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ORIGINAL ARTICLE

Determining Underlying Mechanisms of Early Vascular Ageing by Clustered Analysis: The African-PREDICT Study

Ashleigh Craig, PhD [a], Wayne Smith, PhD [a,b], Catharina M.C. Mels, PhD [a,b], Yolandi Breet, PhD [a,b], Shani Botha-le Roux, PhD [a,b], Adriaan Jacobs, PhD [a,b], Lebo F. Gafane-Matemane, PhD [a,b], Ruan Kruger, PhD [a,b,*]

[a]Hypertension in Africa Research Team (HART); North-West University, Potchefstroom, South Africa
[b]MRC Research Unit for Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa

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Objective Identifying individuals at increased risk of early vascular ageing (EVA) is paramount to inform intervention and prevention strategies and curb the increasing burden of cardiovascular disease.

Methods We stratified and phenotyped pre-screened young apparently healthy South African adults (20–30 yrs) (n=1,041) into vascular ageing profile groups based on carotid femoral pulse wave velocity (cfPWV) percentiles (healthy vascular ageing [HVA]; average vascular ageing [AVA] and EVA). We further compared various anthropometric, cardiovascular (CV), oxidative stress and lifestyle risk factors and determined factor scores to explore associations between CV measures and factor clusters to explore associations in those at risk of EVA.

Results Young adults in the EVA group displayed marked phenotypic characteristics in terms of anthropometry, CV, and lifestyle risk factors, even though cfPWV were within healthy ranges. Blood pressure (brachial and central) and cfPWV were all incrementally higher across all three vascular ageing groups (p-trend $\leq$0.011). Hypertension, lifestyle risk factors such as self-reported smoking and alcohol consumption were all highest in the EVA group (p-trend $\leq$0.046). Additionally, in the EVA group only, cfPWV (adj. R^2=0.028; β=0.171; p=0.042) associated positively with Factor 2 (oxidative stress and antioxidant capacity). No associations existed between Factor 1 (basic lipids) and any anthropometric or CV measures (p>0.050).

Conclusion Young adults with higher cfPWV presented with a less favourable vascular profile and more unhealthy lifestyle behaviours compared to groups with lower cfPWV. In the EVA group, cfPWV positively associated with a cluster of oxidative stress and antioxidant capacity. Early lifestyle behaviours may have the ability to modify the balance between oxidants and antioxidants, potentially contributing to early onset arterial stiffness.

Keywords Arterial stiffness • Early vascular ageing • Lifestyle risk factors • Oxidative stress • Pulse wave velocity

Introduction

Early vascular ageing (EVA) is an evolving concept first described over a decade ago [1]. The phenomenon of EVA has since expanded based on accumulating evidence surrounding arterial stiffness as an intermediate endpoint and independent predictor of cardiovascular disease (CVD) and mortality [2]. Thus, EVA is seen as the premature structural

*Corresponding author at: Prof Ruan Kruger, PhD, Hypertension in Africa Research Team (HART), North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa; Email: ruan.kruger@nwu.ac.za; Twitter: @ProfRuanKruger

and functional alteration within the arterial wall, as measured by carotid femoral pulse wave velocity (cfPWV). Increased cfPWV is an established hallmark of arterial stiffening [2], improves cardiovascular (CV) risk prediction beyond traditional risk factors [3], and has thus been highlighted as the proposed marker of EVA. The clinical implication related to EVA is to identify young individuals with signs of accelerated vascular ageing at an early stage [1]. If left undetected, EVA may ultimately cause those susceptible to early onset CVD to display irreversible CV damage, despite preventive and treatment measures taken later in life. Therefore, as arterial stiffness is seen as a predictor of CVD and CV mortality, assessing EVA in young individuals could be used as a proxy of vascular ageing which may further highlight EVA as an important potentiating factor in the development of CV risk.

Accelerated vascular ageing may be due to several factors, with elevated blood pressure (BP) and unhealthy lifestyle behaviours being among the most relevant [4,5]. As haemodynamic factors play a vital role in functionality, elevated BP and/or hypertension are undoubtedly crucial factors in determining arterial stiffness [6]. Yet it has also been reported that the occurrence of non-haemodynamic factors such as oxidative stress, a key role player in endothelial dysfunction, is also an important factor associated with arterial stiffness [7]. It was previously reported that markers of oxidative stress such as oxidised low-density lipoproteins (ox LDL), reactive oxygen species (ROS) and gamma-glutamyl transferase (GGT) were found to positively associate with arterial stiffness indices (PWV and ambulatory arterial stiffness index) in younger individuals [8–10]. These studies support the notion that early vascular changes are evident in young normotensive individuals and may be related to oxidative stress enhancing early onset arterial stiffness. Previous studies have indicated that various CV risk factors tend to be clustered in certain individuals [11–13] and therefore identifying these clusters to form comprehensive lifestyle interventions may be an effective strategy for controlling risk factors in order to reduce the burden of CVD.

Our study is motivated by the fact that the early identification of young individuals who are at increased risk of EVA is vital to help inform interventions that could alleviate future CVD risk. We therefore aimed to stratify pre-screened young apparently healthy South African adults (20–30 yrs) into vascular ageing profile groups based on cfPWV percentiles. We will further compare various anthropometric, CV and markers of oxidative stress and determined factor scores to explore associations between CV measures and factor clusters in young individuals who are at risk of EVA.

Methodology

The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) is an ongoing longitudinal study, where 1,202 apparently healthy volunteers were screened and assessed at baseline. The population and protocol for the African-PREDICT study have been described elsewhere [14]. Briefly, participants were screened and those with brachial blood pressure >140/90 mmHg, any self-reported diseases or risk factors (other than cigarette use and alcohol consumption) that may influence cardiovascular health, internal ear temperature >37.5°C, human immunodeficiency virus (HIV), diabetes mellitus, liver disease, cancer, tuberculosis or renal disease, who used chronic medication, as well as pregnant or lactating women, were excluded. In this cross-sectional analysis, we included data from 1,041 black and white, men and women (aged 20–30 yrs) participants after the exclusion of participants with missing cfPWV data or whose cfPWV repeated measures differed by more than 0.5 m/s (n=161).

The African-PREDICT study was conducted in line with the ethical principles of the Declaration of Helsinki [15] and was approved by the Health Research Ethics Committee of the North-West University. Participants were fully informed about the objectives of the study and written informed consent was acquired from each participant. The study is registered at clinicaltrials.gov (NCT03292094).

Questionnaire

Basic demographic information was collected with the use of a General Health and Demographic Questionnaire. This questionnaire is seen as a self-administered screening tool that was completed prior to participation. The following information was gathered from the questionnaire: age, sex, ethnicity, and current lifestyle risk factors such as self-reported smoking and alcohol consumption. In the statistical analyses, age (years) was used as a continuous variable while sex (0=women; 1=male), ethnicity (0=Black; 1=White) and lifestyle risk factors (0=no; 1=yes) were captured as binary variables.

Anthropometric Measures

All anthropometric measurements were performed according to specific guidelines set out by the International Society for the Advancement of Kinanthropometry (ISAK) [14,16]. Waist circumference (cm) was obtained using a standard protocol (Lufkin steel anthropometric tape; W606PM; Lufkin; Apex, USA). Body mass index (BMI) (weight [kg]/square height [m^2]) (SECA 813 electronic scale; SECA portable 213 stadiometer; Hamburg, Germany) for each participant was subsequently calculated and used to classify obesity (BMI>30 kg/m^2) [17]. The waist-to-height ratio (waist circumference [cm]/height [cm]) of each participant was also calculated.

Cardiovascular Measures

The SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, NSW, Australia) was used to measure cfPWV [18]. With the participant in a supine position, we located the strongest pulse point on the right carotid artery by means of palpation. 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 with the use of a non-stretchable ruler was used to measure i) the distance from the carotid pulse to the top of the thigh cuff and, ii) the distance from the femoral pulse (mid inguinal point) to the top of the thigh cuff. Eighty per cent (80%) of the distance calculated was entered as an accepted estimate of body surface area, after which the cfPWV was measured along the descending thoracic abdominal aorta using the foot-to-foot velocity method. Any measurements not considered of sufficient quality were repeated based on an operator index and additional quality indices reflecting the degree of variation above acceptable limits [19]. Any participant with repeated measures varying more than 0.5 m/s were excluded from the study (n=14). Furthermore, the built-in generalised transfer function was used to estimate the central haemodynamic variables from the peripheral waveform [20]. From this function, central BP was captured. Since cfPWV is a recognised marker of EVA [21] we used the measurement to stratify our sample population according to different vascular ageing profiles, i.e., healthy vascular ageing (HVA) ($\leq 10^{th}$ cfPWV percentile; n=106), average vascular ageing (AVA) ($>10^{th}$ and $<85^{th}$ cfPWV percentiles; n=769) and those at risk for EVA, which from this point will referred to as EVA ($\geq 85^{th}$ cfPWV percentile; n=166).

With the use of a Dinamap® ProCare 100 Vital Signs Monitor (GE Medical Systems, Milwaukee, WI, USA), brachial blood pressure was measured on the left, then the right upper arms and, after 5 minutes, again on the right, then the left upper arms. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were captured from each measurement. Mean arterial pressure (MAP) was calculated using the formula DBP/(0.4*pulse pressure) [22]. Participants were also fitted with a 24-hour ambulatory blood pressure monitor and electrocardiograph (ECG) apparatus (CardioXplore®, CE0120; Meditech, Budapest, Hungary) with the appropriately sized blood pressure cuff on each participant's non-dominant arm. This device was programmed to take recordings every 30 minutes during the day and every 60 minutes during the night from which 24-hour SBP and DBP measures were captured. Hypertension was defined as 24-hour SBP and/or DBP of ≥130/80 mmHg.

Biochemical Analyses

Participants were required to fast for at least 8 hours before early morning spot urine sample collection, and blood samples were taken by a registered research nurse at the Hypertension Clinic of the North-West University.

Basic Biochemical Analyses

Serum analyses included the lipid profile (total cholesterol, high-density lipoproteins [HDL], LDL and triglycerides) and high-sensitivity C-reactive protein (CRP) (Cobas Integra 400 plus, Roche, Basel, Switzerland). Serum cotinine levels were determined with a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Additionally, sodium fluoride plasma glucose (Cobas Integra 400 plus, Roche) was determined.

Oxidative stress markers

Oxidative stress markers included reactive oxygen species (ROS), measured as serum peroxides (reported as units, where 1 mg H_2O_2/L is equivalent to one unit) using a high-throughput spectrophotometric assay [23] and analysed on a Synergy HT microplate reader (BioTek, Winooski, VT, USA). Whole blood samples were used to measure total glutathione (tGSH) with a Synergy HT microplate reader (BioTek, Winooski, VT, USA) (Kit: BIOXYTECH GSH/GSSG-412). Gamma-glutamyl transferase was measured (Cobas Integra 400 plus, Roche) in serum and malondialdehyde (MDA) in urine by gas chromatography-mass spectrometry [24] and was normalised to creatinine excretion and expressed as μM analyte to mM creatinine (μM/mM). Antioxidant markers included glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant status (TAS) and superoxide dismutase (SOD), which were measured using assay kits (Randox, Co. Antrim, Ireland, UK) and the automated Cobas Integra 400 plus (Roche).

The intra-assay variability and inter-assay variability of all biochemical variables were below 14%.

Statistical Analyses

IBM SPSS version 27 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 5.03 for Microsoft Windows (GraphPad Software, San Diego, CA, USA) were used to analyse and plot the data. Variables were tested for normality using the Kolmogorov-Smirnov test and QQ-plots. Non-Gaussian variables were $\log_{10}$-transformed. Data were expressed as arithmetic mean ± standard deviation if normally distributed and as geometric mean with 5^{th} and 95^{th} percentile boundaries for $\log_{10}$-transformed variables.

For group comparisons, analyses of covariance (ANCOVA), adjusted for sex and ethnicity were used. Proportions were determined with cross-tabs with significant differences indicated by Chi-square tests and presented as numbers and percentages. Partial correlations were used to determine relationships of dependent variables with anthropometric, CV and biochemical variables, adjusting for age, sex, ethnicity and MAP.

Factor analyses were performed using the factor function to determine what factor or cluster of factors potentially contribute to increased cfPWV in young healthy individuals. Only factors with an eigenvalue of >1 were used. With the use of the varimax rotation, independent interpretable factors were obtained and a factor loading of ≥0.30 was applied to interpret factor patterns. The handling of double loading was done by placing the variable in the factor with the strongest loading factor. Only factor scores with a cumulative percentage of >50 was subsequently used in further analyses (multiple regression analyses). As the aim of this

Table 1 General characteristics of young adults stratified according to vascular ageing profiles.

	HVA	AVA	EVA	P-trend
	Lower ≤10th percentile (cfPWV ≤5.3 m/s) (n=106)	Middle <10th & >85th percentile (cfPWV >5.3 m/s & <7.2 m/s) (n=769)	Upper ≥85th percentile (cfPWV ≥7.2 m/s) (n=166)	
Age (years)	23.9±3.08[c]	24.5±3.14[b]	25.5±2.95[b,c]	**<0.001**
Sex, men (*n* %)	9 (8.5)[a]	357 (46.4)[a,b]	139 (83.7)[b]	**<0.001**
Ethnicity, black (*n* %)	46 (43.4)[a]	371 (48.2)[a,b]	98 (59.0)[b]	**0.017**
Body Composition				
Waist circumference (cm)	78.6±12.0	79.5±12.1	81.6±12.0	0.79
Body height (cm)	164±7.60[a,c]	168±9.14[a,b]	174±9.22[b,c]	**<0.001**
Body weight (kg)	70.3±17.2	70.5±16.4	72.7±17.2	0.52
Body mass index (kg/m^2)	26.1±6.08	24.9±5.15	24.0±4.60	0.051
Waist/height ratio	0.48±0.07	0.47±0.07	0.47±0.06	0.40
Obese (*n* %)	24 (22.6)	120 (15.6)	16 (9.6)	**0.014**
Cardiovascular Measures				
Pulse wave velocity (m/s)*	4.98±0.33[a,c]	6.22±0.49[a,b]	7.82±0.81[b,c]	**<0.001**
Brachial systolic blood pressure (mmHg)	110±11[a,c]	118±11[a,b]	126±12[b,c]	**<0.001**
Brachial diastolic blood pressure (mmHg)	73±8[a,c]	78±7[a,b]	85±8[b,c]	**<0.001**
Brachial mean arterial pressure (mmHg)	88±8[a,c]	93±8[a,b]	101±9[b,c]	**<0.001**
Central systolic blood pressure (mmHg)	103±9[a,c]	108±9[a,b]	116±10[b,c]	**<0.001**
24-hr ambulatory systolic blood pressure (mmHg)	113±9	117±9	121±9	0.10
24-hr ambulatory diastolic blood pressure (mmHg)	66±6[a,c]	68±6[a,b]	72±6[b,c]	**<0.001**
Hypertension (*n* %)	6 (5.7)[c]	52 (7.0)[b]	21 (14.0)[b,c]	**0.010**
Oxidative Stress Markers				
Reactive oxygen species (units)	44.9 (12.7-146)	40.0 (12.8-107)	34.5 (12.6-93.4)	0.80
Malondialdehyde (μM/mM creatinine)	1.59 (0.45-5.33)	1.79 (0.61-5.02)	1.73 (0.55-5.02)	0.22
Superoxide dismutase (U/ml)	3.94 (1.72-8.12)	4.01 (1.69-7.780)	4.06 (1.86-6.71)	0.95
Glutathione peroxidase (U/L)	7,681 (3,750-13,978)	8,255 (4,896-14,625)	8,198 (4,785-14,111)	0.81
Glutathione reductase (U/L)	34.2 (12.0-64.9)	35.7 (14.1-68.7)	35.9 (11.1-70.0)	0.80
Total antioxidant status (mmol/L)	1.12±0.32	1.16±0.31	1.20±0.31	0.94
Total glutathione (μM)	758 (472-1477)	722 (432-1,428)	714 (393-1,441)	0.54
Gamma-glutamyl transferase (U/L)	14.9 (5.93-46.4)[a,c]	17.3 (5.80-50.5)[a,b]	23.6 (7.14-88.4)[b,c]	**0.003**
Basic Biochemical Measures				
C-reactive protein (mg/l)	1.15 (0.10-10.8)	0.89 (0.08-9.37)	0.62 (0.07-6.04)	0.62
Total cholesterol (mmol/L)	3.80 (2.28-6.50)	3.55 (1.98-5.75)	3.49 (1.85-5.63)	0.46
High density-lipoproteins (mmol/L)	1.31±0.48	1.15±0.41	1.11±0.42	0.052
Low density-lipoproteins (mmol/L)	2.39 (1.28-4.38)	2.25 (1.08-4.17)	2.18 (0.93-4.10)	0.80
Triglycerides (mmol/L)	0.69 (0.33-1.61)	0.72 (0.33-1.82)	0.73 (0.28-2.01)	0.88
Glucose (mmol/L)	4.11 (2.57-5.44)	3.93 (2.53-4.54)	3.89 (2.30-5.59)	0.47
Cotinine (ng/ml)	1.59 (1.00-222)	2.75 (1.00-315)	4.98 (1.00-435)	0.55
Self-Reported Lifestyle Risk Factors				
Smoking, yes (*n* %)	10 (9.4)[a,c]	187 (24.3)[a,b]	60 (36.1)[b,c]	**<0.001**
Alcohol use, yes (*n* %)	50 (47.2)[a]	408 (53.4)[a,b]	102 (61.8)[b]	**0.046**

Values are arithmetic mean±standard deviation or geometric mean (5th and 95th percentiles) for logarithmically transformed variables. Adjusted for sex and ethnicity.

Bold values denote statistical significance ($p<0.05$).

*Pulse wave velocity and intima-media thickness were additionally adjusted for mean arterial pressure.

[a]Significant difference between HVA and AVA groups.

[b]significant difference between AVA and EVA groups.

[c]significant difference between HVA and EVA groups.

Abbreviations: *n*, number of participants; HVA, healthy vascular ageing; AVA, average vascular ageing; EVA, early vascular ageing.

study is to identify young individuals who are at increased risk of EVA, who present with susceptible risk factors that contribute to the development of EVA, factor scores where only performed in the EVA group.

Standard multiple regression analyses were conducted in the EVA group with CV measures (cfPWV and brachial BP) as dependent variables, and tested separately for their association with each Factor. Covariates included in the model: age, sex, ethnicity, MAP, gamma-glutamyl transferase and self-reported smoking.

Results

The general characteristics of the study population with full cfPWV data (n=1,041), stratified by their vascular ageing profiles are presented in Table 1. Brachial BP (SBP, DBP and MAP), central SBP (cSBP), and cfPWV were all incrementally higher across the vascular ageing groups (all p-trend<0.011). The lipid profile (HDL, LDL, triglycerides and total cholesterol) was comparable across the groups (p>0.05). Gamma-glutamyl transferase was incrementally higher across the vascular ageing groups (p=0.003), while the oxidative stress markers ROS, MDA, SOD, TAS, tGSH, GR and GPx were comparable across the groups (all p≥0.22). Furthermore, hypertension, self-reported smoking and alcohol consumption were all highest in the EVA group as shown by the p-trends (all p<0.05).

We performed factor analyses with separate sets of anthropometric, CV and biochemical marker clusters to determine factor scores (Table 2). Factors with an eigenvalue of <1 and a cumulative % <50 were rejected (Supplementary Table S1). Factors were evident in the EVA group only. Factor 1 included BMI and lipids (total cholesterol, HDL, LDL and triglycerides) (eigenvalue of 2.64; cumulative % of 52.7). Factor 2 included markers of oxidative stress (ROS and MDA) and antioxidants (SOD and TAS) (eigenvalue of 1.62; cumulative % of 54.0).

Partial regression analyses (with adjustments for age, sex, ethnicity and MAP), performed in the EVA group, between CV measures and variables clustered in factors 1 and 2 are illustrated in Figure 1. We found positive associations between cfPWV and ROS (r=0.156; p=0.035). Brachial SBP associated positively with BMI (r=0.210; p=0.004) and inversely with TAS (r=–0.151; p=0.041). Brachial DBP associated positively with HDL (r=0.172; p=0.020).

In the HVA and AVA groups, partial regression analyses (adjusted for age, sex, ethnicity and MAP) that included all anthropometric, CV and biochemical variables across all vascular ageing groups were performed (Supplementary Table S2 and S3). Brachial SBP associated positively with ROS (r=0.193; p=0.035) in the HVA group. Furthermore, cfPWV associated negatively with BMI (r=–0.094; p=0.011) in the AVA group. No associations with cfPWV were found in the HVA group.

Factor scores were subsequently used in multiple regression analyses to determine whether CV measures associated with these factors. Since the factors were identified in the EVA group, multiple regression analyses were only performed in this group. In multivariable-adjusted regression analysis (Figure 2 and Supplementary Table S4), cfPWV associated positively with Factor 2 (adj. R^2=0.028; β=0.171; p=0.042). An inverse relationship was also found between brachial SBP (adj. R^2=0.777; β=–0.103; p=0.011) with Factor 2. No significant associations were found with Factor 1.

Table 2 Cardiovascular risk factor scores in the early vascular ageing group (n=166).

	Factor 1	Factor 2
Eigenvalue	2.64	1.62
Cumulative %	52.7	54.0
Body mass index (kg/m^2)	0.91	
Reactive oxygen species (units)		0.68
Malondialdehyde (μM/mM creatinine)		0.88
Superoxide dismutase (U/mL)		0.69
Glutathione peroxidase (U/L)		
Glutathione reductase (U/L)		
Total antioxidant status (mmol/L)		0.80
Total glutathione (μM)		
Total cholesterol (mmol/L)	0.97	
High-density lipoproteins (mmol/L)	0.59	
Low-density lipoproteins (mmol/L)	0.81	
Triglycerides (mmol/L)	0.57	
Gamma-glutamyl transferase (U/L)		

Discussion

We compared various anthropometric, CV and biochemical variables in a pre-screened biethnic study population of young apparently healthy South African adults (aged 20–30 yrs); stratified into vascular ageing profile groups (HVA, AVA and EVA) based on cfPWV percentiles [21]. We further explored associations between CV measures and factor clusters in young individuals who are at risk of EVA. We found that young healthy adults identified to be at risk of EVA displayed a phenotype of unfavourable CV risk factors. Blood pressure and cfPWV were all incrementally higher across the vascular ageing groups. Additionally, cfPWV associated positively with a cluster that included both oxidative stress and antioxidant enzymes in the EVA group only.

Overall, we found that participants in the EVA group, that is with a mean cfPWV in the upper 15th percentile of the study sample, presented with a less favourable vascular profile. Brachial BP (SBP, DBP and MAP), cSBP and cfPWV were all higher in this group (by ≥6% when compared to the AVA group and ≥11% when compared to the HVA group). The EVA group also consisted of more men (84%), participants of black ethnicity (59%) and participants with BP in the hypertensive range (14%). Studies have consistently reported

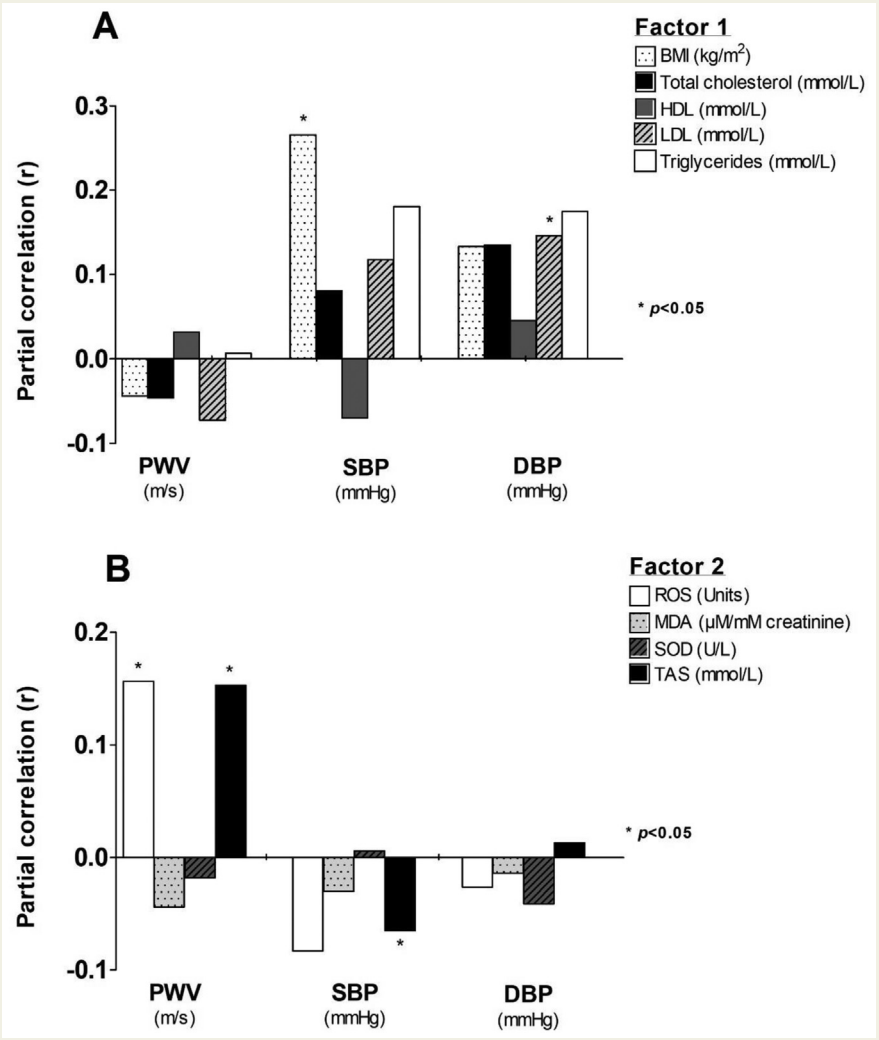

Figure 1 Partial correlations of cardiovascular measures with respective parameters included in (A) Factor 1 and (B) Factor 2 (n=166).
Adjusted for age, sex, ethnicity and mean arterial pressure.
Abbreviations: PWV, pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL, high density lipoproteins; LDL, low density lipoproteins; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; TAS, total antioxidant system.

a higher prevalence of hypertension in black men when compared to their white counterparts, a main reason for the higher incidence of CVD among this population [25]. Likewise, most studies performing ethnic-related arterial stiffness comparisons reported that populations of black ethnicity have higher PWV when compared to white populations [26,27], which was also confirmed in black children from as young as 6 years of age [28].

In addition, an increase in arterial stiffness is often found in groups who frequently follow unhealthy lifestyle behaviours [5]. Participants in the EVA group consistently showed a higher percentage of adverse lifestyle risk factors such as higher alcohol consumption (by ≥11%) and smoking (by ≥8%) when compared to participants stratified in the HVA or AVA groups. It has been reported that smoking, even at low levels, and excessive alcohol consumption associated individually and collectively with arterial changes relevant to atherosclerosis progression [29].

Although the process of EVA is complex, it may seem useful to disentangle factors contributing to increased arterial stiffness in younger populations. Factor 1 consisted largely of lipids (total cholesterol, HDL, LDL and triglycerides) and body mass index (BMI) which have both been previously reported to be independent determinants of arterial stiffness [30,31]. However, no associations were found with any CV measures and this cluster of markers. Only in the EVA group we found arterial stiffness to be positively associated with a cluster of oxidative stress markers including systemic ROS and MDA, a marker of lipid peroxidation indicating whole-body lipid radical formation [32]. Factor 2 additionally included antioxidants (SOD and TAS) which are known to promote HVA [33] and counteract hostile oxidative

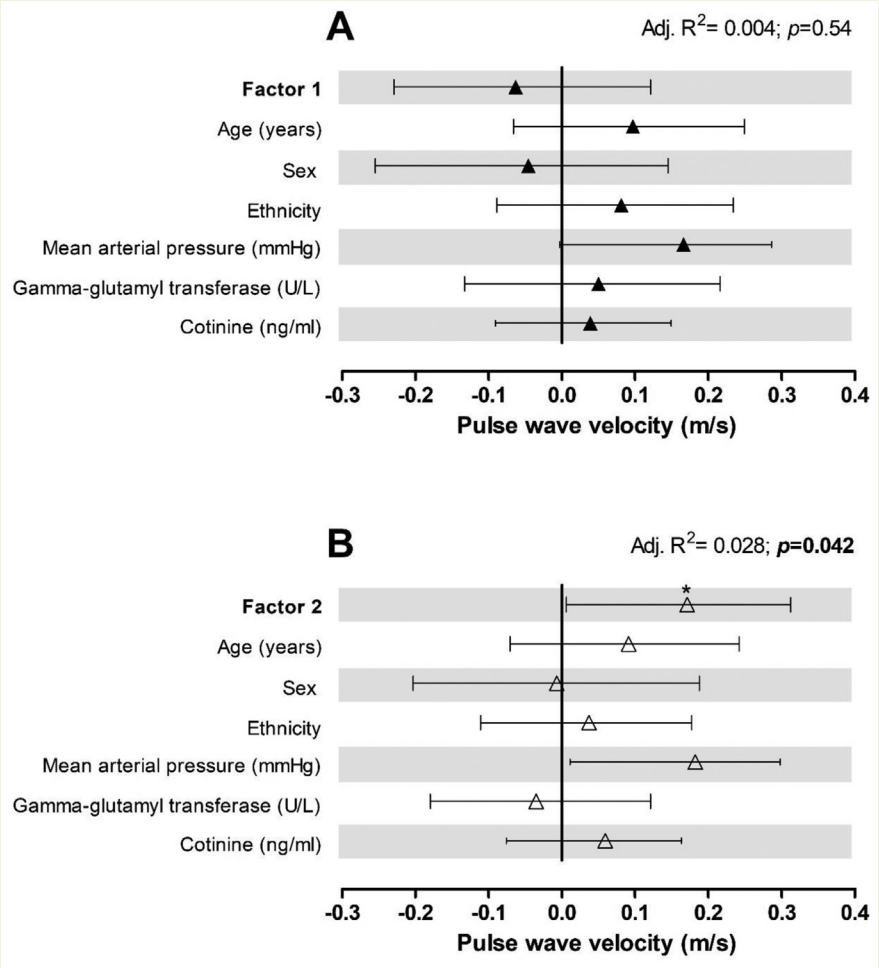

Figure 2 Multiple regression anaylses of pulse wave velocity with respective parameters included in (A) Factor 1 and (B) Factor 2 in the early vascular ageing group (n=166).
Factor 1: body mass index, total cholesterol, high density lipoproteins, low density lipoproteins and triglycerides;
Factor 2: reactive oxygen species, malondialdehyde, superoxide dismutase and total antioxidant status.

environments [7]. Oxidative stress is involved in the development of vascular dysfunction [34] and has been implicated in the pathogenesis of ageing and several CV risk factors such as hypertension [7]. Elevated levels of ROS initiates tissue damage by means of various mechanisms, which include DNA damage and lipid peroxidation (MDA), among others [35], which contribute to increased oxidative stress and the development of pro-inflammation. Although ROS produced by vascular cells act as crucial intracellular signals, physical and lifestyle stressors may also contribute to an overproduction of these molecules [35], ultimately impacting on functional and structural changes within the vasculature [35,36]. These stressors may therefore influence oxidative stress and/or compromise the antioxidant status, which in turn can lead to arterial wall damage and subsequent arterial stiffness [37].

The most prominent finding of our study is the positive association between cfPWV and Factor 2 in the EVA group, suggesting that the effects of both oxidative stress and antioxidant capacity are linked to arterial stiffness development in young, apparently healthy adults. We therefore confirm the findings from a previous study showing a positive association between oxidative stress, as determined by oxidised LDL and PWV in healthy individuals [38]. Although reporting a different marker related to oxidative stress as well as in individuals older than 70 years, Brinkley et al. reported that plasma oxidised LDL associated positively with aortic PWV after applying several adjustments for traditional CV risk factors [38]. Another study reported an expected inverse association between TAS and PWV in a hypertensive cohort, again after adjusting for several CV confounders [39], thus illustrating the beneficial effects of the antioxidant system. To the best of our knowledge, no studies have reported a positive association between antioxidants and PWV. While the exact mechanisms underlying the progression of arterial stiffness are not fully understood, unhealthy lifestyle behavioural risk factors promote oxidative stress and subsequent pro-inflammation and are known to

elevate BP and elicit arterial stiffness [40,41]. Ultimately, the imbalance between oxidants and antioxidants in favour of oxidants (oxidative stress) prompts a counter-regulatory antioxidant response. This could therefore aid in the explanation as to why both markers of oxidative stress and antioxidant capacity clustered together in the formation of Factor 2 and associated positively with cfPWV in the EVA group only.

Our findings should be interpreted within the context of its strengths and limitations. This study was well planned and executed under strict conditions. It highlights the potential to identify young healthy individuals who are at risk of EVA. Studies in young healthy individuals are useful to identify the onset and specific determinants of CV disease. However, we were unable to investigate precise mechanisms and causal relationships due to the cross-sectional design of our study. We included adults from the North West Province of South Africa and may not be representative of the entire country's population. To expand on the present findings, future studies are warranted to verify whether the use of EVA as a potentiating factor in screening programs can be used to identify young healthy adults at risk of accelerated vascular ageing.

To conclude, the present study revealed that healthy young adults at potential risk of EVA presented with a less favourable vascular profile and undesired lifestyle behaviours. We also found oxidative stress and antioxidant capacity-related markers to cluster and contribute to higher cfPWV in the EVA group only. Our results therefore highlight that even young apparently healthy individuals may be susceptible to accelerated vascular ageing, amplified among those with higher BP and adverse lifestyle risk factors.

Conflict of Interest

The authors report that they have no conflict of interest.

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