analysis of co-variance (ANCOVA) after adjusting for age and/or gender where indicated. Statistical significance was set at $p < 0.05$ for all statistical models.

Results

The study enrolled 66 participants (55% female) with a mean age of 35.4 (10.6) years. Continuous demographic, anthropometric, biochemical and vascular data for the whole cohort are depicted in Table 1, in which additional comparisons are made between the male and female subsets. Mean WHR, median BMI and median waist circumference values for the whole cohort fell well within the normal range. In the male subset in particular, BMI and waist circumference values were in the lower margins of the normal range, and male participants had significantly smaller BMI and WHR values compared to females.

Concerning biochemical variables, female participants had significantly higher triglyceride levels compared to males. For males, baseline brachial artery diameter values were higher, and

FMD% values were lower compared to females. Additionally, 38.2% of females and 62.9% of males presented with an FMD% lower than the sample median ($p = 0.05$). Mean retinal vessel diameters were trending higher for CRVE in males, while there were no differences noted for CRAE or AVR.

In a separate set of retinal assessments, qualitative fundus grading and analyses of retinal vessel geometric characteristics showed that retinal tortuosity was present in approximately 18% of the participants, and was predominantly detected in the retinal arterioles. Furthermore, a single micro-aneurysm was identified in one participant, while no cotton wool spots, retinal haemorrhage or telangiectasia were observed in this cohort.

The prevalence of cardiovascular risk factors is shown in Fig. 1. Of the whole cohort, 86.4% of participants indicated that they were current smokers, and 31.8% presented with systolic hypertension, with 40.7% of males being hypertensive. Diastolic hypertension was identified in 23.8% of the study population and in 29.6% of the male subset. Of the female subset, 36.1% were considered overweight or obese and 30.6% presented with central obesity. Reduced HDL-C levels were found in 28.8% of the whole cohort and in 38.9% of females, compared to 16.7% of males.

As expected, an inverse correlation was observed between baseline brachial artery diameter and FMD% (Pearson's correlation coefficient, $r: -0.33$; $p = 0.009$), and a strong positive correlation was noted between the retinal blood vessel equivalents, CRAE and CRVE (Pearson's correlation coefficient, $r: 0.5$; $p < 0.001$).

The correlations between cardiometabolic and vascular (FMD%, CRAE and CRVE) variables in the whole cohort are shown in Table 2. The results indicate that waist circumference showed a positive correlation with FMD%, although there was no correlation with WHR. Both systolic and diastolic blood

Table 1. Population characteristics

Variables	Whole cohort (<i>n</i> = 66)	Females (<i>n</i> = 36)	Males (<i>n</i> = 30)	<i>p</i> -value (<i>F</i> : <i>M</i>)
Age (years)	35.4 (10.6)	34.1 (10.6)	36.9 (10.5)	0.29
WHR	0.8 (0.05)	0.8 (0.05)	0.8 (0.04)	0.01
BMI (kg/m^2)	22.1 (19.3–24.7)	23.4 (19.8–27.2)	20.7 (18.8–23.9)	0.03
WC (cm)	75.5 (69–81)	76 (70.5–80.25)	74 (68–81)	0.49
SBP (mmHg)	129.9 (16.9)	127.8 (16.2)	132.9 (17.7)	0.24
DBP (mmHg)	79.6 (14.1)	78.1 (13.9)	81.5 (14.3)	0.33
Total C (mmol/l)	3.8 (3.3–4.4)	4 (3.2–4.7)	3.7 (3.3–4.3)	0.54
HDL-C (mmol/l)	1.25 (1–1.5)	1.3 (1–1.6)	1.2 (1–1.4)	0.38
LDL-C (mmol/l)	2.1 (1.7–2.6)	2.2 (1.7–2.6)	1.9 (1.5–2.6)	0.17
TG (mmol/l)	0.8 (0.6–1)	0.8 (0.6–1)	0.9 (0.7–1.2)	0.04
Fasting glucose (mmol/l)	4.5 (4.2–4.9)	4.45 (4.05–4.7)	4.5 (4.3–4.9)	0.21
HbA _{1c} (%)	5.4 (0.5)	5.4 (0.5)	5.5 (0.4)	0.27
CRAE (μm)	156.2 (14.5)	155.3 (12.9)	157.2 (16.4)	0.61
CRVE (μm)	250.3 (21)	247.4 (18.6)	253.6 (23.5)	0.07
AVR	0.6 (0.06)	0.6 (0.06)	0.6 (0.05)	0.53
Baseline brachial artery diameter (mm)	3.5 (0.6)	3.2 (0.5)	3.9 (0.5)	< 0.001
FMD (%)	9.6 (6.7–14.1)	11.4 (7.7–15.8)	8.6 (3.4–12.5)	0.06

Parametric data are expressed as mean (standard deviation) and non-parametric data as median (interquartile range). WHR, waist-to-hip ratio; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Total C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HbA_{1c}, glycated haemoglobin; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, retinal arteriolar-to-venular ratio; FMD, flow-mediated dilatation; *F*:*M*, female vs male.

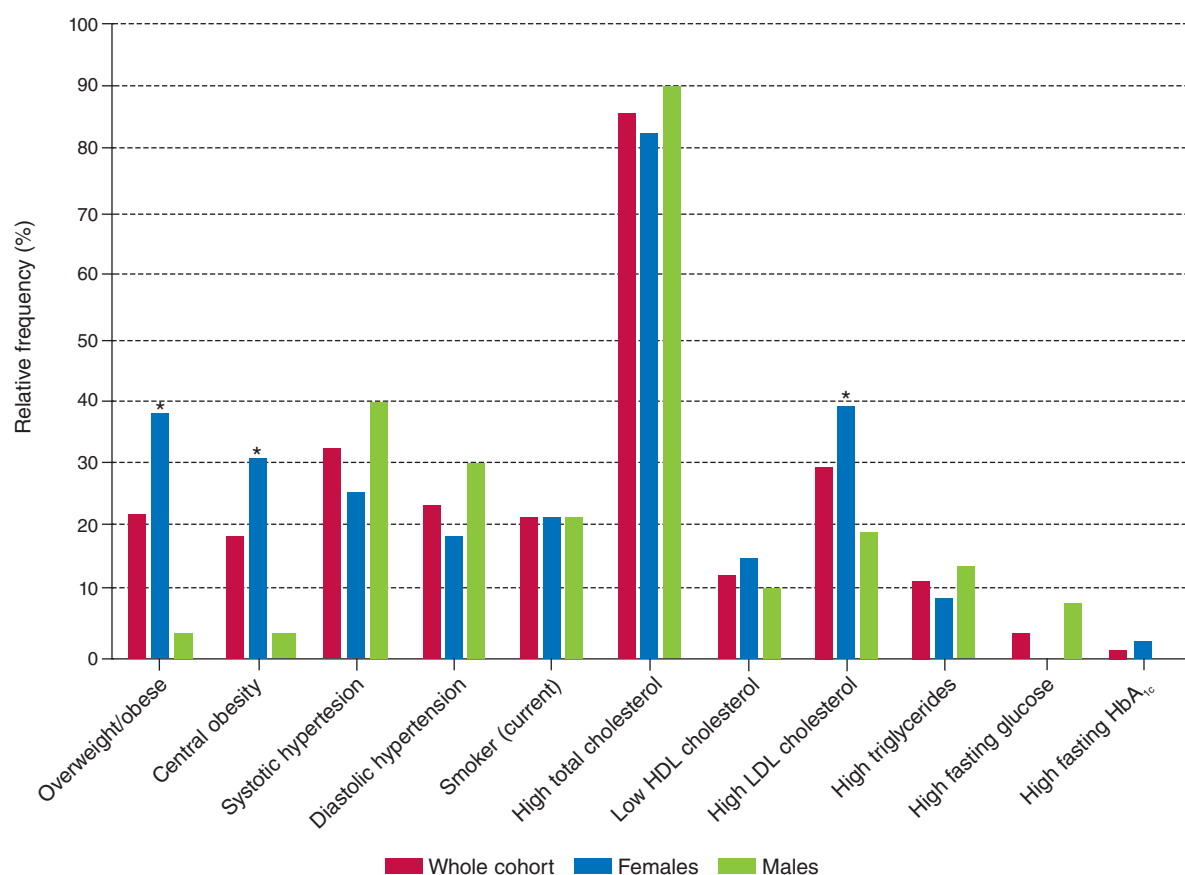


Fig. 1. Relative frequency of cardiovascular risk factors in the whole cohort, and in female and male subsets. Overweight/obese: BMI ≥ 25 kg/m²; central obesity: waist circumference ≥ 94 cm for males and ≥ 80 cm for females; systolic hypertension: ≥ 140 mmHg; diastolic hypertension: ≥ 90 mmHg; high total cholesterol: > 5.1 mmol/l; low HDL-C: < 1 mmol/l for males and < 1.2 mmol/l for females; high LDL-C: > 3 mmol/l; high triglycerides: ≥ 1.7 mmol/l; high fasting glucose: ≥ 7 mmol/l; high HbA_{1c}: $\geq 6.5\%$. Cut-off values for the cardiovascular risk factors are from previously published guidelines adapted from the European and International Societies for Hypertension and the International Diabetes Foundation.^{30,31} * $p < 0.05$ females vs males.

pressure correlated inversely with CRAE, while triglycerides correlated positively with CRVE. None of the cardiometabolic variables correlated with AVR. Furthermore, correlation analyses failed to show a relationship between FMD% and the retinal microvascular calibres (not shown). Participants in whom retinal tortuosity was positively identified, had significantly higher diastolic blood pressure values compared to those without retinal tortuosity [data expressed as mean (95% CI): tortuosity present: 85.4 (80.2–90.6) mmHg vs tortuosity absent: 78.3 (76–80.5) mmHg; $p = 0.01$].

Relationships between cardiovascular risk factors and vascular variables were tested with ANCOVA (all models adjusted for age). Overweight or obese participants (BMI ≥ 25 kg/m²) had significantly higher FMD% compared to normal-weight counterparts [data expressed as mean log FMD% (95% CI): overweight: 1.1 (0.9–1.3) vs normal weight: 0.9 (0.8–0.9); $p = 0.03$]; however the significance disappeared when the model was additionally adjusted for gender. Similarly, participants with high total cholesterol levels (> 5.1 mmol/l) presented with increased FMD% compared to those with normal cholesterol values [mean log FMD% (95% CI): high total cholesterol: 1.2 (0.9–1.5) vs normal total cholesterol: 0.9 (0.8–0.9); $p = 0.03$], but the significance was lost when additionally adjusting

for gender. No other cardiovascular risk factors were associated with changes in FMD%.

The presence of systolic hypertension (systolic blood pressure ≥ 140 mmHg) was associated with significantly decreased CRAE (Fig. 2A), which was not affected by additional adjustment for gender, however, the significance was lost when CRVE was added as an adjustor (not shown). CRVE was significantly lower in participants with systolic hypertension (Fig. 2B), even when additionally adjusting for gender, however, the significance disappeared after including CRAE as a covariate in the model (not shown).

Diastolic hypertension (diastolic blood pressure ≥ 90 mmHg) was associated with significantly decreased CRVE, and although significance was not affected when additionally adjusting for gender, the inclusion of CRAE in the model moderated the significance level to $p = 0.057$ (not shown). There were no associations observed between any of the cardiovascular risk factors and AVR.

Discussion

Evidence emanating from both official statistical sources and

Table 2. Correlations between cardiometabolic and vascular variables in the cohort (*n* = 66)

Variables	FMD%	CRAE	CRVE	AVR
BMI				
<i>r</i>	0.24	0.04	0.04	0.005
<i>p</i>	0.06	0.76	0.78	0.97
WC				
<i>r</i>	0.27*	0.02	0.15	-0.11
<i>p</i>	0.04	0.84	0.24	0.39
WHR				
<i>r</i>	0.02	-0.04	0.2	-0.22
<i>p</i>	0.87	0.74	0.12	0.09
Systolic BP				
<i>r</i>	0.09	-0.35*	-0.23	-0.16
<i>p</i>	0.48	0.01	0.08	0.22
Diastolic BP				
<i>r</i>	0.1	-0.26*	-0.2	-0.09
<i>p</i>	0.44	0.04	0.13	0.5
Total C				
<i>r</i>	0.12	0.02	0.02	0.007
<i>p</i>	0.34	0.85	0.86	0.95
HDL-C				
<i>r</i>	-0.04	-0.01	-0.05	0.03
<i>p</i>	0.75	0.91	0.68	0.84
LDL-C				
<i>r</i>	0.24	0.01	-0.03	0.04
<i>p</i>	0.06	0.92	0.83	0.72
Triglycerides				
<i>r</i>	-0.21	0.18	0.28*	-0.07
<i>p</i>	0.1	0.16	0.02	0.58
Fasting glucose				
<i>r</i>	-0.22	0.05	0.03	0.03
<i>p</i>	0.09	0.67	0.8	0.81
HbA_{1c}				
<i>r</i>	0.08	0.15	0.17	0.02
<i>p</i>	0.55	0.23	0.19	0.88

The following variables with a skewed distribution were logarithmically transformed: FMD%, BMI, waist circumference, total cholesterol, HDL-C, LDL-C, triglycerides and fasting glucose.

BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; BP, blood pressure; Total C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA_{1c}, glycated haemoglobin; FMD, flow-mediated dilatation; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, retinal arteriolar-venular ratio; *r*, Pearson's correlation coefficient; *p*, *p*-value. *Significant.

research studies is pointing to a high prevalence of cardiometabolic diseases and risk factors in Cape Town and the Western Cape Province of South Africa,^{3,6} supporting global trends that low- to middle-income countries carry a high cardiovascular disease burden. The present study collected data from volunteer participants recruited at the Uitsig health clinic, which serves a predominantly low socio-economic status (SES) community outside Cape Town. In recent years, research has increasingly uncovered a link between SES and cardiovascular health, with low-SES individuals being at a greater cardiovascular risk.³²

Detecting early markers of CVD is critical in the management of the disease burden in high cardiovascular risk populations. The assessment of vascular changes can be particularly useful in this regard, with FMD previously being shown to be a marker of future cardiovascular events,^{11,12,14} and the quantitative analysis of retinal microvasculature linked to the development of CVD.³³ Both these non-invasive vascular assessment techniques are new to clinical research in South Africa.

In summary, the results of the present study, conducted

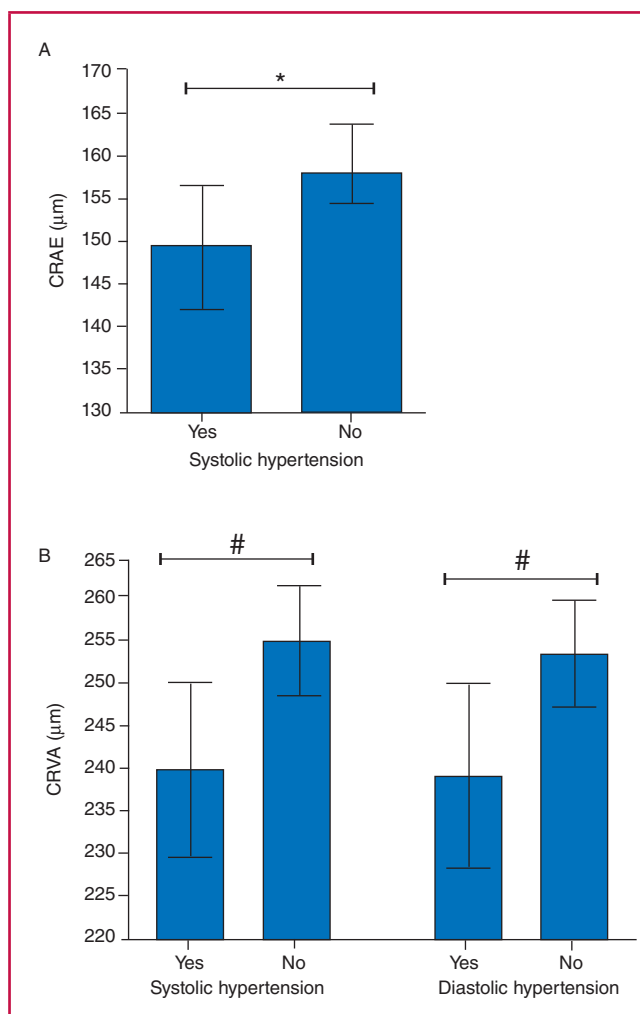


Fig. 2. Relationships between cardiovascular risk factors and retinal vascular variables. A. Effect of systolic hypertension (systolic blood pressure ≥ 140 mmHg) on CRAE; **p* = 0.03. B. Effects of systolic and diastolic hypertension (diastolic blood pressure ≥ 90 mmHg) on CRVE; #*p* = 0.02. Data are expressed as mean (95% CI); all ANCOVA models adjusted for age.

in a relatively young adult population with a high smoking prevalence, show that the FMD% and CRAE values were in line with those previously reported in other populations, whereas comparisons of the CRVE values with those from other studies were more varied. Furthermore, as previously shown by others, female participants had increased FMD% and decreased baseline brachial artery diameters compared to their male counterparts. With the exception of modest relationships with waist circumference and elevated BMI, FMD% showed no association with any of the measured cardiometabolic variables. The retinal microvascular calibres in our study cohort were associated with blood pressure, which is in keeping with findings from previous studies, and the presence of retinal tortuosity was associated with increased diastolic blood pressure. There was no relationship between FMD% and the retinal microvascular calibres in this cohort.

Although no internationally standardised cut-off values exist for FMD, the median FMD% recorded in the present cohort

(9.6%) (Table 1) falls within the range of values reported in a systematic review and meta-analysis comprising 23 studies and over 15 000 participants, where the respective FMD values varied between 2.3 and 13.8%.¹² In our study, females had significantly higher FMD% values compared to males (Table 1). This agrees with findings from the Framingham Heart Study, which showed similar trends in their cohort,³⁴ and emphasises the importance of taking gender differences into account when measuring FMD in study populations. However, this phenomenon may be explained by the significantly larger baseline artery diameters measured in the male participants compared to females, which has also been shown by others.^{16,34}

Body weight parameters appeared to be associated with FMD%, as suggested by a modest correlation with waist circumference (Table 2), and observing higher FMD% values in participants with BMI ≥ 25 kg/m². However, there was no correlation when waist circumference was expressed as a ratio of hip circumference (WHR) (Table 2) and the age-adjusted association with overweight/obesity was lost when additionally adjusting for gender. Furthermore, the presence of central obesity had no effect on FMD% and no correlation was observed between BMI and FMD%. Taken together, the results show that body weight parameters were not strongly associated with FMD%, likely due to the relatively young mean age of the cohort and the fact that waist circumference, WHR and BMI values were well within the normal ranges.

A previous study by Brook *et al.*³⁵ also failed to show a significant relationship between BMI and FMD%, while noting that the WHR was inversely associated with FMD%. Although another study showed an inverse association between BMI and FMD%, the study population included subjects with only severe obesity,³⁴ which was not the case in our cohort. Participants with high total cholesterol levels (> 5.1 mmol/l) had increased FMD% values, however, this age-adjusted relationship disappeared when the model was additionally adjusted for gender, and was therefore unlikely to be of physiological relevance. None of the other lipid and cardiometabolic variables showed relationships with FMD.

In the present study cohort, mean CRAE values corresponded reasonably well with those reported in a systematic review and meta-analysis by Ding *et al.*³⁶ comprising over 10 000 participants, and studies conducted in the North West Province of South Africa.^{37,38} The median CRVE value in our cohort (253.6 μ m) (Table 1) was higher than the range of CRVE values (192.3–231.2 μ m) reported by Ding *et al.*³⁶ On the other hand, the present cohort's CRVE compared reasonably well with that of a study in a cohort from the North West Province in South Africa,³⁸ and tended to be lower than that measured in one of the only other Western Cape Province-based studies investigating retinal microvascular calibres in a Cape Town population.²¹

The apparent inter-study inconsistency may be related to differences in retinal image analysis procedures and methodology, including different software packages used for analysis, as reported previously.³⁹ In addition, the discrepancy may also be ascribed to our cohort's relatively young mean age of around 35 years, compared to a mean age range of between 50 and 61 years in the meta-analysis study of Ding *et al.*,³⁶ as previous reports have shown that retinal venular diameters narrow with increasing age.⁴⁰ The higher smoking prevalence in our cohort (~86%) (Table 1) compared to the smoking rates in the studies reviewed by Ding *et al.*³⁶ (11.5–23.7%) may also explain the higher CRVE,

since cigarette smoking has been shown to be strongly associated with wider retinal venular calibres.²⁰

In our cohort, there was an inverse correlation between CRAE and systolic and diastolic blood pressure (Table 2). In addition, participants with systolic hypertension had lower CRAE values compared to normotensive participants (Fig. 2A), which is in accordance with findings by others, suggesting that narrower retinal arteriolar width may be a marker of hypertension.^{20,36,41,42} This finding underscores the potential value of retinal screening in young adults, such as the current cohort, who may not yet clinically present with hypertension. Results also show decreased CRVE values in participants with systolic and diastolic hypertension compared to normotensive participants (Fig. 2B), which is in agreement with observations made in a previous study.⁴³

However, the relationship between hypertension and retinal venular narrowing is controversial, with some authors arguing that venular narrowing may be confounded by concomitant arteriolar narrowing.⁴⁴ In the present cohort, additional adjustment for CRAE resulted in a loss of significance in the systolic hypertension model, while a borderline significance was maintained in the diastolic hypertension model. Fasting triglyceride levels also showed a significant relationship with wider retinal venules (Table 2), as previously shown by others.⁴²

To further investigate the presence of abnormal retinal features and their potential association with cardiometabolic variables, the retinal images of the present cohort were subjected to qualitative fundus grading, which identified retinal tortuosity (mostly arteriolar) in around 18% of the participants. Results showed that participants with retinal tortuosity had increased diastolic blood pressure compared to participants with no signs of tortuosity. Retinal arteriolar tortuosity has previously been associated with increased systolic and diastolic blood pressure.^{45,46}

The demonstration of an association between retinal microvascular changes and endothelial dysfunction of systemic arteries has important implications, as it may provide an opportunity to use the retinal microvasculature as a surrogate marker of systemic vascular disease. We could not demonstrate an association between CRAE or CRVE and FMD% in our cohort. In the literature, the relationship between retinal microvascular calibre and FMD% remains generally inconclusive, however, one previous study did demonstrate an independent association between CRVE and systemic endothelial dysfunction as measured by brachial FMD.³³

Limitations

The study has shortcomings that need to be considered when interpreting the findings. This was intended to be a pilot study to obtain baseline data in a generally disease-free group of participants, hence the relatively small sample size. This placed limitations on some of the statistical analyses, where a number of borderline significant *p*-values (0.05–0.08) were noted. A larger sample size may have generated more significant outcomes and allowed for the inclusion of more sophisticated and robust association analyses such as multiple regression models.

Furthermore, it has to be acknowledged that the cohort was recruited from a relatively restricted geographical location with limited demographic variability, which limits the extent to which the data can be regarded as representative of the wider South

African population. Despite this, it was interesting to note the comparability of our baseline FMD and retinal measurements with those from previously published studies.

The FMD procedure is technically challenging, requires thorough training and may suffer from operator-dependent variability.^{24,25,47} We would therefore recommend the use of computerised vessel edge-detecting/wall-tracking software systems for future studies, as computerised analysis has been suggested to improve the reproducibility.⁴⁸ Although we have addressed this as far as possible, as described in detail in the methods section, these potential constraints need to be acknowledged.

Conclusion

We present here, for the first time, FMD data in an apparently healthy adult South African cohort from the Western Cape Province. In this cohort, a median FMD of 9.6% was recorded, which compares reasonably well with previously published data in different populations, mainly from Europe and North America. The findings also confirmed previous reports that FMD values appear to be lower in males compared to females. It is proposed that the current FMD values could serve as a starting point of reference for future studies from South Africa and the sub-Saharan African region.

The retinal arteriolar and venular calibre measurements recorded in our cohort are reasonably comparable to those from other studies and will serve to add to a small but growing database of published retinal microvascular data from other South African researchers. In agreement with the literature, narrower retinal microvascular calibres in our cohort were associated with elevated blood pressure, and in a novel finding in the South African context, we showed that participants presenting with retinal tortuosity had increased diastolic blood pressure compared to those without tortuosity. These findings further support the use of non-invasive retinal image analysis in cardiovascular epidemiology research.

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