NT-proBNP and Potential Vascular Calcification in Black and Caucasian African Men: The SAfrEIC Study

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Objective: The N-terminal prohormone B-type natriuretic peptide (NT-proBNP) is a reliable marker of cardiac strain. In hypertensive heart disease, NT-proBNP levels increase and may lose its protective function. Simultaneously, the vasculature is also subject to hemodynamic stress, resulting in vascular matrix remodeling and stiffening which contribute to further cardiac alterations. Alkaline phosphatase (ALP) is a marker of osteoblast activity and is involved in vascular calcification. We explored the link between NT-proBNP and ALP in Black and Caucasian African men.

Design and main outcome measures: This study included 128 Black (mean age, 41.1 years) and 118 Caucasian (mean age, 36.4 years) men. Conventional measurements were acquired along with serum NT-proBNP and ALP.

Results: NT-proBNP correlated positively with ALP ($r=0.29$; $p<0.001$) in Black Africans, but inversely in Caucasians ($r=-0.20$; $p=0.024$). After minimal adjustment (age, body mass index, systolic blood pressure and arterial compliance), the positive significant correlation of NT-proBNP with ALP remained in Black men ($r=0.225$; $p=0.014$), whereas significance was lost in Caucasian men. Multiple regression analyses confirmed the independent association of NT-proBNP with ALP in Black men ($R^2=0.37$; $\beta=0.248$; $p=0.005$), as well as in younger Black men ($R^2=0.26$; $\beta=0.375$; $p<0.001$; $n=96$), with no significance in Caucasians.

Conclusions: NT-proBNP is independently and positively associated with ALP in Black African men. This was however not evident in Caucasian men. These results suggest that African men are susceptible to potential early vascular calcification and may develop increased cardiac afterload prematurely. (*Ethn Dis.* 2012;22[4]:398–403).

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Introduction

The increasing prevalence of hypertensive heart disease is a major concern in Black South Africans.[1,2] One of the contributing risk factors is the progression of arterial stiffness, which seems to be more prominent among Black Africans in comparison with Caucasians.[3] Black South Africans are also subjected to early vascular alterations,[3] making this population group more vulnerable for eminent stiffening of blood vessels and resultant cardiac damage. The subsequent increased hemodynamic afterload on the heart is reliably assessed by the N-terminal prohormone B-type natriuretic peptide (NT-proBNP).[4,5] An elevated level of NT-proBNP indicates left ventricular dysfunction and heart failure.[4–6] NT-proBNP levels are also elevated in the presence of lower-extremity arterial calcification.[7]

Alkaline phosphatase (ALP) is a marker of osteoblast activity[8] and elevated expression of this enzyme may initiate spontaneous calcification in blood vessels.[9] Vascular calcification is more prominent in disease states such as kidney disease, diabetes and hypertension. Under these conditions, vascular smooth muscle cells gain osteoblast-like characteristics and express augmented production of ALP.[10,11] As a result, arterial stiffness increases, which, in turn, augments myocardial afterload and stress.[7] This is supported by NT-proBNP levels, which are elevated in high-risk patients with lower-extremity artery calcification and stiffness.[7] The majority of data on vascular calcification in terms of ALP is available only from animal studies[12] and high-risk Caucasian populations,[13] or African Americans.[14] Little is known about ALP and cardiac function in the African population of South Africa.

Therefore, since the prevalence of arterial stiffness is increasing along with hypertensive heart disease in Black Africans, our study aimed to explore the association between NT-proBNP and a marker of osteoblastic activity in the cardiovascular system in Black and Caucasian African men.

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Methods

Study Population

We selected 186 Black and 160 Caucasian men from a larger South African investigation on the role of sex, age and ethnicity on insulin sensitivity and cardiovascular function (SAfrEIC) study from the North West province of South Africa. We excluded men infected with HIV ($n=55$), those with missing data of relevant variables ($n=4$) and those using medication ($n=41$) for comorbidities, such as diabetes and hypertension. A total of 128 Black and 118 Caucasian men were included in our study. The ethics review board of

the North-West University approved the SAfrEIC study and the protocol conformed to the revised ethical guidelines of the Declaration of Helsinki (revised in 2008) for investigation of human participants.

Clinical Procedures

During March to July, 2007, approximately 20 participants visited the Metabolic Unit facility daily for metabolic assays at the Potchefstroom campus of the North-West University. Each participant was informed about all procedures included in the protocol and gave written informed consent to participate. Basic health and demographic questionnaires were completed during the course of the morning. Each participant received an informative description regarding the results of the health assessment. In the event that abnormalities were identified (eg hypertension or diabetes), the participant was advised to visit their physician.

Cardiovascular Measurements

The OMRON HEM-757 apparatus (Omron, Kyoto, Japan) was used to determine systolic (SBP) and diastolic blood pressure (DBP) with the cuff on the left upper arm in the sitting position. The first blood pressure recording was taken after an initial 10-minute rest and the second after five minutes. Pulse pressure was subsequently calculated by subtracting the DBP from SBP. Participants with a SBP $\geq$140 mm Hg and/or DBP $\geq$90 mm Hg were considered hypertensive.[15] Windkessel arterial compliance in diastole and heart rate were determined with the Finometer apparatus (FMS, Finapres Measurement Systems, Amsterdam, the Netherlands).[16,17]

Anthropometric Measurements

Body height was measured to the nearest 0.1 cm by using the Invicta Stadiometer (Invicta Plastics Ltd. 1465, London, UK) and body weight to the nearest 0.1 kg (Precision Health Scale, A & D Company, Japan), according to standard procedures. Subsequently, the body mass index (BMI) was calculated for each participant as weight (kg) divided by height (m) squared.

Biochemical Measurements

Participants were requested to fast for a minimum of eight hours. In serum, fasting lipids, glucose, γ-glutamyl transferase, ALP, albumin, creatinine and high sensitivity C-reactive protein (CRP) were determined with the Konelab 20i autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland). The Cockcroft–Gault formula was used to determine estimated creatinine clearance.[18] We determined serum cotinine with the IMMULITE 2000 nicotine metabolite assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA) and insulin (ST AIA-PACK IRI, Cat. No. 025260) with a two-site immunoenzymometric assay on the TOSOH AIA System analyzer (San Francisco, CA, USA). The Elecsys proBNP sandwich immunoassay was used on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) to determine serum NT-proBNP.

Statistical Analyses

Statistica software version 10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for database management and statistical analyses. We established the normal distribution of all variables prior to any further statistical analyses. Variables that deviated from normality (NT-proBNP, CRP, insulin, glucose, cotinine and γ-glutamyl transferase) were logarithmically transformed. The association of NT-proBNP with ALP was tested for interaction with ethnicity by introducing the appropriate interaction terms in multiple regression analysis. T-tests and chi-square tests were performed to compare means and proportions between groups, respectively. We investigated associations between NT-proBNP and ALP using single and partial correlations. Forward stepwise multiple regression analyses were plotted to illustrate independent associations between NT-proBNP and ALP in both groups. Several covariates were considered for entry into the model including age, BMI, SBP, heart rate, arterial compliance, albumin, estimated creatinine clearance, glucose, CRP, γ-glutamyl transferase, cotinine and the total cholesterol to high density lipoprotein cholesterol ratio (TC:HDLC). Of these variables, heart rate, serum albumin, estimated creatinine clearance, glucose and cotinine did not enter the model. Probability values of $\leq$.05 were considered statistically significant.

Results

The descriptive characteristics of the Black and Caucasian African men are presented in Table 1. Black men had significantly lower BMI, triglycerides, HOMA index and TC:HDLC ratio compared to Caucasian men. Despite the favorable metabolic profile in Black Africans, they had significantly higher mean systolic and diastolic blood pressures and a higher prevalence of hypertension ($P<.0001$). Self-reported smoking was significantly higher in Black Africans ($P<.0001$), which was supported by the cotinine levels ($P<.0001$). Self-reported alcohol use did not differ between the two groups. The mean γ-glutamyl transferase level was higher in Black Africans compared to Caucasians ($P<.0001$). The mean ALP level was higher in Black men ($P<.0001$) than Caucasian men. ALP levels were also above the normal reference range of 30–120 U/L in Black men, whereas Caucasian men's mean ALP levels were within the normal physiological range. NT-proBNP levels were also significantly higher in Black African men compared to Caucasian men ($P<.0001$). Another comparative feature we considered was socioeconomic status. In this population, 88.5% of the Black men earned less than USD290

Table 1. Characteristics of Black and Caucasian African men

	Blacks *n*=128	Caucasians *n*=118	*P*
Age, years	41.1 ± 13.6	36.4 ± 11.7	<.01
Body mass index, kg/m^2	20.4 ± 4.1	27.8 ± 4.9	<.0001
Biochemical measurements			
NT-proBNP, pg/mL	24.4 (2.5–178.3)	8.4 (2.5–47.9)	<.0001
Alkaline phosphatase, U/L	126.5 ± 45.9	100.0 ± 24.1	<.0001
γ-Glutamyl transferase, U/L	78.7 (20.8–485.9)	35.3 (17.7–86.4)	<.0001
Serum albumin, g/L	45.0 ± 7.2	48.7 ± 8.4	<.001
Serum creatinine, μmol/L	64.3 ± 10.0	71.3 ± 10.5	<.0001
Creatinine clearance, mL/min	1.25 ± 0.38	1.85 ± 0.42	<.0001
C-reactive protein, mg/L	1.55 (0.01–22.9)	1.03 (0.01–9.23)	0.104
Insulin, μU/mL	5.1 ± 5.2	10.1 ± 8.9	<.0001
Serum glucose, mmol/L	5.0 (4.0–6.4)	5.6 (4.6–7.2)	<.0001
HOMA-IR	1.22 ± 1.37	2.63 ± 2.67	<.0001
Triglycerides, mmol/L	1.11 ± 0.58	1.56 ± 0.95	<.0001
TC:HDLC, mmol/L	2.93 ± 1.10	5.24 ± 2.10	<.0001
Cotinine, ng/mL	126.1 (9.0–500.0)	19.3 (9.0–371.0)	<.0001
Cardiovascular measurements			
Systolic blood pressure, mmHg	130.4 ± 20.6	121.8 ± 11.0	<.0001
Diastolic blood pressure, mmHg	84.4 ± 13.8	77.9 ± 8.1	<.0001
Arterial compliance, mL/mmHg	1.68 ± 0.50	2.53 ± 0.50	<.0001
Hypertension status, *n* (%)	50 (39.1)	9 (7.6)	<.0001
Lifestyle			
Current smoking, *n* (%)	97 (75.8)	26 (22.0)	<.0001
Alcohol use, *n* (%)	106 (83.0)	89 (75.4)	0.13
Income, *n* (% above USD 230)	15 (11.5)	108 (96.4)	<.0001

Values are arithmetic mean ± SD, geometric mean (5th and 95th percentile interval) or number of participants.
Abbreviations: HOMA-IR – Homeostatic model assessment insulin resistance score; TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio; USD – United States Dollar.

per month opposed to 76.8% of Caucasian men earning more than USD730 per month (P<.0001). However, these men (both Black and Caucasian) came from the same urbanized environment.

In univariate analysis, we plotted the NT-proBNP by ALP levels for Black and Caucasian African men (Figure 1). We found a strong positive correlation between NT-proBNP and ALP in Black men, but a significant inverse correlation was observed in Caucasian men. After partially adjusting for age, BMI, SBP and arterial compliance (Table 2), the positive association between NT-proBNP and ALP remained in Black men while the negative association in the Caucasians became non-significant.

In multiple regression analysis, we confirmed the positive relationship between NT-proBNP and ALP in Black men (R^2=0.37; β=0.248; P=.005) (Figure 2). However, in order to address the striking difference observed in socioeconomic status between these two male populations, we added income status to the list of independent variables in the multiple adjusted analyses. Income status did not influence the association of NT-proBNP with ALP in either Black (R^2=0.38; β=0.242; P=.006) or Caucasian (R^2=0.18; β=−0.127; P=.16) African men.

In sensitivity analysis to unravel this association and the possibility of early vascular aging, we repeated the analyses in young men. We therefore arbitrarily excluded all men aged 55 years and older. Within the younger Black group, the association between NT-proBNP and ALP remained significant (R^2=0.26; β=0.375; P=.0008; n=96) and again we observed no association in the Caucasian men (R^2=0.11; β=−0.144; P=0.14; n=106). Lastly, even after excluding all hypertensives the results for Black men (R^2=0.34; β=0.386; P=0.004; n=70) and Caucasian men (R^2=0.10; β=−0.167; P=0.11; n=98) were again confirmed.

Our study showed that both NT-proBNP and ALP were higher in Black compared to Caucasian men and were independently associated, even after additionally adjusting for socioeconomic status.

Discussion

We explored the possible link between NT-proBNP as a marker of cardiac strain and ALP as a marker of osteoblastic activity in Black and Caucasian African men. Our study showed that both NT-proBNP and ALP were higher in Black compared to Caucasian men and were independently associated, even after additionally adjusting for socioeconomic status. This independent relationship between NT-proBNP and ALP was consistent with the total Black population as well as in a younger normotensive group. It is noteworthy to mention that this association was completely absent in Caucasian men. Our results suggest that possible vascular calcification and associated cardiac load occurs relatively early in the life of Black African men, even under normotensive conditions. This may contribute to the high cardiovascular risk known to this group.

Most of the health profile variables (such as BMI, CRP, HOMA index and TC:HDLC) were within their reference ranges.[19] However, the positive association of NT-proBNP with ALP together

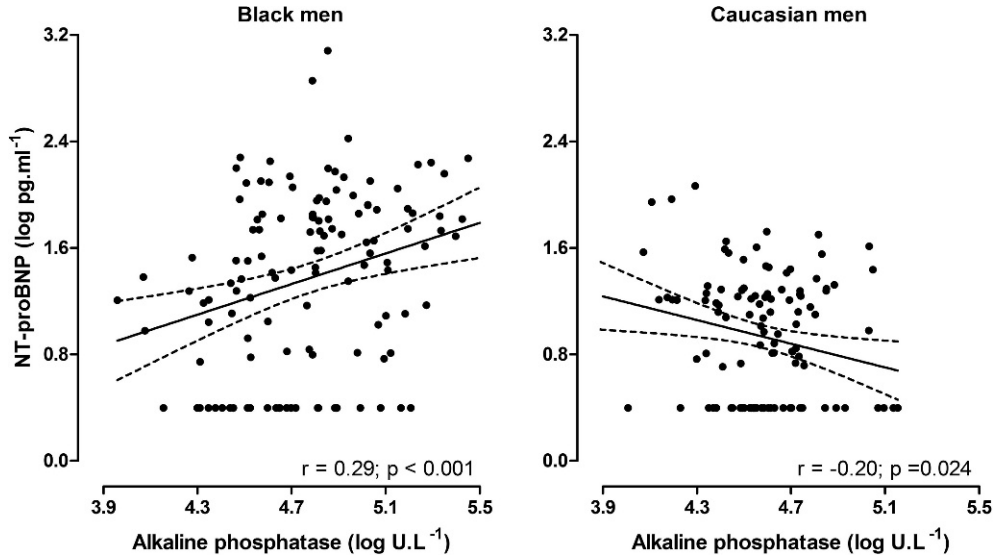

Fig 1. Unadjusted correlations between NT-proBNP and ALP in both the Black and Caucasian African men

with higher SBP in Black men may suggest that the blood vessels are possibly subjected to adverse ectopic osteoblastic activity. This may lead to arterial stiffening due to ectopic calcification, which can escalate into cardiac impairment due to increased afterload on the heart.[20]

The same association of NT-proBNP with ALP was also confirmed in younger normotensive Black men. This result once again underlines the potential cardiovascular risk that is present at a relatively young age, independent of a hypertensive state. Studies indicated that NT-proBNP is directly associated with calcified aortic valves or aortic stenosis,[6,21,22] which merely signifies that NT-proBNP is a reliable biomarker of cardiovascular damage. However, vascular calcification is a complex process of multifactorial origin of which little is known. Beddhu et al and others[14,20,23] concluded that ALP is independently associated with increased mortality in patients subjected to chronic kidney disease. Although kidney function was not extensively measured in our study, the mean serum creatinine levels and estimated creatinine clearance were in the normal physiological range of the groups studied. Vascular calcification contributes to chronic kidney disease, but also plays a role in other conditions including diabetes, hypertension and pressure-related hypertrophy of cardiac tissue.[24,25] However, we found the association between NT-proBNP and ALP in sub-groups without these conditions.

Mineralization *in vivo* is limited to tissues that express both type I collagen and ALP.[26] Type I collagen is present in normal artery walls, but not ALP. Both these components confine as mineral deposits in calcific atherosclerosis[27] and are produced in vascular cells *in vitro*.[28] Therefore, co-expression of these proteins induces ectopic calcification.[29] This ectopic mineralization refers to calcification not taking place in normal bone, but adverse mineralization in blood vessel walls. Membrane-bound ALP normally reduces the expression of inhibitor pyrophosphates and contributes to hydroxyapatite formation by producing free phosphates in bone.[30] However, during this mineralization process matrix vesicles are released, which are rich in ALP and other phospholipids.[31,32] Ectopic calcification is due to increasing uptake of phosphates

Table 2. Adjusted correlations of NT-proBNP with ALP and other biochemical measures

	Blacks *n*=128		Caucasians *n*=118	
	NT-proBNP (pg.ml^{-1})			
	r	*P*	*r*	*P*
Alkaline phosphatase, U/L	**0.23**	**.014**	−0.15	0.14
γ-Glutamyl transferase, U/L	−0.09	.32	.05;	.61
Serum albumin, g/dL	**−0.24**	**.010**	.05	.59
C-reactive protein, mg/L	**0.24**	**.009**	−0.01	.91
Creatinine clearance, mL/min	08	.40	−0.01	.91
TC:HDLC, mmol/L	.01	.93	−0.05	.60
Serum glucose, mmol/L	−0.06	.49	0.03	.80

Adjustments applied for: age, body mass index, systolic blood pressure and arterial compliance.
TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio.

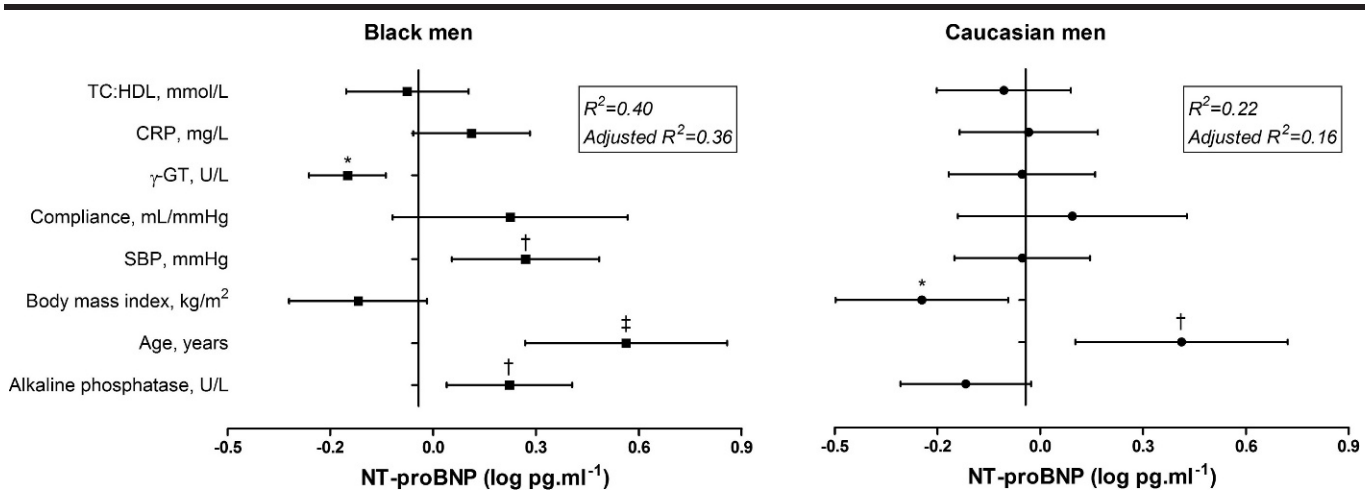

Fig 2. Multiple regression analyses of NT-proBNP with ALP in both Black and Caucasian African men. Values are indicated as standardized β (± 95% confidence interval). CRP – C-reactive protein; γ-GT – gamma glutamyl transferase; SBP –systolic blood pressure; TC:HDLC – total cholesterol and high density lipoprotein cholesterol ratio. * *P* ≤.05; † *P* ≤.01; ‡ *P* ≤.001

into cells by the type III sodium-phosphate co-transporter and up-regulation of type 1 pituitary-specific transcription factor by means of a positive feedback system.[33] This will in turn support osteoblast conversion as well as possible vascular calcification.[11] Calcification of the tunica media is normally the result of adverse alterations in the elastin network of arteries, because elastin acts as a nucleation scaffold for hydroxyapatite deposition.[34] In the process of vascular calcification, the end products of elastin degradation initiate the conversion of smooth muscle cells and dermal fibroblasts into osteoblast-like cells.[35,36] These newly formed cells may be accountable for pathological calcification of blood vessels, increasing the risk of cardiovascular disease and mortality as seen in the Black African men.[37,38]

Although the aforementioned mechanism is merely speculative, the association of NT-proBNP with ALP in our cohort corroborates the possibility of early vascular calcification. Based on our finding, Black men are at risk of developing cardiovascular-related calcification, even in a young normotensive state. Whether this association is merely a reflection of the environmental or classic risk factors contributing to hypertensive heart disease, or whether this population is predisposed to early onset vascular changes remains unanswered. However, the association between a marker of cardiac strain and a marker of calcification in this population may require urgent intervention to curb the ever-increasing trend of cardiovascular morbidity and mortality of Black South Africans.

The findings of this study need to be interpreted within the context of its limitations and strengths. As mentioned previously, this was a cross-sectional study and therefore cause and effect cannot be determined. Furthermore, data on end-organ damage (carotid intima-media thickness, left ventricular hypertrophy, echocardiographic imaging of calcified valvular or vascular sclerosis) were not available in this study. This study was well designed and performed under strong controlled conditions. To our knowledge this was the first study that focused on the association between NT-proBNP and ALP in Black and Caucasian men from South Africa.

In conclusion, we observed a persistent association between NT-proBNP and a marker of calcification in relatively young and normotensive Black African men. This may suggest that these men are subjected to early onset cardiac load, possibly due to enhanced vascular calcification which may escalate in cardiac damage. These findings are clinically relevant and need confirmation in larger prospective studies.

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