Abstract

Background: We explored the association of N-terminal pro-brain natriuretic peptide (NT-proBNP) with metabolic traits in a bi-ethnic African–Caucasian cohort.

Methods: Baseline examinations of the Sympathetic activity and Ambulatory Blood Pressure in African (SABPA) prospective cohort study were performed between 2008 and 2009, and re-examination after a three-year follow up in South African teachers (black African, \(n = 194\); Caucasian, \(n = 203\)).

Results: Each one standard deviation increment of NT-proBNP was significantly inversely associated with body mass index (\(\beta = -1.01\)), glycated haemoglobin (\(\beta = -0.14\%\)), waist circumference (\(\beta = -1.82\)), HOMA-IR (\(\beta = -0.47\)), insulin (\(\beta = -1.66\)) and triglyceride levels (\(\beta = -0.04\)). Each one standard deviation increment of NT-proBNP was also associated with reduced odds of incident diabetes, and subjects within the highest quartile of NT-proBNP were at lowest risk (OR: 0.24; 95% CI: 0.06–0.96; \(p = 0.041\)).

Conclusions: In the SABPA cohort, Africans and Caucasians had similar NT-proBNP levels; however, the associations for Africans were stronger. Those findings suggest that BNP may affect the propensity for metabolic disturbances differently in Africans and Caucasians.

Keywords: Africans, bi-ethnic, Caucasians, metabolic, NT-proBNP

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Natriuretic peptides (NPs) are widely recognised as potent vasoactive hormones that play a key role in volume loading and cardiac remodelling.\(^1\) Their gene transcription and secretion increase as a result of cardiac stress, for example, stretching of the cardiac atria or due to ventricular pressure or volume load.\(^2\) Consequently, BNP levels are elevated in conditions such as heart failure, and BNP are also today extensively used as heart failure biomarkers in clinical routine.\(^3\) In addition to cardiac stress, there is also evidence that neurohormonal factors such as angiotensin II, thyroid hormones, inflammatory cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor \(\alpha\) (TNF-\(\alpha\)) affect and interact with NP secretion.\(^4,5\)

The last two decades of research has also identified NPs as hormones with significant protective metabolic actions, and proposed that genetically predisposed low levels of NPs, the so called ‘natriuretic handicap’, is a cause, rather than a consequence, of metabolic disorders such as obesity, insulin resistance and diabetes.\(^6-9\) Although NP secretion is greatly influenced by volume load on the heart, there is evidence that as much as 40% of the variation in BNP levels could be explained by genetic factors.\(^10,11\) Other studies demonstrate that African Americans have lower NT-proBNP levels than Caucasians.\(^12-14\) However, higher levels independent of diabetes status were observed in an African cohort compared to Caucasian counterparts.\(^15\)

In this study, we aimed to explore the associations of NT-proBNP with obesity, hypertriglyceridaemia, the metabolic syndrome, insulin resistance and diabetes in an African versus a Caucasian gender-matched cohort from South Africa.
Methods
This study is part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study of 409 African and Caucasian school teachers (aged 20–62 years), working in the North-West Province, South-Africa. The examination was conducted in 2008 and 2009 and repeated in 2011 and 2012 (mean follow-up time three years), with an 87.8% successful follow-up rate (Fig. 1).

The study sample was selected to ensure homogeneity regarding socio-economic status and working environment. Exclusion criteria for the SABPA study were: the use of alpha- or beta-blockers, use of psychotropic substances, tympanum temperature > 37.5°C and/or being vaccinated or donating blood within three months prior to participation. Additionally, we excluded participants with missing values of NT-proBNP (n = 12), resulting in 397 participants (194 Africans and 203 Caucasians).

The SABPA study conforms to the principles outlined in the Declaration of Helsinki (revised 2004) (World Medical Association General Assembly 2004) and abided by the institutional guidelines. It was approved by the ethics review board of the North West University, South Africa (0003607S6). All participants provided written informed consent.

The participants were in a semi-recumbent position for at least 30 minutes prior to blood pressure (BP) measurements on the non-dominant arm between 06:15 and 09:00 by a registered nurse and doctor. They used a calibrated sphygmomanometer (Riester CE 0124®) and a 1.3M™ Littman® II SE stethoscope 2205. Two duplicate measures were taken, with a three- to five-minute resting period between each, the second of which was used for statistical analyses.

Body height (stature), weight and waist circumference were measured with calibrated instruments (Invicta Stadiometer, IP 1465, London, UK; Precision Health Scale, A&D Co, Tokyo, Japan; Holtain unstretchable flexible 7-mm-wide metal tape, Crosswell, Wales) while participants were in their underwear. All measurements were done in triplicate by registered anthropometrists according to standard procedures.

A registered nurse obtained fasting blood samples with a sterile winged infusion set from the ante-brachial vein. EDTA whole blood and serum were stored at –80°C. Venous samples for fasting blood glucose were collected in sodium fluoride tubes. All analyses were performed on samples drawn after an overnight fast.

Plasma and serum samples were analysed using two sequential multiple analysers (Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800, Beckman and Coulter®, Germany), doing enzyme-linked immunosorbent assays (Quantikine enzyme-linked immunosorbent assay, R&D Systems, Minneapolis, MN, USA) for serum total and high-density lipoprotein (HDL) cholesterol, serum triglyceride (TG), whole blood glycated haemoglobin (HbA1c), fasting plasma glucose (FPG) and serum insulin levels. The intra- and inter-coefficients of variation for all assays were below 10%.

NT-proBNP serum samples were obtained at both baseline and follow-up examinations and frozen at –80°C until analysis in one batch in 2015 (ECLIA method; Roche Diagnostics, Basel, Switzerland) using Cobas e411 automated platform (inter-batch variability: 4.6%; intra-batch variability: 4.2%).

Prevalent impaired glucose tolerance (IGT) at the baseline examination was defined as FPG > 5.6 mmol/l or HbA1c > 5.7%. The metabolic syndrome (MetS) was defined as any three of the following markers exceeding cut-off points: central obesity (waist ≥ 102 cm in men, ≥ 88 cm in women); raised triglycerides (> 150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality); reduced HDL cholesterol level (< 40 mg/dl (1.03 mmol/l) in men, < 50 mg/dl (1.29 mmol/l) in women or specific treatment for this lipid abnormality); raised BP (systolic BP > 130 or diastolic BP > 85 mmHg, or treatment of previously diagnosed hypertension); raised FPG (> 100 mg/dl (5.6 mmol/l), or previously diagnosed type 2 diabetes mellitus).

Insulin resistance was defined as the upper quartile of homeostatic model assessment of insulin resistance (HOMAIR), which was calculated according to: (glucose x insulin)/22.5. Prevalent and incident diabetes were defined as clinical diagnosis of diabetes and/or use of anti-diabetic medication. History of kidney disease and cardiovascular disease (defined as diseases affecting the heart or blood vessels) were assessed through questionnaires. Hypertension was defined as systolic BP > 140 mmHg or diastolic BP > 90 mmHg or use of antihypertensive medication.

Overweight was defined according to ethnic cut-off points as waist circumference (WC) ≥ 90 cm in African men, and ≥ 88 cm in African women, together with WC ≥ 94 cm in Caucasian men, and WC ≥ 80 cm in Caucasian women.

Statistical analysis
Variables that were skewed (NT-proBNP, TG and FPG) were log-transformed before analysis. Groups were compared using one-way ANOVA tests. We used linear regression analysis adjusted for age and gender to examine the associations per one
standard deviation (SD) increment of log-transformed values of NT-proBNP at baseline with weight, body mass index (BMI), waist circumference, HbA1c, FPG, insulin, HOMA-IR and TG values at baseline and re-examination. In order to get a true perspective of the effect of changes of NT-proBNP in the linear and logistic regression analysis, outcomes were related to one standard deviation of change of the ln-transformed values of NT-proBNP.

Logistic regression models were used to calculate: (1) odds ratios (OR) for prevalent overweight, IGT, hypertiglyceridaemia, the MetS and insulin resistance at baseline examination adjusted for age and gender, and (2) OR for incident diabetes [patients with diabetes (n = 37) at baseline examination excluded] per one SD increment of log-transformed values of NT-proBNP adjusted for age, gender, waist circumference and follow-up time to re-examination. NT-proBNP levels were divided into quartiles to explore the relationship between NT-proBNP and prevalent insulin resistance at baseline (adjusted for age and gender) as well as incident diabetes (adjusted for age, gender, waist circumference and follow-up time to re-examination). All analyses were performed using SPSS Windows version 23.0 and a two-tailed p-value < 0.05 was considered statistically significant.

**Results**

Baseline characteristics of the differences between Caucasians and Africans are listed in Table 1. Africans had overall significantly worse metabolic status at baseline compared to Caucasians, with higher systolic and diastolic BP, BMI, and blood glucose, insulin, HOMA-IR and TG levels, and more prevalent diabetes cases. NT-proBNP levels were significantly higher in women compared to men at baseline examination (Table 1). No significant differences between Caucasians and Africans with regard to age, gender or NT-proBNP levels at baseline examination (Table 1). No significant differences between black and white women were observed (p = 0.861). NT-proBNP and cross-sectional association with continuous metabolic parameters: baseline characteristics of the study samples are listed in Table 1. In cross-sectional linear regression analyses at baseline, each one SD increase in baseline values of NT-proBNP was significantly and inversely associated with reduced odds of prevalent overweight (Caucasians: OR: 0.79; 95% CI: 0.62–1.00; p = 0.045).
and Africans: OR: 0.72; 95% CI: 0.57–0.92; p = 0.009), IGT (glucose: OR: 0.77; 95% CI: 0.60–0.99; p = 0.040 and HbA1c: OR: 0.78; 95% CI: 0.62–0.99; p = 0.038), prevalent MetS (OR: 0.76; 95% CI: 0.60–0.96; p = 0.040), hypertriglyceridaemia (OR: 0.64; 95% CI: 0.47–0.87; p = 0.004) and insulin resistance (OR: 0.57; 95% CI: 0.43–0.76; p < 0.001) (Table 3).

The relative risk of insulin resistance at baseline decreased significantly across quartiles of baseline values of NT-proBNP. Compared with the lowest quartile of NT-proBNP, the OR (95% CI) for prevalent IR in subjects belonging to quartiles two, three and four was 0.83 (0.44–1.57), 0.30 (0.14–0.63) and 0.25 (0.11–0.56; p for linear trend < 0.0001), respectively (Table 4). Fig. 2 illustrates the distribution of baseline HOMA-IR among quartiles of NT-proBNP.

NT-proBNP and prospective association with diabetes: the relative risk of incident diabetes at re-examination decreased significantly across quartiles of baseline values of NT-proBNP. Compared with the lowest quartile of NT-proBNP, the OR (95% CI) for incident diabetes in subjects belonging to quartiles two, three and four was 0.54 (0.18–1.61), 0.43 (0.13–1.41) and 0.24 (0.06–0.96; p for linear trend = 0.041), respectively.

Bi-ethnic differences: in cross-sectional linear regression analyses at baseline of the African subjects, each one SD increase in baseline values of NT-proBNP was inversely associated with body weight (β = –3.52; p = 0.021), BMI (β = –1.25; p = 0.019), HbA1c (β = –0.22; p = 0.027), insulin (β = –1.76; p = 0.035) and TG (β = –0.06; p = 0.002), and borderline associated with FPG (β = –0.01; p = 0.062) (Table 5). In the cross-sectional linear regression analyses at baseline of the Caucasian subjects, each one SD increase in baseline values of NT-proBNP was significantly inversely associated only with insulin (β = –1.57; p = 0.015) and HOMA-IR (β = –0.52; p = 0.014) (Table 5).

In the African study participants, in cross-sectional age- and gender-adjusted analyses at baseline, each one SD increment of NT-proBNP was associated with reduced risk of prevalent IGT (HbA1c: OR: 0.64; 95% CI: 0.44–0.92; p = 0.015), hypertriglyceridaemia (OR: 0.61; 95% CI: 0.40–0.93, p = 0.022) and insulin resistance (OR: 0.52; 95% CI: 0.35–0.77; p = 0.001) (Table 6). In the Caucasian study participants, in cross-sectional age- and gender-adjusted analyses at baseline, each one SD increment of NT-proBNP was associated with reduced risk of prevalent IGT (glucose: OR: 0.62; 95% CI: 0.43–0.89; p = 0.009), the MetS (OR: 0.68; 95% CI: 0.49–0.96; p = 0.028) and insulin resistance (OR: 0.64; 95% CI: 0.42–0.99; p = 0.046) (Table 6).

**Discussion**

In the SABPA study, undertaken in a middle-aged, bi-ethnic cohort, we observed that NT-proBNP was inversely associated with metabolic risk factors such as increased waist circumference...
and BMI, hypertriglyceridaemia, hyperglycaemia and insulin resistance. Moreover, in a prospective analysis, NT-proBNP was inversely associated with incident diabetes, findings in line with previous notions on NPs’ involvement in protection against diabetes. Additionally, ethno-stratified analyses revealed that low NT-proBNP levels in Africans were associated with several metabolic conditions such as obesity, IGT, insulin resistance and hypertriglyceridaemia, whereas low NT-proBNP levels in Caucasians were associated with insulin resistance only.

The last two decades of research have demonstrated that NPs play an important role in the control of energy usage, and have lipolytic properties. Further, atrial NPs exhibit possible favourable effects on chronic inflammation. Cross-sectional studies have demonstrated that NP levels are reduced in subjects with obesity, insulin resistance and type 2 diabetes, conditions that are more common in black Africans. Reduced NP response is also associated with the activation of the renin-angiotensin system in experimental studies, an association that could explain the inverse association of NPs and the MetS/insulin resistance. Moreover, several studies have prospectively shown that low levels of NPs are associated with insulin resistance and diabetes.

NP levels were shown to be higher in women and increased with age, therefore all our analyses were age and gender adjusted. Furthermore, a higher variability in NT-proBNP is seen in African Americans than in Caucasians. As for ethno-stratified analyses, we found cross-sectional associations of low NT-proBNP levels and higher BMI, Hba1c, insulin and TG levels in Africans, whereas in Caucasians, low NT-proBNP levels were associated with insulin resistance and higher insulin levels only. These findings indicate that BNP may affect the propensity for metabolic disturbances differently in Africans and Caucasians, and may play a role in the cause of the higher rates of obesity, insulin resistance, hypertension and diabetes as seen in black Africans. There is evidence that African Americans but not black Africans have lower NT-proBNP levels than Caucasians, and low BNP levels are associated with higher LDL and TG levels.

Although metabolic risk conditions are more common in blacks, the prevalence of the MetS in black American children and adults is likely underestimated due to the notion that hypertriglyceridaemia, one of the characteristics of the MetS, is observed less frequently in blacks than in whites. Our findings on associations of low NT-proBNP levels and hypertriglyceridaemia in Africans but not Caucasians are therefore somewhat contradictory. The differences might be explained in part by higher intake of alcohol in the Africans in general.

It was shown that subjects with a combination of obesity, diabetes and alcohol excess are prone to develop extremely high TG values. Both the Dallas Heart study and the ARIC study demonstrated that NT-proBNP levels were lower in African Americans than in Caucasians. In our study, no significant differences in NT-proBNP levels between Africans and Caucasians were observed, although there was a trend for Africans to have lower NT-proBNP levels (29.9 ng/l) compared with Caucasians (34.1 ng/l). However, NT-proBNP concentration is elevated in hypertension, and a much larger proportion of Africans was hypertensive (n = 116; 59.8%) compared to Caucasians (n = 63; 31.0%), which might have influenced (elevated) NT-proBNP levels in the African population.

Additionally, conditions other than cardiac structural and functional changes influence NT-proBNP levels. Renal dysfunction is known to elevate NT-proBNP levels due to renal clearance of the prohormone. Nonetheless, the proportion of subjects with renal disease in both the Africans and Caucasians was low (2.1 and 2.5%, respectively) and did not differ significantly between the ethnicities. However, prevalence of both cardiovascular and renal disease was assessed through questionnaires, which might be negated by the reporting bias of the participants. It remains to be explored whether the higher diabetes rates in blacks might be, at least partially, explained by genetically predisposed differences in NP levels.

Assessment of NT-proBNP is used in clinical routine to identify subjects with heart failure. There is a need to evaluate, and possibly implement, enforced prevention strategies for early identification of subjects with the MetS and increased risk of diabetes development. This type of preventative strategy that could have a great impact on economic aspects of healthcare might include screening for subjects in the lowest quartiles of NT-proBNP. Together with other preventable risk factors, such as a sedentary lifestyle, NT-proBNP deficiency might help to identify individuals at highest risk of the MetS and diabetes development, and focus on prevention efforts.

**Study limitations**

The data in the SABPA study were collected at a single regional centre and the subjects were matched with regard to age, gender, socio-economic status and ethnicity, which limits the applicability to other populations. Also, as this was a cross-sectional study, it shares the usual limitations of causality and control, as seen for all cross-sectional studies. We had no data on prevalent heart failure, which might have affected the outcome of the analyses.

Furthermore, NPs are unstable hormones that undergo a rapid degradation in plasma. For this reason, immune- assays that target the more stable N-terminal fragments of the prohormones have been developed, and the N-terminal fragments serve as surrogate markers of the biologically active peptides. Nevertheless, one must bear in mind that the measurements of the N-terminal fragments do not necessarily reflect actual levels of the biologically active, mature BNP.

Our samples were stored at −80°C from the baseline examination in 2008–2009 until analysis in 2015, which could be a limitation to our study, considering storage might have affected stability and degradation of NT-proBNP.

**Conclusions**

In a bi-ethnic cohort, NT-proBNP in the high-normal range was associated with a lower prevalence of metabolic risk factors such as high BMI, increased waist circumference, IGT, high insulin levels and hypertriglyceridaemia, with strongest associations for Africans in spite of similar NT-proBNP concentrations. This indicates that BNP may affect the propensity for metabolic disturbances differently in Africans and Caucasians.

The data that support the findings of this study are available from North-West University, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.
Data are however available from the authors upon reasonable request and with the permission of North-West University.

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References


