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Title: Chronic defensiveness and neuroendocrine dysfunction reflect a novel cardiac troponin T cut point: the SABPA study

***Running head**: defensiveness; depression*; *heart-rate-variability; catecholamine, cardiac Troponin T*

Leoné Malan,[1] Mark Hamer,[1,2] Roland von Känel,[1,3], Gavin W. Lambert,[4,5] Rhena Delport,[6] Hendrik S. Steyn,[7] Nicolaas T Malan.[1]

[1]Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[2]National Centre for Sport and Exercise Medicine, Loughborough University, Leicestershire, United Kingdom
[3]Department of Neurology, Inselspital, Bern University Hospital, and University of Bern Switzerland
[4]Iverson Health Innovation Research Institute, Swinburne University of Technology, Hawthorn
[5]Baker Heart & Diabetes Institute, Melbourne, Australia
[6]Department of Chemical Pathology, University of Pretoria, South Africa
[7]Statistical Consultation Services; North-West University; Potchefstroom; South Africa

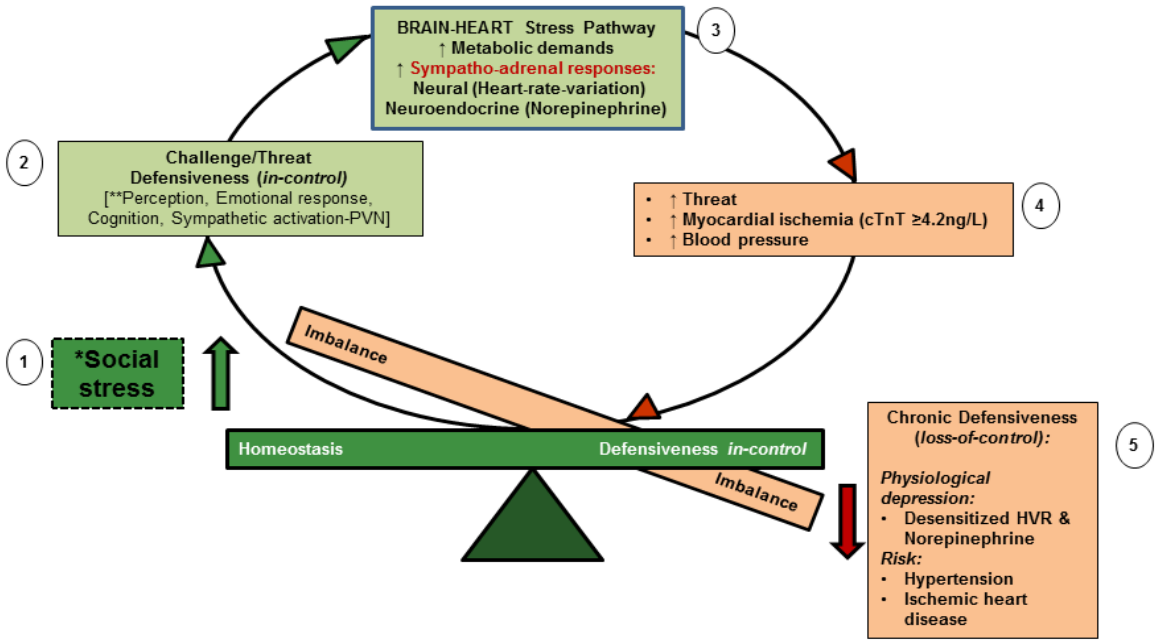
Corresponding author: Prof Leoné Malan (RN, PhD)

Hypertension in Africa Research Team (HART), Faculty of Health Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa

Tel +27 18 2992438; Fax +27 18 2991053; Email: Leone.Malan@nwu.ac.za

Highlights

- Central neural control systems exemplified a brain-heart stress pathway
- Sympatho-adrenal responses are activated as an innate defense coping mechanism
- Desensitization of sympatho-adrenal responses occurred with initial neural- (HRV) followed by neuroendocrine dysfunction (norepinephrine:creatinine) in relation to elevated cardiac Troponin T
- Chronic defensiveness may drive desensitization or a *physiological depression*, reflecting ischemic heart disease risk at a 4.2 ng/L cTnT cut-point



Graphical abstract: Brain-Heart stress pathway exemplifying chronic defensiveness and desensitized sympatho-adrenal responses in relation to cardiac injury. Where: Color coding: Green=Defensiveness in-control/homeostasis; Orange=Defensiveness loss-of-control/imbalance; *Social stress=interpersonal conflict; **Perception=Thalamus; Emotional response=Amygdala, Cognition=Prefrontal cortex; Sympathetic activation-PVN=Hypothalamus-paraventricular nucleus; HRV=heart rate variability.

ABSTRACT

Background: Sympatho-adrenal responses are activated as an innate defense coping (DefS) mechanism during emotional stress. Whether these sympatho-adrenal responses drive cardiac troponin T (cTnT) increases are unknown. Therefore, associations between cTnT and sympatho-adrenal responses were assessed.

Methods: A prospective bi-ethnic cohort, excluding atrial fibrillation, myocardial infarction and stroke cases, was followed for 3 years (N=342; 45.6±9.0 years). We obtained serum high-sensitive cTnT and expossure measures [Coping-Strategy-Indicator, depression/Patient-Health-Questionnarie-9, 24h BP, 24h heart-rate-variability (HRV) and 24h urinary catecholamines].

Results: Blacks showed moderate depression (45% vs. 16%) and 24h hypertension (67% vs. 42%) prevalence compared to Whites. A receiver-operating-characteristics cTnT cut-point 4.2 ng/L predicting hypertension in Blacks was used as binary outcome measure in relation to exposure measures [AUC 0.68 (95% CI 0.60-0.76); sensitivity/specificity 63/70%; P≤0.001]. Bi-ethnic cTnT-incidence was similar (Blacks, 27%, Whites, 25%) with cTnT-recovery better in Blacks (9%) compared to Whites (5%), P=0.001**.** In cross-sectional analyses, elevated cTnT was related to DefS [OR 1.08 (95% CI 0.99-1.16); P=0.06]; 24h BP [OR 1.03-1.04 (95% CI 1.01-1.08); P≤0.02] and depressed HRV [OR 2.19 (95% CI 1.09-4.41); P=0.03] in Blacks, but not in Whites. At 3 year follow-up, elevated cTnT was related to attenuated urine norepinephrine:creatinine ratio in Blacks [OR 1.46 (95% CI 1.01-2.10); P=0.04]. In Whites, a cut point of 5.6 ng/L cTnT predicting hypertension was not associated with exposure measures.

Conclusion: Central neural control systems exemplified a brain-heart stress pathway. Desensitization of sympatho-adrenal responses occurred with initial neural- (HRV) followed by neuroendocrine dysfunction (norepinephrine:creatinine) in relation to elevated cTnT. Chronic defensiveness may thus drive the desensitization or *physiological depression*, reflecting ischemic heart disease risk at a 4.2 ng/L cTnT cut-point in Blacks.

Keywords: *defense; depression; heart-rate-variability; catecholamine, Troponin T*

1. Introduction

Coping with everyday stressors (Amirkhan, 1990) may disturb sympatho-adrenal activity and cardiac rhythmicity as indicated by changes in catecholamine turnover (de Kock et al., 2012) as well as heart-rate variability (HRV) (Malan et al., 2013). Particularly, defensive coping (DefS) or the *fight-flight response* encompassing perception of control and active problem solving, has been suggested as a promoter of health (Amirkhan, 1990). In spite of this view, DefS outcomes have also been linked with pathology and emotional distress related alterations (de Kock et al., 2012; Malan et al., 2008; Malan et al., 2013), in that attenuated sympatho-adrenal responses to acute mental stress in a cross-sectional analysis were associated with wall remodeling and silent myocardial ischemia in a Black male cohort (Malan & Malan, 2017). Therefore, it seems plausible that chronic defensiveness, reflecting emotional distress, may drive direct relationships between sympatho-adrenal activation and markers of cardiac injury (Lazzarino et al., 2013) such as elevated cardiac troponin (cTnT) levels.

A subunit of the troponin complex, namely cTnT is released in response to sympathetic activation or catecholamine overload and myocyte necrosis (Muthu et al., 2014). A decrease in the metabolic supply to the myocardial tissue results in ischemia and resultant cardiomyocyte necrosis of the myocardium (Muthu et al., 2014). Reduced metabolic supply when accompanied by catecholamine vascular responsiveness may further increase myocardial ischemia and cTnT-related damage (Mazzeo et al., 2014; Muthu et al., 2014). Resultant changes in cardiac autonomic modulation and blood pressure may therefore occur to counteract myocardial ischemia, in order to improve perfusion. Accumulative effects of higher chronic metabolic demands may also be taxing if emotional distress is present (Malan et al., 2016). To maintain metabolic homeostasis, central neural control and downstream adrenergic-related signaling will be apparent with either sensitization/upregulation in acute or desensitization/downregulation in chronic situations (Guilliams & Edwards, 2010). Therefore, we aimed to assess sympatho-adrenal exposure measures, including 24h urinary catecholamines, 24h heart-rate-variability (HRV), blood pressure and levels of coping and depression in a bi-ethnic cohort from South Africa. Sympatho-adrenal responses resembling emotional distress might translate to cTnT activity at a certain cut point indicative of future ischemic heart disease risk. Thus, the main aim was to examine prospective associations between binary outcome cTnT and sympatho-adrenal exposure measures.

2. Methods

2.1 Study design

The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective study (Figure 1) was conducted in 2008/9 and 2011/12 and included 409 Black and White teachers (Malan et al., 2015). For the current sub-study, we only included teachers participating in both phases and additionally excluded individuals with atrial fibrillation (N=10), history of or current myocardial infarction or stroke (N=3) and missing cTnT data at baseline (N=4). The final study sample comprised of 342 participants who were fully informed about the objectives and procedures prior to recruitment. All participants provided written, informed consent. The study conformed to the Helsinki Declaration (revised 2004) and was approved by the Ethics Review Board of the North-West University, Potchefstroom Campus, South Africa: Approval number 0003607S6.

The three-year follow up investigation was performed using a similar methodology to the baseline evaluation with clinical assessments done over a 36h period. During the working week, 24h ambulatory blood pressure, -ECG and 24h physical activity devices were fitted to the participants at their working place after which they resumed normal daily activities. After 15:00, participants were transported to the North-West University overnight facilities, where they were introduced to the experimental set-up. Afterwards they enjoyed a standardized dinner, and completed a battery of psychosocial questionnaires under supervision of registered clinical psychologists. The next morning, anthropometric (*E-component*) and sphygmomanometer blood pressure measurements were obtained and registered nursing staff collected overnight fasting blood samples after 07:00.

2.2 Cardiovascular risk measures

2.2.1 Ambulatory BP and ECG monitoring (ABPM)

ABPM devices were attached to participants' non-dominant arm (Cardiotens® CE120 Meditech, Budapest, Hungary) by trained cardiovascular research personnel. The Cardiotens® was programmed to measure BP at 30-min intervals during the day (07:00–22:00) and every hour during night time (22:00–06:00). The successful 24 h inflation rate was 79% (±12) at baseline and 88% (±9) at follow-up. The data were analyzed with the CardioVisions 1.19 Personal Edition software (Meditech, Budapest, Hungary). Any incidents including visual disturbances, headaches, nausea, fainting, palpitations, physical activity and stress were to be recorded on a 24 h diary card. Hypertensive status was defined as 24 h SBP ≥ 130 mm Hg and/or DBP ≥ 80 mm Hg (Piepoli et al., 2016).

2.2.2 Frequency and time-domain heart-rate-variability (HRV) analyses

Ambulatory HRV analyses assessed spontaneous oscillations resulting from sinus node depolarization (Thayer et al., 2012) obtained from analyzable 24h ambulatory 2-lead ECG data. The software program automatically filtered out ventricular and supraventricular ectopic beats as well as artefacts in RR intervals, while HRV outliers were manually removed. HRV measures included the standard deviation of the normal-to-normal (NN) intervals between adjacent QRS complexes (SDNN) which reflected vagus nerve-mediated autonomic control of the heart. SDNN is the best overall prognostic tool to detect depressed HRV (<100ms) (Pizzi et al., 2008). HRV triangular index (HRVti) is an index of the pulse variability based on a triangular interpolation method in the given time interval. In-depth explanations on frequency and time-domain analyses are well-described on-line (*E-component*). Additionally, non-linear analyses plotted each RR interval of a sinus beat as a function of the previous one for a predetermined segment length (Poincaré or Lorenz return maps/plots). Quantitative analyses of these plots have been associated with long-term 24h RR-interval variability (SD2) (Pizzi et al., 2008).

2.2.3 Clinic blood pressure

Participants were in a semi-recumbent position for 20-30 min prior to blood pressure measurements using the Riva-Rocci Korotkoff method by applying a suitable cuff on the non-dominant arm (Riester CE 0124® and 1.3M ™ Littman® II S.E. Stethoscope 2205). Two duplicate measurements were taken, with a 3- to 5-min resting period between each; the second of which was used for statistical analyses. Hypertensive status was defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg (Piepoli et al., 2016).

2.3 Coping Strategy Indicator (CSI)

The CSI is a 33-item self-report measure of coping responses with construct, convergent and discriminant validity (Amirkhan, 1990). Cronbach's alpha (α) reliability coefficients were determined for the three subscales of 11 items each in the SABPA cohort and ranged between 0.61-0.87. DefS implies actively solving problems and in-control responses. Seeking social support supports DefS, with a focus on acquiring advice in stressful times and lastly, emotional avoidance or loss-of-control implies defeat, with psychophysiological withdrawal. When responding to the various questions of the three subscales, the participant had to keep a recent stressful event in mind. The responses were then rated on a three-point Likert scale: a lot (3), a little (2), or not at all (1). The events fell into four broad categories: achievement (work/school related problems); social stressors (interpersonal conflict); personal changes in psychophysiological or spiritual status; and fate events such as accidents and chance

victimization (Amirkhan, 1990). Two research assistants sorted these events with an 88% agreement.

2.4 Depression

Depression symptom scores were obtained via the Patient Health Questionnaire-9 (PHQ-9) (Kroenke & Spitzer, 2002) which has been validated in various ethnic groups for use in primary health care settings (Monohan et al., 2009). Each item evaluated the presence of one of the nine Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-R) criteria for major depression. The Cronbach's alpha-reliability index for the total three-year PHQ-9 score in the current sub-study was 0.80, indicating good reliability. The recommended and established PHQ-9 cut-off point of ≥ 10 indicates the presence of moderately severe depression symptoms.

2.5 Biochemical measurements

2.5.1 Urinary catecholamines

Urine collection was performed overnight, 8 h sampling at baseline and 24 h sampling at follow-up. The sampling periods of 8 h and 24 h compares favorably for detection of stress hormones in urine (Masi et al., 2004). At follow-up, participants began and ended sampling with an empty bladder on Day 1. Urine was collected for the next 24 h in a three liter container, washed with 9 ml of 20% HCl (UriSet24, Sarstedt®, Nümbrecht, Germany). Samples were stored at -80°C until analysis within one year after collection, using the 3-Cat Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). Intra- and inter-assay coefficients for epinephrine were 5.50% and 9.62% respectively and for norepinephrine, 2.70% and 8.59%. Urine creatinine was measured with the calorimetric method.

2.5.2 Blood analyses

A registered nurse obtained fasting blood samples from the ante-brachial vein branches with a sterile winged infusion set, and handled samples according to standardized procedures. Serum and whole blood EDTA samples were analyzed for lipids, high sensitivity C-reactive protein (CRP), cotinine (reflecting smoking status), gamma glutamyl transferase (γ-GT) (reflecting alcohol consumption) and glycated haemoglobin (HbA_{1c}), using Unicel DXC 800, Beckman and Coulter, USA; Modular ROCHE Automized, Switzerland and the Konelab™ 20I Sequential Multiple Analyzer Computer, ThermoScientific, Vantaa, Finland respectively. Citrate fibrinogen was measured by the Viscosity-based clotting method and the Immuno-turbidimetric method (STA Compact, TAGO Diagnostic, Roche; France). The CRP:fibrinogen ratio was used as marker of fibrosis (Jansen van Vuren et al., 2016). Thyroid stimulating hormone (TSH) and high sensitive cTnT were determined with

electrochemiluminescence immunoassay (ECLIA), Elecsys 2010, Roche, Basel, Switzerland. Eighty cTnT values (23.39%) were below detectable limit (<3 ng/L) and substituted with lower than detectable values using log-methods. The cTnT inter- and intra-batch variability was 15% and 5.6%.

2.6 Statistical analyses

Statistica version 13 and IBM SPSS version 23 statistical software packages were used. Power analyses were performed to obtain relevant effect sizes based on differences in ambulatory autonomic dysfunction and biological profiles. Results showed that a sample size of 50 will demonstrate biological differences with a statistical power of 0.8, and significance level of 0.05. Variables with skewed distributions were log-transformed. Covariates were chosen *a priori* (Piepoli et al., 2016) including: age, waist circumference, physical activity, γ-GT, cotinine, hypertension medication as well as TSH for HRV analyses. Considering retinal perfusion deficits and the high hypertension prevalence in Blacks (Malan L et al., 2016; 2017) optimal cTnT cut points associated with clinic and ambulatory 24 h hypertension were computed from the maximum of the Youden index (J) (sensitivity + specificity − 1) using non-parametric receiver operating characteristic (ROC) curves. General linear modelling was used to test a priori hypotheses (Ethnicity x Gender x cTnT) using the derived cTnT cut point as binary outcome measure for all exposure markers, independent of *a priori* covariates and baseline values of the respective risk factors. Sympatho-adrenal exposure measures included 24 h urinary catecholamines, 24 h heart-rate-variability (HRV), blood pressure and levels of coping and depression. Independent *t*-tests and Chi-square (χ^2) tests compared sympatho-adrenal differences and proportions at baseline respectively. ANCOVA analyses compared sympatho-adrenal values at baseline and follow-up; as well as at follow-up in relation to elevated ROC cTnT cut point, controlling for *a priori* covariates and baseline value of the respective risk factors.

McNemar's case-control tests were used to demonstrate changes: a) when cTnT-negative at baseline become positive at follow-up; and cTnT-positive people at baseline recover to negative at follow-up; b) in high DefS and stressful coping events over 3 years. Non-linear HRV analyses, using Poincaré and Lorenz plots, were recorded in a Black male having chronic depressed SDNN (<100ms), DefS (≥31), 24h hypertension and raised cTnT.

Logistic regression analyses calculated odds ratios (OR) and 95% confidence intervals (CI) to determine if sympathy-adrenal exposure measures will increase the risk of raised cTnT.

These analyses were performed to examine cross-sectional and longitudinal associations. For the latter we used the formula: Δ %: (follow-up – baseline)/baseline*100. Sympatho-adrenal

exposure measures were added independently and in combination to multivariate models, considering baseline *a priori* covariates and TSH in HRV models.

Sensitivity analyses were computed to adjust for gender in HRV analyses and excluding hypertension medication users and HIV cases. The statistical significance level was set at $p \leq 0.05$ (two-tailed).

3. Results

3.1 Clinical characteristics

In Table 1, Blacks at baseline were younger, physically less active, consumed more alcohol (γ-GT), and had lower TSH and time-domain HRV values compared to Whites. More Blacks showed moderate depression (45.0% vs. 16.2%) and 24h hypertension (67% vs. 42%) prevalence compared to Whites. Blacks further used more ACE inhibitors, diuretics and calcium channel blockers (P≤0.05).

In Figure 2, a receiver-operating-characteristics (ROC) cTnT cut-point of 4.2 ng/L predicted both clinic [AUC 0.64 (95% CI 0.55-0.72); sensitivity/specificity 63/64%; P≤0.002] and ambulatory 24h hypertension [AUC 0.68 (95% CI 0.60-0.76); sensitivity/specificity 63/70%; P≤0.001] in Blacks. In Whites, the cTnT cut-point of 5.6 ng/L [AUC 0.67 (95% CI 0.58-0.75); sensitivity/specificity 53/78%; P≤0.001] predicted ambulatory 24h hypertension only. An interaction term (ethnicity x cTnT) was fitted for DefS [F (1,324), 6.40; P = 0.01] but not for the other coping strategies or depression. Ethnic differences were evident for cTnT, BP, time-domain HRV and the catecholamines (P≤0.001). Interaction on main effects (gender x cTnT or age x cTnT) for sympatho-adrenal responses resembling emotional distress, were not apparent.

Unadjusted (Table A.1*)* comparisons showed that Blacks consistently sought more social support compared to Whites. Blacks further had depressed HRV (lower time-domain SDNN, increased SDNN risk (≤100ms), lower geometric (HRVti) HRV patterns), higher fibrosis, blood pressure and lower catecholamine levels compared to Whites. In adjusted comparisons, a similar trend was observed in Blacks in relation to elevated cTnT (Table 2, Whites=reference group). Except for SDNN and HRVti, none of the other frequency or time-domain HRV measures showed significant cross-sectional and longitudinal differences or associations with elevated cTnT considering confounders and will not be discussed further. Bi-ethnic cTnT-incidence was similar (Blacks, 27% and Whites, 25%) but cTnT-recovery better in Blacks [9%; OR 3.14 (1.7-5.7)] compared to Whites [5%; OR=4.89 (2.4-10.0)], P=0.001. Coping with social stress or interpersonal conflict (Table A.2) showed increased

changes (3.46%) over time in relation to elevated cTnT in Blacks [OR 0.52 (95% CI: 0.26-1.05), P=0.06].

A composite profile demonstrated a dispersed complex-like pattern with non-linear analysis, i.e. 2-dimensional Poincaré plotting (Figure A.1) and 3-dimensional Lorenz mapping (Figure A.2) in a Black male with high defensive coping (DefS score = 31); hypertension (24h BP = 143/91 mmHg); moderately depressed HRV (SDNN = 73ms); medium cardiovascular risk (HRV triangular index = 9) and raised cTnT (mean 5.1ng/L).

3.2 Cross-sectional associations between sympatho-adrenal responses and elevated cTnT

In Table 3, a trend for cardiac injury risk at baseline was evident in Blacks when habitually using DefS (P=0.06), accompanied by moderately depressed time-domain HRV responses (P=0.03) and increased 24h BP (SBP, DBP) (P≤ 0.02). Apart from fibrosis [OR 0.63 (95% CI: 0.41-0.97); P=0.04], elevated cTnT cut-point (5.6 ng/L) was not associated with any sympatho-adrenal responses in Whites (Table A.3).

3.3 Follow-up associations between sympatho-adrenal responses and elevated cTnT

At follow-up (Table 3), cardiac injury risk was related to desensitized norepinephrine:creatinine ratio [OR 1.46 (1.01-2.10); P=0.04] in Blacks. Sensitivity analyses adjusting for gender in HRV analyses and excluding hypertension medication users or HIV cases did not change the outcomes.

4. Discussion

We assessed cross-sectional and longitudinal associations between cardiac injury (cTnT) and sympatho-adrenal responses in a bi-ethnic cohort of South African teachers. Major central neural control systems exemplified a brain-heart-axis stress pathway. The pathway demonstrated desensitized sympatho-adrenal responses in relation to elevated cTnT levels with initial neural- (HRV) followed by neuroendocrine dysfunction (norepinephrine:creatinine). Chronic defensiveness may drive this desensitization; reflecting a *physiological depression* and ischemic heart disease risk at a novel 4.2 ng/L cTnT cut-point in a Black South African cohort.

4.1 Chronic defensiveness reflecting cardiac injury

Healthy coping strategies integrate central neural responses in order to cope with stress (Vaillant, 2011). Survival circuits and defense responses are activated when threatening conditions are detected in the amygdala and a general arousal state is generated in the paraventricular nuclei of the hypothalamus (Moscarello and LeDoux, 2013). This state is due to widespread fast acting neural-induced involuntary heart rate variations and the release of

slower neuroendocrine aminergic neuromodulators (Moscarello and LeDoux, 2013; Thayer et al., 2012). Individual resiliency will thus depend on the capacity of the individual to recover from the stressor by means of healthy in-control coping strategies with a voluntary mobilizing of social support from friends or family (Amirkhan, 1990; Malan et al., 2017). Currently, the Black cohort sought more social support, which rather supports an in-control DefS response albeit increasing interpersonal conflict and social stress. Indeed, coping defensively with a social stressor showed a trend for elevated cTnT risk in the current Black cohort. Chronic DefS with social stress however showed susceptibility for physiological loss-of-control sympatho-adrenal responses. Previous findings support this notion as an urban-dwelling environment and accompanying acculturation was deemed a psychosocial stressor (Malan et al., 1996). In 1018 Blacks from the North-West Province of South Africa, DefS and seeking social support were associated with loss-of-control cardiometabolic responses (Hamer and Malan, 2010). Our data now additionally showed that chronic DefS further may increase susceptibility for cardiac injury at a cTnT 4.2 ng/L cut-point for Blacks opposed to a suggested cut point of 6ng/L, or lower, for stress-induced cardiomyopathy (Ramaraj et al., 2009). Interestingly, in Whites, the higher 5.6 ng/L cTnT cut-point predicting ambulatory hypertension was not related to disturbed sympatho-adrenal responses, and rather exemplified behavioral in-control DefS responses. Recently F-flurodexoyglucose PET/CTs showed increased amygdalar activity, which was associated with perceived stress and cardiovascular disease risk in 293 patients followed for 3.7 years (Tawakol et al., 2017). These findings are in line with our results in Blacks reflecting chronic defensiveness and cardiac injury over 3 years. Similar cardiac injury incidence might thus reflect acute stress in the Whites and chronic stress in the Blacks (Malan et al., 2016). However, an improved recovery in Blacks may possibly be explained by the protective role of increased estradiol levels against silent myocardial ischemia (Malan NT et al., 2017).

4.2 Heart rate variation reflecting cardiac injury

Chronic defensiveness or more emotional distress will further increase higher metabolic demand and may disrupt homeostasis with activation of central neural control systems (Patil et al., 2015). In the current study, we demonstrated initial neural control where depressed HRV responses in relation to elevated cTnT were accompanied by BP increases as homeostatic reflexes. Indeed, estimators of sympathetic hyperactivity (SDNN <100ms and HRVti) levels were increased in the Black cohort compared to Whites, further underscoring depressed HRV and a risk for cardiac injury in Blacks.

The limbic system modulates cardiac activity in response to stressful events and during homeostatic reflexes (Manea et al., 2015). Sympathetic nervous system (SNS) hyperactivity may thus serve as a pathophysiological event affecting the reciprocal relationships between limbic responses, sympathovagal disturbances and raised cTnT. The possibility of neurocardiogenic injury emerges as a consequence of uncontrolled stress and subsequent catecholamine overload. Indeed, DefS induces SNS hyperactivity and supports the initial neural control (depressed HRV, BP increases or hypertensive status) profile at baseline, reflecting a hypervigilant system. The occurrence of myocardial ischemia and/or myocardial necrosis during chronic defensiveness may further facilitate a mechanical distortion of the afferent and efferent fibers of the autonomic nervous system. This may be due to changes in the geometry related to necrotic and non-contracting segments of the heart (Mazzeo et al., 2014). Changes will also affect local neural regulation and contribute to the resulting diminished HRV. However, contradictory findings were recently reported (Jandackova et al., 2016). In the UK Whitehall II population-based cohort study, cardiac autonomic modulations in 3414 men and women obtained over 10 years and at 3 different time points, were not associated with a higher prevalence of cardiometabolic symptoms but rather with normal ageing (Jandackova et al., 2016).

4.3 Neuroendocrine dysfunction reflecting cardiac injury

Pertaining to neuroendocrine risk, we could not find any bi-ethnic study reporting a longitudinal relationship between the catecholamines and cardiac injury. Our findings therefore emphasize the presence of a novel brain-heart stress pathway. Chronic sympatho-adrenal activation facilitated a later onset of neuroendocrine control with desensitization of norepinephrine:creatinine in relation to elevated cTnT in the Black cohort. Previous findings showed that the trigger enzyme for synthesis of the catecholamines, tyrosine hydroxylase C-824T single nucleotide polymorphism, was not related to hypertension in this Black cohort (van Deventer et al., 2013). Therefore, our findings rather support the role of environmental or social stress demands, which elicited sympatho-adrenal responses in relation to elevated cTnT in Blacks. The risk of an attenuated norepinephrine:creatinine ratio and elevated cTnT levels suggests that overwhelming sustained stress may interfere with behavioral in-control DefS. It may cause distress and hyperactivity of the sympatho-adrenal system, as the body attempts to cope with increasing demands. Two mechanisms may apply: norepinephrine levels may a) decrease due to exhaustion of the autonomic system (Guilliams & Edwards, 2010) where adrenal exhaustion or fatigue will set in, and b) the neuroendocrine system will become non-responsive with desensitization and/or down-regulation of adrenergic receptors,

which has been associated with depression and neural fatigue (Tsigos & Croussos, 2002). The lack of association between chronic depressive symptoms and cardiac injury was therefore surprising. Our findings rather support dissociation of DefS responses where behavioral in-control responses *masked* physiological loss-of-control or desensitized responses (*physiological depression*). Physiological depression may precede psychological depression with detrimental impact on cardiac health in the long-term and increase susceptibility for ischemic heart disease.

4.4 Blood pressure as compensatory mechanism to counteract perfusion deficits and cardiac injury

Coping defensively with overwhelming social stress (Rosengren et al., 2004) may have induced BP increases in relation to elevated cTnT at baseline. Concurrently, the hypertension prevalence rate in the Whites was lower (42%) compared to the Blacks (67%) with no change in hypertension prevalence in the Black Africans at follow-up. Findings from the INTERHEART (Rosengren et al., 2004) and Jackson studies (Abdalla et al., 2016) in African-Americans demonstrated similar findings as social stress was related to myocardial ischemia and elevations in cTnT respectively. Interestingly, the ambulatory hypertension status of 59% African-Americans from the Jackson study (Abdalla et al., 2016) compares reasonably well with the 67% of our cohort. We, therefore, argue that evidence of vascular resistance in Blacks (Malan et al., 2012) and African-Americans (Adefurin et al., 2013) might be driven by chronic defensiveness or a disturbed brain-heart stress response pathway. Indeed, vascular resistance increases cardiac preload in essential hypertensives (Adefurin et al., 2013) and impeded coronary perfusion in a Black cohort (Griffiths et al., 2017; Malan et al, 2012). Inevitable this will increase myocardial oxygen demand, which may result in proteolysis and release of cTnT and increases in blood pressure. Ultimately, loss-of-control DefS in the Blacks facilitated cardiac injury. Previously, the SABPA Whites showed acute emotional stress at follow-up (Malan et al., 2016), which aligns well with the current effective in-control DefS where elevated cTnT was not related to sympatho-adrenal responses. Our findings further suggest that targeting novel markers to explain hypertension prevalence in the African ethnicity should rather consider central neural control or brain-heart stress responses. When chronic conditions such as cardiac injury prevail, dynamic compensatory BP increases may trigger central control mechanisms to maintain homeostatic reflexes in organ systems.

The small sample size prohibited stratification into both ethnic and gender groups thereby limiting an improved understanding of cardiac injury pathology. Therefore expanding data to

other cohorts may confirm findings of a 4.2 ng/l cTnT cut-point when chronic defensiveness is suspected. Early screening for raised cTnT has clinical practice implications as raised cTnT is shown to be related to neural distress, which may precede ischemic heart disease and future heart failure risk.

In conclusion, a novel brain-heart stress pathway exemplified desensitized sympatho-adrenal responses in relation to cardiac injury. Initial desensitized neural- followed by neuroendocrine dysfunction reflected a chronic *physiological depression*. Dissociation of behavioral control (DefS) albeit physiological loss-of-control responses were demonstrated in the Blacks. Chronic defensiveness may have contributed to loss-of-control neuroendocrine responses, facilitating cardiac injury at a 4.2 ng/l cTnT cut-point. Therefore, screening for stress-related cardiac injury at cTnT levels of ≥ 4.2 and ECG ST-segment depression will allow detection of the early dynamics in ischemic heart disease development.

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FINANCIAL DISCLOSURES

No conflicts of interest are declared. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors; therefore, funders do not accept any liability with regard to this study.

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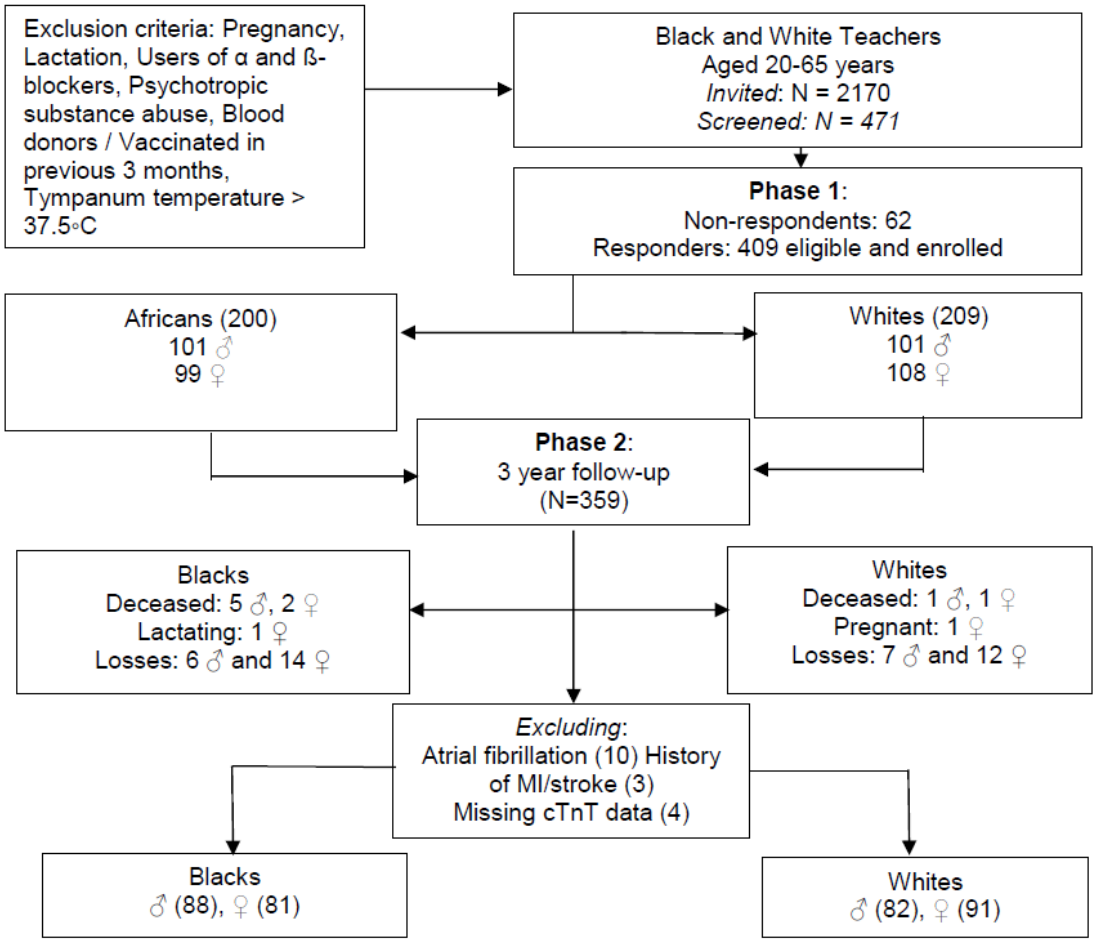

Figure 1: Design of the bi-ethnic gender cohort of the Sympathetic Activity and Ambulatory Blood Pressure in Africans prospective study. Where: cTnT=cardiac troponin T at baseline.

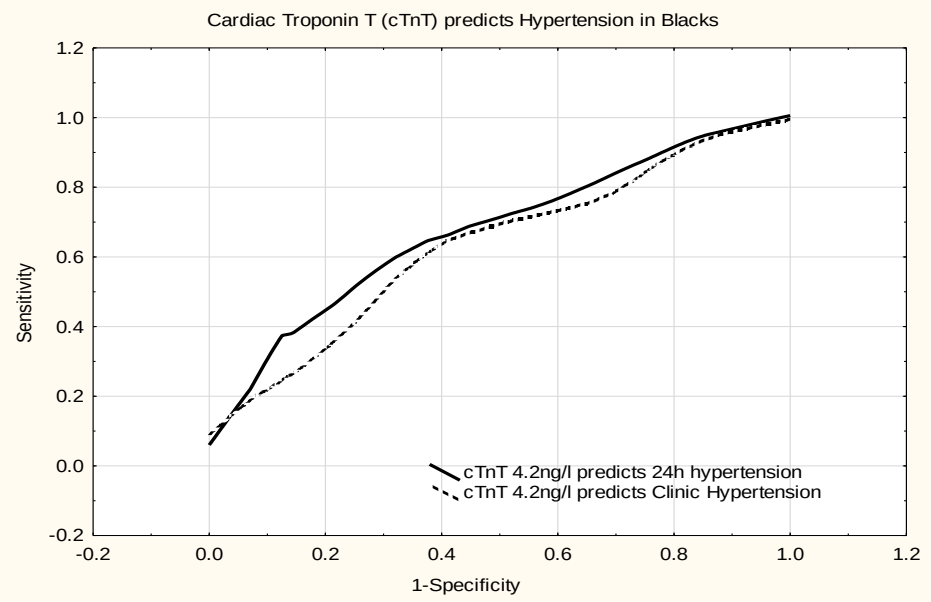

Figure 2a: ROC curves depicting cardiac Troponin T cut points for clinic and 24h hypertension in Black Teachers. The area under the curve (AUC) (95% CI) for 24h hypertension was 0.68 (95% CI 0.60-0.76); sensitivity/specificity 63/70%; P≤0.001; and for clinic hypertension 0.64 (95% CI 0.55-0.72); sensitivity/specificity 63/64%; P≤0.002.

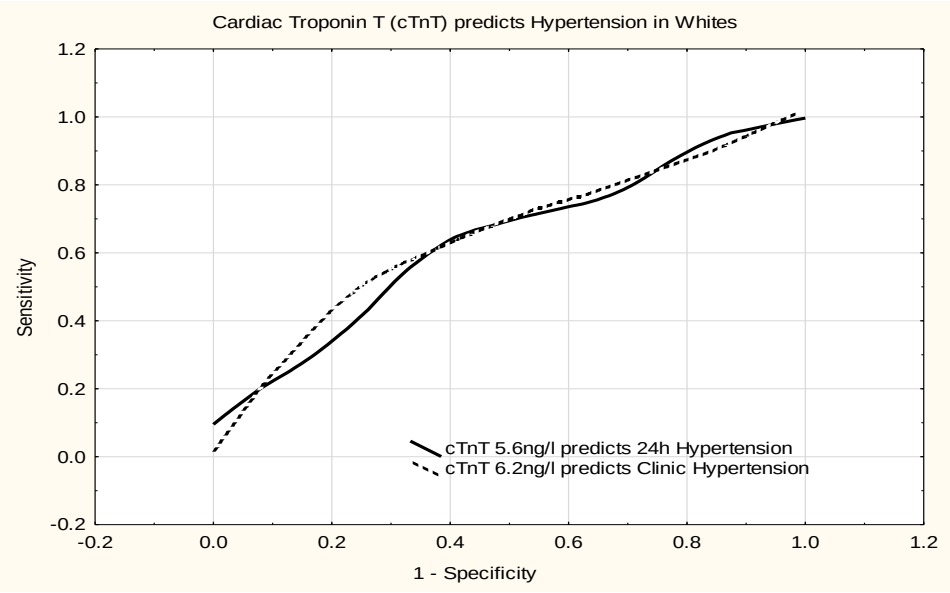

Figure 2b: ROC curves depicting cardiac Troponin T cut points for clinic and 24h hypertension in Whites. The area under the curve (AUC) (95% CI) for 24h hypertension was 0.67 (0.58-0.75); sensitivity/specificity 53/78% (P≤0.001); with an AUC for clinic hypertension of 0.65 (95% CI 0.56-0.74); sensitivity/specificity 52/74%; P≤0.01.

Table 1: Clinical characteristics of a bi-ethnic South African teacher's cohort at baseline.

	Blacks (N=169)	Whites (N=173)	P-values
Age, yrs	44.5 (39.0-51.0)	47.0 (41.0-54.0)	0.04
Women, n (%)	88 (52.1)	82 (47.4)	0.39
Urban living, years	31.8 (19.0-45.0)	20.5 (10.0-30.0)	< 0.001
Cotinine, ng/ml	0.01 (0.01-15.51)	0.01 (0.01-0.01)	0.33
cGGT, U/l	43.5 (28.4-74.4)	18.0 (12.0-28.0)	< 0.001
Physical activity, kcal/24h	2584.6 (2185.9-3118.1)	2968.0 (2370.0-3540.7)	< 0.001
Waist circumference, cm	94.1 (83.6-103.1)	93.6 (80.8, 103.5)	0.10
Coping scores			
Defense coping	29 (15.0-31.0)	30 (27-32)	0.06
Social support coping	26 (23-30)	18 (15-23)	< 0.001
Avoidance coping	21 (18-23)	23 (21-28)	< 0.001
Moderately severe depression, n (%)	76 (45.0)	28 (16.2)	< 0.001
Heart Rate Variability (HRV)			
SDANN, ms	269.0 (234.0-300.5)	263.1 (219.0-295.5)	<0.001
SDNN, ms	112 (85-136)	124 (102-156)	<0.001
rMSSD, ms	29 (21-38)	31 (22-41)	0.26
HRV:Triangular index	29 (22-36)	36 (29-43)	<0.001
Thyroid stimulating hormone, µlU/ml	1.8 (1.3-2.5)	2.1 (1.4-2.9)	0.01
Potential cardiac and neuroendocrine risk markers			
Cardiac Troponin T, ng/L	4.2 (3.1-5.5)	4.9 (3.2-6.9)	0.05
Cholesterol, mmol/l	4.5 (3.8-5.5)	5.5 (4.7-6.4)	< 0.001
CRP:Fibrinogen, g/L:mg/L	1.4 (0.7-2.6)	0.5 (0.4-1.2)	<0.001
24h SBP, mm Hg	131 (122-143)	124 (116-130)	<0.001
24h DBP, mm Hg	82 (77-90)	77 (71-82)	<0.001
24h Heart rate, bpm	79 (73-86)	74 (68-81)	<0.001
24h Hypertension, n (%)[a]	113 (67)	73 (42)	<0.001
24h urinary NE:Cr	18.8 (11.6-29.8)	24.8 (13.2-38.9)	0.07
24h urinary E:Cr	2.9 (1.6-2.9)	2.9 (1.6-4.7)	0.36

HIV, n (%)	15 (8.9)	0 (0)	<0.001
Medications, n (%)			
Statins	2 (1.2)	6 (3.5)	0.16
Aspirin	4 (2.4)	9 (5.2)	0.17
ACE inhibitors	19 (11.2)	3 (1.7)	<0.001
Angiotensin II blockers	1 (0.6)	1 (0.6)	0.99
Diuretics	23 (13.6)	8 (4.6)	<0.001
Calcium channel blockers	13 (7.7)	1 (0.6)	0.001
Beta blockers	5 (3.0)	1 (0.6)	0.09
Alpha blockers	0.0	0.0	-

Values are median (± interquartile range/IQR) or frequencies (%). Where: cGGT=gamma glutamyl transferase; moderately depressed=PHQ-9=≥10; CRP=C-reactive protein; HdL=high density lipoproteins; HIV=Human Immune-deficiency virus infected; SDANN=standard deviation of all the 5 min normal RR intervals (NN); SDNN=Standard deviation of RR interval; rMSSD=the square root of the mean squared difference of successive NNs; NE:Cr=norepinephrine creatinine ratio; E:Cr=epinephrine creatinine ratio.

[a]Hypertensive status classified as 24h SBP ≥ 130 mm Hg and/or DBP ≥ 80 mm Hg (Piepoli et al. 2016).

Table 2: Comparing depression, coping and sympatho-adrenal changes in Blacks vs. Whites (reference group) in relation to cardiac troponin T (cTnT) cut points at 3 year follow-up.

	Blacks vs. Whites (Reference group)	
	cTnT cut point: 4.2 ng/L	**cTnT cut point: 5.6 ng/L**
Depression		
Depressive symptoms	-0.11 (0.10)	-1.62 (0.09)
Coping scores		
Defense coping	0.27 (0.06)	1.54 (0.17)
Heart rate variability (HRV)[a]		
HRV:SDNN, ms	-23.58 (0.71)**	-22.58 (1.73)*
HRV:Triangular index	-4.35 (0.17)*	-4.97 (0.47)
Potential cardiac and neuroendocrine risk markers		
cTnT, ng/L	1.76 (0.09)	1.15 (0.20)
Fibrosis	-0.15 (0.04)	-0.87 (0.12)
24h SBP, mmHg	6 (0.19)**	7 (0.59)*
24h DBP, mmHg	3 (0.11)**	3 (0.33)
24h NE:Cr, nmol/l	-18.25 (0.56)**	-18.18 (0.99)**
24h E:Cr, nmol/l	-3.20 (0.09)**	-3.13 (0.14)**
cTnT (4.2ng/L) incidence and recovery: N (%) (Odds ratio, 95% CI) P		
	Blacks (N=169)	**Whites (N=173)**
Incidence, N (%)	44 (27)	44 (25)
Recovery, N (%)	14 (9)	9 (5)
Odds ratio (95% CI) P	3.14 (1.7-5.74)**	4.89 (2.4-10.0)**

Data presented as means ± SEM (standard error of means). Adjustments were made for *a priori* covariates (age, log waist circumference, log physical activity, log cotinine, log GGT and hypertension medication use) and baseline value of the respective risk marker. Incidence (cTnT-negative at baseline becoming cTnT-positive at follow-up); Recovery (cTnT-positive at baseline becoming cTnT-negative at follow-up); N=cases; SDNN=Standard deviation of RR interval; NE:Cr=urinary norepinephrine creatinine ratio; E:Cr=urinary epinephrine creatinine ratio.

[a]Additional adjustment for thyroid stimulating hormone. *P ≤ 0.05; **P ≤ 0.01.

Table 3: Sympatho-adrenal responses in relation to cardiac Troponin T (cTnT) cut-point (≥ 4.2 ng/L) in a Black cohort.

	Odds ratio	**95% CI**	**P Value**
Cross-sectional			
Coping scores			
Defense coping	1.08	0.99-1.16	0.06
24h Time-domain heart rate variability (HRV)			
SDNN risk cut point (≤ 100 ms)	2.19	1.09-4.41	0.03
Potential cardiac risk			
24h SBP, mmHg	1.03	1.01-1.06	0.01
24h DBP, mmHg	1.04	1.01-1.08	0.02
Follow-up (Δ %)			
Potential neuroendocrine risk			
24h urinary norepinephrine:creatinine ratio.	1.46	1.01-2.10	0.04

Adjustments were made for baseline *a priori* covariates (age, log waist circumference, log physical activity, log cotinine, log GGT and hypertension medication use) and thyroid stimulating hormone in HRV analyses. Where: SDNN=Standard deviation of RR interval; Δ = change; NE:Cr=urinary norepinephrine:creatinine ratio.

E-COMPONENT

Title: Chronic defensiveness and neuroendocrine dysfunction reflect a novel cardiac troponin T cut point: the SABPA study

Methods: Anthropometric and Physical activity, Heart Rate Variability (HRV)

Results: Tables A.1 – A.3; Figure A.1-A.2

Running head*: defensiveness; depression*; *heart-rate-variability; catecholamine, cardiac Troponin T*

[1]Leoné Malan (RN, PhD), [1,2]Mark Hamer (PhD), [1,3]Roland von Känel (MD), [4,5]Gavin W Lambert (PhD), [6]Rhena Delport (RN, PhD), [7]Hendrik S Steyn (DSc), [1]Nicolaas T Malan (DSc).

[1]Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa
[2]National Centre for Sport and Exercise Medicine, Loughborough University, Leicestershire, United Kingdom
[3]Department of Neurology, Inselspital, Bern University Hospital, and University of Bern Switzerland
[4]Iverson Health Innovation Research Institute, Swinburne University of Technology, Hawthorn, and [5]Baker Heart & Diabetes Institute, Melbourne, Australia
[6]Department of Chemical Pathology, University of Pretoria, South Africa
[7]Statistical Consultation Services; North-West University; Potchefstroom; South Africa

E-COMPONENT

a) Method: Anthropometric and Physical activity measures
b) Method: Heart Rate Variability (HRV)
c) Table A.1: Comparing sympatho-adrenal risk factors in Blacks vs. Whites at baseline and at 3yr follow-up.
d) Table A.2: Coping with stressful events in relation to cardiac Troponin T (cTnT) at 3 year follow-up utilizing McNemar computations.
e) Figure A.1 - A.2: Non-linear analysis demonstrates dispersed complex-like pattern with 2-dimensional Poincaré plotting (Figure A.1) and 3-dimensional Lorenz mapping (Figure A.2) in a Black male with a composite profile of high defensive coping (DefS score = 31); hypertension (24h BP = 143/91 mmHg); moderately depressed HRV (SDNN = 73ms); medium cardiovascular risk (HRV triangular index = 9) and raised cTnT (mean 5.1ng/L).
f) Table A.3: Sympatho-adrenal responses in relation to cTnT (5.6 ng/L) in a White cohort.

METHOD

a) Anthropometric and Physical activity measures

Anthropometric measurements were performed in triplicate by qualified personnel according to standardized procedures. Intra- and inter-observer variability was less than 10%. Daily physical activity data, considering metabolic rate, was determined with an Actical® activity device (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada).

b) Heart Rate Variability (HRV) Measures

Frequency- and time-domain analysis (DeGiorgio et al., 2010; Piepoli et al., 2016), was computed to assess spontaneous oscillations resulting from sinus node depolarization obtained from analyzable 24h ambulatory 2-lead ECG data. The software program automatically filtered out ventricular, supraventricular as well as artifacts in RR intervals, and HRV outliers had been manually removed.

Frequency domain (power spectral density) analysis

Frequency domain analysis describes the periodic oscillations of the heart rate signal decomposed at different frequencies and amplitudes; and provides information on the amount of their relative intensity (termed variance or power) in the heart's sinus rhythm. Fast Fourier transformation performed frequency-domain analysis identifying the components in absolute (ms^2) and normalized units (nu) for high frequency (HF), low frequency (LF) and the LF/HF ratio. The LF/HF ratio is indicative of sympathovagal balance.

Time-domain analyses

Time-domain analyses included measures of SDNN and RMSSD. SDNN is a prognostic tool for cardiovascular outcome and defined as the standard deviation of the normal-to-normal (NN) intervals between adjacent QRS complexes, which equal the square root of variance. Since variance is mathematically equal to the total power of spectral analysis, the SDNN reflects all cyclic components responsible for variability in the period of recording. SDNN is regarded as the best overall prognostic tool for values <50 ms are indicative of highly depressed HRV, those between 50-100 ms indicate moderately depressed HRV and those >100 ms are classified as normal. RMSSD, the root mean square of successive differences between adjacent RR intervals is closely related to the high frequency (HF) component of the power spectrum. Both SDNN and RMSSD reflect vagus nerve-mediated autonomic control of the heart.

Geometrical analyses

Geometrical analyses included the HRV triangular index (HRVti), which is an index of the pulse variability based on a triangular interpolation method in the given time interval where

cardiovascular risk 0-15 is high; 15-20 is mid; >20 is low. The histogram assesses the relationship between the total number of RR intervals detected and the RR interval variation. The triangular HRV index considers the major peak of the histogram as a triangle with its baseline width corresponding to the amount of RR interval variability, its height corresponds to the most frequently observed duration of RR intervals, and its area corresponds to the total number of all RR intervals used to construct it. The triangular HRV index is an estimate of the overall HRV.

Non-linear analyses (fractal analysis)

Fractal analysis was furthermore recorded by plotting each RR interval of a sinus beat as a function of the previous one for a predetermined segment length (Poincaré or Lorenz return maps/plots). Quantitative analyses of these plots are associated with non-linear 5 minutes beat-beat- variability (SD1) and long-term 24h RR-interval variability (SD2). Recent data suggest that fractal analysis in comparison to standard HRV measurements seems to detect abnormal patterns of RR fluctuations more efficiently.

DeGiorgio, C.M., Miller, P., Meymandi, S. et al., 2010. RMSSD, a Measure of Heart Rate Variability, is associated with risk factors for SUDEP: The SUDEP-7 Inventory. Epilepsy Behav 19, 78-81.

Piepoli, M.F., Hoes, A,W., Agewall, S. et al., 2016. European Guidelines on cardiovascular diasease prevention in clinical practice. The Sixth Joint Force of the European Society of Cardiology and other Societies on cardiovascular disease prevention in clinical practice. Eur Heart J 2016, 1-78.

c)

Table A.1: Comparing sympatho-adrenal risk markers in Blacks vs. Whites at baseline and at 3yr follow-up.

	Blacks (N=173)	**Whites (N=169)**	**P-values**
Coping			
Defense coping, score			
Baseline	28.3 ± 0.4	28.7 ± 0.4	0.39
Follow-up	28.9 ± 0.4	28.7 ± 0.4	0.82
Social support, score			
Baseline	25.7 ± 4.8	18.8 ± 4.4	≤0.001
Follow-up	20.3 ± 4.1	18.0 ± 4.4	≤0.001
Avoidance, score			
Baseline	21.0 ± 3.8	23.7 ± 5.2	≤0.001
Follow-up	24.4 ± 5.1	24.7 ± 5.5	0.62
Depressive symptoms			
Baseline	9.37 ± 0.5	5.66 ± 0.5	≤0.001
Follow-up	8.63 ± 0.4	5.57 ± 0.4	≤0.001
‡24h Time-domain heart rate variability (HRV)			
SDANN, ms			
Baseline	277.5 ± 6.1	239.8 ± 6.0	≤0.001
Follow-up	198.3 ± 6.9	178.6 ± 6.8	0.33
SDNN, ms			
Baseline	125.2 ± 4.7	140.5 ± 4.5	0.04
Follow-up	109.6 ± 3.4	137.1 ± 3.2	≤0.001
rMSSD			
Baseline	34.0 ± 1.7	34.5 ± 1.7	0.86
Follow-up	30.0 ± 1.4	33.5 ± 1.3	0.10
Triangular index			
Baseline	31.0 ± 0.9	35.3 ± 0.9	≤0.001
Follow-up	30.0 ± 1.0	37.4 ± 0.9	≤0.001
Potential Cardiac risk markers			
Cardiac troponin T, ng/L			

Baseline	4.7 ± 0.3	5.6 ± 0.3	0.04
Follow-up	4.9 ± 0.3	5.1 ± 0.3	0.21
Fibrosis (C-reactive protein:Fibrinogen)			
Baseline	2.1 ± 0.2	1.0 ± 0.2	≤ 0.001
Follow-up	1.5 ± 0.2	0.9 ± 0.2	0.01
24h SBP, mmHg			
Baseline	133 ± 1.1	125 ± 1.1	≤0.001
Follow-up	135 ± 1.2	124 ± 1.2	≤0.001
24h DBP, mmHg			
Baseline	83 ± 0.8	78 ± 0.8	≤0.001
Follow-up	83 ± 0.8	77 ± 0.8	≤0.001
Potential neuroendocrine risk markers			
24h urinary Norepinephrine:Creatinine			
Baseline	24.9 ± 2.2	30.7 ± 2.2	0.10
Follow-up	13.6 ± 1.7	37.2 ± 1.5	≤ 0.001
24h urinary Epinephrine:Creatinine			
Baseline	3.7 ± 0.3	3.9 ± 0.3	0.50
Follow-up	2.3 ± 0.3	6.0 ± 0.3	≤ 0.001

Data presented as means (± standard error of mean). Adjustments were made for *a priori* covariates (age, log waist circumference, log physical activity, log cotinine, log gamma-glutamyl transferase; hypertension medication). Where: SDANN=standard deviation of all the 5 minutes normal RR intervals (NN); SDNN, standard deviation of the normal-to-normal (NN) intervals between adjacent QRS complexes; rMSSD, root mean square of successive differences between adjacent RR intervals. ‡HRV analyses additionally adjusted for thyroid stimulating hormone.

d)

Table A.2: Coping with stressful events at 3 year follow-up in relation to cardiac Troponin T (cTnT) cut-point, 4.2 ng/L, utilizing McNemar computations.

	Blacks: cTnT (≥ 4.2 ng/L)
	Δ % [Odds ratio (95% CI)]
High DefS (≥ 31)	1.88 [1.62 (0.81-3.23)]
Achievement events	0.42 [1.24 (0.50-2.34)]
Personal changes events	0.29 [0.75 (0.26-2.16)]
Social stress events	**3.46 [0.52 (0.26-1.05)],†**
Fate events	2.91 [2.14 (0.87-5.26)]

†, P = 0.06. Where: Δ=change over 3 years; DefS=defensive coping.

e)

Figure A.1: HRV 2D chart

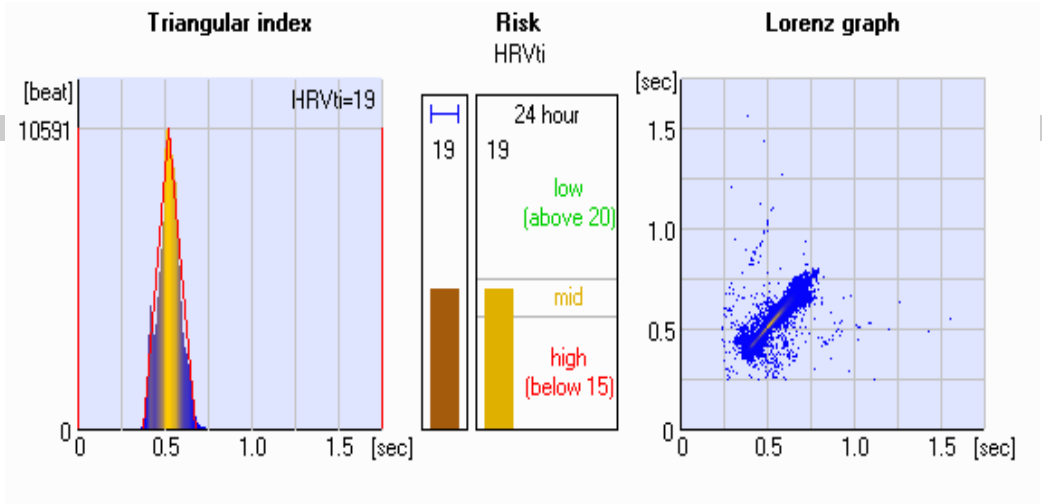

Figure A.2: HRV 3D Lorenz map

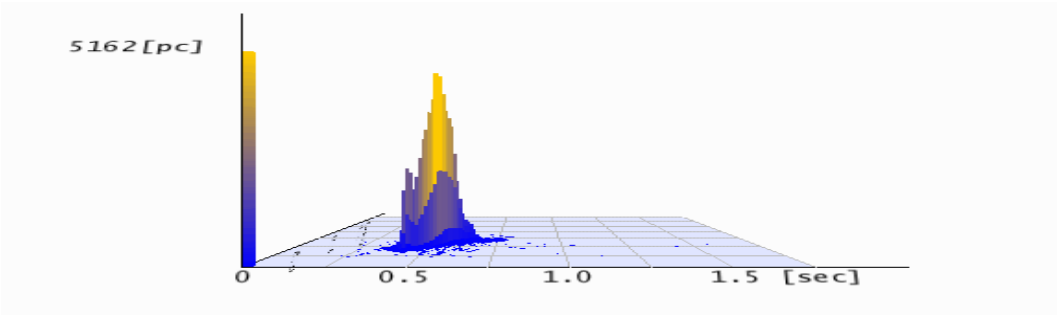

Figure A.1 – A.2: Non-linear analysis demonstrates dispersed complex-like pattern with 2-dimensional Poincaré plotting (Figure A.1) and 3-dimensional Lorenz mapping (Figure A.2) in a Black male with a composite profile of high defensive coping (DefS score = 31); hypertension (24h BP = 143/91 mmHg); moderately depressed HRV (SDNN = 73ms); medium cardiovascular risk (HRV triangular index = 9) and raised cTnT (mean 5.1ng/L).

f)

Table A.3: Fibrosis related to cardiac Troponin T, 5.6 ng/L, in a White cohort.

	Odds ratio	**95% CI**	**P value**
Cross-sectional (N=164)			
Potential cardiac risk			
Fibrosis (CRP:Fibrinogen ratio)	0.63	0.41-0.97	0.05

Adjustments were made for *a priori* covariates (age, log waist circumference, log physical activity, log cotinine, log GGT and hypertension medication use).