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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*

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## 1 ABSTRACT

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

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

11 **Results:** cTnT levels of the cohort remained similar over 3 years but recovery to cTnT-  
12 negative levels was higher in Blacks. Blacks showed moderate depression (45% vs. 16%) and  
13 24h hypertension (67% vs. 42%) prevalence compared to Whites. A receiver-operating-  
14 characteristics cTnT cut-point 4.2 ng/L predicting hypertension in Blacks was used as binary  
15 exposure measure in relation to outcome measures [AUC 0.68 (95% CI 0.60-0.76);  
16 sensitivity/specificity 63/70%;  $P \leq 0.001$ ]. In cross-sectional analyses, elevated cTnT was  
17 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-  
18 1.08);  $P \leq 0.02$ ] and depressed HRV [OR 2.19 (95% CI 1.09-4.41);  $P=0.03$ ] in Blacks, but not  
19 in Whites. At 3 year follow-up, elevated cTnT was related to attenuated urine  
20 norepinephrine:creatinine ratio in Blacks [OR 1.46 (95% CI 1.01-2.10);  $P=0.04$ ]. In Whites, a  
21 cut point of 5.6 ng/L cTnT predicting hypertension was not associated with outcome  
22 measures.

23 **Conclusion:** Central neural control systems exemplified a brain-heart stress pathway.  
24 Desensitization of sympatho-adrenal responses occurred with initial neural- (HRV) followed  
25 by neuroendocrine dysfunction (norepinephrine:creatinine) in relation to elevated cTnT.

1 Chronic defensiveness may thus drive the desensitization or *physiological depression*,  
2 reflecting ischemic heart disease risk at a 4.2 ng/L cTnT cut-point in Blacks.

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

4

## 1 1. INTRODUCTION

2 Coping with everyday stressors (Amirkhan, 1990) may disturb sympatho-adrenal activity  
3 and cardiac rhythmicity as indicated by changes in catecholamine turnover (de Kock et al.,  
4 2012) as well as heart-rate variability (HRV) (Malan et al., 2013). Particularly, defensive  
5 coping (DefS) or the *fight-flight response* encompassing perception of control and active  
6 problem solving, has been suggested as a promoter of health (Amirkhan, 1990). In spite of  
7 this view, DefS outcomes have also been linked with pathology and emotional distress  
8 related alterations (de Kock et al., 2012; Malan et al., 2013), in that attenuated sympatho-  
9 adrenal responses to acute mental stress in a cross-sectional analysis were associated with  
10 wall remodeling and silent myocardial ischemia in a Black male cohort (Malan & Malan,  
11 2017). Therefore, it seems plausible that chronic defensiveness, reflecting emotional  
12 distress, may drive direct relationships between sympatho-adrenal activation and markers  
13 of cardiac injury (Lazzarino et al., 2013) such as elevated cardiac troponin (cTnT) levels.  
14 A subunit of the troponin complex, namely cTnT is released in response to sympathetic  
15 activation or catecholamine overload and myocyte necrosis (Muthu et al., 2014). A  
16 decrease in the metabolic supply to the myocardial tissue results in ischemia and resultant  
17 cardiomyocyte necrosis of the myocardium (Muthu et al., 2014). If reduced metabolic  
18 supply is accompanied by catecholamine vascular responsiveness, myocardial ischemia  
19 and cTnT-related damage may further increase (Mazzeo et al., 2014; Muthu et al., 2014).  
20 Resultant changes in cardiac autonomic modulation and blood pressure may therefore  
21 occur to counteract myocardial ischemia, in order to improve perfusion. Accumulative  
22 effects of higher chronic metabolic demands may also be taxing if emotional distress is  
23 present (Malan et al., 2016) To maintain metabolic homeostasis, central neural control and  
24 downstream adrenergic-related signaling will be apparent with either  
25 sensitization/upregulation in acute or desensitization/downregulation in chronic situations

1 (Guilliams & Edwards, 2010). Therefore, we aimed to assess sympatho-adrenal outcome  
2 measures, including 24h urinary catecholamines, 24h heart-rate-variability (HRV), blood  
3 pressure and levels of coping and depression in a bi-ethnic cohort from South Africa.  
4 Sympatho-adrenal responses resembling emotional distress might translate to cTnT  
5 activity at a certain cut point indicative of future ischemic heart disease risk. Thus, the  
6 main aim was to examine prospective associations between binary exposure cTnT and  
7 sympatho-adrenal outcome measures.  
8

## 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), a history of 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 (*Supplementary Methods*) and sphygmomanometer blood pressure measurements were obtained and registered nursing staff collected overnight fasting blood samples after 07:00.

23

## 1 2.2 *Cardiovascular risk measures*

### 2 2.2.1 *Ambulatory BP and ECG monitoring (ABPM)*

3 ABPM devices were attached to participants' non-dominant arm (Meditech CE120  
4 CardioTens<sup>®</sup>; Meditech, Budapest, Hungary) by trained cardiovascular research personnel.  
5 The Cardiotens<sup>®</sup> was programmed to measure BP at 30-min intervals during the day (07:00–  
6 22:00) and every hour during night time (22:00–06:00). The successful 24h inflation rate was  
7 79% ( $\pm 12$ ) at baseline and 88% ( $\pm 9$ ) at follow-up. The data were analyzed with the  
8 CardioVisions 1.19 Personal Edition software (Meditech, Budapest, Hungary). Any incidents  
9 including visual disturbances, headaches, nausea, fainting, palpitations, physical activity and  
10 stress were to be recorded on a 24-hour diary card. Hypertensive status was classified as 24h  
11 SBP  $\geq 130$  mm Hg and/or DBP  $\geq 80$  mm Hg (Piepoli et al., 2016).

### 12 2.2.2 *Frequency and Time-domain heart-rate-variability (HRV) analyses*

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

1 Quantitative analyses of these plots have been associated with long-term 24h RR-interval  
2 variability (SD2) (Pizzi et al., 2008).

### 3 *2.2.3 Clinic blood pressure*

4 Participants were in a semi-recumbent position for 20-30 minutes prior to blood pressure  
5 measurements using the Riva-Rocci Korotkoff method by applying a suitable cuff on the  
6 non-dominant arm (Riester CE 0124® and 1.3M™ Littman® II S.E. Stethoscope 2205).

7 Two duplicate measurements were taken, with a 3- to 5-minute resting period between each;  
8 the second of which was used for statistical analyses. Hypertensive status was classified as  
9 SBP  $\geq$  140 mm Hg and/or DBP  $\geq$  90 mm Hg (Piepoli et al., 2016).

### 10 *2.3 Coping Strategy Indicator (CSI)*

11 The CSI is a 33-item self-report measure of coping responses with construct, convergent and  
12 discriminant validity (Amirkhan, 1990). Cronbach's alpha ( $\alpha$ ) reliability coefficients were  
13 determined for the three subscales of 11 items each in the SABPA cohort and ranged between  
14 0.61-0.87. DefS implies actively solving problems and in-control responses. Seeking social  
15 support supports DefS, with a focus on acquiring advice in stressful times and lastly,  
16 emotional avoidance or loss-of-control implies defeat, with psychophysiological withdrawal.  
17 When responding to the various questions of the three subscales, the participant had to keep a  
18 recent stressful event in mind. The responses were then rated on a three-point Likert scale: a  
19 lot (3), a little (2), or not at all (1). The events fell into four broad categories: achievement  
20 (work/school related problems); social stressors (interpersonal conflict); personal changes in  
21 psychophysiological or spiritual status; and fate events such as accidents and chance  
22 victimization (Amirkhan, 1990). Two research assistants sorted these events with an 88%  
23 agreement.

24

25

## 1 **2.4 Depression**

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

## 9 **2.5 Biochemical measurements**

### 10 **2.5.1 Urinary Catecholamines**

11 Urine collection was performed overnight, 8h sampling at baseline and 24h sampling at  
12 follow-up. The sampling periods of 8h and 24h compares favorably for detection of stress  
13 hormones in urine (Masi et al., 2004). At follow-up, participants began and ended sampling  
14 with an empty bladder on Day 1. Urine was collected for the next 24 hours in a three liter  
15 container, washed with 9 ml of 20% HCl (UriSet24, Sarstedt®, Nümbrecht, Germany).  
16 Samples were stored at  $-80^{\circ}\text{C}$  until analysis within one year after collection, using the 3-Cat  
17 Urine ELISA Fast Track kit (LDN, Nordhorn, Germany). Intra- and inter-assay coefficients  
18 for epinephrine were 5.50% and 9.62% respectively and for norepinephrine, 2.70% and  
19 8.59%. Urine creatinine was measured with the calorimetric method.

### 20 **2.5.2 Blood analyses**

21 A registered nurse obtained fasting blood samples from the ante-brachial vein branches with  
22 a sterile winged infusion set, and handled samples according to standardized procedures.  
23 Serum and whole blood EDTA samples were analyzed for lipids, high sensitivity C-reactive  
24 protein (CRP), cotinine (reflecting smoking status), gamma glutamyl transferase ( $\gamma$ -GT)  
25 (reflecting alcohol consumption) and glycated haemoglobin ( $\text{HbA}_{1c}$ ), using Unicel DXC 800,

1 Beckman and Coulter, USA; Modular ROCHE Automized, Switzerland and the Konelab™  
2 20I Sequential Multiple Analyzer Computer, ThermoScientific, Vantaa, Finland respectively.  
3 Citrate fibrinogen was measured by the Viscosity-based clotting method and the Immuno-  
4 turbidimetric method (STA Compact, TAGO Diagnostic, Roche; France). The  
5 CRP:fibrinogen ratio was used as marker of fibrosis (Jansen van Vuren et al., 2016). Thyroid  
6 stimulating hormone (TSH) and high sensitive cTnT were determined with  
7 electrochemiluminescence immunoassay (ECLIA), Elecsys 2010, Roche, Basel, Switzerland.  
8 Eighty cTnT values (23.39%) were below detectable limit (<3 ng/L) and substituted with  
9 lower than detectable values using log-methods. The cTnT inter- and intra-batch variability  
10 was 15% and 5.6%.

## 11 **2.6 Statistical analyses**

12 Statistica version 13 and IBM SPSS version 23 statistical software packages were used.  
13 Power analyses were performed to obtain relevant effect sizes based on differences in  
14 ambulatory autonomic dysfunction and biological profiles. Results showed that a sample size  
15 of 50 will demonstrate biological differences with a statistical power of 0.8, and significance  
16 level of 0.05. Variables with skewed distributions were log-transformed. Covariates were  
17 chosen *a priori* (Piepoli et al., 2016) including: age, waist circumference, physical activity,  $\gamma$ -  
18 GT, cotinine, hypertension medication as well as TSH for HRV analyses. Considering retinal  
19 perfusion deficits and the high hypertension prevalence in Blacks (Malan et al., 2016b)  
20 optimal cTnT cut points associated with clinic and ambulatory 24h hypertension were  
21 computed from the maximum of the Youden index (J) (sensitivity + specificity – 1) using  
22 non-parametric receiver operating characteristic (ROC) curves. General linear modelling was  
23 used to test a priori hypotheses (Ethnicity x Gender x cTnT) using the derived cTnT cut point  
24 as binary exposure measure for outcome risk markers, independent of *a priori* covariates and  
25 baseline values of the respective risk factors. Sympatho-adrenal outcome measures included

1 24h urinary catecholamines, 24h heart-rate-variability (HRV), blood pressure and levels of  
2 coping and depression. Independent *t*-tests and Chi-square ( $\chi^2$ ) tests compared sympatho-  
3 adrenal differences and proportions at baseline respectively. ANCOVA analyses compared  
4 sympatho-adrenal values at baseline and follow-up; as well as at follow-up in relation to  
5 elevated ROC cTnT cut point, controlling for *a priori* covariates and baseline value of the  
6 respective risk factors. Positive cTnT cases were determined at cut points 3ng/L and 4.2ng/L  
7 levels; Chi-square ( $\chi^2$ ) tests were used to examine changes in cTnT caseness between baseline  
8 and follow up (i.e., negative results at baseline becoming cTnT-positive at follow-up; and  
9 cTnT-positive people at baseline recovering to cTnT-negative at follow-up). McNemar's  
10 case-control tests were used to demonstrate changes in high DefS and stressful coping events  
11 over 3 years. Non-linear HRV analyses, using Poincaré and Lorenz plots, were recorded in a  
12 Black male having chronic depressed SDNN (<100ms), DefS ( $\geq 31$ ), 24h hypertension and  
13 raised cTnT.

14 Logistic regression analyses calculated odds ratios (OR) and 95% confidence intervals (CI) to  
15 determine if sympathy-adrenal outcome measures will increase the risk of raised cTnT. These  
16 analyses were performed to examine cross-sectional and longitudinal associations. For the  
17 latter we used the formula:  $\Delta \%: (\text{follow-up} - \text{baseline})/\text{baseline} * 100$ . Sympatho-adrenal  
18 outcome measures were added independently and in combination to multivariate models,  
19 considering baseline *a priori* covariates and TSH in HRV models. Sensitivity analyses were  
20 additionally computed to adjust for gender in HRV analyses and excluding HIV cases. The  
21 statistical significance level was set at  $p \leq 0.05$  (two-tailed).

22

### 1 3. RESULTS

#### 2 3.1 *Clinical characteristics*

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

8 In Figure 2, a receiver-operating-characteristics (ROC) cTnT cut-point of 4.2 ng/L predicted  
9 both clinic [AUC 0.64 (95% CI 0.55-0.72); sensitivity/specificity 63/64%;  $P \leq 0.002$ ] and  
10 ambulatory 24h hypertension [AUC 0.68 (95% CI 0.60-0.76); sensitivity/specificity 63/70%;  
11  $P \leq 0.001$ ] in Blacks. In Whites, the cTnT cut-point of 5.6 ng/L [AUC 0.67 (95% CI 0.58-  
12 0.75); sensitivity/specificity 53/78%;  $P \leq 0.001$ ] predicted ambulatory 24h hypertension only.  
13 An interaction term (ethnicity x cTnT) was fitted for DefS [F (1,324), 6.40;  $P = 0.01$ ] but not  
14 for the other coping strategies or depression. Ethnic differences were evident for cTnT, BP,  
15 time-domain HRV and the catecholamines ( $P \leq 0.001$ ).

16 Unadjusted (Table A.1) comparisons showed that Blacks consistently sought more social  
17 support compared to Whites. Blacks further had depressed HRV (lower time-domain SDNN,  
18 increased SDNN risk ( $\leq 100$ ms), lower geometric (HRVti) HRV patterns), higher fibrosis,  
19 blood pressure and lower catecholamine levels compared to Whites. In adjusted comparisons,  
20 a similar trend was observed in Blacks in relation to elevated cTnT (Table 2,  
21 Whites=reference group). Except for SDNN and HRVti, none of the other frequency or time-  
22 domain HRV measures showed significant cross-sectional and longitudinal differences or  
23 associations with elevated cTnT considering confounders and will not be discussed further.

1 cTnT levels remained similar in the bi-ethnic cohort over 3 years. In Table A.2, the recovery  
2 of cTnT-positive people at baseline to cTnT-negative at follow-up was more apparent at  
3 cTnT cut point < 3 ng/L in Blacks (49.7% in Blacks vs. 32.4% in Whites;  $p \leq 0.001$ ).

4 Coping with social stress or interpersonal conflict (Table A.3) showed increased changes  
5 (3.46%) over time in relation to elevated cTnT in Blacks [OR 0.52 (95% CI: 0.26-1.05),  
6  $P=0.06$ ].

7 A composite profile demonstrated a dispersed complex-like pattern with non-linear analysis,  
8 i.e. 2-dimensional Poincaré plotting (Figure A.1) and 3-dimensional Lorenz mapping (Figure  
9 A.2) in a Black male with high defensive coping (DefS score = 31); hypertension (24h BP =  
10 143/91 mmHg); moderately depressed HRV (SDNN = 73ms); medium cardiovascular risk  
11 (HRV triangular index = 9) and raised cTnT (mean 5.1 ng/L).

### 12 **3.2 Cross-sectional associations between sympatho-adrenal responses and elevated** 13 **cTnT**

14 In Table 3, an increased risk for cardiac injury at baseline was evident in Blacks when  
15 habitually using DefS ( $P=0.06$ ), accompanied by moderately depressed time-domain HRV  
16 responses ( $P=0.03$ ) and increased 24h BP (SBP, DBP) ( $P \leq 0.02$ ). Apart from fibrosis [OR  
17 0.63 (95% CI: 0.41-0.97);  $P=0.04$ ], elevated cTnT cut-point (5.6 ng/L) was not associated  
18 with any sympatho-adrenal responses in Whites (Table A.4).

### 19 **3.3 Follow-up associations between sympatho-adrenal responses and elevated cTnT**

20 At follow-up (Table 3), cardiac injury risk was related to desensitized  
21 norepinephrine:creatinine ratio [OR 1.46 (1.01-2.10);  $P=0.04$ ] in Blacks.

22 Sensitivity analyses adjusting for gender in HRV analyses and excluding HIV cases did not  
23 change the outcomes.

24

## 1 **4. DISCUSSION**

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

### 10 **4.1 *Chronic defensiveness reflecting cardiac injury***

11 Healthy coping strategies integrate central neural responses in order to cope with stress  
12 (Vaillant, 2011). Survival circuits and defense responses are activated when threatening  
13 conditions are detected in the amygdala and a general arousal state is generated in the  
14 paraventricular nuclei of the hypothalamus (Moscarello & LeDoux, 2013). This state is due  
15 to widespread fast acting neural-induced involuntary heart rate variations and the release of  
16 slower neuroendocrine aminergic neuromodulators (Moscarello & LeDoux, 2013; Thayer et  
17 al., 2012). Individual resiliency will thus depend on the capacity of the individual to recover  
18 from the stressor by means of healthy in-control coping strategies with a voluntary  
19 mobilizing of social support from friends or family (Amirkhan, 1990; Malan et al., 2017).  
20 Currently, the Black cohort sought more social support, which supports an in-control DefS  
21 response albeit increasing interpersonal conflict and social stress. Indeed, coping defensively  
22 with a social stressor showed a trend for elevated cTnT risk in the current Black cohort.  
23 Chronic DefS with social stress further showed susceptibility for physiological loss-of-  
24 control sympatho-adrenal responses. Previous findings support this notion as an urban-  
25 dwelling environment and accompanying acculturation was deemed a psychosocial stressor

1 (Malan et al., 1996). In 1018 Blacks from the North-West Province of South Africa, DefS  
2 and seeking social support were associated with loss-of-control cardiometabolic responses  
3 (Hamer & Malan, 2010). Our data additionally showed that chronic DefS further may  
4 increase susceptibility for cardiac injury at a cTnT 4.2 ng/L cut-point for Blacks opposed to a  
5 suggested cut point of 6ng/L, or lower, for stress-induced cardiomyopathy (Ramaraj et al.,  
6 2009). Interestingly, in Whites, the higher 5.6 ng/L cTnT cut-point predicting ambulatory  
7 hypertension was not related to disturbed sympatho-adrenal responses, and rather exemplified  
8 behavioral in-control DefS responses. Recently F-fluorodeoxyglucose PET/CTs showed  
9 increased amygdalar activity, which was associated with perceived stress and cardiovascular  
10 disease risk in 293 patients followed for 3.7 years (Tawakol et al., 2017). These findings are  
11 in line with our results in Blacks reflecting chronic defensiveness and cardiac injury over 3  
12 years. **Cardiac injury recovery was more apparent at cTnT levels below 3ng/L and support  
13 our findings that cTnT at  $\geq 4.2$ ng/L impeded recovery as concomitant neuroendocrine  
14 dysregulation occurred at these levels. The protective role of estradiol against silent  
15 myocardial events (Malan NT et al., 2017) might explain underlying mechanisms to cardiac  
16 injury recovery.**

#### 17 **4.2 Heart rate variation reflecting cardiac injury**

18 Chronic defensiveness or more emotional distress further increases higher metabolic demand  
19 and may disrupt homeostasis with activation of central neural control systems (Patil et al.,  
20 2015). In the current study, we demonstrated initial neural control where depressed HRV  
21 responses in relation to elevated cTnT were accompanied by BP increases as homeostatic  
22 reflexes. Indeed, estimators of sympathetic hyperactivity (SDNN <100ms and HRVti) levels  
23 were increased in the Black cohort compared to Whites, further underscoring depressed HRV  
24 and risk for cardiac injury in Blacks.

1 The limbic system modulates cardiac activity in response to stressful events and during  
2 homeostatic reflexes (Manea et al., 2015). Sympathetic nervous system (SNS) hyperactivity  
3 may thus serve as a pathophysiological event affecting the reciprocal relationships between  
4 limbic responses, sympathovagal disturbances and raised cTnT. The possibility of  
5 neurocardiogenic injury emerges as a consequence of uncontrolled stress and subsequent  
6 catecholamine overload. Indeed, DefS induces SNS hyperactivity and supports the initial  
7 neural control (depressed HRV, BP increases or hypertensive status) profile at baseline,  
8 reflecting a hypervigilant system. The occurrence of myocardial ischemia and/or myocardial  
9 necrosis during chronic defensiveness may further facilitate a mechanical distortion of the  
10 afferent and efferent fibers of the autonomic nervous system. This may be due to changes in  
11 the geometry related to necrotic and non-contracting segments of the heart (Mazzeo et al.,  
12 2014). Changes will also affect local neural regulation and contribute to the resulting  
13 diminished HRV. However, contradictory findings were recently reported (Jandackova et al.,  
14 2016). In the UK Whitehall II population-based cohort study, cardiac autonomic modulations  
15 in 3414 men and women obtained over 10 years and at 3 different time points, were not  
16 associated with a higher prevalence of cardiometabolic symptoms but rather with normal  
17 ageing (Jandackova et al., 2016).

#### 18 **4.3 *Neuroendocrine dysfunction reflecting cardiac injury***

19 Pertaining to neuroendocrine risk, we could not find any bi-ethnic study reporting a  
20 longitudinal relationship between the catecholamines and cardiac injury. Our findings support  
21 the presence of a novel brain-heart stress pathway. Chronic sympatho-adrenal activation  
22 facilitated a later onset of neuroendocrine control with desensitization of  
23 norepinephrine:creatinine in relation to elevated cTnT in a Black cohort. Previously we have  
24 established that the trigger enzyme for synthesis of the catecholamines, tyrosine hydroxylase  
25 C-824T single nucleotide polymorphism, was not related to hypertension in this Black cohort

1 (van Deventer et al., 2013). Therefore, our findings rather support the role of environmental  
2 or social stress demands, which elicited sympatho-adrenal responses in relation to elevated  
3 cTnT in Blacks. The risk of an attenuated norepinephrine:creatinine ratio and elevated cTnT  
4 levels suggests that overwhelming sustained stress may interfere with behavioral in-control  
5 DefS. It may cause distress and hyperactivity of the sympatho-adrenal system, as the body  
6 attempts to cope with increasing demands. Two mechanisms may apply: norepinephrine  
7 levels may a) decrease due to exhaustion of the autonomic system (Guilliams & Edwards,  
8 2010) where adrenal exhaustion or fatigue will set in, and b) the neuroendocrine system will  
9 become non-responsive with desensitization and/or down-regulation of adrenergic receptors,  
10 which has been associated with depression and neural fatigue (Tsigos & Crousos, 2002).  
11 The lack of association between chronic depressive symptoms and cardiac injury was  
12 therefore surprising. Our findings rather support dissociation of DefS responses where  
13 behavioral in-control responses *masked* physiological loss-of-control or desensitized  
14 responses (*physiological depression*). This may have a detrimental impact on cardiac health  
15 in the long-term and may increase susceptibility for ischemic heart disease.

#### 16 **4.4 Blood pressure as compensatory mechanism to counteract perfusion deficits and** 17 **cardiac injury**

18 Coping defensively with overwhelming social stress (Rosengren et al., 2004) may have  
19 induced BP increases in relation to elevated cTnT at baseline. Concurrently, the hypertension  
20 prevalence rate in the Whites was lower (42%) compared to the Blacks (67%) with no change  
21 in hypertension prevalence in the Black Africans at follow-up. Findings from the  
22 INTERHEART (Rosengren et al., 2004) and Jackson studies (Abdalla et al., 2016) in  
23 African-Americans demonstrated similar findings as social stress was related to myocardial  
24 ischemia and elevations in cTnT respectively. Interestingly, the ambulatory hypertension  
25 status of 59% African-Americans from the Jackson study (Abdalla et al., 2016) compares

1 reasonably well with the 67% of our cohort. We, therefore, argue that evidence of vascular  
2 resistance in Blacks (Malan et al., 2012) and African-Americans (Adefurin et al., 2013) might  
3 be driven by chronic defensiveness or a disturbed brain-heart stress response pathway.  
4 Indeed, vascular resistance increases cardiac preload in essential hypertensives (Adefurin et  
5 al., 2013) and impeded coronary perfusion in a Black cohort (Griffiths et al., 2017).  
6 Inevitable this may increase myocardial oxygen demand, which may result in proteolysis and  
7 release of cTnT and increases in blood pressure. Ultimately, loss-of-control DefS in the  
8 Blacks facilitated cardiac injury. Previously, the SABPA Whites showed acute emotional  
9 stress at follow-up (Malan et al., 2016), which aligns well with the current effective in-  
10 control DefS where elevated cTnT was not related to sympatho-adrenal responses. Our  
11 findings further suggest that targeting novel markers to explain hypertension prevalence in  
12 the African ethnicity should rather consider central neural control or brain-heart stress  
13 responses. When chronic conditions such as cardiac injury prevail, dynamic compensatory  
14 BP increases may trigger central control mechanisms to maintain homeostatic reflexes in  
15 organ systems.

16 The small sample size prohibited stratification into both ethnic and gender groups thereby  
17 limiting an improved understanding of cardiac injury pathology. Therefore expanding data to  
18 other cohorts may confirm findings of a 4.2 ng/l cTnT cut-point when chronic defensiveness  
19 is suspected. Early screening for raised cTnT has clinical practice implications as raised cTnT  
20 is shown to be related to neural distress, which may precede ischemic heart disease and future  
21 heart failure risk.

22 In conclusion, a novel brain-heart stress pathway exemplified desensitized sympatho-adrenal  
23 responses in relation to cardiac injury. Initial desensitized neural- followed by  
24 neuroendocrine dysfunction reflected a chronic *physiological depression*. Dissociation of  
25 behavioral control (DefS) albeit physiological loss-of-control responses were demonstrated in  
26 the Blacks. Chronic defensiveness may have contributed to loss-of-control neuroendocrine

1 responses, facilitating cardiac injury at a 4.2 ng/l cTnT cut-point. Screening for stress-related  
2 cardiac injury at cTnT levels of  $\geq 4.2$  and ECG ST-segment depression may allow detection  
3 of the early dynamics in ischemic heart disease development.

4

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7 ***FINANCIAL DISCLOSURES***

8 No conflicts of interest are declared. Any opinion, findings and conclusions or  
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16

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## 5. 1 REFERENCES

- 2 Abdalla, M., Booth, J.N., Seals, S.R. et al., 2016. Masked hypertension and incident clinic  
3 hypertension among Blacks in the Jackson Heart Study. *Hypertension* 68, 220-226.
- 4 Adefurin, A., Ghimire, L.V., Kohli, U. et al., 2013. African-Americans have a greater  
5 sensitivity to alpha<sub>1</sub>-adrenoceptor-mediated vasoconstriction compared to Caucasians.  
6 *Hypertension* 61, 915-920.
- 7 Amirkhan, J.H., 1990. A factor analytically derived measure of coping: The Coping Strategy  
8 Indicator. *J Pers Soc Psych* 59, 1066–1074.
- 9 De Kock A, Malan L, Hamer M et al., 2012. Defensive coping and subclinical vascular  
10 disease risk - associations with autonomic exhaustion in Africans and Caucasians: the  
11 SABPA study. *Atherosclerosis* 225, 438-443.
- 12 Griffiths, M., Malan, L., Delport, R., et al. 2017. Lower high-sensitivity cardiac Troponin T  
13 cut-points in Blacks predicted 24h systolic hypertension: the SABPA study. *Eur J Prev*  
14 *Cardiol* DOI:10.1177/2047487317694465.
- 15 Guilliams, T.G., Edwards, L. 2010. Chronic stress and the HPA-axis: Clinical assessment and  
16 therapeutic considerations. *The Standard* 9, 1-12.
- 17 Hamer, M., Malan, L., 2010. Psychophysiological risk markers of cardiovascular disease. In:  
18 *Psychophysiological Biomarkers of Health. Special Edition: Neurosci Biobehav Rev* 35,  
19 76-83.
- 20 Jandackova, V.K., Scholes, S., Britton, A., Steptoe, A., 2016. Are changes in heart rate  
21 variability in middle-aged and older people normative or caused by pathological  
22 conditions? Findings from a larger population-based longitudinal cohort study. *J Am Heart*  
23 *Ass* 1:e002365.
- 24 Jansen van Vuren, E., Malan, L., Cockeran, M., et al., 2016. Fibrosis and reduced perfusion -  
25 a cardiovascular disease risk: The SABPA study. *J Clin Exp Hypertens* 38, 482-8.

- 1 Kroenke, K., Spitzer, R.I., 2002. The PHQ-9: a new depression diagnostic and severity  
2 measure. *Psychiatr Ann*, 1-7.
- 3 Lazzarino, A.I., Hamer, M., Gaze, D. et al., 2013. The association between cortisol response  
4 to mental stress and high-sensitivity cardiac Troponin T plasma concentration in healthy  
5 adults. *J Am Coll Cardiol* 62, 1694-1701.
- 6 Malan, L., Hamer, M., Frasura-Smith, N. et al., 2015. COHORT PROFILE: Sympathetic  
7 activity and Ambulatory Blood Pressure in Africans (SABPA) Prospective Cohort Study.  
8 *Int J Epidemiol* 2015, 1814-1822.
- 9 Malan, L., Hamer, M., Schlaich, M. et al., 2013. Defensive coping facilitates higher blood  
10 pressure and early sub-clinical structural vascular disease via alterations in heart rate  
11 variability: the SABPA study. *Atherosclerosis* 227, 391-7.
- 12 Malan, L., Hamer, M., Schlaich, M. et al., 2012. Facilitated defensive coping, silent  
13 ischaemia and ECG left-ventricular hypertrophy: the SABPA study. *J Hypertens* 30, 543-  
14 550.
- 15 Malan, L., Malan, N.T., 2017. Emotional Stress as a Risk for Hypertension in Sub-Saharan  
16 Africans: Are We Ignoring the Odds? In: Islam, M. (Ed), *Advances in Experimental*  
17 *Medicine and Biology*. Springer: Switzerland, pp. 1-14.
- 18 Malan, L., Hamer, M., von Känel, R. et al., 2016. Chronic depression symptoms and salivary  
19 NOx are associated with retinal vascular dysregulation: the SABPA study. *Nitric Oxide –*  
20 *Biol Chem* 55-56, 10-17.
- 21 Malan, L., Malan, N.T., Wissing, M.P. et al., 2008. Coping with urbanization: A  
22 *Cardiometabolic Risk?* *Biol Psych* 79, 323-328.
- 23 Malan, N.T., Brits, J.S., Eloff, F.C. et al., 1996. The influence of acculturation on endocrine  
24 reactivity during acute stress in urban black males. *Stress Med* 12, 55-63.
- 25

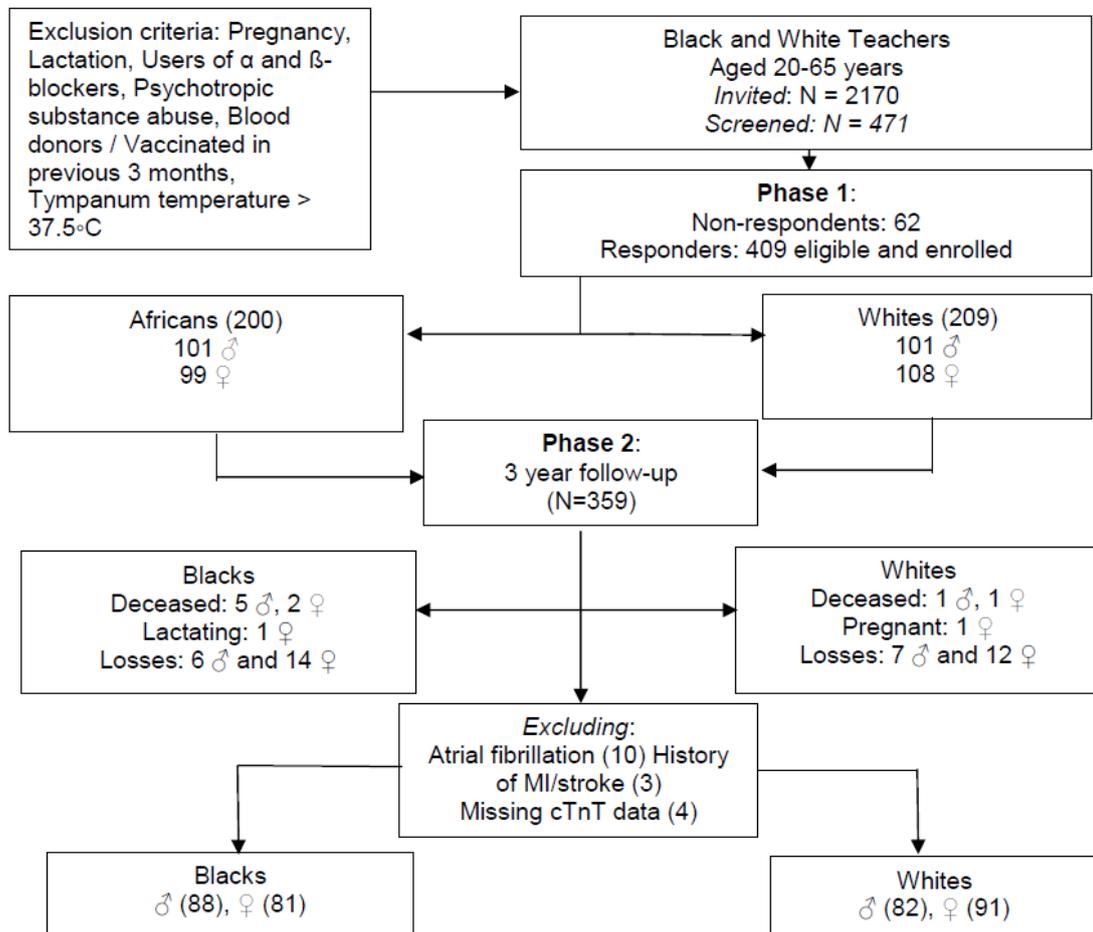
- 1 Malan NT, von Känel R, Kruger R et al., 2017. The protective role of estradiol against silent  
2 myocardial events and hypertensive risk in a Black male cohort: the SABPA Prospective  
3 study. *Inter J Cardiol.* S0167-5273(17)32152-6, DOI:10.1016/j.ijcard.2017.06.025.
- 4 Manea, M., Comsa, M., Minca, A., et al., 2015. Brain-heart axis - Review Article. *J Medicine*  
5 *Life* 8, 266-271.
- 6 Masi, C.M., Rickett, E.M., Hawkey et al., 2004. Gender and ethnic differences in urinary  
7 stress hormones: the population-based Chicago Health, Aging, and Social Relations Study.  
8 *J App Phys* 97, 941-947.
- 9 Mazzeo, A.T., Micalizzi, A., Mascia, L., et al. 2014. Brain–heart crosstalk: the many faces of  
10 stress-related cardiomyopathy syndromes in anaesthesia and intensive care. *Br J Anaesth*  
11 112, 803-815.
- 12 Monohan, P.O., Schacham, E., Reece, M. et al., 2009. Validity/reliability of PHQ-9 and PHQ-  
13 2 depression scales among adults living with HIV/AIDS in Western Kenya. *J Gen Intern*  
14 *Med* 24, 189-197.
- 15 Moscarello, J.M., LeDoux, J.E., 2013. Active avoidance learning requires prefrontal  
16 suppression of amygdala-mediated defensive reactions. *J Neurosci* 33, 3815–3823.
- 17 Muthu, V., Kozman, H., Liu, K. et al., 2014. Cardiac troponins: bench to bedside  
18 interpretation in cardiac disease. *Am J Med Sci* 347, 331-337.
- 19 Patil, K., Singh, M., Singh, G. et al., 2015. Mental stress evaluation using heart rate  
20 variability analysis: a review. *Int J Public Mental Health* 2, 2394-4668.
- 21 Piepoli, M.F., Hoes, A.W., Agewall, S. et al., 2016. European Guidelines on cardiovascular  
22 disease prevention in clinical practice. The Sixth Joint Force of the European Society of  
23 Cardiology and other Societies on cardiovascular disease prevention in clinical practice.  
24 *Eur Heart J* 5, 1-78.

- 1 Pizzi, C., Manzoli, L., Mancini, S. et al. 2008. Analysis of potential predictors of depression  
2 among coronary heart disease risk factors including heart rate variability, markers of  
3 inflammation, and endothelial function. *Eur Heart J* 29, 1110-1117.
- 4 Ramaraj, R., Sorrell, V.L., Movahed, M.R., 2009. Levels of troponin release can aid in the  
5 early exclusion of stress-induced (takotsubo) cardiomyopathy. *Exp Clin Cardiol* 14, 6-8.
- 6 Rosengren, A., Hawken, S., Ounpuu, S. et al., 2004. INTERHEART investigators.  
7 Association of psychosocial risk factors with risk of acute myocardial infarction in 11119  
8 cases and 13648 controls from 52 countries (the INTERHEART study): case-control  
9 study. *Lancet* 364, 953-962.
- 10 Tawakol, A., Ishai, A., Takx, R.A.P. et al., 2017. Relation between resting amygdalar activity  
11 and cardiovascular events: a longitudinal and cohort study. *Lancet* 389, 834-845.
- 12 Thayer, J.F., Åhs, F., Fredrikson, M. et al., 2012. A meta-analysis of heart rate variability and  
13 neuroimaging studies: Implications for heart rate variability as a marker of stress and  
14 health. *Neurosci Biobehav Rev* 36, 747-756.
- 15 Tsigos, C., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine  
16 factors and stress. *J Psychosom Res* 53, 865-871.
- 17 Vaillant, G.E., 2011. Involuntary coping mechanisms: a psychodynamic perspective.  
18 *Dialogues Clin Neurosci* 13, 366-370.
- 19 Van Deventer, C., Van der Westhuizen, F.H., Louw, R. et al., 2013. Tyrosine hydroxylase  
20 polymorphism and hypertension in a selected South-African population: the SABPA  
21 Study. *Clin Exp Hypertens* 35, 614-619.

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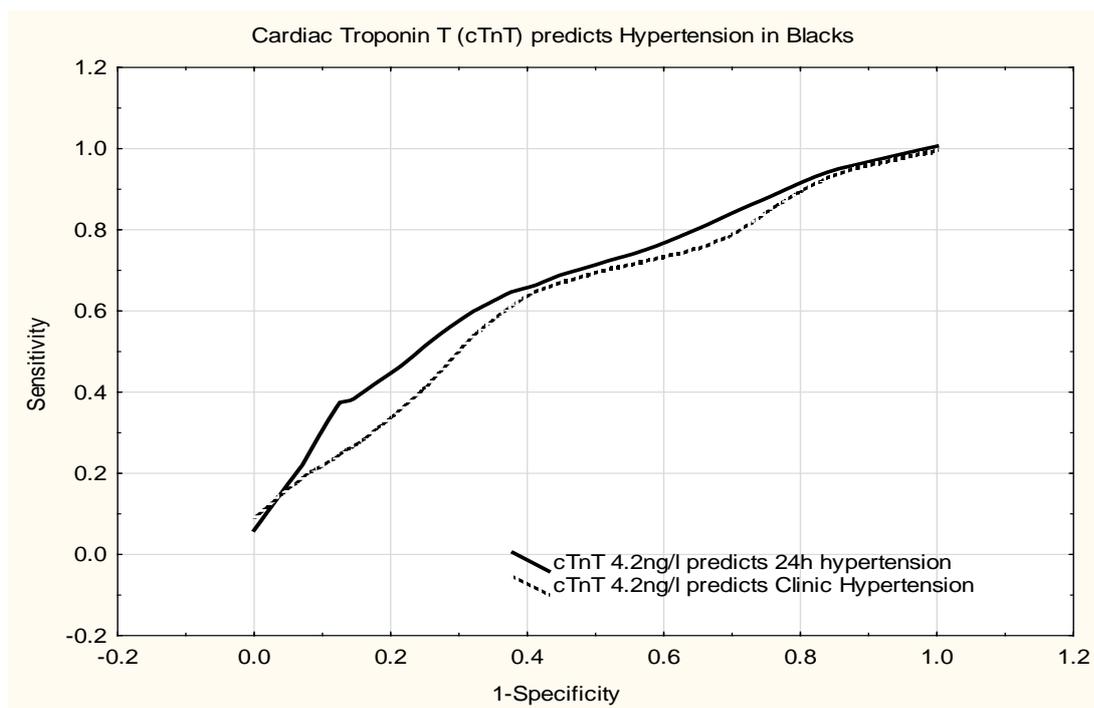


3

4 **Figure 1:** Design of the bi-ethnic gender cohort of the Sympathetic Activity and Ambulatory

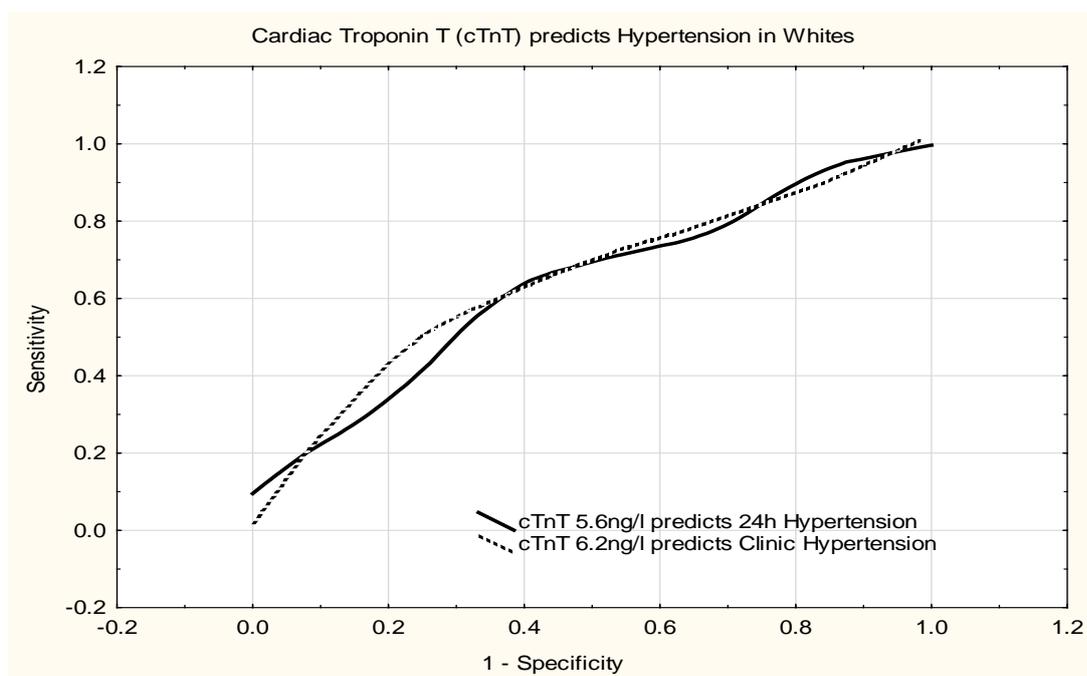
5 Blood Pressure in Africans prospective study. Where: cTnT=cardiac troponin T at baseline.

6



1

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



7

1 **Figure 2b:** ROC curves depicting cardiac Troponin T cut points for clinic and 24h  
2 hypertension in Whites. The area under the curve (AUC) (95% CI) for 24h  
3 hypertension was 0.67 (0.58-0.75); sensitivity/specificity 53/78% ( $P \leq 0.001$ ); with an  
4 AUC for clinic hypertension of 0.65 (95% CI 0.56-0.74); sensitivity/specificity  
5 52/74%;  $P \leq 0.01$ .  
6

**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
<b>Coping scores</b>			
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
<b>Moderately severe depression, n (%)</b>	61 (36.09)	22 (12.72)	< 0.001
<b>Heart Rate Variability (HRV)</b>			
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, μIU/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 (%) <sup>a</sup>	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	-

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1 Values are median ( $\pm$  interquartile range/IQR) or frequencies (%). Where: cGGT=gamma glutamyl  
2 transferase; moderately depressed=PHQ-9= $\geq$ 10; CRP=C-reactive protein; HDL=high density  
3 lipoproteins; HIV=Human Immune-deficiency virus infected; SDANN=standard deviation of all the  
4 5 minutes normal RR intervals (NN); SDNN=Standard deviation of RR interval; rMSSD=the square

1 root of the mean squared difference of successive NNs; NE:Cr=norepinephrine creatinine ratio;

2 E:Cr=epinephrine creatinine ratio.

3 <sup>a</sup>Hypertensive status classified as 24h SBP  $\geq$  130 mm Hg and/or DBP  $\geq$  80 mm Hg.<sup>10</sup>

4

- 1 **Table 2:** Comparing sympatho-adrenal response mean changes in Blacks vs. Whites  
 2 (reference group) in relation to cardiac troponin T (cTnT) cut points at 3 year follow-up.

	<b>Blacks vs. Whites (reference group)</b>	
	<b>cTnT cut point: 4.2 ng/L</b>	<b>cTnT cut point: 5.6 ng/L</b>
<b>Depression</b>		
Depressive symptoms	-0.11 (0.10)	-1.62 (0.09)
<b>Coping scores</b>		
Defense coping	0.27 (0.06)	1.54 (0.17)
<b>Heart rate variability (HRV)<sup>a</sup></b>		
HRV:SDNN, ms	<b>-23.58 (0.71)**</b>	<b>-22.58 (1.73)*</b>
HRV:Triangular index	<b>-4.35 (0.17)*</b>	-4.97 (0.47)
<b>Potential cardiac and neuroendocrine risk markers</b>		
cTnT, ng/L	1.76 (0.09)	1.15 (0.20)
Fibrosis	-0.15 (0.04)	-0.87 (0.12)
24h SBP, mmHg	<b>6 (0.19)**</b>	<b>7 (0.59)*</b>
24h DBP, mmHg	<b>3 (0.11)**</b>	3 (0.33)
24h NE:Cr, nmol/l	<b>-18.25 (0.56)**</b>	<b>-18.18 (0.99)**</b>
24h E:Cr, nmol/l	<b>-3.20 (0.09)**</b>	<b>-3.13 (0.14)**</b>

- 3 Data presented as means  $\pm$  SEM (standard error of means). Adjustments were made for *a*  
 4 *priori* covariates (age, log waist circumference, log physical activity, log cotinine, log GGT  
 5 and hypertension medication use) and baseline value of the respective risk marker.  
 6 SDNN=Standard deviation of RR interval; NE:Cr=urinary norepinephrine creatinine ratio;  
 7 E:Cr=urinary epinephrine creatinine ratio.

8 <sup>a</sup>Additional adjustment for thyroid stimulating hormone. \*P  $\leq$  0.05; \*\*P  $\leq$  0.01.

9

1 **Table 3:** Sympatho-adrenal responses in relation to cardiac Troponin T (cTnT) cut-point ( $\geq$ )  
 2 4.2 ng/L) in a Black cohort.

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

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

7