

Silent myocardial ischemia, cardiac troponins and target organ damage in a bi-ethnic sex cohort: the SABPA study

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Summary

TITLE

Silent myocardial ischemia, cardiac troponins and target organ damage in a bi-ethnic sex cohort:
the SABPA study

MOTIVATION

Cardiovascular disease (CVD) prevalence is escalating and influences general health and well-being. Blacks were more at risk of developing CVD and coronary artery disease (CAD). In addition, silent myocardial ischemia (SMI), which underscores the ischemic burden of CAD and CVD, is an underestimated health risk and unravelling the presence of SMI and reduced perfusion to coronary circulation and the myocardium may therefore be important in Blacks from South Africa. This ethnic group is also more likely to be diagnosed with symptomatic occlusive vascular disease. The determination of the CVD risk factors, insulin resistance (IR) and elevated high-sensitivity cardiac Troponin T (hs-cTnT) for prediction of SMI events in separate ethnic groups, can underpin the prognostic relevance of SMI, and contribute to prognostic screening in healthcare practice. In addition, considering subclinical atherosclerosis and conduction disturbances in relation to these risk markers, may further inform the medical community on the CAD and CVD burden in this cohort.

AIMS

The main aim of this SABPA (Sympathetic activity and Ambulatory Blood Pressure) sub-study was to indicate the prevalence of SMI in an urban South African bi-ethnic sex cohort and its association with hs-cTnT. In addition, to predict the resulting compensatory hypertension, the effect of hs-cTnT was researched. Furthermore, assessing the risk markers IR and hs-cTnT to determine the possible emerging mechanism of SMI was also explored, since possible racial disparity may be evident. When taking conduction disturbances into account, the association between CVD risk

markers hs-cTnT, and IR in relation to subclinical atherosclerosis improved our understanding of myocardial ischemia and CVD burden.

METHODOLOGY

The SABPA is a target population study which encompassed 409 urban black and white South African teachers from the Dr Kenneth Kaunda Education district situated in the North West province, South Africa (13). The cohort ensured homogeneity with regards to socio-economic status and working environment, although diverse cultural backgrounds could not be accounted for. Eligible participants aged between 20 and 65 years partook in this study. The following individuals were excluded for the current sub-study: pregnant or lactating women, individuals who had donated blood or had vaccinations less than 3 months prior to the onset of data collection, dependence on or abuse of psychotropic substances and individuals with a history of any cardiac events or myocardial infarction (heart attack) (n=5). The final cohort used for this sub-study included 198 Blacks and 206 Whites (N=404). For *Manuscript 2*, clinically diagnosed individuals with diabetes (n=17) were excluded. Human immunodeficiency virus- (HIV) infected individuals (n=18) were excluded for *Manuscript 3*. Groups were stratified according to ethnicity and sex after interaction terms were fitted for CVD risk.

Cardiometabolic variables included in this study were: body surface area (BSA), cholesterol, glycated haemoglobin A_{1c} (HbA_{1c}), glucose, homeostasis model of assessment (HOMA IR), high sensitivity C-reactive Protein (hs-CRP), plasma γ -glutamyl transferase (γ -GT), plasma cotinine levels, hs-cTnT and blood pressure (BP). Left carotid intima-media thickness (L-CIMT) of the far wall, left cross sectional wall-area (L-CSWA) and the lumen diameter of the left carotid indicated subclinical atherosclerosis. SMI was automatically detected by 24-hour (24-h) ambulatory electrocardiogram (ECG) measurement, where 1st degree atrioventricular-block (AV block) was determined with 6 cardiac cycles of 12-lead ECG.

Means and proportion were calculated with student T-tests, analysis of covariance (ANCOVA) as well as Chi squares (X^2). Multivariate linear regression analysis determined associations between major variables where receiver-operating characteristic (ROC) curves established cut-points for exacerbated CVD risk.

RESULTS AND CONCLUSIONS:

The results and conclusions of the three manuscripts are as follows:

1. Troponin T release is associated with silent myocardial ischaemia in black men: the SABPA Study

Significant differences were evident in hs-cTnT and its relation with SMI and hypertension in Blacks and Whites from this cohort. These findings indicate that hs-cTnT can possibly be a potential proficient marker of SMI and increases in compensatory systolic blood pressure (SBP). A lower hs-cTnT cut-point ≥ 4.2 pg/ml for 24-h systolic hypertension was predicted in Blacks compared to ≥ 5.6 pg/ml in Whites with a respective sensitivity/specificity of 64/68% and 61/71%. The SBP rises to alleviate myocardial ischemia in the Blacks and risk-factor clustering. The results also underscored the need for ethnic-specific reference values of hs-cTnT, which in turn should be interpreted in consideration of existing risk factors. In-depth assessment of hs-cTnT can thus be a useful improvement in risk prediction research.

2. Silent ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study

Insulin resistance (IR) as determined by elevated homeostasis model of assessment (HOMA IR) levels, was positively related to longer SMI events over 24 hours in White sex groups. Furthermore, White men also showed significant relations between IR and more frequent SMI events. Our findings suggested that IR increases are related to metabolic susceptibility leading to the development of SMI in the Whites but not necessarily in the Blacks. However, the Black men

presented positive associations between hs-cTnT and more frequent and longer SMI events. Findings in Blacks implied a cardiovascular susceptibility to develop ischemic heart disease and underscoring ethnic-related mechanism which could diagnose emergent SMI.

3. Troponin T release is associated with subclinical atherosclerosis in Blacks with first degree AV-block: the SABPA study

In Blacks presenting 1st degree AV-block, elevations in hs-cTnT were positively associated with subclinical atherosclerosis. Similar associations were not evident in Whites. First (1st) degree AV-block in Blacks reflected increases in hs-cTnT and enhanced susceptibility for a compensatory high blood pressure system resulted in carotid hypertrophic remodelling. Thus, by identifying conduction disturbances and perfusion deficits in early screening programs a contribution can be made to CVD prevention programs.

GENERAL CONCLUSION

In Blacks from this cohort, a higher cardiometabolic susceptibility to develop CVD was revealed. This was accentuated with lower hs-cTnT cut-points to predict compensatory SBP hypertension. In addition, when considering electrical conduction disturbances, the use of hs-cTnT cut-points may contribute to the preventive cardiology.

In Whites, IR underscored the development of ischemic heart disease more so than hs-cTnT, and can be translated to health care practice.

The adverse CVD risk in a South African Black cohort is concerning and the contribution of risk factors due to urbanization (poor nutrition, lower activity levels, obesity, alcohol abuse and smoking) further exacerbated a CVD burden.

KEY WORDS

Silent myocardial ischemia, troponin T, insulin resistance, subclinical atherosclerosis, First degree AV-block.

Opsomming

TITEL

Stille miokardiale iskemie, kardiaale troponien en teiken orgaan-skade in 'n bi-etniese kohort: die SABPA-studie

MOTIVERING

Die voorkoms van kardiovaskulêre siekte (KVS) bly beduidend toeneem en beïnvloed algemene gesondheid en welstand. Dit is bewys dat Swartes 'n hoër risiko loop om KVS en koronêre arteriële siekte (KAS) te ontwikkel. Stille miokardiale iskemie (SMI), wat die iskemiese las van KAS en KVS onderskryf, is 'n gesondheidsrisiko wat onderskat word en die vroeë ontknoping van SMI en die verminderde perfusie na koronêre sirkulasie, asook die miokardium, kan van waarde wees in Swartes van Suid-Afrika. Hierdie etniese groep is ook meer geneig om gediagnoseer te word met simptomatiese okklusiewe vaskulêre siekte. Die meet van die risikofaktore insulien weerstandigheid (IW) en hoë sensitiewe kardiaale Troponien T (hs-cTnT) om SMI voorvalle in die verskillende bi-etniese groepe te voorspel, kan moontlik die bepaling van verskille tussen rasse onderskryf en kan ook die prognostiese relevansie van SMI bevestig. Dit sal ook bydra tot prognostiese sifting in gesondheidsorg beoefening. Daarbenewens, deur subkliniese aterosklerose en geleidingsontwrigting in ag te neem in verband met hierdie risikomerkers, kan die KAS en KVS las in hierdie kohort verklaar.

DOELSTELLINGS

Die hoof doelstelling van hierdie SABPA (Simpatiese aktiwiteit en Ambulatoriese Bloeddruk in Afrikane) sub-studie was om aan te dui wat die voorkoms van SMI in 'n stedelike Suid-Afrikaanse bi-etniese kohort is. Gepaard hiermee, om die gevolglike kompensatoriese hipertensie te voorspel, was die effek van hs-cTnT nagevors. Daarbenewens, was die gebruik van die risikomerkers IW en hs-cTnT nagevors, om die moontlik ontluikende meganisme van SMI te bepaal deurdat daar 'n etniese verskil moontlik bestaan. Wanneer geleidingsontwrigting in ag geneem word, het die

verband tussen KVS-risikomerkers hs-cTnT en IW in verband met subkliniese aterosklerose, bygedrae tot 'n beter verstaan van miokardiale ischemi en die KVS las.

METODOLOGIE

Die SABPA-studie was 'n teikenpopulasie-studie wat 409 stedelike swart en wit Suid-Afrikaanse onderwysers, afkomstig van die Dr Kenneth Kaunda Onderwys distrik in die Noordwes provinsie, Suid-Afrika (13) ingesluit het. Hierdie kohort het homogeniteit met betrekking tot sosio-ekonomiese status en werkomgewing gewaarborg, maar kon nie diverse kulturele agtergronde verklaar nie. Wenslike deelnemende individue wat deelgeneem het aan die studie was tussen die ouderdomme 20 en 65 jaar. Die volgende individue was van die studie uitgesluit: swanger of lakterende vroue, individue wat bloed geskenk of inentings ontvang het minder as 3 maande voor die aanvang van data-insameling, afhanklikheid of misbruik van psigotropiese middels asook individue wat 'n geskiedenis het van enige kardiaale voorvalle en miokardiale infarsie (n=5). Die finale kohort wat gebruik is vir hierdie sub-studie sluit in 198 Swartes en 206 Wittes (n=404). Vir *manuskrip 2*, is klinies gediagnoseerde individue met diabetes (n=17) uitgesluit., Vir *Manuskrip 3*, is menslike immunitetsgebrekvirus- (MIV) geïnfecteerde individue (n=18) uitgesluit. Groepe was ingedeel ooreenkomstig etnisiteit en geslag, soos aangedui deur statisties betekenisvolle interaksierme te pas vir KVS-risiko.

Kardiometaboliese veranderlikes wat by hierdie studie ingesluit was, is: liggaamsoppervlaks-area, cholesterol, glikeerde hemoglobien A_{1c} (HbA_{1c}), glukose, homeostase-model van assessering (HOMA IR), hoë sensitiewe C-reaktiewe proteïen (hs-CRP), plasma γ -glutamiel transferase (γ -GT), plasma-kotonienvlakke, hs-cTnT en bloeddruk (BD). Linker karotis intima-media verdikking (L-CIMT) van die ver wand, die deursnit van die linker wandarea (L-CSWA) en die lumen diameter van die linkerkarotis het subkliniese arteriosklerose aangedui. SMI was outomaties bepaal aan die hand van die 24-uur ambulatoriese elektrokardiogram- (EKG) meting. Eerstegraadse atrioventrikulêre blok (AV-blok) is bepaal deur 6 kardiaale siklusse van die 12-afleiding EKG.

Gemiddeldes en proporsies was bereken deur middel van studente- *T*-toetse, analise van kovariansie (ANCOVA) en Chi-kwadraattoetse (X^2). Meerveranderlike lineêre regressie-analise het die assosiasies van hoofveranderlikes bepaal waar die "receiver operating characteristics"- (ROC) kurwes die afsnypunt bepaal het vir die verhoogde KVS-riisiko.

RESULTATE EN GEVOLGTREKKINGS

Die resultate en gevolgtrekkings van elk van drie manuskripte is soos volg:

1. Troponien T-vrystelling is geassosieer met stille miokardiale iskemie in swart mans: die SABPA-studie

Betekenisvolle verskille was sigbaar in hs-cTnT en die verband daarvan met SMI en hipertensie in Swartes en Wittes van hierdie kohort. Hierdie bevindinge wys dat hs-cTnT potensieel 'n gesaghebbende merker van SMI kan wees, asook in verhogings in kompensatoriese sistoliese bloeddruk (SBD). 'n Laer hs-cTnT afsnypunt ≥ 4.2 pg/ml vir 24-uur sistoliese hipertensie is voorspel in die Swartes vergeleke met die ≥ 5.6 pg/ml in Wittes, met 'n afsonderlike sensitiwiteit/spesifisiteit van 64/68% en 61/71%. Die SBD in Swartes verhoog om die miokardiale iskemie te versag, asook die groepering van risikofaktore. Hierdie resultate onderskryf die belangrikheid van etnies-spesifieke verwysingswaardes van hs-cTnT, wat weer geïnterpreteer moet word met inagneming van bestaande risiko faktore. Deurtastende assessering van hs-cTnT kan 'n bruikbare verbetering wees in risikovoorspelling-navorsing.

2. Stille iskemie, insulien weerstandigheid en kardiovaskulêre risiko in 'n bi-etniese geslagskohort: die SABPA-studie

Insulien weerstandigheid (IW), soos bepaal deur die verhoogde homeostase-model van assesserings- (HOMA IR) vlakke, was betekenisvol in verband gebring met langer SMI-voorvalle oor die verloop van 24 uur in wit geslagsgroepe. Desnieteenstaande, het wit mans ook betekenisvolle verband aangedui tussen IW en meer gereelde SMI-voorvalle. Ons bevindinge stel

voor dat IW verhogings in verband gebring word met metaboliese vatbaarheid wat lei tot die ontwikkeling van SMI in die Wittes, maar nie noodwendig in die Swartes nie. Hierteenoor het die swart mans betekenisvolle verbande getoon tussen hs-cTnT en meer gereelde en langer SMI-voorvalle. Bevindinge by Swartes impliseer 'n kardiovaskulêre vatbaarheid om iskemiese hartsiektes te ontwikkel en dit onderskryf die etnies-verwante meganisme wat die ontluikende SMI kan diagnoseer.

3. Troponien T vrystelling hou verband met subkliniese arteriosklerose in Swartes met eerstegraadse AV-blok: die SABPA-studie

In Swartes met 1^{ste}-graadse AV-blok was verhogings in hs-cTnT positief verbind met subkliniese arteriosklerose. Dienooreenkomstige assosiasies was nie in Wittes sigbaar nie. Eerste- (1^{ste}) graadse AV-blok in Swartes weerspieël verhogings in hs-cTnT en verhoogde vatbaarheid vir kompensatoriese hoëbloeddruksisteem wat lei tot karotis hipertrofiese hermodulering. Die identifisering van die geleidingsafwyking en perfusie-tekortkominge tydens vroeë siftingsprogramme kan dus 'n bydrae lewer tot KVS-voorkomingsprogramme.

ALGEMENE GEVOLGTREKKING

In Swartes van hierdie kohort is 'n hoër kardiometaboliese vatbaarheid om KVS te ontwikkel blootgelê. Dit is benadruk deur die lae hs-cTnT afsnyppunte om kompensatoriese SBD-hipertensie te voorspel. Ook wanneer geleidingsafwyking in ag geneem word, kan die gebruik van hs-cTnT betekenisvol tot voorkomingskardiologie bydra.

In Wittes word die ontwikkeling van iskemiese hartsiektes onderskryf deur IW meer as hs-cTnT en kan in gesondheidsorg-beoefening omgesit word.

Die ongunstige KVS-risiko in 'n Suid-Afrikaanse Swart kohort is kommerwekkend, en die bydrae van risikofaktore as gevolg van verstedeliking (swak voeding, laer aktiwiteitsvlakke, obesiteit, alkoholmisbruik en rook) kan die CVD-las vererger

SLEUTELWOORDE

Stille miokardiale iskemie, troponien T, insulienweerstandigheid, Homeostase-model van assessering, subkliniese atherosklerose, eerstegraadse AV-blok.

Preface

This thesis is written in article format and comprise of 3 published or submitted for publication peer-reviewed original research papers. A comprehensive literature overview of the main topics from this thesis is presented in *Chapter 1*. Also included in this chapter are the aims and hypotheses of the entire study as well as for the separate manuscripts followed by the references according to the Vancouver style. Each of the manuscripts can be found in *Chapter 2,3* and *4* respectively. Each of these manuscripts include abstracts, introductions, methods, results, discussions and conclusions followed by appropriate referencing formats according to the guidelines set out by each specific peer-reviewed journal. *Chapter 5* entails of the main findings and conclusions of the thesis as well as limitations and recommendations for future research. Please note, that black South Africans are referred to as Blacks whereas white South Africans are referred to as Whites throughout the thesis as these are internationally recognized terminology. The web-based citation management program Endnote™ was used to finalise reference listings. Graphs were created by using Microsoft® Excel computer software. Tables and figures were allocated Arabic numerals consecutively in order of appearance and according to the respective chapter of the thesis.

All manuscripts have been submitted to peer-reviewed journals for publication.

The following article: *Troponin T release is associated with silent myocardial ischaemia in black men: The SABPA study*, has been published in the journal *European Journal of Preventive Cardiology* with an impact factor of 4.542. Results of this manuscript were also presented at the 45th Conference of the Physiology Society of Southern Africa held at University of Pretoria during August 2017.

The second article: *Silent Ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study*, has been submitted to the peer-reviewed journal *Heart, Lung and Circulation* with an impact factor of 1.921. The manuscript has been assigned a number HLC-S-18-00984 and is currently under review.

The third research article: *First degree AV-block and cardiometabolic predictors of subclinical atherosclerosis in a bi-ethnic cohort: The SABPA study*, has been submitted to the peer-reviewed journal *Atherosclerosis* with an impact factor of 4.542. The manuscript has been assigned a number ATH-D-18-01482 and is currently under review.

The promotor and co-promoters agreed on co-authorship in all three papers. Their permission was granted for the use of these papers as part of the final thesis. *Manuscript 1* and *2* were validated by a statistical consultant, which was also included as a co-author for her expert input. However, the first author was solely responsible for planning, writing, literature searches, all initial statistical analysis, interpretation of results of the three manuscripts and the entire thesis. This author also contributed to the collection and interpretation of data in the SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans) study as well as collection of data during the PURE (Prospective Urban Rural Epidemiology) and AFRICAN PREDICT (African PRospective study on the Early Detection and Identification of Cardiovascular disease and HyperTension) study (see *Postgraduate Student Skills*).

The Ethics Review Board of the North-West University (Potchefstroom Campus: NWU-00036-07-S6) approved this SABPA sub-study, and procedures obeyed with terms and guidelines of the Declaration of Helsinki. Before recruitment, participants were informed about the study protocol by a staff member. Hereafter willing participants signed an informed consent form. Any participant-identifying information do not form part of the Statistica database used in this sub-study. This Statistica database is stored on password protected computers. Only the study leader, postgraduate student and statistician will have access to the database. The proposed sub-study will have no direct benefits to the participants, but the knowledge gained from this study may assist in our understanding of cardiovascular disease in a bi-ethnic cohort from South Africa (see *Ethical Approval – Appendix 1*).

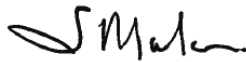
Postgraduate student skills 2018





POSTGRADUATE STUDENT SKILLS

STUDENT NAME:	Tick if accomplished		
Undergraduate teaching (indicate number of courses)	N = 0		
Optional: Clinical Pharmacology course (16 credit module)	x		
Optional: Honours student mentorship (indicate number of students)	N = 0		
Ethical consent: Sub-study application under Umbrella-study	✓		
Obtained and interpreted medical history, medication status: Socio-economic (medical aid access, education; job), marital, family history, health and cardio-metabolic incidents/events; medications	✓		
Dietary habits questionnaire	✓		
Good Clinical Practice (GCP) course: Year obtained	x		
¹Observed collection/²Interpreted psychosocial battery measures: Measures with known heritability: Life orientation, Personality Predictors of developing/worsening hypertension: Coping, Depression, Cognitive distress Moderating effects of the environment: Fortitude, Mental Health, Self-regulation, Job stress	1 ✓	2 ✓	
¹Observed/²Interpreted anthropometry measurements Height, Body mass, Waist circumference, Physical activity	✓		
¹Cardiovascular assessments, ²download and ³interpretation of data Resting Blood Pressure [Riester CE 0124® & 1.3M™ Littman® II S.E. Stethoscope 2205] *Finometer [Finapres Medical Systems®] 12-lead resting ECG [NORAV PC-ECG 1200®] 24 ambulatory BP & -ECG [Cardiotens® & Cardiovisions 1.19®, Meditech] Pulse Wave Velocity and Pulse Wave Analysis [Sphygmocor EXCEL, AtCor]	✓		
Laboratory skills (sample handling and analyses) 24h Urine/blood/saliva/hair: ¹ collection/ ² sampling/ ³ aliquoting/ ⁴ waste material Rapid tests (cholesterol, glucose, urine dipstick and blood type) Laboratory analyses of samples (ELISA, RIA, COBAS Integra, E411) Whole blood HIV status [PMC Medical, Daman, India; Pareekshak test, BHAT Bio-Tech, Bangalore, India]	1 x	2 x	
¹Accomplished training & ²measuring of ultrasound Carotid Intima Media Thickness (CIMT) [SonoSite Micromaxx®, SonoSite Inc., Bothell, WA] ³Retinal Vessel Assessment, ⁴Data download & Interpretation (Imedos®)	1 ✓	2 x	
Statistical analyses ¹ Normal distribution & T-tests, ² General linear models, ³ Multiple regression analyses ⁴ ROC analyses; ⁵ Prospective data analyses and risk prediction	3 x	4 x	
Successful grant/funding application/s: NRF ¹ /MRC ² South Africa	1 ✓	2 ✓	3 ✓
Publications: Prepared, submitted, handled rebuttal of manuscript in a peer-reviewed journal	4 ✓	5 ✓	
Conference meetings: ¹ National, ² International ³ oral/ ⁴ poster presentation	1 N=1	2 N=0	
	N=2		
	1 N=2	2 N=0	
	3 N=1	4 N=0	

N=number; *Inclusive of sympathetic nervous system (SNS) responses (acute mental laboratory stressors e.g. cold pressor & colour-word-conflict)


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Herewith the contribution of each author towards the study, manuscripts and entire thesis:

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- Prof R Delport** *Co-promotor* – Provided critical feedback as well as expert critical review of the thesis.
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- Dr M Reimann** *Co-author* - Provided critical feedback as well as expert input to the written material for manuscript 1 and 2. She is presently not able to sign-off the thesis. Please contact Prof Tjalf Ziemssen for further enquiries at Tjalf.Ziemssen@uniklinikum-dresden.de OR Ziemssen@web.de OR ziemssen@me.com.

Herewith is a statement of all co-authors verifying their actual contribution to the study and giving permission that all three manuscripts may form part of the thesis.

I hereby declare that my role as indicated is representative of my actual contribution to the study and/or thesis. I approve the manuscripts and give my consent that these manuscripts may be published as part of the thesis for the degree Philosophiae Doctor of Ms Madelein Elizabeth Griffiths.

			<i>Not available</i>
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List of symbols and abbreviations

The following symbols and abbreviations have been used for this thesis:

%	:	Percentage
24-h	:	24 hours
ABPM	:	Ambulatory Blood Pressure Monitoring
ACS	:	Acute Coronary Syndrome
AFRICAN PREDICT	:	African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension
ANCOVA	:	Analysis of Covariance
ARIC	:	Atherosclerosis Risk in Communities
ATP	:	Adeno-Triphosphate
AUC	:	Area Under the Curve
AV-block	:	Atrioventricular block
AV-node	:	Atrioventricular node
BMI	:	Body Mass Index
BP	:	Blood Pressure
BSA	:	Body Surface Area
CAD	:	Coronary Artery Disease
CI	:	Confidence Intervals
CIMT	:	Carotid Intima-Media Thickness
CIMTf	:	Carotid Intima-Media Thickness of the far wall
cm	:	centimeter
CRP	:	C-Reactive Protein
cTnI	:	cardiac Troponin I

cTnT	:	cardiac Troponin T
CV	:	Coefficient of Variation
CVD	:	Cardiovascular Disease
DBP	:	Diastolic Blood Pressure
ECG	:	Electrocardiogram
ECLA	:	Electrochemiluminescence Assay
ESC	:	European Society of Cardiology
ESH	:	European Society of Hypertension
et al.	:	<i>et alia</i> (and others)
G-proteins	:	Guanine nucleotide-binding proteins
h	:	hour
HART	:	Hypertension in Africa Research Team
HbA _{1c}	:	Glycated haemoglobin
HdL	:	High-density Lipoprotein
HEC	:	Hyperinsulinemic Euglycemic Clamp
HIV	:	Human Immunodeficiency Virus
HOMA IR	:	Homeostasis Model of Assessment
HR	:	Heart Rate
hs-CRP	:	high-sensitivity C-Reactive Protein
hs-cTnT	:	high-sensitivity cardiac troponin T
HT	:	Hypertension
IL	:	Illinois
IR	:	Insulin Resistance
ISAK	:	International Society of Advancement of Kinantropometry
kcal	:	kilocalories

kg	:	kilogram
l	:	litre
L-CIMT	:	Left Carotid Intima-Media Thickness
L-CSWA	:	Left Cross Sectional Wall-Area
LdL	:	Low-density Lipoprotein
LOD	:	Limit of Detection
m ²	:	Square meter
MAP	:	Mitogen-Activated Protein
MetS	:	Metabolic Syndrome
mg/dl	:	milligram per desilitre
mg/l	:	milligrams per litre
mHz	:	megaHertz
MI	:	Myocardial Infarct
min	:	minute
ml	:	millilitre
mm	:	millimeter
mmHg	:	millimeter of mercury
mmol/l	:	millimoles per litre
mmol/mol	:	millimoles per mole
ms	:	milliseconds
mU/l	:	milliunits per litre
mV	:	milliVolt
n	:	number
ng/l	:	nanograms per litre
ng/ml	:	nanograms per millilitre

NWU	:	North-West University
pg/ml	:	picograms per millilitre
PP	:	Pulse Pressure
PURE	:	Prospective Urban Rural Epidemiology
r	:	Correlation coefficient
R ²	:	Relative predictive power of a model
ROC	:	Receiver Operating Characteristics
s	:	seconds
SABPA	:	Sympathetic Activity and Blood Pressure in Africans
SBP	:	Systolic Blood Pressure
SD	:	Standard Deviation
SE	:	Standard Error
SMI	:	Silent Myocardial Ischemia
STATS SA	:	Statistics South Africa
THUSA	:	Transition, Health and Urbanisation in South Africa
U/l	:	Units per litre
USA	:	United States of America
WHO	:	World Health Organization
X ²	:	Chi-square
α	:	Alpha
β	:	Beta
γ	:	Gamma
γ-GT	:	Gamma-Glutamyl Transferase

Afrikaans

ANCOVA	:	Analise van kovariansie
BD	:	Bloeddruk
CIMTf	:	Karotis intima-media verdikking
CSWA	:	Deursnit van die wand area
EKG	:	Elektrokardiogram
HbA _{1c}	:	Glikeerde hemoglobien A _{1c}
HOMA IR	:	Homeostase Model van Assessering
hs-CRP	:	hoë sensitiewe C-Reaktiewe Proteïen
hs-cTnT	:	hoë sensitiewe kardiaale Troponien T
IW	:	Insulien Weestandigheid
KAS	:	Koronêre Arteriële Siekte
KVS	:	Kardiovaskulêre Siekte
SABPA	:	Simpatiese Aktiwiteit en Ambulatoriese Bloeddruk in Afrikane
SBD	:	Sistoliese Bloeddruk
SMI	:	Stille Miokardiale Iskemie
X ²	:	Chi-kwadraattoetse
γ-GT	:	γ-Glutamiel Transferase

Chapter 1



Literature, overview, aims and hypotheses

Illustration used with permission from Prof. L. Malan – The SABPA study principal investigator.

Literature, overview, aims and hypotheses

1. INTRODUCTION

The World Health Organization (WHO) established that cardiovascular disease (CVD) is the primary cause of deaths globally (1). Annually, the mortality rate due to CVD is higher than the mortality rate for any other cause (1). In the South-African context, according to Statistics South Africa (STATS SA), CVD is the fourth leading cause of death (2), where ischemic heart disease ranked 11th among the leading causes of death in 2016 (2). In urban Blacks from South-Africa the prevalence of CVD keeps rising despite growing awareness and focussed healthcare (3-7) and an adverse progression of CVD risk markers are observed among this race (8). In addition, the Heart of Soweto Study revealed that heart failure is the leading diagnosis in this ethnic group but they were less likely to be diagnosed with coronary artery disease (CAD) (9). Also, Malan et al. (10) previously reported a higher cardiometabolic risk in urban Blacks from the THUSA (Transition, Health and Urbanisation in South Africa) study (1996-1998: North West Province, South Africa) where higher metabolic risk was displayed when Blacks were compared with another ethnic population ten years later (the SABPA (Sympathetic and Ambulatory Blood Pressure in Africans) study) (11). One of the most common manifestations of CVD, is silent myocardial ischemia (SMI) (12), which can occur in a wide spectrum of individuals (13). In addition, SMI occurred in approximately 25% to 50% of individuals with CAD (14). CAD was a leading cause of morbidity and mortality and it was apparent that the burden in sub-Saharan Africa is progressing (4, 15). Therefore, considering SMI as an independent predictor of mortality in the detection of CAD may improve preventive treatment strategies and lessen cardiac deaths (7). SMI has also been associated with subclinical atherosclerosis (16) in a black male cohort (17) and was enhanced by the prevalence of cardiovascular risk factors, i.e. diabetes and hypertension (HT) (8, 18). Indeed, the most common underlying cause responsible for myocardial ischemia, was atherosclerotic coronary disease (19), and especially men were more susceptible to myocardial perfusion defects (20). Also, ischemic heart disease has been described as one of the most common

causes of First (1st) degree atrioventricular block (AV-block) (21). In black men from South Africa, 1st degree AV-block can be induced by low-grade inflammation which can be ascribed to mechanisms involving ischemia (22).

The measurement of high-sensitivity cardiac troponin T (hs-cTnT) is a highly validated measurement for the detection of myocardial infarct (MI) (23), which in turn may be predicted by SMI (14). Not only is hs-cTnT used for the diagnosis of MI because of its vital diagnostic sensitivity and negative predictive value; it is also used in cases of primary myocardial ischemia due to an imbalance between supply and demand (23, 24), and relates to known risk factors of atherosclerosis (25).

In addition, insulin resistance (IR) may further contribute to the development of SMI (26) and CAD (27). Using the homeostasis model of assessment (HOMA IR), as a reliable marker of IR (28, 29) may add significance as independent predictor of CVD risk (30, 31).

2. CONTRIBUTING RISK FACTORS OF CARDIOVASCULAR DISEASE (CVD) AND CORONARY ARTERY DISEASE (CAD)

Urbanization in South Africa has been associated with the adoption of a westernized lifestyle, which was shown to be a foremost contributing factor for the development of CVD especially in Blacks (9, 10, 15, 32, 33). Also, urbanization in this population created an increased prevalence of various risk factors contributing to the development of CAD and CVD (10). These factors included: abdominal obesity (3, 34), HT (3, 34, 35), dyslipidaemia (3), diabetes (3), prediabetes (36), IR (30), subclinical atheroma (34), less physical activity (37), lack of proper diets (38), psychosocial stress (39, 40), poor socio-economic and geographic conditions (39, 40), alcohol abuse (39-41) and cigarette smoking (3, 39, 40). Interventions directed to white populations may not be effective in Blacks (42) and lifestyle modification regimes were proposed (4) to reduce the CVD burden increase in this ethnic group (8).

2.1 Hypertension (HT)

HT prevalence was higher in urbanizing individuals (38) and was the leading contributing factor for the development of CVD (43). In addition, HT was more prevalent in Blacks than in Whites (6, 44) and was also poorly controlled and inadequately treated in Blacks (45). Factors contributing to the susceptibility of developing HT included: changes in plasma renin levels (46); sodium impairment (46); higher peripheral vascular resistance (47); obesity, lower socio-economic status (38); and defensive coping (48).

2.2 Diabetes and prediabetes

The rise of urbanization in sub-Saharan Africa and the adoption of lifestyle factors associated with urbanization increased the risk of developing diabetes (49) and its associated complications (32, 50). In addition, in individuals with diabetes, CVD was the leading cause of mortality (51) and SMI was also more common (52). Furthermore, it was proposed that partial or complete autonomic denervation in individuals with diabetes may lead to the development of SMI (53, 157). SMI is associated with higher glycated haemoglobin (HbA_{1c}) levels (53) and is also associated with other CAD risk factors (54, 55). Metabolic disorders as a result of diabetes as well as higher HT prevalence elevates the risk of developing atherosclerosis (56). Furthermore, CAD is responsible for approximately 65-85% of deaths in individuals with diabetes (57) and the INTERHEART Africa study revealed that cardiometabolic risk factors i.e. diabetes and HT, underscored a population-attributable risk of approximately 90% for the occurrence of heart attacks (3, 39).

Additionally, atherosclerosis and plaque vulnerability were more progressed in prediabetic individuals (determined by fasting plasma glucose levels and HbA_{1c}) than nondiabetic individuals (58, 59). In support, Rubin et al. (60) demonstrated that hyperglycaemia, as measured by HbA_{1c}, was associated with myocardial injury determined by elevations in hs-cTnT but not necessarily driven by atherosclerosis. This was confirmed by Selvin et al. (61) who concluded in the Atherosclerosis Risk in Communities Study (ARIC-study) that diabetes

and prediabetes were independently associated with elevated hs-cTnT as a measure of subclinical myocardial damage and suggested microvascular damage as a deleterious result of hyperglycaemia. Alternative mechanisms proposed contributing to the myocardial damage included: coronary microvascular dysfunction induced by hyperglycaemia; oxidative stress; fibrosis of the myocardium; and progressive glycation end-products (60).

2.3 Insulin resistance (IR)

IR is defined as the incapability of insulin (exogenous or endogenous) to improve glucose uptake and utilization (62) and reflects the weakened suppressive function of insulin on hepatic glucose production (63). IR was an independent risk factor contributing to the development of SMI, myocardial damage (26) and rank among the major cardiovascular disease risk factors (64). IR posed a higher risk of congestive heart failure (64) and promoted atherosclerosis before the onset of diabetes mellitus (64). In addition, earlier evidence revealed that IR contributed more to the development of CVD in Whites than in Blacks (65). IR was also a contributing factor to the prevalence of Metabolic Syndrome (MetS) (66), (defined by a clustering of cardiometabolic risk factors which include HT, dyslipidaemia, abdominal obesity, diabetes and elevated fasting plasma glucose (67)) which in turn manifested into CAD (66).

An earlier study conducted by Howard and co-workers (68), revealed that IR positively relates with thicker carotid intima-media thickness (CIMT) in Whites but not in Blacks. Bertoni et al. (69) established positive associations between IR and subclinical atherosclerosis in Blacks and Whites (70). In addition, black women from South Africa revealed significantly higher levels of IR than their white counterparts, which on the other hand were more insulin sensitive (71).

Other studies revealed that individuals with SMI and diabetes had a poor prognosis imitated by adverse cardiac events or death (72). As early as 1990, Saad et al. (65) established a link

between IR and blood pressure (BP) especially in Whites, which was confirmed by more recent studies (70, 73). The deranged glucose metabolism in Whites can possibly be due to structural and cellular defects as well as enhanced adrenergic tone (18, 65).

2.3.1 Detection of insulin resistance (IR)

Several methods and indices are used as measures of IR (63, 74). The recognized gold-standard and most reliable method for the determination of IR is the hyperinsulinemic euglycemic clamp (HEC) method (75), with the intravenous glucose tolerance test also considered as being reliable (63). As these methods are time and money consuming and seemed impractical for epidemiological studies (63, 74), the method more commonly used for clinical and epidemiological research, due to the robust, safer and less invasive technique applied, is the HOMA IR (74) method. This method, first described in 1985 (29), is considered a reliable and more convenient method for determining IR (28) and correlated well with the HEC method (76). HOMA IR quantifies insulin resistance from the relationship between fasting glucose and fasting insulin concentrations (63) and measured the hepatic component of IR (74, 76).

The following equations are used to determine HOMA IR (76):

$$\text{HOMA IR} = \text{insulin (mU/l)} \times \text{glucose (mmol/l)} / 22.5$$

Or

$$\text{HOMA IR} = \text{glucose (mg/dl)} \times \text{insulin} / 405$$

Where,

mU/l = milliunits per litre

mmol/l = millimoles per litre

mg/dl = milligram per decilitre.

Even though HOMA IR may not identify peripheral IR, which may correlate more with adverse metabolic disturbances of IR i.e. inflammation, HT and dyslipidaemia (74, 76), several studies

found correlations among these metabolic disturbances and higher HOMA IR levels (77-80), which clustered together with IR as part of MetS risk factors (64). In addition, HOMA IR strongly predicted the development of type 2 diabetes more than fasting insulin did alone (81), was independently associated with SMI in individuals with type 2 diabetes and also had a greater predictive value than MetS for detecting SMI (28).

2.3.2 Insulin resistance (IR) and silent myocardial ischemia (SMI)

IR contributed to the development of SMI (74) and featured dysregulation of autonomic nervous homeostasis, which possibly can explain its pathogenesis (82). IR was also associated with myocardial damage in normal individuals (26), due to a disparity between coronary perfusion and myocardial metabolism as IR is detrimental to the coronary microcirculation (83, 84). Individuals with IR already exhibited myocardial perfusion defects without any symptomatic cardiac illness (85), implying evidence of myocardial injury in early stages of IR before the onset of diabetes.

Also, SMI, which may be due to autonomic neuropathy (86) or sympathetic nerve dysfunction (38, 51, 72, 87), occurred more frequently in individuals with diabetes (28). It was also possible that the mechanism underlying the development of SMI in these individuals, may be due to a decrease in vascular supply and a higher cardiac demand (38, 51, 72, 87).

The myocardium is disposed to diabetic glucose/insulin homeostatic alterations, due to its foremost insulin-responsive nature (88). Both IR and hyperglycaemia can distress myocardial myocyte metabolism leading to the progression of cardiomyopathy (88). Structural and functional alterations in the myocardium of diabetic individuals also render it predisposed to ischemia (88). In addition, during ischemia, the disturbance of glycolytic adenosine triphosphate (ATP) production may occur due to a decreased glucose transport into myocardial myocytes, further underscoring the contribution of IR in the development of CVD

(88). Furthermore, myocardial damage may occur (30), which in turn can be predicted by hs-cTnT (89, 90).

3. HIGH-SENSITIVITY CARDIAC TROPONIN T (hs-cTnT)

3.1 Overview

Hs-cTnT is a non-invasive biomarker of subclinical myocardial damage (89, 90) and irreversible necrosis (24), and may precede the development of HT (61, 91). Additionally, hs-cTnT is a validated biomarker for the clinical diagnosis of acute coronary syndrome in individuals with chest discomfort (89, 92, 93) and the detection of MI (93), which in turn may be predicted by SMI (86) due to an imbalance between supply and demand (23, 24). Elevated hs-cTnT levels was also independently associated with mortality (94). Circulating cardiac Troponin T (cTnT) can be found in plasma due to transient ischemia or inflammatory myocardial damage (95) and can remain elevated for up to 14 days after MI (96). This is confirmed by Ohman et al. (97) who stated that cTnT is an invaluable risk marker in individuals with acute myocardial ischemia. More recently, Turer et al. (98) concluded that cTnT release is evident in individuals with myocardial ischemia induced by rapid atrial pacing. Also, cTnT can also improve risk stratification and add significant prognostic value in acute and chronic heart failure (61, 99).

Hs-cTnT further related to known risk factors of atherosclerosis (25) and has been shown to improve the prediction of CAD and mortality in an apparently healthy population (100, 101). Higher hs-cTnT levels may possibly reflect subclinical ischemia because of atherosclerosis (102). Even minor elevations in troponin levels are associated with adverse outcomes in individuals with acute coronary syndrome and is an important predictor of cardiovascular events in hypertensive individuals (99, 103, 104). Identification of individuals at risk for HT secondary to myocardial ischemia may aid in preventing exacerbation of the ischaemic burden (25).

Myocardial injury, as detected by elevated hs-cTnT levels, was significantly related with a higher risk to develop incident diabetes (105). This may explain the relation among hyperglycaemia, microvascular dysfunction as well as lipotoxicity with one another (106). Common risk factors such as inflammation, platelet activation and endothelial dysfunction can underscore this association (106).

3.2 Mechanism of troponin action

Troponin (a protein complex) consists of three subunits – troponin I, T and C. These troponins are involved in the contractile processes of cardiac and skeletal muscle and are included in the thin sarcomere filaments essential for contraction and relaxation (107). Troponin T and troponin I are expressed cardiac specific and control the calcium mediated interaction between actin and myosin (108), whereas troponin C is expressed by both cardiac and skeletal muscle (109). Cardiac troponin T (cTnT) is the tropomyosin-binding protein of the regulatory complex which is situated on the contractile apparatus of cardiac myocytes (107) and is responsible for contraction (110). Cardiac troponins are bound to actin filaments of sarcomeres via tropomyosin, whereas a small portion (3-8%) can be detected in the sarcoplasmic or cytosolic pool (107) acting as a precursor pool for myofibrillar assembly (111). cTnT and cardiac troponin I (cTnI) are released in the circulation by the cardiomyocytes due to inflammatory myocardial damage or transient ischemia (95), in relation to the degree of damage (112). Due to irreversible myocyte damage (as a result of intracellular acidosis and activation of proteolytic enzymes (111)), the free cytoplasmic pool is immediately released (113, 114) and depends on the disintegration of the contractile apparatus (111), trailed by a continuing slow release of the myofibril-bound proteins resulting in subsequent troponin increases (113). The increases in circulating troponin levels are evident within 2 to 10 hours and remain elevated for up to 14 days (troponin I: 4-7 days; troponin T: 10-14 days) (96, 107, 111). Cardiac troponins are detected in serum via monoclonal antibodies, which are highly specific for cTnT isoforms without cross-reactivity to skeletal muscle troponins; thus reflecting elevations of cTnT, pertaining specifically to myocardial damage (115).

Increased circulating cardiac troponin concentrations were also evident in individuals presenting: cardiomyopathy (108), unstable angina pectoris (107), coronary and cardiac intervention (including cardioversion and ablation) (108), peri-myocarditis (115), supraventricular tachycardia (115), renal insufficiency (108, 115) which may be associated with left ventricular hypertrophy (99), “silent” micro-infarctions and a reduced renal troponin elimination (115), stroke (107), pulmonary embolism (108), septicaemia (108), chemotherapy (108), and ultra-endurance athletes (107). In these cases, the underlying troponin release mechanism needed further exploration.

Potential factors which may contribute to the release of circulation troponins included: cardiomyocyte apoptosis (99, 116, 117), increased transmural wall stress and stiffening of the myocardium resulting in subendocardial ischemia (99, 116), increased transmural cell wall permeability due to stress stretch increased by cavity dilation, and elevated filling pressure resulting in higher oxygen demands (99, 116), cell release of proteolytic products that contain troponin probably due to reversible injury (99, 116, 117). However, in an “apparently healthy” general population, higher hs-cTnT levels predicted imminent cardiovascular events and were associated with structural cardiac disease (100, 101).

3.3 Detection of high-sensitivity cardiac troponin T (hs-cTnT)

Troponin assays have been used as biochemical marker for the diagnosis of acute coronary syndromes since 1999 with a moderate sensitivity for cardiomyocyte injury (118, 119). A high-sensitivity assay for the detection of cTnT has entered the diagnostic scene in the last 10-years, detecting troponin concentrations and improving diagnostic precision at lower levels (99, 116, 120), which proved to be cost-effective (119, 121) and added sensitivity for cardiomyocyte necrosis (116). Interpretation of hs-cTnT is based in the 99th percentile value in the general population optimizing the sensitivity and specificity of troponin while decreasing false-positive testing (119, 122). The coefficient of variation (CV) should be less than 10% at or below this level. A CV of 10 – 20% is clinically acceptable (122). The 99th percentile upper

limit of normal in the South African context for cTnT is 14 ng/l (119). Gore et al. (123) revealed that this cut-point value can over-diagnose MI in men and older individuals and is generally higher in Blacks than in non-Blacks. However, this study did not infer for uniformly recording of ethnicity and recommend sex and age specific cut-off values (123). It is recommended nationally (119) and internationally (122) that at least two troponin samples be acquired for the determination of MI and tested for the determination of diagnosis at least 3 hours apart (119), reported within 60 minutes (119), and should be used in co-operation with other clinical evaluation tools i.e. the ECG. (110, 119). Inadequate evidence exists to deliver more stringent guidelines to distinguish between acute coronary syndrome (ACS) and non-ACS ischemia-related troponin elevations without considering the clinical presentation of symptoms (124). It is also true that increases in cTnT will not be capable of determining the pathophysiological mechanism of myocardial necrosis or injury, may not be related to ischemia (110) and will prompt further investigation if myocardial ischemia is absent (110, 124). The possibility of false high and false test results also do exist due to heterophile antibodies and human auto-antibodies interfering with the assay, but it is rare (119). As multiple cardiac and non-cardiac conditions were associated with mild-to-moderate hs-cTnT elevations, these hs-cTnT levels can be used as risk stratification in individuals with stable CAD, heart failure and non-cardiac disease conditions even at levels below the limit of detection of previous cTnT assays (120). Therefore, the measurement of cTnT in addition to the determination of SMI can have additive value in early recognition of CAD.

4. SILENT MYOCARDIAL ISCHEMIA (SMI)

4.1 Overview

SMI can be prevalent in a variety of individuals with CAD (13) and diabetes (19), and has a substantial prognostic implication (125, 126). SMI was thus considered the most common manifestation of coronary heart disease (12, 16). SMI was also a predisposing influence for unexpected cardiac death as a result of ventricular arrhythmia (127). In the 1970's, it was recorded that silent ischemia during ambulatory blood pressure monitoring (ABPM) occurred more frequently than symptomatic ischemic episodes in individuals with CAD (125) which was also more recently confirmed by Stone et al. (128).

T-wave abnormalities and ST-segment depression episodes are indicators of myocardial ischemia (129) and 70-80% of these episodes have been classified as 'silent' (130). The definition of SMI is the occurrence of ST-segment depression (ischaemic) episode in the absence of associated chest pain or any other angina-matching indication (dyspnoea, arrhythmia) (130). Transient ST-segment abnormalities may also be present during ABPM or electrocardiogram (ECG) stress testing (127). Thus, SMI is the documentation of myocardial ischemia in the absence of accompanying chest pain (86).

As early as the 1980s, Nesto et al. (131) determined that ECG and left ventricular mechanical abnormalities preceded the development of symptoms after coronary artery occlusion and the development of an ischemic event characterized the increasing impact of a sequence of pathophysiologic events. Myocardial perfusion is determined by coronary blood supply and myocardial oxygen demand and each ischemic episode is initiated by any disparity between myocardial oxygen supply and demand (131), which occurs more often throughout activities not requiring effort (132).

Cohn et al. (133) described 3 ways to classify a population presenting SMI:

- 1) Total asymptomatic individuals.
- 2) Asymptomatic individuals after having a MI.
- 3) Individuals with symptomatic and asymptomatic episodes (i.e. individuals with unstable and stable angina).

SMI was also more prevalent in individuals with diabetes (19). However, in individuals with confirmed CAD as well as individuals with or without diabetes, the risk of developing SMI during exercise was similar (134). Furthermore, SMI was shown to be more prevalent in individuals with impaired glucose tolerance and individuals with higher levels of HbA_{1c} (\geq 7.6%) (53, 56).

4.2 The Ischemic Cascade

The ischemic cascade is defined as a sequence of predictable events which occurred in the myocardium after the onset of ischemia (135). In 1985, Hauser et al. (135) described this sequence of events (mechanical, electrographic and clinical) and suggested that myocardial ischemia occurred in a predictable sequence prior to clinical symptoms (135).

The proposed order of events were as follows (Figure 4.2.1):

After perfusion defects:

- Metabolic abnormalities occur.
- Abnormal diastolic function (i.e. slowed ventricular relaxation).
- Abnormal systolic function – characterized by regional wall motion disparity and further comprehensive abnormalities which led to reduced ejection fraction and seldom to a fall in BP.
- ST-segment depression.
- Angina pectoris – clinical manifestation of myocardial ischemia (136).

However, later research by Detry et al. (136) concluded that silent ischemia in this cascade of events remained an undiagnosed area of research. Leong-Poi et al. (137) further concluded that localized perfusion defects preceded localized function defects during ischemic demand. More recently, Maznyczka et al. (138) further explored the use of this “ischemic cascade” to diagnose ischemia. These coworkers concluded that these events occur often out of sequence and proposed an “ischaemic constellation” to evaluate ischemia.

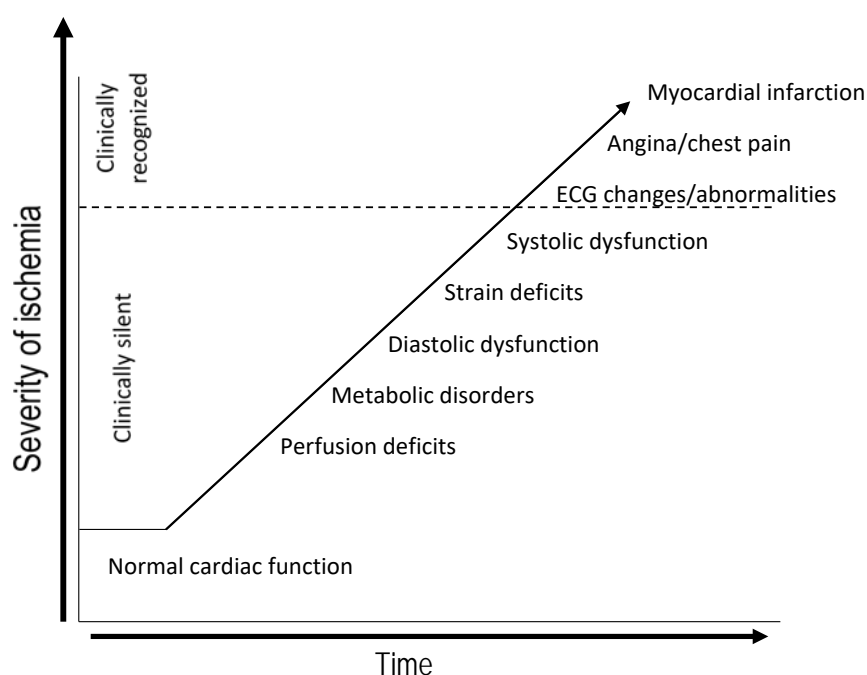


Figure 4.2.1: Depiction of the ischemic cascade from clinically silent to clinically recognized symptoms. Adapted from Ansari A, Puthumana J. The “Ischemic Cascade”. In: Herzog E, Chaudhry F, editors. *Echocardiography in Acute Coronary Syndrome: Diagnosis, Treatment and Prevention*. London: Springer London; 2009. p. 149-60. (139)

4.3 Detection of Silent Myocardial Ischemia (SMI)

SMI can be detected during non-invasive ECG or a pharmaceutical stress test (140) as well as with the use of ABPM monitors (128). Even though the use of ABPM to determine cardiovascular prognosis is rising (141), the best method of detection seems to be the combination of 24-h BP and ECG monitoring as they accentuate each other (142). Non-specific and false positives may occur during 24-h BP measurement (125); hence, the following strict criteria are set to diagnose SMI by reducing the false positives to a mere 6% (127).

The 1-1-1 rule:

1. More than 1 mm horizontal or descending ST-segment depression.
2. The ST-segment depression lasted for more than 1 minute (min).
3. Two consecutive ST-segment episodes were counted as independent episodes if the interval between these are at least 1 min (127).

Gutterman (7) also proposed strict criteria where ST-segment depression is at least 0.5 mV and the episode lasted for more than 60 seconds. Another method for detecting SMI is via intra-cardiac electrocardiogram signals, by placing a pacemaker lead at the right ventricular apex (143). ABPM and implanted devices evaluate silent ischemia during everyday life and longer periods of time than the stress testing (127). Several emerging measures to detect SMI were also proposed including: intramyocardial temperature monitoring, tissue oxygen tension, near-infrared spectroscopy and computed topography (14). These measures may be reliable in the diagnosis of SMI, but were mostly invasive and costly (14).

Risk factors contributing to the development of SMI included HT (14), diabetes (14), preceding MI (14), surgical revascularization (14), aging (14), smoking (144), hypercholesterolemia (144), male sex (145) and diabetic retinopathy (145). On the contrary, another study revealed an increased the risk of developing SMI in females (146).

However, SMI was more prevalent in hypertensive men than in normotensives (147). Additionally, vascular remodelling resulting from HT contributed significantly to SMI development (148). Vascular remodelling in HT contributed to elevated systemic vascular resistance due to structural changes of resistance vessels (149). In addition, in individuals with HT, microalbuminuria and salt sensitivity were associated with the increased presence of SMI, possibly due to higher sympathetic nervous system activity or more extensive myocardial microvascular injury (150). SMI was also independently related to elevations in troponin levels and can predict mortality in critically ill individuals (14).

4.4 Proposed Mechanisms of silent myocardial ischemia (SMI)

Several mechanisms have been proposed for the occurrence of SMI. Advanced and more recent scientific research papers (1988 – 2009) suggested it can vary from autonomic neuropathy (133), cerebral cortical dysfunction (due to abnormal neural dispensation by the afferent pain impulse from the heart) (14), as well as coronary microvascular dysfunction in combination with a lower sensitivity to painful input (7, 14). Also a dysfunctional perception of symptoms can lead to an absent pain stimuli recognition (51). This may be due to an impaired perception of pain, a higher threshold for pain, or a surplus of circulating endogenous endorphins (51). A decline in cortical activation was also proposed in non-diabetics, where extracardiac influences may affect the central dispensation of the stimulus i.e. emotional status and personality characteristics (151). Also, mental stress can trigger ischemia in individuals with CAD (in 40 – 70% of cases) (152) and was a frequent trigger for the development of SMI (153). Wall motion dysfunction was related to myocardial ischemia induced by mental stress (19) and ischemia can also be predicted by cardiac autonomic dysfunction (19).

Myocardial oxygen demand played a prominent role in the development of SMI (154). SMI occurred frequently in hypertensive individuals due to reduced vascular supply and increased cardiac demand (38) and associations between SMI prevalence and CVD have been linked to

higher 24-h BP values in hypertensive individuals (17, 129). In support, the incidence of SMI was related to elevations in BP and heart rate (HR) (129, 155), where the HR rose due to changes in myocardial oxygen demand and supply (156) and contributed to the development of the ischemic event (156). Myocardial oxygen demand is reliant on the contractibility of the myocardium, HR, SBP afterload as well as the preload tension of the ventricular wall (19). If HR elevations were absent, the ischemia may possibly be a result of lower vasoconstriction-induced coronary blood flow (156). Also, due to higher oxygen demand in the morning, SMI was more prevalent in these early hours (157). This can possibly be a result of elevations in BP, HR, catecholamines, coronary vasomotor tone and platelet aggregation responses as well as inhibited intrinsic fibrinolytic processes (51). An elevated pulse pressure was also associated with SMI (129). The lowering of diastolic blood pressure (DBP) limited coronary perfusion, where elevated pulse pressure and left ventricular hypertrophy contributed to a higher oxygen demand as a result of limited coronary flow reserve leading to ischemia (129). The induction of SMI could also be ascribed to CIMT thickening and arterial plaques as well as rises in vasomotor tone as a result of impaired endothelium-derived relaxation (148). Another plausible explanation was that SMI can also be a result of endothelial dysfunction as measured by flow-mediated dilation and inflammation (detected by high-sensitivity c-reactive protein (hs-CRP)) in individuals with CAD (158). Endothelial dysfunction however independently predicted SMI and was not influenced by genetics (159). In Blacks from South Africa, SMI and left ventricular structural variations may be due to vascular responsiveness and explain a higher risk for ischemic stroke (160). Downstream signalling in beta-adrenergic receptors, adenylyl cyclase and G-proteins (guanine nucleotide binding proteins) has also been implicated during the ischemic process of SMI (7).

Myocardial ischemia further contributed to the development of conduction impairment and caused disturbances in the atrioventricular node (AV-node) and intra-nodal structures (21). A plausible explanation may be due that it is caused by biochemical and ionic deviations which are characteristics of myocardial ischemia. This led to unstable electric substrates which

caused and sustained arrhythmias. In addition, MI possibly caused electrical deficiencies and blocked conduction, further contributing to the development of arrhythmias (21). Also, SMI may be the underlying mechanism of low-grade-inflammation (hs-CRP > 3 mg/l) which induced 1st degree AV-block in black men from South Africa (22).

5. FIRST DEGREE ATRIOVENTRICULAR BLOCK (1ST DEGREE AV-BLOCK)

AV-block is defined by the delayed or disrupted conduction between the atria and the ventricles and can be divided into three different degrees of AV-block (Table 5.1): First- (1st), second- (2nd)- and third (3rd) degree AV-block. Table 5.1 presents the definition and characteristics/symptoms of the different degrees of AV-block.

Table 5.1: Different degrees of atrioventricular block (AV-block) (88, 161, 162).

Degree of AV-block	Definition	Characteristics/symptoms
First (1st) degree AV-block	<ul style="list-style-type: none"> PR-interval more than 200 milliseconds. All the impulses are conducted between the atria and ventricle. 	<ul style="list-style-type: none"> Asymptomatic. Present in 14% of individuals with myocardial infarction (MI).
Second (2nd) degree AV-block <i>Mobitz type I (Wenckebach)</i>	<ul style="list-style-type: none"> Progressive prolonged PR-interval until complete blocked atrial conduction. P-wave evident without QRS complex on ECG. “Dropped beat”. 	<ul style="list-style-type: none"> Often asymptomatic. Possible reduction in cardiac output leading to reduced perfusion and bradycardia. Irregular heartbeat. Elevated vagal tone without evident structural cardiac disease.

Degree of AV-block	Definition	Characteristics/symptoms
<p>Second (2nd) degree AV-block</p> <p><i>Mobitz type II</i></p>	<ul style="list-style-type: none"> • Constant PR-interval evident on ECG before and after non-conducted atrial beat. • Single or intermittent non-conducted P-waves and absent QRS complex. 	<ul style="list-style-type: none"> • Uncommon in individuals without structural cardiac disease. • Related to sclerosis or fibrosis of myocardium and myocardial ischemia. Bradycardia and lowered cardiac output. • Fatigue, Syncope. • Angina. • Dyspnoea. • May progress to complete heart block.
<p>Third (3rd) degree (complete) AV-block</p>	<ul style="list-style-type: none"> • Complete block. No conduction evident between atria and ventricles. • More P-waves than QRS complexes are evident on ECG characterized by own regular rhythm with no association to each other (atrioventricular dissociation). <p>PR-interval variable.</p>	<ul style="list-style-type: none"> • Bradycardia (< 40 beats per minute) with reduced cerebral perfusion (cognitive impairment, irritability, dizziness, syncope). • Heart failure. • Dyspnoea. • Cardiac arrest.

First (1st) degree AV-block as earlier described, was associated with heart failure and death in individuals with CAD (163). In about 14% of individuals with MI, 1st degree AV-block was detected and was the most common presentation of conduction impairment (21). First (1st) degree AV-block is diagnosed when the PR-interval of an electrocardiogram (ECG) is extended even though all the impulses were conducted (88). In addition, ischemic heart disease was described as one of the most common causes of 1st degree AV-block (21). Myocardial ischemia triggered interactions between ionic and biochemical factors resulting in deleterious effects on myocardial cellular electrophysiology, which led to conduction disturbances (21). In addition, this conduction disturbance may be due to an enhanced vagal tone or functional injury of the AV-node related to inferior MI (21) proximal to the bundle of His. Additionally, this disturbance can be a result of AV-node ischemia, Bundle of His ischemia, or both, which in turn was a result of occlusion of the right coronary artery proximal to the AV-node artery (164). Also, if the AV-block was localized below the Bundle of His, it was related to anterior MI and worse outcomes (21). It also comprised infarction of the septum and necrosis of the bundle branches (164). In individuals with CAD, perfusion of the atrioventricular nodal artery can possibly be impaired and a longer or shorter PR-interval may be related to worse outcome (165). However, the role if ischemia, autonomic dysfunction or structural remodeling as the underlying cause could not be clearly established (165).

First (1st) degree AV-block was also more common in men (165, 166) and African-American individuals compared to Whites (166). In addition, 1st degree AV-block was more common in the elderly and can be due to impairment of the left ventricular conduction as a result of left ventricular conduction sclerosis (166). As early as 1970, Lev et al. (167) histologically found atherosclerotic narrowing of coronary arteries as well as structural myocardial hypertrophy in individuals presenting acute and chronic 1st degree AV-block (167). Uhm and co-workers (168) found that 1st degree AV-block in hypertensive individuals is an independent predictor of left ventricular dysfunction, atrial fibrillation and advancing AV-block. The study by Uhm et al. (168) also proposed that the underlying mechanisms possibly contributing to the occurrence of

1st degree AV-block included coronary atherosclerosis, neuromuscular illness, dilated and infiltrative cardiomyopathy, left ventricular dysfunction, atrial fibrosis and poor BP control (168). Other factors contributing to the prevalence of 1st degree AV-block are: increased genetic factors, medication, enlargement of right atrium, delay of the AV-node conduction, fibrosis of right atrium and a delay in the bundle branch or Purkinje fibre (165). Structural remodelling of electrophysiological cardiac conduction was related to heart failure and led to a decline in RR variability, longer QRS- and PR-intervals as well as atrial fibrillation (165). First- (1st) degree AV-block can also contribute to dysfunctional hemodynamics, atrial fibrillation and regurgitation (165).

First- (1st) degree AV-block can thus be considered a significant marker of underlying heart disease (169), even though earlier studies considered it benign with no prognostic implications (170). A prolonged PR-interval was also considered an adverse prognostic measure in individuals with CAD and heart failure (165). The development of CAD in the form of atherosclerosis (atherogenesis), may additionally be ascribed to endothelial dysfunction (171, 172) and/or injury (172). Its onset is during childhood and progresses with aging (173) and is also distinguished by the thickening of the intima or media (174).

6. SUBCLINICAL ATHEROSCLEROSIS

Measuring thickening of carotid intima and media layers, delivered prognostic info in addition to traditional CVD risk factors (171). Thickening of the CIMT showed hypertrophy of the intima or media due to an adaptive response to variations in flow, wall stiffness or lumen diameter (175), and comprised an continuing inflammatory response (176). These changes in the CIMT included many factors associated with the pathogenesis and progression of atherosclerotic plaque. These factors encompassed: dysfunction of the endothelium (171); an enhanced pro-coagulant state (177), molecules responsible for vasoconstriction (172) and inflammation (176, 178); higher systemic cytokines and chemokines (179); higher oxidative stress levels (180); and smooth muscle cell proliferation and migration (181).

Age, total cholesterol, cigarette smoking and diabetes were independently associated with the severity of atherosclerotic lesions (182). The pathogenesis of atherosclerosis influenced by aging was due to the vascular changes with older age as well as higher exposure to known risk factors (182). Men were also more likely to develop atherosclerosis (182), however after menopause the likelihood of women to develop atherosclerosis increased (182). In black men from South Africa, adverse high-density lipoprotein (HdL) profile contributed to the development of subclinical atherosclerosis, where blood glucose levels in women from this ethnic group were positively related to subclinical atherosclerosis (183). Stensland-Bugge et al. (184) explained that the effect of triglycerides, smoking and physical activity on the development of subclinical atherosclerosis differs between sexes. However age, BP, cholesterol (total and HdL) and body mass index (BMI) were significant independent risk factors to predict the development of subclinical atherosclerosis. In addition, HT was independently associated with atherosclerosis due to a reduction in the compliance of larger arteries and the aorta; thus leading to higher SBP and lower DBP (184). Weber et al. (185) further concluded that SBP was an independent strong predictor of atherosclerosis in healthy individuals. Additionally, BP elevations promoted functional and structural changes leading to endothelial damage resulting in atherosclerosis (186). Other risk factors (non-traditional)

include: family history of CAD (187), renal disease (188), high coronary artery calcium score (189) abnormal ankle brachial index (189), elevated homocysteine (190), elevations in microproteinuria albumin/creatinine (190), MetS (191), human immunodeficiency virus (HIV) (192), elevated levels of c-reactive protein (189), fibrinogen (193), and lipoprotein (193).

In a study conducted by Hamer et al. (194), Blacks from South Africa had higher levels of subclinical atherosclerosis possibly due to some of the above mentioned risk factors and poorer health behaviours, which included less physical activity, more smoking and alcohol abuse. However, other studies revealed that the black South African population have favourable lipid profiles (45) and genetically lower homocysteine levels (195). Hence, the clinical significance of risk factor differences between races should be considered (196).

6.1 Detection of subclinical atherosclerosis

CIMT is widely used as an independent surrogate non-invasive, sensitive and dependable marker for atherosclerosis (197-199) and also predicted future risk for CVD and CAD (197, 198, 200). Measuring CIMT by ultrasound was reported since 1986 (201). B-mode two-dimensional ultrasound assessment of CIMT is indicative of arterial wall abnormalities (202), systemic vessel pathology (178) and can assess the degree of atherosclerotic lesion formation (202) in all stages of atherosclerosis (199). The sensitivity of CIMT measurement as surrogate for subclinical atherosclerosis was confirmed by arterial wall histology compared with ultrasound far wall measurements of the carotid (202). The CIMT of the common carotid artery is thinner than the CIMT at the bifurcation and thickens with aging (203). This may be due to low shear stress, although oscillations in shear stress is high (204). According to the European Society of Hypertension (ESH) an IMT of > 0.9 mm is indicative of vascular damage (atherosclerosis and vascular hypertrophy) in the absence of associated symptoms (205). However Lacroix et al. (206) showed that a CIMT of 0.7 mm was associated with an increased risk of cardiovascular events after coronary angioplasty. The American Society of Echography indicated that a CIMT $\geq 0.75^{\text{th}}$ percentile is considered abnormal as it predicted an increase in

vascular disease risk (207). Knoflach et al. (178) considered a CIMT \geq 0.90th percentile as high where de Groot et al. (199) stated that disruption of the arterial wall emerges at a thickness of 0.8 mm. In addition, a thicker CIMT is independently associated with IR (208).

6.2 Subclinical atherosclerosis and insulin resistance (IR)

IR is independently related to atherosclerosis in non-diabetic individuals (64, 209), even with absent hyperglycaemia (210) and is associated with an increased CIMT (208) or calcification of coronary arteries (211, 212). IR may exhibit pro-atherogenic consequences on the arterial wall that lead to plaque development as a result of several deficient cellular activities (213). IR has a significant part in every phase of atherosclerosis from the commencement and progression of primary atherosclerosis to the development of significant plaques (214, 215), by proliferation of atherogenesis (69) and atherosclerotic plaque volatility (216). The mechanisms responsible for the mentioned altered states of the arterial wall due to IR, likely involved systemic factors including HT (79, 217), dyslipidaemia (213, 218), low-grade systemic inflammation (78), obesity (219), disrupted insulin signalling of endothelial cells (220) and macrophages (221), decreases in the inhibitory effect of insulin on the synthesis of fibrinogen (222), and insulin-mediated inhibition of migration of vascular smooth muscle cells (218). The partial function of insulin in endothelial cells is signal transduction and vasodilation (223). Insulin resistant and obese individuals experienced endothelial dysfunction and a resistance to the effect of insulin on the endothelium-dependent vasodilation, which could increase the risk of atherosclerosis (224). A study by Rask-Madsen et al. (225) underscored this conclusion by stating that insulin therapy re-established insulin-stimulated endothelial function in individuals with ischemic heart disease and type 2 diabetes (225). Also, the numerous anti-atherogenic mechanism effects on the arterial wall of insulin included the increased production of nitric oxide (226) (important for regulation of BP and vascular tone (227)), and decreased endothelial cell apoptosis (228). IR reduced the anti-atherogenic and anti-inflammatory effects of nitric oxide produced by the endothelium (209). In addition, the pro-atherogenic consequence of vascular and endothelial smooth muscle cells mitogen-

activated protein (MAP) kinase pathway may be heightened (229), which was exerted by endothelin (220). This in turn was a prominent feature of IR (230). IR may also downregulate insulin-mediated AKt activation (231), which prevented cell apoptosis, and played a major role in the integrity of the endothelium (228).

Circulatory macrophages and monocytes have insulin receptors that are impaired by IR (209). This contributed to macrophage apoptosis due to the activation of the endoplasmic reticulum stress-apoptosis pathway located in atherosclerotic abrasions (209). In addition, efferocytosis (phagocytic process involved in removal of apoptotic cells) was also impaired in IR (232). As both these processes were impaired in IR, they could possibly contribute to an increase in plaque necrosis, and may significantly contribute to the development of atherosclerosis (233).

Low-grade inflammation as detected by hs-CRP > 3mg/l, is a contributing factor leading to the development of type 2 diabetes (234). Also, inflammation contributed to the development of atherosclerosis (64, 235). Even though several studies also established independent associations between low-grade inflammation and IR (236, 237) in diverse ethnic populations, including Blacks and Whites (236), several studies concluded that no causative interaction exists between these two leading to the progression of subclinical atherosclerosis (209, 238).

6.3 Subclinical atherosclerosis and silent myocardial ischemia (SMI)

Atherosclerosis may lead to the development of lesions limiting blood flow leading to impaired oxygen delivery to tissue, causing ischemia (239). This invariably showed that increases of the CIMT was associated with the prevalence and/or extend abnormal myocardial perfusion (240). Indeed, such a profile was observed in individuals with coronary heart disease, cerebrovascular disease, CVD and peripheral vascular disease (241). In addition, Anand et al. (146) concluded that SMI was more evident in individuals with moderate coronary atherosclerosis, where Griffiths et al. (17) established that SMI in black men was associated with subclinical atherosclerosis not necessarily due to HT. The lack of oxygen as a result of

SMI (86), and cardiomyocyte injury (as measured by hs-cTnT), can also contribute to ventricular strain and facilitate thickening of the carotid intima-media (17). Atherosclerotic lesions in coronary arteries contributed to intermittently impaired coronary blood supply as an underlying cause of SMI (242). Also, when stenotic lesions as a result of atherosclerosis (luminal narrowing between 50- and 70%) were formed, a threshold level can be reached where higher myocardial oxygen demand leads to myocardial ischemia (19).

7. MOTIVATION FOR THE CURRENT INVESTIGATION

The SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) study is a target population comparative study in sub-Saharan Africa which was designed to investigate the link between neural activity and cardiometabolic risk markers in black individuals (243). The contribution of this study is of importance as CAD and CVD is the leading cause of mortality and morbidity in the South African context. The prevalence of CVD is escalating and impacts general well-being; and Blacks seem more susceptible to develop CVD and CAD (244) than their white peers.

For the first time in Africa, the simultaneous 24-h ambulatory monitoring of BP and electrocardiogram (ECG) allowed the detection of SMI. The current study is the first to relate SMI to cardiac troponins in a well-controlled cohort. SMI is an underestimated health risk and the premature detection of SMI and the possibility of reduced perfusion to the heart or coronary circulation may be important in black individuals from South Africa who are more likely to be diagnosed with symptomatic occlusive vascular disease (244), and expanding this knowledge may lessen the mortality rate annually. Using the measurements of elevated hs-cTnT and IR as risk factors in the prediction of SMI events in separate ethnic groups can also [1] contribute to the determination of ethnic disparity; and [2] underpin the prognostic relevance of SMI screening in health-care practice as target organ damage marker. Furthermore, [3] when considering conduction disturbances and subclinical atherosclerosis in

relation to these risk markers (hs-cTnT and IR), it may improve our understanding of the CVD and CAD burden in this bi-ethnic cohort.

8. MOTIVATION FOR COHORT SUBDIVISIONS

In each manuscript the cohort has been divided into specific ethnic or ethnic/sex groups after interaction terms have been calculated, which was also discussed accordingly as part of the statistical analyses in the result section of each manuscript.

The design of the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) is provided in Figure 9.1.

9. STUDY DESIGN

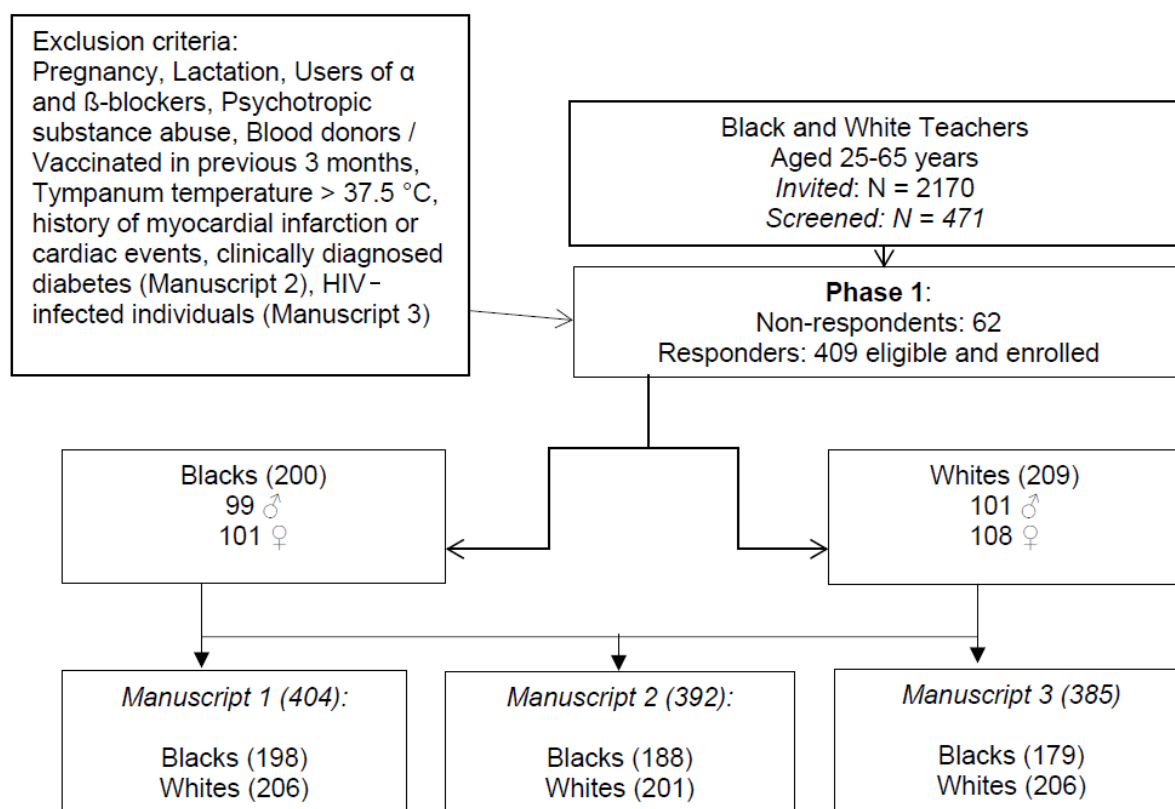


Figure 9.1: The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study population. Adapted from Malan L, Hamer M, Frasere-Smith N, Steyn HS, Malan NT. Cohort Profile: Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study. *Int J Epidemiol.* 2015;44(6):1814-22.

The following questions still need to be answered:

1. Why are urban Blacks from South Africa more vulnerable to the development of CVD and CAD than their white counterparts?
2. What is the prevalence of SMI in this bi-ethnic cohort from South Africa?
3. Can hs-cTnT and IR be used as reliable cardiometabolic risk markers to determine the prevalence of SMI and subclinical atherosclerosis in a bi-ethnic cohort?
4. Do the prognostic risk markers hs-cTnT and IR differ between ethnic groups in the prediction of atherosclerosis, especially when conduction disturbances are present?

10. AIMS

10.1 Troponin T release is associated with silent myocardial ischemia in black men: the SABPA study

- To compare high-sensitivity cardiac troponin T levels in white and black South Africans and determine its relationship with silent myocardial ischemia; and
- To explore the capability of high-sensitivity cardiac troponin T to predict compensatory systolic hypertension in a bi-ethnic cohort.

10.2 Silent ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study

- To compare possible associations between silent myocardial ischemia and high-sensitivity cardiac troponin T; and
- To compare possible associations between silent myocardial ischemia and insulin resistance in a bi-ethnic sex cohort.

10.3 Troponin T release is associated with subclinical atherosclerosis in Blacks with first degree AV-block: the SABPA study

- To determine the associations between subclinical atherosclerosis and high-sensitivity cardiac troponin T in a bi-ethnic cohort when 1st degree AV-block is present; and
- To determine the associations between subclinical atherosclerosis and insulin resistance in a bi-ethnic cohort when 1st degree AV-block is present.

11. HYPOTHESES

11.1 Main hypotheses of this study

Considering the literature and the aims of this specific study, the following main hypotheses were proposed: Urban blacks reveal a cardiovascular susceptibility (as measured by high sensitivity cardiac troponin T (hs-cTnT)) whereas Whites reveal a cardiometabolic susceptibility (as measured by insulin resistance (IR)). In addition, hs-cTnT may predict compensatory systolic hypertension in this bi-ethnic cohort. Associations between hs-cTnT and subclinical atherosclerosis in the Blacks will be more evident when 1st degree AV-block is present, whereas associations between IR and subclinical atherosclerosis will be observed in the Whites when 1st degree AV-block is present.

11.2 Detailed hypotheses of each manuscript

11.2.1 Manuscript 1: Troponin T release is associated with silent myocardial ischaemia in black men: the SABPA study

- High sensitivity cardiac Troponin T is associated with silent myocardial ischemia in the Blacks from South Africa; and
- High sensitivity cardiac Troponin T will predict systolic hypertension differently in races.

11.2.2 Manuscript 2: Silent ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study

- Positive associations will be evident between high-sensitivity cardiac Troponin T and silent myocardial ischemia in Blacks; and
- Positive associations will be evident between silent myocardial ischemia and insulin resistance in Whites.

11.2.3 Manuscript 3: Troponin T release is associated with subclinical atherosclerosis in Blacks with first degree AV-block: the SABPA study

- Positive associations will be evident between subclinical atherosclerosis and high-sensitivity cardiac Troponin T in Blacks presenting 1st degree AV-block; and
- Subclinical atherosclerosis will be positively associated with insulin resistance in Whites presenting 1st degree AV-block.

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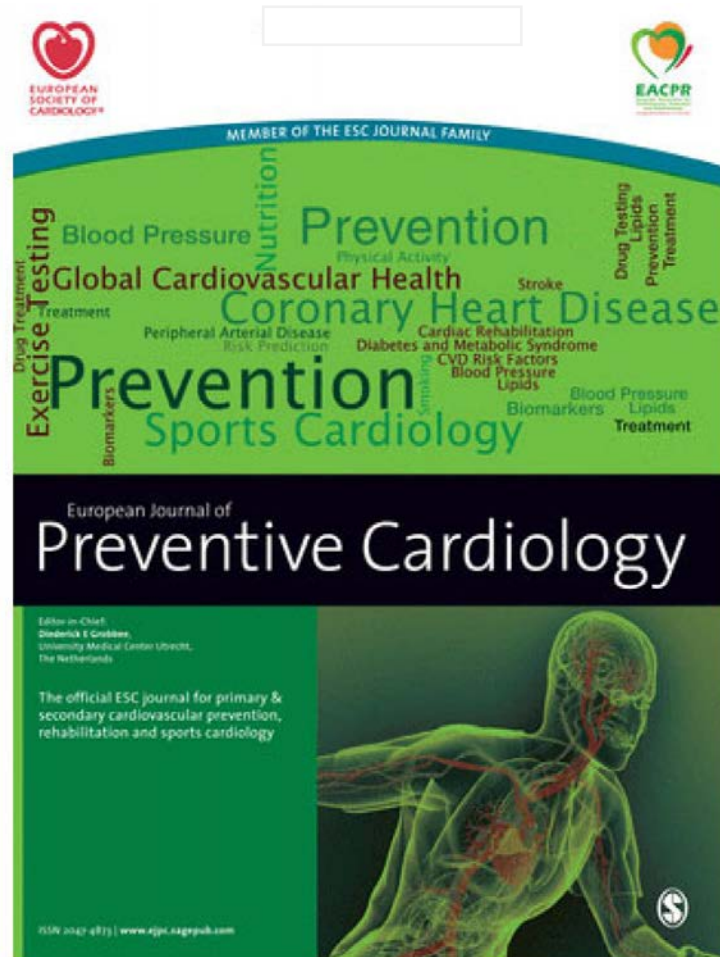
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Chapter 2



Manuscript 1: Troponin T release is associated with silent myocardial ischemia in black men: the SABPA study

Manuscript 1

Manuscript 1 has been published in the peer-reviewed journal: EUROPEAN JOURNAL OF PREVENTIVE CARDIOLOGY

Impact Factor: 4.542

This journal publishes original basic research articles from all the scientific, clinical and public health disciplines. These include studies which address the cause and prevention of cardiovascular disease, cardiovascular rehabilitation and exercise physiology.

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- Maximum of 5000 words (each table or figure reducing the word count by 250).
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 - Use of abbreviations should be kept to an absolute minimum.
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- Title Page**
- The title page should carry the full title of the paper, consisting of no more than 20 words.
 - All authors' names: the full first name, middle name/initial (optional) and last name of each author should appear.
 - The affiliations of all the authors.
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- Structured** – Not exceeding 250 words.
- Abstract**
- Background, Design, Methods, Results and Conclusions.
 - Abstract word count at the end of the abstract.
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 - Double spacing, beginning on a separate page.
 - All abbreviations used in the illustration must be defined in the legend
- References**
- SAGE Vancouver style.
 - Up to 30 references.

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Troponin T release is associated with silent myocardial ischemia in black men: the SABPA study

Running head: High-sensitivity troponin, silent myocardial ischemia and hypertension

Madelein E Griffiths, Leoné Malan, Rhena Delport, Marike Cockeran, Manja Reimann

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Troponin T release is associated with silent myocardial ischaemia in black men: The SABPA Study

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Marike Cockeran³ and Manja Reimann⁴

Abstract

Background: High sensitivity cardiac troponin T (hs-cTnT) is a validated marker of myocardial damage and may reflect the degree of silent myocardial ischaemia (SMI) and ventricular strain. Our aim was to compare hs-cTnT levels in black and white South Africans taking SMI into consideration. We further explored the capability of hs-cTnT to predict the presence of compensatory systolic hypertension in this South African cohort.

Methods: A bi-ethnic sex cohort ($n = 404$) with similar socioeconomic status (198 black participants and 206 white participants, aged 20–65 years) participated in this target population study where 24 h ambulatory blood pressure, electrocardiogram and overnight fasting cardiometabolic variables were measured.

Results: Hypertension, higher glycated haemoglobin levels and more frequent and longer SMI events were observed more often in the black participants. Multivariate linear regression analysis showed positive associations between SMI events [Adj. $R^2 = 0.19$; β 0.35 (0.08–0.62); $p < 0.01$], SMI event maximum duration [Adj. $R^2 = 0.17$, β 0.43 (0.16–0.70), $p < 0.01$], SMI total duration [Adj. $R^2 = 0.12$; β 0.37 (0.10; 0.65), $p = 0.05$] and hs-cTnT in black males only. A lower hs-cTnT cut-point ≥ 4.2 pg/ml for 24 h systolic hypertension was predicted in the black participants compared with ≥ 5.6 pg/ml in the white participants (area under the curve 0.66–0.67 (95% CI: 0.57–0.75), $p < 0.001$) with a respective sensitivity/specificity of 64/68% and 61/71%.

Conclusions: hs-cTnT may be a potential marker of SMI in the prediction of systolic blood pressure increases, as well as clusters of risk factors for cardiovascular disease. Ethnic- and possibly sex-specific reference values for hs-cTnT should be considered for risk stratification.

Keywords

hs-cTnT, hypertension, ethnicity, sex, silent myocardial ischaemia

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Introduction

Silent myocardial ischaemia (SMI) contributes to the total ischaemic burden in people with coronary artery disease.^{1–3} It is more evident in black people from South Africa than in the white population⁴ and occurs more often in people with hypertension resulting from a reduced vascular supply and increased cardiac demand.^{5,6} High sensitivity cardiac troponin T (hs-cTnT), which is often measured in primary myocardial ischaemia^{7–9} and minor myocardial injury,¹⁰ may be useful in detecting chronic subclinical myocardial damage before the development of hypertension.¹¹ The identification of people at risk of hypertension secondary to myocardial ischaemia may aid in preventing exacerbation

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ABSTRACT

Background: High-sensitivity cardiac troponin T (hs-cTnT) is a validated marker of myocardial damage and may reflect the degree of silent myocardial ischemia (SMI) and ventricular strain. Our aim was therefore to compare hs-cTnT levels in black and white South Africans taking SMI into consideration. We further explored the capability of hs-cTnT to predict the presence of compensatory systolic hypertension in this South African cohort.

Methods: A bi-ethnic sex cohort (n=404) with similar socio-economic status (Blacks n=198, Whites n=206, aged 20-65) participated in this target population study where 24-h ambulatory blood pressure, -ECG and overnight fasting cardiometabolic variables were measured.

Results: Hypertension (HT), higher glycosylated haemoglobin (HbA_{1c}) and more frequent and longer SMI events were more frequently observed in Blacks. Multivariate linear regression analysis showed positive associations among SMI events [Adj. R² = 0.15; β 0.29 (0.01 0.57); p <0.05], SMI event maximum duration [Adj. R² = 0.15, β 0.41 (0.13; 0.68), p <0.05], SMI total duration [Adj. R² = 0.12; β 0.37 (0.10; 0.65), p = 0.01] and hs-cTnT in black males only. A lower hs-cTnT cut-point ≥ 4.2 pg/ml for 24-h systolic hypertension was predicted in Blacks compared with ≥5.6 pg/ml in Whites (AUC 0.66-67 (95% CI: 0.57-0.75), p<0.001) with a respective sensitivity/specificity of 64%/68% and 61%/71%.

Conclusion: High-sensitivity cardiac Troponin T (hs-cTnT) may be a potential marker of SMI to predict systolic blood pressure increases, as well as cardiovascular disease risk factor clusters. Ethnic and possibly sex-specific reference values for hs-cTnT should be considered for risk stratification.

Word count: 249

Key words: hs-cTnT, hypertension, ethnicity, sex, silent myocardial ischemia

1. INTRODUCTION

Silent myocardial ischemia (SMI) contributes to the total ischemic burden in individuals with coronary artery disease (CAD) (1-3), is more evident in Blacks from South Africa (4) and occurs more frequently in hypertensive individuals due to a reduced vascular supply and increased cardiac demand.(5, 6) In addition, high-sensitivity cardiac Troponin T (hs-cTnT), which is frequently measured in conditions such as primary myocardial ischemia (7-9) and minor myocardial injury (10), may be useful to detect chronic subclinical myocardial damage, preceding the development of hypertension (11). Identification of individuals at risk for hypertension secondary to myocardial ischemia may aid in preventing exacerbation of the ischaemic burden (12). We compared hs-cTnT levels in a black and white South African cohort to predict ethnic-related prevalence towards elevated hs-cTnT in relation to SMI. In support, we further investigated the capability of hs-cTnT to predict risk for compensatory systolic hypertension in this South African cohort.

2. METHODS

The SABPA (Sympathetic and Ambulatory Blood Pressure in Africans) was a target population study including 409 urban black and white South African teachers from the Dr Kenneth Kaunda Education district of the North West province, South Africa.(13) The study sample was selected to predict homogeneity regarding socio-economic status and working environment, although participants were from diverse cultural backgrounds. Eligible participants between ages 20 and 65 years were requested to partake. Exclusion criteria for this study included: pregnancy or lactation, blood donation or vaccinations less than 3 months prior to data collection, psychotropic substance dependence or abuse and individuals with a history of myocardial infarction or cardiac events (n=5). The final sample included 198 Blacks and 206 Whites (N=404). Before enrolment, participants were informed about the study protocol and signed an informed consent form. The Ethics Review Board of North-West University (Potchefstroom Campus: NWU-00036-07-S6) approved this study, and procedures abided with terms and guidelines of the Declaration of Helsinki.(14) The study was conducted from February to May in 2008/9.

2.1 Ambulatory blood pressure measurements

Participants were fitted with a 24-hour (24-h) blood pressure (BP) and electrocardiographic (ECG) device (Cardiotens CE120®, Meditech, Budapest, Hungary) as well as an accelerometer (Actical®, Montreal, Quebec) during workdays (Mondays to Thursdays) at approximately 08:00. The British Hypertension Society validated Cardiotens CE120® device (B/B) provided oscillometrically measured 24-h ambulatory BP monitoring (ABPM) and 2-lead ECG measurements. The device measured BP every 30 minutes between 08:00 and 22:00 and every 60-minutes (min) between 22:00 and 06:00 (15). Suitable cuff sizes were fitted on the non-dominant arm. Inflation rates of successful measurements for the 24-h BP period were 72.8% ($\pm 0.8\%$) in Blacks and 84.7% ($\pm 0.7\%$) in Whites. Hypertension was classified as an average 24-h BP of ≥ 130 mmHg SBP and/or ≥ 80 mmHg DBP (16, 17).

Before the start of the 24-h ECG investigation, the isoelectric reference point (PQ-segment), J-point, L-point (80 ms after the J-point), and an ST-segment detection interval of at least 3 mm as the initial ST-level, were calculated for each patient. Two-lead 24-h ECG recordings were obtained according to a pre-set program for 20 seconds at 5-min intervals assessing silent ischemic events (ST-segment depression). Silent ischemic events were documented according to the 1-1-1 rule: 1) more than 1 mm horizontal or descending ST-segment, 2) the ST-segment event occurs for at least 1 min (duration), and 3) two consecutive ST-episodes must have an in-between interval of at least 1 min to be counted as independent episodes.(18) In case of horizontal or descending ST-depression, an ECG tracing lasting 60 seconds was recorded and an additional blood pressure measurement was automatically initiated by the trigger mechanism of the device. The BP and ECG data were analysed using the CardioVisions 1.19.2 Personal Edition software (Meditech®, Budapest, Hungary).

Participants continued their usual daily activities and were asked to record (on their ambulatory dairy card) stressful events, headaches, fainting, nausea, palpitations and visual disturbances on their ambulatory diary card. At 16:30 of the same day participants were transported to the Metabolic Unit of North-West University where they reviewed the protocol and were informed about the experimental setup at the facilities. Participants reported information regarding medication use and medical history. In addition, they completed psychosocial questionnaires under the supervision of registered clinical psychologists. A standardized dinner was served, and participants were requested to go to bed at 22:00 whilst fasting overnight. At 05:45 the following morning, anthropometric measurements were performed. Hereafter the participants were in a semi-recumbent position for 12-lead ECG monitoring and blood sampling. After completion of these measurements, participants received breakfast and were transported back to school.

2.2 *Assessment of lifestyle risk factors*

Physical activity or total daily energy expenditure (kcal/day), considering resting metabolic rate, were obtained using the Actical® omnidirectional accelerometer (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) which measures movement at 15-second intervals. Data were converted into 1-second intervals for analysis. Plasma γ -glutamyl transferase (γ -GT) and plasma cotinine levels were analysed to indicate possible alcohol abuse and smoking status respectively. Cotinine values exceeding 14.99 ng/ml² classified subjects as regular smokers. Anthropometric measurements were performed in triplicate by registered level II ISAK-accredited Anthropometrists (International Society for the Advancement of Kinanthropometry) using standardized methods. Height and body mass were determined to the nearest 0.1cm and 0.1kg respectively (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer IP 1465, Invicta, London, UK), whereas body surface area (BSA) in m² was calculated using the Mosteller formula.³ Inter and intra variation was less than 10% (13, 19).

2.3 *Biochemical sampling and analyses*

Serum γ -GT was determined by a high-sensitivity enzyme rate method. Serum cotinine was measured by a homogenous immunoassay method (Modular ROCHE Automized, Switzerland). Whole blood glycated haemoglobin A_{1C} (HbA_{1C}) was determined using a turbidimetric inhibition immunoassay (Cobas Integra 400plus, Roche, Basel, Switzerland). Prediabetes was defined as HbA_{1C} \geq 5.7% according to the American Diabetes Association.⁽²⁰⁾ Human Immunodeficiency Virus (HIV) infection was determined in EDTA plasma by the First Response kit (RPM Plus, USA) and confirmed by the Pareekshak test (Bhat Biotech, India). HIV pre- and post-counselling was done by trained counsellors. High-sensitivity Troponin T (hs-cTnT) levels were determined using the electrochemiluminescence immunoassay (ECLA) Modular ROCHE Automized, Switzerland. High-sensitivity Troponin T (hs-cTnT) inter- and intra-assay coefficients of variation were less than 10% and the lower limit of detection was 3 pg/ml.

2.4 Statistical analyses

Statistica version 13.0 (Statsoft Inc., Oklahoma, USA, 2013) and SPSS software package version 22 for Windows (IBM, SPSS Inc., Chicago, IL, USA) were used. Distribution of data was tested using the Shapiro-Wilks W -test for normality, and non-normal distributions were logarithmically transformed (cotinine, physical activity, γ -GT, HbA_{1c}, SMI events, hs-cTnT, SMI maximum duration). Data are presented as mean \pm SD unless stated otherwise. Students' T -test were used to describe the population by ethnic status. Chi-square (χ^2) test was used to compare proportions of hypertension, SMI, HIV, prediabetes, use of statins and antihypertensive medication.

Multivariate analyses of covariance (ANCOVA), were used to determine interaction on main effects (ethnicity \times sex) for cardiovascular risk markers, independent of *a priori* confounders (age, body surface area, physical activity, serum cotinine levels and γ -GT) Subsequently, univariate ANCOVAs were applied to compare the number of SMI events, SMI total duration and maximum duration, as well as cardiovascular variables between ethnic groups. Partial correlations were controlled for *a priori* confounders and performed to analyse associations among SMI events, total SMI duration in 24 hours and the maximum duration (the longest SMI event), and cardiovascular variables in ethnic groups. Forward stepwise regression analysis predicted relationships between SMI and hs-cTnT (F to enter 2.50), independent of *a priori* confounders, in the total group and in the separate bi-ethnic sex groups. Ethnicity and sex were added as co-variates in the total cohort analyses. Optimal hs-cTnT cut-points associated with systolic hypertension were computed from the maximum of the Youden index (J) (sensitivity + specificity - 1) using non-parametric receiver-operating characteristic (ROC) curves. The statistical significance level was set at $p \leq 0.05$ (two-tailed). *Sensitivity analyses* were conducted by excluding HIV infected individuals and repeating forward stepwise regression analyses.

3. RESULTS

The Blacks and Whites were well-matched for sex and age dispersal. Cardiovascular disease risk factors were more prevalent in Blacks from South Africa and included higher 24-h blood pressure, -body surface area, -HbA_{1c} levels, -cholesterol levels, - pulse pressure and -heart rate (Table 3.1). Blacks revealed higher γ -GT values and were less active than the Whites (Table 3.1).

Table 3.1: Demographic and clinical characteristics.

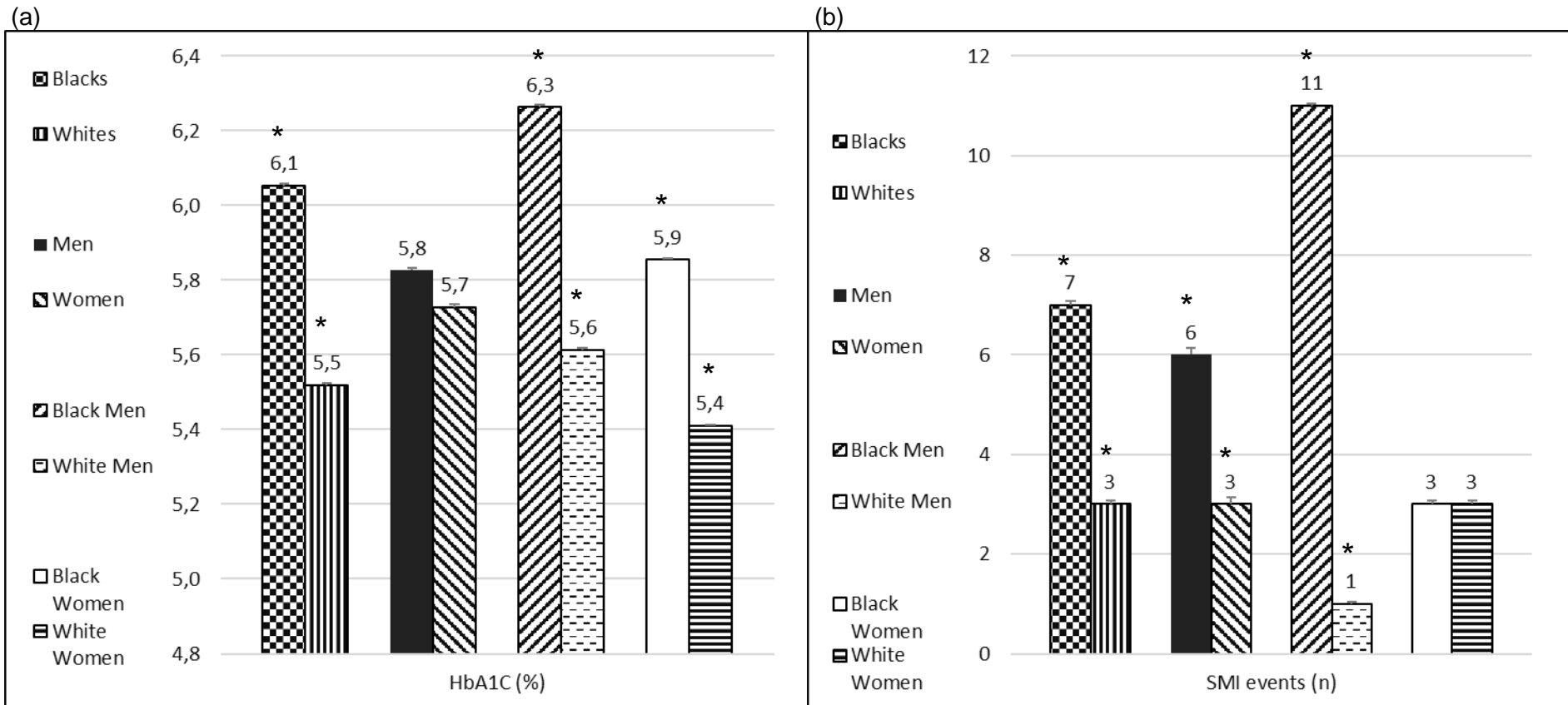
	Blacks (n=198)	Whites (n=206)	p-values
Men, n (%)	100 (50.5)	98 (47.6)	0.56
<i>Lifestyle factors</i>			
Age, years	44 (8)	45 (11)	0.54
Body Surface area, m ²	1.92 (0.2)	2.00 (0.3)	0.002
Physical activity, kcal/day [†]	2599.2 (2.163.7; 3118.1)	2927.2 (2356.0; 3487.7)	0.001
Cotinine, ng/ml [†]	0.010 (0.01; 16.01)	0.010 (0.01; 0.01)	0.59
γ-GT, U/L [†]	41.11 (28.1; 73.0)	17.50 (12.0; 28.0)	<0.001
HbA _{1c} , %	6.08 (1.2)	5.50 (0.4)	<0.001
Cholesterol, mmol/l	4.60 (1.2)	5.53 (1.3)	<0.001
24-h SBP, mmHg	133 (16)	124 (12)	<0.001
24-h DBP, mmHg	83 (11)	77 (8)	<0.001
24-h PP, mmHg	50 (9)	48 (7)	<0.001
24-h HR, beats/min	80 (11)	74 (10)	0.01
24-h SMI events, n (SD)	6 (16)	3 (6)	0.002
24-h SMI max duration, min [†]	3.00 (1.5; 17.0)	2.50 (1.0; 7.8)	0.49
24-h SMI events total duration, min [†]	7.0 (2.0; 39.5)	5.5 (1.0; 24.0)	0.60
hs-cTnT, pg/ml	4.78 (2.7)	5.49 (3.5)	0.02
hs-cTnT LOD>3 pg/ml, n (%)	135 (69.6)	153 (75.0)	0.22
<i>Cardiovascular disease risk</i>			
24-h Hypertension, n (%)	131 (66.2)	79 (38.4)	<0.001
24-h SBP Hypertension, n (%)	112 (56.6)	51 (24.7)	<0.001
24-h DBP Hypertension, n (%)	119 (60.1)	64 (31.1)	<0.001

	Blacks	Whites	p-values
	(n=198)	(n=206)	
HIV, n (%)	18 (9.1)	0 (0)	<0.001
Prediabetes, n (%)	122 (62.2)	57(27.7)	<0.001
Silent Ischemia, n (%)	101 (51.1)	87 (42.2)	0.07
<i>Medications</i>			
Hypertension, n (%)	68 (34.3)	25 (12.1)	<0.001
Statins, n (%)	2 (1)	8 (3.9)	0.06

Data are reported as mean (SD) or proportions (%) unless otherwise indicated. † - Data are reported as median (Interquartile range). γ -GT = Gamma glutamyl transferase; SBP = Systolic Blood Pressure; DBD = Diastolic Blood Pressure; PP = Pulse Pressure; HR = Heart Rate; SMI-Events = Silent Myocardial Ischemic events determined according to the 1-1-1 rule (18), SMI events max duration = The maximum duration of Silent Myocardial Ischemic events; hs-cTnT = High-sensitivity cardiac Troponin T; hs-cTnT LOD>3 pg/ml = participants with a high-sensitivity troponin T Level above the Limit of Detection (LOD) of 3 pg/ml; Prediabetes = HbA_{1c} \geq 5.7% (20).

In addition, they displayed more SMI events over a 24-h period than their white counterparts (Figure 3.1). Furthermore, hypertension, self-reported use of antihypertensive medication and HIV infection were more prevalent in Blacks than in Whites.

A significant ethnicity x sex interaction existed for SMI [$F(1,359) = 12.78$ $p = <0.001$]. High-sensitivity cardiac Troponin T (hs-cTnT) was only significantly different in men and women [$F(1,386) = 17.28$; $p = <0.001$] independent of ethnicity. In general, the men revealed more SMI events, longer SMI events and higher hs-cTnT levels than women did (Figure 3.1). Sex and ethnic differences were only evident in the number and duration of SMI events.



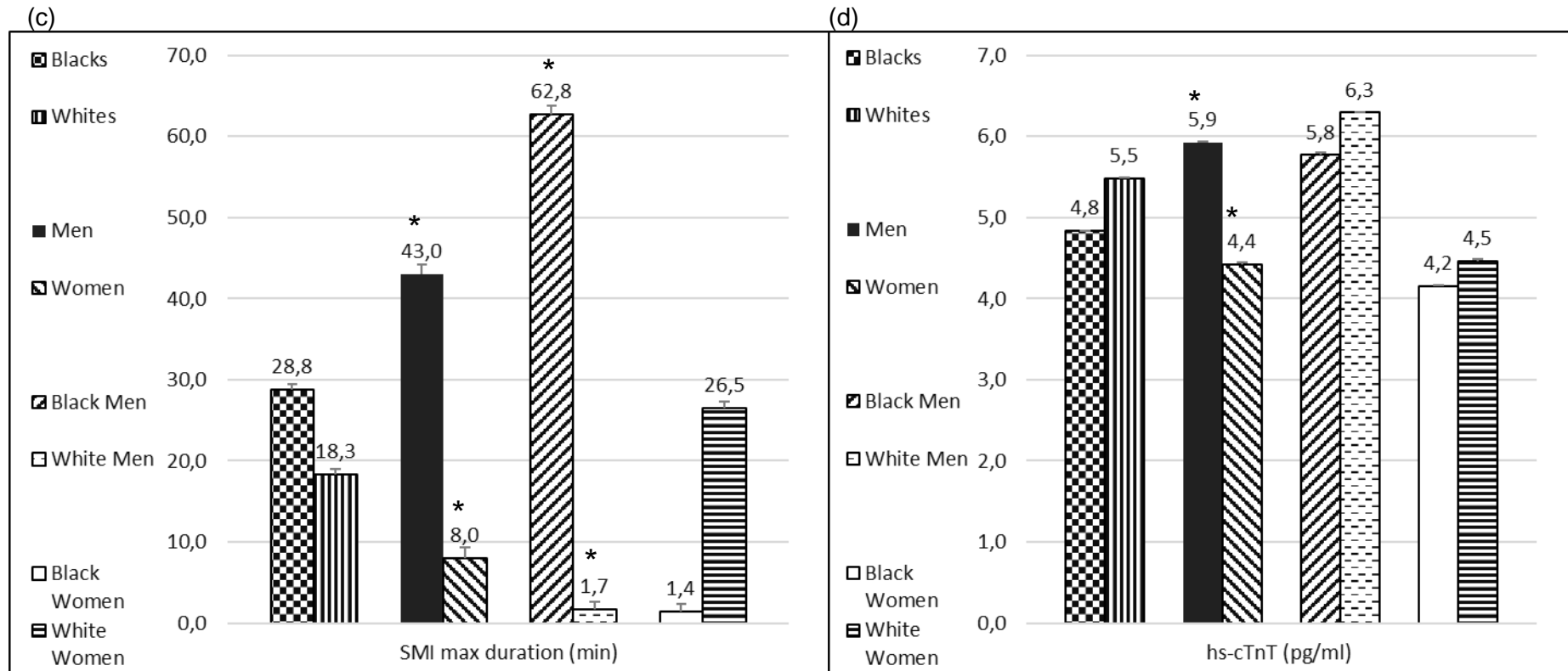


Figure 3.1a – 3.1d: Analysis of covariates revealing cardiovascular risk markers comparing bi-ethnic groups from South Africa. Data are presented as adjusted means \pm SE (Adjusted for *a priori* confounders). Glycated haemoglobin levels (Hb_{A1c}) (fig 3.1a), Number of silent myocardial ischemic events (SMI events) (Fig 3.1b), maximum duration of silent myocardial events (SMI max duration) (fig 3.1c) and high-sensitivity Troponin T levels (hs-cTnT) (fig 3.1d). * $p \leq 0.05$.

Significant partial correlations in the Blacks were observed among hs-cTnT levels, cardiovascular disease risk factors and SMI events, as reported in Table 3.2 ($R > 0.30$ and $p \leq 0.05$).

Table 3.2: Partial correlations between high-sensitivity cardiac troponin T (hs-cTnT) and cardiovascular risk markers.

Variable	Blacks		Whites	
	R-Value	<i>p</i> -value	R-value	<i>p</i> -value
	hs-cTnT			
HbA _{1c}	0.40	<0.001	0.18	0.111
Cholesterol (mmol/l)	0.16	0.144	0.05	0.655
24-h SBP (mmHg)	0.30	0.004	-0.01	0.944
24-h DBP (mmHg)	0.22	0.040	-0.11	0.327
24-h pulse pressure (mmHg)	0.27	0.011	0.12	0.313
Heart rate (beats/min)	-0.08	0.408	-0.01	0.944
SMI events	0.18	0.085	0.04	0.728
SMI event max duration	0.35	0.001	0.01	0.979
SMI total duration	0.34	0.001	-0.00	0.977

Abbreviations: hs-cTnT = high-sensitivity cardiac troponin T; SBP = systolic blood pressure; DBP = diastolic blood pressure. SMI events = silent myocardial ischemic events. SMI events max duration = The maximum duration of SMI events. SMI total duration = The total duration of SMI events over 24-h. Values in bold indicate significant associations ($R > 0.30$ and $p \leq 0.05$). Adjusted for *a priori* confounders (age, body surface area (BSA), total energy expenditure (TEE), cotinine levels, gamma-glutamyl transferase (γ -GT) levels).

The ROC area under the curve (AUC) demonstrated an hs-cTnT cut-off for Blacks at ≥ 4.2 pg/ml (AUC 0.67 (95% CI: 0.59; 0.74), $p < 0.001$) to predict systolic hypertension with 64% sensitivity and 68% specificity (Figure 3.2). A hs-cTnT of ≥ 5.6 pg/ml (AUC 0.66 (95% CI: 0.57; 0.75), $p < 0.001$) predicted systolic hypertension with 61% sensitivity and 71% specificity in Whites (Figure 3.2).

High Sensitivity cardiac Troponin T predicts 24-h systolic hypertension in Blacks and Whites

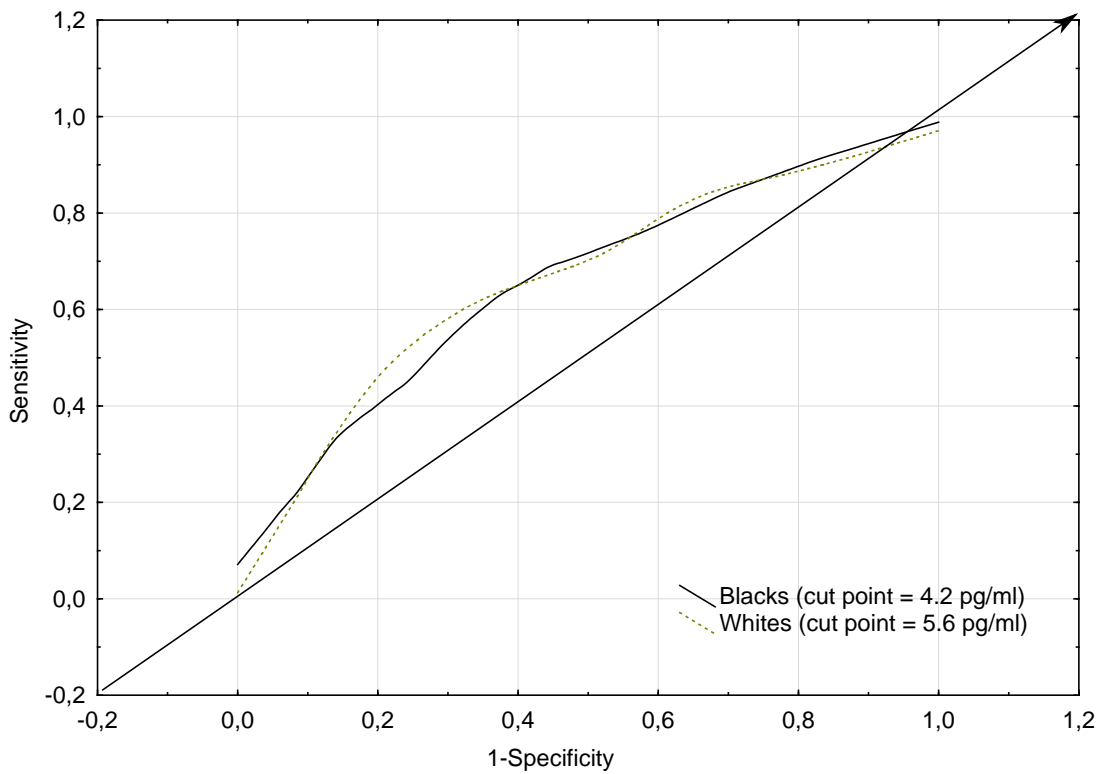


Figure 3.2: ROC curve depicting the high-sensitivity cardiac troponin T (hs-cTnT) cut-point for 24-h systolic hypertension in Blacks and Whites. The area under the curve (AUC) (95% CI) was 0.67 (95% CI 0.59; 0.74) for the Blacks and 0.66 (95% CI 0.57; 0.75) for the Whites. The specificity/sensitivity was determined at 64/68% for the Blacks and 61/71% for the Whites.

Linear regression analyses revealed significant positive associations between SMI events, SMI events duration, SMI max duration and hs-cTnT in Blacks and Black men only, controlling for a *priori* confounders (Table 2.3). Hs-cTnT explained 11% of the variation in SMI in Blacks. After further stratification into sex groups, SMI events were significantly associated with hs-cTnT in black males but not in black females. Excluding HIV infected individuals did not change the main outcomes.

Table 3.3: Associations among silent myocardial ischemia (SMI) events, SMI duration and hs-cTnT in Blacks.

	Log SMI events	Log SMI maximum duration	Log SMI Total duration
Adjusted R²	0.06	0.04	0.05
	β (95% CI)	β (95% CI)	β (95% CI)
hs-cTnT	0.19 (0.09; 0.29)**	0.22 (0.08; 0.36)**	---
Race	-0.19 (-0.47; 0.09)**	---	-0.17 (-0.31;-0.02)*
Blacks			
	Log SMI events	Log SMI maximum duration	Log SMI Total Duration
Adjusted R²	0.14	0.19	0.14
	β (95% CI)	β (95% CI)	β (95% CI)
hs-cTnT	0.33 (0.13; 0.53)**	0.44 (0.26; 0.62)**	0.24(0.03; 0.44)**
Sex	-0.22 (-0.41; -0.02)*	-.23 (-0.43; -0.03)*	-0.23(-0.44; -0.03)**
Black men			
	Log SMI events	Log SMI maximum duration	Log SMI Total duration
Adjusted R²	0.19	0.17	0.12
	β (95% CI)	β (95% CI)	β (95% CI)
hs-cTnT	0.35 (0.08; 0.62)**	0.43 (0.16; 0.70)**	0.37 (0.10; 0.65)*

β denotes standardized regression coefficient. Independent covariates included in models were: age, body surface area (BSA), log total energy expenditure (TEE), log cotinine, log gamma glutamyl transferase (γ -GT). F to enter 2.5. * $p \leq 0.05$; ** $p \leq 0.001$.

4. DISCUSSION

The objective of this study was to compare the prevalence of SMI in relation to hs-cTnT, in a bi-ethnic South African cohort. Further, we investigated the capability of hs-cTnT to predict systolic hypertension in this cohort. Our main findings revealed that elevated hs-cTnT levels in Blacks were associated with more frequent and lengthier SMI events, particularly in black men. In this group, high-sensitivity cardiac Troponin T (hs-cTnT) might thus be a useful marker of silent myocardial ischemia. Further, hs-cTnT predicted secondary systolic blood pressure increases to alleviate myocardial ischemia in Blacks as well as risk factor clustering in general. However, the need for ethnic-specific reference values of hs-cTnT, interpreted in relation to the number of existing risk factors, exists.

4.1 Potential burden of silent myocardial ischemia (SMI) in Blacks

Silent myocardial ischemia (SMI) is the documentation of myocardial ischemia in the absence of accompanying chest pain (21) and occurs more frequently in hypertensive individuals.(5, 6) This may be due to reduced vascular supply and increased cardiac demand.(1-3) In addition, the prevalence of hypertension and burden of CVD are escalating in Blacks.(22, 23) Findings in the Blacks from the present study, underscore this notion where higher mean 24-h SBP values (reaching hypertensive status) were evident, than the cas was with their white counterparts. Also, the Blacks had significantly more SMI events than the Whites, but no ethnic differences were evident in the total duration and maximal duration of SMI event comparisons. Associations between SMI prevalence and CVD have been linked to higher 24-h BP values in hypertensive individuals (4, 18), and the higher number of SMI events may be detrimental to the higher 24-h BP values.

In support, Blacks from this study revealed cardiometabolic susceptibility with elevated glycated haemoglobin (HbA_{1c}). Malan et al. (22) previously reported a higher cardiometabolic risk in urban black Africans from the THUSA study (1996-1998: North West Province, South Africa).

Urban Blacks displayed higher metabolic risk when compared with another ethnic population 10 years later (the SABPA study) (24). Pertaining to diabetes risk, Ford et al. (25) further suggested that prediabetes is associated with a higher CVD risk. The mean HbA_{1C} levels exceeded the prediabetes cut-off point (20) (according to the American Diabetes Federation) in the Blacks from this study. Compared to the 27.7% of Whites, 62.2% of the Blacks were prediabetic, which further emphasize the high cardiometabolic risk profile of this ethnic group. In another study (26), SMI was shown to be prevalent in individuals with impaired glucose tolerance and individuals with higher levels of HbA_{1C} ($\geq 7.6\%$). This may further explain the higher number of SMI events among the Blacks and further supports the altered cardiac profile of this vulnerable group.

4.2 High-sensitivity troponin T (hs-cTnT)

In the current study, 47% of Blacks and 53% of Whites had detectable hs-cTnT levels. Rubin et al. (12) demonstrated independent associations of detectable hs-cTnT with traditional cardiovascular risk factors even in individuals with “ideal cardiovascular health”. In addition, hs-cTnT is considered a validated marker for the detection of myocardial damage (27) and may prove useful in the detection of SMI. The Blacks, especially the men in the SABPA study, revealed positive associations between elevated hs-cTnT levels, 24-h SMI events, and both the SMI maximum duration of a single event and the total duration of SMI events over 24 hours. The underlying mechanism may be due to reduced vascular supply and increased cardiac demand (1-3). Compensatory blood pressure increases will occur to improve perfusion, thereby increasing volume loading and ventricular strain (11). Rubin et al. (12) supported this notion, as both sex and diabetes were associated with elevated hs-cTnT levels, which contributed to the higher burden of cardiovascular risk factors.

Additionally, prediabetes has been independently associated with deleterious effects on the myocardium as measured via hs-cTnT (28). As previously mentioned, SMI is also more prevalent in individuals with impaired glucose tolerance and higher levels of HbA_{1C} (26) which

further enhances the relevance of predicting SMI and hs-cTnT associations. We have revealed positive independent associations between HbA_{1C} and hs-cTnT in the Blacks indicating that they are more susceptible to the damaging effects of elevated glucose levels on the myocardium (28).

The lower hs-cTnT cut-point to predict systolic hypertension observed in Blacks may reflect the higher cumulative cardiovascular risk burden in this ethnic group than that in the Whites. McEvoy et al. (11) support our data which showed associations between hs-cTnT and hypertension. As stated, they explained that hs-cTnT may have predictive value to identify individuals at risk of developing hypertension and hypertensive end-organ damage (11). SMI may be considered hypertensive end-organ damage as SMI in hypertensives develops due to a higher cardiac demand (i.e. atherosclerotic obstruction) and reduced vascular supply (11). If SBP increase is secondary to ventricular distress, the lower cut-point for association with systolic hypertension in Blacks demonstrates racial disparity. The supply-demand mismatch will progressively worsen as vascular resistance increases with the secondary rise in BP, probably in concerted action with other determinants of ventricular demand. We therefore, determined ethnic-specific cut-points for hs-cTnT in the prediction of compensatory systolic hypertension as hypertension further contributes to the SMI burden. The Blacks indicated a higher risk for CVD with lower cut-points of 4.2 pg/ml as opposed to the higher hs-cTnT cut-point of 5.6 pg/ml for Whites for predicting systolic hypertension. The lower cut-off in Blacks may imply greater myocardial damage probably due to a higher prevalence of cardiovascular risk factors and/or higher mean blood pressure. In fact, we previously demonstrated a higher SMI incidence in black men (4). However, the higher incidence and maximum duration of one SMI event or the increased intensity of all SMI events over 24 hours in black men may be the driving force for injury of the myocardium with lower hs-cTnT cut-points and compensatory systolic blood pressure increases to alleviate myocardial ischemia.

Limitations to this study include the small sample size and the cross-sectional design which cannot infer causality. However, the strength of the study lies in its well-controlled design to include individuals from similar socio-economic status although cultural differences can't be accounted for.

5. CONCLUSIONS

We demonstrated significant differences in hs-cTnT and its associations with SMI and hypertension in black and white South African individuals. Our findings indicate that hs-cTnT may be a potential useful marker of SMI and compensatory systolic blood pressure increases. The BP increased to alleviate myocardial ischemia in Blacks from South Africa and of risk factor clustering in general. Our results also highlighted the need for ethnic-specific reference values of hs-cTnT, which should be interpreted in relation to the number of existing risk factors. An in-depth assessment of hs-cTnT may therefore be a useful enhancement in risk prediction studies.

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Author contribution

MEG and LM contributed to the design and conception of the manuscript; acquisition, analysis and interpretation of data. RD, MR and MC contributed to the analysis and interpretation of the data presented. MEG, LM, RD, MR and MC critically revised the document whereas MC contributed to the statistical analysis and critical revision of the statistical data presented. All the authors gave their final approval of the manuscript and accept full accountability for all the aspects of the work further ensuring the accuracy and integrity of the work presented.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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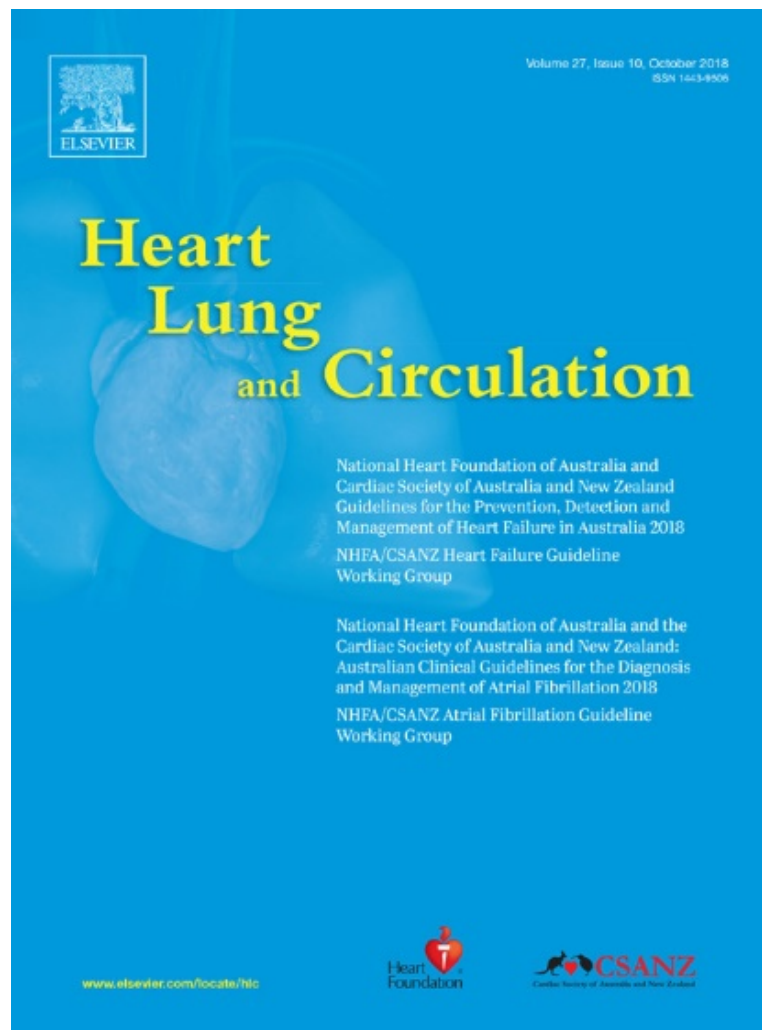
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Chapter 3



Manuscript 2: Silent ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study

Manuscript 2

Manuscript 2 has been submitted for publication in the peer-reviewed journal:

HEART, LUNG and CIRCULATION

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Please note that some of the format requirements were changed to ensure uniformity throughout the thesis.

**Silent Ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the
SABPA study**

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ABSTRACT

Background: Insulin resistance (IR) and elevated high-sensitivity cardiac troponin T (hs-cTnT) levels are risk factors contributing to silent myocardial ischemia (SMI). We aimed to determine associations between IR, hs-cTnT and SMI in a bi-ethnic sex South-African cohort to establish an ethnic-related mechanism diagnosing emergent SMI.

Methods: A bi-ethnic sex cohort (n=392) with similar socio-economic status (aged 20-65 years) participated in this study where 24-h ambulatory blood pressure (BP), -electrocardiogram (ECG) and overnight fasting cardiometabolic variables were measured. Additionally, IR was determined by calculating the homeostasis model of assessment (HOMA IR) score.

Results: Multivariate linear regression analyses showed positive associations ($p \leq 0.05$) among SMI events [Adj. $R^2 = 0.25$; β 0.57 (0.26; 0.88)], SMI total duration [Adj. $R^2 = 0.22$; β 0.49 (0.19; 0.79)], SMI maximum duration [Adj. $R^2 = 0.12$; β 0.38 (0.07; 0.70)] and HOMA IR in White men. Additionally, positive ($p \leq 0.05$) associations existed between SMI total duration [Adj. $R^2 = 0.22$; β 0.55 (0.23; 0.87)] and HOMA IR in White women. The Black men revealed significant associations ($p \leq 0.05$) among SMI events [Adj. $R^2 = 0.24$; β 0.30 (0.03; 0.56)], SMI max duration [Adj. $R^2 = 0.15$; β 0.41 (0.13; 0.68)], SMI total duration [Adj. $R^2 = 0.21$; β 0.37 (0.09; 0.63)] and hs-cTnT.

Conclusions: White men and women from South Africa may have a higher metabolic susceptibility to the development of ischemic heart disease; whereas black men revealed a cardiovascular susceptibility to the development of ischemic heart disease.

Word count: 241

Keywords: insulin resistance, troponin T, ethnicity, sex, silent myocardial ischemia

1. INTRODUCTION

Silent myocardial ischemia (SMI) is defined as the presence of myocardial ischemia in the absence of accompanying chest pain, discomfort or any other angina equivalents (1) and is prominent amongst individuals with cardiovascular disease (CVD) (2). In support, approximately 25-50% of individuals with coronary artery disease (CAD) (1) has SMI and CAD is mostly silent in individuals with insulin resistance (IR) or diabetes (3).

IR is a risk factor contributing to the development of SMI (4). In addition, IR contributed more to the development of CVD in white individuals than in black individuals (5). The homeostasis model of assessment (HOMA IR) is a reliable marker of IR (3, 6) and is a significant independent predictor of CVD risk (7, 8).

Troponin T concentrations, measured with a high-sensitivity method may reflect myocardial ischemia (9-12), and could be employed to detect chronic subclinical myocardial damage (13). The burden of CVD is rapidly increasing in sub-Saharan Africa (14, 15). We therefore sought to investigate 1) the possible association between SMI and IR and 2) the association between SMI and high-sensitivity troponin T (hs-cTnT) in a bi-ethnic sex cohort, with a view to establish an ethnic-related mechanism diagnosing emergent SMI.

2. MATERIALS AND METHODS

Urban black and white teachers (n=409) from the Dr Kenneth Kaunda Education district of the North West province, South Africa were included in The SABPA (Sympathetic and Ambulatory Blood Pressure in Africans) target population study (16) during February to May in 2008/9. To predict homogeneity, teachers (aged 20 – 65) were recruited with regard to similar education, socio-economic status and working environment, although cultural backgrounds may be diverse. Exclusion criteria included pregnancy or lactation, vaccinations or blood donation less than 3 months prior to data collection, psychotropic substance dependence or abuse, individuals with a history of myocardial infarction (MI) or cardiac events and clinically diagnosed diabetes (n=17). The final model included 94 black men, 97 white men, 94 black women and 107 white women (n=392). Before enrolment, recruited individuals were informed about the study protocol and they signed an informed consent form. The Ethics Review Board of North-West University, Potchefstroom Campus, approved this study (NWU-00036-07-S6), and procedures were performed in conformity with the terms and guidelines of the Declaration of Helsinki (17).

2.1 Ambulatory blood pressure measurements

Individuals were fitted with an 24-h ambulatory blood pressure (BP) and electrocardiographic device (Cardiotens CE120®, Meditech, Budapest, Hungary). Additionally, an accelerometer (Actical®, Montreal, Quebec) was also fitted during workdays (Mondays to Thursdays) at approximately 08:00. The Cardiotens CE120® device (B/B) (validated by the British Hypertension Society) provided oscillometrically measured 24-h blood pressure (BP) monitoring and 2-lead electrocardiogram (ECG) measurements. BP was recorded every 30 minutes between 08:00 and 22:00 and every 60 minutes between 22:00 and 06:00 (18). The non-dominant arm of each individual was fitted with suitably sized cuffs. Successful measurement inflation rates for the ambulatory BP period were 72.8% (\pm 0.8%) in black individuals and 84.7% (\pm 0.7%) in white individuals. An average 24-h BP of \geq 130 mmHg SBP and/or \geq 80 mmHg DBP defined hypertensive status (19).

The device calculated the isoelectric reference point (PQ-segment), J-point, L-point (80 ms after the J-point), and an ST-segment detection interval of at least 3 mm as the initial ST-level before the start of the 24-h ECG investigation. A pre-set program for 20 seconds at 5-min intervals assessing SMI events (ST-segment depression) were obtained by the 2-lead 24-h ECG recordings. This automated device documented the SMI according to the 1-1-1 rule: 1) at least 1 mm horizontal or descending ST-segment or more; 2) the ST-segment event continued for more than 1 min (duration); and 3) an interval of at least 1 min between two successive ST-segment episodes must be present to be counted as independent episodes (20). In the event of additional horizontal or descending ST-depression, a 60-second ECG tracing was recorded, and an additional BP measurement was recorded. The BP and ECG data were analysed using the CardioVisions 1.19.2 Personal Edition Software (Meditech®, Budapest, Hungary).

Individuals maintained their normal daily activities and recorded, on their ambulatory diary card, any stressful events, headaches, fainting, nausea, palpitations, and visual disturbances. Individuals were transported to the Metabolic Unit of North-West University at 16:30 (same day) where the protocol was reviewed and the experimental setup at the facilities was explained. Medication use and medical history were recorded. A standardized dinner was served, and the individuals were requested to go to bed at 22:00 whilst fasting overnight. At 06:00 the following morning after the last 24-h BP measurement, the Cardiotens apparatus was removed and anthropometric measurements were performed. Hereafter, 12-lead ECG monitoring followed, and blood sampling was performed while individuals were in a semi-recumbent position for 30 minutes. Once these measurements were completed, individuals received breakfast, feedback on data, and were transported back to school.

2.2 Assessment of lifestyle risk factors

The Actical® omnidirectional accelerometer (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada), which measures movement at 15-second intervals, determined physical activity or total daily energy expenditure (TEE) (kcal/day), considering resting metabolic rate. Data were

converted into 1-second intervals for analysis. Plasma γ -glutamyl transferase (γ -GT) and plasma cotinine levels were analysed to indicate possible alcohol abuse (21) and smoking status respectively. Cotinine values exceeding 14.99 ng/ml (22) classified subjects as regular smokers. Standardized anthropometric measurements (in triplicate) were performed by registered level II ISAK-accredited (*International Society for the Advancement of Kinanthropometry*) Anthropometrists. Height and body mass were determined to the nearest 0.1 cm and 0.1 kg respectively (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer IP 1465, Invicta, London, UK), whereas body surface area (BSA) in m² was calculated using the Mosteller formula (23). Inter- and intra-variation was less than 10%.

2.3 Biochemical sampling and analyses

A high-sensitivity enzyme rate method determined serum γ -GT and serum cotinine by a homogenous immunoassay method (Modular ROCHE Automized, Switzerland). Whole blood glycated haemoglobin A_{1c} (HbA_{1c}) was determined using a turbidimetric inhibition immunoassay (Cobas Integra 400plus, Roche, Basel, Switzerland). Sodium Fluoride glucose (in mmol/l) was determined by applying the timed-end-point method (Unicel DXC 800 - Beckman and Coulter, Germany), where serum insulin levels (uU/ml) were determined with the Elecsys 2010 (Roche, Basel, Switzerland) (Intra-assay precision: 2%; inter-assay precision: 2.8%). Insulin sensitivity was calculated by using the HOMA IR score according to the formula: fasting glucose (mmol/l) x fasting insulin (uU/ml)/405 (6). The Human immunodeficiency virus- (HIV) infection prevalence was determined in EDTA-plasma by the First Response kit (RPM Plus, USA) and confirmed by the Pareekshak test (Bhat Biotech, India). Pre- and post-counselling for HIV were done by trained counsellors. Hs-cTnT levels were determined using the electrochemiluminescence immunoassay (ECLA) Modular Roche Automized, Switzerland. Hs-cTnT inter- and intra-assay coefficients of variation were less than 10%. Of the 387 values, 105 values were lognormal computed according to Croghan et al. (24).

2.4 Statistical analyses

Statistical analyses were completed by using Statistica version 13.2 (Statsoft Inc., Oklahoma, USA, 2016). Data distribution was tested using the Shapiro-Wilk's *W*-test for normality, and non-normal distributions were logarithmically transformed (cotinine, physical activity (TEE), γ -GT, HbA_{1c}, HOMA IR, SMI events, hs-cTnT, SMI maximum duration). Data are presented as median (interquartile range) unless stated otherwise.

Interaction on main effects (ethnicity \times sex) for SMI, independent of *a priori* confounders (age, BSA, physical activity (TEE), serum cotinine levels and γ -GT), were determined by multivariate analysis of covariance (ANCOVA).

Student *T*-tests determined demographic and clinical characteristics of the bi-ethnic sex groups and described the population by ethnic and sex status. Chi-square (χ^2) tests compared proportions of CVD prevalence, hypertension (HT), SMI, HIV-infection status, prediabetes, use of statins and antihypertensive medication. Subsequently, univariate ANCOVAs compared the number of SMI events, SMI total and maximum duration, glucose, insulin and HOMA IR between sex and ethnic groups, considering *a priori* confounders. Partial correlations (controlling for *a priori* confounders) were performed to analyse associations between number of SMI events, total and maximum duration of SMI events, and other cardiovascular variables within bi-ethnic sex groups. Statistical significance was set at $p \leq 0.05$ and $R > 0.35$. Forward stepwise regression analysis predicted relationships among SMI, HOMA IR (F to enter 2.50) and hs-cTnT (F to enter 2.50), independent of *a priori* confounders, within bi-ethnic sex groups. The statistical significance level was set at $p \leq 0.05$ (two-tailed) and adjusted R^2 for regression analysis was set at $R^2 > 0.25$.

Sensitivity analysis was done by excluding HIV infected individuals and repeating forward stepwise regression analysis.

3. RESULTS

A significant ethnicity x sex interaction existed for SMI [$F(1,167) = 11.35; p \leq 0.001$]. In table 3.1, the bi-ethnic sex individuals were well matched for age dispersal. CVD risk factors were more prevalent in Black than White men from South Africa and included higher 24-h BP reaching hypertensive status, higher HbA_{1c} levels, and more SMI events over a period of 24 hours. Also, black men were less active than white men (Table 3.1) and more alcohol use was reflected by higher γ -GT levels in this group. Black men also revealed a higher prevalence of self-reported use of antihypertensive medication (black men: 31%; white men: 12%), HT (black men: 73%; white men: 52%) and HIV-infection status (black men: 12%; white men: 0%) in comparison to white men (Table 3.1).

Black women revealed a similar trend than black men and more cardiometabolic disease risk factors were evident in this sub-group – such as higher 24-h BP, HbA_{1c} levels, HOMA IR, 24-h heart rate (HR) and a significant higher BSA and waist circumference than in their white counterparts (Table 3.1). In addition, black women displayed a 53.6% HT prevalence as opposed to the 24.1% of the white women. Also, there were significant more black women with self-reported use of antihypertensive medication (black women: 31%; white woman: 13%) and HIV-infection status (black women: 5%; white women: 0%) than their white counterparts (Table 3.1).

However, IR risk stratification revealed no significant differences in bi-ethnic sex groups (Figure 3.1).

Table 3.1: Demographic and clinical characteristics.

	Black men (n=94)	White men (n=97)	p-value	Black women (n=94)	White women (n=107)	p-value
Age, years	42 (38; 49)	47 (39; 53)	0.22	45 (39; 52)	47 (38; 53)	0.72
<i>Lifestyle factors</i>						
Body Surface area, m ²	2.0 (1.7; 2.1)	2.2 (2.1; 2.3)	<0.001	1.9 (1.7; 2.0)	1.8 (1.7; 1.9)	0.04
Waist Circumference, cm	92.0 (80.7; 104.3)	98.7 (92.0; 108.0)	<0.001	93.0 (83.6; 102.3)	82.7 (74.6; 94.6)	<0.001
Physical activity, kcal/day	2739.1 (2185.9; 3234.6)	3412.5 (2990.5; 3726.7)	<0.001	2646.7 (2076.0; 3060.3)	24389.0 (2105.5; 2896.2)	0.41
Cotinine, ng/ml ^a	31.2 (59.2)	32.2 (98.5)	0.93	17.3 (54.2)	15.2 (53.2)	0.78
γ-GT, U/L	54.2 (37.3; 84.8)	25.0 (17.0; 38.0)	<0.001	30.2 (22.7; 53.2)	13.0 (9.0; 19.0)	<0.001
hs-cTnT, pg/ml	5.0 (3.9; 6.0)	6.3 (4.7; 8.2)	0.003	3.5 (2.9; 4.3)	3.5 (2.9; 5.0)	0.72
HbA _{1C} , %	5.9 (5.6; 6.3)	5.6 (5.4; 5.8)	<0.001	5.7 (5.3; 6.0)	5.3 (5.2; 5.6)	<0.001
HbA _{1C} , mmol/mol	41 (38; 45)	38 (36; 40)	<0.001	39 (34; 42)	34 (33; 38)	<0.001
NaF Glucose, mmol/l	5.3 (4.9; 5.9)	5.8 (5.5; 6.2)	0.88	4.9 (4.5; 5.5)	5.3 (5.0; 5.7)	0.01
Insulin, uU/ml	11.2 (8.1; 19.5)	12.4 (8.9; 15.9)	0.69	12.9 (9.0; 16.0)	7.9 (5.9; 11.6)	<0.001

	Black men	White men	p-value	Black women	White women	p-value
	(n=94)	(n=97)		(n=94)	(n=107)	
HOMA IR	3.1 (1.8; 5.5)	2.6 (1.9; 3.7)	0.90	2.7 (1.9; 3.7)	1.8 (1.4; 2.7)	<0.001
24-h SBP, mmHg	138 (126; 148)	127 (117; 135)	<0.001	127 (117; 135)	119 (112; 125)	<0.001
24-h DBP, mmHg	87 (79; 95)	78 (73; 84)	<0.001	78 (73; 84)	73 (70; 78)	<0.001
24-h HR, beats/min	79 (72; 86)	81 (73; 79)	<0.001	81 (73; 88)	75 (69; 81)	<0.001
24-h SMI events, n ^a	11 (22)	2 (6)	<0.001	3 (5)	3 (6)	0.48
24-h SMI Total duration, min	21.5 (3.5; 164.0)	5.3 (1.0; 20.8)	0.08	4.5 (2.0; 12.5)	6.5 (1.0; 25.0)	0.17
SMI maximum duration, min	6.8 (1.8; 59.5)	2.3 (1.0; 7.5)	0.07	2.5 (1.0; 5.5)	3.0 (1.0; 9.3)	0.26
SMI maximum deviation, mm	-6.4 (-11.0; -5.0)	-6.8 (-10.8; -4.8)	0.45	-6.4 (-8.8; -5.2)	-4.8 (-6.4; -4.4)	0.06
<i>Cardiovascular disease risk</i>						
CVD History	7 (7.5)	10 (10.3)	0.49	7 (7.5)	11 (10.3)	0.48
24-h Hypertension, n (%)	73 (77.7)	52 (53.6)	<0.001	50 (53.2)	26 (24.3)	<0.001
SMI, n (%)	44 (46.8)	37 (38.1)	0.23	52 (55.3)	49 (45.8)	0.18
HIV, n (%)	12 (12.8)	0 (0)	<0.001	5 (5.32)	0 (0)	0.02

	Black men	White men	p-value	Black women	White women	p-value
	(n=94)	(n=97)		(n=94)	(n=107)	
<i>Medications</i>						
Hypertension, n (%)	31 (33.0)	12 (12.4)	<0.001	31 (33.0)	13 (12.2)	<0.001
Statins, n (%)	1 (1.1)	5 (5.2)	0.11	1 (1.1)	3 (2.8)	0.38

Data are reported as median (interquartile range) or proportions (%) unless otherwise indicated. (a) Data reported as mean (standard deviation). Abbreviations: n = number of individuals; γ -GT = gamma glutamyl transferase; hs-cTnT = high-sensitivity cardiac Troponin T; HbA_{1c} = glycated haemoglobin; HOMA IR = homeostasis model of assessment; SBP = systolic blood pressure; DBD = diastolic blood pressure; HR = heart rate; SMI events = silent myocardial ischemic events determined according to the 1-1-1 rule (20), SMI events max duration = The maximum duration of silent myocardial ischemic events; hs-cTnT LOD>3 pg/ml = participants with a high-sensitivity cardiac troponin T level above the Limit of Detection (LOD) of 3 pg/ml; CVD = cardiovascular disease; HIV = human immunodeficiency virus. $p \leq 0.05$.

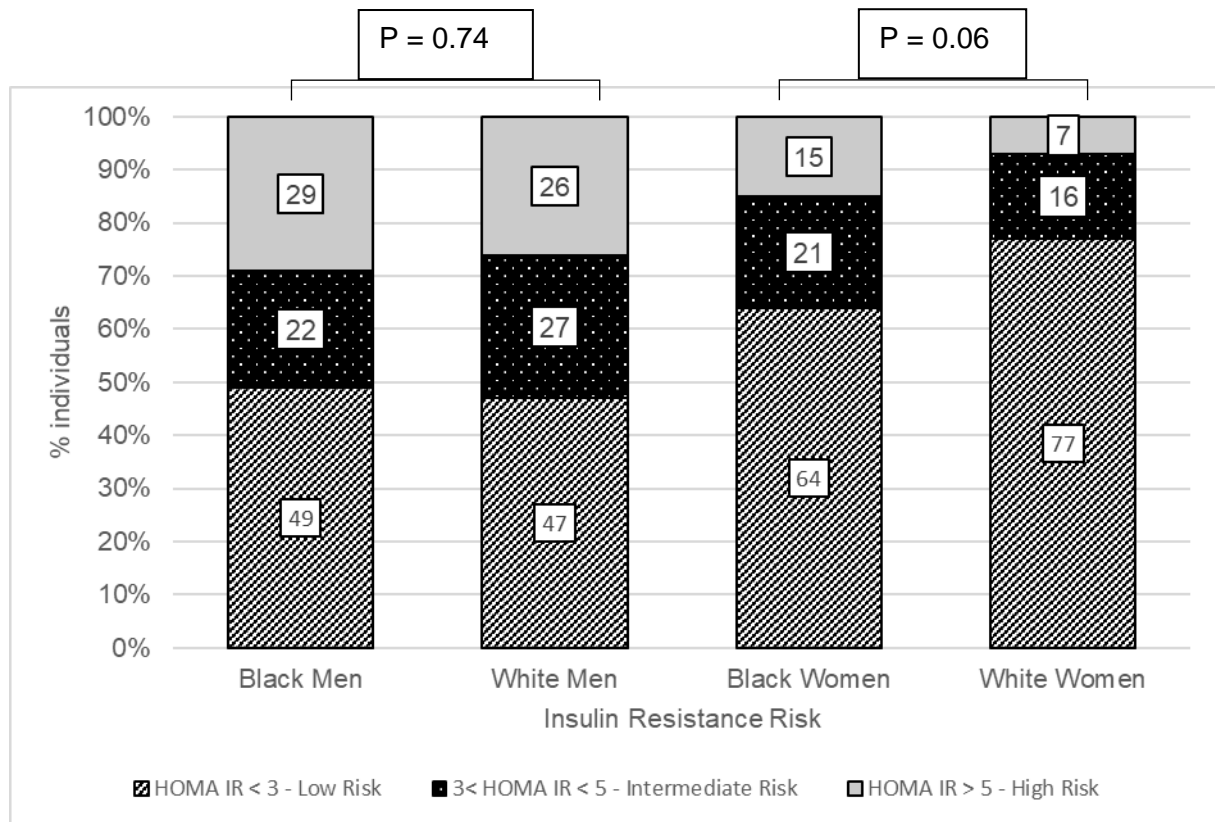


Figure 3.1: Bi-ethnic sex insulin resistance risk stratification. Data is presented in percentage. HOMA IR = homeostasis model of assessment. HOMA IR < 3 Low insulin resistance risk; HOMA IR > 3 < 5 Intermediate insulin resistance risk; HOMA IR > 5 High insulin resistance risk (6).

In general, when adjusting for *a priori* confounders black men revealed a higher number of SMI events, longer duration of SMI events, higher glucose levels and lower hs-TnT levels compared to white men (Supplementary figure 1a*). Black women, revealed higher insulin levels, glucose levels and HOMA IR levels than did white women (Supplementary figure 1b*).

Significant partial correlations between number of SMI events and HOMA IR, glucose and insulin were only evident in white men as reported in supplementary table 1a* ($R > 0.30$ and $p \leq 0.05$). On the contrary, for the number of SMI events, SMI total duration and SMI max duration significant correlations existed for hs-cTnT in black men ($R > 0.30$ and $p \leq 0.05$) (Supplementary table 1a – 1c*).

Black women showed significant correlations between SMI total duration and plasma glucose ($R > 0.30$ and $p \leq 0.05$), whereas white women showed significant correlations of SMI events, SMI max duration and SMI total duration with insulin and HOMA IR (Supplementary table 1a - 1c*). Additionally, white women also showed significant correlations between SMI total duration and HbA_{1c} and plasma glucose (Supplementary table 1c*) ($R > 0.30$ and $p \leq 0.05$).

In Table 3.2, multivariate linear regression analyses, independent of confounders, mostly showed positive associations ($p \leq 0.05$) among SMI events [Adj. $R^2 = 0.24$; β 0.30 (0.03; 0.56)], SMI max duration [Adj. $R^2 = 0.15$; β 0.41 (0.13; 0.68)], SMI total duration [Adj. $R^2 = 0.21$; β 0.37 (0.09; 0.63), $p \leq 0.05$] and hs-cTnT in black men. No significant associations were evident in Black women.

Footnote: * Supplementary figure 1a – 1b and supplementary table 1a – 1c are included after the references.

In white men, positive associations ($p \leq 0.05$) were evident among SMI events [Adj. $R^2 = 0.25$; β 0.57 (0.26; 0.88)], SMI total duration [Adj. $R^2 = 0.22$; β 0.49 (0.19; 0.79)], SMI max duration [Adj. $R^2 = 0.12$; β 0.38 (0.07; 0.70)] and HOMA IR. In addition to this, positive ($p \leq 0.05$) associations existed between SMI total duration [Adj. $R^2 = 0.22$; β 0.55 (0.23; 0.87)] and HOMA IR in white women. Excluding HIV infected individuals in regression analyses did not change the main outcomes.

Table 3.2: Forward stepwise regression analysis associations between silent myocardial ischemia (SMI) events, -duration and cardiometabolic risk factors.

	Log SMI events		Log SMI max duration		Log SMI total duration	
	Black men	White men	Black men	White men	Black men	White men
Adjusted R²	0.24	0.25	0.15	0.12	0.21	0.22
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Log hs-cTnT	0.30 (0.03; 0.56)*	--	0.41 (0.13; 0.68)*	--	0.37 (0.09; 0.63)*	--
Log HOMA IR	--	0.57 (0.26; 0.88)*	--	0.38 (0.07; 0.70)*	--	0.49 (0.19; 0.79)*
	Black women	White women	Black women	White women	Black women	White women
Adjusted R²	N.S.	--	--	--	--	0.22
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Log HOMA IR	--	--	--	--	--	0.55 (0.23; 0.87)*

β denotes standardized regression coefficient. Independent covariates included in models were: age, body surface area (BSA), log total energy expenditure (TEE), log cotinine, log gamma-glutamyl transferase (γ -GT). Abbreviations: SMI = silent myocardial ischemia; hs-cTnT = high-sensitivity cardiac troponin T; HOMA IR = homeostasis model of assessment; N.S. = not significant. F to enter 2.5. * Significance $p \leq 0.05$.

4. DISCUSSION

The aim of this study was to establish the possible associations among SMI, IR and hs-cTnT in a bi-ethnic sex cohort. Our main findings revealed that IR, determined by elevated HOMA IR levels, were significantly associated with lengthier SMI events over 24 hours in White men and women. Also, more frequent SMI events were associated with IR in white men. Black men revealed significant associations between hs-cTnT and more frequent and lengthier SMI events which may be due to hypertension-induced ventricular strain (4).

4.1 Silent myocardial ischemic burden and insulin resistance (IR)

Previous studies indicated that individuals with SMI and diabetes have a poor prognosis which is imitated by adverse cardiac events or death (25). This may be due to the fact that SMI occurs more frequently in individuals with diabetes (3). Several studies proposed that the development of SMI in individuals with diabetes may be caused by reduced vascular supply and increased cardiac demand or a sympathetic nerve dysfunction or neuropathy (25-28). However, individuals with IR already exhibit myocardial perfusion defects without any symptomatic cardiac disease (29). IR can impair coronary microcirculation characterized by disparity between myocardial metabolism and coronary blood flow (30, 31). The myocardium is a foremost insulin-responsive tissue and is susceptible to diabetic glucose/insulin homeostatic modifications (32). Both IR and hyperglycemia, which were more prevalent in Whites, affect myocardial cellular metabolism thereby facilitating the development of cardiomyopathy (32).

Structural and functional modifications in the myocardium of individuals with diabetes also render it susceptible to ischemia. During ischemia, disruption of glycolytic ATP generation may also occur due to a reduced glucose transport into cardiac myocytes further enhancing the role of IR in CVD (32). In support, a contributing risk factor for developing SMI is IR (33), which contributed more to the development of cardiovascular disease in white individuals

than in black individuals (5, 33). Saad et al., (5) established a link between insulin resistance and BP especially in white individuals. This deranged glucose metabolism in our white individuals may thus be due to structural and cellular defects as well as enhanced adrenergic tone (5). However, enhanced adrenergic tone in the current white cohort did not contribute to the apparent deranged glucose metabolism (16).

Our data thus aligns with studies conducted (5, 33) since a metabolic susceptibility to develop ischemic heart disease is visible in white individuals with IR, who also displayed a higher IR risk than their black counterparts.

4.2 High-sensitivity cardiac troponin T (hs-cTnT)

Independent associations of detectable hs-cTnT (a diagnostic marker of myocardial injury (34)) with prevalent traditional cardiovascular risk factors are evident even in individuals with “ideal cardiovascular health” (4, 35), which may prove useful in the detection of SMI, especially in our black male cohort. Rubin et al. (35) also reported higher hs-cTnT levels in males, which purportedly contributed to the higher burden of cardiovascular risk factors. In addition, this study suggested that detectable hs-cTnT levels may be a result of microvascular damage (36), which may also contribute to the development of SMI (35).

Black men in this study revealed positive associations between higher hs-cTnT levels, 24-h SMI events, and both the SMI maximum duration and total duration of SMI events. The higher cardiac demand due to a lower vascular supply may have contributed to the development of SMI in this ethnic group (12, 27). A lower hs-cTnT cut-point to predict SBP in the black individuals, despite higher SBP levels, may explain the lower hs-cTnT levels (12) than those in white individuals. This lower cut-point can be due to chronic adrenergic signalling and neuroendocrine dysfunction as observed in previous studies conducted in the SABPA black individuals (36). Compensatory BP elevation will occur to recover perfusion

defects, where volume loading as well as ventricular strain may increase (13). This reveals a cardiovascular strain with an hs-cTnT cut-point as low as 4.2 pg/ml (12), reflecting ischemic heart disease risk in a black male cohort from South Africa.

5. CONCLUSION

We conclude that IR, determined by elevated HOMA IR levels, is significantly associated with lengthier SMI events over a period of 24 hours in White men and women. In addition, White men also revealed a significant association between IR and more frequent SMI events. These findings underline the notion that IR increases metabolic susceptibility to develop SMI in the White population but not necessarily in the Black population. On the contrary, the Black men revealed significant associations between hs-cTnT and more frequent and lengthier SMI events suggesting a cardiovascular susceptibility to the development of ischemic heart disease in the Black population, establishing a ethnic-related mechanism which could diagnose emergent SMI.

This study is limited by the small sample size and the cross-sectional design, which cannot deduce causality. A well-controlled design was followed to include individuals from similar socio-economic status contributing to the strength of this study. However cultural differences cannot be accounted for.

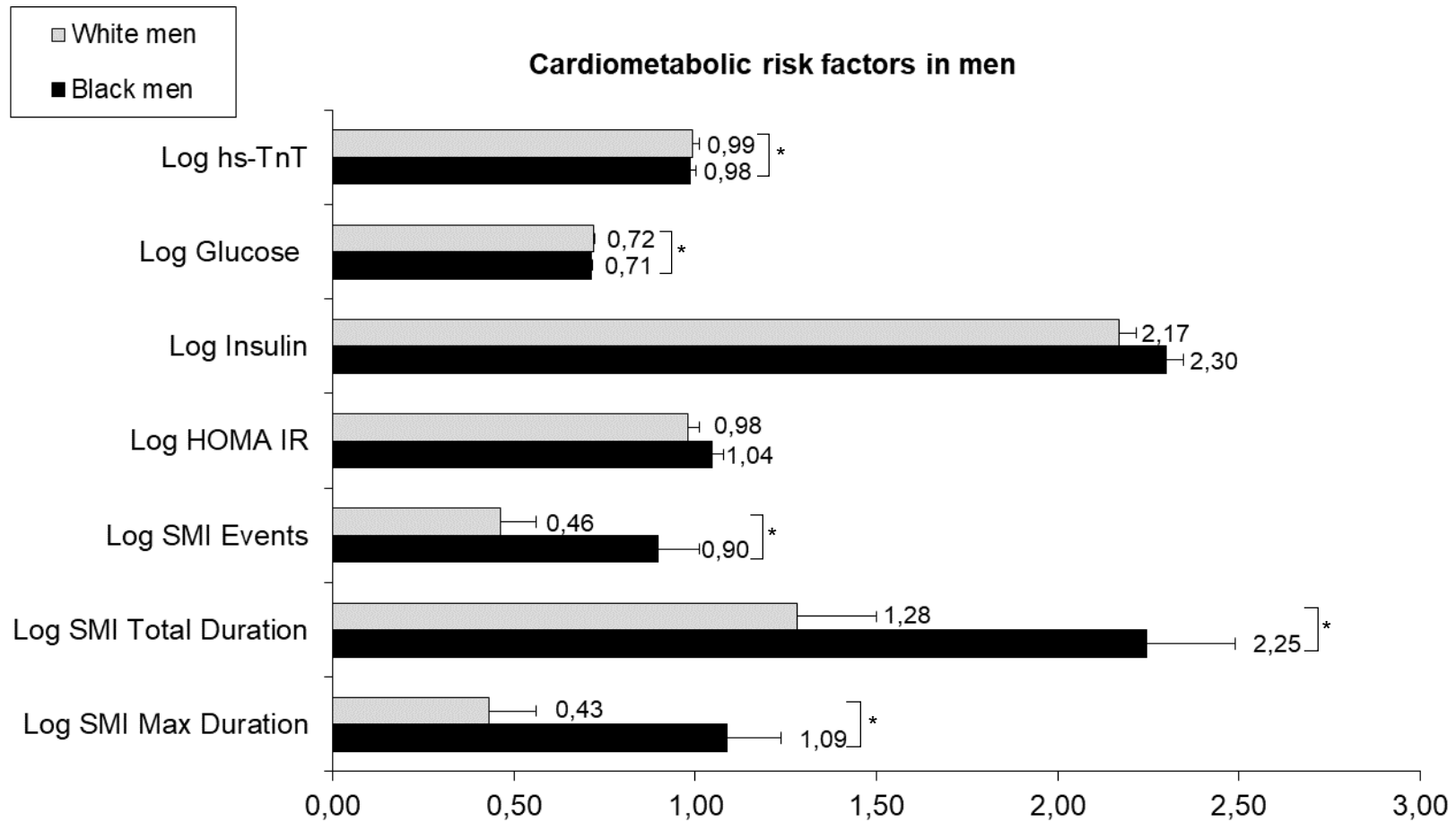
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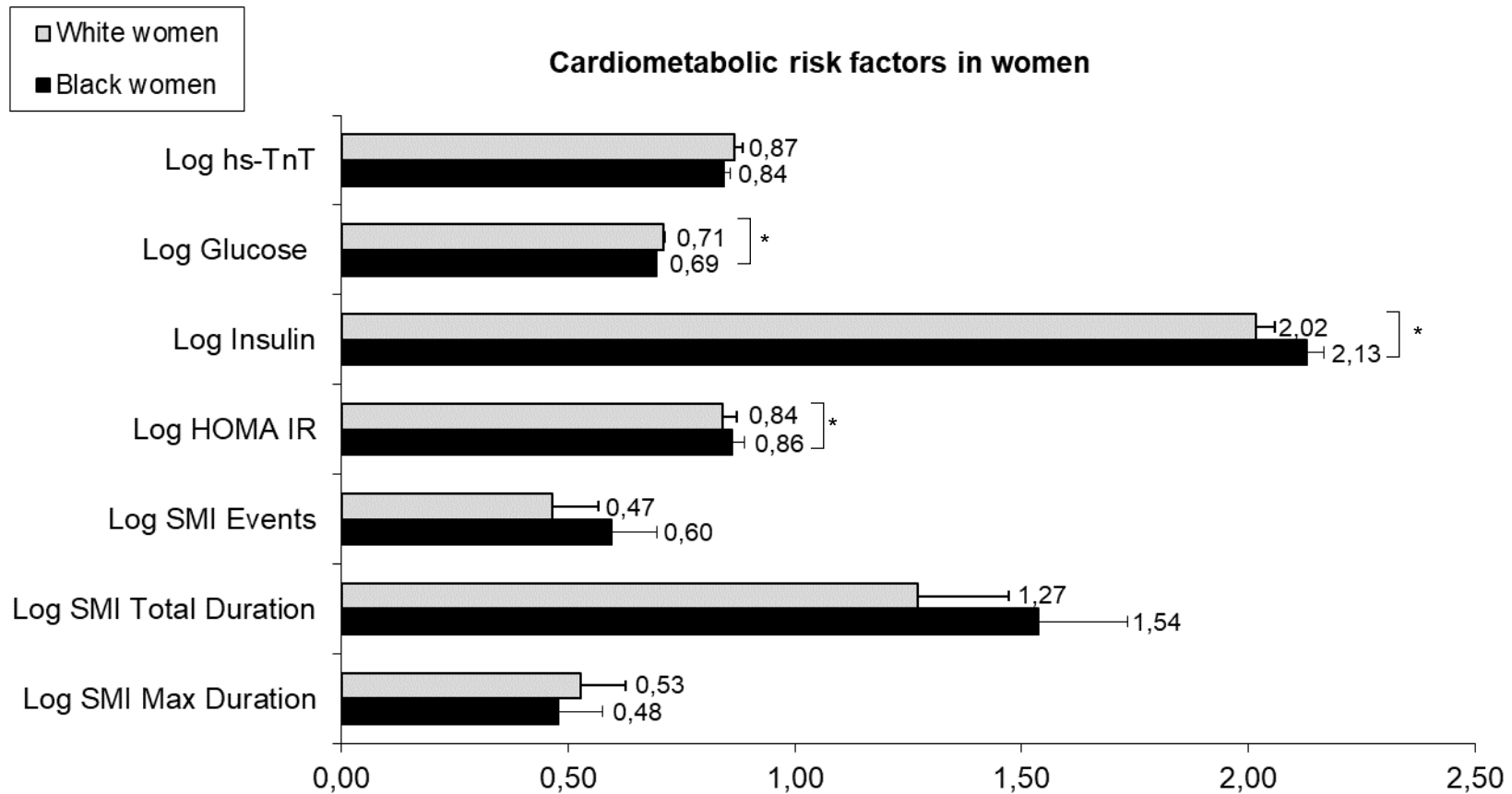
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Supplementary figure 1a: Analysis of covariance revealing cardiometabolic risk in bi-ethnic male groups. Variables were log-transformed. Abbreviations: hs-cTnT = high-sensitivity troponin T; HOMA IR = homeostasis model of assessment; SMI = silent myocardial ischemia. Data are presented as adjusted mean \pm SE values (adjusted for *a priori* confounders - age, body surface area (BSA), log total energy expenditure (TEE), log cotinine levels, log gamma-glutamyl transferase (γ -GT) levels). * Significance $p \leq 0.05$



Supplementary figure 1b: Analysis of covariance revealing cardiometabolic risk in bi-ethnic female groups. Variables were log-transformed. Abbreviations: hs-cTnT = high-sensitivity troponin T; HOMA IR = homeostasis model of assessment; SMI = silent myocardial ischemia. Data are presented as adjusted mean \pm SE values. Adjusted for *a priori* confounders (age, body surface area (BSA), log total energy expenditure (TEE), log cotinine levels, log gamma-glutamyl transferase (γ -GT) levels). * Significance $p \leq 0.05$.

Supplementary table 1a: Partial correlations between silent myocardial ischemia (SMI) events and cardiometabolic risk markers.

	Black men		White men		Black women		White women	
Silent myocardial ischemia (SMI) events								
Variable	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
Log HbA _{1c}	0.18	0.29	0.16	0.43	0.12	0.47	0.050	0.74
Log Insulin	-0.001	0.99	0.39	0.04	0.18	0.27	0.33	0.03
Log NaF Glucose	0.19	0.25	0.41	0.03	0.27	0.10	0.14	0.39
Log HOMA IR	0.09	0.59	0.48	0.01	0.22	0.18	0.31	0.04
24-h SBP, mmHg	0.25	0.14	0.30	0.11	0.03	0.87	0.12	0.47
24-h DBP, mmHg	0.10	0.54	0.37	0.05	-0.05	0.78	-0.05	0.76
Log hs-cTnT	0.40	0.01	0.18	0.34	-0.28	0.08	-0.05	0.73

Variables were log-transformed. Abbreviations: HbA_{1c} = glycated haemoglobin, HOMA IR = homeostasis model of assessment, SBP = systolic blood pressure; DBP = diastolic blood pressure; hs-cTnT = high-sensitivity cardiac Troponin T. Values in bold indicate significant associations. Adjusted for *a priori* confounders (age, body surface area (BSA), log total energy expenditure (TEE), log cotinine levels, log gamma-glutamyl transferase (γ -GT) levels). Significance: $p \leq 0.05$; $R > 0.35$.

Supplementary table 1b: Partial correlations between silent myocardial ischemia (SMI) maximum duration and cardiometabolic risk markers.

	Black men		White men		Black women		White women	
	Silent myocardial ischemia (SMI) maximum duration							
Variable	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
Log HbA _{1c}	0.12	0.49	-0.11	0.58	0.22	0.18	0.25	0.11
Log Insulin	0.08	0.65	0.27	0.16	0.04	0.81	0.46	0.002
Log NaF Glucose	0.15	0.37	0.05	0.80	0.28	0.09	0.24	0.12
Log HOMA IR	0.14	0.41	0.27	0.15	0.22	0.19	0.45	0.003
24-h SBP, mmHg	0.20	0.24	-0.02	0.92	0.07	0.67	0.27	0.08
24-h DBP, mmHg	0.07	0.70	0.09	0.66	0.004	0.98	0.0002	0.99
Log hs-cTnT	0.39	<0.001	0.04	0.83	0.03	0.83	-0.03	0.86

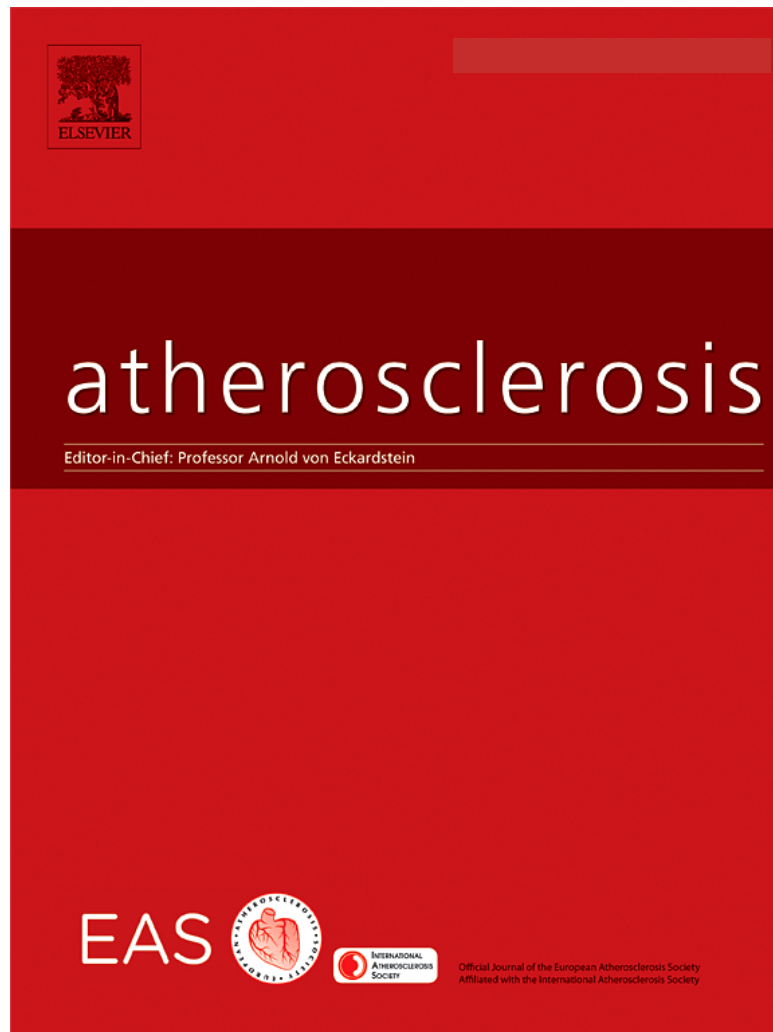
Variables were log-transformed. Abbreviations: HbA_{1c} = glycated haemoglobin, HOMA IR = homeostasis model of assessment; SBP = systolic blood pressure; DBP = diastolic blood pressure; hs-cTnT = high-sensitivity cardiac Troponin T. Values in bold indicate significant associations. Adjusted for *a priori* confounders (age, body surface area (BSA), log total energy expenditure (TEE), log cotinine levels, log gamma-glutamyl transferase (γ -GT) levels). Significance: $p \leq 0.05$; $R > 0.35$.

Supplementary table 1c: Partial correlations between silent myocardial ischemia (SMI) total duration and cardiometabolic risk markers.

	Black men		White men		Black women		White women	
	Silent myocardial ischemia (SMI) total duration							
Variable	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
Log HbA _{1c}	0.16	0.35	-0.07	0.73	0.21	0.20	0.31	0.05
Log Insulin	0.05	0.76	0.35	0.07	0.19	0.25	0.44	0.004
Log NaF Glucose	0.16	0.33	0.12	0.55	0.33	0.04	0.35	0.02
Log HOMA IR	0.12	0.47	0.36	0.06	0.25	0.12	0.46	0.002
24-h SBP, mmHg	0.04	0.81	0.06	0.75	0.04	0.82	0.08	0.59
24-h DBP, mmHg	-0.05	0.75	0.32	0.19	-0.05	0.77	-0.11	0.50
Log hs-cTnT	0.31	0.05	0.02	0.91	-0.18	0.27	0.02	0.91

Variables were log-transformed. Abbreviations: HbA_{1c} = glycated haemoglobin, HOMA IR = homeostasis model of assessment, SBP = systolic blood pressure; DBP = diastolic blood pressure; hs-cTnT = high-sensitivity cardiac Troponin T. Values in bold indicate significant associations. Adjusted for *a priori* confounders (age, body surface area (BSA), log total energy expenditure (TEE), log cotinine levels, log gamma-glutamyl transferase (γ -GT) levels). Significance: $p \leq 0.05$; $R > 0.35$.

Chapter 4



Manuscript 3: Troponin T release is associated with subclinical atherosclerosis in blacks with first degree AV-block: the SABPA study

Manuscript 3

Manuscript 3 has been submitted for publication in the accredited peer-reviewed journal: ATHEROSCLEROSIS

Impact Factor: 4.467

This journal publishes original basic research articles covering basic and translational, clinical and population research approaches to arterial and vascular biology and disease. scientific, clinical and public health disciplines. These include studies which investigate atherosclerosis, its risk factors and clinical manifestations. These risk factors include disturbances of lipid and lipoprotein metabolism, diabetes, hypertension, thrombosis and inflammation.

Guide for authors:

- Manuscript**
 - Main text 4000 words (including legends to figures and tables).
 - It should be divided into sections headed (Abstract, Keywords, Introduction, Materials and methods, Results, Discussion, Conclusions, Tables, Artwork).
 - Abbreviations and abbreviated phrases should be written out at first mention followed by the abbreviation in parentheses.
 - Include source of funding and conflicts of interests, if any.
- Title Page**
 - Include an informative concise title without abbreviations.
- Abstract**
 - Not exceeding 250 words.
 - Structured (Background and aims, Methods, Results, Conclusions).
- Keywords**
 - 3-7 keywords.
- Tables**
 - Tables should be provided with legends as Word files.
 - Double spacing on separate pages.

- Footnotes to tables must be listed with superscript lowercase letters, beginning with “a”.
 - Footnotes must not be listed with numbers or symbols.
- Highlights**
- Highlights should consist of 3-5 bullet points of the main findings.
- Artwork**
- Use uniform lettering and sizing (Arial or Times New Roman).
 - Number illustrations according to appearance in text.
- Figure captions**
- Each illustration should have a caption with a brief title and description (explain all symbols and abbreviations used).
- p values**
- p values must be consistently formatted according to the below style throughout the manuscript (including figures and tables):
 $p < X$
 - All p's are italicized and lower case.
- References**
- There are no strict requirements on reference formatting at submission.
 - References can be in any style format as long as the style is consistent.
 - Maximum 50 references.
 - Include DOI where applicable.
 - For authors' names, the general rule is up to 5 names before et al.
 - Include author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present.

Please note that some of the format requirements were changed to ensure uniformity throughout the thesis.

Troponin T release is associated with subclinical atherosclerosis in Blacks with first degree AV-block: the SABPA study

Madelein E. Griffiths, Rhena Delport, Leoné Malan

Submitted for publication to the peer-reviewed journal: **ATHEROSCLEROSIS**

Under review

ATH-D-18-01482

Highlights:

- Increases in high-sensitivity cardiac troponin T (hs-cTnT) are associated with subclinical atherosclerosis in Blacks presenting 1st degree AV-block.
- Findings in Blacks suggested an ischemic heart disease and hypertrophic remodelling susceptibility.
- Similar associations were not evident in Whites from this cohort.

ABSTRACT

Background and aims: First (1st) degree heart block is commonly observed in black people. The purpose of this study was to assess the clinical significance of sub-clinical atherosclerosis, myocardial distress, deranged glucose metabolism and common cardiovascular disease (CVD) risk factors in the presence of 1st degree atrioventricular block (AV-block).

Methods: A bi-ethnic cohort (n=385) with similar socio-economic status (Blacks n=179, Whites n=206, aged 20 - 65 years) partook in this study. Ambulatory blood pressure (BP) and -electrocardiogram (ECG), 12-lead ECG and overnight fasting cardiometabolic variables were measured. High-sensitivity cardiac troponin T (hs-cTnT) was measured to reflect degree of myocardial distress. IR was calculated using the homeostasis model of assessment (HOMA IR) score. B-mode ultrasound images of the left carotid intima-media thickness (L-CIMT) of the far wall (L-CIMT), left cross-sectional wall area (L-CSWA) and left carotid lumen diameter were obtained as measures of subclinical atherosclerosis.

Results: Blacks showed a 64% hypertensive prevalence compared with 38% in Whites. Also, Blacks had significantly thicker L-CIMT than their white counterparts. Multivariate linear regression analyses in Blacks with 1st degree AV-block, revealed positive associations between L-CIMT and hs-cTnT [Adj. $R^2 = 0.36$; $\beta=0.46$ (0.17; 0.75), $p \leq 0.05$] as well as L-CSWA and hs-cTnT [Adj. $R^2 = 0.30$; $\beta=0.42$ (0.12; 0.71), $p \leq 0.05$]. Similar associations were not found in Whites.

Conclusions: Increases in hs-cTnT is associated with subclinical atherosclerosis in Blacks presenting 1st degree AV-block, suggesting an increased susceptibility for hypertrophic remodelling and ischemic heart disease. Early screening programs may identify conduction problems and perfusion deficits in CVD prevention programs.

Word count: 256

Key words: first degree AV-block; troponin T; insulin resistance, subclinical atherosclerosis

1. INTRODUCTION

The burden of coronary artery disease (CAD) is progressing in sub-Saharan Africa and is a leading cause of morbidity and mortality (1, 2). High-sensitivity cardiac troponin T (hs-cTnT) and insulin resistance (IR) are well-known risk factors in the development of CAD (3, 4). It was proposed that coronary atherosclerosis may be one of the underlying mechanisms contributing to the occurrence of 1st degree atrioventricular block (AV-block) (5), which was previously considered benign (6) and is commonly observed in Blacks (7).

The measurement of hs-cTnT is a highly valued measurement for the detection of myocardial infarction (8) and relates to known risk factors of atherosclerosis (9). Hs-cTnT additionally indicates risk for cardiovascular disease (CVD) of non-atherosclerotic origin (9) and may have prognostic value in recognizing individuals at risk of developing hypertensive end-organ damage (10). This may relate especially to Blacks from South Africa due to the observed lower hs-cTnT cut-points in the prediction of systolic blood pressure (SBP) hypertension (11).

Furthermore, the homeostasis model of assessment (HOMA IR) is a dependable indicator of insulin resistance (IR) (12) and a risk factor contributing to atherosclerosis (13) and hypertension (14) and may increase coronary perfusion deficits. Indeed, fifty-three white individuals with IR in a study conducted by Saad et al. (15) were more prone to develop CVD than their black counterparts (15).

In individuals with CAD, 1st degree AV-block was related to heart failure and death (16), which also occurred in about 14% of individuals with myocardial infarction (17) and was therefore considered a serious complication of myocardial infarction (18). Also, in hypertensive individuals, 1st degree AV-block occurred as a result of insufficient blood pressure (BP) control, and was considered an independent risk factor for the development of left ventricular dysfunction, atrial fibrillation and advanced AV-block in future (5). First (1st) degree AV-block is diagnosed when the PR-interval of an electrocardiogram (ECG) is

extended even though all the impulses are conducted (19) and can be considered a marker of underlying heart disease (5). First (1st) degree AV-block is also more common in men (7, 20) and African-American individuals than in Whites (7). Thus, the clinical significance of myocardial distress, deranged glucose metabolism, common CVD risk factors and subclinical atherosclerosis in the presence of 1st degree AV-block, in a bi-ethnic cohort from South-Africa, is not clear and were investigated. This may improve our understanding of the CVD and CAD burden in this cohort.

2. METHODS

The SABPA (Sympathetic and Ambulatory Blood Pressure in Africans) was a target population study, conducted in February to May 2008/9. This study included 409 urban black and white teachers (aged 20 – 65 years) from the Dr Kenneth Kaunda Education district, North West province in South Africa (21). Homogeneity in this population was established by regarding socio-economic status and the working milieu, although participating individuals were from diverse cultural backgrounds. We excluded the following individuals: individuals with a history of psychotropic substance dependence or abuse, pregnant or lactating women, individuals who donated blood or had vaccinations less than 3 months prior to data collection, human immunodeficiency virus- (HIV) positive individuals (n=18) individuals with a history of myocardial infarction or cardiac events (n=5). The final sample included 179 Blacks and 206 Whites. Participating individuals were informed about the study protocol before recruitment and they afterwards signed an informed consent form. This study was approved by the Ethics Review Board of North-West University (Potchefstroom Campus: NWU-00036-07-S6) and the terms and guidelines of the Declaration of Helsinki (22) were followed.

2.1 Ambulatory measurements

At approximately 08:00 during week workdays (Mondays to Thursdays), participating individuals were fitted with an 24-h Blood Pressure (BP), an electrocardiographic device (Cardiotens CE120®, Meditech, Budapest, Hungary) and an accelerometer (Actical®, Montreal, Quebec). The Cardiotens CE120® reported oscillometrically measured ambulatory blood pressure BP and 2-lead electrocardiogram (ECG) measurements (validated by the British Hypertension Society; 2003). On the non-dominant arm of each participating individual, suitable cuff sizes were fitted and blood pressure (BP) was measured every 30 minutes between 08:00 and 22:00 and every 60 minutes between 22:00 and 06:00 (23). Successful inflation and measurement rates for the 24-h period were 72.8% (\pm 0.8%) in Blacks and 84.7% (\pm 0,7%) in Whites. Hypertension was determined as an average 24-h BP of \geq 130 mmHg SBP and/or \geq 80 mmHg diastolic blood pressure (DBP) (24). Ambulatory

ECG registrations were determined via two independent channels by the ECG unit of the Cardiotsens®. The isoelectric reference point (PQ segment), J point, L point (80 ms after the J point), and an ST-segment detection interval of at least 3 mm as the initial ST level before the onset of the 24-h ECG examination, were determined by the device for each participant. Approximately 4 hours of 2-lead ECG recordings were recorded during the 24 hours, as a pre-set program measured the ECG every 5 minutes for 20 seconds. Silent myocardial ischemia (SMI) were detected automatically by the Cardiotsens® apparatus by means of the following criteria (the 1-1-1 rule) (25-27), namely: (i) ST-segment depression by 1 mm horizontal or descending; (ii) ST-segment episode of 1 minute (duration); and (iii) ST-segment episodes with a 1-minute interval from preceding episodes. If additional horizontal or descending ST-depression episodes occurred, a 60-second ECG tracing and an additional BP measurement were recorded. The BP and ECG data were analyzed using the CardioVisions 1.19.2 Personal Edition software (Meditech®, Budapest, Hungary).

Participating individuals continued their typical daily activities and were asked to record distinguishable incidences on an ambulatory diary card. These included incidences of stress, headaches, fainting, nausea, palpitations, and visual disturbances. Data analyses were performed using CardioVisions 1.19.2 Personal Edition (Meditech, Budapest, Hungary). At 16:30 the participating individuals were transported to North-West University's Metabolic Unit research facility where the experimental setup of the study was explained to them. Participating individuals recorded info regarding their medical history and medicine use. Psychosocial questionnaires were completed under the supervision of registered clinical psychologists. Dinner was served (standardized) and participating individuals were requested to go to bed at 22:00 whilst fasting overnight. At 05:45 the subsequent morning, the participating individuals were woken, and anthropometric measurements were completed. The participating individuals remained in a semi-recumbent position for blood sampling and 12-lead ECG monitoring. Carotid intima-media thickness (CIMT) measures in supine position followed.

2.2 Assessment of lifestyle risk factors

The Actical® omnidirectional accelerometer (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada), which measured movement at 15-second intervals, recorded the physical activity or total daily energy expenditure (TEE) (kcal/day) levels of participating individuals, considering resting metabolic rate. Alcohol abuse was determined by the biochemical marker gamma-glutamyl transferase (γ -GT) (28). Smoking status of participating individuals was determined by measuring cotinine levels and were classified as smokers if cotinine values exceeded 14.99 ng/ml (29). Registered level II ISAK-accredited (*International Society for Advancement of Kinanthropometry*) Anthropometrists performed anthropometric measurements in triplicate by using standardized methods. Body mass and height of participating individuals were measured to the nearest 0.1 kg and 0.1 cm respectively (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer IP 1465, Invicta, London, UK). The Mostellar formula was used to calculate body surface area (BSA) in m² (30). The intra- and inter-observer variability was less than 10%.

2.3 Biochemical sampling and analyses

A registered nurse obtained fasting blood samples before 09:00 from the antebraial vein branches via a sterile winged infusion set. The high sensitivity enzyme rate method determined serum γ -GT levels and high-sensitivity C-reactive protein (hs-CRP), where serum cotinine was measured by the homogenous immunoassay method (Modular Roche Automized, Switzerland). Whole blood glycated haemoglobin A_{1c} (HbA_{1c}), was determined by using a turbidimetric inhibition immunoassay (Cobas Integra 400plus, Roche, Basel, Switzerland) where cholesterol analyses were performed with a sequential multiple analyser computer (Konelab 20i; Thermo Scientific, Vantaa, Finland). Hs-cTnT was determined using the electrochemiluminescence immunoassay (ECLA), Modular Roche Automized, Switzerland. The inter- and intra-assay coefficients of variation were less than 10%. Of the 387 values 105 values were lognormal computed (31).

2.4 Target-organ damage

Left carotid intima-media thickness (L-CIMT), left cross-sectional wall area (L-CSWA) and carotid luminal diameter were done via B-mode imaging with a Sonosite Micromaxx ultrasound system (SonoSite, Bothell, WA) and a 6-13 MHz linear array transducer. At least two ideal angle images of the left and right common carotid artery were captured and measured. The Rudy Meijer protocol was followed (32). These digitized images were imported into the Artery Measurement Systems automated software for dedicated analyses of the carotid measures. A maximal 10 mm segment was considered good quality and was used for analyses. The software automatically recognized the borders of the intima-media of the near and far wall, and the inner diameter of the vessel, and analysed the CIMT and diameter from around 100 distinct measurements through the 10 mm segment. The far wall measurements were used for purposes of this study. The far wall intra-observer variability was 0.04 mm between two measurements made 4 weeks apart on 10 participating individuals.

2.5 First degree AV-block

Resting 12-lead ECG was registered with the Norav NHH-1200® ECG (NORAV medical Ltd PC 1200, Isreal, Software version 5.030) where 1st degree AV-block was automatically detected by the software.

2.6 Statistical analyses

All statistical analyses were calculated using Statistica version 13.3 (TIBCO Software Inc., Palo Alto, USA, 2018). The Gaussian distribution of data was tested, and non-normal distributions were logarithmically transformed (γ -GT, physical activity (TEE), cotinine, HbA_{1c}, hs-CRP, hs-cTnT, HOMA IR).

Multivariate analyses of variance, corrected for *a priori* covariates, were used to determine interaction on main effects (ethnicity \times L-CIMT \geq 0.75 mm) for subclinical atherosclerotic risk markers. *A priori* covariates included age, body surface area (BSA), physical activity (total energy expenditure (TEE), γ -GT, cotinine (33). Hereafter, the sample group was described

using students' *T*-tests by ethnic status. Proportions and percentages were calculated using Chi-square (χ^2) tests. One-way analyses of covariance (ANCOVA) calculated differences among ethnic groups with and without 1st degree AV-block for L-CIMT, L-CSWA, left carotid lumen diameter, hs-cTnT, HOMA IR, and SMI events, considering *a priori* covariates as well as 24-h SBP, cholesterol and hs-CRP.

Uni- and multivariate regression analyses were performed controlling for *a priori* covariates, 24-h SBP, cholesterol and hs-CRP in ethnic groups with and without 1st degree AV-block. Adjusted partial correlations were determined between L-CIMT, L-CSWA, left carotid lumen diameter and cardiovascular variables. Forward stepwise regression analysis predicted relationships among dependent markers L-CIMT, L-CSWA, left carotid luminal diameter, hs-cTnT and HOMA IR in separate bi-ethnic groups with 1st degree AV-block. Other independent risk markers included *a priori* covariates, 24-h SBP, cholesterol and hs-CRP. Statistical significance level was set at $p \leq 0.05$ (two-tailed) and adjusted $R^2 > 0.25$ for regression analysis. F to enter was set at 2.50.

3. RESULTS

Table 3.1 lists the demographic and clinical characteristics of the Blacks and Whites. Interaction terms were fitted for L-CIMT in ethnic groups [$F(1,360) = 5.50; p \leq 0.05$]. The ethnic groups were notably well-matched for age dispersion. Blacks from South Africa revealed a higher cardiometabolic risk as several of these risk factors were more prevalent, including: higher 24-h BP reaching hypertensive status, HbA_{1c} levels, HOMA IR score, 24-h heart rate (HR), higher inflammation levels as determined by hs-CRP and more SMI events in 24 hours. Blacks were less active and used more alcohol (as reflected by higher γ -GT levels) than their white counterparts (Table 3.1). Also, a higher self-reported use of hypertension (HT) medication was evident in the black cohort than the Whites (Table 3.1). We aimed to determine the associations of cardiometabolic risk markers and subclinical atherosclerosis in a bi-ethnic cohort presenting 1st degree AV-block. Therefore, ethnic groups were further stratified into individuals with and without 1st degree AV-block.

Table 3.1: Baseline demographic and clinical characteristics.

	Blacks (n=179)	Whites (n=206)	p-values
Men, n (%)	87 (48.6)	98 (47.6)	
<i>Lifestyle factors</i>			
Age, years	44 (8)	45 (11)	0.609
Body Surface area, m ²	1.92 (0.2)	2.00 (0.3)	0.005
Physical activity, kcal/day _a	2585.5 (2163.8; 3070.6)	2927.2 (2356.0; 3487.7)	0.002
Cotinine, ng/ml _a	0.01 (0.01; 13.0)	0.01 (0.01; 0.01)	0.864
γ-GT, U/L _a	40.70 (27.6; 71.7)	17.50 (12.0; 28.0)	<0.001
<i>Cardiometabolic risk factors</i>			
HbA _{1c} , %	6.08 (1.2)	5.50 (0.4)	<0.001
hs-CRP, mg/l	8.69 (10.4)	3.12 (3.9)	<0.001
Cholesterol, mmol/l	4.66 (1.2)	5.53 (1.3)	<0.001
HdL Cholesterol, mmol/l	1.15 (0.4)	1.21 (0.4)	0.122
L-CIMT mean, mm	0.69 (0.2)	0.65 (0.2)	0.014
L-CSWA mean, mm ²	13.88 (5.2)	13.15 (4.5)	0.144
L-CIMT mean Lumen diameter, mm	5.85 (0.7)	5.81 (0.6)	0.624
24-h SBP, mmHg	133 (17)	124 (12)	<0.001
24-h DBP, mmHg	83 (11)	77 (8)	<0.001
24-h HR, beats/min	80 (11)	74 (10)	<0.001
24-h SMI events, n	6 (15)	3 (6)	0.004
hs-cTnT, pg/ml	4.77 (2.7)	5.49 (3.5)	0.025
HOMA IR score	3.81 (3.4)	3.16 (2.8)	0.039

Cardiovascular disease risk

	Blacks (n=179)	Whites (n=206)	p-values
24-h Hypertension, n (%)	114 (63.7)	79 (38.4)	<0.001
24-h SMI, n (%)	94 (52.5)	87 (42.2)	0.044
L-CIMT cut-point, n (%)	50 (28.9)	51 (25.1)	0.410
1 st degree AV-Block, n (%)	34 (19.0)	40 (19.4)	0.916
hs-cTnT cut point, n (%)	82 (47.6)	76 (37.1)	0.060
<i>Medications</i>			
Hypertension, n (%)	63 (35.2)	25 (12.1)	<0.001
Statins, n (%)	2 (1.1)	8 (3.9)	0.089

Data are reported as mean (SD) or proportions (%) unless otherwise indicated. a - Data are reported as median (Interquartile range). Abbreviations: n = number of individuals; γ -GT = gamma glutamyl transferase; HbA_{1c} = glycated haemoglobin; hs-CRP = high sensitivity c-reactive protein; HDL cholesterol = high-density lipoprotein cholesterol; L-CIMT = left carotid intima-media thickness; L-CSWA = left cross-sectional wall area; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; SMI events = silent myocardial ischemia events in 24 hours determined according to the 1-1-1 rule (25); hs-cTnT = high sensitivity cardiac troponin T; HOMA IR = homeostasis model of assessment determined by fasting glucose x fasting insulin/405. 24-h hypertension was determined as an average 24-h SBP of \geq 130 mmHg and/or DBP \geq 80 mmHg (33). L-CIMT cut point \geq 0.75 mm (34). hs-cTnT cut point \geq 4.2 pg/ml Blacks; hs-cTnT cut point \geq 5.6 pg/ml Whites (11). Significance set at $p \leq 0.05$.

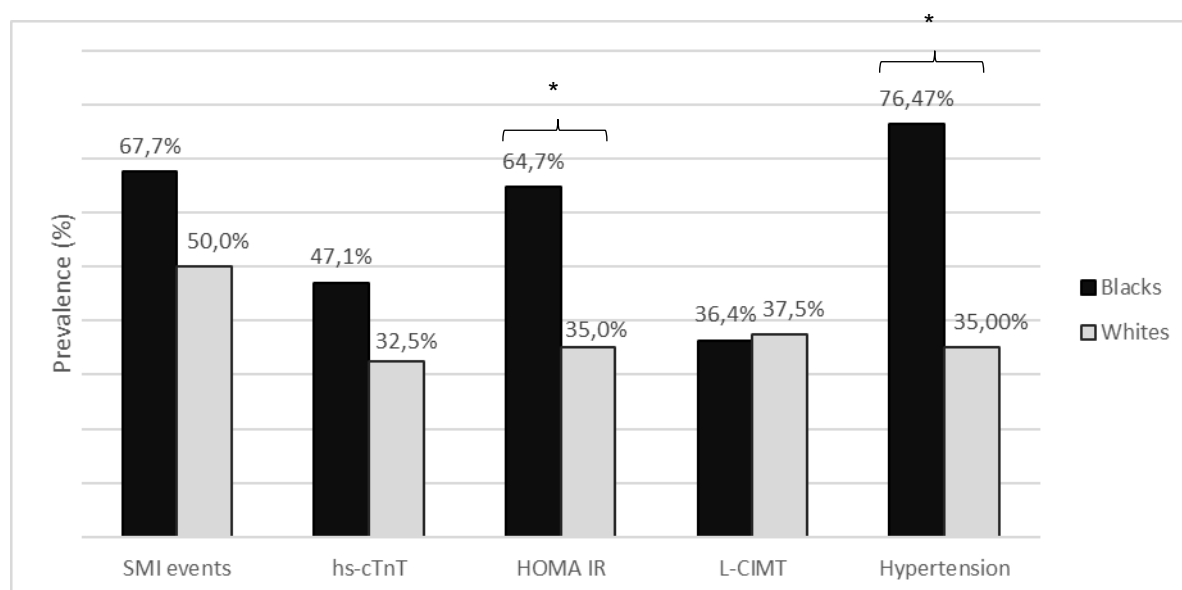


Figure 3.1: Number of cardiovascular disease risk factors in individuals presenting first degree AV-Block (Blacks, n=34; Whites, n=40). Factors included: silent myocardial ischemia (SMI) events in 24 hours, homeostasis model of assessment (HOMA IR) ≥ 3 ; high-sensitivity cardiac troponin T (hs-cTnT) ≥ 4.2 pg/ml Blacks; hs-cTnT ≥ 5.6 pg/ml Whites (11); left carotid intima-media thickness (L-CIMT) ≥ 0.75 mm (34). Hypertension was determined as an average 24-h systolic blood pressure (SBP) of ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg (33). *Significance set at $p \leq 0.05$.

Figure 3.1 emphasises the higher cardiometabolic risk profile with a higher prevalence of HOMA IR and hypertension in Blacks with 1st degree AV-block than their white counterparts. Even after adjusting for *a priori* covariates, 24-h SBP, cholesterol and hs-CRP, the Blacks with 1st degree AV-block still indicated a higher risk profile with an elevated HOMA IR ($p \leq 0.05$), thicker L-CIMT ($p \leq 0.05$) and smaller L-CSWA ($p \leq 0.05$) than did their white peers (Figure 3.2a). Additionally, in individuals without 1st degree AV-block, Blacks revealed significant L-CIMT thickening and more SMI events. However, significantly lower hs-cTnT levels were evident in Blacks without 1st degree AV-block than in Whites (Figure 3.2b).

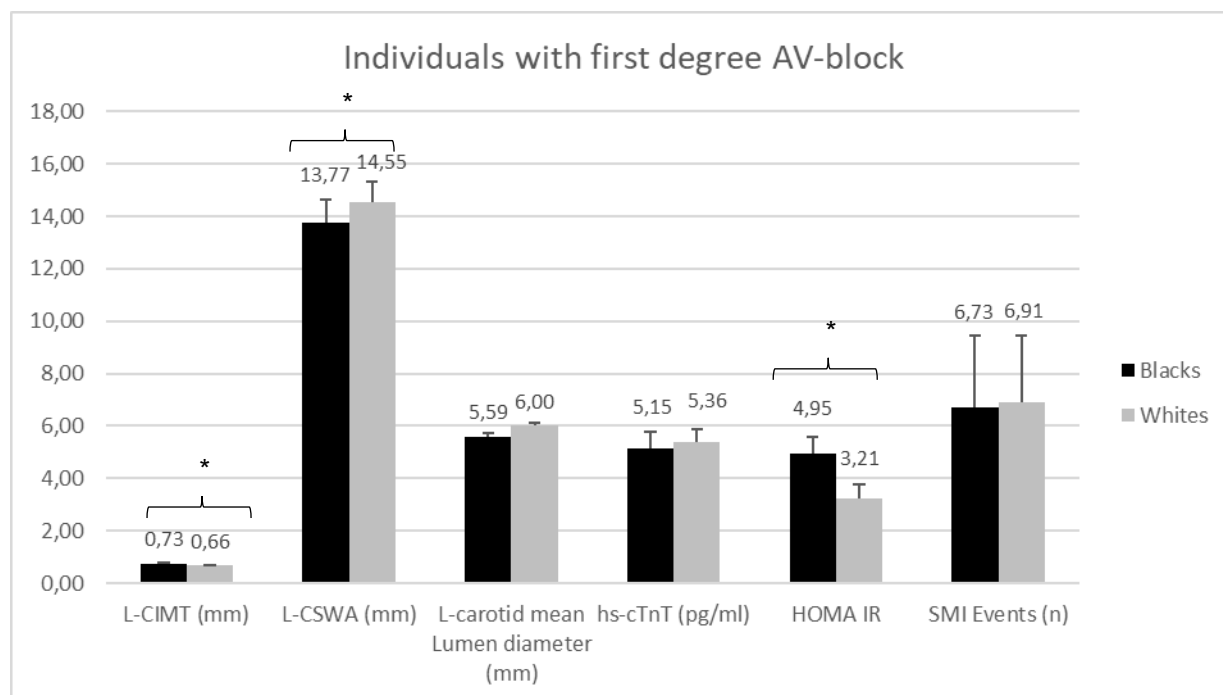


Figure 3.2a

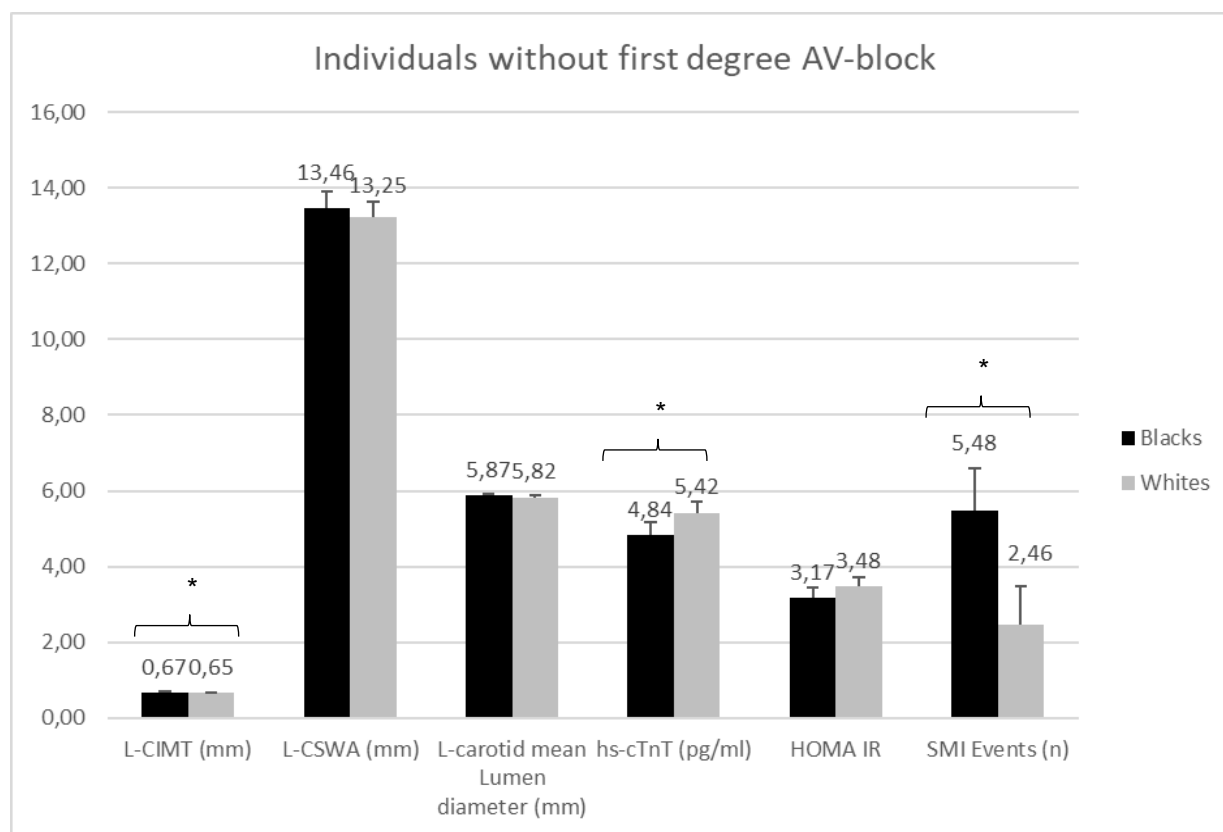


Figure 3.2b

Figure 3.2a – 3.2b: Analysis of covariates revealing cardiovascular risk markers comparing bi-ethnic groups with (figure 3.2a) (Blacks, n=34; Whites, n=40) and without 1st degree AV-block (figure 3.2b) (Blacks, n=136; Whites, n=162). Data are presented as adjusted means ± SE (Adjusted for *a priori* confounders, 24-h systolic blood pressure, cholesterol, and c-reactive protein). * Significance = $p \leq 0.05$. Abbreviations: 1st degree AV-block = first degree atrioventricular block; L-CIMT = left carotid intima-media thickness of the far wall; L-CSWA = left cross-sectional wall area; hs-cTnT = high-sensitivity troponin T; HOMA IR = homeostasis model of assessment; SMI events = silent myocardial ischemia events in 24 hours.

In Supplementary table 1a*, independent of *a priori* covariates, SBP, hs-CRP and cholesterol, positive associations existed between L-CIMT and hs-cTnT in Blacks with 1st degree AV-block. Additionally, the L-CSWA of Blacks also correlated positively with hs-cTnT. No correlations were evident in any of white groups as well as in Blacks without 1st degree AV-block (Supplementary table 1a – 1c*).

Stepwise multiple linear regression analyses in the total group revealed significant relationships between ethnicity and L-CIMT (Table 3.2). After stratification of the cohort into ethnic groups, hs-cTnT was positively associated with L-CIMT and L-CSWA respectively if 1st degree AV-block was present - only in the Blacks. No associations were found between subclinical atherosclerotic risk markers and HOMA-IR in any of the groups.

Footnote: * Supplementary table 1a – 1c are included after the references.

Table 3.2: Associations between subclinical atherosclerosis and adjusted cardiovascular disease risk markers in a bi-ethnic cohort presenting first degree AV-block.

	Individuals with 1 st degree AV-block (n=72)		Individuals with 1 st degree AV-block (n=72)	
	L-CIMT		L-CSWA	
Adjusted R²	0.39		0.34	
	β (95% CI)		β (95% CI)	
Ethnicity	-0.21 (-0.39; -0.03)*		N.S.	
	Blacks with 1 st degree AV-block (n=32)	Whites with 1 st degree AV-block (n=40)	Blacks with 1 st degree AV-block (n=32)	Whites with 1 st degree AV-block (n=40)
Adjusted R²	0.36	---	0.30	---
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Log hs-cTnT	0.46 (0.17; 0.75)*	---	0.42 (0.12; 0.71)*	---

β denotes standardized regression coefficient. F to enter 2.5. Independent covariates included in models were: age, body surface area (BSA), log total energy expenditure (TEE), log cotinine, log gamma-glutamyl transferase (γ -GT), 24-h systolic blood pressure, cholesterol, log high-sensitivity c-reactive protein. Abbreviations: 1st degree AV-block = first degree atrioventricular block; N.S. = non-significant; L-CIMT = left carotid intima-media thickness far wall; L-CSWA = left cross-sectional wall area; hs-cTnT = high-sensitivity cardiac troponin T. * Significance = $R^2 > 0.25$; $p \leq 0.05$.

4. DISCUSSION

The main objective of this study was to examine the associations among subclinical atherosclerosis, a deranged glucose metabolism, myocardial distress and common CVD risk factors in a bi-ethnic cohort in the presence of 1st degree AV-block. Subclinical atherosclerosis markers were positively associated with elevated hs-cTnT in Blacks presenting 1st degree AV-block, suggesting an increased susceptibility for hypertrophic remodelling and ischemic heart disease.

Findings in a black cohort from South-Africa revealed higher cardiometabolic susceptibility. They revealed significantly more SMI events and higher mean 24-h SBP levels, reaching hypertensive status (24), and concomitant higher DBP and HR. Not only were their HbA_{1c} levels elevated; also their HOMA IR levels, which may reflect a moderate risk for IR were elevated. The Blacks also had significantly higher inflammation levels and thicker CIMT's than their white counterparts, further emphasizing potential cardiovascular morbidity in this cohort (35). These results are supported by findings in a study conducted by Malan et al. (36) who revealed that Blacks from South Africa presented a higher cardiometabolic risk than their white counterparts, which was supported by Jansen van Vuren et al. (37).

Insulin is partly responsible for vasodilation and signal transduction in endothelial cells (38). A study performed by Steinberg et al (39), suggested that obese and insulin resistant individuals have endothelial dysfunction and a resistance to the effect of insulin on endothelium-dependent vasodilation, which could increase the risk of atherosclerosis. This was confirmed by research done by Rask-Madsen et al. (40) in which it was indicated that insulin therapy restored insulin-stimulated endothelial function in individuals with ischemic heart disease and type 2 diabetes. Also, IR is an independent risk factor for vascular disease and has several anti-atherosclerotic mechanisms, i.e. an increased production of Nitric Oxide (41) and decreased endothelial cell apoptosis (42). However, we could not replicate findings of IR being positively associated with subclinical atherosclerosis in Blacks or Whites with 1st degree AV-block.

Blacks presenting with 1st degree AV-block and hs-cTnT levels above 4.2 ng/ml (11) were 60.6%, whereas the Whites presenting 1st degree AV-block with levels of hs-cTnT above 5.6 pg/ml (11) were 39.4%. Hs-cTnT has been associated with atherosclerotic risk factors and can also specify risk for cardiovascular disease (CVD) of non-atherosclerotic origin (9). Additionally, hs-cTnT possibly increased the prognostic value in detecting individuals at risk of incurring end-organ injury due to hypertension (10). The ARIC (Atherosclerosis Risk in Communities) study explained that relations existed between detectable hs-cTnT and well-known cardiovascular risk factors, even in individuals who exhibit an 'ideal cardiovascular health' profile (9).

Indeed, hs-cTnT was associated with SMI in Blacks from South Africa (11). Additionally, in 2012, Griffiths et al. (26) demonstrated that SMI was associated with subclinical atherosclerosis in black men from the same cohort increasing their stroke risk. SMI reflecting myocardial ischemia in the absence of associated painful chest events (43) may occur more often in hypertensive individuals (44, 45) and increase the ischemic load burden of CAD (44, 45). In addition to this, ischemic heart disease is one of the most common causes of 1st degree AV-block (46) while 1st degree AV-block in turn can be attributed to disturbances in BP control (5). Low-grade inflammation in the Blacks (hs-CRP > 3 mg/L) further contributed to the development of 1st degree AV-block possibly through mechanisms which included SMI, thereby increasing the cardiac burden (47). The Blacks in the current study revealed a higher ischemic cardiovascular burden than their white counterparts. As a result, it is possible that the ischemic burden due to sclerosis of coronary arteries or inadequate blood pressure control may have induced 1st degree AV-block which exacerbated the cardiac burden; and in turn the elevated cardiac demand facilitating less vascular supply (48). Compensatory BP increases to alleviate perfusion deficits, explained ventricular strain and volume loading. Additionally, it is feasible that perfusion deficits (43), which were associated with hs-cTnT elevations (11), can also contribute to ventricular strain as a result of thickening of the carotid intima (26) and contribute to the occurrence of 1st degree AV-block. Furthermore, BP elevations promote functional and structural changes leading to

endothelial damage and subclinical atherosclerosis (49), further exacerbating the problem. A composite profile of low-grade inflammation, a high-pressure system and lower hs-cTnT levels in Black compared to their white counterparts emerges. The established lower hs-cTnT cut-points to predict SBP hypertension (10) nevertheless may underscore the ischemic burden, 1st degree AV-block and apparent subclinical atherosclerosis presumably indicative of inadequate BP control in Blacks. In addition, since the Blacks from South Africa have a prognosis of CVD worse than their white counterparts (36), this can possibly explain racial disparity due to psychosocial stress and increased adrenergic signalling (21).

5. CONCLUSIONS

To conclude, elevated hs-cTnT levels were associated with subclinical atherosclerosis in Blacks from South Africa presenting 1st degree AV-block, suggesting an increased CVD burden. However, other underlying mechanisms should be explored, i.e. chronic stress and adrenergic signalling (50).

Limitations of this study encompass the small sample size and cross-sectional design which cannot infer causality. The measurement of AV-block may not accurately localize lesions in the conduction system. However, the strength of the study lies in the well-controlled design which included teachers with similar socio-economic status and who all had access to medical aids. However, we could not account for cultural diversity.

Conflict of interest

None

Author contributions

MEG and LM contributed to the design and conception of the manuscript; acquisition, analysis, and interpretation of data. RD contributed to the analysis and interpretation of the data presented. MEG, RD and LM critically revised the document, the statistical analysis and critical revision of the statistical data presented. All the authors gave their final approval of the manuscript and accept full accountability for all the aspects of the work further ensuring the accuracy and integrity of the work presented.

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Supplementary table 1a: Partial correlations between the left carotid intima-media thickness and cardiometabolic risk markers.

Variable	Blacks with 1 st degree AV-block (n=34)		Whites with 1 st degree AV-block (n=40)		Blacks without 1 st degree AV-block (n=136)		Whites without 1 st degree AV-block (n=162)	
	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
Left carotid intima-media thickness (far wall)								
Log hs-cTnT	0.661	0.019	-0.283	0.399	0.150	0.266	-0.019	0.891
Log HOMA IR	0.466	0.127	-0.104	0.761	0.238	0.074	-0.012	0.930
Log SMI events	0.300	0.344	-0.172	0.613	0.185	0.167	-0.149	0.273

Abbreviations: hs-cTnT – high sensitivity cardiac troponin T; HOMA IR – homeostasis model of assessment; SMI events – silent myocardial ischemia events in 24 hours. Values in bold indicate significant associations. Significance set at $R > 0.35$ and $p \leq 0.05$. Adjusted for *a priori* confounders (age, body surface area (BSA), total energy expenditure (TEE), cotinine levels, gamma glutamyl transferase (γ -GT) levels), 24-h systolic blood pressure, cholesterol and high-sensitive c-reactive protein.

Supplementary table 1b: Partial correlations between the left cross-sectional wall area and cardiometabolic risk markers.

	Blacks with 1st degree AV- block (n=34)		Whites with 1st degree AV- block (n=40)		Blacks without 1st degree AV-block (n=136)		Whites without 1st degree AV-block (n=162)	
	Left cross-sectional wall area (L-CSWA)							
Variable	R-value	<i>p</i> -value	R-value	<i>p</i> -value	R-value	<i>p</i> -value	R-value	<i>p</i> -value
Log hs-cTnT	0.712	0.009	-0.358	0.279	0.171	0.203	-0.019	0.891
Log HOMA	0.425	0.168	-0.022	0.948	0.161	0.230	-0.012	0.930
Log SMI events	0.467	0.126	0.034	0.921	0.185	0.168	-0.149	0.273

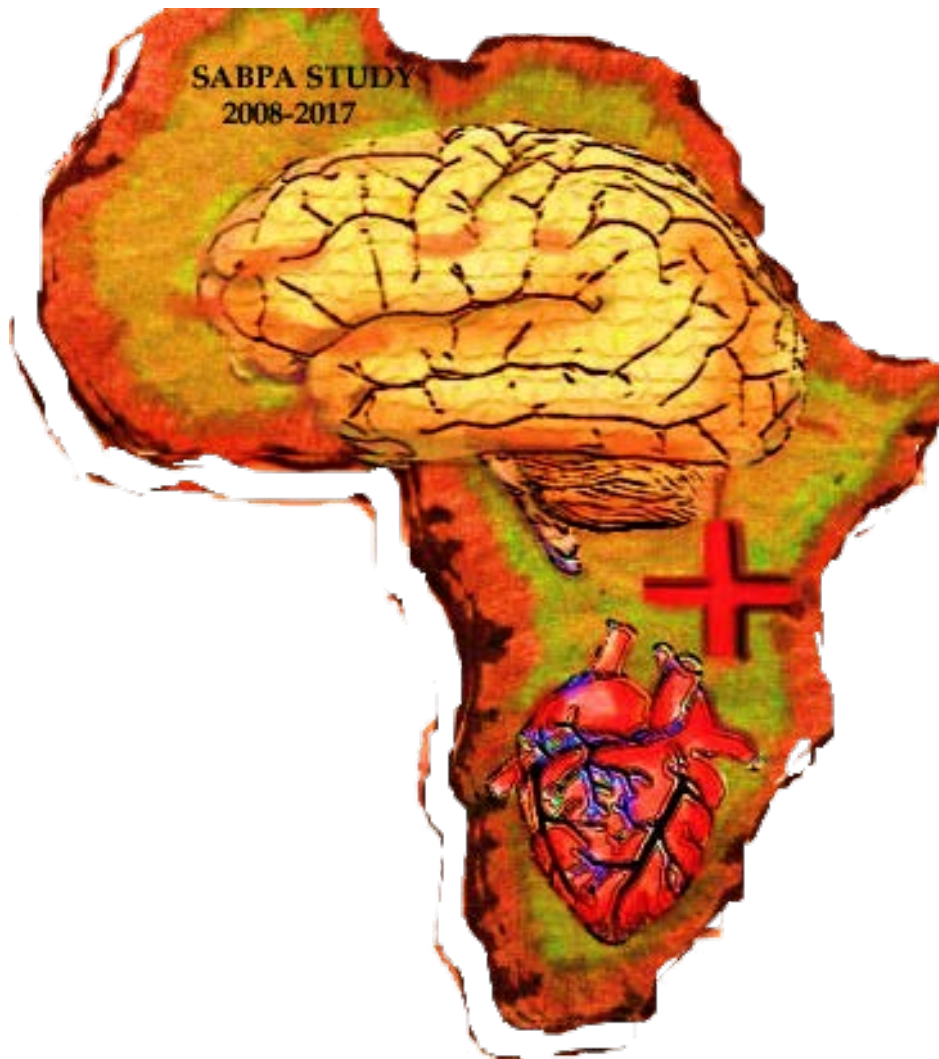
Abbreviations: hs-cTnT – high sensitivity troponin T; HOMA IR – homeostasis model of assessment; SMI events – silent myocardial ischemia events in 24-h. Values in bold indicate significant associations. Significance set at $R > 0.35$ and $p \leq 0.05$. Adjusted for *a priori* confounders (age, body surface area (BSA), total energy expenditure (TEE), cotinine levels, gamma glutamyl transferase (γ -GT) levels), 24-h systolic blood pressure, cholesterol and high-sensitive c-reactive protein.

Supplementary table 1c: Partial correlations between left carotid lumen diameter and cardiometabolic risk markers.

Variable	Blacks with 1 st degree AV-block (n=34)		Whites with 1 st degree AV-block (n=40)		Blacks without 1 st degree AV-block (n=136)		Whites without 1 st degree AV-block (n=162)	
	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
Left carotid lumen diameter								
Log hs-cTnT	0.287	0.365	-0.147	0.665	0.099	0.465	0.062	0.648
Log HOMA	-0.016	0.961	0.115	0.737	-0.131	0.330	-0.014	0.921
Log SMI events	0.553	0.062	0.326	0.327	-0.021	0.876	0.076	0.578

Abbreviations: hs-cTnT – high sensitivity troponin T; HOMA IR – homeostasis model of assessment; SMI events – silent myocardial ischemia events in 24-h. Values in bold indicate significant associations. Significance set at $R > 0.35$ and $p \leq 0.05$. Adjusted for *a priori* confounders (age, body surface area (BSA), total energy expenditure (TEE), cotinine levels, gamma glutamyl transferase (γ -GT) levels), 24-h systolic blood pressure, cholesterol and high-sensitive c-reactive protein.

Chapter 5



General Findings and Conclusions

Illustration used with permission from Prof. L. Malan – The SABPA study principal investigator.

General Findings and Conclusions

1. INTRODUCTION

The main findings of the three manuscripts as presented in this thesis, are included in this chapter. An in-depth discussion and conclusion are included, elucidating the interpretation of all the results and comparisons with the relevant literature reviewed in Chapter 1. Recommendations for future research are also presented, specifically to the Blacks and Whites from South Africa pertaining to cardiovascular risk.

2. SUMMARY OF MAIN FINDINGS

The primary aim of this study was to assess the role of high-sensitivity cardiac troponin T (hs-cTnT) as marker to detect silent myocardial ischemia (SMI) and target organ damage (subclinical atherosclerosis) in an urban South African bi-ethnic cohort. In addition, the predictive value of hs-cTnT in compensatory systolic hypertension (HT) was investigated. Furthermore, we endeavoured to determine the contribution of an additional cardiovascular disease (CVD) risk marker, namely insulin resistance (IR) and associated SMI. Pertaining to CVD risk the associations between subclinical atherosclerosis, hs-cTnT and IR were explored when 1st degree atrioventricular block (AV-block) was found to be present.

Key findings of the three manuscripts:

2.1 Troponin T release is associated with silent myocardial ischemia in black men: the SABPA study

The aims of the first manuscript were to compare hs-cTnT levels in black and white South Africans and to determine its relationship with SMI. In addition, the capability of hs-cTnT to predict systolic HT in this South African cohort was explored.

The first hypothesis postulated that hs-cTnT is positively associated with hs-cTnT in Blacks from South Africa. This hypothesis was accepted, since hs-cTnT was positively associated with SMI especially in the Black men.

The second hypothesis assumed that hs-cTnT predicts systolic HT between ethnic groups. As a defence response, compensatory SBP increased secondary to myocardial damage to alleviate myocardial ischemia. Thus, this hypothesis was accepted as a cut-point for hs-cTnT was established in risk prediction for compensatory SBP rises. This cut-point was set at 4.2 pg/ml for Blacks and 5.6 pg/ml for Whites, implying greater myocardial damage prevalence in Blacks at lower hs-cTnT levels and which should thus be considered for risk stratification.

2.2 Silent ischemia, insulin resistance and cardiovascular risk in a bi-ethnic sex cohort: the SABPA study

The second sub-study set out to explore whether the underlying susceptibility to CVD in this bi-ethnic sex cohort, was of a cardiovascular- or metabolic origin. Thus, the aims of the second study were to compare possible associations between SMI and hs-cTnT in a bi-ethnic sex cohort. In addition, the possible associations between SMI and insulin resistance (IR) in this bi-ethnic sex cohort was explored to determine an ethnic/sex-related mechanism for emerging SMI.

The first hypothesis postulated that only Black men from South Africa will exhibit positive associations between SMI and hs-cTnT. This hypothesis was accepted and suggested that Black men have a higher cardiovascular susceptibility to develop ischemic heart disease than their White peers.

The second hypothesis postulated that in White sex groups, SMI will be positively associated with IR. This hypothesis was accepted, as IR (determined by the homeostasis model of assessment (HOMA IR) levels) was significantly associated with SMI in both White sex groups. Not only was IR associated with lengthier SMI events in both sexes; it was also related to more frequent SMI events, underscoring the role of metabolic perturbation in the development of ischemic heart disease in this White cohort. Thus, an ethnic-specific mechanism may reflect emerging SMI.

2.3 Troponin T release is associated with subclinical atherosclerosis in Blacks with first degree AV-block: the SABPA study

For the third manuscript we set out to explore associations between subclinical atherosclerosis, cardiometabolic risk markers and cardiac conduction symptoms.

Thus, the aims of this sub-study were to determine associations between subclinical atherosclerosis and hs-cTnT in a bi-ethnic-cohort when 1st degree AV-block is present. Also, the associations between subclinical atherosclerosis and IR were determined in a bi-ethnic cohort presenting 1st degree AV-block.

The hypotheses of this manuscript were built on the second manuscript which stated that ethnic-specific mechanisms exist in the emergence of SMI (indicator of coronary artery disease(CAD)). IR and hs-cTnT may thus be ethnic-specific associated with subclinical atherosclerosis. Whites may be more prone to cardiometabolic perturbations, whereas Blacks may be more prone to cardiovascular disturbances when conduction disturbances are prevalent. The first hypothesis suggested that positive associations will be evident between subclinical atherosclerosis and hs-cTnT in Blacks presenting 1st degree AV-block. In the presence of 1st degree AV-block in Blacks revealed that increases in hs-cTnT and an enhanced susceptibility for a compensatory high-pressure system and carotid hypertrophic remodelling; hence acceptance of the first hypothesis. The second hypothesis postulated that subclinical atherosclerosis will be positively associated with IR in Whites with 1st degree AV-block. However, no positive associations were evident between carotid markers of subclinical atherosclerosis and IR in Whites presenting 1st degree AV-block, thus rejecting the second hypothesis.

3. DISCUSSION OF MAIN FINDINGS AND COMPARISON WITH THE LITERATURE

The SABPA study contributed to an understanding of CVD and CAD risk in Blacks and Whites from a sub-Saharan cohort and it deemed it to be essential to expand this knowledge. The novel findings from this study contributed meaningfully to the available literature, either confirming or contradicting research as previously conducted.

The prevalence of CVD keeps escalating in urban Blacks from South-Africa even though focussed healthcare and awareness programs exist (1-4) and inauspicious progression of CVD risk factors are observed among this ethnic group (5). SMI is the most common manifestation of CVD (6, 7), was also related to subclinical atherosclerosis (8) in black males (9) and was enhanced by the prevalence of cardiovascular risk markers, i.e. diabetes and HT (5, 10). SMI preceded myocardial infarction, which can be detected by the validated biochemical marker hs-cTnT (11), and reflecting risk markers of atherosclerosis (12). In addition, insulin resistance (IR) also underscored the development of SMI (13) and was a prominent independent predictor of coronary artery disease (CAD) (14). Furthermore, ischemic heart disease was also found to be the most common cause of 1st degree AV-Block (15).

The findings of this study can be a fundamental starting point for a larger prospective study, even though it may be difficult to extrapolate these results to the general black population of South Africa. These findings may however contribute to addressing and curbing worse outcome of CVD in this population. The higher prevalence of SMI in the black cohort is underscored by the higher cardiovascular susceptibility to develop CVD and CAD as a raised hs-cTnT level was positively associated with SMI in black men. This was confirmed by the higher mean 24-h SBP values (reaching hypertensive status) and lower hs-cTnT cut-point (Blacks hs-cTnT \geq 4.2 pg/ml; Whites hs-cTnT \geq 5.6 pg/ml) in Blacks, predicting compensatory BP rises. Previous studies revealed that associations between SMI

prevalence and CVD have been linked to higher 24-h BP values in hypertensive individuals (9, 16), and the higher number of SMI events may be detrimental to the higher 24-h blood pressure (BP) values. In addition to this, Rubin et al.(12) also showed independent relations of detectable hs-cTnT with known cardiovascular risk factors even in people with “ideal cardiovascular health”. The underlying mechanism can be ascribed to a lower vascular supply and a higher cardiac demand (17). BP will compensate by increasing to improve perfusion, thereby elevating volume loading and ventricular strain (18). Rubin et al. (12) also revealed that both sex and diabetes as risk factors were related to elevated hs-cTnT levels and can possibly contribute to the CVD risk factor burden. Blacks were also more susceptible to metabolic perturbations with elevated glycated haemoglobin (HbA_{1c}), which was confirmed by several studies (19, 20). In addition, Rubin et al. (21) also stated that hyperglycemia, as measured by HbA_{1c}, is related to myocardial injury determined by elevations in hs-cTnT.

Therefore, a possible mechanism for emergent SMI considering the CVD risk factors hs-cTnT and IR in this bi-ethnic sex cohort, was explored. Black men revealed a cardiovascular susceptibility (as measured by hs-cTnT) to the development of CVD. The lower hs-cTnT cut-point to predict systolic HT observed in Blacks which may reflect the elevated snowballing cardiovascular burden in this ethnic group. McEvoy et al.(18) found relationships between hs-cTnT and HT and explained that hs-cTnT can possibly have a predictive worth to identify individuals at risk of developing HT and hypertensive end-organ damage (18). It is possible that SMI can be considered to be hypertensive end-organ damage as SMI in hypertensive individuals develops due to an increased cardiac demand (i.e. atherosclerotic obstruction) and a reduced vascular supply (18). If systolic blood pressure- (SBP) increases are secondary to ventricular distress, the lower cut-point for the relation with systolic HT in Blacks shows racial disparity. The supply-demand discrepancy will increasingly deteriorate as vascular resistance rises with the secondary elevation in BP, probably in combined action with other causes of ventricular demand.

Additionally, a metabolic susceptibility to develop CVD was more evident in white men and women from this cohort who displayed a higher IR risk in relation to SMI than their black counterparts. IR is a risk factor underscoring the development of SMI (22) which also contributed more to the development of CVD in Whites than in Blacks (22, 23) confirming the results from this study. Saad et al. (23) also recognized the link between IR and BP especially in White individuals.

Individuals with IR already have myocardial perfusion deficits without any symptomatic cardiac disease (24). IR also impairs coronary microcirculation which is characterized by discrepancy between myocardial metabolism and coronary blood flow (25, 26). As the myocardium is an insulin-responsive tissue it is susceptible to diabetic glucose/insulin homeostatic modifications (27).

The CAD burden of Blacks was further explained by the positive associations between raised hs-cTnT and subclinical atherosclerosis when 1st degree AV-block is present. Ischemic heart disease is one of the most common causes of 1st degree AV-block (28), which in turn can be ascribed to discrepancies in BP control (29). It may be possible that the ischemic burden because of coronary atherosclerosis or impaired blood pressure control contributed to the prevalence of 1st degree AV-block, which enhanced the cardiovascular burden. As a result, cardiac demand increased and reduced the vascular supply (17). Furthermore, it is also feasible that the oxygen deficiency due to the presence of SMI events (30), which related to elevated hs-cTnT, can also underwrite ventricular strain as a result of thickening in the carotid-intima thickness (CIMT) (9) and can therefore contribute to the presence of 1st degree AV-block. In addition, the ischemic burden underscoring 1st degree AV-block, as a result of subclinical atherosclerosis, may be caused by compensatory BP rises as well as deficient BP control. This possibly caused raises in hs-cTnT levels in Blacks presenting 1st degree AV-block, which were significantly related to subclinical atherosclerosis.

4. CONCLUSIONS

The Blacks from this cohort have a higher cardiovascular susceptibility to the development of CVD. This was underscored by a lower hs-cTnT cut-point to predict compensatory SBP HT. Furthermore, in Blacks with 1st degree AV-block, subclinical atherosclerosis was positively associated hs-cTnT. Thus, the use of hs-cTnT cut-points (Blacks hs-cTnT \geq 4.2 pg/ml; Whites hs-cTnT \geq 5.6 pg/ml) may contribute significantly to preventive cardiology especially in Blacks from South Africa.

In addition, in Whites, IR contributed more to the development of ischemic heart disease than did hs-cTnT and can be translated to health care practice.

The adverse CVD risk in a South African black cohort is concerning and the contribution of risk factors due to urbanization (poor nutrition, lower activity levels, obesity, alcohol abuse and smoking) further exacerbated a CVD burden.

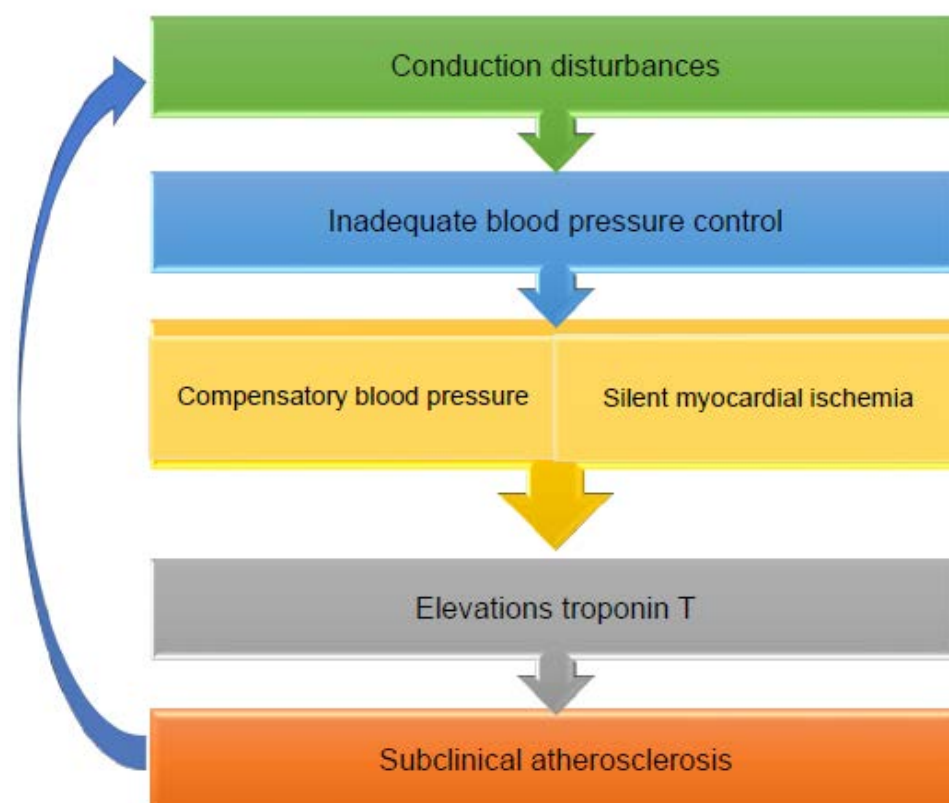


Figure 4.1: Illustration depicting the effect of conduction disturbances and elevations in high-sensitivity cardiac troponin T resulting target organ damage in Blacks from South Africa.

5. CHANCE AND CONFOUNDING FACTORS

It is important to reflect on factors which may have influenced or confounded the presented results. Firstly, causality could not be concluded as this was a cross-sectional designed study. Conclusions made were therefore carefully considered.

Two-way and three-way analyses of covariance (ANCOVAs) determined interactions on the main effects of all three the papers and defined grouping variables for further statistical analysis. Ethnicity (*Manuscript 1, 2 and 3*) and sex (*Manuscript 1 and 2*) had pronounced impact in the sub-studies and showed interactions with SMI, CAD and CVD risk factors.

In the statistical analysis, *a priori* confounders were adjusted for. These *a priori* confounders include: age, body surface area (BSA), total energy expenditure (TEE), smoking (cotinine) and alcohol use (gamma glutamyl transferase (γ -GT)). For *Manuscript 3* adjustments were also included for 24-h SBP, high-sensitivity c-reactive protein (hs-CRP) and total cholesterol. Individuals excluded from the analysis were those with a history of myocardial infarction of cardiac events, clinically diagnosed diabetes (*Manuscript 2*) and individuals with human immunodeficiency virus (HIV)-infection (*Manuscript 3*). Sensitivity analyses were done in *Manuscripts 1 and 2*, by excluding HIV infected individuals. However, these analyses did not change the outcomes.

The above-mentioned adjustments and exclusions were important so as to diminish erroneous interpretation of results. In conjunction with the well-controlled design of the SABPA study, small intra- and inter-variability in the standardised protocols, this added to the overall worth of the final results attained.

6. RECOMMENDATIONS FOR FUTURE RESEARCH

To validate this sub-study's results it is important to conduct this research in other urban African cohorts. This will contribute to extrapolation to the broader South African population and the following recommendations are subsequently suggested:

- Repeating this study in the form of a longitudinal study can possibly contribute to the establishment of cause and effect. The possibility to include larger groups of different ethnicities can possibly clarify results from this study.
- The measurement of biomarkers (i.e. hs-cTnT) and end-organ risk markers in the general population are costly. Thus, cost-effective screening methods should be developed to predict future cardiac events. As a result, early detection could possibly decrease the cardiovascular burden.
- The inclusion of intrinsic (i.e. mental stress), genetic and modifiable factors (i.e. medication use) in relation to the above-mentioned CVD markers, 1st degree AV-block and SMI can contribute to an understanding of the increasing burden of this population.
- Furthermore, including the measurement of SMI during ECG-stress testing could clarify the pathology and conduction disturbances of this cohort.

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Appendix 1 –Ethics Approval



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

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Dr L Malan

Ethics Committee
Tel +27 18 299 2542
Fax +27 18 297 5308
Email Ethics@nwu.ac.za

Dear Dr Malan

6 February 2008

ETHICS APPROVAL OF PROJECT

The North-West University Ethics Committee (NWU-EC) hereby approves your project as indicated below. This implies that the NWU-EC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans)																														
Ethics number: <table border="1"><tr><td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>0</td><td>3</td><td>6</td><td>-</td><td>0</td><td>7</td><td>-</td><td>S</td><td>6</td></tr><tr><td colspan="3">Institution</td><td colspan="5">Project Number</td><td colspan="2">Year</td><td colspan="5">Status</td></tr></table>	N	W	U	-	0	0	0	3	6	-	0	7	-	S	6	Institution			Project Number					Year		Status				
N	W	U	-	0	0	0	3	6	-	0	7	-	S	6																
Institution			Project Number					Year		Status																				
Approval date: 12 November 2007 Expiry date: 11 November 2012																														

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-EC:
 - annually (or as otherwise requested) on the progress of the project,
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-EC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-EC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-EC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the NWU-EC or that information has been false or misrepresented,
 - the required annual report and reporting of adverse events was not done timely and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)



Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

Faculty of Health Sciences

Tel: 018-299 2092
Fax: 018-299 2088
Email: Minrie.Greeff@nwu.ac.za

14 Augustus 2015

Prof L Malan
Physiology


Dear Prof Malan

APPROVAL LETTER: ETHICS APPLICATION: NWU-00036-07-A6 (L MALAN-ME GRIFFITHS) "NWU-00036-07-A6 SYMPATHETIC ACTIVITY AND AMBULATORY BLOOD PRESSURE IN AFRICANS (SABPA) STUDY"

Thank you for amending your application. All ethical concerns have now been addressed and ethical approval is granted for the sub-study, entitled "Silent myocardial ischemia, cardiac troponins and target organ damage in a bi-ethnic sex cohort: THE SABPA STUDY" until 01/11/2017.

Please note that any changes to the approved application must be submitted to the Health Research Ethics Committee for approval before implementation.

Yours sincerely



Prof Minrie Greeff
HREC Chairperson

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2015 Applications\Applications 04 - 14 May 2015\NWU-00036-07-A6 (L Malan-ME Griffiths)\NWU-00036-07-A6 (L Malan-ME Griffiths) - AL\NWU-00036-07-A6 (L Malan-M Griffiths) - AL.docm
14 August 2015

File reference: 9.1.5.3



Appendix 2 - SABPA participant Information and Consent Forms

**NORTH-WEST
UNIVERSITY
POTCHEFSTROOM
CAMPUS**

**SCHOOL FOR PHYSIOLOGY, NUTRITION AND CONSUMER
SCIENCES PARTICIPANT INFORMATION AND CONSENT FORM**

PART 1

PRINCIPAL RESEARCHER: Dr Leoné Malan, Subject Group Physiology

PROJECT LEADER: Dr. Leoné Malan, Subject Group Physiology

Associate Researcher(s): The postdoctoral fellow involved in this trial is Dr. Szabolcs Péter. Other persons assisting in the study are Dr. Hugo W. Huisman, Prof. Johannes M. van Rooyen, Prof. Nico T. Malan, Dr R Schutte, Mrs. Carla M.T. Fourie, Mrs. Tina Scholtz (Cardiovascular research group, Physiology), Prof. Salomé Kruger & Dr. Ramoteme Mamabolo, (Physical activity), Proff. Hans de Ridder (Anthropometry), Marié Wissing (Psychology), Linda Brand & Brian Harvey (Pharmacology), Kobus Mentz (Education), Francois van der Westhuizen (Biochemistry), Hester Klopper (Nursing), Nancy Frasure-Smith & Francois Lespérance (Psychology, Canada), Alaa Alkerwi (Epidemiology, Luxembourg), Yackoob Seedat (ECG, Kwazulu Natal), Paul Rheeder (Sonar, Pretoria Univeristy), Drs. Johan Potgieter & Michael Temane & Mr Thumi Khumalo (Psychology), Mrs Gedina de Wet (Nursing).

This Participant Information and Consent Form is 7 pages long. Please make sure you have all the pages.

Your Consent

You are invited to take part voluntarily in this research project.

This participant information document contains detailed information about the research project which has been explained to you verbally. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part.

Please read this *Participant Information Form* carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

What is the study about?

The aim of this project is to have an impact on the eventual prevention and treatment of lifestyle diseases in Africans from South Africa. New knowledge regarding the relationship between higher nervous system activity implicating cardiovascular, metabolic and psychological well-being will improve understanding and change strategies at the roots of treatment and prevention of lifestyle diseases.

Our research has shown that lifestyle diseases in urbanised Africans present higher obesity levels, high blood pressure or hypertension prevalence rates and the experiencing of more stress. This pattern is enhanced during psychosocial stress/urbanisation in participants with a specific coping style.

Hence the planned SABPA project, which is the first study in South Africa where coping and direct markers of nervous system activity in Africans will be measured.

Purpose of study

The purpose of this study is to investigate biological markers associated with higher sympathetic nervous system activity in urbanised teachers with a specific coping style.

To investigate the relationship between blood pressure, inflammation, obesity, stress and coping in more detail we are going to perform this study in 400 men and women from the North West province, aged 25-60 years. A comprehensive assessment of the cardiovascular and nervous systems by means of non-invasive painless techniques will be performed and a blood sample will be taken by an experienced research doctor and nurse to determine your blood sugar, cardiovascular, inflammation and stress hormone levels amongst other health markers.

Procedures

All measurements are performed in the Metabolic Unit (lipid clinic) of the University. A researcher has explained the entire procedure in detail and while you are reading this information document you have time to ask questions and to have clarified matters. If you are fine with the explained procedure you are requested to sign a *consent form (at the end of this document). Remember all personal data will be handled with care and remain confidential.

**By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for the purposes noted above. Your blood will also be tested for preliminary results on HIV status, since your HIV status may directly influence the main purposes of this study. If you would like to know what your HIV-status is, we will provide it. If tested positive we will refer you to your doctor and he/she will perform the necessary tests which will allow you to apply for chronic medication benefit. Also, the blood cells from your donated blood sample will be used to investigate the molecular genetics of higher nervous system activity and type 2 diabetes in order to enable pre- symptomatic diagnosis of hypertension and diabetes in the long term.*

Why was I chosen? Teachers are exposed to changing curricula and disciplinary problems whilst living in an urbanised environment adding to higher stress experiencing and nervous system activity.

How was I chosen?

Inclusion criteria:

Phase I: 200 black Africans aged 25-60 years (male=100, female = 100)

Phase II: 200 white Africans (n = male, 100 = female) aged 25-60 years.

Exclusion criteria: pregnancy, lactation, any acute/chronic medication (e.g. high blood pressure, TB/tuberculosis, high sugar/diabetes, arthritis, anti-clotting/stroke factors, epilepsy/mental diseases

or being treated for it as well being addicted to the medicine). You can not be included if you have been vaccinated in the previous 3 months and if you are a regular blood donor.

What will be expected of me?

You, as participant will be screened once by a registered nurse to be eligible complying to the inclusion criteria. The following procedures will be followed:

- Recruitment, screening and informed sessions with all participants will be done two months prior to the study (October - November 2007, Phase I, and November, 2008, Phase II) and informed consent forms will be signed.
- After selection of all participants, the details of the project will be discussed with you in English or your home language, i.e. what the exact objectives of the study are, what procedures will be taken and what will be expected from each of you (e.g. overnight stay, resting blood pressure procedures and fasting urine and blood samples are required, importance of complying with the correct sampling methods, incentives). You will be given the opportunity to ask questions.
- Data collection for each participant will involve two days (15min in the morning and 2½ hours in the evening) on Day I; and 2 hours on Day II):

DAY I

- On day I at 07:00, the blood pressure apparatus, which will measure your blood pressure and heart function as well as a physical activity meter will be applied to your arm and waist at your school and you can then resume your normal daily activities. In the afternoon you must complete the Neethling Brain Instrument questionnaire which measures thought processes of the brain.
- At the end of Day I (\pm 16:30) you will be transported from your schools to overnight in the Metabolic Unit Research Facility of the North-West University. This unit is a research unit for human studies and equipped with 10 well furnished bedrooms, a kitchen, two bathrooms and a television room. Each of you will be subjected to the following procedures:
 - At the end of Day I between \pm 17:15 and 18:00 you will be welcomed and each of you will receive your own private bedroom.
 - The procedures, which will be done, will be explained again and each of you will then complete a general socio-demographic health questionnaire. Afterwards you will receive dinner.
 - After dinner, psychological questionnaires will be completed under supervision of registered education specialists and psychologists. Completion of questionnaires will take approximately 40 min, including a break of 20 minutes with coffee/tea and biscuits. This will be your last meal for Day I as you must be fasting on Day II for obtaining good results.
 - Thereafter, you can relax and watch television or socialise with your c-participants. It will be wise to go to bed not later than 22:00 as the blood pressure apparatus will take measurements every hour during the night and it can be tiring.

DAY II

- At 06:45 on Day II the AMBP will be removed and an urine sample collected. Once this has been done you will be directed to the anthropometric station where your weight, height and body circumferences will be measured.
- The next station involves the blood pressure measurement station. Whilst in a sitting position your blood pressure will be taken in duplicate with the sphygmomanometer (the same as used at clinics) with a resting period of 5 minutes in between. Our registered research doctor/nurse will take a fasting saliva sample as well as a blood sample of 45ml from a vein in your dominant arm. The infusion set will be left in your arm to lessen the effect of inserting a needle again for blood sampling after exposure to the two stressors. A small amount of diluted heparin will be left in the infusion set in

your arm to prevent clotting.

Next the cardiovascular measurements will follow consisting of three separate procedures:

- The 1st measurement involves an ECG apparatus, which measures heart function, with 12 leads, which will be placed into position on your rib cage/front part of the body.
- The 2nd measurements are non-invasive and will be done by means of the Finometer device which also involves the assessment of heart functioning such as pulse (beats per minute), stroke volume (blood volume ejected by the heart per beat), cardiac output (blood volume ejected by the heart per minute), total peripheral resistance (resistance against the blood flow created by small arteries), central resistance (resistance against which the heart has to work while ejecting the blood into the aorta) as well as the elasticity of your large arteries (compliance). For this procedure a blood pressure cuff will be placed around your left arm and middle finger which will be inflated and stepwise deflated. You will not have more discomfort than during a common blood pressure measurement. This will take about 5 minutes.
- The stressor application procedure follows: You will now be exposed to a stressor for 1 minute whilst your blood pressure and ECG will still be taken. After exposure a saliva and blood sample (45ml) will be taken. After 10 minutes another saliva sample will be taken. Then the stressor application procedure will be repeated with the second stressor.
- At another station your 3rd measurement includes the assessment of pulse wave velocity, i.e. how fast your blood travels through your arteries. This measurement gives us an indication about how stiff your vessel walls are. The stiffer your vessel wall is the faster the blood travels from one point of your body to another. These painless measurements will require two technicians using blunt probes (tonometer) putting light pressure on the neck and on the foot to measure the velocity of the pulse waves. This takes only a few minutes. An ultrasound device will be taken of your arteries in the neck with a blunt probe to indicate the intrinsic thickness of your arteries which contributes to high blood pressure.

The two stressors you will be exposed to for one minute include:

1. The *Colour-Word-Conflict Chart (applied for 1 minute)* is written in various colours. You must say or select the ink colour rather than the name of the colour spelled out by the word. A sliding scale with monetary incentives (maximum of R55.00) will be given if you can complete reading the chart.
 2. The *Cold Pressor Test (Foot) (applied for 1 minute)*: Immersion of your foot up to the wrist in ice water (4 degrees Celcius). As the cold can make you hold your breath you must quietly count to yourself during cold exposure to breath more rythmic.
- You have reached the end of the sampling phase.
 - Thank you for your participation! You now will have the opportunity to shower and a take away breakfast will be given.
 - Immediate feedback on your HIV/AIDS status, obesity, blood pressure and blood glucose/sugar values will be given. *HIV/AIDS post-test counselling will be arranged if you are tested positive.*
 - You are now transported back to your school and after one week you will receive your Neethling Brain Instrument and 24-hour blood pressure reports.

Possible Risks

The measurements performed in our study will include only non-invasive techniques that are not expected to reveal any risks but might cause little discomfort. The taking of blood samples is an invasive procedure with a minimal risk of bleeding. Thus the procedure may cause only a few seconds of light discomfort. All tests will be performed by experienced research nurses of our department. There may be additional unforeseen or unknown risks.

Precautions to protect the participant

The Metabolic Unit facility of the NWU is fully equipped, and in case of an emergency which could not be handled by the registered nurse, the supervising medical doctor Emile Kotzé will be contacted. Dr. Kotzé was notified before the study commenced that this study will be taking place, and that there is a slight possibility that he may be contacted. Supporting medical treatment care facilities will be at hand anytime if needed.

Other Treatments Whilst on Study

It is important to tell the research staff about any treatments or medications you may be taking, including non-prescription medications, vitamins or herbal remedies during your participation in the study.

Incentives

1. All teachers will receive feedback on their health profile and if necessary references will be given to physicians/clinics/hospitals.
2. Printout feedback on 24 hour blood pressure monitoring report (normally costing R637.60), sonar of the artery (R1200.00), resting ECG (R600.00) and other variables (R500.00). Your benefit of participation is a comprehensive assessment of the cardiovascular and metabolic condition including investigation of blood pressure, inflammatory status and psychological well-being. These examinations will help us to assess the degree of vascular impairment of the arteries and to predict your risk of possible cardiovascular events such as heart attacks and stroke. The results may assist your doctor in decision making for further treatment or for instituting preventive measures. Our study will also contribute to the identification of possible factors leading to high blood pressure. As 24 hour ambulatory blood pressure monitoring is required for the diagnosis of hypertension, medical aids insist on this method of diagnosis to qualify for chronic medication. Additional testing could also reveal illnesses of a chronic nature and would serve as a motivation to qualify for chronic medication, such as metabolic syndrome, anti-inflammatory and cholesterol-lowering drugs.

Monetary incentive on completion of the colour word conflict chart (\pm R55.00).
Dinner and breakfast (\pm R24.00).

Neethling Brain Instrument profiles done by registered user of the Whole Brain (normally

Privacy, Confidentiality and Disclosure of Information

By consenting to participate in this study, you consent to the storage and later analysis and testing of your stored blood samples for purposes noted above. Your blood samples will be discarded immediately after analysis. All information provided by you and the results of tests will be treated in the strictest confidence, and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law.

The results of your medical tests will be labelled only with a code number, and will be stored separately from any identifying information. When the results are analysed we will be looking for differences between groups of people, not at the results of individuals. No information that could identify any person taking part in the study will be revealed when the results are reported.

Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the North- West University.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.

Ethical Guidelines

This project will be carried out according to Ethical Guidelines of the Helsinki declaration from 2000, with additional notes in 2002. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of **North-West University Potchefstroom**.

Further Information or Any Problems

If you require further information or if you have any problems concerning this project, you can contact the principal researcher or *the other* researchers responsible for this project.

Dr Leoné Malan (018-299 2438)

Signature:

Sr. Chrissie Lessing (018-299 2480)

Project Leader:

Dr Leoné Malan

PART 2

To the subject signing the consent as in part 3 of this document

You are invited to participate in a research project as described in paragraph 2 of Part 1 of this document. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project: Participation in this project is voluntary.

1. It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.
2. You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.
3. The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.
4. We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.
5. We require that you indemnify the University from any liability due to detrimental effects of treatment by University staff or students or other subjects to yourself or anybody else. We also require indemnity from liability of the University regarding any treatment to yourself or another person due to participation in this project, as explained in Part 1. Lastly it is required to abandon any claim against the University regarding treatment of yourself or another person due to participation in this project as described in Part 1.
6. If you are married, it is required that your spouse abandon any claims that he/she could have against the University regarding treatment or death of yourself due to the project explained in Part 1.

PART 3

Consent

Title of the project:

“THE SABPA STUDY (**S**YMPATHETIC ACTIVITY AND **A**MBULATORY **B**LOOD **P**RESSURE IN **A**FRICANS)”.

I, the undersigned (full names) read/listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

(Signature of the subject)

Signed at on2008/9

Witnesses

1.

2.

Signed at on2008/9



Appendix 3 - Originality Report



Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

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Submission ID: 1043523142

Literature, Overview, Aims and Hypotheses

1. INTRODUCTION

The World Health organization (WHO) established that cardiovascular disease (CVD) is the primary cause of deaths globally (1). Annually, the mortality rate due to CVD's is higher than the mortality rate for any other cause (1). In the South-African context, according to Statistics South Africa (STATS SA), CVD is the second leading cause of death (2), where ischemic heart disease ranked 8th among the leading causes of death in 2008 (3). In urban Blacks from South-Africa the prevalence of CVD keeps rising despite growing awareness and focussed healthcare (4-7) and an adverse progression of CVD risk markers are observed amongst this race (8). In addition, the Heart of Soweto Study revealed that heart failure is the leading diagnosis in this ethnic group but they were less likely to be diagnosed with coronary artery disease (CAD) (9). Also, Maian et al. (10) previously reported a higher cardiometabolic risk in urban Blacks from the THUSA study (1999-1998, North-West Province, South Africa) where higher metabolic risk was displayed when Blacks were compared to another ethnic population ten years later (the SABPA study) (11). One of the most common manifestations of CVD, is silent myocardial ischemia (SMI) (12), which can occur in a wide spectrum of individuals (13). In addition, SMI occurred in approximately 25 % to 50 % of individuals with CAD (14). CAD was a leading cause of morbidity and mortality and it was apparent that the burden in sub-Saharan Africa is progressing (4, 15). Therefore, considering SMI as an independent predictor of mortality in the detection of CAD may improve preventive treatment strategies and lessen cardiac deaths (7). SMI has also been associated with subclinical atherosclerosis (16) in a Black male cohort (17) and was enhanced by the prevalence of cardiovascular risk factors, i.e. diabetes and hypertension (8, 18). Indeed, the most common underlying cause responsible for myocardial ischemia, was

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Appendix 4 – Declaration of language editing



20 NOVEMBER 2018

I, **Ms Cecilia van der Walt**, hereby declare that I took care of the editing of the dissertation of **Ms Madelein Griffiths** titled ***Silent myocardial ischemia, cardiac troponins and target organ damage in a bi-ethnic sex cohort: the SABPA study.***

MS CECILIA VAN DER WALT

BA (*Cum Laude*)

THED (*Cum Laude*),

Plus Language editing and translation at Honours level (*Cum Laude*),

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