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A Challenged Sympathetic System Is Associated with Retinal Vascular Calibre in a Black Male Cohort: The SABPA Study

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Additional information is available at the end of the chapter

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Abstract

Sympathetic system hyperactivity and depression are related to cardiac remodelling in Black men. We investigated whether sympathetic system hyperactivity and depressive symptoms are related to retinal vascular dysregulation. A total of 76 Black and 83 White men (23–68 years of age) from the SABPA study were included. Depressive symptoms, 24h pulse pressure (PP), fasting blood and 24-hour urinary catecholamine data were obtained. Retinal vascular calibre was quantified from digital photographs using standardized protocols. Black men demonstrated increased ($p < 0.05$) hyperpulsatile pressure (PP > 50 mmHg), hypertension (78.9 % vs 48.4%) and depression (34.2% vs. 13.3%) prevalence compared to White men. Despite lower epinephrine levels, epinephrine was associated with arteriolar narrowing and venular widening in the Black men [Adj R² -0.37 (95% CI: -0.66, -0.09), $p=0.013$; Adj R² 0.35 (95% CI: 0.13, 0.57), $p=0.003$]. This might suggest β -adrenergic hyporesponsivity to epinephrine, which was accompanied by hyperpulsatile blood pressure in the Black group. In the White group, depressive symptoms and norepinephrine were associated with retinal arteriolar narrowing. A profile of β -adrenergic hyporesponsivity, indicative of a chronically challenged sympathetic system, was associated with retinal vascular remodelling in Black men. β -adrenergic hyporesponsivity as a result of chronic stress emphasized central control of the brain on the circulatory system irrespective of the vascular bed.

Keywords: Africans, retinal microvascular calibre, 24 h urinary epinephrine, depressive symptoms, ethnicity

1. Introduction

South Africa is facing an epidemic of hypertension (HT) and vascular disease but there still is inadequate information on the physiological factors that are contributing to this process [1, 2]. Microvascular disease seems to play an important role in the development of HT, arterial stiffness and structural remodelling [3]. Currently, HT is regarded as the most important modifiable risk factor for stroke and major macrovascular cerebral complications, but it may also predispose to more subtle cerebral processes based on, amongst others, the microcirculation [4, 5]. Both the ophthalmic artery and the anterior cerebral artery originate from the internal carotid artery and most likely will share common characteristics [6]. Therefore the retinal microvasculature may be an ideal structure to study these abnormalities [7]. Longitudinal studies have shown that an inverse association exists between reduced retinal arteriolar calibre and HT in ageing populations, whilst retinal venular dilation is associated with stroke risk [7, 8]. A higher ratio from either wider retinal arteriolar calibre or narrower retinal venular calibre or both is an index of a better retinal vessel profile [9]. Ref. [8] found racial differences in retinal microvascular calibre of various Asian population groups but whether that is also true for Black and White African men is not clear [8]. In a study using Doppler imagery and iontophoresis of acetylcholine and sodium nitroprusside, it was, however, reported that, after correcting for skin resistance in a Black African group, endothelium-independent microvascular function of Black Africans is attenuated compared to that of White Africans [10]. This might be a contributing factor to the ethnic differences in microvascular disease risk in South Africa.

Enhanced peripheral resistance vascular α -adrenergic responses on exposure to a laboratory stressor, i.e. the handgrip test, were shown in Black Africans during urbanisation when compared to their rural counterparts [11]. Thus overstimulation of the sympathetic nervous system (SNS) and the sympathetic adrenal cortex and medullary stress hormone pathway may explain some of the observed ethnic differences [11–13]. Intense emotional stress may induce sympathetic hyperactivity with persistent increases in catecholamine and cortisol levels, which is detrimental to normal physiological processes [13]. However, during chronic stress this initial hyperactivity may be followed by autonomic exhaustion or depression, receptor hyporesponsivity and decreases in catecholamines and cortisol [14–18]. Phenylethanolamine N-methyltransferase (PNMT) is an enzyme found in the adrenal medulla which converts norepinephrine to epinephrine. PNMT is known to be regulated by glucocorticoids synthesised in the adrenal gland [19]. One-way PNMT expression can be regulated by corticosterone's positive influence on the maintenance of PNMT mRNA [20]. Chronic depression has been related to attenuated cortisol levels which will lead to a decrease in the synthesis of epinephrine [21]. These alterations in autonomic function are of importance as they have been associated with both depression and cardiovascular pathology [14, 15]. Moreover, chronic psychosocial stress often precedes depression [22] which, in turn, has recently been acknowledged as a risk factor for cardiac remodelling and poor prognosis in patients with coronary heart disease [23]. Indeed, decreased cortisol and catecholamine metabolite responses to a mental stressor were risk factors for the development of vascular diseases in a Black African cohort

exhibiting symptoms of depression [24]. There still remains no clear cut or generally accepted model for cortisol responses in depression, as both blunted and increased cortisol activities have previously been noted [21, 25]. Blunted cortisol responses were apparent in individuals with depressive symptoms *after* exposure to the Stroop test [13]. This could imply that the presence of depressive symptoms sensitises the individual to stress and the subsequent development of vascular disease and/or other lifestyle illnesses. Blunted cortisol responses to laboratory and psychosocial stressors have been demonstrated in both clinical and subclinical depression [26, 27]. However, it could be speculated that since depression is a constant state of perceived stress, further exposure to a challenging urban environment or psychosocial stress may result in habituation of the neuroendocrine pathways [28].

The 24 h urinary catecholamines and depressive symptoms might, therefore, indicate a challenged SNS associated with retinal microvascular calibre in an urban-dwelling cohort. Whether sympathetic innervation of the retinal vessels exists, is still being debated although it was recently demonstrated that the choroid of the uvea is densely innervated by the sympathetic system and that both α - and β -adrenergic innervations were demonstrated in the precular central retinal artery (CRA) in humans [29]. The optic canal is a regular conduit for autonomic nerves of the internal carotid plexus to the eye. However, the possible distribution of α - and β -adrenergic receptors in the arterioles of the CRA is still unknown. Generally, in resistance vessels, vasoconstriction is mediated via α_1 - and α_2 -adrenergic receptors whilst β_2 -adrenergic receptors mediate vasodilation [2]. It was recently shown that the CRA receives adrenergic and cholinergic innervation supporting autoregulation of intra-retinal vessels [29]. Systemic sympathetic transmitter spillover (epinephrine and norepinephrine) in the carotid and retinal vasculature may thus impact on retinal perfusion. Indeed, Ref. [30] reported associations of psychosocial risk factors and depression with retinopathy signs (microaneurysms, retinal or vitreous haemorrhages, soft or hard exudates or intra-retinal microvascular abnormalities) and suggested the presence of adrenergic receptors in retinal vessels.

They further demonstrated that heterogeneity in psychosocial effects could result from greater vulnerability of subjects with diabetes and HT due to underlying vascular damage associated with these conditions. This appeared to be the case for symptoms of depression, which had a stronger association with retinopathy in subjects with HT compared with those without, 60% versus 30% greater odds of retinopathy [30].

Chronic stress, as presented by depressive symptoms, may thus induce chronic stimulation of the SNS and initial hyperactivity may be followed by autonomic exhaustion, receptor hyporesponsivity and decreases in catecholamines resulting in hyperkinetic blood pressure (BP) values and receptor hyporesponsivity and decreases in catecholamine levels [15, 16, 31]. The main purpose of this study was, therefore, to assess the associations between retinal microvascular calibre, as primary endpoint and systemic adrenergic neurotransmitters and depressive symptoms, in a bi-ethnic cohort of South African men.

2. Main body of paper

2.1. Materials and methods

2.1.1. *Design and participants*

Urban Black and White African teachers were recruited as part of the prospective Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study [32]. All participants of the first phase of SABPA (2007–2008) were invited to participate in the follow-up. Their ages varied between 23 and 68 years. Of the initial 204 male participants in the first phase, 180 men reported for the second phase where, additionally, retinal blood vessel measurements were obtained. Men are more prone to the development of cardiovascular disease (CVD); therefore, only men were included in order to obtain a homogenous high CVD risk cohort [1, 2].

We excluded one participant with a history of epilepsy and 20 participants who did not have usable retinal microvascular images. Finally we included a total of 76 Black and 83 White Africans in the study. Participants were fully informed about the objectives and procedures of the study prior to their recruitment. All participants provided written, informed consent. The study conformed to the Helsinki Declaration (2007) and was approved by the Ethics Review Board of the North-West University, Potchefstroom Campus (approval number 0003607S6).

2.1.2. *Assessment of health behaviour*

Participants were in a semi-recumbent position from 07 h15 for at least 2 h during which the 12-lead ECG (NORAV PC 1200) registration was performed followed by blood sampling. Physical activity was assessed with the Actiheart® (GB0/67703, CamNtech Ltd., Cambridge-shire, UK) monitors considering resting metabolic rate. The 12-lead ECG resting heart rate was used to calculate the sleep heart rate required by the Actiheart programme. Quantitative assessment of some markers was done to determine smoking status (cotinine, a nicotine metabolite) and alcohol consumption levels (gamma glutamyl transferase, γ -GT) [33]. All anthropometric measurements were performed in triplicate by registered level II anthropometrists according to standardised procedures. The body mass index (BMI) as well as body surface area (BSA) was calculated. BSA was based on the Mosteller formula [34]. Intra- and inter-variability was less than 5%.

2.1.3. *Depressive symptoms*

The Patient Health Questionnaire (PHQ-9) was used to determine the depressive symptom score of the participants [35]. The PHQ-9 is a measure of depressive symptom severity and has been validated in various ethnic groups including sub-Saharan Africans [36]. The questionnaire is designed for use in primary health-care settings adapting diagnostic criteria from the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition). Each item of the PHQ-9 evaluates the presence of one of the nine DSM-IV criteria of major depression [35]. In the current study, the Cronbach alpha-reliability index for the total PHQ-9 score was 0.80. Items on the questionnaire are scored to reflect the frequency of symptom occurrence during

the prior two weeks on a scale of zero to three, with zero reflecting “not at all” and three “nearly every day,” thus providing continuous score between 0 and 27 [35]. Examples of questions are: “Feeling down/depressed/hopeless; feeling bad about yourself OR that you are a failure/that you have let yourself or your family down, thoughts that you would be better off dead/of hurting yourself in some way” [35]. The recommended and established PHQ-9 cut-off point of ≥ 10 was used to indicate the presence of depressive symptoms [35].

2.1.4. Cardiovascular measurements

On the morning of the first clinical assessment day, ABPM and 2-lead electrocardiograph monitors were attached to participants on the non-dominant arm at their workplace between 07 h00 and 07 h30 (Meditech CE120 CardioTens®; Meditech, Budapest, Hungary). The ABPM was programmed to measure BP at time intervals shown for assessing sympathetic activity at 30-min intervals during the day (07 h00–22 h00) and every hour during night time (22 h00–06 h00) [37]. The successful inflation rate over this period was 85.8% (± 9.14) in Africans and 90.4% (± 8.61) in Whites. Hypertensive status and CVD risk were classified from 24 h ABPM as systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg [38]. Hyperpulsatile pulse pressure (PP) was defined as 24 h SBP–24 h DBP > 50 mmHg [39]. The apparatus was removed after the last BP measurement at 07 h30 the next day.

2.1.5. Measurement of retinal vascular calibre

Static retinal microvascular measurements were performed in a well-controlled light and temperature regulated laboratory using an Imedos Retinal Vessel Analyser (Germany) with a Zeiss FF450^{Plus} camera and the VesselMap 1 Version 3.10 software. No intake of food or caffeine containing beverages, alcohol, smoking or exercise was allowed one hour prior to retinal vessel measurements. Participants were introduced to the procedure and screened for Acute angle-closure glaucoma risk with a small light source by a trained registered nurse. Mydriasis was induced in the right eye of the participant by means of a drop containing tropicamide 1% and benzalkonium chloride 0.01% (m/v). In the event of previous injury to the right eye, the left eye was used (Black men N = 3; White men N = 1). Retinal vascular calibre was measured in the monochrome images by manually selecting first-order vessel branches in a measuring zone located between 0.5 and 1.0 optic disc diameters from the margin or the optic disc. Upon selection of the vessel, the VesselMap 2, Version 3.02 software, automatically delineated the vessels' measuring area. The colour photograph was used as a reference to ascertain correct identification of venules and arterioles. Identification of vessels was done by two experienced scientists who had to agree on the vessel type before selection. Automated software calculations, based on the Knudtson revision of the Parr-Hubbard formulas, determined estimates from the six largest arterioles and venules and were summarised as the central retinal arterial equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively [40]. AVR was also calculated (CRAE/CRVE). Arterio-venular nicking was defined when a small arteriole crossed a small venule and resulted in the compression of the vein with bulging on either side of the crossing. A higher ratio from either wider retinal arteriolar calibre or narrower retinal venular calibre or both is an index of a better retinal vessel profile [9]. As the image scale of

each eye was unknown, the values of CRAE and CRVE were expressed as measuring units (MU). 1 MU is equivalent to 1 μm when the dimensions of the eye being examined correspond to those of the normal Gullstrand eye. Reproducibility was computed for a randomly selected cohort with a correlation coefficient of 0.84. The ICC analysis involved a mixed-model framework, whereby random effects were assumed for subjects and fixed effects were assumed for the graders. The Cronbach's alpha-reliability index for the AVR was 0.91 for this randomised cohort. Retinal pathology as seen in hypertensive/diabetic retinopathy and including optic nerve cup/disc ratio and arterio-venular nicking was diagnosed by a registered ophthalmologist.

2.1.6. 24 h urinary catecholamines

A three-litre container, washed with 9 ml of 20% HCl, ensured preservation of urinary metanephrines and an accurately 24 h timed specimen (Sarstedt®, Nümbrecht, Germany). Sampling began and ended with an empty bladder and participants were instructed to complete a 24 h diary to indicate voiding time, volume and fluid intake.

2.1.7. Biochemical analyses

Sodium fluoride blood samples, serum and whole blood EDTA samples were analysed for glucose, lipids, C-reactive protein (CRP), cotinine, γ -GT and glycated haemoglobin (HbA_{1c}), using Unicel DXC 800 (Beckman and Coulter, USA), Modular ROCHE Automized (Switzerland) and the Konelab™ 20I Sequential Multiple Analyzer Computer (ThermoScientific, Vantaa, Finland), respectively. An acidified sample from the 24 h urine collection was stored at -80°C until analysis within one year after collection [41]. Urinary epinephrine and norepinephrine values were determined using the 3-Cat Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). Intra- and inter-assay coefficients for epinephrine were 5.50% and 9.62%, respectively, and for norepinephrine 2.70% and 8.59%.

2.1.8. Statistical methods

Data were analysed using Statistica® software version 12.0 (Statsoft Inc., Tulsa, USA, 2012). Skewness of data was tested and γ -GT and CRP values were logarithmically transformed. Independent T-tests determined participant characteristic differences. A priori covariates which are implicated in higher sympathetic activity and CVD risk included age, BSA, physical activity, log γ -GT, log CRP and cholesterol [33, 38]. Chi-square (χ^2) statistics compared proportions. General linear model analyses, independent of a priori covariates, were computed to test interactions with race for depressive symptoms, norepinephrine-to-creatinine ratio (NECR), epinephrine-to-creatinine ratio (ECR) and potential cardiovascular risk markers (i.e. PP) and retinal vasculature markers, and, as a result of the high correlation between CRAE and CRVE, CRAE was adjusted for CRVE and vice versa [42]. ANCOVA's determined significant differences by comparing ethnic male groups from least square means analyses whilst adjusting for covariates (age, BSA, physical activity, log γ -GT, log CRP, cholesterol).

Multiple linear regression analyses were computed in the total male cohort and in separate race groups. Unadjusted associations between retinal vessel calibre markers, depressive symptoms and catecholamines were computed in the male cohorts. Forward stepwise multiple regression analyses were performed in various models based on significant interactions for race. Dependent variables were AVR, CRVE and CRAE. Independent covariates included age, BSA, physical activity, log γ -GT, log CRP, cholesterol 24 h PP, depressive symptoms, NECR and ECR. As a result of the high correlation between CRAE and CRVE, CRAE was added as covariate for CRVE and vice versa.

Sensitivity analyses: Forward stepwise regression analyses with similar dependent and independent covariates were repeated in several models in both ethnic male groups, by (a) excluding HIV-positive status participants (N = 16) (b) including only 24 h hypertensive participants and (c) adding HT medication users, cotinine and/or serum glucose as independent covariates. Significance was noted as $p \leq 0.05$.

2.2. Results

General linear model analyses showed ethnic differences for principal variables investigated, NECR and ECR ($F_{1,151} = 20.66$, $p < 0.0001$), depressive symptoms ($F_{1,165} = 4.45$, $p = 0.04$) as well as AVR ($F_{1,150} = 9.09$, $p = 0.003$), independent of a priori covariates.

Table 1 shows unadjusted baseline characteristics of the Black and White men. The Black men displayed lower waist circumference, BSA, BMI and physical activity but a larger metabolic risk with higher glucose, HbA_{1c}, cholesterol, CRP and γ -GT than their White counterparts. They also had a higher depressive symptom score with 34.2% of the Black men above the cut-off point for modestly severe depressive symptoms [36] compared to 13.3% of the White men. Despite their higher depressive symptom score, the Black men had lower 24 h urine NECR and 24 h urine ECR ratios than the White men. The Black group had higher BP, PP, arterio-venular nicking, optic nerve cup/disc ratio and CRVE values, whilst their retinal AVR was smaller compared to that of the White group.

	Black men (N = 76)	White men (N = 83)	P
Lifestyle and biochemical variables			
Age (years)	45.4 \pm 6.9	48.8 \pm 10.2	0.016
BMI (kg/m ²)	28.4 \pm 5.77	30.4 \pm 5.24	0.026
BSA (m ²)	1.99 \pm 0.22	2.24 \pm 0.21	<0.0011
Waist circumference (cm)	98.3 \pm 14.8	106.2 \pm 13.2	<0.001
Physical activity (kcal/24 h)	3464.2 \pm 1284.5	4101.2 \pm 1859.2	0.015
Cholesterol (mmol/L)	4.63 \pm 1.02	4.22 \pm 1.00	0.012
HDL cholesterol (mmol/L)	0.93 \pm 0.35	0.84 \pm 0.22	0.054

	Black men (N = 76)	White men (N = 83)	P
Glucose (mmol/L)	5.75 ± 1.71	4.65 ± 1.30	<0.001
Glycated haemoglobin (%)	6.18 ± 1.40	5.70 ± 0.88	0.010
γ-Glutamyl transferase (U/L)	71.7 ± 61.9	36.7 ± 41.2	<0.001
C-reactive protein (mg/L)	5.91 ± 12.2	2.84 ± 10.0	0.084
Cotinine (ng/mL)	48.3 ± 97.8	29.5 ± 98.7	0.232
Depressive symptoms score	7.59 ± 4.51	4.86 ± 4.23	<0.0011
Depressive symptoms (PHQ-9 ≥ 10) N (%)	26 (34.2)	11 (13.3)	<0.001
24 h endocrine variables			
24 h urine norepinephrine/creatinine ratio (nmol/mmol)	15.3 ± 10.7	28.4 ± 20.5	<0.001
24 h urine epinephrine/creatinine ratio (nmol/mmol)	2.61 ± 1.70	4.71 ± 3.17	<0.001
Cardiovascular variables			
24 h SBP (mmHg)	137 ± 15	128 ± 11	<0.001
24 h DBP (mmHg)	87 ± 10	80 ± 7	<0.001
24 h PP (mmHg)	51 ± 8	48 ± 6	0.034
24 h heart rate (beats/min)	78 ± 9	72 ± 10	<0.001
Central retinal arterial equivalent (MU)	147.9 ± 13.2	150.4 ± 11.9	0.218
Central retinal venular equivalent (MU)	251.4 ± 19.2	237.9 ± 18.6	<0.001
Retinal arteriolar-to-venular ratio	0.59 ± 0.06	0.63 ± 0.04	<0.001
Optic nerve cup/disc ratio (right eye)	0.37 ± 0.19	0.28 ± 0.24	0.011
Hypertensive/diabetic retinopathy (%)	75.0	36.3	<0.001
Arterio-venular nicking (N (%))	59 (77.6)	20 (24.1)	<0.001
HT (SBP > 130 and/or DBP > 80 mmHg) (N (%))	60 (78.9)	40 (48.4)	0.001
HT medication (% of hypertensives)	36.6	34.1	0.274

Data presented as unadjusted means with standard deviation or percentages. Where BMI, body mass index; BSA, body surface area; PHQ, Patient Health Questionnaire; 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; 24 h PP, 24 h pulse pressure; HT, hypertension; MU, measuring units, equal to μm in the normal Gullstrand eye.

Table 1. Comparing unadjusted mean (\pm SD) baseline characteristics of Black and White men.

In **Table 2**, principal variables were compared considering a priori covariates. In the Black male cohort, a similar trend was revealed with increased hyperpulsatile PP (>50 mmHg) accompanied by more depressive symptoms, lower AVR and also lower urine NECR and ECR values

compared to their White counterparts. However, only the AVR was smaller in the Blacks whilst neither CRAE nor CRVE was different between the race groups.

	Black men (N = 76)	White men (N = 83)	P
24 h SBP (mmHg)	140 (137, 144)	126 (123, 130)	<0.001
24 h DBP (mmHg)	88 (86, 90)	79 (77, 81)	<0.001
24 h PP (mmHg)	52 (50, 54)	47 (45, 50)	<0.001
Central retinal arterial equivalent (MU)	147.4 (144.0, 150.8)	150.9 (147.6, 154.1)	0.21
Central retinal venular equivalent (MU)	248.0 (242.9, 253.1)	241.2 (236.4, 246.1)	0.11
Retinal arteriolar-to-venular ratio	0.60 (0.58, 0.61)	0.63 (0.61, 0.64)	0.01
24 h urine ECR (nmol/mmol)	14.3 (9.76, 18.9)	30.0 (25.7, 34.3)	<0.001
24 h urine NECR (nmol/mmol)	2.08 (1.14, 3.02)	5.82 (4.94, 6.69)	<0.001
Depressive symptom score	7.11 (6.02, 8.21)	4.71 (3.84, 5.79)	0.006

Comparing adjusted mean (\pm SD) pulse pressure, retinal vessel calibre, depressive symptoms and 24 h urinary catecholamines in a cohort of Black and White men. Values were adjusted for age, body surface area, physical activity, log γ -glutamyl transferase, log C-reactive protein and cholesterol. Where 24 h SBP, 24 h systolic blood pressure; 24 h DBP, 24 h diastolic blood pressure; 24 h PP, 24 h pulse pressure; MU, measuring units, equal to μ m in the normal Gullstrand eye; 24 h NECR ratio, 24 h urinary norepinephrine-to-creatinine ratio; 24 h ECR ratio, 24 h urinary epinephrine-to-creatinine ratio.

Table 2. Comparing adjusted mean (\pm 95% CI) baseline characteristics of Black and White men.

Forward stepwise linear regression analyses (**Table 3**) revealed expected patterns of associations between the dependent retinal microvascular calibre variables (AVR, CRAE and CRVE) and independent variable, PP, in the total group (Model 1). AVR and CRAE were negatively associated with PP, whilst CRVE showed a positive association with PP. In the total group, negative associations were found between AVR, CRAE and depressive symptoms, whilst no associations were found between any of the retinal microvascular variables and NECR or ECR. In the separate ethnic groups, AVR was negatively associated with PP in both racial groups. CRAE was negatively associated with PP in the White men whilst positively associated with CRVE in the Black men. In the White group, AVR and CRAE were negatively associated with depressive symptoms, whilst AVR was, rather unexpectedly, positively associated with NECR. In the Black group, AVR and CRAE were negatively associated with ECR, whilst a positive association existed with CRVE. No unadjusted or adjusted associations between depressive symptoms and the catecholamines were revealed (data not shown).

No changes in the outcome of the data occurred with sensitivity analyses after excluding HIV-positive status participants or including 24 h hypertensive participants. Adding HT medication users, cotinine and serum glucose as independent covariates also did not alter any of the associations.

Model 1: Total group (N = 159)

	AVR	CRAE	CRVE
<i>Adjusted R²</i>	0.28	0.36	0.39
β (95% CI)			
Race	0.16 (0.002, 0.32), p = 0.049	–	–0.20 (–0.36, –0.04), p = 0.017
24 h PP (mmHg)	–0.24 (–0.38, –0.10), p = 0.001	–0.24 (–0.38, –0.11), p = 0.001	0.17 (0.04, 0.31), p = 0.014
Depressive symptoms	–0.19 (–0.34, –0.05), p = 0.014	–0.23 (–0.30, –0.15), p = 0.001	–
24 h urine ECR	–	–	NS
24 h urine NECR	–	NS	–

Model 2: Separate ethnic groups

	Black men (N = 76)			White men (N = 83)		
	AVR	CRAE	CRVE	AVR	CRAE	CRVE
<i>Adjusted R²</i>	0.24	0.15	0.29	0.27	0.59	0.52
β (95% CI)						
24 h PP (mmHg)	–0.24 (–0.46, –0.01), p = 0.048	–	0.23 (0.01, 0.45), p = 0.045	–0.30 (–0.48, –0.11), p = 0.003	–0.22 (–0.36, –0.08), p = 0.003	–
Depressive symptoms	–	–	–	–0.27 (–0.46, –0.08), p = 0.007	–0.18 (–0.32, –0.04), p = 0.014	–
24 h urine ECR (nmol/mmol)	–0.35 (–0.57, –0.12), p = 0.004	–0.37 (–0.66, –0.09), p = 0.013	0.35 (0.13, 0.57), p = 0.003	–	–	–
24 h urine NECR (nmol/mmol)	–	NS	–	0.19 (0.0, 0.38), p = 0.050	–	–

Covariates included age, body surface area, physical activity, log γ -glutamyl transferase, log C-reactive protein and cholesterol. In models with CRAE as a dependent variable, adjustment for CRVE was made and vice versa. Where AVR, arteriolar-to-venular ratio; CRAE, central retinal arterial equivalent; CRVE, central retinal venular equivalent; 24 h PP, 24 h pulse pressure; ECR, epinephrine-to-creatinine ratio; NECR, norepinephrine-to-creatinine ratio; NECR, norepinephrine-to-creatinine ratio.

Table 3. Forward stepwise regression analyses predicting relationships between the 24 h urinary catecholamine levels, depressive symptoms and retinal vessel parameters.

2.3. Discussion

The aim of this study was to evaluate the association between the retinal microvascular calibre as primary endpoint and systemic adrenergic transmitters and depressive symptoms as independent variables, comparing a Black and White male cohort from South Africa.

The main novel finding suggests a cardiometabolic vulnerable profile in terms of more depressive symptoms, PP, arterio-venular nicking, optic nerve cup/disc ratio and CRVE values, whilst their retinal AVR was smaller in the Black men. Despite lower catecholamine levels, epinephrine was positively associated with arteriolar narrowing, venular widening and hyperpulsatile BP (indicative of arterial stiffness) in the Black men.

2.3.1. *Ethnicity and retinopathy*

Although cultural differences exist between the Black and White groups, all the participants were teachers with the same educational background, income and working conditions. Despite these similarities, the Black group clearly exhibited a poorer health profile than their White counterparts with regard to cardiometabolic and mental health characteristics. They presented with increased cardiometabolic risk markers such as hyperglycaemia, cholesterol, inflammation, alcohol consumption and depressive symptoms. The Black group's mean BP values were above the cut-off point for HT (ABPM $\geq 130/80$) [38], which reflect in the HT prevalence of nearly 80% in this group. Elevated BP and PP are associated with structural microvascular changes and our findings are in line with those references [43, 44]. Indeed, elevated BP and PP were associated with attenuated retinal arteriolar and increased venular diameter values and consequently also the AVR. This may impact on vascular wall remodelling as is evident from the presence of arteriolar narrowing, AV nicking, retinopathy [45] and possibly progression towards subclinical atherosclerosis. If the effect of elevated glucose as well as HbA_{1c} levels is added which, in the case of the Black men, are both, according to the American Diabetes Association, above the cut-off point indicative of a prediabetic state, changes in the retinal vessels comparable to those in diabetic subjects could be expected. The prevalence of AV nicking and hypertensive/diabetic retinopathy is a clear indication.. that the retinal vasculature is showing signs of structural changes and reduced microvascular health in both groups but especially in the Black group.

2.3.2. *Retinal vessel calibre and depressive symptoms*

Depression has recently been acknowledged as a major risk factor for poorer prognosis in patients with coronary heart disease by the American Heart Association [23]. The depressive symptom score of the Black men was significantly higher than that of the White men with 34 % of the group exceeding the cut-off point for moderately severe depression, thereby worsening their CVD risk. Although underlying stress levels, as assessed using the depressive symptoms risk score, were elevated in the Black men, both their 24 h ECR and NECR levels were lower compared to their White counterparts. During chronic stress the initial hyperactivity may be followed by autonomic exhaustion, receptor hyporesponsivity and decreases in catecholamines and cortisol [14–18]. PNMT converts norepinephrine to epinephrine and is regulated by glucocorticoids synthesised in the adrenal gland [19]. One way that it can regulate PNMT expression is by corticosterone's positive influence on the maintenance of PNMT mRNA [20]. Therefore a reduction in cortisol will lead to a decrease in the synthesis of epinephrine. These alterations in autonomic function are of importance as they have been associated with both depression and cardiovascular pathology [14, 15]. It is known that depression is often preceded by psychosocial stress [22] which might, therefore, also be associated with the risk for cardiac remodelling as well as a poor prognosis in individuals with coronary heart disease [23]. This notion is enhanced by the finding that in a Black cohort with symptoms of depression, attenuated cortisol and catecholamine metabolites were identified as risk factors for the development of vascular diseases [24]. Even though depression [46], diabetes and HT are associated with activation of the SNS [31], we could not replicate these findings.

Our results, therefore, oppose the findings from Ref. [47], showing a positive association between NECR excretion and moderate depressive symptoms. As more depressive symptoms and a hypertensive state are evident in the Black men, the SNS and adrenal medulla may present neural fatigue or “burnout.” Our findings could, therefore, indicate a possible down-regulation of norepinephrine and epinephrine secretion as a consequence of long-term overstimulation of the SNS and possible β -adrenergic hyporesponsivity in the Black men. In support of this notion, depressed heart rate variability (HRV) was associated with increased parasympathetic dominance albeit cardiac contractility (24-h heart rate and SBP) in the current African men at baseline, rather suggesting β -adrenergic receptor activation [1, 48]. Conversely, increased SNS activity and a possible vagal-impaired HR profile may however contribute to disturbed endothelial function, possibly because of activation of β -adrenergic receptors [49]. When α -adrenergic responsiveness though prevails [48], dysregulation or desensitisation of β -adrenergic receptors may occur. This was evident in the clustering of increased 24-h heart rate, SBP and depressed HRV values which indicated a possible diminished β -adrenergic responsiveness and vagal-impaired response [1]. A plausible explanation may be that depressed HRV as a reflection of α -adrenergic sympathetic overdrive could also be due to poor ventricular performance as was observed in another study [50].

It supports previous findings in these SABPA Black men, where blunted neuroendocrine responses were associated with vascular wall remodelling concurring with a profile of autonomic exhaustion and emotional distress [14, 16]. Our subsample of White men showed a 13% prevalence of depressive symptoms which were inversely associated with the retinal vessel calibre. Findings from the ARIC study compare favourably with the White group where the depressive symptom score was associated with retinal arteriolar narrowing. In contrast, we could not replicate these findings in the Black group. Clearly prospective studies are needed to determine causality [30].

2.3.3. Retinal microvascular calibre and catecholamines

SNS activation is present in both diabetes and HT [31] and may be associated with microvascular calibre. Increased perfusion pressure enforces.... contraction in the ocular arteries, which are resistance vessels and regulated by myogenic mechanisms (Bayliss effect) [51]. Retinal microvascular calibre associations with the adrenergic transmitters revealed different profiles in the two ethnic groups. Chronic SNS activation will desensitise the baroreceptors with compensatory increases in BP and PP as was shown in the Black group [1]. In the Black group, the smaller CRAE and a larger CRVE are both associated with epinephrine but not with norepinephrine levels. This may imply that epinephrine will reduce blood flow to the retina by stimulating arteriolar contraction but also increasing the draining of blood away from the retina by stimulating venular dilation. Myogenic tone may however be impaired in Blacks and increase retinal venular widening especially during chronic pressure overload with increased hyperpulsatility. An overactive sympathetic system and/or chronic depression symptoms might therefore explain part of the mechanism. Presently, instead of epinephrine's normal arterial vasodilatory response [52], it induces vasoconstriction, which may suggest hyporesponsivity or down-regulation of the β_2 -adrenergic receptors as was also shown previously [1].

Therefore, this hyporesponsivity may be a homeostatic reaction to protect the retina from SNS-stimulated increases in hyperpulsatile pressure in a cohort who has more depressive symptoms. This may be true for both the retina and the brain as emotional stress can also provoke reversible cerebral vasoconstriction similar to retinal vasoconstriction [53].

Both a smaller AVR and a larger CRVE are associated with a greater risk for stroke mortality [7]. This also suggests that β -adrenoceptor hyporesponsivity due to SNS hyperactivity as reflected in lower catecholamine levels might constitute an increased risk for vascular hypertrophy and eventually stroke in the Black male cohort. The same associations were not seen in the White men, maybe as result of their lower depressive symptom scores as well as their lower BP and PP levels. The prevalence of depressive symptoms and possible down-regulated catecholamine profile presuming chronic distress in the Black men compared to their White counterparts, therefore, may explain the differences or lack of association between the catecholamine levels and AVR in the Whites.

2.3.4. Retinal microvascular calibre and local or systemic sympathetic activation

Whether local or systemic catecholamine levels are associated with retinopathy is hotly debated [29, 30]. Recently, both α - and β -adrenergic innervation was demonstrated in the preocular CRA in humans [29]. It seems clear that some aspects of sympathetic transmission regulate choroidal and CRA blood flow by way of changes in vascular smooth muscle tone [54]. The inverse association between AVR and ECR may support a vasodilatory (venular) or vasoconstrictive (arteriolar) tone in the retinal vessels. A notion for vasoconstriction is suggested as a hypertensive state increases peripheral vascular resistance in the retinal arterioles [7]. Therefore, increased or hyperpulsatile PP exerting mechanical stress on the vessel walls may contribute to a diminished β -adrenergic albeit an augmented α -adrenergic responsiveness in Black men [1, 33] and subsequent risk of vascular hypertrophy [1] and possibly arteriolar narrowing. The profile of β -adrenergic hyporesponsivity in Black men emphasises central control of the brain on the circulatory system irrespective of the vascular bed.

Several limitations should be noted. The cross-sectional design of the current study prevents us from being able to infer causality. Studies showing direct evidence of sympathetic tone and retinal vascular remodelling in human models could greatly contribute to our knowledge in this field. Larger sample sizes and more diverse data on autonomic and endothelial function are needed to delineate possible physiological mechanisms and the role of the ageing process. Only an indirect measure of SNS activity via 24 h catecholamine concentrations was measured and more direct measurements should be implemented, along with the determination of the corticosteroid profile. A more representative sample of the whole population is necessary to draw generalised conclusions.

2.4. Conclusions

A profile of β -adrenergic hyporesponsivity was evident in Black men. They revealed more depressive symptoms, indicative of a chronically challenged SNS, which were associated with retinal vascular remodelling and possible vascular hypertrophy. Whether these changes

precede or result from hyperpulsatile pressure impacting on retinal autoregulation is still debatable.

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Abbreviations

ambulatory blood pressure measurement (ABPM)

arteriolar-to-venular ratio (AVR)

blood pressure (BP)

body surface area (BSA)

body mass index (BMI)

cardiovascular disease (CVD)

central retinal arterial equivalent (CRAE)

central retinal artery (CRA)

central retinal venular equivalent (CRVE)

C-reactive protein (CRP)

diastolic blood pressure (DBP)

electrocardiogram (ECG)

epinephrine-to-creatinine ratio (ECR)

gamma glutamyl transferase (γ -GT)

glycated haemoglobin (HbA_{1c})

heart rate variability (HRV)

hypertension (HT)

measuring units, equal to μm in the normal Gullstrand eye (MU)

norepinephrine-to-creatinine ratio (NECR)

Patient Health Questionnaire (PHQ-9)

phenylethanolamine N-methyltransferase (PNMT)

pulse pressure (PP)

Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study

sympathetic nervous system (SNS)

systolic blood pressure (SBP)

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