Ethnic-specific relationships between haemostatic and oxidative stress markers in black and white South Africans: The SABPA study

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Abstract

Haemostatic- and oxidative stress markers are associated with increased cardiovascular risk. In the black population, evidence exists that both an imbalance in the haemostatic system and oxidative stress link with the development of hypertension. However, it is unclear whether these two risk components function independently or are related, specifically in the black population, who is known to have a high prevalence of stroke. We aimed to investigate associations between the haemostatic system and oxidative stress in black and white South Africans. We performed a cross-sectional study including 181 black (mean age, 44; 51.4% women) and 209 white (mean age, 45; 51.7% women) teachers. Several markers of the haemostatic- (von Willebrand factor, fibrinogen, plasminogen activator inhibitor-1, D-dimer and clot lysis time) and oxidant–antioxidant (serum peroxides, total glutathione, glutathione peroxidase- and glutathione reductase activities) systems were measured. Along with a worsened cardiovascular profile, the black group had higher haemostatic-, inflammation- and oxidative stress markers as well as decreased glutathione peroxidase activity. In multiple regression analyses, fibrinogen was positively associated with serum peroxides (p < 0.001) in both ethnic groups. In the black population, we found negative associations of von Willebrand factor and clot lysis time with glutathione peroxidase activity (p < 0.008), while a positive association existed between clot lysis time and serum peroxides (p = 0.011) in the white population. We conclude that in the black population, decreased GPx activity accompanies an altered haemostatic profile, while in the white population associations may suggest that serum peroxides impair fibrin clot lysis.

Keywords

Antioxidant capacity, cardiovascular, fibrinogen, glutathione peroxidase, hydrogen peroxide

Introduction

Cardiovascular disease is increasing in South Africa, especially in the black population who has a high prevalence of hypertension and stroke (1–3). Cardiovascular disease development is associated with a thrombotic component initiated at least in part by underlying endothelial dysfunction (4). An imbalance between the oxidative/antioxidant system in favour of oxidative stress as reflected by elevated reactive oxygen species (ROS) (5–7) and/or a decreased antioxidant capacity (8,9) can influence the haemostatic balance either directly or indirectly towards a procoagulant state. Glutathione peroxidase (GPx) utilises glutathione (GSH) to metabolise hydrogen peroxides to water and in doing so, results in the formation of glutathione disulphide (GSSG). GSSG in turn is reduced back to GSH by glutathione reductase (GR) (10). GPx and GR are therefore crucial enzymes in the regulation of the GSH/GSSG system and are important in the protection of the vascular wall (11,12). Both animal and human studies have shown that a compromised antioxidant capacity, as reflected by decreased GPx activity, predisposes an increased cardiovascular and thrombotic risk (8,13,14).

Ethnic differences in the functioning of the haemostatic system and oxidative stress seems to exist in blacks with higher thrombotic- (von Willebrand factor (vWF) (15), fibrinogen (16,17) and D-dimer (18), but lower plasminogen activator inhibitor-1 (PAI-1) (19)) and oxidative stress markers as compared to whites (20). This has been associated with the development and progression of cardiovascular disease (7,21–23). Evidence in the black population is limited; however associations of ROS (24) and D-dimer (25) with blood pressure have been reported. It is still unknown whether associations exist between components of the haemostatic system and oxidative stress in this population group and if these associations are comparable to what has been observed in whites (8,13,26).

We therefore investigated whether associations exist between components of the haemostatic system and oxidative...
stress, and if these associations differ between black and white populations in South Africa.

Materials and methods

Study population

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study was a cross-sectional study that was performed during 2008 and 2009, involving school teachers ($n = 409$) between the ages of 25–60 years from the North West Province, South Africa. Participants consisted of 200 black (101 men and 99 women) and 209 white (101 men and 108 women) participants. Exclusion criteria were an elevated body temperature, dependence or abuse of psychotropic substances, regular blood donors, or individuals vaccinated in the previous 3 months. For this sub-study, 19 participants were excluded due to human immunodeficiency virus infection. Written informed consent was obtained prior to the commencement of measurements. The study complied with all applicable international regulations and the Helsinki declaration for investigation of human participants. The Ethics Review Board of the national regulations and the Helsinki declaration for investigation of human participants. The Ethics Review Board of the North-West University at Potchefstroom Campus approved the study. Each participant completed a lifestyle questionnaire, including questions about their cardiovascular health history, smoking and alcohol habits as well as chronic medication use.

Clinical measurements

A 24-h ambulatory blood pressure (ABPM) and electrocardiogram apparatus (Meditech CE120® Cardiotens, Budapest, Hungary) was attached to the participants at their workplace. Each participant’s energy expenditure during the day was calculated with a validated accelerometer device (Actical® accelerometers, Montréal, Québec). Participants reported to the Metabolic Research Unit of the North-West University at 16:30 where they were informed of the procedures of the following day. They received a standardised meal at 18:00, and final snacks and drinks at 20:30, and were requested to go to bed at around 22:00. At 06:00, the ABPM apparatus was removed, followed by anthropometric measurements and blood sampling. The 24-h blood pressure and electrocardiogram data were downloaded onto a database using the CardioVisions 1.9.0 Personal Edition software (Meditech, Budapest, Hungary).

Anthropometric measurements

All measurements were taken in triplicate with calibrated instruments. Stature was measured to the nearest 0.1 cm with a stadiometer (Invicta Stadiometer, IP 1465, London, UK), body mass to the nearest 0.1 kg (Precision Health Scale, A & D Company, Tokyo, Japan) and waist circumference to the nearest 0.1 cm with a unstretchable flexible 7 mm wide metal tape (Holtain, Crosswell, Wales) (27,28). Body mass index (BMI) was calculated for each participant using the standard formula of weight/(height)$^2$.

Blood sampling

A registered nurse collected fasting blood samples with a sterile winged infusion set from the participants’ antebrachial vein branches before 10:00 in the morning. The blood samples were centrifuged at $2000 \times g$ for 15 min to obtain serum, sodium fluoride and citrate plasma samples. All samples were stored at $-80^\circ$C until analysis.

Biochemical analyses

High-sensitivity C-reactive protein (hs-CRP), total cholesterol (TC), high-density lipoprotein (HDL-C) cholesterol, low-density lipoprotein (LDL-C), gamma glutamyltransferase ($\gamma$-GT), glycylated haemoglobin A1c and fasting glucose were determined with methods described elsewhere (25). A Quantikine high-sensitivity enzyme-linked immunosorbent assay from R&D Systems (R&D Systems, Minneapolis, MN) was used to determine Interleukin-6 (IL-6) from serum.

Citrated plasma samples were used for the analysis of von Willebrand factor antigen (vWF$_{ag}$), fibrinogen, plasminogen activator inhibitor-1 antigen (PAI-1$_{ag}$), fibrin D-dimer and clot lysis time (CLT). vWF$_{ag}$ levels were determined with a sandwich enzyme-linked immunosorbent assay. Polyclonal rabbit anti-vWF antibody and rabbit anti-vWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark) were used to perform the assay. The 6th International Standard for vWF/FVIII was used to create the standard curve against which the samples were measured. Plasma fibrinogen levels were determined by means of a modified Clauss method using the FIB kit from STAGO diagnostics (STAGO diagnostics, Asnières, France). The Liestat D-dimer kit from STAGO diagnostics (STAGO diagnostics, Asnières, France) was used to determine fibrin D-dimer levels with a immuno-turbimetric method with a 540 nm detection limit. PAI-1$_{ag}$ levels were determined with the TrinILIZE PAI-1$_{ag}$ (Trinity Biotech, Bray, Ireland) kit using an enzyme-linked immunosorbent assay. CLT was determined by studying the lysis of a tissue factor-induced clot by exogenous tissue-plasminogen activator. Changes in turbidity during clot formation and lysis were monitored as described by Lismay et al. (29). Tissue factor and tissue plasminogen activator concentrations were slightly modified to obtain comparable CLTs of about 60 min. The modified concentrations were 17 mmol/L calcium chloride, 60 ng/mL tissue plasminogen activator (Actilyse, Boehringer Ingelheim, Ingelheim, Germany) and 10 $\mu$mol/L phospholipids vesicles (Rossix, Mölndal, Sweden). Tissue factor was diluted 3000 times (Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany). CLT was defined as the time from the midpoint in the transition from the initial baseline to maximum turbidity, which is representative of clot formation, to the midpoint in the transition from maximum turbidity to the final baseline turbidity, which represents the lysis of the clot.

The method described by Hayashi et al. (30) was used to determine one of the measurable ROS species, namely total peroxides that includes hydrogen peroxides and lipid peroxides, in serum samples. In this assay 1.0 mg/L hydrogen peroxide represents one unit of ROS. Total GSH were determined on ethylenediaminetetraacetic whole blood sample with the BIOXYTECH® GSH/GSSG-412™ kit supplied by OxisResearch, a division of OXIS Health Products Inc. (Foster City, CA). GPx and GR were measured with kits from Cayman Chemical Company (Cayman Chemical Company, Ann Arbor, MI) on ethylenediaminetetraacetic plasma samples.
ROS, GSH, GPx and GR were measured with the Bio-Tek Synergy HT microplate reader (Bio-Tek Instruments., Inc., Winooski, VT).

**Statistical analyses**

Statistica software v12.0 was used for database management and statistical analyses (Statsoft, Inc., Tulsa, OK). Variables not normally distributed (fibrin D-dimer, hs-CRP, HbA1c, glucose, γ-GT, and energy expenditure) were logarithmically transformed. The central tendency and spread of these variables were represented by the geometric mean and the 5th and 95th percentile intervals. We tested for interaction with ethnicity by investigating associations between haemostatic markers and markers of oxidative stress with multiple regression analyses (Table S1). Results found served as motivation for ethnic split. Means and proportions were compared by an independent *t*-test and chi-square test, respectively. We used partial correlations to determine associations between oxidative and haemostatic markers, followed by forward stepwise multiple linear regression analyses. Covariates included in the models were age, body mass index, gender, γ-GT, current smoking, physical activity, HbA1c, TC:HDL-C, 24-h systolic blood pressure and contraception usage. Sensitivity analyses were performed to test for the influence of inflammation by adding hs-CRP and IL-6 separately into the regression models. All *p*-values refer to a two-sided hypothesis.

**Results**

The general characteristics of the study population are listed in Table 1. Although the groups were of similar age, the black participants were generally more obese (*p* < 0.001), had higher mean systolic and diastolic (*p* < 0.001) blood pressures as well as an increased inflammatory state (*p* < 0.001) compared to their white counterparts. The black participants also had a more detrimental haemostatic profile with higher vWF, fibrinogen, PAI-1ag, D-dimer levels and a longer CLT (*p* < 0.001), as well as a mixed oxidant–antioxidant profile.

**Table 1. Characteristics of the study population.**

|                           | Black (*n* = 181) | White (*n* = 209) | *p*  |
|---------------------------|-------------------|-------------------|------|
| **Women, n (%)**          | 93 (51.4)         | 108 (51.7)        | 0.95 |
| **Age, years**            | 44.4 ± 8.3        | 45.0 ± 10.9       | 0.52 |
| **Body mass index, kg/m²**| 30.3 ± 7.1        | 27.6 ± 5.9        | <0.001|
| **Waist circumference, cm (men)** | 93.6 ± 15.8 | 101.5 ± 14.4 | <0.001|
| **Waist circumference, cm (women)** | 93.9 ± 15.8 | 85.0 ± 13.3 | <0.001|
| **Haemostatic markers**   |                   |                   |      |
| von Willebrand factor, %  | 92.2 ± 24.7       | 63.1 ± 15.0       | <0.001|
| Fibrinogen, g/L           | 3.58 ± 0.89       | 3.07 ± 0.55       | <0.001|
| Plasminogen activator inhibitor-1, ng/mL | 35.2 ± 9.6 | 21.6 ± 6.5 | <0.001|
| Fibrin D-dimer, µg/L      | 295 (80–1175)     | 208 (68–610)      | <0.001|
| Clot lysis time, min      | 84.2 ± 18.0       | 75.3 ± 10.7       | <0.001|
| **Oxidative stress markers** |               |                   |      |
| Reactive oxygen species, mg/L | 93.8 ± 24.6       | 87.8 ± 21.9       | 0.012|
| Glutathione, µM           | 904.0 ± 183.4     | 823.9 ± 134.0     | <0.001|
| Glutathione peroxidase, nmol/min/mL | 32.2 ± 12.3       | 36.0 ± 7.62       | <0.001|
| Glutathione reductase, nmol/min/mL | 7.60 ± 3.46      | 3.59 ± 2.08       | <0.001|
| **Biochemical measurements** |               |                   |      |
| C-reactive protein, mg/L  | 4.48 (0.65–31.70) | 2.03 (0.99–8.99)  | <0.001|
| Interleukin-6, pg/mL      | 1.25 ± 0.69       | 0.98 ± 0.56       | <0.001|
| Total cholesterol, mmol/L | 4.65 ± 1.20       | 5.54 ± 1.28       | <0.001|
| Total cholesterol: high-density cholesterol | 4.44 ± 2.10 | 4.99 ± 1.62 | 0.003|
| High-density lipoprotein cholesterol, mmol/L | 1.15 ± 0.35 | 1.20 ± 0.44 | 0.15|
| Low-density lipoprotein cholesterol, mmol/L | 2.87 ± 1.00 | 3.80 ± 1.09 | <0.001|
| Triglycerides, mmol/L     | 1.41 ± 1.31       | 1.20 ± 0.76       | 0.042|
| Glycosylated haemoglobin A1c, % | 5.92 (5.11–7.40) | 5.47 (5.00–6.10) | <0.001|
| Glucose, mmol/L           | 5.27 (3.93–7.18)  | 5.61 (4.70–6.81)  | <0.001|
| Gamma glutamyl transferase, U/L | 45.3 (20.0–154.5) | 19.0 (7.0–74.0) | <0.001|
| **Cardiovascular measurements** |               |                   |      |
| 24-h Systolic blood pressure, mmHg | 132.4 ± 15.4 | 123.5 ± 10.7 | <0.001|
| 24-h Diastolic blood pressure, mmHg | 82.8 ± 10.3 | 76.4 ± 7.5 | <0.001|
| 24-h Pulse pressure, mmHg | 49.6 ± 8.7       | 47.3 ± 7.0        | 0.003|
| **Lifestyle**             |                   |                   |      |
| Physical activity, kcal/d | 2564 (1702–4018)  | 2910 (1914–4405)  | <0.001|
| Current smoking, n (%)    | 29 (16.0)         | 29 (13.9)         | 0.57 |
| **Intake of medications** |                   |                   |      |
| Anti-hypertensive medication, n (%) | 40 (22.1)       | 18 (8.6)          | <0.001|
| Anti-coagulant medication, n (%) | 2 (1.1)         | 0 (0)             | 0.13 |
| Contraception, n (%)      | 17 (9.4)          | 7 (3.4)           | 0.013|

Values are arithmetic mean ± SD, geometric mean (5th to 95th percentile interval), or number of subjects (%). Normal reference ranges: (46).

von Willebrand factor, %: 75–125.
Fibrinogen, g/L: 1.5–4.0.
Plasminogen activator inhibitor-1, ng/mL: 4.0–43.0.
Fibrin D-dimer, µg/L: 0–500.
with higher serum peroxides ($p < 0.012$) and lower GPx activity ($p < 0.001$) and GR activity ($p < 0.001$) were associated with lower GPx activity ($p < 0.001$) were associated with lower GPx activity ($p < 0.001$) and positively associated with GR activity ($p < 0.001$) and positively associated with GR activity ($p < 0.001$).

### Associations of the haemostatic markers with peroxides and GPx activity

In partial regression analyses (adjusted for age, gender and body mass index), a positive association was found between fibrinogen and serum peroxides ($p < 0.001$) in both ethnic groups. In the black population, higher vWF and longer CLT were associated with lower GPx activity ($p < 0.001$ and $p = 0.030$). No associations were found between the haemostatic components and GPx activity in the white population.

After full adjustment (age, gender, body mass index, current smoking, γ-GT, physical activity, HbA1c, TC:HDL, 24-h SBP and contraception usage) in multiple regression analyses, the association between fibrinogen and serum peroxides ($p < 0.001$) remained in both ethnic groups, as well as the negative associations of vWF and CLT with GPx activity ($p < 0.008$) in the black population. We found additional associations between vWF and serum peroxides ($p = 0.021$) in the black population and CLT with serum peroxides ($p = 0.011$) in the white population.

### Associations of haemostatic markers with the glutathione/glutathione disulphide system

In partial regression analyses (Table 2), vWF, PAI-1$_{ag}$ and CLT were negatively associated with total GSH ($p < 0.012$) and positively associated with GR activity ($p < 0.030$) in the black population. In the white population a positive association was found between vWF and D-dimer, and GR activity ($p < 0.050$).

After full adjustments (Table 3), the negative associations of vWF and PAI-1 with total GSH ($p < 0.002$) and the positive associations of vWF, PAI-1$_{ag}$ and CLT with GR activity ($p < 0.030$) remained in the black population, while only the association between vWF and GR activity ($p = 0.006$) remained in the white population.

### Sensitivity analyses

Since oxidative stress is usually accompanied by a pro-inflammatory state, which may also influence haemostatic functioning, we additionally added hs-CRP (Table S2) and IL-6 (Table S3), separately to the models (31–33). After adjusting for hs-CRP, both vWF and fibrinogen were no longer significantly associated with serum peroxides, or vWF with GR activity in the black population. After adjusting for IL-6, only vWF was no longer associated with serum peroxides in the black population and GR activity in the white population. The positive associations of fibrinogen and CLT with ROS remained in the white population after adjusting for CRP and IL-6, respectively.

### Discussion

We investigated ethnic differences with respect to the associations between several components of the haemostatic and oxidant–antioxidant systems. The black population with elevated ambulatory blood pressure had increased serum peroxides and decreased GPx activity that was associated with several haemostatic components, suggesting that oxidative stress may alter their haemostatic profile. In the white population, associations between CLT and ROS suggest that oxidative stress may impair the lysis of a fibrin clot.

The link found between higher vWF and lower GPx activity in the black population confirms previous findings by Blann et al. (8), reporting lower GPx-1 activity and increased oxidative stress, which may impede platelet inhibitory mechanisms leading to increased platelet aggregation and ultimately a prothrombotic state. The decreased GPx levels of the black population could therefore be an indication of a decreased antioxidant capacity, while the associations between vWF, CLT and GPx suggest that diminished GPx activity levels are associated with a prothrombotic profile.

Furthermore, vWF, PAI-1 and CLT were negatively associated with total GSH and positively with GR in the black population. Limited information is available on the relationship between the antioxidant markers and the components of the antioxidant system.

### Table 2. Partial regression analyses between the haemostatic markers and markers of oxidative stress.

|        | vWF, %     | Fibrinogen, g/L | PAI-1, ng/mL | D-dimer, µg/L | CLT, min |
|--------|------------|-----------------|-------------|---------------|----------|
| Black  |            |                 |             |               |          |
| ROS, mg/L | $r = 0.14$; $p = 0.078$ | $r = 0.27$; $p < 0.001$ | $r = -0.06$; $p = 0.45$ | $r = 0.11$; $p = 0.16$ | $r = 0.04$; $p = 0.61$ |
| GSH, µM  | $r = -0.22$; $p = 0.007$ | $r = -0.06$; $p = 0.46$ | $r = -0.22$; $p = 0.006$ | $r = -0.01$; $p = 0.87$ | $r = -0.20$; $p = 0.012$ |
| GPx, nmol/min/mL | $r = -0.26$; $p < 0.001$ | $r = 0.02$; $p = 0.86$ | $r = -0.08$; $p = 0.30$ | $r = -0.04$; $p = 0.60$ | $r = -0.18$; $p = 0.030$ |
| GR, nmol/min/mL | $r = 0.17$; $p = 0.030$ | $r = 0.05$; $p = 0.57$ | $r = 0.19$; $p = 0.014$ | $r = -0.04$; $p = 0.65$ | $r = 0.23$; $p = 0.004$ |
| White  |            |                 |             |               |          |
| ROS, mg/L | $r = 0.08$; $p = 0.27$ | $r = 0.33$; $p < 0.001$ | $r = 0.02$; $p = 0.77$ | $r = 0.08$; $p = 0.30$ | $r = 0.12$; $p = 0.097$ |
| GSH, µM  | $r = -0.04$; $p = 0.63$ | $r = 0.04$; $p = 0.54$ | $r = 0.00$; $p = 0.98$ | $r = -0.09$; $p = 0.20$ | $r = -0.08$; $p = 0.28$ |
| GPx, nmol/min/mL | $r = 0.07$; $p = 0.30$ | $r = 0.03$; $p = 0.64$ | $r = -0.02$; $p = 0.81$ | $r = 0.04$; $p = 0.54$ | $r = 0.02$; $p = 0.84$ |
| GR, nmol/min/mL | $r = 0.14$; $p = 0.050$ | $r = 0.03$; $p = 0.65$ | $r = 0.07$; $p = 0.35$ | $r = 0.16$; $p = 0.025$ | $r = 0.07$; $p = 0.34$ |

vWF, von Willebrand factor; PAI-1, Plasminogen activator inhibitor-1; CLT, Clot lysis time; hs-CRP, C-reactive protein; IL-6, Interleukin-6; ROS, Reactive oxygen species; GSH, Glutathione; GPx, Glutathione peroxidase; GR, Glutathione reductase.

Adjusted for age, gender and body mass index. Bold values indicate statistical significance $p < 0.05$. 

Reactive oxygen species; GSH, Glutathione; GPx, Glutathione peroxidase; GR, Glutathione reductase.
Table 3. Multiple regression analyses of haemostatic markers with oxidative stress markers.

| Plasminogen activator inhibitor-1, ng/mL | Fibrinogen, g/L | von Willebrand factor, % | Clot lysis time, min |
|-----------------------------------------|----------------|-------------------------|---------------------|
| Plasmogen activator inhibitor-1, ng/mL  | 0.18 (0.02 to 0.42) | 0.14 (0.017 to 0.319) | 0.12 (0.08 to 0.17) |
| Fibrinogen, g/L                         | 0.14 (0.032 to 0.36) | 0.13 (0.017 to 0.319) | 0.12 (0.08 to 0.17) |
| von Willebrand factor, %                | 0.12 (0.08 to 0.17) | 0.12 (0.08 to 0.17) | 0.12 (0.08 to 0.17) |
| Clot lysis time, min                    | 0.10 (0.02 to 0.26) | 0.10 (0.02 to 0.26) | 0.10 (0.02 to 0.26) |

Adjusted for age, body mass index, gender, gamma glutamyl transferase, current smoking, physical activity, glycosylated haemoglobin A1c, total cholesterol: high-density lipoprotein ratio, 24-h systolic blood pressure and contraception usage.

ROS, Reactive oxygen species; GSH, Glutathione; GPx, Glutathione peroxidase, GR, glutathione reductase.

The present study should be interpreted within the context of its limitations and strengths. Additional markers of haemostatic markers. However, in agreement with our findings a previous study investigating relationships between vWF and reduced GSH in diabetic patients reported an inverse relationship between vWF and GSH (26). A study by Martina et al. (34) who evaluated the effect of GSH treatment on PAI-1 levels also reported an inverse relationship. Even though we used total GSH instead of reduced GSH it is possible that the total GSH levels of the black population could consist out of a decreased GSH/GSSG ratio. We could argue that GR activity may be up-regulated in the black population in an attempt to restore the redox balance. However, this is highly speculative and needs to be confirmed. A previous study by Rybka et al. (35) who investigated the glutathione antioxidant defence system also reported that decreased GPx activity levels are accompanied by a concomitant increase in GR activity in patients with hypertension. When oxidative stress supersedes GPx availability, damage may occur to the surrounding cells which may at least in part, result in the release of several prothrombotic factors (36). The decreased GPx availability in the black population could alter their haemostatic profile and thereby their susceptibility to stroke. No associations were found between vWF and ROS, GSH or GPx in the white population, this could be due to the fact that their mean vWF levels were significantly lower than their black counterparts.

The positive association initially found between fibrinogen and ROS in both ethnic groups disappeared in the black population after adjusting for hs-CRP, while the initial associations remained after adjusting for IL-6. Although several in vitro studies exist regarding the relationship between fibrinogen and oxidative stress (37,38), epidemiological data regarding the relationship is lacking. Fibrinogen and hs-CRP are both markers of the acute phase response and their dependence on each other in the black population suggests that the association between fibrinogen and ROS could be mediated through the acute phase response (39). Overall, the black population had a worsened cardiovascular profile that may contribute to increased low-grade inflammation or vice versa. The more pronounced inflammatory state in the black population could possibly explain the influence of hs-CRP on the fibrinogen and ROS relationship. Furthermore, fibrinogen is known to be highly susceptible to oxidative modifications which may affect changes in the formation and architecture of the fibrin network (38,40,41). However, controversy exists as to whether oxidised fibrinogen induces a prothrombotic (42,43) or anti-thrombotic (37,44) state. The positive association between CLT and ROS in the white population may serve as an indicator that oxidative stress and inflammatory markers as well as a worsened cardiovascular profile. Further studies are needed to gain insight into the haemostatic markers and oxidative stress interactions in different ethnicities.
oxidative stress such as superoxide dismutase and catalase could add valuable insight into the antioxidant capacity. Residual confounding cannot be excluded even though the results were consistent after multiple adjustments. Causality cannot be inferred, due to the cross-sectional nature of the study. Since our population group was only recruited from urban areas of the Potchefstroom district in the North West province of South Africa, our findings are not seen as representative of the entire South African population. Overall, this was a well-designed study under controlled conditions in two different ethnic groups, with a relatively similar socio-economic status.

We conclude that in the black population, decreased GPx activity accompanies an altered haemostatic profile, while in the white population associations may suggest that serum peroxides impair fibrin clot lysis.

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Declaration of interest

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Supplementary material available online
Table S1–3