

**Procoagulant reactivity to laboratory acute mental stress in Africans and Caucasians,  
and its relation to depressive symptoms: The SABPA Study**

**Running title:** Procoagulant stress reactivity in Africans

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## SUMMARY

The risk of cardiovascular disease is dramatically increasing in Africans (black). The prothrombotic stress response contributes to atherothrombotic disease and is modulated by depressive symptom. We examined coagulation reactivity to acute mental stress and its relation to psychological well-being in Africans relative to Caucasians (white). 102 African and 165 Caucasian school teachers underwent the Stroop Color-Word Conflict test. Circulating levels of von Willebrand factor (VWF) antigen, fibrinogen, and D-dimer were measured before and after the Stroop. Cardiovascular reactivity measures were also obtained. All participants completed the Patient Health Questionnaire-9 and the General Health Questionnaire-28 for the assessment of depressive symptoms and total psychological distress, respectively. After controlling for covariates, resting levels of VWF, fibrinogen, and D-dimer were higher in Africans than in Caucasians (all p-values  $\leq 0.006$ ). Depressive symptoms and psychological distress were not significantly associated with resting coagulation measures. Stress reactivity in VWF ( $p < 0.001$ ) and fibrinogen ( $p = 0.016$ ), but not in D-dimer ( $p = 0.27$ ), were decreased in Africans relative to Caucasians with Africans showing greater reactivity of total peripheral resistance ( $p = 0.017$ ). Depressive symptoms, but not general psychological distress, were associated with greater VWF increase ( $p = 0.029$ ) and greater fibrinogen decrease ( $p = 0.030$ ) in Africans relative to Caucasians. In conclusion, Africans showed greater hypercoagulability at rest but diminished procoagulant reactivity to acute mental stress when compared with Caucasians. Ethnic differences in the vascular adrenergic stress response might partially explain this finding. Depressive symptoms were associated with exaggerated VWF reactivity in Africans relative to Caucasians. The clinical implications of these findings for Africans need further study.

**Keywords:** Cardiovascular disease, coagulation, depression, ethnicity, psychological stress

## INTRODUCTION

Enhanced coagulation, impaired fibrinolysis, endothelial activation, and hyperactive platelets play an important role in the development of atherogenesis, atherothrombosis, and acute coronary syndromes (ACS) (1). During acute mental stress, healthy individuals show a prothrombotic state that is viewed as an adaptive fight-flight response protecting the organism from excessive bleeding should injury occur (2, 3; for review). Fibrinogen, von Willebrand factor antigen (VWF:Ag) and D-Dimer, the latter indicating fibrin turnover, are particularly responsive to acute mental stress (4, 5). Excessive stress procoagulant changes might potentially increase the risk of incident **cardiovascular disease (CVD)** and recurrent CVD events (3). For instance, stress-induced fibrinogen increase was associated with carotid artery stiffness (6) and predicted an increase in ambulatory systolic blood pressure (SBP) over three years in healthy individuals (7). Owing to impaired endothelial anticoagulant function, patients with atherothrombotic diseases showed greater platelet activation (8) and D-dimer increase (9) than controls free of CVD. Moreover, patients with emotional triggers like depressive feelings in the two hours before ACS onset, showed greater platelet aggregation during laboratory mental stress than patients reporting non-emotional triggers (10). Depressive symptoms have also been associated with stress-induced elevation in platelet reactivity (11) and D-dimer (12) in elderly subjects, thereby supporting the notion that hypercoagulability is one mechanism linking depression with an increased CVD risk (13).

Potential ethnical differences in the acute procoagulant response to stress and their relation to depressive symptoms have not previously been explored. Such biobehavioral research seems important for urbanized Africans in whom a concerning increase in CVD can be observed, much of it being a consequence of their transition from a traditional African to a modernized “Western” lifestyle (14). For instance, in Africans, the CVD risk predicted by fibrinogen becomes stronger with an increasing degree of urbanization (15). Moreover, subclinical atherosclerosis, as measured by increased carotid intima media thickness (CIMT),

was greater in Africans than in Caucasians (16) as well as in those with more severe depressive symptoms of both these ethnicities (17).

The autonomic nervous system and hypothalamic pituitary adrenal axis are the two major physiologic stress response systems in humans. As cortisol peaks between 15 and 30 minutes after stress onset, prothrombotic changes within a few minutes of acute stress are largely governed by the sympathetic nervous system through release of catecholamines and adrenergic receptor stimulation (18, 19). Stress-hormone mediated stimulation of vascular beta2-adrenergic receptors releases VWF from endothelial cells (19-21). Stress-induced increases in D-dimer and in alpha-receptor activating norepinephrine are correlated with each other (5), but beta-adrenergic stimulation did not result in D-dimer increase (20). Compared to Caucasians, Africans show a greater alpha-adrenergic vascular response, which is reflected by a greater total peripheral resistance (TPR) (22). One specific hypothesis following from the above literature is that Africans would show greater D-dimer reactivity but lower VWF reactivity to acute stress than Caucasians. More severe depressive symptoms would hypothetically relate to greater procoagulant activity in both Africans and Caucasians.

The primary aim of this study was to investigate procoagulant reactivity between Africans and Caucasians through measurements of changes in circulating levels of VWF, fibrinogen and D-dimer to acute standardized laboratory stress. A secondary aim was to explore the association of depressive symptoms both continuously and categorically (i.e., based on a clinical cut-off level, with stress-induced coagulation changes. We further examined whether procoagulant reactivity would specifically relate to depressive mood or would rather be associated with elevated levels of general psychological distress.

## **MATERIALS AND METHODS**

### **Study participants and design**

The participants of this blood coagulation reactivity study were recruited as part of the

Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study conducted between February 2008 and May 2009. The study protocol was conducted in accordance with the Declaration of Helsinki (23) and was approved by the Ethics Review Board of the North-West University, Potchefstroom Campus (0003607S6). All participants provided written informed consent before participation. The SABPA study has a target population comparative design and recruited 409 teachers, aged 25–65 years, working in the Dr Kenneth Kaunda Education district in the North West Province, South Africa. This selection assured a homogenous sample from a similar socio-economic class. Exclusion criteria included pregnancy, lactation, and vaccination or blood donation within 3 months prior to participation.

For purposes of the present study, we excluded participants with a history of atherothrombotic disease (myocardial infarction, n=4; stroke, n=1), atrial fibrillation (n=16), and HIV positive status (n=18), all of which are associated with hypercoagulability at rest and/or in response to acute stress (10, 24, 25), as well as participants with an ear temperature  $\geq 37.5^{\circ}\text{C}$  (n=3) because of a probable acute phase response that might increase coagulation factors like fibrinogen. Users of oral anticoagulants (n=2), aspirin (n=12), cortisone (n=3), oral contraceptives (n=21), antidepressants (n=3), anxiolytics (n=1), beta blockers (n=4), and calcium antagonists (n=12) were also excluded, as all of these might affect resting coagulation activity and/or coagulation reactivity with acute mental stress and/or mood (21, 26-29). To allow full multivariate statistical analyses, subjects with we also excluded subjects who missed data for coagulation measures (n=22), hemodynamic reactivity (n=10), psychological questionnaires (n=1), hemoglobin A1c (HbA1c) (n=1), creatinine (n=2), alcohol consumption (n=1), and physical activity (n=1). Finally, we excluded 4 participants because of excessively high D-dimer levels after stress ( $\geq 10'000$  ng/ml), yielding a final study sample of 267 subjects. Excluded participants (n=142) were significantly more often Africans, women, and antihypertensive drug users, and they also had significantly higher screening systolic blood pressure (SBP), HbA1c, and gamma glutamyl transferase ( $\gamma$ -GT) levels than those included.

The participants were admitted at 4:30 PM to the multi-bedroomed Metabolic Unit Research Facility of the North-West University on Monday through Thursday. All received a standardized dinner at 06:00 PM after which they completed psychological questionnaires. They had their last beverages (tea/coffee) and biscuits at 8:30 PM followed by recreational activities such as reading, watching television, or interacting socially. We also obtained information on demographic data, general health, including medication, and health behaviors. All participants went to bed at around 10:00 PM and were woken at 05:45 AM. After the completion of anthropometric and manual BP measurements, a registered nurse obtained fasting blood samples which were handled according to standardized procedures. A sterile winged infusion set was left in situ with a heparin block (0.5ml of a Heparin Sodium-Fresenius 5000 IU/ml in 50ml normal saline solution; Fresenius Kabi, Port Elizabeth, South Africa) to prevent clotting. Thereafter, all participants underwent the laboratory stress.

### **Acute laboratory stress testing**

***Mental stress test:*** All participants underwent the Stroop Color-Word Conflict test for 1 minute. The Stroop is a standardized laboratory stressor showing reproducibility on cardiovascular reactivity (30). The Stroop requires identification of the ink color of the word rather than the name of the color spelled by the word, under time pressure. The participants received a monetary motivation reward in line with performance. Perceived stress triggered by the Stroop was rated on a Likert scale (1="not at all stressful", 7="very stressful").

***Cardiovascular data collection:*** With participants in a semi-recumbent position a registered nurse took a 5-minute continuous measurement of resting cardiovascular parameters with the validated Finometer® device (Finapres Medical Systems, Amsterdam, the Netherlands) (31). The Finometer provides beat-by-beat BP and thus detects the full contour of the cardiovascular responses. The Finometer recorded the SBP, diastolic blood pressure (DBP) and heart rate (HR) and computed an integrated age dependent aortic flow

curve from the surface area beneath the pressure/volume curve determining cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR) online (32); data were stored in the results files. Calculations of SV, CO, and TPR yield reliable measures (33, 34). We stabilized BP to resting state before participants underwent the Stroop. We obtained beat-to-beat BP responses throughout mental stress and the 5 min recovery phase. For analyses, we used the average of the last 2 minutes of the resting recordings and the averages of the last 15 seconds of the stressor recordings as well as of the recovery recordings at 1, 3, and 5 minutes after the Stroop had ended. Cardiovascular reactivity was calculated as the area under the curve (AUC) with respect to increase from rest (35) across these five time points for SBP, DBP, HR, CO, SV, and TPR.

**Blood collection:** Citrated blood samples were taken before the Stroop to determine resting levels of coagulation measures. At 10 minutes poststress, the infusion set was thoroughly flushed with 2-3 ml of saline and the first 2 ml of blood were discarded before sampling was done for coagulation measures. Coagulation reactivity was calculated for each participant as the percentage change from the resting value.

### **Cardiometabolic risk factors**

**Body mass index:** With participants in their underwear, we measured height and weight to the nearest 0.1 cm and 0.1 kg to calculate the body mass index (BMI, kg/m<sup>2</sup>).

**Screening blood pressure:** After 5 minutes of rest, using a stethoscope and a mercury sphygmomanometer (auscultatory method), duplicate BP readings were taken 5 minutes apart and measurements averaged to obtain screening SBP and DBP.

**Blood lipids:** Total cholesterol (T-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured in serum with the Konelab 20i (Thermo Fisher Scientific, Vantaa, Finland). For statistical analysis, we computed the T-C/HDL-C ratio.

**Hemoglobin A1c:** HbA1c levels were determined by a turbidometric inhibition

immunoassay method from EDTA plasma (Integra 400, Roche, Basel, Switzerland).

**Renal function:** We used the Modification of Diet in Renal Disease (MDRD) Study equation to estimate glomerular filtration rate (eGFR) from creatinine levels, age, sex, and ethnicity (36). Creatinine was measured in serum using an enzymatic colorimetric test (Cobas Integra 400 plus, Roche, Basel, Switzerland).

### Health behaviors

**Smoking:** Participants who indicated that they currently smoked and/or had smoked at least one cigarette per day during one year in the past were categorized as ever smokers.

**Alcohol consumption:** Serum levels of  $\gamma$ -GT activity were used as a marker of alcohol abuse (37) and measured with an enzymatic colorimetric assay (Cobas Integra 400 plus).

**Physical activity:** We used the Actical<sup>®</sup> accelerometers (Montréal, Québec) to quantify and index physical activity with 3 (vigorous intensity), 2 (moderate intensity) or 1 (light intensity) (38).

### Coagulation measures

**Blood processing:** Citrated blood samples were centrifuged at 1,500 rpm (2000g) for 15 minutes at room temperature. Citrated plasma samples were aliquoted and frozen at  $-80^{\circ}\text{C}$  until analysis. Fibrinogen and D-dimer were determined by an accredited laboratory.

**Von Willebrand factor:** Plasma VWF:Ag (%) were measured with a "sandwich" ELISA assay. A polyclonal rabbit anti-VWF antibody and a rabbit anti-VWF-HRP antibody (DAKO, South Africa) were used to form the assay. The 6th International Standard for VWF/FVIII was used to set the standard curve against which the samples were measured (39).

**Fibrinogen:** Plasma fibrinogen levels (g/l) were determined using a viscosity-based method (STA Compact, STAGO Diagnostic, Roche, France).

**D-dimer:** Plasma D-dimer levels (ng/ml) were determined with an immuno-based



method (STA Compact, STAGO Diagnostic, Roche, France). **There were respectively 47.6% and 39.7% of D-dimer values at rest and after stress** below the limit of detection (<220 ng/ml). **Undetectable values were** substituted using the maximum likelihood method that generates values from the lognormal distribution of uncensored data under the assumption that censored values follow a lognormal distribution.

### **Psychological questionnaires**

**Depressive symptoms:** We used the 9-item Patient Health Questionnaire (PHQ-9) to measure the frequency of depressive symptoms during the prior two weeks corresponding to criteria in the **Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition** (40). Each item is rated on a 4-point scale (0=“not at all“, 1=“several days“, 2= “more than half the days“, 3=“nearly every day“) giving a possible global score of 0-27. PHQ-9 scores  $\geq 10$  have 88% sensitivity and specificity for major depression (40). In our sample, Cronbach’s  $\alpha$  was 0.82 for Africans and 0.84 for Caucasians for the total scale.

**Psychological distress:** With the 28-item General Health Questionnaire (GHQ-28), we quantified perceived **psychological distress in general** related to symptoms of depression, anxiety, somatic complaints and social withdrawal over the past few weeks applying the binary scoring method. The global score provides a severity measure of total psychological distress. If exceeding the threshold of 4, subjects are classified as achieving „psychiatric caseness“ (41). In our sample, Cronbach’s  $\alpha$  for the total scale was 0.91 for Africans and 0.90 for Caucasians.

### **Statistical analysis**

We used SPSS version 21.0 for Windows to analyze the data with significance level at  $p < 0.05$  (2-tailed). In case of a non-normal distribution, dependent variables were log transformed; for clarity, all data are given in original units. Chi-square test and Student’s t-test

with Levene's correction in the case of unequal variances were applied for data comparison between groups. We used multivariate analysis of variance (MANOVA) and of covariance (MANCOVA) to test for a significant association of ethnicity, **PHQ-9 measures, GHQ-28 measures**, and cardiovascular reactivity measures with coagulation measures. We only deemed results of individual coagulation measures to be significant if the MAN(C)OVA test statistic was significant, as this procedure protects against inflated Type I errors due to multiple tests of (likely) correlated dependent variables. Covariates were selected a priori based on the literature about demographic, cardiometabolic and life style factors affecting circulating levels of coagulation measures (42-44). Effect sizes are expressed as partial eta squared ( $\eta_p^2$ ) or partial correlation coefficients.

## RESULTS

### Participant characteristics

Table 1 shows the demographic and health characteristics of the 267 study participants per ethnic group. The percentage of men was higher in Africans than in Caucasians. In terms of cardiometabolic risk factors, Africans showed higher BMI, HbA1c, and screening BP – despite using more antihypertensives – than Caucasians, while Caucasians had a higher T-C/HDL-C ratio than Africans. **Of the 20 subjects who took antihypertensive medications, 9 had thiazide diuretics, 6 had angiotensin-converting enzyme (ACE) inhibitors, 2 had angiotensin receptor blockers (ARB), and 3 had combination therapy (i.e., thiazide diuretic plus ACE inhibitor or ARB).** Regarding health behaviors, Africans were physically less active and had higher  $\gamma$ -GT indicating more alcohol abuse than Caucasians. **Africans were significantly more depressed and they also perceived more distress than Caucasians.**

### Coagulation measures at rest

**Ethnicity:** Both MANOVA ( $F_{3,263}=43.28$ ,  $\eta_p^2=0.330$ ,  $p<0.001$ ) and MANCOVA ( $F_{3,252}=36.31$ ,  $\eta_p^2=0.302$ ,  $p<0.001$ ) showed a significant association between ethnicity and resting levels of the three coagulation measures. Table 2 shows that compared with Caucasians, Africans had significantly higher resting levels of VWF, fibrinogen, and D-dimer in the unadjusted as well as in the adjusted analyses.

**Psychological questionnaires:** To test for an association of PHQ-9 and GHQ-28 measures with resting levels of coagulation measures, we reran the above MANCOVA with continuous and categorical PHQ-9 and GHQ-28 measures as additional covariates (four separate models). Ethnicity remained associated with coagulation measures in all of these models (all p-values  $<0.001$ ). However, coagulation measures showed no significant association with continuous PHQ-9 scores ( $p=0.46$ ), categorical PHQ-9 scores ( $p=0.071$ ), continuous GHQ-28 scores ( $p=0.59$ ), and GHQ-28 caseness ( $p=0.47$ ).

**Interaction between ethnicity and psychological questionnaires:** Taking into account main effects of ethnicity and PHQ-9 and GHQ-28 scores, the interaction terms between ethnicity and the continuous as well as categorical PHQ-9 and GHQ-28 scores were not significant (all p-values  $>0.10$ ).

### Stress-induced changes across all participants

The mean level of perceived stress from the Stroop was  $3.70\pm 1.64$  (range 0-7). Mental stress provoked significant responses in coagulation and hemodynamic measures. While VWF levels increased from  $74.9\pm 26.1\%$  to  $99.9\pm 40.1\%$  ( $p<0.001$ ), fibrinogen levels decreased from  $3.18\pm 0.69$  g/l to  $2.88\pm 0.68$  g/l ( $p<0.001$ ). D-dimer levels showed no significant change ( $320\pm 339$  ng/ml vs.  $376\pm 503$  ng/ml,  $p=0.44$ ). Moreover, SBP ( $134.4\pm 16.2$  mmHg vs.  $154.2\pm 20.8$  mmHg,  $p<0.001$ ), DBP ( $78.6\pm 9.3$  mmHg vs.  $88.6\pm 11.4$  mmHg,  $p<0.001$ ), HR ( $66.8\pm 10.7$  bpm vs.  $87.4\pm 16.6$  bpm,  $p<0.001$ ), and CO ( $6.59\pm 1.81$  l/min vs.  $8.12\pm 2.26$  l/min,

$p < 0.001$ ) all increased and SV ( $99.6 \pm 23.9$  ml vs.  $94.2 \pm 23.1$  ml,  $p < 0.001$ ) and TPR ( $1.02 \pm 0.46$  mmHg/ml/s vs.  $0.99 \pm 0.51$  mmHg/ml/s,  $p = 0.013$ ) both decreased.

**Association between hemodynamic and coagulation reactivity:** Reactivity measures of VWF:Ag, fibrinogen, and D-dimer were not significantly correlated with each other (all  $p$ -values  $> 0.25$ ). Separate MANOVA models for AUC measures of hemodynamic variables showed that reactivity in DBP ( $F_{3,263} = 3.83$ ,  $\eta_p^2 = 0.042$ ,  $p = 0.010$ ), SV ( $F_{3,263} = 3.35$ ,  $\eta_p^2 = 0.037$ ,  $p = 0.020$ ), and TPR ( $F_{3,263} = 2.74$ ,  $\eta_p^2 = 0.030$ ,  $p = 0.044$ ) were significantly associated with coagulation reactivity, whereas reactivity in SBP ( $p = 0.44$ ), HR ( $p = 0.83$ ), and CO ( $p = 0.52$ ) were not. Correlation analysis on individual measures revealed that VWF reactivity increased with greater SV reactivity ( $r = 0.18$ ,  $p = 0.003$ ), but decreased with greater reactivity in DBP ( $r = -0.18$ ,  $p = 0.003$ ) and TPR ( $r = -0.14$ ,  $p = 0.022$ ). Fibrinogen and D-dimer reactivity were not significantly associated with DBP, SV, and TPR reactivity (all  $p$ -values  $> 0.12$ ).

### Stress-induced changes and ethnicity

**Perceived stress:** Africans ( $3.69 \pm 1.87$ ) and Caucasians ( $3.70 \pm 1.49$ ) perceived the Stroop protocol as similarly stressful ( $p = 0.94$ ).

**Coagulation reactivity:** Using MANOVA ( $p = 0.19$ ) and MANCOVA ( $p = 0.62$ ) tests, there were no significant associations between ethnicity and poststress levels of coagulation measures (cf. Table 2 for unadjusted and adjusted poststress levels of VWF, fibrinogen, and D-dimer). However, there were significant association between ethnicity and coagulation reactivity in MANOVA ( $F_{3,263} = 13.73$ ,  $\eta_p^2 = 0.135$ ,  $p < 0.001$ ) and MANCOVA ( $F_{3,251} = 16.86$ ,  $\eta_p^2 = 0.168$ ,  $p < 0.001$ ) tests. Table 1 shows that relative to Caucasians, Africans experienced lower procoagulant reactivity with less of an increase in VWF reactivity and more of a decrease in fibrinogen reactivity; D-dimer reactivity showed no significant difference between Africans and Caucasians.

**Hemodynamic reactivity:** MANCOVA showed a significant association between ethnicity and AUC measures of hemodynamic variables ( $F_{6,248}=3.18$ ,  $\eta_p^2=0.072$ ,  $p=0.005$ ). Relative to Caucasians, Africans had lower SBP AUC ( $29.3\pm 5.8$  vs.  $45.1\pm 4.3$ ,  $\eta_p^2=0.016$ ,  $p=0.043$ ), and lower SV AUC ( $-32.3\pm 8.1$  vs.  $5.4\pm 6.1$ ,  $\eta_p^2=0.049$ ,  $p<0.001$ ), but greater TPR AUC ( $0.40\pm 0.27$  vs.  $-0.30\pm 0.20$ ,  $\eta_p^2=0.029$ ,  $p=0.007$ ). Ethnicity was not related to AUC measures of DBP ( $p=0.46$ ), HR ( $p=0.12$ ), and CO ( $p>0.09$ ). Ethnicity did not significantly interact with AUC measures of any cardiovascular parameter to determine coagulation reactivity (all  $p$ -values  $>0.18$ ).

**Psychological questionnaires:** After controlling for ethnicity MANCOVA tests showed no significant associations of **continuous PHQ-9 scores** ( $p=0.71$ ), categorical **PHQ-9 scores** ( $p=0.94$ ), **continuous GHQ-28 scores** ( $p=0.18$ ), and GHQ-28 caseness ( $p=0.91$ ) with coagulation reactivity. However, there were significant interactions of ethnicity with **continuous PHQ-9 scores** ( $F_{3,249}=3.03$ ,  $\eta_p^2=0.035$ ,  $p=0.030$ ) and categorical **continuous PHQ-9 scores** ( $F_{3,249}=3.80$ ,  $\eta_p^2=0.044$ ,  $p=0.011$ ), but not with **continuous GHQ-28 scores** ( $p=0.24$ ) and GHQ-28 caseness ( $p=0.62$ ).

Between-subject analysis revealed significant associations between ethnicity and **continuous PHQ-9 scores** for reactivity of VWF ( $\eta_p^2=0.016$ ,  $p=0.044$ ) and fibrinogen ( $\eta_p^2=0.020$ ,  $p=0.026$ ), but not for D-dimer reactivity ( $p=0.54$ ). Partial correlation coefficients differed significantly between ethnic groups for VWF reactivity ( $p=0.029$ ) and for fibrinogen reactivity ( $p=0.030$ ). With greater **continuously scaled PHQ-9 scores, indicating greater** severity of depressive symptoms, VWF increased more in Africans ( $r=0.202$ ) than in Caucasians ( $r=-0.074$ ), whereas fibrinogen decreased more in Africans ( $r=-0.215$ ) than in Caucasians ( $r=0.058$ ).

Similarly, ethnicity interacted with categorical **PHQ-9 scores** in determining VWF reactivity ( $\eta_p^2=0.023$ ,  $p=0.015$ ) and fibrinogen reactivity ( $\eta_p^2=0.018$ ,  $p=0.035$ ), but not D-dimer reactivity ( $p=0.28$ ). Figure 1 illustrates these interactions. Partial correlation

coefficients significantly differed between groups for VWF reactivity ( $p=0.035$ ) and for fibrinogen reactivity ( $p=0.047$ ). PHQ-9 scores  $\geq 10$  were directly correlated with VWF reactivity in Africans ( $r=0.149$ ), but inversely so in Caucasians ( $r=-0.119$ ). Moreover, PHQ-9 scores  $\geq 10$  were inversely correlated with fibrinogen reactivity in Africans ( $r=-0.187$ ), but directly so in Caucasians ( $r=0.064$ ).

We performed six complementary univariate analyses of covariance to test whether sympathetic activity as indicated by hemodynamic reactivity might relate to the direct association between continuous PHQ-9 scores and VWF reactivity in Africans versus Caucasians. However, all six three-way-interactions between ethnicity, continuous PHQ-9 scores and AUC measures of any hemodynamic parameter were non-significant (all  $p$ -values  $\geq 0.12$ ).

## DISCUSSION

The main finding from our study is that Africans showed lower procoagulant reactivity in response to standardized acute laboratory mental stress when compared with Caucasians, controlling for a range of potentially confounding variables. Relative to Caucasians, VWF levels had increased less and fibrinogen levels had decreased more in Africans after stress relative to resting levels. One explanation for the diminished increase in VWF in Africans could be a “ceiling effect” as Africans started out with higher resting levels of VWF than Caucasians. For instance, previous studies in apparently healthy individuals reported stress-induced increases of VWF of 6%, 9%, and 30% from resting levels which were respectively 91%, 98%, and 95%, in these studies (4, 45, 46). This amount of an increase in VWF is clearly lower than in our Caucasian subjects who showed an increase in VWF of 60% starting out from average resting values of 64%, but similar to our African subjects who showed an increase in VWF of 18% from average resting levels of 93%. In contrast, a “floor effect” in Caucasians who started out on relatively lower resting fibrinogen

levels might account for the greater decrease in poststress fibrinogen levels relative to resting levels in Africans. Another explanation for the blunted VWF reactivity in Africans might be their alpha-adrenergic vascular stress response, **as was suggested by their increased TPR reactivity** (22). Across all participants, lower VWF reactivity correlated with both greater TPR reactivity and lower SV reactivity, **the latter likely reflecting increased afterload due to elevated TPR** (22). Moreover, in Caucasians, the systemic vasodilation response during the Stroop test is largely mediated by the beta2-adrenergic receptor (47) whose stimulation through stress hormones will result in endothelial release of VWF into the circulation (19-21).

A decrease in fibrinogen levels between rest and 10 minutes after the Stroop was seen across all study participants, and to an even greater extent in Africans than Caucasians. This is contrary to expectations, as fibrinogen levels were shown to increase in response to acute mental stress in several previous studies (2-4). The Stroop protocol provoked an average level of psychological distress that compares to similar protocols (10). However, previous studies suggest that the Stroop alone might be less effective in provoking a significant fibrinogen response (48) than combinations of the Stroop with other stressors (e.g., mental arithmetic) (45) or speech stressors (4). As there was no correlation of VWF reactivity with both fibrinogen and D-dimer reactivity in the present study, but in a previous one (49), it is also possible that the 1-minute stressor was too short to evoke a significant fibrinogen increase with fibrin formation and degradation further downstream. **Nevertheless, previous studies showed coagulation and fibrinolysis activation within 2 to 5 minutes of acute mental stress** (50, 51) **and infusion with the stress hormone epinephrine** (52). D-dimer did not increase in a previous study that combined the Stroop with mental arithmetic (53), but in studies that applied speech stressors to inflict social evaluative threat (5, 9). The sensitivity of the various D-dimer assays used in previous stress studies might also explain heterogeneous results (54). **Saying that, previous stress reactivity studies also used different methods to measure fibrinogen (e.g., the functional Clauss method) (4) and VWF (e.g., enzyme-linked**

immunosorbent assays) (46). To our knowledge, a direct comparison of the sensitivity between different assays to detect reactivity in prothrombotic measures has not been performed, but would seem important to reconcile heterogeneous study findings.

Coagulation reactivity was specifically associated with depressive symptoms (i.e., PHQ-9 scores) as opposed to more general psychological distress (i.e., GHQ-28 scores). More severe depressive symptoms were associated with greater VWF reactivity in Africans compared with Caucasians. This concurs with the notion that depressive mood along a continuum of severity is associated with an increased risk of incident CVD and recurrent cardiac events (55). However, poststress levels of VWF and fibrinogen were similar in Africans and Caucasians, fibrinogen decreased more in depressed Africans than depressed Caucasians, and VWF reactivity was, on the whole, lower in Africans than in Caucasians; therefore, a depression-associated increase in CVD risk in Africans through a pathway of stress-induced VWF increase would need to be shown in prospective studies.

Ethnic differences in coagulation are still poorly understood with studies showing enhanced clotting but also increased bleeding tendency in various clinical settings in Africans compared with Caucasians (56). We found resting levels of VWF:Ag, fibrinogen, and D-dimer to be higher in Africans than Caucasians, which is in agreement with some previous studies (57-59). Theoretically, this potential predisposition to hypercoagulability might contribute to the CVD risk in Africans. In contrast, lower increase in VWF levels and greater decrease in fibrinogen levels both with acute mental stress could be viewed a hypoactive fight-flight response (60). As opposed to constitutively released VWF, the VWF released from the endothelium after stimulation consists of large and hemostatically highly active multimers (61). Moreover, although not investigated in our study, acute stress also activates fibrinolysis (2, 45, 50). Therefore, the inability to mount physiologic hypercoagulability might subject Africans to an increased risk of bleeding in fight-flight situations either upon injury or even spontaneously. Therefore, the almost absent VWF response in the non-depressed



Africans might be as harmful as the increased VWF reactivity in the depressed Africans for the maintenance of the haemostatic balance between thrombosis and haemorrhage during acute mental stress.

Rigorous selection of participants on medical characteristics to minimize confounding of coagulation measures was a strength of our study, but also might reduce generalizability of study findings to the larger African population and those with established CVD. We selected three coagulation measures that were previously shown to be stress-responsive. However, they do not cover the entire dynamics of the coagulation and fibrinolysis pathways during acute mental stress. A speech stressor inflicting social evaluative threat and measuring coagulation measures longer into the recovery period from stress might have provided additional informative data. The Stroop test provoked substantial increases in BP and HR, but there is an ongoing debate as to whether cardiovascular reactivity data gained from standardized lab stressor are generalizable to real life situations (62). We controlled our analysis for antihypertensive medications as a group but due to insufficient statistical power could not take into account potential class effects of the different antihypertensives on outcomes. We did not include a non-stress control group. Therefore we were unable to account for circadian changes in coagulation measures, particularly so a decrease in plasma fibrinogen levels during the morning hours. We are unable to account for the possibility that stress-related changes in clearance impacted plasma concentrations of coagulation measures. For instance, glycosylation of VWF influences its clearance by the liver, which, moreover, occurs more rapidly in individuals with blood group O than in non-O individuals (63); unfortunately, information on ABO blood group was not available in our study. The difference in liver function between Africans and Caucasians seems not an apparent explanation for the ethnic difference in VWF reactivity because the relation between ethnicity and procoagulant reactivity persisted after adjustment for  $\gamma$ -GT activity.

Taken together, Africans seem to have lower procoagulant reactivity with acute mental stress than Caucasians. This observation might partially be explained by ethnic differences in the vascular stress response. Depressive symptoms may modulate coagulation reactivity against an ethnic background. The potential clinical implications of our findings for CVD risk and bleeding disorders in Africans need further studies.

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### **Legend to Figure 1**

The figure depicts the multivariate-adjusted interaction effects between ethnicity and categorical depression (i.e., score  $\geq 10$  on the PSQ-9 total depressive symptom scale) on von Willebrand factor (VWF) and fibrinogen reactivity (i.e., relative change in levels of coagulation measures in % from resting levels to 10 minutes after the Stroop test). Relative to non-depressed individuals (Dep -) of the respective ethnicity, VWF reactivity was significantly greater in Africans vs. Caucasians with depression (Dep +). Fibrinogen reactivity showed a significantly greater decrease in depressed Africans than in depressed Caucasians (cf. text for detailed statistics). Africans/Dep + (n=47), Africans/Dep - (n=55), Caucasians/Dep + (n=29), Caucasians/Dep - (n=136).

**Table 1. Characteristics of 267 study participants**

Variable	Africans (n=102)	Caucasians (n=165)	P-value
Gender (% men)	62.7	49.7	0.037
Age (yrs)	43.4±8.2	44.1±11.0	0.580
Body mass index (kg/m <sup>2</sup> )	29.9±7.8	27.6±5.6	0.008
Total cholesterol (mmol/l)	4.76±1.17	5.50±1.22	<0.001
HDL-cholesterol (mmol/l)	1.15±0.36	1.20±0.41	0.339
Total cholesterol/HDL-cholesterol ratio	4.51±1.86	4.98±1.60	0.028
Systolic blood pressure (mmHg)	134.4±19.6	126.7±13.5	<0.001
Diastolic blood pressure (mmHg)	88.4±12.6	83.1±9.6	<0.001
Hemoglobin A1c (%)	6.05±1.12	5.48±0.41	<0.001
Creatinine (µmol/l)	77.6±13.5	74.2±15.3	0.069
Estimated GFR (ml/min/1.73m <sup>2</sup> )	114.7±29.3	112.0±27.2	0.453
Ever smoker (%)	25.5	21.2	0.419
Gamma glutamyl transferase (U/L)	64.6±75.8	26.9±36.2	<0.001
Physical activity index	1.33±0.57	1.62±0.70	0.001
Antihypertensive drugs (%)	12.7	4.2	0.010
Patient Health Questionnaire-9 (scores)			
Total depressive symptoms	9.40±5.60	5.70±4.73	<0.001
Moderate depression (cut-off ≥10) (%)	46.1	17.6	<0.001
General Health Questionnaire-28 (scores)			
Total psychological distress	8.50±6.71	3.79±4.80	<0.001
Cases (cut-off >4) (%)	64.7	33.3	<0.001

Data are given as means±SD or percentage values. P-value refers to group differences.

GFR, glomerular filtration rate; HDL, high-density lipoprotein

**Table 2. Coagulation measurements per ethnic group at rest and after mental stress**

Condition	Adj.	von Willebrand factor				Fibrinogen				D-dimer			
		Africans	Caucasians	ES	P	Africans	Caucasians	ES	P	Africans	Caucasians	ES	P
Rest	–	93.2± 28.4%	63.6±16.5%	0.330	<0.001	3.35±0.82g/l	3.07±0.57g/l	0.030	0.004	354±340ng/ml	298±338ng/ml	0.015	0.044
	+	94.2±2.4%	62.9±1.8%	0.291	<0.001	3.33±0.06g/l	3.09±0.05g/l	0.029	0.006	379±37ng/ml	283±28ng/ml	0.030	0.005
Poststress	–	101.8±34.1%	98.7±43.4%	0.010	0.101	2.95±0.71g/l	2.84±0.65g/l	0.006	0.208	398±515ng/ml	363±496ng/ml	0.004	0.302
	+	97.5±4.4%	101.4±3.3%	<0.001	0.817	2.91±0.07g/l	2.86±0.05g/l	0.001	0.593	426±56ng/ml	345±42ng/ml	0.006	0.217
Reactivity	–	18.1±53.6%	60.1±70.2%	0.110	<0.001	–11.2±11.6%	–7.4±12.6%	0.022	0.016	64.5±241.9%	74.5±319.9%	0.002	0.499
	+	10.2±7.0%	65.0±5.2%	0.140	<0.001	–11.7±1.4%	–7.2±1.0%	0.023	0.016	48.1±32.6%	84.7±24.6%	0.005	0.270

Data are given as means±SD for unadjusted values (–) and as means±SEM for adjusted values (+) with P-values for group comparisons.

Adjustment was made for gender, age, body mass index, total cholesterol/high-density cholesterol ratio, screening systolic blood pressure, hemoglobin A1c, estimated glomerular filtration rate, smoking, gamma glutamyl transferase, physical activity, and antihypertensive drugs for resting values and additionally for perceived stress with the Stroop test for post-stress and reactivity values. Reactivity measures are expressed as percentage change of poststress values from resting values.

Adj., adjustment; ES, effect sizes (partial eta-squared).

## Extra Table

<b>What is known about this topic?</b>
<ul style="list-style-type: none"><li>• Acute mental stress induces a hypercoagulable state that may contribute to atherothrombotic diseases.</li><li>• Depressive symptoms are associated with the procoagulant stress response.</li><li>• There are ethnic differences in coagulation between <b>Africans</b> and <b>Caucasians</b>.</li></ul>
<b>What this paper adds</b>
<ul style="list-style-type: none"><li>• Africans show lower procoagulant reactivity in response to acute laboratory mental stress than Caucasians</li><li>• Explanation might be higher resting levels of coagulation factors in Africans than Caucasians and ethnic differences in the vascular stress response.</li><li>• Depressive symptoms are associated with greater stress reactivity of von Willebrand factor in Africans relative to Caucasians.</li></ul>

**Figure 1: Coagulation reactivity related to ethnicity and depression**

