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Chronic depression symptoms and salivary NOx are associated with retinal vascular dysregulation: The SABPA study

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ABSTRACT

Background: Depression has been associated with impaired nitric oxide (NO)-mediated vasodilation and vascular dysregulation (VD). Whether depression and NO levels will disturb retinal haemodynamics is not clear.

Objectives and methods: Associations between the retinal vasculature, diastolic ocular perfusion pressure (DOPP) as measure of hypoperfusion, NO metabolites (NOx) and depression symptoms were assessed. Chronic VD risk markers [depression symptoms (Patient Health Questionnaire/PHQ-9 $\geq$ 10) and 24 h pulse pressure] were determined in a bi-ethnic cohort (n = 313; 48.6 ± 9 years; 53.9% men). At 3 year follow-up, retinal vessel calibre and retinopathy signs were quantified from digital images. Salivary NOx was obtained pre- and post-flicker light-induced provocation (FLIP). DOPP was defined as diastolic blood pressure minus intraocular pressure.

Results: Chronic VD risk was evident in Blacks opposed to acute risk in Whites ($P < 0.05$). At follow-up, retinopathy (Blacks 60.4%/Whites 39.6%), lower pre-FLIP (μM) and higher post-FLIP NOx (changes from baseline, %), arteriolar narrowing and wider venular calibre values were evident in Blacks compared to Whites, independent of confounders. A wider venular calibre, an index of stroke risk, was associated with chronic depression symptoms [cut point 248 MU: Area under the curve 0.61 (95% CI: 0.51, 0.72); 71% sensitivity; 55% specificity] as well as with hypoperfusion in the Blacks. In this group, arteriolar narrowing was associated with hypoperfusion; and attenuated arteriolar dilation with increased post-FLIP NOx responses.

Conclusions: Chronic depression symptoms may alter NO regulation and facilitate VD. NO-mediated vasoconstriction presumably impeded perfusion, retinal haemodynamics and −remodelling; potentiating stroke risk in Blacks.

1. Introduction

The retinal microvasculature shares many features with the cerebral circulation, such as the presence of a blood-retinal barrier

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which is similar to the blood-brain-barrier and provides insight into vascular dysregulation (VD) of the brain [1]. However, the relationship between chronic conditions such as VD, depression and hypertension is complex as indicated by opposing findings [2–5]. Most of these chronic conditions may involve an increased cardiometabolic risk with apparent depression, inflammation and perfusion deficits [6–9]. Perfusion deficits increases metabolic demands in the microvasculature [10], and might explain a mechanism linking depression with VD. Taylor et al. indeed demonstrated that higher metabolic demands and subcortical ischaemia emerge if VD is evident [11].

VD risk may furthermore be an important pathophysiological mechanism in South African Blacks as they are more likely to be diagnosed with symptomatic occlusive vascular disease [12], hypertension, depression and exhibition of enhanced sympathetic activity, vascular and attenuated norepinephrine responses [13,14]. Indeed, in depressed patients, glucose metabolism in the brain and norepinephrine turnover was shown to be impaired in subcortical limbic structures controlling emotions [15]. Therefore, if depression symptoms are present, vascular pathology in the brain will impose powerful restraints on ocular perfusion pressure and haemodynamics [16].

Ocular perfusion pressure is the difference between arterial and venous blood pressure and is the driving force of ocular blood flow [17]. Diastolic ocular venous pressure is similar or slightly higher than intraocular pressure (IOP) [17,18]. Diastolic ocular perfusion pressure, as marker of hypoperfusion, may therefore be estimated as the difference between diastolic BP and IOP (DOPP) [18]. In patients with essential hypertension, increased DOPP via higher blood pressure and/or lower IOP will enhance vascular resistance and reduce ocular blood flow to the optic nerve head, thereby inducing ischaemia and/or hypoxia [19]. VD will thus be present when blood flow does not adapt to the need of the respective tissues. Retinal flicker-light-induced provocation (FLIP) therefore might be one way to directly investigate the dynamic nature of vascular regulation involving the vasodilatory actions of nitric oxide (NO). Parasympathetic innervation of the salivary glands contains the NO synthesizing enzyme, NO synthase [20]. NO secretion of parasympathetic origin however, does not seem to take part in the regulation of the secretory activity [20]. Instead, NO of intracellular origin is mobilized particularly upon sympathetic nerve activity, e.g. FLIP and might relate to flicker-induced retinal vasodilation [20–23]. In Black men, *increased* α-adrenergic-driven blood pressure responses, less serum NO bio-availability and perfusion deficits were shown during mental stressor exposure [21]. We therefore hypothesize that less salivary NOx, as non-invasive measure, may support VD at pre- and post-FLIP phases. A number of clinical studies additionally support a causal role for neural NO in depression as altered NO-cyclic guanosine monophosphate (cGMP) signalling has been proposed as putative biomarker of the depression response and VD [11,22]. Importantly, this pleiotropic transmitter has diverse biological effects in the brain and in VD [23].

Whilst retinal arteriolar narrowing has been associated with VD and risk for hypertension, a wider venular calibre was associated with stroke risk [23]. Depression symptoms may relate to VD and perfusion deficits, thereby accelerating vascular remodelling. Therefore, we aimed to assess associations between baseline depression symptoms and retinal vascular regulation under well-controlled conditions at 3 year follow-up.

2. Materials and methods

2.1. Design and participants

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective study (Fig. 1) was conducted in 2008/9 and 2011/12 and described elsewhere [9]. A teachers' cohort included 409 Black and White men and women from South Africa of which 359 participated in the 3-year follow-up. Exclusion criteria [9], for the purpose of the current study, additionally included epilepsy ($n = 4$), and poor quality retinal vessel images ($n = 42$) yielding a final study sample of 313. Participants were fully informed about the objectives and procedures prior to recruitment and provided written, informed consent. The study conformed to the Helsinki Declaration and was approved by The Ethics Review Board of the North-West University, Potchefstroom Campus: Approval number 0003607S6.

Similar to the baseline, clinical assessments were done over a 48 h period at the three-year follow-up [4,9,13]. During the working week, 24 h ambulatory blood pressure apparatuses were fitted at school, a 24 h standardized diet commenced and participants resumed their normal daily activities. At 15:00, participants were transported to the North-West University for clinical measures including retinal vessel imaging and completion of a psychosocial battery under the supervision of registered clinical psychologists. Anthropometric measures, 8 h overnight fasting blood sampling and physical activity measures were obtained the next morning after 07:00.

2.2. Cardiovascular measures

Ambulatory blood pressure monitoring (ABPM) devices were attached to participants' non-dominant arm (Meditech CE120 CardioTens®; Meditech, Budapest, Hungary) at school by trained cardiovascular research personnel. The Cardiotens® was programmed to measure BP at 30-min intervals during the day (07:00–22:00) and every hour during night time (22:00–06:00). The 24 h successful inflation rate at follow-up was 85.99% (±9.29) in Africans and 91.80% (±7.95) in Whites. Hypertensive status was classified as 24 h SBP $\geq$ 130 mm Hg and/or DBP $\geq$ 80 mm Hg [24] and 24 h pulse pressure (24 h PP) reflecting a hyperpulsatile pressure, was defined as 24 h SBP − 24 h DBP as a measure of arterial stiffness. If 24 h PP $\geq$ 50 mmHg, the hyperpulsatile pressure impacts on arterial tone and wall permeability [25].

2.3. Anthropometric and physical activity measures

Anthropometric measurements were performed in triplicate by level II anthropometrists according to standardized procedures. Intra- and inter-observer variability was less than 10%. From 07:15 onwards, participants were in a semi-recumbent position. The ambulatory blood pressure apparatus was removed after the last recording at 07:30 followed by blood sampling. After all clinical measures were completed the Actiheart® monitor (GB0/67703, CamNtech Ltd., Cambridgeshire, UK) was fitted to assess 7 days physical activity data.

2.4. Depression

Depression symptoms scores were obtained via the PHQ-9 which has 9 items and was validated in various ethnic groups for use in primary health care settings [26,27]. Each item evaluates the presence of one of the nine Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-R) criteria for major depression. The Cronbach's alpha-reliability index for the total

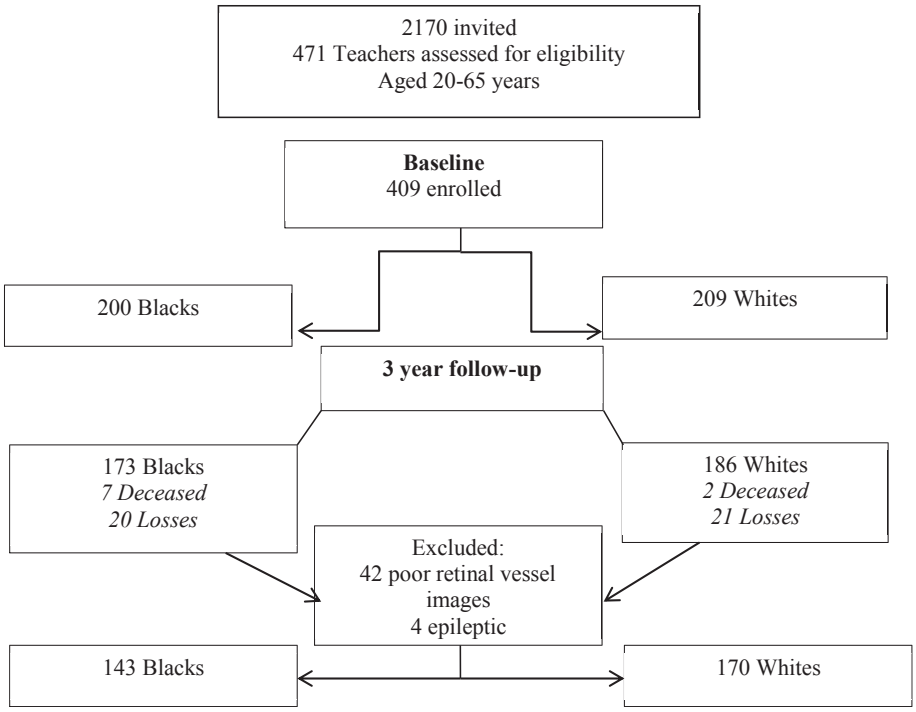

Fig. 1. A South African bi-ethnic sex cohort.

three-year PHQ-9 score in the current sub-study was 0.80 indicating good reliability. The recommended and established PHQ-9 cut-off point of ≥10 indicates the presence of moderately severe depression symptoms.

2.5. Retinal vessel analyses (function and structure)

Participants abstained from the intake of food or caffeine containing beverages, alcohol, smoking or exercising at least 1 h prior to measurements. They were introduced to the experimental setup and screened for acute angle anterior chamber glaucoma risk by a registered nurse. The retinal vessel analyser (Imedos Systems UG, Jena, Germany) was used for digital fundus imaging with a Carl Zeiss FF450^{Plus} camera (Carl Zeiss, Medictech Jena, Germany) to perform static and dynamic retinal vessel analysis. Mydriasis was induced in the right eye by a drop containing tropicamide, 1% and benzalkonium chloride 0.01% (m/v).

a) Dynamic retinal vessel analysis

Flicker-light-induced-provocation (FLIP) (Video clip A.1) commenced allowing non-invasive measuring of the retinal arterioles and venules diameter responses to the provocation. An optoelectronic shutter was inserted in the retinal camera which interrupted the observation light with a frequency of 12.5 Hz and wavelength of 530–600 nm. The baseline diameter was measured for 30 s in continuous light followed by three cycles of 20-s flicker provocation and 80-s steady fundus illumination. An arteriolar and venular segment with an average length of 498.7 measuring units (MU) was subsequently measured. 1 MU is equivalent to 1 μm when the dimensions of the eye being examined correspond to those of the normal Gullstrand eye. Changes in ocular haemodynamic markers were expressed as percentage change over baseline values.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.niox.2016.02.008.

Event blood pressure (Meditech CE120 CardioTens®; Meditech, Budapest, Hungary) and - salivary sampling were done simultaneously, at pre- (baseline, μM) and post-FLIP phases (changes from baseline calculated in μM and in %). The cuffed arm was relaxed and placed along the side whilst a salivette cotton swab was simultaneously placed in the mouth and passive drooling sampling was done for 1–2 min (Salimetrics®). The tweezers used to place the saliva cotton swab (Sarstedt Inc., Leicester, UK) in the mouth, was kept in a 0.5% chlorhexidine gluconate solution and rinsed with distilled water before sampling.

b) Static retinal vessel analysis

The Visualis software was used to delineate all vessels' calibre automatically (VesselMap 2, Version 3.10 software, IMEDOS, FF450plus, 535–561 nm, 30° image, 1840 × 1360 pixel) (Figure A.1). The arteriolar calibre (central retinal arteriolar equivalent, CRAE) and the venular calibre diameters (central retinal venular equivalent, CRVE) were computed and based on the Knudtson big six formula [28,29] and identified by two experienced scientists. As the image scale of each eye was unknown, the values of CRAE and CRVE were expressed as MU. The reproducibility correlation coefficient was 0.84 and intra-class correlation Cronbach's alpha-reliability index for the arteriolar:venular ratio was 0.91 for a randomly selected cohort.

2.6. Diastolic ocular perfusion pressure

A local anaesthetic drop (Novasine Wander 0.4% Novartis) was inserted in the right eye prior measuring IOP with the Tono-Pen Avia Applanation Tonometer (Reichert 7-0908, ISO 9001, New York, USA). The right eye DOPP was calculated (Pre-FLIP DBP − IOP mmHg) and general retinopathy was diagnosed by a registered ophthalmologist (Table 3).

Table 1
Clinical characteristic profile of a South African bi-ethnic sex cohort at baseline.

	Blacks (n = 143)	Whites (n = 170)	P-values
	Mean (± SD)		
Men	77 (53.9)	81 (47.7)	0.28
Age (years)	44.5 (7.6)	46.4 (9.9)	0.06
Urban living (years)	31.2 (16.1)	20.8 (12.8)	<0.001
Cholesterol (mmol/l)	4.7 (1.2)	5.6 (1.3)	<0.001
Cardiometabolic clinical characteristics	*Count (prevalence %)*		
CVD History	13 (40.6)	19 (59.4)	0.54
Active smoking	38 (26.8)	22 (12.9)	0.002
Alcohol abuse	77 (54.2)	24 (14.1)	0.001
Central obesity	91 (63.6)	108 (63.5)	0.98
Depression symptoms (PHQ-9)	50 (35.0)	18 (10.6)	<0.001
Pre-diabetes	87 (61.7)	53 (31.2)	<0.001
Low grade inflammation	92 (64.8)	48 (28.2)	0.001
Arterial stiffness	49 (8.8)	48 (7.1)	0.33
Hypertension	94 (65.7)	73 (42.9)	<0.001
Hypertension with Depression symptoms	41 (28.67)	9 (5.29)	<0.001
Medications	*Count (prevalence %)*		
Statins	2 (1.4)	8 (4.7)	0.1
Aspirin	2 (1.4)	12 (7.1)	0.02
Hypertension	50 (13.9)	58 (16.2)	<0.001

Abbreviations: CVD, history of cardiovascular disease; Active smoking (Cotinine > 50 ng/ml) [31]; Alcohol abuse (gamma-glutamyl transferase ≥ 41 U/l) [32]; PHQ-9 ≥ 10, Patient Health Questionnaire [26]; Central obesity, waist ≥94 cm men and ≥80 cm women [30]; Pre-diabetes, HbA1C ≥ 5·7 [30]; Low grade inflammation, CRP ≥ 3U/L [24]; Arterial stiffness, 24 h pulse pressure ≥ 50 mmHg [25]; Hypertension, SBP ≥ 130 and/or DBP ≥ 80 mmHg [24].

Table 3
Ocular media and fundus assessment at 3 year follow-up.

	Blacks (n = 143)	Whites (n = 170)	P-values
	Count (prevalence %)		
Men	77 (53.9)	81 (47.7)	0.28
Age (years)	44.5 (7.6)	46.4 (9.9)	0.06
Acute anterior glaucoma risk	0 (0.00)	14 (8.24)	<0.001
Retinopathology	99 (69.23)	65 (38.24)	<0.001
Retinal atrophy	0.(0.0)	2 (1.18)	0.20
Retinal scarring	1 (0.70)	0 (0.00)	0.26
Drusen	1 (0.70)	0 (0.00)	0.27
Ciliary blood vessels	1 (0.70)	0.(0.00)	0.27
Microaneurysm	7 (4.90)	0 (0.00)	0.003
Exudates	2 (1.40)	0 (0.00)	0.12
Arteriovenous nicking	90 (62.94)	38 (22.35)	<0.001
Cotton wool spots	2 (1.40)	0 (0.00)	0.11
Focal narrowing	6 (4.20)	0 (0.00)	0.06
Macula scarring	0 (0.00)	1 (0.59)	0.37
Optic head (cup:disc ratio > 0.50)	29 (20.28)	32 (18.82)	0.55

2.7. Biochemical analyses

Sodium fluoride glucose, serum and whole blood plasma samples were analysed for lipids, high sensitivity C-reactive protein (CRP), gamma-glutamyl transferase (γ-GT), cotinine, and glycated haemoglobin (HbA1C) respectively [30–32] using the Cobas Integra 400 Plus and the Modular ROCHE Automized systems (Basel, Switzerland). Intra- and inter-assay coefficients were less than 10%. Saliva NOx analyses based on the enzymatic conversion of nitrate to

Table 2
Cardiometabolic profile changes over 3 years in a Black and White South African cohort.

	Blacks follow-up (n = 143)	Whites follow-up (n = 170)	
	Δ % [OR: 95% CI], P		
CVD History	2.8 [1.7: 0.6–4.6], 0.46	6.5 [2.8: 1.1–7.2], 0.04	
Active smoking	7.2 [6.0: 1.3–26.8], 0.06	1.8 [4.0: 0.5–35.8)], 0.38	
Alcohol abuse	9.4 [2.4: 1.1–5.3], 0.02	4.0 [4.5: 1.0–20.8], 0.67	
Central obesity	5.0 [2.4: 0.9–6.8], 0.14	11.2 [5.8: 2.0–16.6], 0.001	
Depression symptoms	1.4 [1.1: 0.6–1.9], 0.89	6.6 [2.6: 1.1–6.2], 0.04	
Pre-diabetes	2.9 [1.3:0.6–2.5], 0.61	10.7 [2.8: 1.4–5.8], 0.01	
Low grade inflammation	5.7 [1.8:0.8–3.9], 0.19	8.2 [2.6: 1.2–5.5], 0.02	
Arterial stiffness	8.4 [2.2: 1.0–4.7], 0.05	1.8 [1.2: 0.6–2.2], 0.81	
Hypertension	1.4 [1.2: 0.6–2.4], 0.85	7.1 [5.8: 1.0–3.6], 0.88	
[a]**Retinal vascular regulation characteristics**	Mean (±SD)		P-Values
Retinopathology, n (%)	99 (60.4)	65 (39.6)	<0.001
R-Arteriolar calibre (MU)	149.64 ± 12.98	151.22 ± 11.48	0.28
R-Venular calibre (MU)	250.34 ± 19.88	237.01 ± 18.02	<0.001
R-DOPP (mmHg)	71.17 ± 12.43	68.17 ± 10.74	0.02
R-Arteriolar dilation (%)	4.38 ± 2.58	4.02 ± 2.05	0.17
R-Venular dilation (%)	5.15 ± 2.36	4.56 ± 1.96	0.02
Pre-FLIP SBP/DBP (mmHg)	140/88 ± 20/12	133/83 ± 15/11	<0.001
Post-FLIP SBP/DBP (mmHg)	139/88 ± 19/13	132/83 ± 14/10	<0.001
Pre-FLIP NOx (μM)	253.01 ± 200.15	334.63 ± 243.05	0.02
Post-FLIP NOx (μM)	308.94 ± 212.08	342.48 ± 213.13	0.11
Post-FLIP NOx (%)	42.54 ± 76.72	14.70 ± 48.34	<0.001
Medications	*Δ % [OR: 95% CI], P*		
Hypertension	3.5 [1.4: 0.7–2.8], 0.47	7.7 [2.6: 1.2–5.9], 0.02	
Statins	0.0 [1.0: 0.1–7.0], 0.1	11.8 [21.0: 2.8–156.0], 0.001	
Aspirin	14.0 [21.0: 2.8–156.1], 0.001	5.9 [3.5: 1.2–10.6], 0.03	
Anti-depressants	0	0	

Abbreviations: CVD, history of cardiovascular disease; Active smoking (Cotinine > 50 ng/ml) [31]; Alcohol abuse (gamma-glutamyl transferase ≥ 41 U/l) [32]; PHQ-9 ≥ 10, Patient health Questionnaire [26]; Central obesity, waist ≥94 cm men and ≥80 cm women [30]; Pre-diabetes, HbA1C ≥ 5·7 [30]; Low grade inflammation, CRP ≥ 3U/L [24]; Arterial stiffness, 24 h pulse pressure ≥ 50 mmHg [25]; Hypertension, SBP ≥ 130 and/or DBP ≥ 80 mmHg [24]; R, right; DOPP, diastolic ocular perfusion pressure; FLIP, flicker light-induced provocation; NOx, nitric oxide provocation.

[a] Available at follow-up only.

nitrite by nitrate reductase included clorimetric detection of nitrite (Griess Reaction, 540 nm) with inter- and intra-assay variability of 2.4–7.7% and 1.5–5.5% respectively (product code 10752345; Thermo Scientific, Pierce Biotechnology, IL, USA).

2.8. Statistical analysis

Statistica® software version 12.0 (Statsoft Inc., Tulsa, USA, 2012) and SPSS (SPSS Inc., IBM SPSS Statistics Version 22, Release 22.0.0, 2015, Copyright© IBM Corporation and its licensors) were used for data analyses. Normal distribution was evident when standard errors of kurtosis and skewness did not exceed the value itself by twofold. Logarithmical transformation was deemed appropriate for CRP, cotinine and γ-GT. Independent t and Chi-square (χ^2) tests compared ethnic differences and prevalence at baseline. McNemar's case-control tests were used to demonstrate potential VD risk markers prevalence and change in risk (Δ %) over 3 years. Recognized a priori confounders included sex, age, log γ-GT, log cotinine, log CRP, glucose and cholesterol [24]. General linear models determined interaction on main effects (ethnic by sex) at follow-up for depression symptoms and retinal VD risk markers, independent of *a priori* confounders. After a priori hypotheses for all tests were performed, general linear models followed by comparing depression symptoms and retinal VD risk markers in ethnic groups. We used least square means and considered *a priori* confounders.

Forward stepwise regression analyses investigated associations between retinal vascular function and structure, perfusion, NOx and chronic depression symptoms. Dependent variables included static and functional analyses of retinal arterioles and venules. Independent covariates included: *a priori*, baseline depression symptoms (indicative of chronic depression), DOPP, 24 h pulse pressure, retinopathy prevalence, pre- and post-FLIP NOx values. As result of the high correlation between CRAE and CRVE, CRAE was added as covariate for CRVE and vice versa. Sensitivity analyses were conducted to additionally control for cardiovascular disease (CVD) history, hypertension medication and HIV infection.

Optimal venular cut points associated with chronic depression symptoms were computed from the maximum of the Youden index (J) (sensitivity + specificity − 1) using non-parametric receiver operating characteristic (ROC) curves. The statistical significance level was set at $p \leq 0.05$ (two-tailed).

3. Results

Ethnic differences were evident for retinal arteriolar narrowing ($F_{1,289} = 11.11$, $P < 0.001$) as well as chronic depression symptoms ($F_{1,313} = 10.26$, $P = 0.002$). In Table 1, Blacks showed cardiometabolic susceptibility with more smoking, alcohol consumption, depression symptoms, increased pre-diabetes, low-grade inflammation and hypertension compared to Whites at baseline.

In Table 2, it is clear that Blacks showed chronic as opposed to acute VD risk in the Whites for depression symptoms, inflammation, pre-diabetes and 24 h hypertension. The Blacks showed increased alcohol abuse and arterial stiffness (-tone) risk over 3 years. This was accompanied by wider venular diameter and venular dilation, DOPP, pre-FLIP BP and -Nox (μM), as well as post-FLIP BP values and -NOx (%) responses, at follow-up. In Table 3, higher retinopathy prevalence (60.4%) compared to Whites (39.6%; $P < 0{\cdot}001$) included more arteriovenous nicking, micoraneurysms but less acute anterior glaucoma risk.

General linear models (ANCOVAs) considering *a priori* confounders in Fig. 2, revealed more depression symptoms, potential vascular disease with arterial narrowing and wider venular calibres, lower pre-FLIP NOx values and higher post-FLIP NOx (%) responses in Blacks.

In Fig. 3, a wider venular calibre cut point was associated with chronic depression symptoms in the Blacks only [248 MU: Area under the curve 0·61 (95% confidence interval/CI: 0·51, 0·72); 71% sensitivity; 55% specificity]. In Table 4, inverse associations existed between increased DOPP and arteriolar calibre in Blacks and Whites. A wider venular calibre, with cut point of 248 MU (Fig. 3) was associated with both hypoperfusion (increased DOPP) and chronic depression symptoms in the Blacks only. In this group, arteriolar narrowing was associated with hypoperfusion whilst attenuated arteriolar dilation was associated with increased post-FLIP NOx responses in the Blacks only [(Adj R^2 0.21; β −0.16 (−0.3, 0.0), $P = 0.05$)]. Repeating multiple regression analyses to control for cardiovascular disease (CVD) history, hypertension medication and HIV infection did not change the outcome.

4. Discussion

Firstly, chronic depression in the Blacks rather than acute depression in the Whites were accompanied by retinal vascular pathology. Secondly, VD seems more apparent in the Black cohort, where increased post-FLIP NOx responses were associated with attenuated arteriolar vasodilation, a notion of endothelial dysfunction. Lastly, a wider venular calibre (248 MU cut point), an index of stroke risk, was associated with hypoperfusion and chronic depression in Blacks. These three factors (chronic depression, dysregulated NO and retinal vessel haemodynamics) can facilitate VD [11] in the Blacks.

4.1. Depression and VD

The presence of a blood-retinal barrier may provide some insight into cerebrovascular pathology [1,8,11,18,19,33], subcortical ischaemia [11] and perfusion deficits in the ocular media [10]. Therefore, chronic symptoms of depression, pre-diabetes, low-grade inflammation and hyperpulsatile pressure (vascular tone/stiffness) prevalence in the Black cohort may potentiate retinal vascular pathology. Sustained VD susceptibility in this cohort may have emerged due to higher metabolic demands (oxygen and glucose) in the brain when elevated symptoms of depression are present. Lambert et al. support this notion, having demonstrated reduced cerebral respiratory quotient in depressed subjects [15]. Chronic depression symptoms thus impair metabolism in limbic areas associated with depression [11,34] that in turn may be associated with altered NO metabolism leading to central and retinal perfusion deficits and -structural changes.

Meier et al. [34] and Jensen et al. [8] support the associations between depression and structural changes in the retinal vascular calibres. However, for the first time we now show that chronic depression symptoms and bio-available salivary NO may facilitate retinal functional and structural dysregulation. Over-demanding emotional challenges activate sympatho-adrenal medullary (SAM) and hypothalamic-pituitary-adrenal cortical axis (HPAA) stress pathway responses and can facilitate chronic depression symptoms [11,35]. Indeed, evidence of chronic disturbed metabolism seems plausible in the Blacks, as they presented cardiometabolic vulnerability, depression symptoms and compensatory post-FLIP induced neural NO release. On related note, higher sympathetic activity was shown to be present in around 30% of depressed patients [35]. In agreement, the SABPA Black cohort has demonstrated attenuated acute stress pathway reactivity responses when having depression symptoms [14], which support the notion of habituation of neuro-endocrine pathways, resulting in disturbed metabolism and inevitable neural fatigue [9]. Ultimately, chronic and not acute depression symptoms accompanied by a hyperpulsatile pressure/

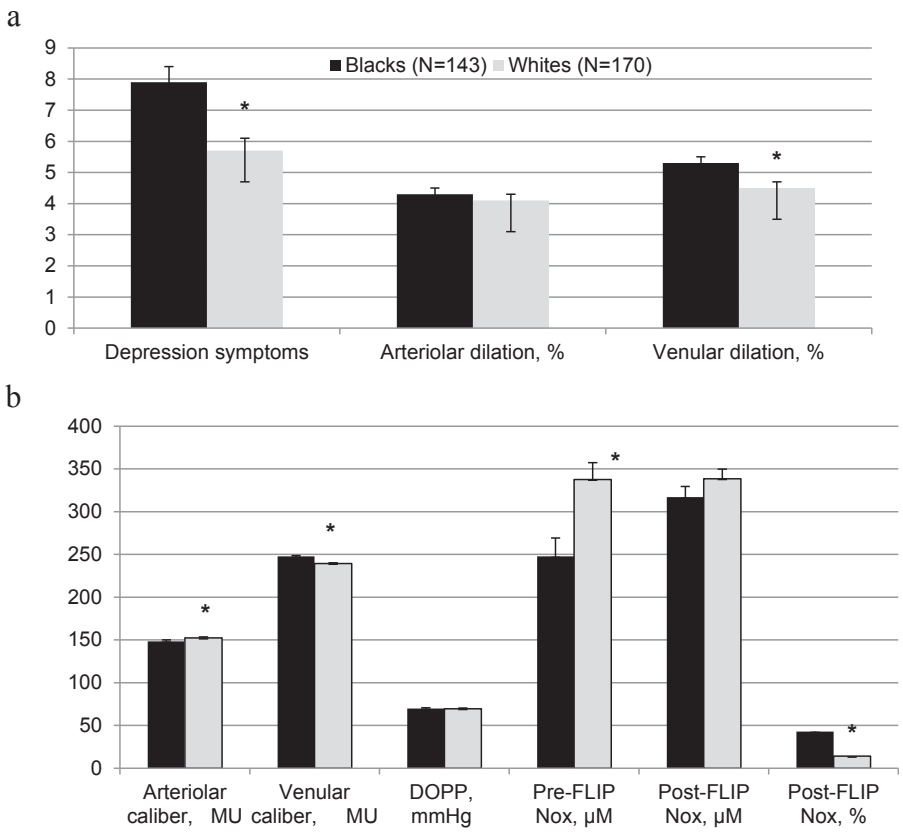

Fig. 2. (a & b): Depression symptoms and vascular disease risk in Black vs. White Africans (mean ± standard error of mean), independent of covariates. Covariates included age, sex, log cotinine, log GGT, log CRP, cholesterol and glucose. When retinal arteriole calibre was a dependent variable, retinal venular calibre was included and vice versa. Abbreviations: DOPP, diastolic ocular perfusion pressure; FLIP, flicker light-induced provocation; %, salivary NOx mean changes during FLIP and adjusted for baseline values; NOx, nitric oxide metabolites. *P ≤ 0.05.

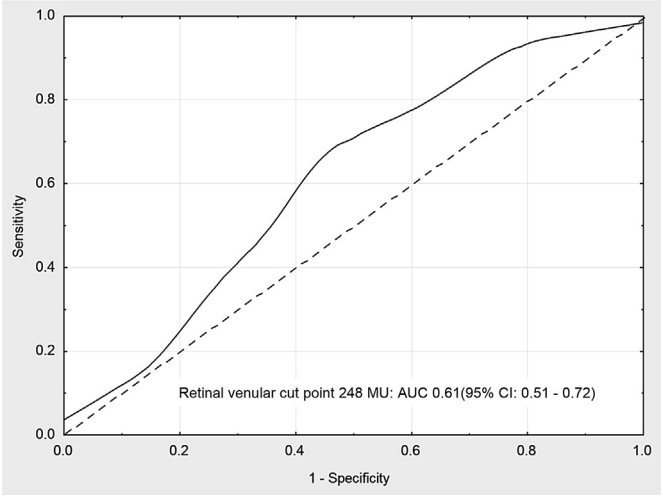

Fig. 3. The optimal retinal venular cut point associated with chronic depression symptoms in a Black sex cohort. Where: AUC, area under the curve; CI, confidence interval with 71% sensitivity, 55% specificity.

vascular tone in the Black cohort may have facilitated VD. Our findings underscore the importance of chronic and not acute increased metabolic demands for associated VD as similar associations were not found in the Whites who demonstrated acute depressive symptoms.

VD may further increase if ocular perfusion pressure [28] is disturbed as it is the driving force to ascertain sufficient blood flow through the ocular vasculature to nourish retinal neurons [17]. Blood flow in the ocular blood vessels will be modified by changes in the microvascular calibre. Therefore any changes in the retinal vascular calibre may affect ocular blood flow which was demonstrated in our Black cohort who presented a mean profile of chronic hypertension and more retinopathy signs, such as more microaneurysms and arteriovenous nicking. Their chronic higher hyperpulsatile pressure may further increase DOPP, augment vascular resistance and reduce ocular blood flow to retinal tissue thereby inducing ischaemia and/or hypoxia. Over time it may manifest as functional arteriolar narrowing [18,19,22].

A profile of chronic depression symptoms and disturbed vascular function may further impede perfusion, driven by chronic hyperpulsatile pressure. The notion of increased perfusion pressure enforces contraction in the ocular arteries, which are resistance vessels and regulated by myogenic mechanisms (Bayliss effect) [35]. In the Blacks however, myogenic tone may be impaired during chronic hypertension and -depression symptoms when less bioavailable NO is evident, as distal capillaries are not protected against deleterious increases in hyperpulsatile pressure [18]. To maintain autoregulation and perfusion, compensatory increases in neural to blood vessels signalling or neurovascular coupling, will enhance vascular tone (pulse pressure) and increase post-FLIP NOx with arteriole dilation. However, our findings contradict this mechanism and we reject the hypothesis of higher NOx coupled to increased retinal arteriolar vasodilation. The potent vasodilatory effects of NO, modulating neurovascular coupling, seems to be inhibited and vasoconstriction [36] or impaired arteriolar dilation

Table 4
Forward stepwise regression analyses depicting associations between retinal vasculature, chronic depression symptoms, hypoperfusion and NOx responses.

	Blacks (n = 143)			
	Artery dilation, %	Artery calibre, MU	Vein dilation, %	Vein calibre, MU
Adjusted R^2	0.21	0.37	<0.10	0.30
β (95% CI), P				
Sex	–	–	–	−0.22 (−0.4,−0.1), 0.01
Chronic depression symptoms	–	–	–	0.18 (0.0,0.3), 0.03
DOPP, mmHg	–	−0.46 (−0.6,−0.3), <0.001	–	0.20 (0.0,0.4), 0.04
Saliva Pre-FLIP NOx (μM)	–	–	–	0.15 (0.0,0.3), 0.07
Saliva Post-FLIP NOx (μM)	−0.16 (−0.3,0.0), 0.05	–	–	–
	Whites (n = 170)			
Adjusted R^2	<0.10	0.47	<0.10	0.47
β (95% CI), P			–	
Sex	–	–	–	–
Chronic depression symptoms	–	–	–	–
DOPP, mmHg	–	−0.20 (−0.3,−0.1), <0.001	–	–
Salivary Pre-FLIP NOx (μM)	–	–	–	–
Salivary Post-FLIP NOx (μM)	–	–	–	–

Where F to enter = 2.5. Covariates included age; log -GGT; log cotinine; log CRP; cholesterol; glucose; when retinal arteriole calibre was a dependent variable, retinal venular calibre was included and vice versa. Abbreviations: DOPP, diastolic ocular perfusion pressure; chronic depression symptoms, baseline depression symptoms; FLIP, flicker light induced provocation, NOx, nitric oxide metabolites.

occurs. The mechanism by which NO suppresses flicker-evoked vasodilation is not certain. Impaired myogenic tone may increase retinal venular widening especially during chronic pressure overload and we cautiously suggest that an overactive sympathetic system and/or chronic depression symptoms might explain part of the mechanism.

4.2. NOx bio-availability and microvasculature susceptibility

In patients with depression, a reduction in NO bio-availability was associated with impairment in vasodilation [37] as they showed less uptake and utilisation of amino acids such as L-arginine, the precursor of NO [38]. Indeed, we have previously demonstrated that the L-arginine/NO system in the black SABPA cohort is affected by emotional distress [39]. Therefore, the inability to generate sufficient NO to overcome vascular resistance in retinal vessels may increase the risk in Blacks for diseases such as stroke and peripheral occlusive vascular disease [22,40].

Increased neural responses in the Blacks via activation of the SAM and HPAA stress pathways during the FLIP may thus have facilitated NOx release to maintain perfusion. In chronic cardiometabolic vulnerable conditions, bio-availability of NO can further be attenuated as was observed in Blacks compared to Whites. Indeed, the lower bio-available pre-FLIP saliva NOx levels in the Blacks appear to be the regulating factor for retinal VD and subsequent ischaemia. A compromised NO synthase system may affect downstream nitrite production in glia cells and strain subcortical metabolism. With higher central metabolic demands, superoxide increases that in turn lowers NOx and NO to induce VD [41]. The increased NOx post-FLIP levels oppose findings of lower serum NOx mental stress levels from another bi-ethnic SABPA male cohort [21]. Therefore, saliva NOx as neural source of NO might prove to be an improved marker as opposed to serum or urine for retinal vascular regulation assessment during a challenged sympathetic system.

4.3. Central control: compensatory haemodynamics and stroke risk

Esler et al. proposed that chronic mental stress is a cause of essential hypertension [41]. We support these findings as chronic cardiometabolic vulnerability i.e., depression symptoms in conjunction with a hypertensive state is more prevalent in Blacks than in Whites (28.67% vs. 5.29·%; $P \leq 0.001$). This profile will increase neural drive in the brain during demanding chronic situations [7], decreasing sensitivity in organ structures such as the retina to prevent vascular pathology [11,18]. The high prevalence of hypertension in Blacks (66%) may thus influence autoregulation and vasomotor tone, favouring increased vascular resistance (arteriolar vasoconstriction) and possibly, vessel closure in the microcirculation [42]. On-going uncontrolled systemic hypertension and perfusion pressure in the Blacks may thus pose a risk of injury to the optic nerve head, retinal ganglion cells and thus lead to the development of retinopathy [43].

A key limitation is that retinal vascular data were not available at baseline and therefore we can't confirm that these vascular changes were not already apparent earlier on. Flammer et al. also suggested that ocular venous pressure rather than DOPP is preferred as measure of hypoperfusion and should be obtained when VD is present [18]. The contribution of autonomic dysfunction to delineate possible physiological mechanisms at play needs to be explored. More objective measures such as functional MRI's to substantiate subcortical ischaemia and perfusion deficits are needed.

In conclusion, a profile of chronic depression and cardiometabolic susceptibility may disturb the regulation of NO synthesis resulting in the observed changes in NOx and thus facilitate VD [7]. Subsequent increases in hyperpulsatile pressure may counteract reduced ocular perfusion thereby impairing myogenic tone and vascular regulation. Screening of diastolic ocular perfusion pressure and for the presence of depression symptoms is recommended to detect early onset of vascular pathology and potential subcortical ischaemia.

Conflicts of interest and source of funding

WV is chief executive officer of the Imedos Systems UG (Jena, Germany) and a freelance researcher. All other researchers declare no conflict of interest.

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Author contributions

L.M. had full access to the data; takes responsibility for the integrity of the data and accuracy of the data analyses. All authors contributed to the concept and design of the sub study, draughting and critical revision of the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.niox.2016.02.008.

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