Original Article

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Central systolic pressure and a nonessential amino acid metabolomics profile: the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension

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Objectives: Early-life exposures to cardiovascular risk factors may manifest as early vascular ageing, a phenomenon to which black populations are more prone. The metabolome provides insight into the current state and regulation of physiological processes and was used to investigate the early molecular determinants of arterial stiffness.

Methods: Black ($N=80$) and white ($N=80$) men and women (aged 20–30 years, clinic blood pressure <140 and 90 mmHg) across the arterial stiffness spectrum were included. Carotid–femoral pulse wave velocity, central SBP (cSBP) and central pulse pressure (cPP) were measured. NMR spectroscopy, liquid chromatography tandem mass spectrometry and gas chromatography–time of flight-mass spectrometry methods produced metabolomic data.

Results: Differences ($d \geq 0.3$) in 34 metabolites between black and white groups were found (adjusted for multiple comparisons). Only cSBP were higher in the black group ($P=0.003$). Lower dietary protein intake ($P<0.001$), but higher urinary nonessential amino acid levels were found in the black group ($q \leq 0.05$). In multivariable-adjusted regression models cSBP and cPP inversely correlated with various nonessential amino acids, but only in black adults. These include associations of cSBP with 4-hydroxyproline ($\beta = -0.24$; $P=0.042$), alanine: ($\beta = -0.29$; $P=0.015$), glutamine ($\beta = -0.25$; $P=0.028$), glycine ($\beta = -0.26$; $P=0.027$), histidine ($\beta = -0.30$; $P=0.009$), serine ($\beta = -0.29$; $P=0.012$), and associations of cPP with alanine ($\beta = -0.31$; $P=0.005$) and serine ($\beta = -0.26$; $P=0.019$).

Conclusion: These amino acids play pivotal roles in collagen metabolism, glucose metabolism and oxidative stress and this ethnic-specific finding suggests that biosynthesis of nonessential amino acids may be upregulated to protect the vasculature against the onset of early vascular deterioration.

Keywords: arterial stiffness, black race, carotid–femoral pulse wave velocity, central blood pressure, metabolomics, nonessential amino acids, young adults

Abbreviations: ABPM, ambulatory blood pressure monitoring; African-PREDICT, African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension; BP, blood pressure; cfPWV, carotid–femoral pulse wave velocity; cPP, central pulse pressure; cSBP, central systolic blood pressure; GC–TOF-MS, gas chromatography–time of flight-mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry

INTRODUCTION

Since 1975 the worldwide prevalence of hypertension increased from 594 million to 1.13 billion in 2015. This increase is greater in low and middle-income countries and is the result of population growth and ageing [1]. This was confirmed by the WHO's Study on Global Ageing and Adult Health conducted in low and middle income countries, which reported the prevalence of hypertension in older South Africans (>50 years) to be 77.9% [2]. In an ageing world, the attention is therefore shifting towards the effects of lifetime exposure to cardiovascular risk factors which already starts in early life, with consequent vascular damage manifesting already in early adulthood [3].

Increased arterial stiffness, a measure of vascular ageing, along with aortic (central) blood pressure (BP) is intricately linked with hypertension, cardiovascular events and cardiovascular mortality [4,5]. It is well known that arterial stiffness is greater in black than white populations [6–8]. This phenomenon is already evident in 6–8-year-old boys [8] and was found to be independent of traditional cardiovascular risk factors [7,8]. Whether this is a result of genetic predispositions or early life exposures is still unclear [9].

Although multiple metabolomic studies in relation to cardiovascular disease have been conducted, the populations often involved patients already at advanced stages of cardiovascular disease, including hypertension, atherosclerosis and coronary heart disease [10–13]. A few metabolomic studies in the general population were also conducted and include a study in aged individuals (70.6 ± 11.2 years) recruited from the Singapore Chinese Health Study [14] and a study conducted in women (aged 18–84 years) enrolled in the TwinsUK Registry [15]. In the Singapore Chinese Health Study the amino acid, histidine, related positively with arterial stiffness, whereas the amino acids, methionine and valine, related inversely with arterial stiffness in unadjusted analyses [14]. In women from the TwinsUK Registry the authors reported inverse associations between arterial stiffness and various amino acids including methionine, glutamine, glycine, serine and *trans*-4-hydroxyproline [15]. Clearly none of these studies focused on the identification of early metabolomic features related to arterial stiffness and central BP in young black populations with a known predisposition for early vascular ageing [8,16].

In this hypothesis generating study we compared metabolomic profiles between young healthy black and white adults (aged 20–30 years) to identify distinct metabolite features and to determine whether BP and arterial stiffness are associated with these metabolites.

MATERIALS AND METHODS

Study population and protocol

The current study is part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT). The central aim of African-PREDICT is to follow young, apparently healthy adults over a 10-year period to identify novel markers related to early cardiovascular disease development. The baseline phase of African-PREDICT was completed in 2017 and included 1202 participants. Participant recruitment took place in the Potchefstroom and surrounding areas of South Africa by making use of active contact via field workers, access through the workplace and advertisements in local newspapers and radio stations. The study population therefore represents a convenience sample, while attempting to balance race (black and white), sex and socioeconomic status.

Participants were screened for their eligibility to take part in African-PREDICT before inclusion into the study. The inclusion criteria were young (20–30 years of age), apparently healthy black and white men and women with a normal clinic BP (<140/90 mmHg), HIV uninfected, no self-reported chronic disease (or treatment thereof) and not pregnant or breast feeding at the time of inclusion. The study complies with all applicable requirements as set out in the Declaration of Helsinki for investigations on human participants and is registered on ClinicalTrials.gov (NCT03292094). Participants gave written informed consent and the Health Research Ethics Committee of the North-West University approved the study (NWU-00001-12-A1).

For this cross-sectional substudy the study population was selected from the first 426 participants who enrolled in 2013–2014 (Fig. 1). Carotid–femoral pulse wave velocity (cfPWV) data were sorted ascending and equal proportions of black and white participants in the lower ($n = 80$) and higher ($n = 80$) arterial stiffness range were selected, while maintaining a normal distribution for cfPWV in the selected group of participants (Fig. S1, http://links.lww.com/HJH/B58).

Questionnaires

Participants completed a general health and demographic questionnaire to assess alcohol use, smoking habits and socio-economic status. The Kuppuswamy socio-economic status scale was adapted for a South African environment to derive socio-economic status using a scoring system. Three categories including skills level, education and household income were graded after which participants were divided into a low, middle or high socio-economic class [17]. Three 24-h dietary recall interviews using the five-step multiple-pass approach [18] were conducted, with at least one of these interviews on a week-end day. A standardized dietary collection kit containing example pictures, packages, measurement tools and food models were used during the interview. Data were coded according to the South African Medical Research Council Food Composition Tables [19] and the Food Quantities Manual [20] was used to convert household measures to grams. Nutrient and food analysis of the dietary data was conducted by the South African Medical Research Council at the Biostatistics Unit.

Anthropometric and physical activity measurements

Anthropometric measurements were performed according to standard methods [21] and included weight (SECA 813 electronic scale; SECA, Hamburg, Germany), height (SECA 213 portable stadiometer, SECA), waist circumference (Lufkin steel tape, W606PM; Lufkin, Apex, USA) and BMI was calculated. The ActiHeart device (CamNtech, Cambridge, UK) was used to measure total energy expenditure as an estimate of physical activity for a maximum of 7 consecutive days.

Cardiovascular measurements

Blood pressure

The Card(X)plore CE120 device (MediTech, Budapest, Hungary) was used for ambulatory BP monitoring (ABPM). The ABPM devices were fitted to participants and BP was recorded every 30 min during the day (0600–2200 h) and every hour during the night (2200–0600 h). The mean successful inflation rate over the 24-h period was 87%.

Pulse wave velocity and analyses

The SphygmoCor XCEL device (AtCor Medical Pty. Ltd., Sydney, New South Wales, Australia) was used to measure cfPWV [22]. With the participants in supine position the right carotid artery was located by means of palpation to identify the strongest pulse point. The carotid pulse was measured using a tonometer while the femoral pulse was measured by a femoral cuff placed around the thigh of the participant. The transit-distance method was used and 80% of the distance calculated and entered after which the
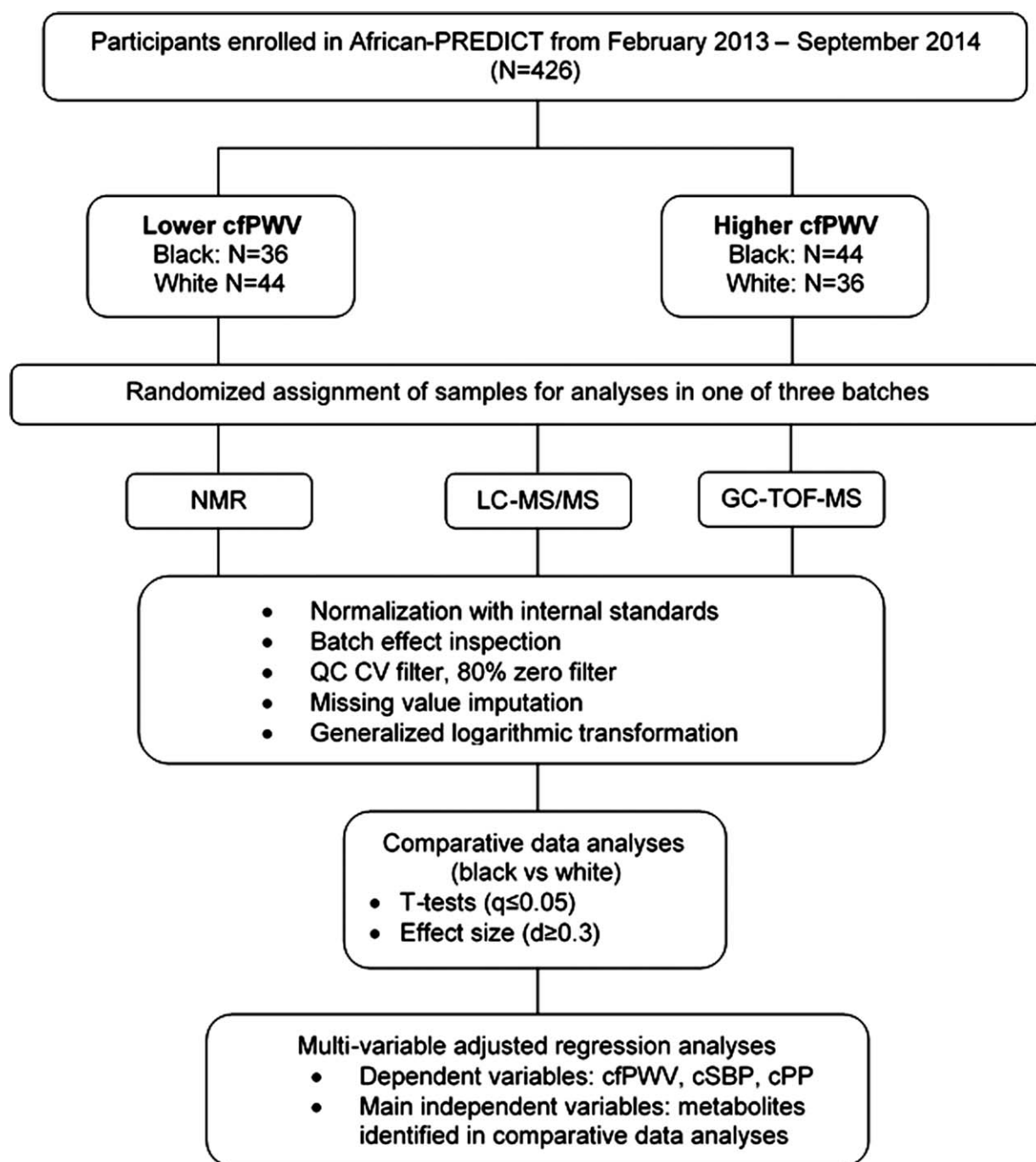
FIGURE 1 Experimental design indicating the selection of black ($n = 80$) and white ($n = 80$) participants across the arterial stiffness spectrum for metabolomic analyses on three different analytical platforms. Statistical data analyses included comparative and multivariable adjusted regression analyses. African-PREDICT, African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension; cfPWV, carotid–femoral pulse wave velocity; cPP, central pulse pressure; cSBP, central SBP; CV, coefficient of variation; GC–TOF-MS, gas chromatography–time of flight-mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; QC, quality control.

cfPWV was measured along the descending thoracic-abdominal aorta using the foot-to-foot velocity method. Duplicate measurements were taken and the mean value used in subsequent analyses. Any measurement not considered of sufficient quality were repeated based on an operator index and additional quality indices reflecting the degree of variation above acceptable limits [23]. The augmentation index was measured using pulse wave analysis with a cuff at the brachial artery. A central arterial waveform was produced that provided a central SBP (cSBP) reading, obtained from the peripheral arterial waveform [24], and central pulse pressure (cPP) was calculated.

Biochemical analyses

Participants were required to fast for at least 8 h before a registered nurse took blood samples from the antebrachial vein. An early-morning spot urine sample was also collected. All biological samples were taken to the on-site

laboratory for further preparation before stored in bio-freezers (−80 °C) until analyses. Basic biochemical analyses included serum total cholesterol (TC), HDL cholesterol, LDL cholesterol, triglycerides, high sensitivity C-reactive protein, creatinine, alanine aminotransferase, aspartate aminotransferase, total protein, creatinine (Cobas Integra 400 plus; Roche, Basel, Switzerland), cotinine (Immulite; Siemens, Erlangen, Germany) and plasma glucose (Cobas Integra 400 plus). Estimated glomerular filtration rate was calculated from serum creatinine values [25]. Whole ethylenediaminetetraacetic acid blood samples were used to obtain full blood counts (Coulter AcT 5 diff Analyzer; Beckman Coulter, Porterville, California, USA). Oxidative stress markers included serum γ-glutamyl transferase, uric acid (Cobas Integra 400 plus), glutathione peroxidase (Randox, Co. Antrim, UK) and serum peroxides, as an indicator of reactive oxygen species which was determined using a high-throughput spectrophotometric assay (Synergy HT microplate reader; BioTek, Winooski, Vermont, USA) [26].

Three analytical platforms including NMR spectroscopy (500-MHz Bruker, Avance III HD NMR spectrometer), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Agilent 6410 LC–MS/MS system with 1200 series LC front-end) and gas chromatography–time of flight-mass spectrometry (GC–TOF-MS) (Leco Pegasus HT GC–TOF-MS system with Agilent 7890A GC front-end) were used to gather metabolomic data. The methods used were previously described [27]. In short, the volume of urine samples used on the different analytical platforms for each sample was calculated by taking the creatinine level, as measure of urine concentration into account. All samples were randomly assigned to be analyzed in one of three batches on each of the different analytical platforms. Data generated from the various analytical platforms were analyzed with appropriate software and the identity of metabolites were confirmed against spectral libraries including the Bruker pH7.0 BBO REFCODE spectral library for NMR as well as the National Institute of Standards and Technology (2011) and an in-house created library for GC–TOF-MS. The data were normalized and relatively quantified by using the appropriate internal standards to minimize any technical variance. Abundance of metabolites are reported as arbitrary units. The data were examined for within-batch and between-batch effects/drifts using quality control samples (Fig. 1). As no drifts were observed the data were processed further with standard procedures.

Statistical analyses

Data was managed using the Research Electronic Data Capture system, a secure, web-based system designed to support data capture for research studies, hosted at the North-West University [28]. Statistical analyses were performed with Statistica 13.2 (Dell, Round Rock, Texas, USA) and MetaboAnalyst (www.metaboanalyst.ca) [29]. Nonmetabolomic variables were tested for normality by visual inspection (quintile-quintile plots). Normally distributed variables were expressed as arithmetic mean and SD and data that were not normally distributed were logarithmically transformed and presented as geometric means with 5th and 95th percentile boundaries. Means and proportions were compared between black and white groups using

independent *t* tests or analyses of covariance and chi-square tests, respectively.

Metabolomic data processing was done in MetaboAnalyst. A quality control coefficient of variance filter was applied. Data were uploaded as a peak intensity table and an 80% zero filter was applied after which zero value imputation was done by replacing zero values with the minimum value of each column. Hereafter, the data were transformed by using a generalized logarithmic transformation. Metabolomic data were also explored using both unsupervised (principal component analysis) and supervised (partial least square discriminant analysis) methods (Fig. S2, http://links.lww.com/HJH/B58). Independent *t* tests were done on the transformed data to compare the geometric means (with the 5th and 95th percentile boundaries) between black and white groups, *P* values were adjusted for multiple comparisons ($q \leq 0.05$) to lower the false discovery rate using the Benjamini–Hochberg procedure, and Cohen's *d* effect size was calculated (Fig. 1). Due to the young normotensive nature of the study population, we did not expect large metabolic differences between the groups and therefore selected an effect size cut-off value of *d* at least 0.3. Duplicate metabolites obtained from different analytical platforms were evaluated and those with the largest effect size were kept in the dataset. Metabolites identified to differ significantly between the black and white groups ($q \leq 0.05$) with an effect size *d* at least 0.3 were further explored in subsequent regression analyses.

Interactions of race on the relationship between cfPWV, cSBP and cPP as dependent variables and the identified metabolites were tested using multiple regression analyses. Partial regression analyses (while adjusting for sex, age and waist circumference) were performed to investigate associations of arterial stiffness and central BP (cfPWV, cSBP and cPP) with identified metabolites. Partial regression analyses with cfPWV as dependent variable were additionally adjusted for 24 h mean arterial pressure. Multivariable adjusted linear regression analyses were used to determine independent associations between cardiovascular measures and identified metabolites. The following covariates were considered for entry in the multiple regression models: age, sex, BMI, waist circumference, total energy expenditure, 24 h SBP, 24 h DBP, 24 h mean arterial pressure, TC, HDL cholesterol, LDL cholesterol, triglycerides, C-reactive protein, cotinine, socio-economic status, total energy intake, total protein intake and total energy expenditure. Based on the strongest bivariate correlations with the dependent variables, the following covariates were entered in multiple regression models: age, sex, waist circumference, total energy expenditure, cotinine and total protein intake. Models with cfPWV as dependent variable were additionally adjusted for 24 h mean arterial pressure. Correlation analyses of residual values with main independent variables (respective metabolite) were nonsignificant ($P \geq 0.18$), indicating valid regression models.

RESULTS

Interactions of race on the following associations were found: cfPWV and serine ($P = 0.048$); cfPWV and 4-hydroxyproline ($P = 0.018$); cSBP and glycine ($P = 0.047$);

TABLE 1. Characteristics of black and white groups

	Black, $n=80$	White, $n=80$	P value
Age (years)	24.1 ± 3.15	25.8 ± 2.66	<0.001
Sex (men/women)	49/31	52/28	0.62
BMI (kg/m^2)	24.3 ± 5.50	25.1 ± 5.68	0.38
Waist circumference (cm)	75.9 ± 10.5	79.5 ± 14.0	0.064
Cardiovascular measurements			
24-h SBP (mmHg)	116 ± 9.76	118 ± 8.78	0.10
24-h DBP (mmHg)	69 ± 6.04	69 ± 6.41	0.73
24-h Pulse pressure (mmHg)	46 ± 6.66	49 ± 8.29	0.083
24-h Mean arterial pressure (mmHg)	88 ± 7.03	89 ± 6.24	0.27
Central SBP (mmHg)	113 ± 5.66	110 ± 4.37	0.003
Central pulse pressure (mmHg)	34 ± 6.43	35 ± 5.18	0.15
Carotid–femoral pulse wave velocity (m/s)[a]	6.51 (6.31; 6.71)	6.33 (6.13; 6.54)	0.23
Augmentation index (%)[b]	7.07 (5.35; 8.78)	9.17 (7.51; 10.8)	0.96
Biochemical analyses			
Total cholesterol (mmol/l)	3.63 (2.50; 4.97)	4.74 (3.30; 7.22)	<0.001
HDL cholesterol (mmol/l)	1.24 (0.80; 1.75)	1.43 (0.83; 2.30)	0.001
LDL cholesterol (mmol/l)	2.11 (1.24; 3.44)	2.96 (1.67; 5.18)	<0.001
Triglycerides (mmol/l)	0.79 (0.39; 1.58)	0.96 (0.44; 2.18)	0.004
Glucose (mmol/l)	3.79 ± 0.92	4.77 ± 0.76	<0.001
C-reactive protein (mg/l)	1.31 (0.19; 9.37)	1.10 (0.10; 8.58)	0.39
Alanine aminotransferase (U/l)	14.7 (7.60; 51.8)	14.8 (7.20; 50.2)	0.93
Aspartate aminotransferase (U/l)	19.2 (11.9; 44.8)	17.8 (11.2; 34.1)	0.21
Total protein (g/l)	73.8 ± 5.87	70.8 ± 4.57	<0.001
Creatinine (μmol/l)	64.3 ± 13.9	75.5 ± 14.5	<0.001
eGFR (ml/min per 1.73 m^2)	121 ± 13.8	106 ± 13.8	<0.001
Haemoglobin (g/dl)	14.0 ± 1.84	14.0 ± 1.59	0.12
Haematocrit (%)	42.2 ± 4.70	41.8 ± 4.01	0.62
Oxidative stress markers			
γ-Glutamyl transferase (U/l)	26.9 (12.4; 101)	16.4 (7.50; 55.9)	<0.001
Total glutathione (μmol/l)	1260 ± 276	954 ± 242	<0.001
Glutathione peroxidase (U/l)	18.2 ± 1.93	20.2 ± 1.20	<0.001
Reactive oxygen species (U)[c]	182 (90.5; 329)	166 (98.4; 386)	0.17
Uric acid (μmol/l)	289 ± 63.8	339 ± 83.1	<0.001
Lifestyle			
Cotinine (ng/ml)	68.3 ± 124	38.7 ± 89.3	0.089
Self-reported smoking, n (%)	24 (30.0)	13 (16.0)	0.039
Self-reported alcohol use, n (%)	59 (74.0)	55 (66.0)	0.30
Total energy expenditure (kCal)	2125 ± 379	2347 ± 513	0.004
Socio-economic score	16.6 ± 5.07	25.5 ± 5.03	<0.001
Diet			
Total energy intake (kJ)	7238 ± 2878	9080 ± 2856	<0.001
Total protein intake (g)	59.7 ± 24.4	85.0 ± 37.4	<0.001
Plant protein intake (g)	21.2 ± 10.1	17.6 ± 7.40	0.012
Animal protein intake (g)	29.5 ± 18.2	56.7 ± 31.9	<0.001

Values are arithmetic mean and SD, geometric mean with 5th and 95th percentiles, frequency and percentage, or adjusted mean (least square) with 95% confidence interval. eGFR, estimated glomerular filtration rate.
[a]Adjusted for 24 h mean arterial pressure.
[b]Adjusted for body height and heart rate.
[c]Reactive oxygen species measured as serum peroxides in which $1 U = 1.0$ mg/l H_2O.

cPP and serine ($P = 0.044$); cPP and alanine ($P = 0.034$); cPP and 4-hydroxyproline ($P = 0.044$). Based on the aim of this study and these interactions, the study population were divided into black and white groups. As described in Table 1, the black participants were younger ($P < 0.001$) than the white participants, but their cSBP was higher ($P = 0.003$), while 24-h BP measurements, cfPWV, augmentation index and cPP were similar (Table 1 and Fig. 2). The black group displayed a more favourable lipid profile with lower TC, LDL cholesterol and triglycerides ($P \le 0.004$) while glucose levels ($P < 0.001$) were also lower when compared with their white counterparts. Renal function as indicated by estimated glomerular filtration rate was also higher in the black group ($P < 0.001$). Regarding oxidative stress markers, the black group had higher γ-glutamyl transferase

activity and lower glutathione peroxidase activity and uric acid, while total glutathione levels were higher (all $P < 0.001$) when compared with the white group. When comparing lifestyle and behavioural risk factors between these groups, the black group reported to smoke more ($P = 0.039$) and their total energy expenditure ($P = 0.004$) and socio-economic score ($P < 0.001$) were lower than that of whites. Although total reported dietary energy intake along with total protein intake and animal protein intake were lower ($P < 0.001$) in the black group, their total serum protein levels were higher ($P < 0.001$).

As expected (due to the nature of the study population) natural separation based on metabolomics data in principal component analyses was not achieved, while the supervised partial least square discriminant analyses showed
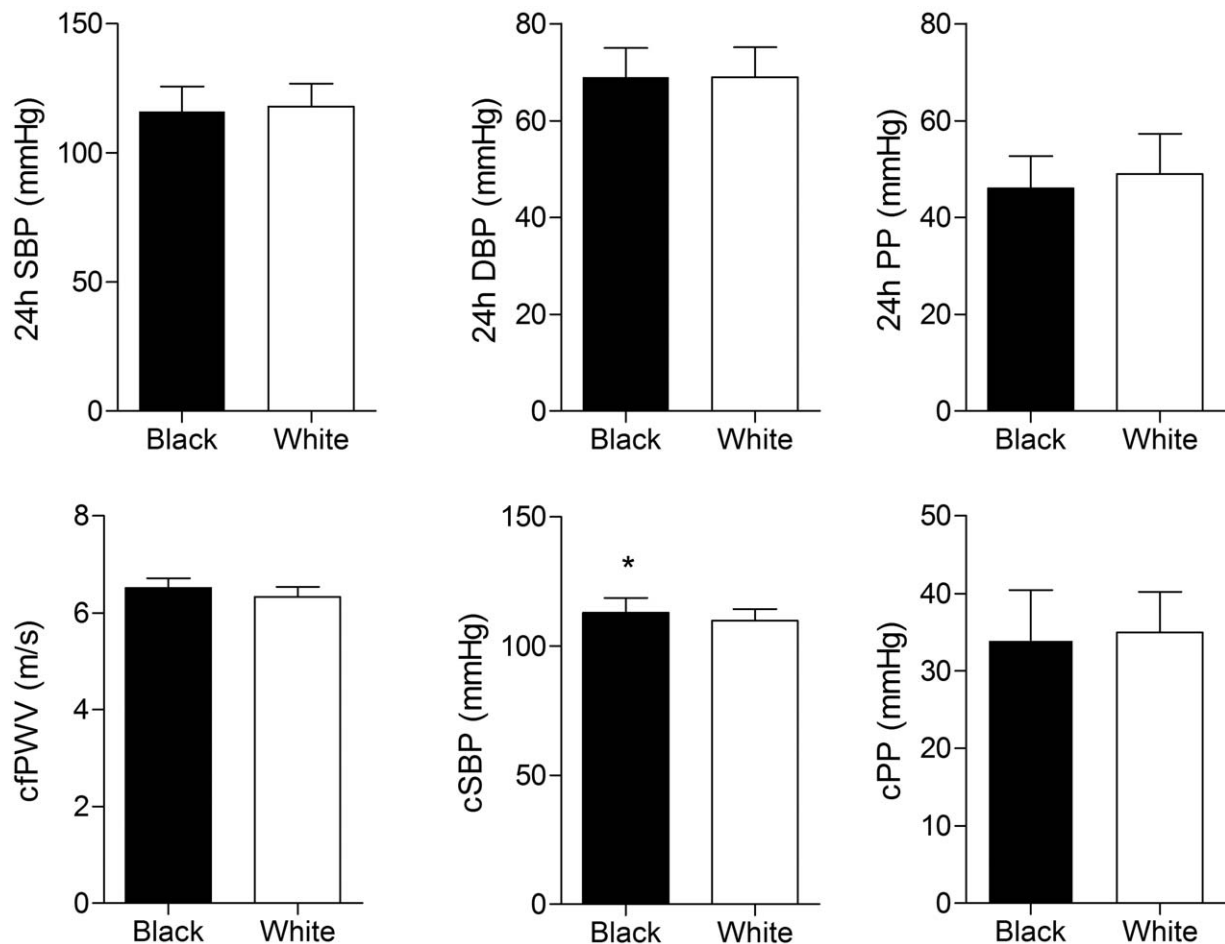
FIGURE 2 Comparison of cardiovascular measures between black (*n* = 80) and white (*n* = 80) groups. *Indicate statistical significance. 24 h PP, 24 h pulse pressure; cfPWV, carotid–femoral pulse wave velocity; cPP, central pulse pressure; cSBP, central SBP.

better separation between the two racial groups (Fig. S2, http://links.lww.com/HJH/B58). All metabolomic data obtained from the different analytical platforms were compared between black and white groups (Tables S1–S3, http://links.lww.com/HJH/B58).

In Table S4, http://links.lww.com/HJH/B58 metabolites with an adjusted *P* value (*q* ≤ 0.05) to lower the false discovery rate and an effect size (*d* ≥ 0.3) were compared between black and white groups. Various amino acids were found to be more abundant in the black group including alanine, dimethylglycine, glycine, histidine, serine, glutamine, 4-hydroxyproline and methionine (Fig. 3).

In partial (Table S5, http://links.lww.com/HJH/B58) and multivariable-adjusted regression analyses (Table S6, http://links.lww.com/HJH/B58 and Fig. 4) associations of cfPWV, cSBP and cPP with the identified metabolites were determined. In the black group cSBP and cPP inversely correlated with various amino acids or amino acid derivatives: cSBP and 4-hydroxyproline (β = −0.24; *P* = 0.042); cSBP and alanine: (β = −0.29; *P* = 0.015); cSBP and glutamine (β = −0.25; *P* = 0.028); cSBP and glycine (β = −0.26; *P* = 0.027); cSBP and histidine (β = −0.30; *P* = 0.009); cSBP and serine (β = −0.29; *P* = 0.012); cPP and alanine (β = −0.31; *P* = 0.005); cPP and serine (β = −0.26; *P* = 0.019). These associations were absent in the white

group. No associations with cfPWV were found in either of the groups.

DISCUSSION

The prevalence of hypertension is increasing in African populations [1,2] and greater arterial stiffness is experienced by black populations at younger ages than white populations [8,16]. In this hypothesis generating study, we investigated this phenomenon by comparing metabolomic profiles to identify a metabolite signature related to arterial stiffness and central BP. Despite similar arterial stiffness, cSBP was higher in the black than white group. We also found various urinary nonessential amino acids to be more abundant and inversely related to central BP in these young black adults. An increased abundance of nonessential amino acids may relate to either increased protein catabolism or increased endogenous amino acid biosynthesis. In light of a lower dietary protein intake, and especially animal protein intake observed in this group and as inverse relationships of nonessential amino acids with central BP were found, the upregulation of biosynthesis pathways as a vascular protective mechanism to prevent or delay early vascular compromise seems more plausible. Notably, these links were absent in the young white adults who may be at
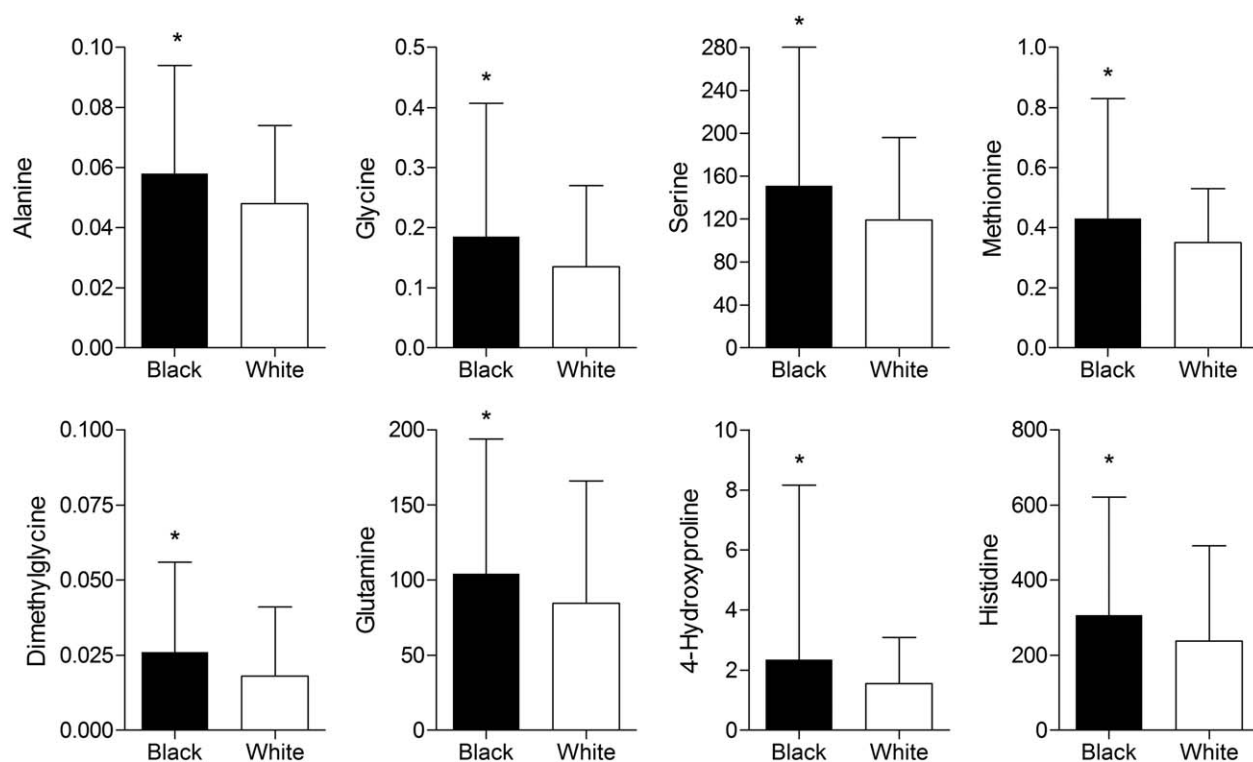
FIGURE 3 Comparison of identified amino acids between black ($n = 80$) and white ($n = 80$) groups. *Indicate statistical significance after adjustment of multiple comparisons ($q \leq 0.05$).

lower risk for the future development of elevated BP, arterial stiffness and ultimately cardiovascular disease.

Various nonessential amino acids including alanine, glycine, serine, methionine and glutamine as well as amino acid derivatives such as dimethylglycine and 4-hydroxyproline and one essential amino acid, histidine, were more abundant in the black group. When comparing our findings with those from previous metabolomics studies in diseased populations such as patients with hypertension [13], stable carotid atherosclerosis [30], stroke [31] and acute coronary syndrome [32], our findings are in contradiction to these studies, which indicated alanine, glycine, serine, glutamine,

proline and 4-hydroxyproline to be lower when compared with controls. However, when taking the young normotensive nature of our study population into account the contradiction to previous findings in diseased populations is not surprising. In addition to the more abundant amino acids levels we also found inverse associations of central BP with these amino acids adding to the controversy in the literature as some previous findings are in agreement with ours [14,15] and others not [10,33]. In women enrolled in the TwinsUK Registry, pulse wave velocity was inversely related to glycine, serine, glutamine and trans-4-hydroxyproline [15]. On the other hand, results from a large
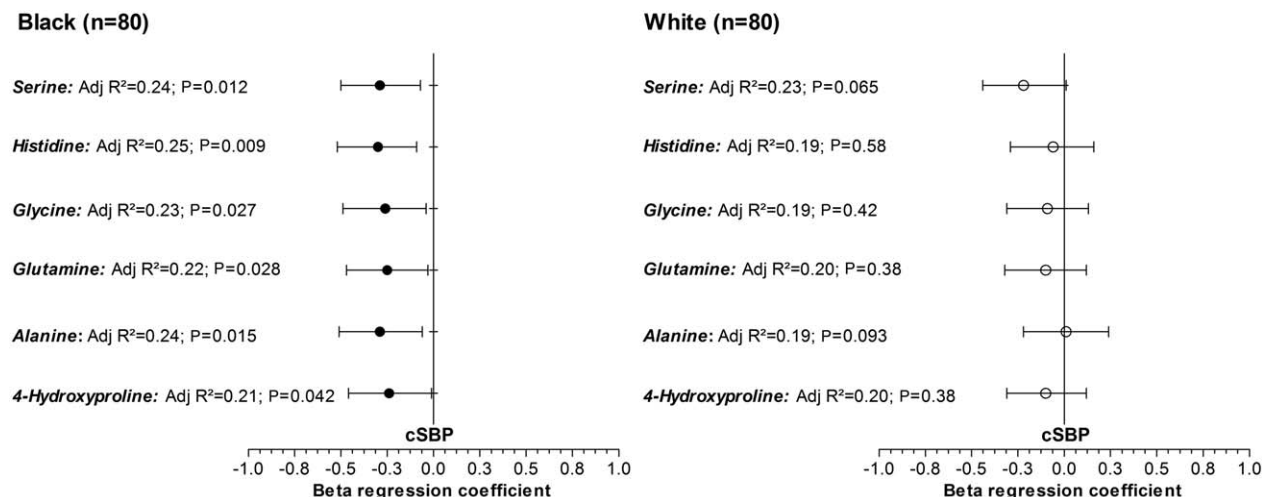

FIGURE 4 Forest plots indicating independent inverse associations of central SBP with identified amino acids in black ($n = 80$) and white ($n = 80$) groups.

population-based study showed higher serum glutamine to have predictive value for carotid wall thickening [10], and findings from the International Study of Macro-and-Micro-Nutrients (INTERMAP) found positive associations of SBP and DBP with alanine [33].

The absence of a link between aortic stiffness (cfPWV) and the identified nonessential amino acids is also noteworthy. The general understanding is that aortic stiffness is represented well by either cfPWV or central BP as listed in the 2018 European Society of Cardiology/European Society of Hypertension guidelines [34]. However, findings from the Framingham Heart study indicated that central pulsatile haemodynamics and aortic stiffness should be interpreted separately as each reflect different aspects of central arterial structure and function [35]. This is supported by the findings of a recent study done in black South Africans ($N = 1232$) in which it was indicated that cPP and cfPWV are differentially associated with cardiovascular risk [36]. Aortic stiffness and central pulsatile haemodynamics may therefore contribute differently to cardiovascular risk and the absence of a link between aortic stiffness and the identified nonessential amino acids in the black group of this study may suggest a role for these nonessential amino acids in pulsatile haemodynamics. Nonetheless, both aortic stiffness and increased mechanical stretch as a result of altered central pulsatile haemodynamics are both associated with altered collagen metabolism [37–39].

We propose an altered metabolic pathway in these young black adults as a possible mechanism to maintain collagen biosynthesis and stability and delay the onset of early vascular ageing (Fig. 5). Lower glucose levels in the black group, may reflect lower total energy intake or increased flux through the glycolysis pathway. Pyruvate, the end-product of glycolysis, can be converted to alanine (KEGG 00250) [40], an important regulator of glucose metabolism [41,42]. Both pyruvate and alanine were more abundant in the black group, which support the increased flux through the glycolysis pathway. Serine with BP-lowering effects [43], was found to be higher in the black group and can be actively synthesized from 3-phosphoglycerate (Fig. 5), a glycolysis intermediate (KEGG 00260) [40]. Serine may act as an one-carbon donor to the folate cycle, during which 1,5-methylene tetrahydrofolate and glycine are formed. Glycine, which was also higher in the black group,
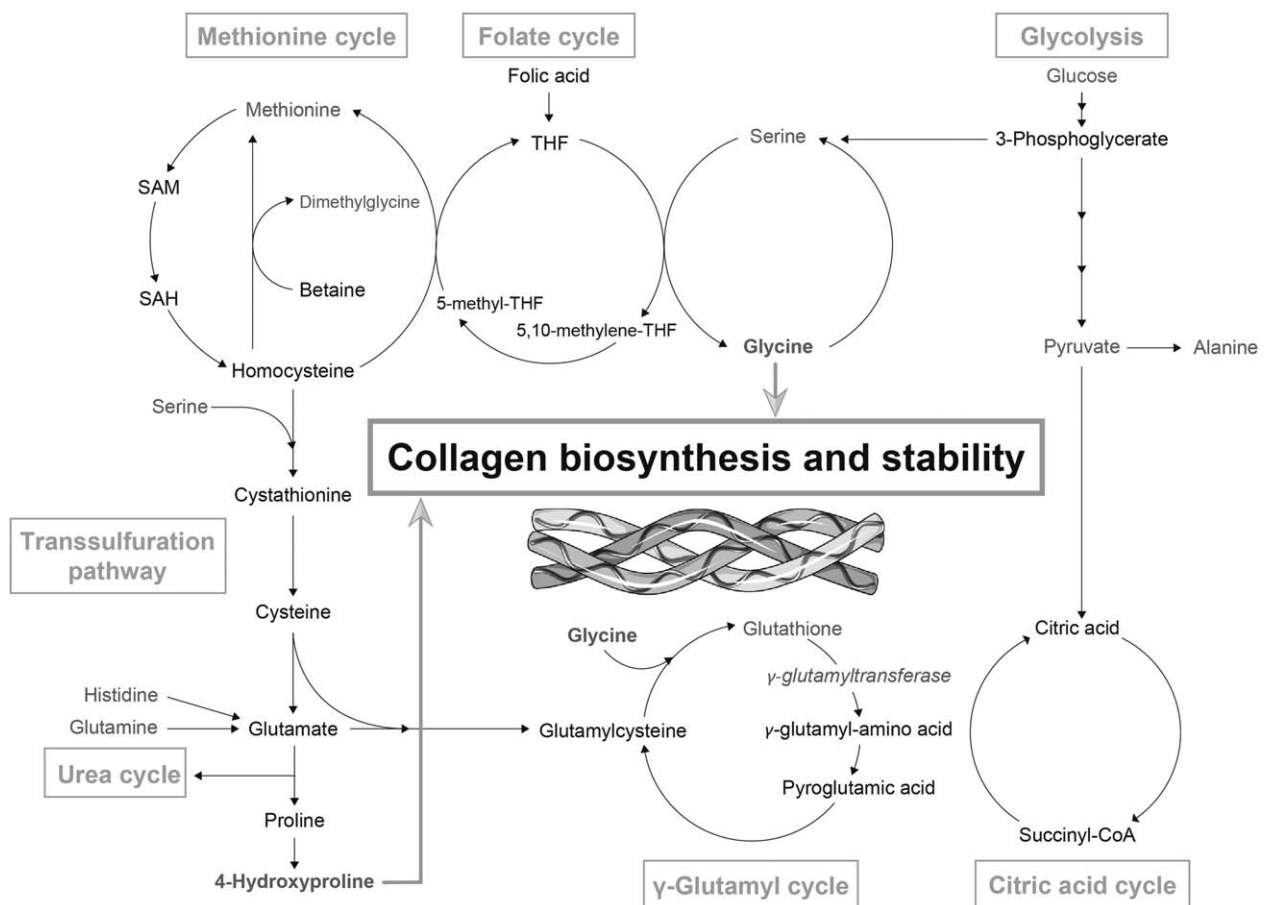

FIGURE 5 Proposed altered metabolic pathway in the black group. The lower glucose levels along with the higher pyruvate and alanine levels may indicate increased flux through the glycolysis pathway. One of the intermediates of the glycolysis pathway, 3-phosphoglycerate can be used to actively synthesize serine (more abundant in the black group) and during one-carbon-metabolism serine acts as an one-carbon donor to the folate cycle, during which 1,5-methylene tetrahydrofolate and glycine (higher in the black group) are formed. In turn, the folate cycle is coupled to the methionine cycle in which both methionine and dimethylglycine was found to be more abundant in the black group. The methionine cycle is coupled to the transsulfuration pathway leading to the formation of cysteine. Cysteine along with glutamate which can be synthesized from glutamine (more abundant in the black group) and glycine are involved in the synthesis of glutathione. Alternatively, if not used for glutathione synthesis glutamate can be converted to proline and upon hydroxylation 4-hydroxyproline is formed. Both glycine and 4-hydroxyproline are important for collagen biosynthesis and stability. Metabolites in blue and red text indicate lower and higher abundance respectively. SAH, S-adenosyl-homocysteine; SAM, S-adenosyl methionine; THF, tetrahydrofolate.

is involved in various anabolic pathways including the synthesis of collagen of which glycine constitutes one-third of the total amino acid residues. This implies an important requisite for glycine bioavailability to support healthy collagen turnover [44] and consequently arterial elasticity [45].

The folate cycle is coupled to the methionine cycle (Fig. 5) responsible for the conversion of methionine, which was higher in the black group, to S-adenosylhomocysteine and then to homocysteine. Homocysteine can be converted back to methionine and during this reaction betaine is converted to dimethylglycine (higher in the black group) (KEGG 00260) [40]. Homocysteine can also enter the transsulfuration pathway (Fig. 5), leading to the formation of cysteine. Cysteine and glutamate (which can be formed from glutamine and histidine, both found to be more abundant in the black group) and glycine are involved in glutathione synthesis (KEGG00480) [40]. Glutathione synthesis is dependent on the availability of its containing amino acids and the action of γ-glutamyl transferase (which was also higher in the black group) (Fig. 5) to cleave extracellular glutathione to increase the availability of its containing amino acids for intracellular glutathione synthesis [46]. Despite its important role in glutathione homeostasis, elevated γ-glutamyl transferase is associated with an increased risk for the development of cardiovascular disease, all-cause and cardiovascular mortality [46]. Alternatively, if not used for glutathione synthesis, glutamate may be converted to glutamate-5-semialdehyde which in turn may be converted to either ornithine (urea cycle) or proline (KEGG 00330) [40]. Proline may then be converted to 4-hydroxyproline which play an important role in collagen stability [47] (Fig. 5). Taken together, the proposed altered metabolic pathway may be the result of lower protein intake and may reflect a compensatory mechanism to maintain healthy collagen turnover and stability required to delay early vascular compromise in these young black adults with a known predisposition for early vascular ageing.

These findings should be interpreted within the context of the strengths and limitations of the study. The study population consisted of a modest bi-ethnic sample of individuals selected to include participants across the arterial stiffness spectrum, which resulted in similar arterial stiffness between the race groups. Participants were recruited from the North West Province of South Africa and may not represent the entire population. However, this study included participants from the understudied black population, prone to the development of early vascular ageing and is one of the first studies to investigate the early molecular features associated with central BP and arterial stiffness. Although the results were consistent after several adjustments, we cannot exclude residual confounding. The study was conducted under highly controlled conditions in a well equipped research facility and included metabolomic data from three different analytical platforms. Apart from the metabolites discussed in association with central BP some other metabolites were also identified to differ between the black and white groups of this study. These metabolites should be investigated in subsequent studies in relation with other measures of cardiovascular structure and function.

In conclusion, in young black adults with normal BP and arterial stiffness profiles, we identified a unique metabolomic signature consisting of more abundant levels of nonessential amino acids, which are inversely associated with central BP. These amino acids may be more abundant as a result of increased protein breakdown or upregulated biosynthesis. However, in light of the lower dietary protein intake observed in young black adults, along with the pivotal roles these amino acids play in collagen metabolism, glucose metabolism and oxidative stress, the amplification of biosynthesis pathways as a vascular protective mechanism against early vascular compromise seems conceivable. Future studies should aim to confirm the hypotheses generated in this study, both in independent black cohorts and over time.

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Conflicts of interest
There are no conflicts of interest.

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