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ORIGINAL ARTICLE

The association of endothelin-1 with markers of oxidative stress in a biethnic South African cohort: the SABPA study

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Both endothelin-1 and oxidative stress have important roles in the development of cardiovascular diseases such as hypertension and atherosclerosis. Limited information is available on the interaction between oxidative stress, the glutathione system and endothelin-1 in humans. We aimed to investigate the association of endothelin-1 with markers of oxidative stress and the antioxidant capacity in a biethnic South African cohort. This cross-sectional study included 195 black and 198 white South Africans. Serum endothelin-1 levels and oxidative stress-related markers such as reactive oxygen species (measured as serum peroxides), glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase were measured. In single, partial and multiple regression analyses endothelin-1 correlated positively with glutathione reductase activity (adj. $R^2 = 0.10$; $\beta = 0.232$; $P = 0.020$) and negatively with antihypertension medication ($P = 0.02$) and tended to correlate with glutathione reductase-to-glutathione peroxidase ratio (adj. $R^2 = 0.10$; $\beta = 0.19$; $P = 0.057$) in black men. In white men, endothelin-1 correlated positively with ROS (adj. $R^2 = 0.09$; $\beta = 0.26$; $P = 0.01$) and negatively with glutathione peroxidase activity (adj. $R^2 = 0.05$; $\beta = -0.23$; $P = 0.02$). In black women, endothelin-1 correlated negatively with total glutathione (adj. $R^2 = 0.22$; $\beta = -0.214$; $P = 0.026$). Endothelin-1 may contribute to glutathione reductase upregulation through increased reactive oxygen species production mediated via endothelin-1 in black men. In white men, we observed a negative association between glutathione peroxidase and endothelin-1, describing the expected physiological relationship between endothelin-1 and reactive oxygen species. Higher total glutathione levels may act as a counter-regulatory mechanism to protect against oxidative vascular damage attributed by endothelin-1 in black women.
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INTRODUCTION

Endothelin-1 has an important physiological role in the maintenance of vascular tone.[1] Under pathophysiological conditions, plasma endothelin-1 is elevated and causes enhanced vasoconstriction and endothelial dysfunction.[2–5] Endothelial dysfunction is described as a precursor in the development of cardiovascular diseases related to hypertension, atherosclerosis and arteriosclerosis.[6–9]

In addition to the role of endothelin-1 in the regulation of vascular tone, reactive oxygen species (ROS) are also important modulators in this regard.[1] In response to increased production of ROS, antioxidant enzymes such as superoxide dismutase, catalase and the glutathione system (glutathione peroxidase (GPx) and glutathione reductase (GR)) are activated to maintain the balance between oxidants and antioxidants.[10,11] However, when the production of ROS exceeds the availability of antioxidant defense mechanisms, it may have detrimental effects such as endothelial injury.[10,11] Oxidative stress may, therefore, also have an important role in the development and progression of cardiovascular disease such as atherosclerosis.[9–13]

The regulation of vascular tone via ROS is achieved through different mechanisms. The first involves the inactivation of the vasodilator, nitric oxide, by binding with the superoxide.[14] ROS also regulates the vascular tone by increasing intracellular Ca^{2+} uptake in vascular smooth muscle cells, thereby inducing smooth muscle contraction and proliferation.[14] Finally, experimental results indicated ROS can lead to increased production of endothelin-1,[12,15] which may result in vasoconstriction by binding to ET_A receptors.[16] Increased endothelin-1 may in turn lead to increased production of superoxide radicals.[12,15,17]

Previous results from the sympathetic activity and ambulatory blood pressure in Africans (SABPA) study indicated that higher ROS levels in black men and lower GPx activity in black women are associated with higher blood pressure.[9,18] It was also found that

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increased carotid intima–media thickness were associated with higher GR levels in black men and decreased total glutathione levels in hypertensive black men.[18,19] Additionally, increased endothelin-1 were also found to be independently associated with blood pressure and inflammatory markers in this population.[20] These findings suggest that endothelin-1- and oxidative stress-related markers may contribute to the development of hypertension and subclinical atherosclerosis in the sub-Saharan population. Although the link between endothelin-1 and oxidative stress were demonstrated in experimental studies (*in vitro* and *in vivo*), limited information is available on humans, especially in a South African context. We therefore aimed to investigate the association of endothelin-1 with markers of oxidative stress and antioxidant capacity in a cohort of black and white individuals.

METHODS

Study population and protocol

The SABPA study was a cross-sectional study that included 202 black and 208 white teachers from the Dr Kenneth Kuanda Education District of the North-West Province of South Africa. Detailed information regarding the procedure of the SABPA study were published previously.[21] Exclusion criteria for the SABPA study were pregnant or lactating women, individuals using α- and β-blockers, participants with an ear temperature $\geqslant 37$ °C and those who had a vaccination or donated blood 3 months before participation. In the substudy, we included 195 black (men: $n=99$; women: $n=96$) and 198 white (men: $n=99$; women: $n=99$) South Africans. Excluded from the substudy were outliers of endothelin-1 ($n=10$) by residual statistics ($3\times$s.d.) as well as participants with missing endothelin-1 data ($n=6$). A standard health survey was used for the collection of demographic information and antihypertension medication usage. The Health Research Ethics Committee of the North-West University, Potchefstroom campus, granted approval for this substudy. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki for the investigation on human subjects.

Anthropometric and physical activity measurements

Waist circumference was measured with a non-stretchable metal flexible measuring tape (Holtain, Dyfed, UK) and body mass index was determined.[22] The total energy expenditure was obtained in kcal per 24 h by the Actical omnidirectional accelerometer (Mini Mitter, Bend, OR, USA and Montreal, QC, Canada) taking the resting metabolic rate into account.

Biochemical analyses

A fasting blood sample was collected from each participant and serum and plasma were prepared according to standard procedures. Serum and plasma samples were frozen at −80 °C until analyzed. Endothelin-1 was determined with a Quantikine enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Intra- and interassay variability for endothelin-1 were 2.7% and 17.2%, respectively. Serum interleukin-6 was determined with a high-sensitivity Quantikine enzyme-linked immunosorbent assay (R&D Systems). Intra- and interassay variation of interleukin-6 were 4.2% and 6.4%, respectively. Serum cotinine was determined with a homogenous immunoassay on a Roche Modular System (Roche, Basil, Switzerland). Fasting lipids (total and high-density lipoprotein cholesterol), glycated hemoglobin A1c and γ-glutamyl transferase were determined using two sequential multiple analyzers in serum samples (Konelab 20i (Thermo Scientific, Vantaa, Finland) and Unicel DXC 800 (Beckman and Coulter, Germany)). Intra- and interassay variability were <10%. Low-density lipoprotein cholesterol was calculated with the Friedewald formula: low-density lipoprotein cholesterol = total cholesterol − high-density lipoprotein cholesterol − (triglycerides/2.2) provided that no values of triglycerides inserted is higher than 4000 mmol l^{-1}.[23] Human immunodeficiency virus status was measured, using the First Response Kit (Premier Medical Corporation, Mumbai, India) as well as the Pareekshak test (Bhat Biotech, Bangalore, India). Estradiol levels were determined using an electrochemiluminescence immunoassay (Elecsys 2010; Roche, Basel, Switzerland). Intra- and interassay variability was <10%.

One of the measurable ROS, namely total peroxides, was determined in serum samples.[24] Total glutathione levels were determined with the BIOXYTECH GSH/GSSG-412 supplied by OxisResearch (Foster City, CA, USA). GPx and GR (EDTA plasma) and serum superoxide dismutase activities were determined with Assay Kits (Cayman Chemical Company, Ann Arbor, MI, USA), whereas serum catalase activity was determined with a Oxiselect Fluorometric Kit from Cell Biolabs (San Diego, CA, USA) with appropriate apparatus (Synergy H_4hybrid Microplate Reader; BioTek, Winooski, VT, USA). The intra- and interassay variability of these analyses were <10%. Antioxidant enzyme ratios were calculated to assess antioxidant defenses and included the glutathione reductase-to-glutathione peroxidase ratio (GR-to-GPx ratio) and the glutathione peroxidase-to-superoxide dismutase ratio (GPx-to-SOD ratio).

Cardiovascular measurements

The cardiovascular measurements were taken in a semirecumbent position for each participant. Five minute continuous measurements of cardiovascular variables were recorded using the validated Finometer (Finapres Medical Systems, Amsterdam, The Netherlands), based on the vascular unloading technique of Peñáz and were processed with the Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, The Netherlands) to obtain systolic blood pressure and diastolic blood pressure.[25] The Complior SP Acquisition System (Artech-Medical, Pantin, France) was used to measure pulse wave velocity from the carotid to dorsalis–pedis pulse sites.[26]

Statistical analysis

G*Power version 3.1.9.2 software (University of Kiel, Kiel, Germany) was used to compute the achieved power in *post hoc* analysis.[27] At a probability of 0.05, effect size of 0.5 and one-tailed input method, the achieved power ($1-\beta$ error probability) was estimated at 96.86% in men and 96.66% in women. Statistical analyses were carried out using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA; 2016). Main effects of race and sex were tested based on the association between endothelin-1- and oxidative stress-related markers by means of multiple regression. *T*-tests were used to compare means and χ^2 tests to compare proportions between the groups. Single and partial correlations were used to determine correlations of endothelin-1 with cardiovascular-, biochemical- and oxidative stress-related variables. Forward stepwise multiple regression analyses were performed to determine independent associations between endothelin-1- and oxidative stress-related variables. The main independent variables included GR and GPx in black men (Models 1 and 2, respectively), ROS and GPx in white men (Models 3 and 4, respectively) and total glutathione in black women (Model 5). Covariates considered for entry in the models included age, body mass index, total energy expenditure, interleukin-6, γ-glutamyl transferase, high-density lipoprotein cholesterol and antihypertension medication. We applied a sensitivity analysis for glycated hemoglobin A1c, human immunodeficiency virus infection status, estradiol, oral contraceptives and testosterone by adding these variables as covariates in applicable multiple regression models.

RESULTS

Basic descriptive characteristics of this study population are listed in Table 1. Owing to significant interactions of race ($F(391)=2.33$; $P<0.05$) and sex ($F(391)=2.22$; $P<0.05$) on the association of endothelin-1 with GR, we stratified the population accordingly.

There were no differences in endothelin-1 levels between the black and white groups. ROS was higher in black men compared with that in white men ($P=0.008$), but similar in women. Total glutathione, GR, catalase and GR-to-GPx ratio were higher in black men and women compared with that in their white counterparts (all $P\leqslant 0.009$). GPx was similar when comparing black and white men, whereas lower values were observed in black women ($P<0.001$) compared with white women. GPx-to-SOD ratio were higher in black men compared with white men ($P<0.003$), with no differences when comparing women. Interleukin-6, glycated hemoglobin A1c and γ-glutamyl transferase were higher in black men compared with women

Table 1 Population characteristics stratified by sex and race

	Men (n = *198*)			*Women* (n = *195*)		
	Black (n = *99*)	*White* (n = *99*)	P-*value*	*Black* (n = *96*)	*White* (n = *99*)	P-*value*
Age (years)	43.1 ± 8.08	45.0 ± 11.1	0.18	45.6 ± 7.95	44.7 ± 10.7	0.51
Body mass index (kg m^{-2})	27.6 ± 5.81	29.1 ± 5.23	0.061	32.9 ± 7.29	26.0 ± 5.62	<0.001
Waist circumference (cm)	93.6 ± 15.5	101.7 ± 14.5	<0.001	93.9 ± 15.6	84.8 ± 13.0	<0.001
Total energy expenditure (kcal per day)	2723.3 ± 805.8	3659.3 ± 2069.5	<0.001	2664.2 ± 800.1	2577.7 ± 620.0	0.40
Human immunodeficiency virus, n (%)	13 (13)	1 (1)	<0.001	5 (5.21)	0 (0)	0.021
Antihypertension medication, n (%)	35 (35.4)	14 (14.1)	<0.001	33 (34.4)	12 (12.1)	<0.001
Biochemical variables						
Endothelin-1 (pg ml^{-1})	2.26 ± 0.81	2.16 ± 1.02	0.41	2.02 ± 0.96	2.19 ± 1.22	0.27
Interleukin-6 (pg ml^{-1})	1.07 (0.93–1.23)	0.87 (0.76–0.99)	0.032	1.24 (1.06–1.46)	0.95 (0.82–1.10)	0.016
Glycated hemoglobin A1c (%)	6.25 ± 1.23	5.67 ± 0.48	<0.001	5.85 ± 1.00	5.37 ± 0.30	<0.001
γ-Glutamyl transferase (U l^{-1})	63.0 (54.7–72.4)	27.5 (24.2–31.3)	<0.001	35.6 (31.4–40.4)	14.2 (12.5–16.2)	<0.001
Cotinine (ng ml^{-1})	62.9 (43.4–91.2)	77.3 (31.1–192.3)	0.60	48.5 (26.8–87.4)	91.7 (36.1–233.4)	0.21
Total cholesterol (mmol l^{-1})	4.72 ± 1.17	5.59 ± 1.21	<0.001	4.46 ± 1.21	5.54 ± 1.31	<0.001
High-density lipoprotein cholesterol (mmol l^{-1})	1.04 ± 0.34	1.00 ± 0.27	0.36	1.20 ± 0.31	1.41 ± 0.43	<0.001
Low-density lipoprotein cholesterol (mmol l^{-1})	2.86 ± 0.95	3.91 ± 1.07	<0.001	2.80 ± 1.02	3.71 ± 1.07	<0.001
Oxidative stress-related variables						
Reactive oxygen species (U[a])	81.9 (78.1–85.9)	75.2 (72.2–78.4)	0.008	104.1 (98.6–109.8)	98.3 (93.4–103.5)	0.13
Total glutathione (μM)	929.5 ± 194.1	859.7 ± 180.4	0.009	868.8 ± 127.6	782.6 ± 163.0	<0.001
Glutathione peroxidase (U ml^{-1})	34.6 ± 14.0	34.9 ± 7.83	0.85	31.9 ± 14.0	37.4 ± 7.90	<0.001
Glutathione reductase (U ml^{-1})	7.71 (6.82–8.71)	2.19 (1.75–2.75)	<0.001	6.43 (5.62–7.34)	2.81 (2.34–3.40)	<0.001
Superoxide dismutase (U ml^{-1})	3.92 (3.16–4.86)	4.23 (3.92–4.57)	0.50	4.69 (3.83–5.74)	4.09 (3.67–4.55)	0.23
Catalase (U ml^{-1})	4.29 (3.92–4.57)	4.25 (4.23–4.28)	0.009	4.29 (4.28–4.30)	4.23 (4.20–4.25)	<0.001
GR-to-GPx ratio	0.34 ± 0.30	0.09 ± 0.07	<0.001	0.34 ± 0.46	0.10 ± 0.07	<0.001
GPx-to-SOD ratio	17.2 ± 27.1	8.87 ± 4.6	0.003	10.9 ± 12.5	10.6 ± 6.95	0.85
Cardiovascular variables						
Systolic blood pressure (mm Hg)	146.2 ± 20.6	136.5 ± 12.8	<0.001	136.4 ± 14.4	132.1 ± 15.2	0.042
Diastolic blood pressure (mm Hg)	85.8 ± 11.0	80.2 ± 8.36	<0.001	77.3 ± 7.69	73.4 ± 6.72	<0.001
Pulse wave velocity (m s^{-1})	9.18 ± 2.29	8.62 ± 1.34	0.039	8.19 ± 1.39	7.47 ± 1.20	<0.001

Abbreviations: GPx-to-SOD ratio, glutathione peroxidase-to-superoxide dismutase; GR-to-GPx ratio, glutathione reductase-to-glutathione peroxide ratio; H_2O_2, hydrogen peroxide.
Values are arithmetic mean plus/minus s.d., geometric mean (5th and 95th confidence interval).
[a]1 U = 1mg l^{-1} H_2O_2.

(all $P \leqslant 0.032$) than their white counterparts. Total cholesterol and low-density lipoprotein cholesterol were higher in white men and women (all $P < 0.001$) compared with their black counterparts. Systolic blood pressure, diastolic blood pressure and pulse wave velocity were higher in black men and women compared with the white groups (all $P \leqslant 0.042$). The prevalence of human immunodeficiency virus was higher among the black groups and both black men and women were more likely to use antihypertension medication than their white counterparts.

In both single (Supplementary Table 1) and partial regression analyses after adjusting for age, body mass index, total energy expenditure and antihypertension medication (Table 2), endothelin-1 correlated positively with GR ($P \leqslant 0.02$) and GR-to-GPx ratio ($P < 0.05$) in black men only. In white men, a positive correlation existed between endothelin-1 and ROS ($P \leqslant 0.03$) and an inverse correlation between endothelin-1 and GPx ($P \leqslant 0.03$). In black women, a borderline correlation were found between endothelin-1 and total glutathione ($P = 0.06$). No correlation existed in white women.

In forward stepwise multiple regression analysis, we performed a separate model for each group based on previous findings in single and partial regression analyses (Table 3). In black men, an independent positive association of endothelin-1 with GR (Model 1: adj. $R^2 = 0.10$; $\beta = 0.23$; $P = 0.02$) was confirmed. Additionally, in black men a borderline association between endothelin-1 and GR:GPx ratio (Model 2: adj. $R^2 = 0.05$; $\beta = 0.19$; $P = 0.057$) was observed. A negative association of endothelin-1 with antihypertension medication (Model 1: adj. $R^2 = 0.10$; $\beta = -0.24$; $P = 0.015$; Model 2: adj. $R^2 = 0.05$; $\beta = -0.24$; $P = 0.016$) was also confirmed in black men. An independent positive association between endothelin-1 and reactive oxygen species (Model 3: adj. $R^2 = 0.09$; $\beta = 0.26$; $P = 0.010$) and a negative association between endothelin-1 and interleukin-6 (Model 3: adj. $R^2 = 0.09$; $\beta = -0.24$; $P = 0.016$) were confirmed in white men. Additionally, a negative association between endothelin-1 and GPx (Model 4: adj. $R^2 = 0.045$; $\beta = -0.23$; $P = 0.02$), in white men, was confirmed. In Model 5, an independent negative association between endothelin-1 and total glutathione (adj. $R^2 = 0.22$; $\beta = -0.21$; $P = 0.026$), as well as a positive association between endothelin-1 and age (adj. $R^2 = 0.22$; $\beta = 0.23$; $P = 0.016$), body mass index (adj. $R^2 = 0.22$; $\beta = 0.34$; $P = 0.001$) and high-density lipoprotein cholesterol (adj. $R^2 = 0.22$; $\beta = 0.23$; $P = 0.019$) was found in black women. No significant correlations existed in white women.

Table 2 Partial correlations of endothelin-1- with oxidative stress-related and inflammatory markers

	Endothelin-1 (pg ml^{-1})			
	Men (n = *198)*		*Women* (n = *195)*	
	Black (n = *99)*	*White* (n = *99)*	*Black* (n = *96)*	*White* (n = *99)*
Biochemical variables				
Interleukin-6 (pg ml^{-1})	r=0.043; P=0.68	r=–0.19; P=0.07	r=0.12; P=0.27	r=–0.10; P=0.32
Glycated hemoglobin A1c (%)	r=–0.080; P=0.44	r=–0.035; P=0.74	r=0.020; P=0.85	r=–0.071; P=0.50
γ-Glutamyl transferase (U l^{-1})	r=0.19; P=0.07	r=–0.13; P=0.21	*r=0.22; P=0.04*	r=–0.046; P=0.66
Total cholesterol (mmol l^{-1})	r=0.10; P=0.31	r=0.081; P=0.43	r=–0.070; P=0.51	r=–0.10; P=0.34
High-density lipoprotein cholesterol (mmol l^{-1})	r=0.14; P=0.18	r=0.23; *P=0.03*	r=0.23; P=0.03	r=0.042; P=0.69
Low-density lipoprotein cholesterol (mmol l^{-1})	r=0.025; P=0.81	r=0.061; P=0.56	r=–0.15; P=0.17	r=–0.14; P=0.19
Oxidative stress and antioxidant variables				
Reactive oxygen species (U[a])	r=–0.17; P=0.10	r=0.23; P=0.03	r=0.18; P=0.08	r=0.039; P=0.71
Total glutathione (μM)	r=0.023; P=0.83	r=–0.007; P=0.95	r=–0.20; P=0.06	r=0.063; P=0.55
Glutathione peroxidase (U ml^{-1})	r=–0.020; P=0.85	r=–0.22; P=0.03	r=–0.009; P=0.93	r=–0.13; P=0.22
Glutathione reductase (U ml^{-1})	*r=0.23; P=0.02*	r=–0.13; P=0.23	r=0.024; P=0.82	r=–0.14; P=0.16
Superoxide dismutase (U ml^{-1})	r=–0.18; P=0.10	r=–0.16; P=0.13	r=0.073; P=0.50	r=0.060; P=0.56
Catalase	r=–0.10; P=0.34	r=–0.024; P=0.82	r=–0.035; P=0.74	r=–0.004; P=0.97
GR-to-GPx ratio	r=0.20; P=0.047	r=–0.13; P=0.23	r=0.001; P=0.99	r=–0.10; P=0.33
GPx-to-SOD ratio	r=0.19; P=0.07	r=0.055; P=0.60	r=–0.090; P=0.39	r=–0.13; P=0.23
Cardiovascular variables				
Systolic blood pressure (mm Hg)	r=0.10; P=0.35	r=0.013; P=0.90	r=0.13; P=0.21	r=0.083; P=0.43
Diastolic blood pressure (mm Hg)	r=0.022; P=0.84	r=0.11; P=0.29	r=0.11; P=0.32	r=–0.100; P=0.34
Pulse wave velocity (m s^{-1})	r=0.15; P=0.16	r=0.13; P=0.20	r=0.11; P=0.31	r=0.12; P=0.27

Abbreviations: GPx-to-SOD ratio, glutathione peroxidase-to-superoxide dismutase ratio; GR-to-GPx ratio, glutathione reductase-to-glutathione peroxide ratio; H_2O_2, hydrogen peroxide.
Adjustments applied for age, body mass index, total energy expenditure and antihypertension medication.
[a]1 U = 1mg l^{-1} H_2O_2.

Sensitivity analyses

After performing the same multiple regression analyses and additionally correcting for human immunodeficiency virus infection, glycated hemoglobin A1c, estradiol, hormonal contraceptive usage and testosterone, no change in the relationships between endothelin-1- and oxidative stress-related markers were found in men or women. An additional sensitivity analysis was performed by removing the participants using antihypertension medication and we found that a positive association of endothelin-1 with glutathione reductase (Model 1: adj. R^2 = 0.141; β = 0.303; P = 0.014), GR-to-GPx ratio (Model 2: adj. R^2 = 0.150; β = 0.317; P = 0.010) and interleukin-6 (Model 1: adj. R^2 = 0.141; β = 0.288; P = 0.019; Model 2: adj. R^2 = 0.150; β = 0.300; P = 0.015) was observed in black men.

DISCUSSION

To our knowledge, we are the first to describe a link of endothelin-1 with markers of oxidative stress and antioxidant capacity in a black and white cohort. Our results indicated an independent positive association of endothelin-1 with GR and GR-to-GPx ratio in black men. A previous study from our cohort linked higher GR levels with increased carotid intima–media thickness.[19] Increased endothelin-1 levels may lead to an increase in ROS production; however, increased ROS production can also lead to increased endothelin-1 level, which in turn leads to an increase in antioxidant enzyme activity, such as GR.[28] Therefore, our results suggest that endothelin-1 may have an indirect role in the upregulation of GR activity in this group and therefore contribute to the increased risk for the development of atherosclerosis often seen in the black population. Blood glutathione concentrations is an useful indicator of glutathione status in humans with cardiovascular diseases such as atherosclerosis and hypertension, and GR activity and GR-to-GPx ratio similarly gives an indication of glutathione regeneration.[19,29] Under normal physiological conditions, an increase in GR activity are indicative of increased regeneration potential to recycle GSSG to GSH and thereby make more GSH available for use in other enzyme reactions such as the inactivation of hydrogen peroxide by GPx.[29] However, under pathophysiological conditions, such as hypertension, when there is an increase in hydrogen peroxide production, more GSH is consumed by GPx, which may lead to an even further upregulation of GR in an attempt to maintain the redox balance.[29] In this black male cohort, the positive association between endothelin-1 and GR activity may therefore be because of the upregulation of GR as a result of increased ROS production via endothelin-1-mediated stimulation of the NAD(P)H oxidase enzyme.[28] Additionally, the increase in vascular ROS production may also impair endothelium-dependent NO-mediated relaxation by inactivating endogenous NO.[30,31] A previous study has suggested that increased oxidative stress may counteract NO bioavailability by increasing NO inactivation in black men.[32] This might be due to endothelin-1 also having the ability to counteract NO bioavailability,[33] which suggests an interconnected role between endothelin-1 and oxidative stress. Even though antihypertension medication can protect the vasculature against increased endothelin-1-mediated vasoconstriction, we still observed an association between endothelin-1 and GR and GR-to-GPx ratio, when taking antihypertension medication usage into account. Although the black men and women were more likely to take

Table 3 Forward stepwise multiple regression analyses of endothelin-1 with measures of oxidative stress-related markers

	Endothelin-1 (pg ml^{-1})	
Black men ($n=99$)	Adj. $R^2=0.10$	
Model 1	*Std β (95% CI)*	P-*value*
Glutathione reductase (U ml^{-1})	0.232 (0.039–0.424)	0.020
Antihypertension medication	–0.243 (–0.435 to –0.051)	0.015
Model 2	*Adj.* $R^2=0.051$	
GR-to-GPx ratio	0.191 (0.003–0.384)	0.057
Antihypertension medication	–0.247 (–0.443 to –0.051)	0.016
White men ($n=99$)		
Model 3	*Adj.* $R^2=0.085$	
Reactive oxygen species (U[a])	0.257 (0.065–0.449)	0.010
Interleukin-6 (pg ml^{-1})	–0.240 (–0.432 to –0.048)	0.016
Model 4	*Adj.* $R^2=0.045$	
Glutathione peroxidase (U ml^{-1})	–0.233 (–0.427 to –0.039)	0.020
Black women ($n=96$)		
Model 5	*Adj.* $R^2=0.22$	
Total glutathione (μM)	–0.214 (–0.400 to –0.028)	0.026
Age (years)	0.232 (0.046–0.418)	0.016
Body mass index (kg m^{-2})	0.342 (0.156–0.528)	0.001
High-density liporprotein cholesterol (mmol l^{-1})	0.226 (0.040–0.412)	0.019

Abbreviations: CI, confidence interval; GR-to-GPx ratio, glutathione reductase-to-glutathione peroxidase ratio; H_2O_2, hydrogen peroxide; NS, not significant; std β, standardized regression β-coefficients.
Main independent variables included glutathione reductase, GR-to-GPx ratio, reactive oxygen species, glutathione peroxidase and total glutathione, respectively, for Models 1–5. Covariates considered for entry: age, body mass index, total energy expenditure, interleukin-6, γ-glutamyl transferase, high-density lipoprotein cholesterol, systolic blood pressure and antihypertension medication.
[a]1 U = 1 mg l^{-1} H_2O_2.

antihypertension medication, it is noteworthy to mention that black people do not get the correct hypertension treatment. Thus, antihypertension medication is effective enough to lower blood pressure and still show a similar physiological association between endothelin-1 and antioxidant capacity to maintain homeostasis. Even after removing the participants using antihypertension medication, the results remained robust in the black male group. This might suggest that antihypertension medication protects the vasculature against inflammatory markers released in response to the presence of endothelin-1. Endothelial damage together with reduced NO bioavailability may alter the balance between vascular injury and repair, increasing the risk for atherosclerotic disease in black men of this population.

In white men, endothelin-1 associated positively with ROS and negatively with GPx. The GPx enzyme has a critical role in the reduction of lipid peroxides and hydrogen peroxide.[34,35] In this white male cohort, the positive association between endothelin-1 and ROS as well as the negative association between endothelin-1 and GPx activity may be as a result of nuclear factor-κB-mediated endothelin-1 synthesis. In turn, endothelin-1 then binds to ET_A receptors on nuclear factor-κB and it may lead to the activation of angiotensin II stimulation of NADPH oxidase leading to ROS production[36,37] as well as the induction of an inflammatory response in human vascular smooth muscle cells without the release of interleukin-6.[36] The negative association between endothelin-1 and interleukin-6 is contradictory to previous findings.[36,38,39] This could be because of a chance finding or that some confounding variable, which we are unaware of, might contribute to this finding. Although similar GPx activity were observed in the white and black men of this study, white men had lower ROS levels, suggesting that the black men may be at a disadvantage as they have to scavenge more ROS with similar GPx activity, which may exaggerate the effect of endothelin-1 in the black men. Therefore, the opposite associations of endothlin-1 with ROS and GPx activity may indicate the physiological relationship between these factors.

In black women, we found a negative association of endothelin-1 with total glutathione. Glutathione levels is determined by the synthesis of GSH in the cell *vs.* the efflux of GSH out of the cell.[40] The most important determinant of GSH synthesis is the availability of cysteine[41] while elevated cysteine levels inside endothelial cells may lead to injury, increasing inflammation in blood vessels and in turn leads to atherogenesis.[42,43] A previous study demonstrated that endothelin-1 increases the uptake of cysteine into cells, but reduce the efflux of GSH out of the cell[41] favoring the accumulation of cysteine in the cells. Furthermore, it was demonstrated that black premenopausal women also had higher plasma total homocysteine levels than white women, possibly because of lifestyle factors, and as homocysteine levels can also be converted to cysteine, it may further increase their risk for coronary artery disease such as atherosclerosis.[44] Previous studies demonstrated that age and body mass index are associated with enhanced endothelin-1-mediated vasoconstriction that contributes to endothelial vasodilator dysfunction and may have a role

in the increased prevalence of hypertension often seen in black men and women.[45–47] On the other hand, black women also have elevated high-density lipoprotein cholesterol levels that provides a protective mechanism against oxidative stress, reducing the risk to cardiovascular diseases such as atherosclerosis.[44] This antiatheroprotective role might decrease the additional release of endothelin-1 and vasoconstriction in the smooth muscle cells in comparison with the black men in our cohort. Flagg *et al.*[48] found that men had higher levels of plasma glutathione compared with women and that the use of estrogen-containing oral contraceptives was associated with lower plasma glutathione levels. However, after we adjusted for estradiol and hormonal contraceptive usage, our results remained the same. Despite the negative role of endothelin-1 on GSH synthesis, this may suggest that the combined protective nature of GSH and high-density lipoprotein cholesterol are still sufficient to counter regulate proinflammation, protecting the black women against vascular damage. A schematic representation of the possible mechanisms found from our results can be seen in Supplementary Figure 1.

The results of this study need to be interpreted within the context of its limitations and strengths. This was a cross-sectional study and we cannot pinpoint any cause or effect. Although the results were consistent after multiple adjustments, we cannot exclude residual confounding. It is known that renin–angiotensin–system inhibitors, calcium antagonists, diuretics, β-blockers and aldosterone antagonists can also lower blood pressure;[49–53] however, the amount of participants who use different types of medications were statistically incomparable in this study, and larger samples are needed to test the effects of blood pressure treatment on oxidative stress and antioxidant capacity markers. Therefore, a collective variable of overall antihypertension medication usage were used as a covariate in sensitivity analysis. This study lacked dietary data to quantify amino-acid (such as cysteine) and antioxidant intake. The strength of this study can be measured on the basis of its design and implementation under controlled conditions (two ethnic and homogenous socioeconomic groups). The inclusion of various factors involved in oxidative stress aided to elucidate the mechanistic relationships between endothelin-1- and oxidative stress-related markers within this population.

In conclusion, our study suggests that in black men, endothelin-1 may contribute to GR upregulation through increased ROS production mediated via endothelin-1, whereas the expected physiological tendency between endothelin-1 and ROS was observed in white men. In black women, higher total GSH levels may act as a counter-regulatory mechanism to protect against oxidative vascular damage attributed by endothelin-1.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Hypertension Research website (http://www.nature.com/hr)