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Antioxidant enzyme activity is associated with blood pressure and carotid intima media thickness in black men and women: The SABPA study

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ABSTRACT

In the urbanized black population of South Africa, oxidative stress may play a crucial role in the development of hypertension. Since oxidative stress may result from impaired antioxidant capacity we aimed to investigate antioxidant enzyme activity as well as its associations with vascular function and structure in a bi-ethnic population. Participants included 409 subjects almost equally stratified by ethnicity and sex. Blood pressure and carotid intima media thickness (cIMT) were measured and glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) enzyme activities were determined. GR activity was significantly higher in black men (7.71 nmol/min/ml vs 2.23 nmol/min/ml) and women (6.46 nmol/min/ml vs 2.86 nmol/min/ml) ($p < 0.001$) when compared to their white counterparts. In black women, GPx activity was significantly lower ($p < 0.001$) when compared to white women (31.9 nmol/min/ml vs 37.1 nmol/min/ml). In black men, cIMT was positively and independently associated with GR activity ($R^2 = 0.30$; $\beta = 0.18$; $p = 0.048$). In black women, systolic blood pressure ($R^2 = 0.21$; $\beta = -0.24$; $p = 0.014$), diastolic blood pressure ($R^2 = 0.11$; $\beta = -0.20$; $p = 0.044$) and mean arterial pressure ($R^2 = 0.20$; $\beta = -0.31$; $p = 0.002$) were inversely associated with GPx activity. No associations were found in the white groups. The positive association between GR activity and cIMT in black men may be the result of a compensatory response to prevent arterial remodelling. The inverse association between GPx activity and blood pressure in black women may indicate a role for decreased GPx activity in hypertension development in this population.

1. Introduction

Hypertension is a severe problem and the prevalence thereof is increasing [1]. Sub-Saharan Africa is no exception, with an increased incidence in hypertension, especially among the rapidly urbanizing black populations [2]. Oxidative stress has been implicated to play a role in the development of hypertension [3,4], whereas hypertension may also lead to oxidative stress [4].

Oxidative stress occurs as a result of an increase in the generation of reactive oxygen species (ROS) or a decrease in the antioxidant defence mechanisms, or a combination thereof [3,5]. ROS is produced in all vascular cell types by the actions of various enzymes which are balanced by the counteractions of endogenous antioxidant molecules and antioxidant enzymes to prevent oxidative stress [5]. Antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), while endogenous antioxidants include glutathione [4,6]. In turn, glutathione reductase (GR) plays an important role to maintain redox balance as it is involved in the recycling of oxidized glutathione (GSSG) to reduced glutathione (GSH) [7].

We previously reported higher ROS levels in black women compared to black men, and higher ROS levels in hypertensive men when compared to normotensive men [8]. It was also indicated that both systolic blood pressure (SBP) and pulse pressure (PP) are positively associated with ROS levels in black men [8]. In another study it was found that decreased total glutathione (tGSH) levels are associated with increased carotid intima media thickness (cIMT) in hypertensive black men [9].

The aforementioned studies did not investigate decreased antioxidant enzyme activity as a possible origin for the excessive

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ROS levels in this population. The aim of the present study is therefore to compare antioxidant enzyme activity (GPx, GR, SOD and CAT) between black and white groups stratified by sex, and to investigate relationships between vascular function (blood pressure) and structure (cIMT), with antioxidant enzyme activity in black and white men and women.

2. Materials and methods

2.1. Study population and protocol

This study is embedded in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, a cross-sectional target population study. Detail on the study population and protocol was described elsewhere [10]. In short, we included 101 black and 101 white male and 99 black and 108 white female teachers from the Dr. Kenneth Kaunda Education District in the North West Province of South Africa between the ages of 20 and 65 years. Exclusion criteria included pregnancy, lactation, elevated ear temperature (>37 °C), and blood donation or vaccination 3 months prior to the commencement of the study.

This study was conducted in line with the ethical principles of the Helsinki Declaration and was further approved by the Ethics Review Board of the North-West University (Potchefstroom campus) (NWU-00036-07-S6). This sub-study was also approved by the Health Research Ethics Committee of the North-West University (Potchefstroom campus) (NWU-00036-07-A6).

2.2. Anthropometric and physical activity measurements

Participants' height and weight were measured in triplicate using calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan and Invicta Stadiometer, IP 1465, Invicta, London, UK) using standard methods [11]. Body mass index (BMI) was calculated as kg/m^2. Total energy expenditure (TEE) was calculated using the Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) over a 24 h period.

2.3. Cardiovascular measurements

The validated Finometer device was used to measure SBP, diastolic blood pressure (DBP) and mean arterial pressure (MAP) (FMS, Finapres Medical Systems, Amsterdam, The Netherlands) [12–14]. The Finometer device was connected, and after a 10 min resting period, a 5 min continuous measurement of resting cardiovascular parameters was taken. During the recording, after 2 min, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial arterial pressure. The highest precision in cardiovascular measurements is obtained only after this calibration. The average of the recordings of the last minute was used.

B-mode ultrasonography (SonoSite Micromaxx, SonoSite Inc., WA, USA) and a 6–13 MHz linear array transducer was used to determine the cIMT according to the Mannheim Consensus [15]. The Meijer carotid arc was used to identify segments for imaging on the common carotid artery at 90°, 120°, 150° and 180° degrees on the right side, and 180°, 210°, 240° and 270° degrees on the left side. Images from at least two optimal angles with the clearest views of the left and right common carotid artery were obtained. These segments were imaged and measured and were imported into the Artery Measurement Systems (AMS) II v1.139 (Gothenburg, Sweden) automated software for dedicated analysis of cIMT. A maximal 10 mm segment with good image quality was chosen for offline analysis to assess the near and far wall. The program automatically identifies the borders of the intima-media of the near and far wall and the inner diameter of the vessel, and calculates the cIMT and diameter from around 100 discrete measurements through the 10 mm segment. In this study the far wall measurements of cIMT were used. The offline analysis of the cIMT was done by one observer. Intra-observer variability was 0.04 mm between two measurements made four weeks apart ($n = 10$). The above mentioned method is suitable for the detection of early stages of atherosclerotic disease. Since intima-media thickness is associated with cardiovascular risk factors and incident cardiovascular disease, it has been used successfully to monitor arterial wall alterations [15]. Cross-sectional wall area (CSWA) was calculated using the formula $CSWA = \pi(d/2 + CIMT)^2 - \pi(d/2)^2$.

2.4. Biochemical measurements

After an overnight fast, blood samples (for serum and ethylenediaminetetraacetic acid (EDTA) plasma) were obtained from each participant by a registered nurse from the ante brachial vein branches, and serum and plasma were prepared according to standardised procedures. Since previous results indicated efficient long term storage stability of antioxidant enzymes at −80 °C [16], all samples were stored accordingly until various biochemical analyses were performed.

Assay kits from Cayman Chemical Company (Ann Arbor, MI, USA) were used to measure GPx, GR (EDTA plasma) and SOD (serum), while CAT (serum) was measured using a fluorometric OxiSelect catalase activity assay kit (Cell Biolabs Inc., San Diego, CA, USA). All the antioxidant enzyme activities were measured on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). The ratio of GR to GPx was calculated. Total glutathione (tGSH) was measured with the BIOXYTECH GSH/GSSG-412 kit on a Bio-Tek FL600 microplate reader in EDTA whole blood samples. One of the measureable ROS species (serum peroxides) was measured with a high-throughput automated analysis based on the principle of the derivatives of reactive oxygen metabolites (D-ROM) test [17] on a Bio-Tek FL600 microplate reader (BioTek, Winnoski, VT, USA). Both intra- and inter assay variation were less than 10%.

Glycated haemoglobin (HbA1c) was measured in EDTA whole blood via a turbidimetric inhibition immunoassay (Integra 400, Roche, Switzerland). Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, high sensitivity C-reactive protein (CRP) and γ-glutamyl transferase (γGT), as a measure of increased alcohol abuse [18], were analysed in serum (Unicel DXC 800 device, Beckman & Coulter, Germany and Konelab™ 20I Sequential Multiple Analyser Computer, Thermo Scientific, Vantaa, Finland). The total cholesterol to HDL cholesterol ratio was calculated. Serum cotinine was measured with a homogenous immunoassay (Automated Modular, Roche, Basel, Switzerland), while tumor necrosis factor-α (TNF-α) was measured using a high sensitivity enzyme linked immunosorbent assay (ELISA) (R&D systems, Minneapolis, MN, USA). Both intra- and inter assay variation were less than 10%.

2.5. Statistical analyses

In agreement with our aims we divided our population into four groups, namely black men, white men, black women and white women. Statistica 12 (Statsoft Inc., Tulsa, OK, USA) was used to perform the statistical analyses. The central tendency and spread for normally distributed data were expressed as arithmetic mean and standard deviation. Variables not normally distributed were logarithmically transformed (CRP, GR, SOD, CAT and γGT) and expressed as the geometric mean and the 5th and 95th percentile intervals. Mean values were compared using independent T-tests, while proportions were compared using Chi-square tests. Differences in cardiovascular variables and antioxidant enzyme activity