Vascular function, oxidative stress and inflammation in South Africans with an active-and inactive lifestyle: The SABPA study

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PREFACE

For this dissertation the article-format was followed, as this format is recommended and approved by the North-West University for postgraduate studies. The layout of this dissertation is as follows:

Chapter 1: Introduction and literature study

Chapter 2: Methodology

Chapter 3: Research article which comprises an abstract, introduction, methods, results, discussion, conclusion, and acknowledgements.

Chapter 4: Summary and conclusions.

References are provided at the end of each chapter in the Vancouver referencing style as required by the *European Journal of Preventive Cardiology* to which we will submit the manuscript. This entire MHSc is formatted according to the guidelines of the journal to ensure uniformity. Figures in this MHSc were designed by the student with Servier Medical ART (https://smart.servier.com).

AFFIRMATIONS OF AUTHORS

The contribution of each of the authors to the research was as follows:

E van Niekerk: Responsible for conducting the literature search. Assisted in data collection in research projects conducted by the Hypertension in Africa Research Team by preparing the blood and urine samples for storage. The candidate performed statistical analyses, designed, wrote, and compiled the dissertation.

Prof CMC Mels (Supervisor): Supervised the writing of the manuscript. Responsible for collection of data, reviewing the dissertation and giving recommendations. Provided guidance to obtain ethical approval and assisted in the statistical analyses.

Prof JM van Rooyen (Co-supervisor): Responsible for collection of data, reviewing the dissertation and giving recommendations. Assisted in statistical analysis and writing of the manuscript.

Dr S Botha (Co-supervisor): Responsible for collection of data, reviewing the dissertation and giving recommendations. Assisted in statistical analysis and writing of the manuscript.

STATEMENT BY THE AUTHORS

The following statement of the authors is to verify their individual contributions and involvement in this study and to grant their permission that the relevant research article may form part of this dissertation:

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SUMMARY

Motivation

Cardiovascular disease was responsible for 17.5 million deaths in 2014, which made it the main cause of mortality among non-communicable diseases. Statistics from low- and middle-income countries demonstrated that South Africa had the highest prevalence of hypertension (78%), obesity (45%), and physical inactivity (59%) among individuals who are older than 50 years, compared to countries such as China, Ghana, India, Mexico, and Russia. These figures are a cause for concern, considering that there is a dose-dependent relationship between physical inactivity and cardiovascular disease. A sedentary lifestyle may for instance cause endothelial dysfunction by means of increased oxidative stress and inflammation, as well as a subsequent decrease in nitric oxide bioavailability. Physical inactivity also contributes to increases in arterial stiffness (arteriosclerosis) and the development of atherosclerosis. Pharmacological treatments for cardiovascular disease have potential side effects and can be a financial burden, which limits the use of this treatment in low- and middle-income countries such as South Africa. Physical activity has been shown to be a cost-effective preventative, alternative, and conjoining therapy, which is associated with lower cardiovascular disease mortality, independent of other cardiovascular disease risk factors. In fact, the therapeutic effects of physical activity were shown to be as effective as drug treatment for cardiovascular disease, highlighting the necessity for research in this regard. Previous research demonstrated that high intensity physical activity is required to obtain optimal cardiovascular risk-lowering benefits. However, this physical activity prescription may be difficult to adhere to, especially in older populations. Limited research is available that explores the relationship between physical inactivity and vascular dysfunction, along with these associative factors in a South African population. Further, research on physical inactivity and cardiovascular disease is generally obtained with subjective measures such as self-reported questionnaires, therefore emphasising the need for more objective research obtained by physical activity measuring devices.

Aim

The aim of this study was to investigate the interplay of vascular function measures, including twenty-four hour blood pressure, total peripheral resistance, and Windkessel compliance, with oxidative stress, inflammation, and nitric oxide synthesis capacity markers in physically active and inactive South Africans.

Methodology

This cross-sectional study formed part of the second phase of the Sympathetic activity and Ambulatory Blood Pressure in Africans study. This phase of the study was conducted between February 2011 and May 2012, and included 359 black and white school teachers, between the ages of 25 and 65 years, from the Dr Kenneth Kaunda Educational District, North West Province, South Africa. After the exclusion criteria were met, a total of 216 black and white men and women were included. Exclusion criteria included an ear temperature >37.5°C, α- and β- blocker/psychotropic substance users, pregnant/lactating women, and individuals vaccinated/donated blood within three months prior to participation. Additional exclusion criteria for our study are participants with physical activity recordings that did not last the entire seven days, or recordings with more than 40 minutes daily lost time.

Standardised methods were used to capture data, which included physical activity measurements with a validated Actiheart® (CamNtech Ltd., Cambridge, UK), anthropometric measurements, and questionnaires. Cardiovascular measurements comprised Windkessel compliance and total peripheral resistance measured with a validated Finometer device (Finapres Medical Systems®, Amsterdam, Netherlands). Twenty-four hour blood pressure, including systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure were also measured with a validated Meditech Cardiotens CE120® apparatus (Budapest, Hungary). Biochemical analyses included markers of oxidative stress such as glutathione reductase, glutathione peroxidase, total glutathione, gamma-glutamyltransferase, and thiobarbituric acid reactive substances. Inflammatory markers such as interleukin-6, C-reactive protein, monocytes, and neutrophil/lymphocyte ratio, as well as nitric oxide synthesis capacity markers such as L-homoarginine, asymmetric dimethylarginine, and symmetric dimethylarginine were also measured.

Participants were divided into physically active (n=84) and physically inactive (n=132) groups according to the 2008 United States Physical Activity Guidelines. Means between groups were compared with the use of analyses of covariance whereas proportions were compared with Chi-square tests. Relationships of cardiovascular variables with markers of oxidative stress, inflammation and nitric oxide synthesis capacity were investigated by means of partial and multiple regression analyses.

Results and conclusions

The physically active group consisted of 84 (38.9%) participants, of whom only 3 (3.57%) participants achieved high intensity physical activity levels. The physically active group included 18.3% fewer black participants (p=0.009) than the inactive group, but sex

distribution was similar. Despite higher total energy expenditure and activity-related energy expenditure levels (both p≤0.001) in the physically active group, body mass index was higher (p=0.046) than in the physically inactive group.

Physically active participants had higher Windkessel compliance (p=0.041) and L-homoarginine (p=0.006) while gamma-glutamyltransferase was lower (p=0.034) when compared to the inactive group. Unexpectedly, thiobarbituric acid reactive substances (p=0.043) were higher in the physically active group. Both partial and multiple regression analyses revealed associations between twenty-four hour diastolic blood pressure and total glutathione (β=0.18; p=0.037), as well as L-homoarginine (β=0.21; p=0.028) in the physically active group. Additionally, in multiple regression analyses, twenty-four hour systolic blood pressure (β=0.18; p=0.04) and twenty-four hour mean arterial pressure (β=0.20; p=0.025) correlated with L-homoarginine. In both analyses, the physically inactive group showed relationships of twenty-four hour systolic blood pressure (β=0.25; p=0.001), twenty-four hour diastolic blood pressure (β=0.20; p=0.013), twenty-four hour mean arterial pressure (β=0.23; p=0.003), and twenty-four hour pulse pressure (β=0.21; p=0.012) with symmetric dimethylarginine. In further analyses, since cardiovascular variables did not associate with markers of inflammation in multiple regression analyses, we repeated the above analyses, with additional inclusion of C-reactive protein to the models. Associations remained unchanged, indicating that the results are independent of inflammation.

In conclusion, even moderate physical activity alters vascular risk, possibly by means of modifications in nitric oxide synthesis capacity. These results suggest that increased nitric oxide synthesis capacity due to physical activity may mitigate the development of cardiovascular disease in a South African population.

Key words: L-homoarginine, asymmetric dimethylarginine, symmetric dimethylarginine, oxidative stress, inflammation, physical activity.

LIST OF ABBREVIATIONS

AEE	Activity-related energy expenditure
ABPM	Ambulatory blood pressure measurement
ADMA	Asymmetric dimethylarginine
BMI	Body mass index
CVD	Cardiovascular diseases
Cwk	Windkessel compliance
CRP	C-reactive protein
DBP	Diastolic blood pressure
EDTA	Ethylenediaminetetraacetic acid
eNOS	Endothelial nitric oxide synthase
eGFR	Estimated glomerular filtration rate
GGT	Gamma-glutamyltransferase
GPx	Glutathione peroxidase
GR	Glutathione reductase
HbA1c	Glycated haemoglobin
HDL-C	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
IL-6	Interleukin-6
LDL-C	Low-density lipoprotein cholesterol
MAP	Mean arterial pressure
METs	Metabolic equivalents
NADPH	Nicotinamide adenine dinucleotide oxidase
NLR	Neutrophil/lymphocyte ratio

NO	Nitric oxide
PP	Pulse pressure
ROS	Reactive oxygen species
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure
SDMA	Symmetric dimethylarginine
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
tGSH	Total glutathione
TEE	Total energy expenditure
TNF-α	Tumour necrosis factor alpha
TPR	Total peripheral resistance
WC	Waist circumference

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INTRODUCTION AND LITERATURE STUDY

1. Introduction

Cardiovascular disease (CVD) was responsible for 17.5 million deaths in 2014, which made it the main cause of mortality among non-communicable diseases [1]. Future projections show that annual CVD mortality will increase to 22.2 million by the year 2030 [1]. A vast majority of CVD can be attributed to endothelial dysfunction [2], and contributing factors include, but are not limited to, alcohol abuse, smoking, increased blood glucose, dyslipidaemia, obesity, metabolic syndrome, and physical inactivity [3,4,5]. Lloyd-Sherlock *et al.,* [6] compared cardiovascular risk factors in low- and middle-income countries which included China, Ghana, India, Mexico, Russia, and South Africa. Data obtained during the 2007-2010 time period demonstrated that South Africa had the highest prevalence of hypertension (78%), obesity (45.1%), and physical inactivity (59.4%) among individuals who are 50 years and older [6]. Given these statistics, Lloyd-Sherlock *et al.,* recommended that dietary and physical activity awareness be given top priority in countries like South Africa [6].

Physical inactivity is a more powerful predictor of chronic disease in comparison to risk factors such as obesity, hypertension, diabetes, and hyperlipidaemia [7,8]. In effect, physical inactivity is responsible for 6-10% of deaths caused by non-communicable diseases and is one of the leading risk factors for CVD due to its deleterious effects on the vasculature [5,8]. A survey conducted by the World Health Organisation indicated that 42.2% of South African men and 51.6% of South African women do not meet the prescribed minimum recommended physical activity requirements [1]. Another study demonstrated similar findings with a 59.7% overall physical inactivity in South Africa [9]. These figures are a cause for concern, considering that there is a dose-dependent relationship between physical inactivity and CVD [10]. Previous findings from the Sympathetic activity and Ambulatory Blood Pressure in Africa (SABPA) study by Hamer *et al.* [11] supported these findings. This study concluded that hypertensive participants (blood pressure 140±17 / 93±10 mmHg) spend considerably more (10.7%) sedentary waking hours per day compared to the normotensive group (blood pressure 117±10 / 79±8 mmHg). In another study on the same South African cohort it was further demonstrated that black men had more sedentary time and less moderate physical activity time when compared to white men [12]. Socio-economic similarities in the SABPA study was ensured as all participants where teachers [12]. Physical inactivity, alongside other contributing risk factors, may therefore partially explain why black men have a higher prevalence of hypertension when compared to white men [12].

It was further concluded that vascular disease strongly correlated with increased body mass index [13,14]. These findings are supported by the fact that adults with higher physical activity levels have lower serum cholesterol, systolic blood pressure (SBP) and body mass

index compared to their counterparts who had lower physical activity levels [10]. Additionally, it was indicated that habitual physical activity improved endothelial function in obese and overweight participants, even in the absence of changes in body composition [5]. This suggests that physical activity may exclusively be considered as a general determinant of vascular health, independent of alterations in adipose tissue [5].

Physical inactivity can increase levels of oxidative stress and inflammation, as well as decrease nitric oxide synthesis capacity, which all play an essential role in the development of vascular disease [15]. A sedentary lifestyle is further associated with cardiometabolic risk factors which are known to worsen oxidative stress and inflammation [16,17]. These vascular changes can result in endothelial dysfunction and increased stiffness of the arteries [5,18]. The following literature will underpin the effects of physical activity on different aspects related to the vasculature, including vascular function, nitric oxide synthesis capacity, oxidative stress, and inflammation.

2. Vascular function/dysfunction

Endothelial dysfunction is characterised by an imbalance between vasodilator and vasoconstrictor substances, caused by either increased levels of vasoconstrictors or decreased levels of vasodilators [19]. Endothelial dysfunction is also associated with a bi-directional relationship between oxidative stress and inflammation [20,21]. As illustrated in **Figure 1.1**, increased levels of oxidative stress and inflammation may decrease the bioavailability of nitric oxide, which is central to endothelial dysfunction [22,23]. The bioavailability of nitric oxide plays an essential role in endothelial health by functioning as a vasodilator [23]. Nitric oxide facilitates vasodilation by serving as an intracellular messenger for guanylate cyclase stimulation, and subsequently leads to relaxation of smooth muscle cells in the blood vessels [24]. In addition, nitric oxide has several other protective functions in the vasculature such as the prevention of leukocyte and platelet adhesion [23]. Nitric oxide thus has antihypertensive, antithrombotic, and anti-atherosclerotic properties [23,25].

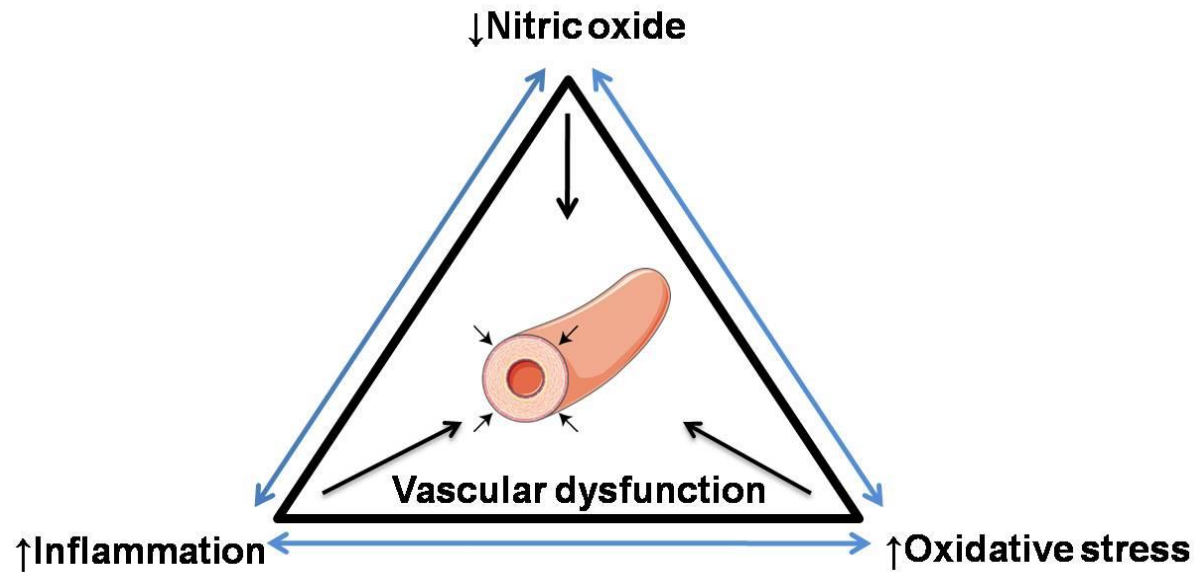

Figure 1.1: Vascular triad of oxidative stress, inflammation, and vascular dysfunction

Nitric oxide is produced by calcium dependent endothelial nitric oxide synthase (eNOS), located on the endothelial cell membrane [25]. Endothelial nitric oxide synthase makes use of the amino acid, L-arginine, as a substrate for nitric oxide production [25]. Decreased availability of L-arginine may therefore play a role in endothelial dysfunction [26]. L-homoarginine, an analogue of L-arginine, may serve as an arginase inhibitor [27]. The arginase enzyme functions as a catalyst to convert arginine into ornithine and urea [27]. Homoarginine-mediated arginase inhibition may result in increased levels of L-arginine, and subsequently increased nitric oxide synthesis [27], which favours vasodilation [24]. However, this is more likely to occur at elevated levels and it has been suggested that L-homoarginine's function as a substrate for nitric oxide synthase or its ability to facilitate nitrate excretion may be responsible for the cardioprotective effects of L-homoarginine [27]. Conversely, it was also proposed that L-homoarginine is a weak substrate for nitric oxide synthase and thus competes with L-arginine as L-homoarginine has a 10 to 20-fold lower binding affinity compared to L-arginine [28]. Despite L-homoarginine's weak binding affinity, it may have a more prolonged effect than L-arginine. Experimental studies done in mouse models showed eight hours of nitric oxide synthase activity after L-homoarginine supplementation, and only four hours of nitric oxide synthase activity after arginine supplementation [28].

Other factors inhibiting nitric oxide production include endogenous eNOS inhibitors, symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) [26,29]. Asymmetric dimethylarginine competes with L-arginine for binding to eNOS with consequent inhibition of nitric oxide synthesis [24], whereas SDMA indirectly inhibits nitric oxide

synthesis by decreasing L-arginine transport from plasma into endothelial cells [29,30]. Symmetric dimethylarginine levels are also an indication of renal function, which affects cardiovascular health [29]. The kidney plays a role in cardiovascular function as it contains an aminotransferase that catalyses L-homoarginine synthesis from the amino acid, L-lysine [28]. Research therefore shows a correlation between L-homoarginine and kidney function measured from creatinine and estimated glomerular filtration rate [28]. Ultimately, reduced nitric oxide bioavailability in the vasculature is not only associated with vasoconstriction, but also with leukocyte adhesion, platelet adhesion and aggregation, as well as proliferation of vascular smooth muscle cells [22].

Arterial stiffness is a cardiovascular risk factor [31], characterised by decreased compliance and increased resistance of the vasculature, which is caused by functional changes in vascular smooth muscle tone and structural changes in the vascular wall [32,33,34]. Increased arterial stiffness is age dependent and accompanied by increased oxidative stress and a pro-inflammatory state [20,23,32,35,36]. However, physical inactivity and dietary habits have been shown to be a larger predictor of CVD than age [36]. Endothelial dysfunction may be the initial step in the development of arteriosclerosis [20]. Ultimately, vascular remodelling due to endothelial dysfunction may increase the development of arteriosclerosis and arterial stiffness, which is well known to accompany the ageing process [34].

2.1 Vascular function and physical activity

Improved vascular function and smooth muscle tone, induced by physical activity, occur due to decreased oxidative stress and inflammation, along with increased nitric oxide bioavailability [5,32,33,37]. Higher levels of physical activity induce structural improvements evidenced by decreased intima-media thickness, decreased collagen deposition, and decreased fragmentation of elastin [5,37]. It is implied that increased nitric oxide synthesis capacity is primarily responsible for the advantageous effects which physical activity has on the vasculature [5]. As shown in **Figure 1.2**, the beneficial effects of physical activity on nitric oxide synthesis capacity and vascular function may in part be explained by the effects of increased shear stress during physical activity [24]. Increased shear stress during physical activity can activate calcium dependent eNOS to produce nitric oxide from L-arginine [24]. Physical activity also improves L-arginine transport from the plasma into the endothelial cells, thereby increasing L-arginine availability for nitric oxide synthesis [38].

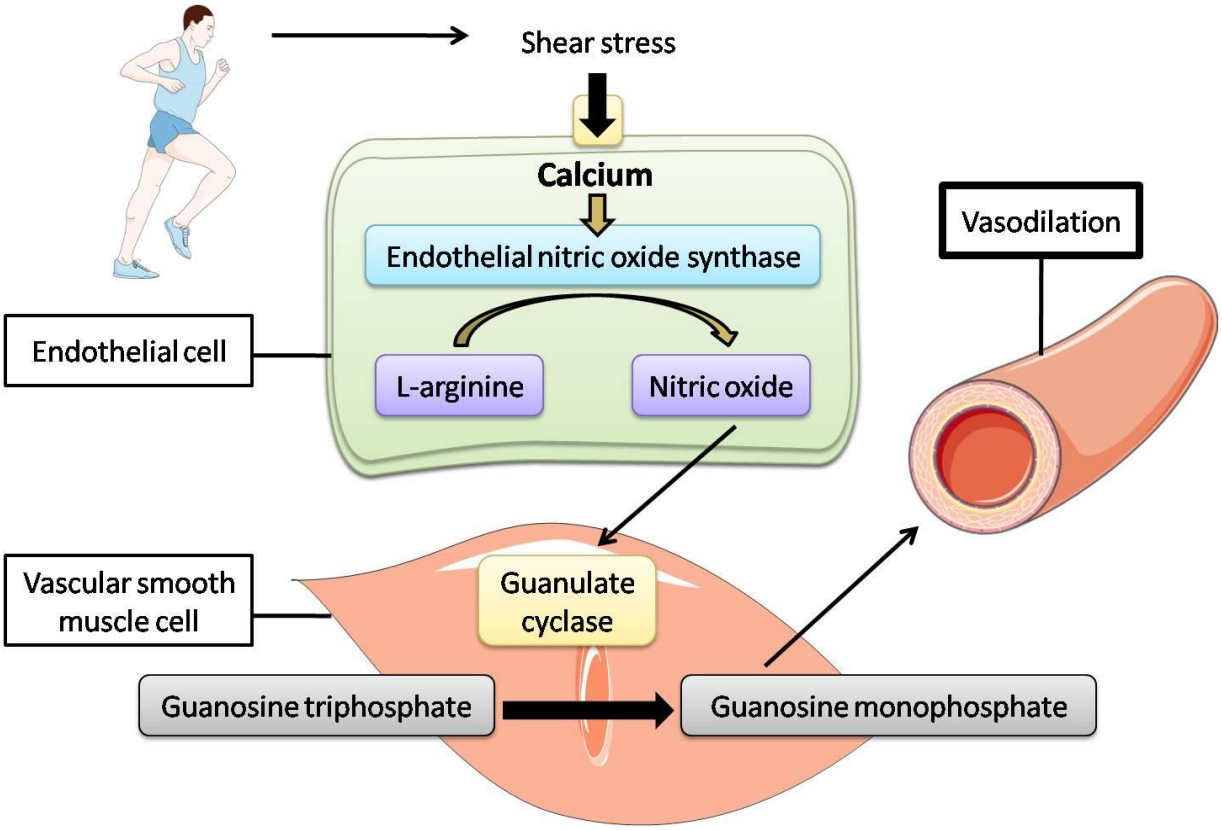

Figure 1.2: Mechanism by which physical activity increases vasodilation

In addition to increasing L-arginine, physical activity further increases nitric oxide bioavailability by lowering SDMA and ADMA [26,29]. The production of ADMA and SDMA is increased by oxidative stress and inflammation [39]. Therefore, habitual physical activity decreases ADMA and SDMA levels resulting from decreased oxidative stress and inflammation [39]. In addition, dimethylarginine dimethylamino-hydrolase is known to break down ADMA, and physical activity enhances the messenger ribonucleic acid gene expression thereof [39]. Research revealed these benefits of physical activity in postmenopausal women where it resulted in decreased ADMA, which was also inversely correlated with arterial compliance [39].

Physical activity is well known to reduce arterial stiffness and improve endothelial function, even in individuals with a family history of hypertension [5]. Research indicated that master endurance athletes have enhanced vascular function and decreased arterial stiffness characteristics, including higher vascular compliance, lower total peripheral resistance (TPR) and lower pulse pressure, compared to their sedentary peers [40]. This is supported by another study which found that endurance-trained men had 70%-120% higher venous compliance when compared to their physically inactive counterparts [26].

In contrast, physically inactive individuals have increased levels of oxidative stress and inflammation with a concomitant decrease in nitric oxide, which leads to endothelial dysfunction, and ultimately vasoconstriction [32,41]. Additionally, ADMA and SDMA both increase due to oxidative stress and inflammation [42]. Oxidative stress, in particular, can activate the enzyme arginine methyltransferase-2 which is needed for ADMA and SDMA synthesis [43]. It seems feasible to propose that inadequate nitric oxide bioavailability may be central to this triad of oxidative stress, inflammation, and endothelial dysfunction which takes place in individuals who are physically inactive [23,44].

Physical inactivity is associated with increased arterial stiffness, which is caused and aggravated by endothelial dysfunction [20,23,32]. Research revealed that physical inactivity among ageing individuals results in lower venous compliance [26]. Physical inactivity causes endothelial damage due to oxidative stress and inflammation, and therefore cell adhesion molecules emerge [22]. Consequently, monocytes are attracted and transformed into macrophages [22]. Macrophages become foam cells as they engulf oxidised low-density lipoprotein cholesterol, stimulating the proliferation of smooth muscle cells, and eventually lead to the formation of atherosclerotic plaque [23]. The foam cells additionally secrete inflammatory cytokines, which leads to a further increase in reactive oxygen species production [44]. Reactive oxygen species therefore both initiates the inflammatory process and is also a consequence thereof, causing a downward spiral of vascular dysfunction to occur [44]. In the long term, arterial stiffness associates with increased morbidity and mortality in physically inactive individuals [45].

3. Oxidative stress

Free radicals are molecules with the ability to remove electrons from other substrates and thereby generate reactive species [35]. Free radicals play a central role as regulatory mediators in cell signalling and are required in moderation to upregulate endogenous anti-oxidants as a result of physical activity [46].However, at high levels reactive oxygen species may have detrimental effects [46]. Superoxide is the primary source of reactive oxygen species and plays a role in the formation of secondary reactive oxygen species, such as hydroxyl radicals and hydrogen peroxide [23,35,47]. Sources of superoxide production are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, uncoupled eNOS, xanthine oxidases, cyclooxygenases, lipoxygenases, cytochrome P450s, and oxidative phosphorylation [2,35]. Further to this, superoxide has a propensity to react with nitric oxide and form peroxynitrite [23]. Oxidative stress therefore plays a key role in vascular dysfunction [32]. Many factors are associated with increased oxidative stress such as

increased adipose tissue, hyperglycaemia, dyslipidaemia, inflammation, hypertension, and physical inactivity [18,26,35,48].

Anti-oxidant substances are capable of mitigating the oxidation of other substrates by donating electrons [35]. **Figure 1.3** illustrates how the endogenous anti-oxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), are used to scavenge reactive species and lower oxidative stress [15,35,49]. Superoxide dismutase is used to catalyse superoxide and produce hydrogen peroxide [20]. In addition, hydrogen peroxide can be neutralised by catalase or glutathione peroxidase to form water and oxygen [35,25]. An imbalance, which favours pro-oxidants over anti-oxidants, results in oxidative stress and leads to disruptive cell signalling and molecular damage [35].

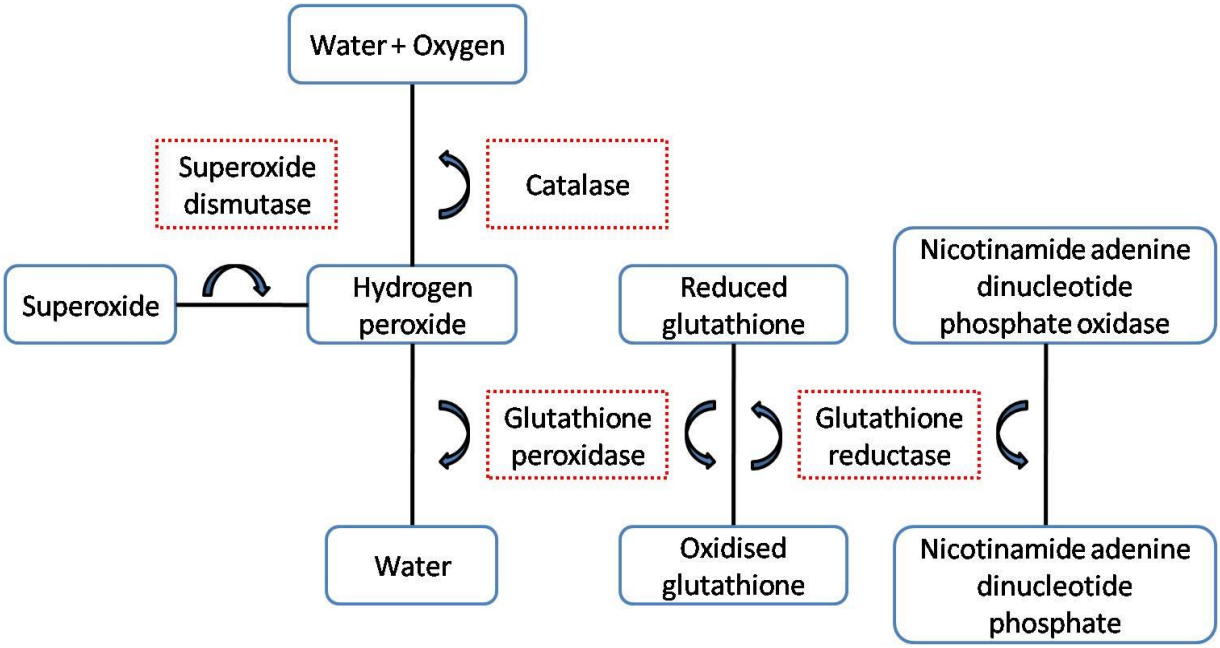

Figure 1.3: The mechanism by which endogenous anti-oxidant enzymes inactivate reactive oxygen species

3.1 Oxidative stress and physical activity

According to the Hormesis theory illustrated in **Figure 1.4**, increased production of reactive oxygen species due to physical activity seems to be necessary to up-regulate the endogenous anti-oxidant system and alter the body's redox system into a more reduced state [46]. An acute session of exercise increases reactive oxygen species by means of the mitochondrial electron transport chain and uncoupled eNOS [15]. The exercise-induced production of reactive oxygen species then serves as a signal to induce the up-regulation of the enzymatic antioxidant defence system [15] by stimulating the expression of genes which

regulate anti-oxidant enzymes [49]. Instead of exercise, even a minimum weekly session of moderate to high intensity physical activity has been shown to be beneficial to cardiovascular disease [5]. However, other studies suggested that high intensity physical activity is needed to up-regulate anti-oxidant enzymes [5,22]. Long term physical activity can therefore mitigate the production of reactive species and enhance the body's endogenous anti-oxidant defence system [48]. In addition, well-trained athletes are more resistant to acute oxidative stress from strenuous exercise than sedentary individuals [46]. Various studies supported the fact that habitual physical activity increases vascular expression of these anti-oxidant enzymes and decreases reactive oxygen species-producing enzymes such as NADPH oxidase [15,26]. Decreased oxidative stress from habitual physical activity may be partly responsible for improvements in endothelial function and arterial stiffness [32].

Physically inactive individuals also display lower levels of endogenous anti-oxidant enzymes, such as SOD, GPx, CAT, and GR when compared to physically active individuals [50]. In addition, physical inactivity is characterised by oxidative stress which causes damage to the vascular endothelium [5,32]. Physical inactivity increases oxidative stress by means of increased NADPH oxidase activity [51], a significant source of superoxide [5]. In turn, the overproduction of superoxide can result in endothelial dysfunction, as nitric oxide is scavenged by superoxide [5].

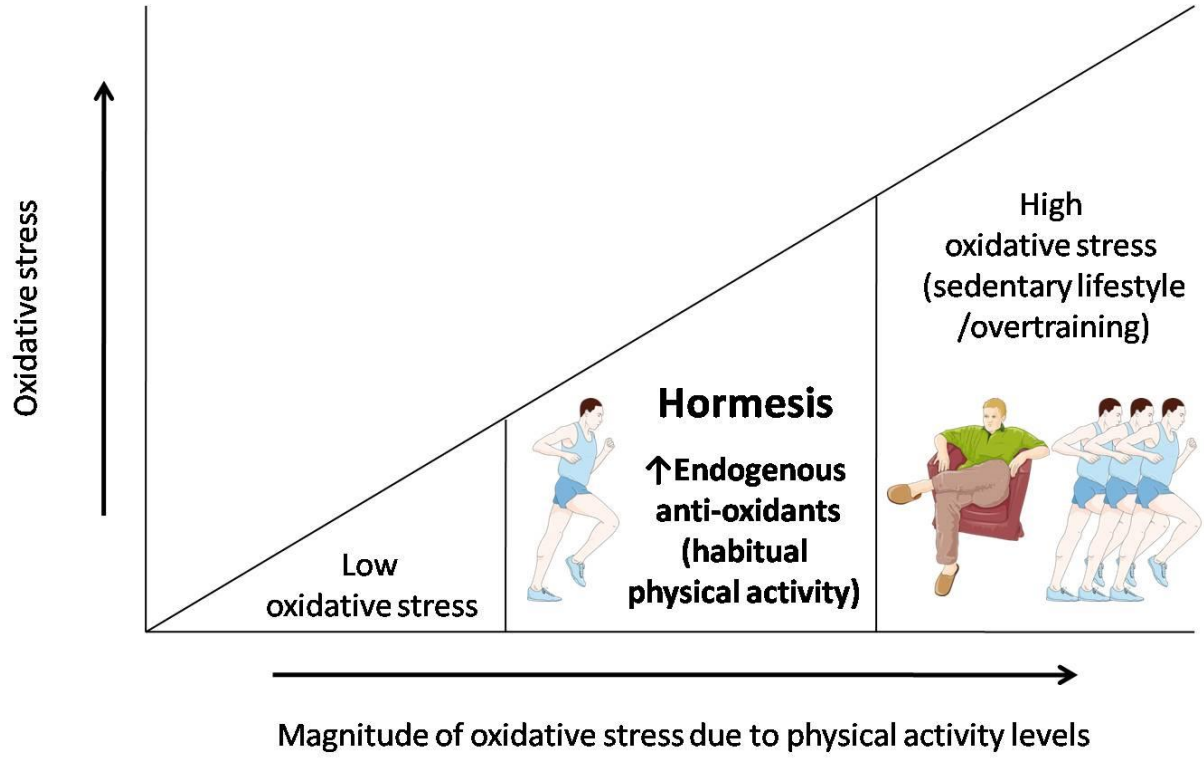

Figure 1.4: Increased endogenous anti-oxidants from optimal physical activity, as illustrated by means of the Hormesis theory

4. Inflammation

Inflammation is known to play a role in the body's defence mechanism against infection or tissue damage [23]. Vascular inflammation results from factors such as increased reactive oxygen species, triglycerides, low-density lipoprotein cholesterol, hyperglycaemia, and cotinine from smoking, which all have deleterious effects on the endothelium and may contribute to atherosclerotic plaque formation [44,52,53]. Inflammation can predict the development of a vast array of chronic diseases [54]. As such, endothelial health is associated with low levels of inflammation [22].

4.1 Inflammation and physical activity

Depending on the circumstances, exercise can worsen or attenuate inflammation [55]. An acute session of exercise may cause muscle and soft tissue damage, which activates an inflammatory response and result in increased levels of interleukin-6 (IL-6) [8,44,55]. However, this type of inflammatory response, caused by an acute session of exercise, diminishes with long term physical activity as the muscle and soft tissue adapt [55,22]. Although not as strenuous as exercise, regular physical activity can lead to decreased systemic inflammation which is associated with improved vascular health [55,5]. The effect of physical activity on systemic inflammation is often measured by levels of cytokines such as C-reactive protein (CRP), IL-6, and tumour necrosis factor-alpha. Habitual physical activity decreases CRP and various pro-inflammatory cytokines such as interleukin-1, IL-6, tumour necrosis factor-alpha, along with inflammatory cells such as lymphocytes, monocytes, and neutrophils [8,44,56,57]. Furthermore, an inverse relationship between CRP and physical activity is evident, independent of other cardiovascular disease risk factors such as obesity or insulin resistance [55]. Another manner, by which physical activity lowers inflammation, is by lowering oxidative stress [55] and atherogenic activity in the vasculature [10]. Additionally, age progression has been linked to increased inflammation and cardiovascular disease [55]. Physical activity may decrease inflammation in ageing individuals without the potential side effects of pharmacological treatment [55]. However, as with oxidative stress, research suggests that high intensity physical activity is needed to decrease inflammation [5,22].

In contrast to physically active individuals, more inflammatory mediators, such as IL-6 and tumour necrosis factor alpha, are being released in physically inactive individuals [23,41]. Chronically increased systemic inflammation in physically inactive individuals ultimately

contributes to the development of vascular pathology [23,41]. Physical inactivity may also play a role in low-grade inflammation by secreting tumour necrosis factor-alpha, which in turn increases reactive oxygen species production through activation of NADPH oxidase [58].

5. Other cardiovascular disease risk factors and physical inactivity

Physical inactivity may increase the prevalence of cardiometabolic risk factors such as hyperglycaemia, obesity, dyslipidaemia [5], and gamma-glutamyltransferase [59].These risk factors are associated with increased oxidative stress and inflammation, as well as reduced nitric oxide [5,47]. In addition to vasoconstriction from decreased nitric oxide, cardiometabolic risk factors may play a role in the development of endothelial dysfunction and arterial stiffness [5].

5.1 Hyperglycaemia and physical inactivity

Vascular changes from type 2 diabetes mellitus may result in adverse changes within the vascular wall, such as increased intima-media thickness, which is known to exacerbate arterial stiffness [32]. It is evident from the literature that endothelial exposure to high levels of glucose is also accompanied by reduced nitric oxide availability [5]. Hyperglycaemia has shown to promote the uncoupling of eNOS and endothelial NADPH oxidase [47], which is a major source of the nitric oxide scavenger, superoxide [5]. Intracellular hyperglycaemic conditions are further known to reduce the anti-oxidant substance, glutathione [47].

5.2 Obesity and physical inactivity

Physical inactivity may further contribute to an increase in adipose tissue and the development of obesity, which is also associated with oxidative stress, inflammation, and consequently endothelial dysfunction [5,17,18]. Endothelial dysfunction in individuals with obesity may be a consequence of altered signalling between adipose tissue and the endothelium [5]. Adipose tissue operates as a metabolic and endocrine organ as it produces pro-inflammatory cytokines, chemokines, and hormones [5]. Damage caused to the endothelial cells by increased adiposity may result in the release of cell adhesion molecules, and subsequently atherosclerosis [22]. A positive correlation therefore occurs between body mass index and pro-inflammatory markers such as IL-6 and CRP [60]. Pro-inflammatory cytokines released by adipocytes ultimately result in decreased nitric oxide synthesis capacity [5]. Moreover, increased adipose tissue and impaired lipolysis also result in NADPH oxidase activation which leads to excess reactive oxygen species production [5].

5.3 Dyslipidaemia and physical inactivity

Endothelial exposure to high levels of lipids is accompanied by increased oxidative stress and reduced nitric oxide availability [5,47]. Dyslipidaemia, due to physical inactivity [17] have shown to play a role in NADPH oxidase expression and thus increased reactive oxygen species production [47]. Reactive oxygen species from physical inactivity may oxidise low-density lipoprotein cholesterol by means of lipid peroxidation and result in an increased production of the by-product thiobarbituric acid reactive substances (TBARS) [25]. Physical activity has been shown to reduce TBARS and this may be a result of nitric oxide's ability to inhibit lipid peroxidation [25]. In turn, oxidised low-density lipoprotein cholesterol may increase NADPH oxidase activity and further aggravate superoxide formation [22,47].

5.4 Gamma-glutamyltransferase and physical inactivity

Gamma-glutamyltransferase (GGT) is not only related to alcohol use and liver function, but is also a biomarker for oxidative stress and glutathione metabolism [59]. This independent risk marker of cardiovascular disease inversely correlates with physical activity [59]. Also, GGT positively correlates with cardiometabolic risk factors such as high triglycerides, increased body mass index, high blood pressure, low-density lipoprotein cholesterol, and fasting blood glucose [3,59]. Correlations between GGT and oxidative stress are evident in the ability of GGT to degrade glutathione, an important anti-oxidant [59]. However, GGT is required to hydrolyse extracellular glutathione into glutamate and cysteinylglycine-dipeptide, which are reused for intracellular glutathione synthesis [59].

6. Motivation

In 2011, it was estimated that South Africa had 3.3 million diagnosed hypertensive cases, of which 2.1 million received treatment [61]. Hypertension prevalence is the highest in low- and middle-income countries such as South Africa, where individuals often remain uninformed, undiagnosed, and untreated [62]. In fact, about 80% of hypertensive cases occur in low- and middle-income countries, with an incidence of just 20% in high-income countries [63]. Mortality caused by CVD has been significantly reduced in most high-income countries due to governmental policies which assist with the implementation of a healthier lifestyle, along with the provision of reasonable health care [1].

Physical inactivity is an independent risk factor for cardiovascular disease and may play a role in the impairment of vascular function, which partially explains the dose-dependent relationship among physical inactivity and cardiovascular disease incidence [10]. A sedentary lifestyle may cause endothelial dysfunction by means of increased oxidative stress and inflammation, as well as a subsequent decrease in nitric oxide bioavailability [15]. Additionally, physical inactivity increases cardiometabolic risk factors which may further contribute to endothelial dysfunction [5].

Pharmacological treatments for cardiovascular disease have potential side effects and can be a financial burden, which limits the use of treatment [54]. Furthermore, the 2011-2012 South African National Health and Nutrition Examination Survey study found that 13.5% of hypertensive individuals who received treatment still had uncontrolled blood pressure [64]. A multi-disciplinary approach may be more effective for these individuals where treatment strategies are combined. Such an approach has been proven to be effective atlowering blood pressure in other diseases such as type 2 diabetes mellitus, which included dietary changes, increased physical activity and psychological counselling along with medication use [65].On the other hand, habitual physical activity was proven to be as effective as pharmacological treatments for some CVD [66], even in the absence of weight loss [7]. It is suggested that shear stress from physical activity leads to higher levels of nitric oxide synthesis [24], which is mainly responsible for the beneficial effects of physical activity on the vasculature [5]. This is due to the ability of nitric oxide to improve vasodilation by means of vascular smooth muscle relaxation [24]. Physical activity also increases nitric oxide bioavailability by lowering the eNOS inhibitors, ADMA and SDMA [26,29]. The production of these eNOS inhibitors are increased by oxidative stress and inflammation [39], which are also lowered by physical activity [5,15]. Lower levels of oxidative stress [5] and inflammation [22,23] from physical activity is further important as both are nitric oxide scavengers. Strategies proposed to increase physical activity are therefore one of the most cost-effective ways to improve cardiovascular health and related co-morbidities [61].

7. Aim

The aim of this study was to investigate the interplay of vascular function with oxidative stress, inflammation, and nitric oxide synthesis capacity in physically active and inactive South Africans.

8. Objectives

Our objectives were to:

- Explore the difference between markers of vascular function (Windkessel compliance (Cwk), TPR, twenty-four hour (24h)SBP, 24h diastolic blood pressure (DBP), 24h mean arterial pressure (MAP), and 24h pulse pressure (PP)), oxidative stress (total glutathione (tGSH), GPx, GR, and TBARS), inflammation (CRP, IL-6, neutrophil-lymphocyte ratio (NLR), and monocytes), and nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) between physically active and inactive South Africans; and to

- Investigate whether markers of vascular function (Cwk, TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP), are associated with markers of oxidative stress (tGSH, GPx, GR, and TBARS), inflammation (CRP, IL-6, NLR, and monocytes), and/or nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) in physically active and inactive South Africans.

9. Hypotheses

From the literature we hypothesised that:

- Physically inactive South Africans will display worse vascular function (lower Cwk, higher TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP), oxidative stress (higher TBARS and lower tGSH, GPx, and GR), inflammation (higher CRP, IL-6, neutrophil-lymphocyte ratio, and monocytes), and nitric oxide synthesis capacity (higher L-homoarginine, as well as lower ADMA and SDMA) profiles when compared to physically active individuals.

- Markers of vascular function (Cwk, TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP) will adversely associate with markers of oxidative stress (TBARS, tGSH, GPx, and GR), inflammation (CRP, IL-6, NLR, and monocytes), and nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) in the physically inactive group. Furthermore, markers of vascular function (Cwk, TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP) will beneficially associate with markers of oxidative stress (TBARS, tGSH, GPx, and GR), inflammation (CRP, IL-6, NLR, and monocytes), and nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) in the physically active group.

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METHODOLOGY

1. The Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study

The SABPA study originally aimed to investigate the role of a hyperactive sympathetic nervous system on cardiometabolic disease burden in black and white South African men and women [1].

The SABPA study comprised of two phases, where the first phase was undertaken between February 2008 and May 2009, while the second phase was undertaken three years later between February 2011 and May 2012. Data were collected over the same time every year to control for seasonal changes. This is particularly important to our study as seasonal changes may affect physical activity levels [2], coagulation markers, inflammatory biomarkers, and inflammatory cells [3]. However, controlling for seasonal changes limited participants to only 200 individuals per year. School teachers were used to ensure similar socio-economic status. This cross-sectional sub-study formed part of the second phase of the SABPA study. Data from the second phase were used as it contains additional measurements such as twenty-four hour diet, seven-day objective physical activity, and differential blood cell count. The second phase of the study included 359 black and white school teachers, between the ages of 25 and 65 years. Participants were recruited from the Dr Kenneth Kaunda Educational District, North West Province, South Africa, as indicated in **Figure 2.1**.

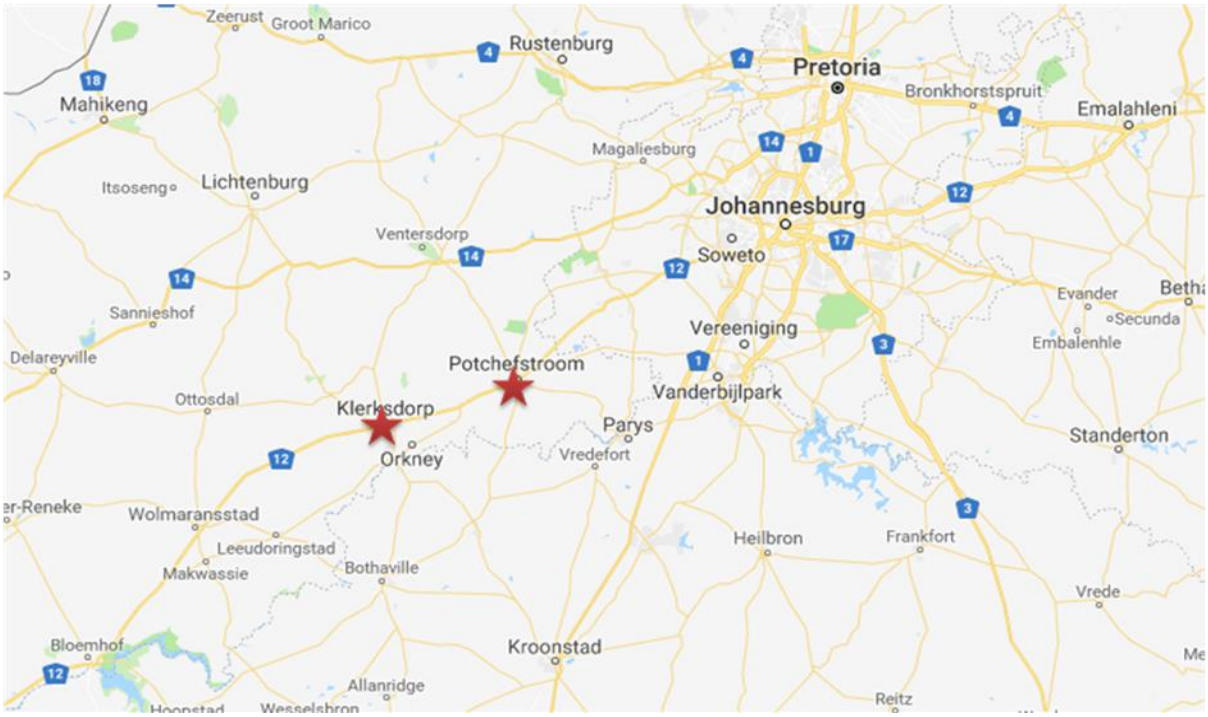

Figure 2.1: Areas involved in this study from the North-West Province, South Africa are marked with a star.

2. Recruitment

Recruitment was done over a three-month period before the clinical assessments were done. Headmasters of 43 schools from the designated area were informed about the SABPA study to gain their support and cooperation. Altogether, 2170 teachers were invited to participate in the first phase of the SABPA study and they were recruited by the principal investigator and a fieldworker. The participants were informed about the purpose and procedure of the study before recruitment commenced and provision was made for participants to be informed in their home language. Two months post-recruitment, the selected participants voluntarily signed an informed consent form and were informed that they could withdraw at any given time.

The screening process of the volunteered participants was assembled by a registered nurse to assess the possibility of participation by applying the inclusion and exclusion criteria. Exclusion criteria included individuals with a tympanum temperature higher than 37.5°C, those who used α and β blockers or psychotropic substances, pregnant or lactating women, and individuals who have been vaccinated or donated blood within three months prior to participation. The aforementioned factors were excluded because of their direct and indirect influence on vascular markers. Human immunodeficiency virus infected patients, and the use of anti-hypertensive, anti-diabetic, statin and anti-oxidant medication were indicated as these factors may also influence the markers analysed in this study; for e.g. inflammation, vascular function, glucose and cholesterol profiles, and oxidative stress status are some of the markers that may be influenced.

Additionally, all participants were excluded with physical activity recordings that did not last for the entire seven days, or recordings with more than 40 minutes daily lost time (n=143). As indicated in **Figure 2.2**, the total sample (n=216) for this study therefore included black (n=109) and white (n=107) men (n=104) and women (n=112). Race and sex were equally distributed in this study. Participants were divided into physically active and -inactive groups by means of metabolic equivalents (METs) categories: physically active (n=84) and physically inactive (n=132) groups, as according to the 2008 United States Physical Activity Guidelines [4].

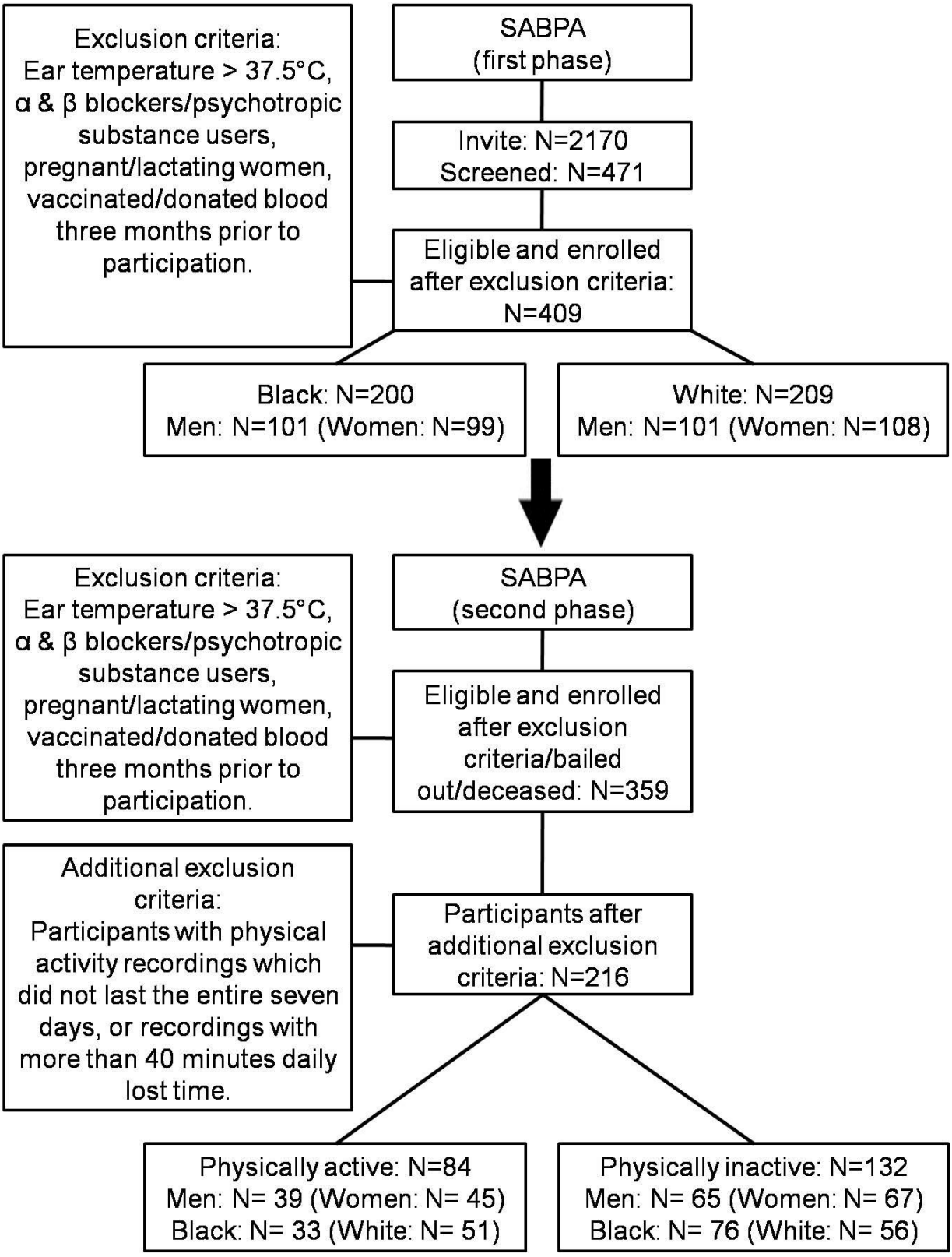

Figure 2.2: Flow diagram of SABPA Phase I and SABPA Phase II with additional exclusion criteria for this study

Details regarding the study protocol were discussed in English or in the home language of participants who met the inclusion criteria. This discussion included the purpose, procedure, and expectations from each of the participants such as incentives, accommodation, stressor application, resting blood pressure, fasting urine and blood samples required, as well as the importance of correct sampling methods. Thereafter, participants were offered an opportunity to ask questions relevant to the study.

The SABPA study met the requirements as stated by the Helsinki Declaration of 1975 (revised in 2008) for investigation on human participants. Further to this, approval for the overarching SABPA study was granted by the North-West University's Health Research Ethics committee (NWU-00036-07-S6), together with endorsements from the North West Department of Education, and the South African Democratic Teachers' Union. Approval was also granted by the North-West University's Health Research Ethics committee (NWU-00106-17-S1) for completion of this MHSc (see Appendix A).

3. Data collection

For the second phase of the SABPA study, data were collected from four participants per weekday from February 2011 to May 2012, with the clinical assessments performed over a two-day period. On the first day, at 07h00, a Meditech Cardiotens CE120® apparatus (Budapest, Hungary) which was validated by the British Hypertension Society, was fitted to each participant at their schools to obtain an ambulatory blood pressure measurement. Participants resumed their normal daily activities for the rest of the day and were transported to the North-West University at approximately 15h00 for clinical assessments. An introduction to the experimental set-up was given with the aim to avoid white coat effect, mitigate overall anticipation stress, and maintain high quality data [5]. Feedback reports from the participants indicated that they were comfortable with the experimental set-up. A well-controlled environment was provided for an overnight stay at the Metabolic Unit Research Facility of the North-West University where they had a standardised dinner and were asked to avoid any beverages after 22h00. On the second day, participants were woken at 07h00 whereafter the Meditech Cardiotens CE120® apparatus was disconnected and the anthropometric measurements were obtained. This was followed by measurements of total peripheral resistance and Windkessel compliance with a validated Finometer device (Finapres Medical Systems®, Amsterdam, Netherlands) [6,7]. Afterwards, a resting blood sample of 65 ml was obtained by a registered nurse from the antebrachial vein of the dominant arm using a winged infusion set after which it was immediately sent to the laboratory for preparation and storage. Samples were prepared according to standardised methods and stored in a laboratory bio-freezer at -80ºC until analysis. The participants then

showered and the Actiheart® (CamNtech Ltd., Cambridge, UK) device was fitted for the seven-day physical activity measurement. Each participant received four extra electrodes to ensure that the Actiheart® was refitted as soon as possible, should it become disconnected during the seven days. Participants were instructed to continue with their habitual daily activities wearing the monitor at all times whilst awake and asleep. The Actiheart® was collected from each participant at the various schools on the eighth day and the data downloaded onto the computer for storage, viewing, and analysis.

4. Physical activity measures

Habitual physical activity was measured over a period of seven consecutive days with a validated Actiheart®, chest-worn heart-rate and accelerometer device [8,9]. The accelerometer device was used to obtain an objective measure of physical activity which is a better measure than self-report physical activity [10]. Self-reported questionnaire data are often associated with over- or under reporting [11]. Over-reporting often occurs due to social desirability, whereas under-reporting is possibly a result of not including activities which last less than ten minutes [11]. Other measures such as pedometers and accelerometers are unable to obtain upper extremity activities as it is worn on the waist, or activities such as swimming as it is not waterproof [11,12]. Despite the fact that the Actiheart® device is waterproof, in the combined accelerometer and heart rate monitors, other disadvantages outweigh its benefits [11,12]. Barreira *et al.* compared energy expenditure, movement counts, and heart-rate measurements from the Actiheart with that of an AEI Moxus Metabolic Cart, Actigraph accelerometer, Polar heart-rate monitor, and electrocardiogram, respectively [8]. This device was found to be valid and reliable for the accurate estimation of energy expenditure of humans at rest and at low, moderate, and vigorous activities [8,9]. Even more so, the Actiheart® has been validated for habitual physical activity measurement in sub-Saharan Africans [13].

A step test was not performed for calibration, as the participants included were at cardiovascular risk. A biokineticist questioned each participant to assess their physical activity levels and accordingly chose an appropriate activity level on the Actiheart programme. The Actiheart® software (Version 4.0.116) was used to capture and analyse data. Sleep time was distinguished from awake time by means of heart rate and METs. One MET was regarded as being asleep along with a decrease in heart rate and zero movement. The end of sleep was established by an increase of heart rate by 10 to 20 beats per minute along with an increase in METs and movement. The software calculated the time spent in different METs categories which consisted of daily awake sedentary time ($\leqslant$1.5 METs), daily awake light-activity time (1.5 to 2.99 METs), daily awake moderate-activity time (3 to <6

METs), and daily awake vigorous activity time (⩾6 METs) as demonstrated in **Figure 2.3** [14].

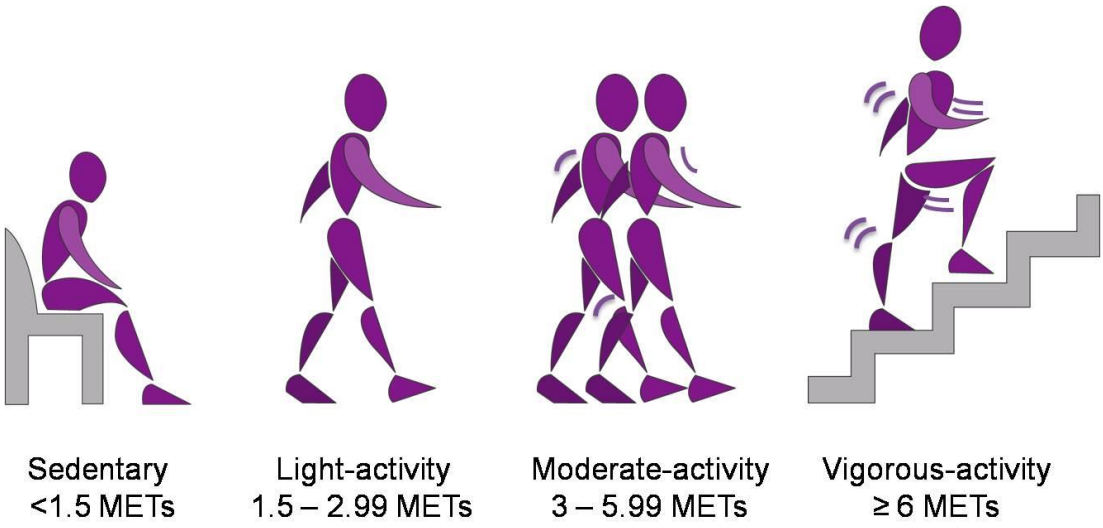

Figure 2.3: Illustration of METs categories

We classified physical activity as ≥150 minutes per week and physical inactivity as <150 minutes per week of the following: moderate intensity physical activity at 3 to <6 METs/<75minutes, vigorous intensity physical activity at ≥6 METs, or a combination which results in equivalent energy expenditure, as recommended by the 2008 United States Physical Activity Guidelines [4]. The heart rate and accelerometer measured the participant's physical activity and energy expenditure in kilocalories (kcal). Total energy expenditure is composed of resting energy expenditure, dietary-induced energy expenditure, and activity-induced energy expenditure.

5. Questionnaires

Participants completed a Sociodemographic questionnaire that was validated for the specific population, which contained questions regarding their lifestyle and demographic factors such as sex, age, race, medication use, alcohol consumption, and smoking habits (see Appendix B).

6. Anthropometric measurements

Standardised methods of the International Society for Advancement of Kinanthropometry were used for anthropometric measurements [15]. Measurements were obtained in triplicate and were also obtained in a private room by an accredited anthropometrist [15]. Intra- and inter-observer variability was less than 10%. Calibrated instruments were used to measure body height (Invicta Stadiometer IP 1465, Leicester, UK) and weight (calibrated Precision Health Scale, A&D Company, Japan). Subsequently, body mass index was calculated [16].

A calibrated instrument was also used to measure waist circumference to the nearest 0.1 cm (Holtain non-stretchable metal tape, A&D Company, Tokyo, Japan). Waist circumference was included as increased muscle mass from physical activity can increase body mass index and give a misperception regarding the participant's adipose tissue. In addition, specifically abdominal adipose tissue has a substantial effect on oxidative stress and inflammation [17].

7. Cardiovascular measurements

Ambulatory blood pressure measurements were obtained with a Meditech Cardiotens CE120® device (Budapest, Hungary) which was validated by the British Hypertension Society. The device was programmed to take measurements at 30-minute intervals between 07h00 and 22h00hours during the day and at one-hour intervals between 22h00 and 06h00 hours during the night [18]. An appropriate-sized cuff was fitted on the non-dominant arm of the participants at the same time every day. Data were analysed with CardioVisions 1.15.2 Personal Edition software (Meditech®, Budapest, Hungary). Participants were asked to make use of their ambulatory diary cards to record abnormalities such as headaches, visual disturbances, fainting, nausea, physical activity, and emotional stress.

The validated Finometer device (Finapres Medical Systems®, Amsterdam, Netherlands) was connected to the left upper arm and middle finger, respectively, with the participant in the semi-Fowlers position [6,7]. After a ten-minute resting period, a five-minute continuous resting cardiovascular measurement was executed. Two minutes into this recording, a systolic return-to-flow calibration was completed. The mean of the recordings in the last minute was used as results. Finometer (Finapres Medical Systems®, Amsterdam, Netherlands) results were analysed with Beatscope 1.1 software (Finapres Medical Systems®, Amsterdam, Netherlands) in order to obtain total peripheral resistance (mmHg/s/ml) and Windkessel compliance (ml/mmHg).

8. Biochemical analyses

A qualified nurse drew fasting blood samples from each participant's antebrachial vein with a sterile winged infusion set and sterile syringes in order to mitigate the risk of infection. The infusion set was flushed with two to three millilitre saline and the first two millilitres of blood discarded before samples were obtained. The infusion set was kept *in situ* with a heparin block in order to prevent clotting (0.5 ml of heparin sodium-fresenius 5000 IU/ml in 50 ml saline solution; Fresenius Kabi, Port Elizabeth, South Africa). Blood samples were taken while participants were at rest. The serum and plasma samples were prepared according to standardised methods. Serum was left in the blood sample vacutainer at room temperature for 30 minutes to enable coagulation to come about; thereafter the sample was centrifuged

by means of a bench top centrifuge. Afterwards, the prepared serum was transferred to Eppendorf tubes. Ethylenediaminetetraacetic acid (EDTA) plasma was also prepared by centrifugation of the blood sample. All serum, plasma, and twenty-four hour urine samples were stored in a laboratory bio-freezer at -80ºC until analysis.

Given the short half-life of nitric oxide [19], other markers were used to determine nitric oxide synthesis capacity. Mass spectrometric determinations of L-homoarginine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were performed as described elsewhere [20] by using liquid chromatography tandem mass spectrometry (LC-MS/MS). In short, 25 µL of plasma was diluted with stable isotope-labelled internal standards (i.e. [$^{13}C_6$]-homoarginine and [$^{2}H_6$]-ADMA). Thereafter, methanol was used to precipitate proteins and guanidine compounds were converted to their butyl esters. Concentrations were calculated with calibration curves (four levels, triplicates), and plate-wise quality controls were performed (two levels, duplicates). Intra- and inter-assay coefficients of variation were ≤7.5% for all analyses. Samples were re-analysed for coefficients of variation and bias of quality controls ≥15%.

Anti-oxidant enzyme activities, including glutathione peroxidase (GPx) and glutathione reductase (GR) represented the body's redox status and were measured from EDTA plasma by means of assay kits (Caymen Chemical Company, Ann Arbor, MI, USA). Thiobarbituric acid reactive substances (TBARS) was included as a marker of oxidative stress, which is formed from lipid peroxidation, given that reactive oxygen species has a short half-life [19]. Thiobarbituric acid reactive substances were measured spectrophotometrically from urine with a Synergy multimode microplate reader (BioTek, Winooski, VT, USA) [21]. Total glutathione measurements was measured from EDTA whole blood with the use of BIOXYTECH GSH/GSSG-412 kits from OXIS International Inc. (Beverly Hills, CA) as part of the endogenous anti-oxidant system. C-reactive protein (CRP) serves as an acute phase inflammatory biomarker and independent biomarker of cardiovascular disease. Gamma-glutamyltransferase was also added as participants may over- or under-estimate alcohol use [22]. Gamma-glutamyltransferase (GGT) and high-sensitivity CRP were measured from serum with a CobasIntegra 400 plus (Roche, Basel, Switzerland) apparatus. The intra- and inter-coefficients of variation for the assays were below 10%.

Inflammatory cells and the inflammatory cytokine interleukin-6 (IL-6) were also used as markers of inflammation [17,23]. Neutrophil, lymphocyte and monocyte counts were analysed by means of a Coulter AcT 5 diff Analyser (Beckman Coulter, California, United States). The intra- and inter-coefficients of variation for the assays were below 10%. Interleukin-6 was measured from serum through the use of high sensitivity Quantikine

Enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN USA) with intra assay variability at 5.9% and inter assay variability at 18.9%.

Cotinine was added as participants may over- or under estimate smoking exposure, and second-hand smoke is also included in smoking exposure. Other confounders included glucose and lipids. Serum cotinine levels were measured with a homogeneous immunoassay kit (Automated Modular, Roche, Basel, Switzerland). Glucose, triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured in serum with a Cobas Integra 400 plus (Roche, Basel, Switzerland) apparatus. Low density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula: LDL-C=TC-HDL-C-VLDL-C (VLDL-C=0.456*TG) [24]. Glycated haemoglobin was measured from EDTA whole blood with the Integra 400 plus (Roche, Switzerland). The intra- and inter-coefficients of variation for these variables were below 10%.

Creatinine was analysed using sequential multiple analysers (Cobas Integra 400 plus, Roche, Basel, Switzerland). Estimated glomerular filtration rate (eGFR) was measured as an indication of kidney function, as it plays a role in cardiovascular health [20,25]. Results for kidney function may also add clarity to our study, as SDMA is also a marker of kidney function [26]. The eGFR was calculated from serum creatinine with the Chronic Kidney Disease Epidemiology Collaboration formula as it is a more accurate measure of kidney function than creatinine alone [27]. Human immunodeficiency virus tests were performed by means of a First Response kit (Premier Medical Corporation, Daman, India) and confirmed with a Pareekshak test (Bhat Biotech, Bangalore, India). Participants received pre- and post-counselling for the human immunodeficiency virus tests.

9. Statistical analyses

Data management, statistical analyses and graphical illustrations were completed with the use of TIBCO® Statistica™ version 13.3 (TIBCO Software Inc.) and GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA). Participants were divided into physically active and inactive groups. Means between groups were compared with the use of analyses of covariance whereas proportions and categorical differences were compared by means of Chi-square tests. Relationships of cardiovascular variables with markers of oxidative stress, nitric oxide synthesis, and inflammation were investigated by means of single and partial regression analyses.

Furthermore, we investigated independent associations of cardiovascular variables with markers of oxidative stress, nitric oxide synthesis capacity, and inflammation with multiple regression analyses. Models additionally included race, sex, age, waist circumference,

cotinine, GGT, glycated haemoglobin, total cholesterol, and eGFR. Covariates included in the models were selected based on the strongest bivariate relationships with cardiovascular variables, markers of oxidative stress, inflammation and nitric oxide synthesis. Other covariates that we considered included body mass index, self-reported smoking, self-reported alcohol use, glucose, LDL-C, and triglycerides. Multiple regression analyses were repeated to test for the contribution of inflammation by additionally including CRP as an independent variable in models with oxidative stress and nitric oxide synthesis capacity markers as main independent variables.

10. The student's contribution to data collection and competence regarding the subject.

During her studies, the student assisted in data collection for the African Prospective study on the Early Detection and Identification of Cardiovascular disease and HyperTension study by preparing blood (Stabilyte, Citrate, Serum, EDTA, and NaF) and urine samples for storage. The student is also an International Society for the Advancement of Kinanthropometry certified anthropometrist and has experience in this field. As a sportscientist, experience was gained in laboratory exercise testing which included VO_2max, lactate threshold, and heart rate recovery, among others.

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MANUSCRIPT

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Article should include the following sections:	(1) Title page, (2) Abstract and Keywords, (3) Introduction, (4) Methods, (5) Results, (6) Discussion, (7) Acknowledgements, (8) Funding, (9) Conflict of interest, (10) Authors' Contributions, (11) References, (12) Figure legends, (13) Appendices, (14) Tables, (15) Figures. Please note: For the purpose of this MHSc the tables and figures will be kept in text for the examiners' convenience.
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Abstract number of words	≤250
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Title page	-Title should consist of ≤20 words (common abbreviations should be used when necessary); titles should be clear and brief, conveying the message of the paper. -Authors' names full first name, middle name/initial (optional) and last name should appear and department or institution's full name and location should be included. -The affiliations of all the authors should be connected using a,b,c. -Sources of any support for the work in the form of grants, equipment, or drugs. -Disclaimers, if any. -Name and address of the author responsible for correspondence. -Full word count, including references.
Abbreviations	-Should be kept to a minimum. -Should be written out at first mention followed by the abbreviation.
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Blood pressure and nitric oxide synthesis capacity in physically active and -inactive groups: The SABPA study

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Conflict of interest: None

Word count: 4 803

Abstract

Aim: Physical activity affects the vasculature through mechanisms related to nitric oxide bioavailability, oxidative stress, and inflammation; with endothelial function at the centre of this triad. In a South African setting, with the prevalence of hypertension and physical inactivity being alarmingly high, we aimed to investigate relationships of vascular function measures with markers of oxidative stress, inflammation and nitric oxide synthesis capacity in physically active and -inactive groups.

Methods: Physically active (n=84) and physically inactive (n=132), black and white school teachers, as classified by the 2008 United States Physical Activity Guidelines, were included in this cross-sectional study. We measured twenty-four hour blood pressure, total peripheral resistance and Windkessel compliance, along with markers of oxidative stress, inflammation and nitric oxide synthesis capacity including L-homoarginine, asymmetric dimethylarginine, and symmetric dimethylarginine.

Results: Twenty-four hour blood pressure, oxidative stress, and inflammatory profiles were similar between the two groups, except for Windkessel compliance (p=0.041) and L-homoarginine (p=0.006) which were higher in the physically active group. In the same group, twenty-four hour diastolic blood pressure (β=0.19; p=0.034) associated with total glutathione, and twenty-four hour blood pressure measures (systolic blood pressure: β=0.18, p=0.039; diastolic blood pressure: β=0.21, p=0.026) associated with L-homoarginine. In the physically inactive group, twenty-four hour blood pressure (systolic blood pressure: β=0.25, p=0.0001; diastolic blood pressure: β=0.20, p=0.013) associated with symmetric dimethylarginine. These associations were independent of inflammation.

Conclusion: Moderate physical activity may improve vascular function through modifications in nitric oxide bioavailability, which may mitigate vascular dysfunction in physically active populations.

Keywords: L-homoarginine, asymmetric dimethylarginine, symmetric dimethylarginine, oxidative stress, inflammation.

(Word count: 247/250)

Introduction

In South Africa, 40.5% of men and 53.1% of women are physically inactive [1]. A sedentary lifestyle may contribute to increased endothelial dysfunction and arterial stiffness [2], as noted in a South African population [3]. Physical activity levels affect the vasculature through mechanisms related to nitric oxide bioavailability, oxidative stress, and inflammation [4].

Habitual physical activity protects the integrity of the vascular wall by maintaining a healthy redox and inflammatory state [4]. In this regard, oxidative stress is lowered by increased expression of endogenous anti-oxidant enzymes, such as glutathione peroxidase (GPx) and superoxide dismutase, thereby reducing oxidative damage [5]. Decreased oxidative stress is further accompanied by reduced inflammation, with resultant lower circulating levels of biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6), as well as lower inflammatory cell counts [5].

Habitual physical activity enhances nitric oxide bioavailability. This is achieved not only by a decrease in the inactivation of nitric oxide when inflammation and oxidative stress is kept low [6], but also by enhanced nitric oxide synthesis [7]. Evidently, higher levels of physical activity are associated with increased L-arginine availability [7,8], which is an analogue of the arginase inhibitor, L-homoarginine [9]. Physical activity can further increase endothelial nitric oxide synthase activity and decrease levels of endothelial nitric oxide synthase inhibitors such as asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) [7,8].

Endothelial dysfunction closely associates with inadequate nitric oxide bioavailability and may be the link in this triad of oxidative stress, inflammation, and vascular dysfunction, which is initiated and exacerbated by physical inactivity [2,5,6]. Moreover, it was suggested that high levels of physical activity are required to obtain vascular health such as increased endogenous anti-oxidant enzyme activity and decreased inflammation, with a subsequent increase in nitric oxide synthesis capacity [4,6]. We aimed to investigate the relationship of vascular function measures, including twenty-four hour (24h) blood pressure, total peripheral resistance (TPR), and Windkessel compliance (Cwk), with markers of oxidative stress, inflammation, and nitric oxide synthesis capacity in physically active and inactive South Africans.

Methods

Study population

This cross-sectional study formed part of the second phase of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study [10]. The second phase of the SABPA study was conducted between February 2011 and May 2012, and included 359

black and white school teachers, between the ages of 25 and 65 years, from the Dr Kenneth Kaunda Educational District, North West Province, South Africa.

Exclusion criteria included individuals with a tympanum temperature higher than 37.5°C, those who used alpha- and beta-blockers or psychotropic substances, pregnant or lactating women, and individuals who have been vaccinated or donated blood within three months prior to participation. In this sub-study, we additionally excluded all participants with physical activity recordings which did not last for the entire seven days, or recordings with more than 40 minutes daily lost time (n=143), resulting in a total of 216 black and white men and women. Participants were divided into physically active (n=84) and physically inactive (n=132) groups, according to the 2008 United States Physical Activity Guidelines [11].

All participants signed an informed consent form. This study was approved by the Health Research Ethics Committee of the North-West University and adheres to the ethical guidelines of the Declaration of Helsinki for investigation on human participants.

Demographic data and anthropometric measurements

General health questionnaires were completed to obtain data regarding sex, age, race, smoking habits, alcohol, and medication use. Standardised methods were used for anthropometric measurements. Measurements were obtained in triplicate, as prescribed by a standard international protocol [12]. Calibrated instruments were used to measure body height (Invicta Stadiometer IP 1465, Leicester, UK), weight (calibrated Precision Health Scale, A&D Company, Japan), and waist circumference (Holtain non-stretchable metal tape, A&D Company, Tokyo, Japan). Subsequently, body mass index (BMI) was calculated.

Physical activity measurements

Habitual physical activity was measured over a period of seven consecutive days with an Actiheart® (CamNtech Ltd., Cambridge, UK); a chest worn heart rate and accelerometer device. This device is valid and reliable for the accurate estimation of energy expenditure on humans at rest, low, and moderate activities [13]. A biokineticist questioned each participant to assess their physical activity levels and accordingly chose an appropriate activity level on the Actiheart program (CamNtech Ltd., Cambridge, UK). The Actiheart® software (Version 4.0.116) was used to capture and analyse data. The software calculated the time spent in different metabolic equivalents (METs) categories which consisted of daily awake sedentary time ($\leqslant$1.5 METs), daily awake light-activity time (1.5 to 2.99 METs), daily awake moderate-activity time (3 to <6 METs), and daily awake vigorous activity time ($\geqslant$6 METs). This device also measured total energy expenditure (TEE), which comprised resting energy expenditure, dietary and activity-induced energy expenditure (AEE).

Cardiovascular measurements

Ambulatory blood pressure measurements (ABPM) were obtained with a Meditech Cardiotens CE120® device (Budapest, Hungary), validated from the British Hypertension Society. The device was programmed to take measurements at 30-minute intervals between 07:00 and 22:00 hours during the day and at one-hour intervals between 22:00 and 06:00 hours during the night [14]. An appropriate sized cuff was fitted on the non-dominant arm. Data were analysed with CardioVisions 1.15.2 Personal Edition software (Meditech®, Budapest, Hungary). Participants were asked to make use of their ambulatory diary cards to record abnormalities such as headaches, visual disturbances, fainting, nausea, physical activity, and emotional stress.

Further cardiovascular measurements were obtained with a validated Finometer device (Finapres Medical Systems, Amsterdam, Netherlands) [15]. The cuffs of the Finometer device was secured and connected to the left upper arm and middle finger, respectively, with the participant in the semi-Fowlers position. After a ten-minute resting period, a five-minute continuous resting cardiovascular measurement was executed. Two minutes into this recording, a systolic return-to-flow calibration was completed. The highest precision of cardiovascular measurements was obtained only after this calibration. Finometer results were analysed with Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, Netherlands) to obtain TPR and Cwk.

Biochemical analyses

Serum and plasma samples were collected and stored at -80°C until analysed. Mass spectrometric determinations of L-homoarginine, ADMA, and SDMA were performed [16], by using liquid chromatography tandem mass spectrometry (LC-MS/MS). In short, 25 µL of plasma was diluted with stable isotope-labelled internal standards (i.e. [$^{13}C_6$]-homoarginine and [$^{2}H_6$]-ADMA). Thereafter, methanol was used to precipitate proteins and guanidine compounds which were converted to their butyl esters. Concentrations were calculated with calibration curves (four levels, triplicates) and plate-wise quality controls were run (two levels, duplicates). Intra- and inter-assay coefficients of variation were ≤7.5% for all. Samples were re-analysed for coefficients of variation and bias of quality controls ≥15%.

Anti-oxidant enzyme activities, including GPx and glutathione reductase (GR) were measured by means of assay kits (Caymen Chemical Company, Ann Arbor, MI, USA). Thiobarbituric acid reactive substances were measured spectrophotometrically with a Synergy multimode microplate reader (BioTek, Winooski, VT, USA) [17]. We obtained total glutathione measurements from ethylenediaminetetraacetic acid (EDTA) whole blood with the use of BIOXYTECH GSH/GSSG-412 kits (OXIS International Inc., Beverly Hills, CA).

Gamma-glutamyltransferase (GGT) and high-sensitivity CRP were measured with a Cobas Integra 400 plus (Roche, Basel, Switzerland) apparatus. Neutrophil, lymphocyte and monocyte counts were analysed by means of a Coulter AcT 5 diff Analyser (Beckman Coulter, California, United States). The intra- and inter-coefficients of variation for the assays were below 10%. Interleukin-6 was measured with high sensitivity Quantikine Enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN USA) with intra assay variability at 5.9% and inter assay variability at 18.9%.

Serum cotinine levels were measured with a homogeneous immunoassay kit (Automated Modular, Roche, Basel, Switzerland). Glucose, triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured with a Cobas Integra 400 plus (Roche, Basel, Switzerland) apparatus. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula: LDL-C=TC-HDL-C-VLDL-C (VLDL-C=0.456*TG) [18]. Glycated haemoglobin (HbA1c) was measured from EDTA whole blood with the Integra 400 plus (Roche, Basel, Switzerland).The intra- and inter-assay coefficients of variation for these variables were below 10%. Creatininewas analysed in serum (Cobas Integra 400 plus, Roche, Basel, Switzerland) and estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration formula [19]. Human immunodeficiency virus (HIV) tests were performed by means of a First Response kit (Premier Medical Corporation, Bangalore, India) and positive results were confirmed with a Pareekshak test (Bhat Biotech, Bangalore, India). Participants received pre- and post-counselling with HIV testing.

Statistical analyses

Data management, statistical analyses and graphical illustrations were completed with the use of TIBCO® Statistica™ version 13.3 (TIBCO Software Inc.) and GraphPad Prism version 5.03 (GraphPad Software Inc., California, USA). Participants were divided into physically active and inactive groups.

Variables with a non-Gaussian distribution (TEE, AEE, GGT, neutrophil-lymphocyte ratio (NLR), CRP, IL-6, thiobarbituric acid reactive substances (TBARS), total glutathione (tGSH), GPx, GR, HbA1C, HDL-C, and triglycerides) were logarithmically transformed and represented as geometric means with 5th and 95th percentiles. Means between groups were compared with the use of analyses of covariance, while adjusting for race, sex, and age. Proportions between groups were compared by means of Chi-square tests. Relationships of cardiovascular variables with markers of oxidative stress, nitric oxide synthesis, and inflammation were investigated by means of single and partial regression analyses, the latter adjusted for race, sex, and age, in both groups.

Furthermore, we investigated independent associations of cardiovascular variables 24h systolic blood pressure (SBP), 24h diastolic blood pressure (DBP), 24hmean arterial pressure (MAP), 24h pulse pressure (PP), TPR, and Cwk with markers of oxidative stress (TBARS, tGSH, GPx, and GR), nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA), and inflammation (NLR, monocytes, CRP, and IL-6)with multiple regression analyses. Models additionally included race, sex, age, waist circumference, cotinine, GGT, HbA1c, total cholesterol, and eGFR. Covariates included in the models were selected based on the strongest bivariate relationships with cardiovascular variables, markers of oxidative stress, inflammation and nitric oxide synthesis. Other covariates which we considered included BMI, self-reported smoking, self-reported alcohol use, glucose, LDL-C, and triglycerides. Multiple regression analyses were repeated to test for the contribution of inflammation by additionally including CRP as an independent variable in models with oxidative stress and nitric oxide synthesis capacity markers as main independent variables.

Results

Characteristics of the study population

Characteristics of the total group, as well as comparisons between the physically active and physically inactive groups, independent of race, sex and age, are presented in **Table 3.1**. The total group comprised of 48.1% men, 50.5% blacks, and 6.02% HIV infected participants, who were on average 49.7 years old. In the physically active group (≥150 minutes of 3-6 METs) of 84 (38.9%) participants, only 3 (3.57%) achieved high intensity physical activity levels (≥75 minutes of >6 METs). The physically active group included 18.3% fewer black participants (p=0.009) than the inactive group, but sex distribution was similar. As expected, the physically active group had higher TEE and AEE levels (both p≤0.001).Despite similar waist circumferences between groups (p=0.25), BMI was higher (p=0.046) in the physically active group. With regards to cardiovascular measurements, physically active participants had higher Cwk (p=0.041), while blood pressure and TPR were similar (p≥0.38). When comparing markers of oxidative stress and nitric oxide synthesis, we found that TBARS (p=0.043) and L-homoarginine (p=0.006) were higher and GGT lower (p=0.034) in the physically active- compared to the inactive group.

Regression analyses

In the physically active group, both partial (**Supplementary Table 3.1**) and multiple regression (**Supplementary Table 3.2**) analyses revealed associations of 24h DBP with tGSH (β=0.18; p=0.037), as well as L-homoarginine (β=0.21; p=0.028). Additionally, in multiple regression analyses, 24h SBP (β=0.18; p=0.04) and 24h MAP (β=0.20; p=0.025) correlated with L-homoarginine in the physically active group. In both analyses

(**Supplementary Table 3.1 and 3.3**), the physically inactive group, showed relationships of 24h SBP (β=0.25; p=0.001), 24h DBP (β=0.20; p=0.013), 24h MAP (β=0.23; p=0.003), and 24h PP (β=0.21; p=0.012) with SDMA. In partial regression analyses, correlations of TPR and Cwk with markers of oxidative stress (TBARS and GR) and inflammation (NLR, monocytes, CRP, and IL-6) were found, but these relationships lost significance in multivariate regression analyses (all p>0.05).

Further, as cardiovascular variables did not associate with markers of inflammation in multiple regression analyses, we repeated the above multiple regression analyses, but additionally included CRP to the models (**Figure 3.1** and **Supplementary Table 3.4**). Similar to previous results, **Figure 3.1** illustrates that 24h DBP (β=0.19; p=0.034) associated with tGSH and that 24h SBP (β=0.18; p=0.039), 24h DBP (β=0.21; p=0.026), and 24h MAP (β=0.21; p=0.023) associated with L-homoarginine in the physically active group only. The physically inactive group showed an association of 24h SBP (β=0.25; p=0.001), 24h DBP (β=0.20; p=0.013), 24h MAP (β=0.23; p=0.003), and 24h PP (β=0.21 p=0.014) with SDMA only.

Discussion

We investigated relationships of vascular function measures with markers of oxidative stress, inflammation and nitric oxide synthesis capacity in physically active and inactive groups. The physically active group had better arterial compliance and higher L-homoarginine levels than the physically inactive group. Twenty-four-hour DBP associated with L-homoarginine in the physically active group, while all 24h blood pressure measures adversely associated with SDMA in the physically inactive group. Twenty-four-hour SBP, DBP, and MAP also correlated with tGSH in the physically active group.

Our findings agree with previous findings which suggested that L-arginine, ADMA, and SDMA work in concert to alter vascular function [7]. In a study by Riccioni *et al.*, coronary artery disease patients, on average 52 ± 4.5 years of age, underwent a 10-minute walking exercise that resulted in higher L-arginine, while ADMA and SDMA were decreased when compared to baseline measures [7]. In another study aerobic exercise in healthy, postmenopausal women resulted in decreased ADMA that inversely correlated with arterial compliance [20]. The higher arterial compliance we found in the physically active group is supported by a study which proved that master endurance athletes have healthier vascular function, as evident by increased arterial compliance and decreased TPR, when compared to their sedentary peers [21]. Despite the fact that most studies investigated the vascular effects of high intensity physical activity [6,21], we found better vascular function and nitric oxide synthesis capacity in the physically active group, who were only moderately physically

active compared to the physically inactive group. Other studies have shown that changes in nitric oxide synthesis capacity are mainly responsible for the beneficial effects of physical activity on the vasculature [6], which underlines our results.

The beneficial effects of physical activity on nitric oxide synthesis capacity and vascular function may in part be explained by the effects of increased shear stress during physical activity. Increased shear stress during physical activity can activate calcium-dependent endothelial nitric oxide synthase to produce nitric oxide from L-arginine [22]. Physical activity may further improve L-arginine transport and consequently nitric oxide bioavailability [7,22]. In our study we investigated L-homoarginine, an analogue of L-arginine. At elevated levels, L-homoarginine has an inhibiting effect on the arginase enzyme, which functions as an enzyme responsible for the conversion of arginine to ornithine and urea [9]. Research suggests that homoarginine-mediated arginase inhibition may lead to increased levels of L-arginine, and a subsequent increase in nitric oxide synthesis [9]. On the contrary, our study showed a positive relationship between the arginase inhibitor, L-homoarginine, and blood pressure. This was explained in a previous study where exercise was found to increase arginase activity and nitric oxide synthase, thereby decreasing DBP [23]. The authors also found reduced L-arginine and argued that increased arginase activity and nitric oxide synthase may consume L-arginine after exercise [23]. Another possible mechanism may be that L-homoarginine, as weak substrate for nitric oxide synthase, competes with L-arginine [24].

Moreover, our study revealed that all 24h blood pressure measures were adversely associated with SDMA in the physically inactive group. Asymmetric dimethylarginine inhibits nitric oxide synthesis by competing with L-arginine for binding to endothelial nitric oxide synthase [22]. Symmetric dimethylarginine acts as an indirect inhibitor of nitric oxide synthase by increasing the production of reactive oxygen species, thereby decreasing L-arginine transport from plasma into the endothelial cells [7]. Asymmetric dimethylarginine and SDMA have been shown to increase in response to oxidative stress and inflammation [25]. It may be due to the fact that ADMA and SDMA synthesis depends on the activity of protein arginine methyltransferase-2, which is activated by oxidative stress [26]. Decreased oxidative stress and inflammation from physical activity may therefore result in decreased ADMA and SDMA levels [20]. Furthermore, physical activity enhances messenger ribonucleic acid gene expression of dimethyl-arginine-dimethylaminohydrolase, which is known to break down ADMA [20].

Oxidative stress and inflammation are important factors in vascular dysfunction as it can lower nitric oxide synthesis and availability [6]. It is evident from the literature that exercise is

effective in enhancing the body's endogenous anti-oxidant enzymes and lowering inflammation [4]. Unexpectedly, in our study both GPx and GR were similar when comparing physically active and inactive groups. Results also remained unchanged when we additionally adjusted for inflammation in multiple regression analysis. We therefore speculate that the cardiovascular benefits from moderate physical activity may not be dependent on redox- or inflammatory status, but rather on increased nitric oxide synthesis capacity as discussed previously. Indeed, previous studies indicated that high intensity physical activity is needed to activate an acute inflammatory and oxidative stress response, which only then will result in adaptations to lower chronic inflammation and oxidative stress [4,6]. Another unexpected finding was the higher levels of TBARS in the physically active group compared to the inactive group. This is contradictory to other research which showed a negative association between physical activity and TBARS [27]. This may be explained by the hierarchical oxidative stress model described by Xiao *et al.* [28]. The model proposes that dose-dependent adaptations to oxidative stress lead from a protective, up-regulated anti-oxidant system to a pro-inflammatory, and eventually a cytotoxic response [28]. In our study population with physical activity levels in the moderate category, the higher TBARS may indicate that oxidative stress was at a level to cause more lipid peroxidation, but not yet high enough to activate the protective response of endogenous anti-oxidant synthesis.

Results from another SABPA study suggested that GSH may have a hypotensive effect in normotensive individuals [29]. However, in the physically active group of our study, we found a positive association between 24h DBP and tGSH. In corroboration, another SABPA study showed that black men have increased tGSH and L-arginine, despite having higher blood pressure, when compared to white men [30]. It was suggested that increased oxidative stress and a subsequent up-regulated redox status in black men may counteract nitric oxide bioavailability, and that nitric oxide inactivation may be of greater importance than redox status for blood pressure control [30]. The inclusion of black men in our study may have influenced our results and it may therefore also be more relevant to focus on nitric oxide inactivation than redox status in our study. However, we did adjust for sex and race in attempt to mitigate the influence thereof but still found significant correlations of blood pressure with tGSH and L-homoarginine. Moreover, increased oxidative stress from chronic physical inactivity may up-regulate synthesis of endogenous anti-oxidants, such as tGSH, in an attempt to counteract oxidative stress.

The study should be interpreted in context of its strengths and limitations. This study was based on cross-sectional data, from which causality could not be determined. Only moderate, and not high-intensity physical activity was achieved by most of the participants in the physically active group. High intensity physical activity may be required for improvements

in inflammation and oxidative stress status, as well as further improvements in nitric oxide synthesis capacity and vascular function [4,6]. Moderate physical activity is, however, a more reasonable prescription to adhere to, especially for elderly individuals. This sample population stemmed from urban areas of Potchefstroom in the North West Province and may not necessarily represent the entire South African population. However, this study included black and white men and women with a similar socio-economic status. Our results were independent of various confounders, such as waist circumference, which are known to affect oxidative stress and inflammation. Data were obtained under strictly controlled conditions and gold-standard measures were used.

In conclusion, even moderate physical activity may lower vascular risk, possibly by means of modifications in nitric oxide synthesis capacity. In turn, increased nitric oxide synthesis capacity may mitigate the development of cardiovascular disease in this physically active population.

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Conflict of interest

The authors of this study have no conflict of interest to declare.

Table 3.1. Characteristics of the total study population, as well as comparisons between physically active and –inactive groups.

	Total group	Physically Active*	Physically Inactive*	p-value
N	216	84	132	
Demographic factors				
Sex, male, n (%)	104 (48.1)	39 (46.4)	65 (49.2)	0.69
Race, black, n (%)	109 (50.5)	33 (39.3)	76 (57.6)	0.009**
Age, years	49.7 (48.5; 50.8)	50.0 (48.3; 51.7)	49.4 (47.9; 50.9)	0.62
HIV infected, n (%)	13 (6.02)	1 (1.19)	12 (9.01)	0.017*
Lifestyle factors				
Alcohol use, n (%)	104 (48.1)	42 (50.0)	62 (47.0)	0.66
Smoking, n (%)	30 (13.9)	13 (15.5)	17 (12.9)	0.59
TEE (kCal/day)	3037 (1918;5807)	3719 (3487; 4967)	2670 (2564; 2843)	<0.001***
AEE (kJ/kg/day)	46.4 (11.6; 162)	73.8 (62.4; 87.3)	34.6 (30.3; 39.6)	<0.001***
Anthropometric measurements				
Body mass index (kg/m^2)	29.4 (28.5;30.2)	30.4 (29.0; 31.7)	28.6 (27.5; 29.7)	0.046*
Waist circumference (cm)	96.4 (94.3; 98.5)	97.7 (1.60; 94.6)	95.3 (92.8; 97.8)	0.25
Cardiovascular measurements				
24h SBP (mmHg)	129 (127; 132)	129 (126; 133)	129 (126; 131)	0.74
24h DBP (mmHg)	80 (78.4; 81.3)	79 (77; 81)	80 (78; 81)	0.75
24h MAP (mmHg)	96 (94.7; 98.1)	96 (94; 98)	96 (94; 98)	0.98
24h PP (mmHg)	50 (48.3; 50.8)	50 (48; 52)	49 (47; 51)	0.38
TPR (mmHg/ml/s)	1.07 (1.03; 1.12)	1.08 (1.01; 1.15)	1.06 (1.01; 1.12)	0.78
Cwk (ml/mmHg)	1.87 (1.81; 1.93)	1.93 (1.86; 2.01)	1.83 (1.77; 1.89)	0.041*
Oxidative and nitric oxide synthesis				
TBARS (mg/g creatinine)	0.11 (0.03; 0.35)	0.12 (0.10; 0.14)	0.10 (0.08; 0.11)	0.043*
tGSH (µM)	818 (476; 1428)	801 (734; 875)	828 (772; 889)	0.57
GGT (U/l)	28.3 (8.40; 140)	24.4 (21.0; 28.4)	30.1 (26.7; 33.9)	0.034*
GPx (nmol/min/ml)	32.2 (16.8; 61.1)	32.7 (30.5; 35.1)	31.7 (30.0; 33.5)	0.49
GR (nmol/min/ml)	3.00 (0.51; 17.8)	3.24 (2.55; 4.13)	2.80 (2.30; 3.40)	0.36
L-homoarginine (µmol/l)	3.49 (3.29; 3.68)	3.79 (3.50; 4.01)	3.25 (3.01; 3.50)	0.006**
ADMA (µmol/l)	0.62 (0.60; 0.64)	0.62 (0.59; 0.65)	0.62 (0.60; 0.64)	0.95
SDMA (µmol/l)	0.35 (0.34; 0.36)	0.34 (0.33; 0.36)	0.35 (0.34; 0.37)	0.53
Inflammation markers				
NLR (x10^3/µl)	1.00 (0.40; 3.06)	0.91 (0.80; 1.04)	1.05 (0.95; 1.17)	0.10
Monocytes (x10^3/µl)	0.39 (0.37; 0.41)	0.39 (0.36; 0.42)	0.38 (0.36; 0.41)	0.84
C-reactive protein (mg/l)	1.98 (0.18; 15.7)	1.88 (1.47; 2.40)	1.94 (1.60; 2.34)	0.84
Interleukin-6 (pg/ml)	1.02 (0.30; 0.35)	1.05 (0.88; 1.25)	0.98 (0.85; 1.13)	0.54
Other biochemical markers				
Cotinine (ng/ml)	18.8 (11.1;26.4)	23.4 (11.1; 35.7)	15.3 (5.56; 25.0)	0.31
Glycated haemoglobin (%)	5.79 (5.17; 7.40)	5.78 (5.64; 5.92)	5.77 (5.66; 5.89)	0.97
Glucose (mmol/l)	5.02 (4.77; 5.28)	4.90 (4.42; 5.27)	5.06 (4.76; 5.36)	0.51
Cholesterol (mmol/l)	4.46 (4.32; 4.60)	4.39 (4.17; 4.61)	4.48 (4.31; 4.66)	0.51
LDL-C (mmol/l)	2.85 (2.74; 2.97)	2.85 (2.67; 3.04)	2.84 (2.69; 3.00)	0.93
HDL-C (mmol/l)	0.99 (0.59; 1.64)	0.99 (0.93; 1.054)	0.99 (0.94; 1.04)	0.99
Triglycerides (mmol/l)	1.07 (0.44; 3.04)	1.00 (0.90; 1.11)	1.10 (1.12; 1.20)	0.14
eGFR (ml/min/1.73m^2)	163 (143; 183)	153 (124; 183)	165 (142; 189)	0.54
Medication use				
Anti-hypertensive, n (%)	67 (31.0)	24 (28.6)	43 (32.6)	0.54
Anti-diabetic, n (%)	22 (10.2)	5 (5.95)	17 (12.9)	0.10
Statins, n (%)	27 (12.5)	10 (11.9)	17 (12.9)	0.83
Anti-oxidant, n (%)	22 (10.2)	5 (5.95)	17 (12.9)	0.10

Values are expressed as arithmetic mean with 95% confidence intervals, geometric mean with 5th and 95th percentiles, or proportions. Groups are compared by means of analysis of covariance, adjusted for race, sex, and age. Categorical differences are compared by means of Chi-square.
*Physically active denotes ≥150minutes 3-6 METs and physically inactive denotes <150minutes 3-6 METs.*P, P<0.05; **P, P<0.01; ***P, P<0.001.

N, number of participants; HIV, human immunodeficiency; TEE, total energy expenditure; AEE, activity-related energy expenditure; 24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure; Cwk, Windkessel compliance; TPR, total peripheral resistance; TBARS, thiobarbituric acid reactive substances; GPx, glutathione peroxidase; GR, glutathione reductase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; NLR, neutrophil/lymphocyte ratio; GGT, gamma-glutamyltransferase; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol, eGFR, estimated glomerular filtration rate; and METs, metabolic equivalents.

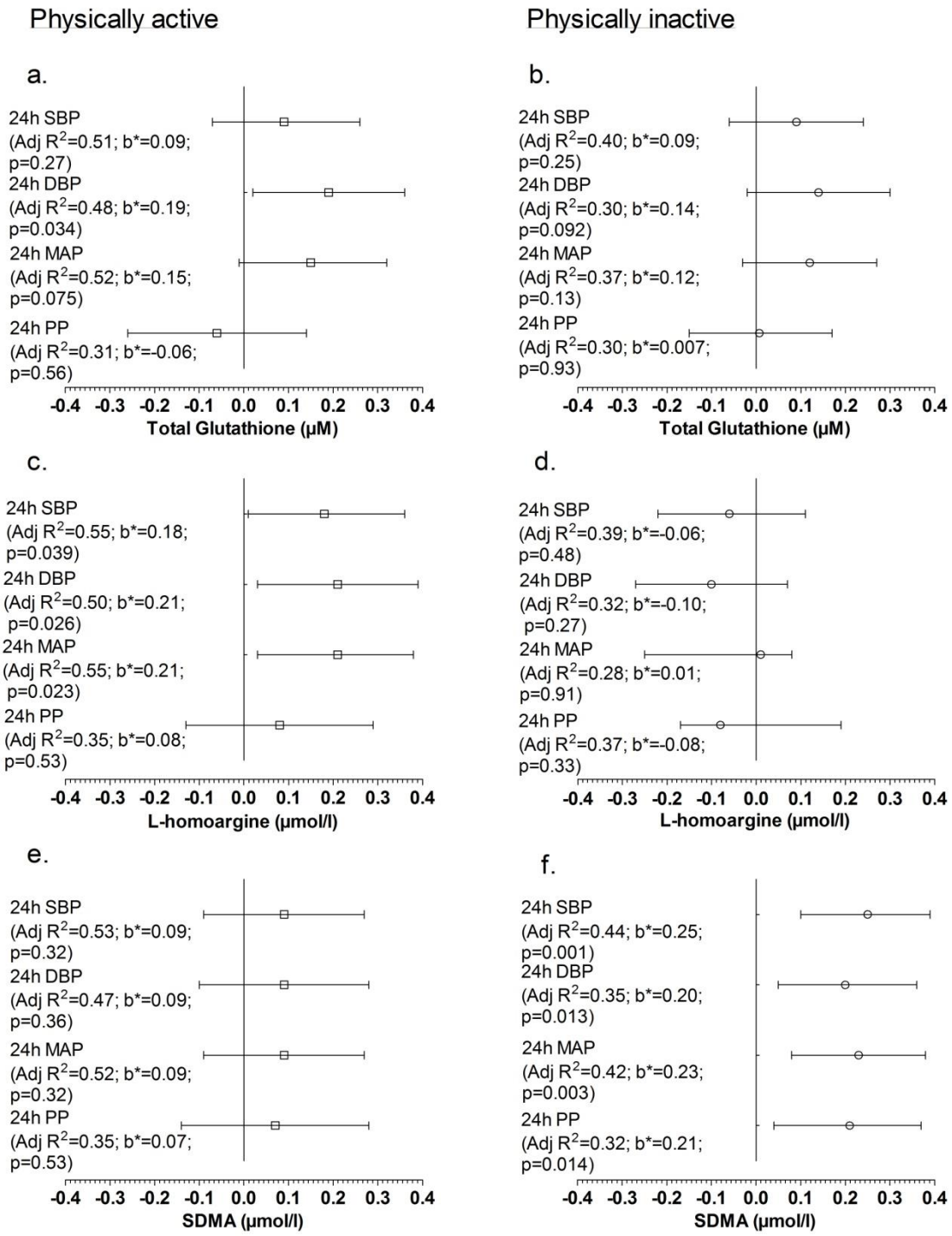

Figure 3.1. Independent associations of cardiovascular markers with L-homoarginine, total Glutathione, and SDMA in the physically active and inactive participants, respectively

As in Supplementary Table 3.3, models were adjusted for race, sex, age, waist circumference, cotinine, GGT, glycated haemoglobin; cholesterol, eGFR, and C-reactive protein. 24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure. GGT; gamma-glutamyltransferase; and eGFR; estimated glomerular filtration rate. P-values ≤ 0.05 regarded as significant.

Supplementary Table 3.1. Partial regression analyses of cardiovascular markers with markers of oxidative stress and inflammation in physically active and – inactive groups.

	Physically active* (n=84)						Physically inactive* (n=132)					
	24h SBP (mmHg)	24h DBP (mmHg)	24h MAP (mmHg)	24h PP (mmHg)	TPR (mmHg/ ml/s)	Cwk (ml/mm Hg)	24h SBP (mmHg)	24h DBP (mmHg)	24h MAP (mmHg)	24h PP (mmHg)	TPR (mmHg/ ml/s)	Cwk (ml/mm Hg)
TBARS (mg/gCrn)	r=-0.01	r=-0.02	r=-0.02	r=0.01	r=0.07	-0.08	r=-0.14	r=-0.02	r=-0.08	r=-0.20	r=0.20	r=-0.18
	p=0.92	p=0.84	p=0.87	p=0.92	p=0.56	p=0.53	p=0.15	p=0.82	p=0.42	p=0.045	p=0.041	p=0.068
tGSH (µM)	r=0.24	r=0.31	r=0.29	r=0.06	r=0.09	r=0.0004	r=0.02	r=0.05	r=0.04	r=-0.02	r=0.11	r=-0.04
	p=0.043	p=0.009	p=0.014	p=0.60	p=0.46	p=1.00	p=0.85	p=0.60	p=0.69	p=0.86	p=0.26	p=0.69
GPx (nmol/min/ml)	r=0.09	r=0.12	r=0.12	r=0.02	r=0.19	r=-0.16	r=0.05	r=0.05	r=0.05	r=0.03	r=-0.06	r=0.01
	p=0.43	p=0.30	p=0.33	p=0.85	p=0.11	p=0.18	p=0.64	p=0.62	p=0.61	p=0.80	p=0.55	p=0.87
GR (nmol/min/ml)	r=-0.06	r=0.07	r=0.01	r=-0.22	r=0.05	r=-0.24	r=0.11	r=0.13	r=0.13	r=0.04	r=0.21	r=-0.13
	p=0.59	p=0.55	p=0.91	p=0.062	p=0.69	p=0.047	p=0.28	p=0.18	p=0.19	p=0.70	p=0.040	p=0.21
L-homoarg (µmol/l)	r=0.21	r=0.24	r=0.23	r=0.09	r=-0.01	r=-0.08	r=-0.02	r=-0.07	r=-0.05	r=0.04	r=-0.08	r=0.10
	p=0.078	p=0.049	p=0.052	p=0.47	p=0.93	p=0.52	p=0.84	p=0.49	p=0.62	p=0.67	p=0.43	p=0.33
ADMA (µmol/l)	r=0.20	r=0.10	r=0.15	r=0.23	r=0.006	r=0.17	r=0.14	r=0.06	r=0.11	r=0.16	r=-0.19	r=0.19
	p=0.10	p=0.40	p=0.22	p=0.054	p=0.96	p=0.16	p=0.15	p=0.54	p=0.30	p=0.11	p=0.057	p=0.052
SDMA (µmol/l)	r=0.02	r=0.04	r=0.03	r=-0.01	r=0.03	r=-0.02	r=0.29	r=0.18	r=0.25	r=0.28	r=-0.001	r=-0.01
	p=0.88	p=0.76	p=0.80	p=0.94	p=0.80	p=0.87	p=0.003	p=0.069	p=0.013	p=0.005	p=0.99	p=0.90
NLR (x103µl)	r=-0.06	r=-0.09	r=-0.08	r=-0.006	r=-0.09	r=0.03	r=0.09	r=-0.11	r=-0.02	r=0.24	r=-0.22	r=-0.13
	p=0.63	p=0.47	p=0.52	p=0.96	p=0.48	p=0.91	p=0.40	p=0.26	p=0.81	p=0.017	p=0.025	p=0.20
Monocytes (x103µl)	r=0.16	r=0.14	r=0.15	r=0.12	r=-0.07	r=0.04	r=0.02	r=-0.10	r=-0.05	r=0.13	r=-0.20	r=0.18
	p=0.19	p=0.25	p=0.21	p=0.30	p=0.54	p=0.76	p=0.81	p=0.30	p=0.63	p=0.18	p=0.040	p=0.073
CRP (mg/l)	r=0.24	r=0.21	r=0.23	r=0.18	r=-0.26	r=0.29	r=0.06	r=0.04	r=0.05	r=0.06	r=-0.31	r=0.26
	p=0.045	p=0.083	p=0.056	p=0.12	p=0.032	p=0.015	p=0.54	p=0.69	p=0.60	p=0.52	p=0.002	p=0.008
IL-6 (pg/ml)	r=-0.02	r=-0.02	r=-0.02	r=-0.02	r=-0.001	r=0.07	r=0.09	r=0.07	r=0.08	r=0.07	r=-0.18	r=0.24
	p=0.85	p=0.85	p=0.85	p=0.89	p=0.99	p=0.54	p=0.40	p=0.50	p=0.43	p=0.51	p=.078	p=0.014

Adjusted for race, sex, and age; and in the case of TPR and Cwk, also for 24h MAP.
*Physically active denotes ≥150minutes 3-6 METs and physically inactive denotes <150minutes 3-6 METs.

24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure; Cwk,Windkessel compliance; TPR, total peripheral resistance; TBARS, thiobarbituric acid reactive substances; tGSH, total glutathione, GPx, glutathione peroxidase; GR, glutathione reductase; L-homoarg, L-homoarginine; ADMA, asymmetric dimethylarginine; SDMA,

symmetric dimethylarginine; NLR, Neutrophil/Lymphocyte ratio, and CRP, c-reactive protein; IL-6, interleukin-6; N, number of participants; and METs, metabolic equivalents.

Supplementary Table 3.2. Multiple regression analyses of cardiovascular markers with markers of oxidative stress and inflammation in the physically active group.

	Physically active (n=84)											
	24h SBP (mmHg)			24h DBP (mmHg)			24h MAP (mmHg)			24h PP (mmHg)		
	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value
TBARS (mg/g Crn)	0.53	0.01 (-0.16; 0.19)	0.87	0.46	-0.001 (-0.19; 0.18)	0.99	0.51	0.005 (-0.17; 0.18)	0.95	0.36	0.04 (-0.16; 0.24)	0.71
tGSH (µM)	0.52	0.09 (-0.07; 0.26)	0.27	0.49	0.18 (0.01; 0.35)	0.037	0.52	0.15 (-0.01; 0.31)	0.078	0.32	-0.06 (-0.25; 0.14)	0.57
GR (nmol/min/ml)	0.53	-0.008 (-0.17; 0.15)	0.93	0.48	0.11 (-0.07; 0.28)	0.23	0.52	0.06 (-0.11; 0.22)	0.49	0.38	-0.16 (-0.34; 0.03)	0.10
GPx (nmol/min/ml)	0.53	0.048 (-0.12; 0.22)	0.58	0.48	0.077 (-0.10; 0.26)	0.40	0.52	0.067 (-0.10; 0.24)	0.45	0.36	0.004 (-0.19; 0.20)	0.97
L-homoarg (µmol/l)	0.56	0.18 (0.01; 0.35)	0.04	0.50	0.21 (0.03; 0.39)	0.028	0.55	0.20 (0.03; 0.37)	0.025	0.36	0.08 (-0.12; 0.29)	0.44
SDMA (µmol/l)	0.54	0.09 (-0.09; 0.27)	0.33	0.47	0.08 (-0.10; 0.27)	0.39	0.52	0.09 (-0.09; 0.27)	0.34	0.36	0.07 (-0.14; 0.28)	0.52
NLR (x103µl)	0.54	-0.10 (-0.26; 0.05)	0.20	0.48	-0.10 (-0.27; 0.06)	0.23	0.53	-0.10 (-0.26; 0.05)	0.20	0.37	-0.08 (-0.26; 0.11)	0.42
Monocytes (x103µl)	0.54	0.09 (-0.08; 0.26)	0.31	0.47	0.06 (-0.12; 0.24)	0.54	0.52	0.07 (-0.10; 0.24)	0.42	0.37	0.11 (-0.09; 0.30)	0.30
CRP (mg/l)	0.53	0.03 (-0.16; 0.23)	0.74	0.47	0.06 (-0.14; 0.27)	0.56	0.36	-0.02 (-0.15; 0.25)	0.88	0.52	0.05 (-0.24; 0.21)	0.62
IL-6 (pg/ml)	0.55	-0.13 (-0.30; 0.04)	0.13	0.48	-0.11 (-0.29; 0.07)	0.22	0.53	-0.12 (-0.29; 0.05)	0.16	0.37	-0.11 (-0.31; 0.08)	0.27

Models were adjusted for race, sex, age, waist circumference, cotinine, gamma-glutamyltransferase, glycated haemoglobin, cholesterol, and estimated glomerular filtration rate.

N, number of participants; 24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure; Adj, adjusted; TBARS, thiobarbituric acid reactive substances; tGSH, total glutathione, GR, glutathione reductase; GPx, glutathione peroxidase; L-homoarg, L-homoarginine; SDMA, symmetric dimethylarginine; NLR, Neutrophil/Lymphocyte ratio; CRP, C-reactive protein, and IL-6, interleukin-6.

Supplementary Table 3.3. Multiple regression analysis of cardiovascular markers with markers of oxidative stress and inflammation in the physically inactive group.

	Physically inactive (n=132)											
	24h SBP (mmHg)			24h DBP (mmHg)			24h MAP (mmHg)			24h PP (mmHg)		
	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value
TBARS (mg/g Crn)	0.40	-0.01 (-0.16; 0.14)	0.37	0.37	0.03 (-0.13; 0.19)	0.72	0.37	0.01 (-0.14;0.16)	0.88	0.30	-0.05 (-0.05; 0.11)	0.53
tGSH (µM)	0.40	0.09 (-0.09; 0.19)	0.37	0.37	0.06 (-0.09; 0.20)	0.093	0.37	0.12 (-0.03; 0.27)	0.13	0.31	0.007 (-0.15; 0.17)	0.93
GR (nmol/min/ml)	0.40	0.05 (-0.11; 0.16)	0.37	0.37	0.37 (-0.11; 0.19)	0.44	0.37	0.06 (-0.08; 0.20)	0.43	0.29	0.02 (-0.16; 0.14)	0.78
GPx (nmol/min/ml)	0.40	0.08 (-0.05; 0.22)	0.24	0.30	0.05 (-0.10; 0.20)	0.50	0.37	0.07 (-0.07; 0.21)	0.35	0.30	0.10 (0.06; 0.25)	0.22
L-homoarg (µmol/l)	0.39	-0.06 (-0.22; 0.01)	0.38	0.38	-0.10 (-0.27; 0.07)	0.27	0.38	-0.08 (-0.25; 0.08)	0.32	0.28	0.006 (-0.17; 0.18)	0.95
SDMA (µmol/l)	0.45	0.25 (0.10; 0.39)	0.001	0.42	0.20 (0.05; 0.36)	0.013	0.42	0.23 (0.08; 0.38)	0.003	0.32	0.21 (0.05; 0.37)	0.012
NLR (x103µl)	0.40	0.01 (-0.14; 0.16)	0.37	0.37	-0.10 (-0.26; 0.06)	0.21	0.37	-0.05 (-0.21; 0.10)	0.49	0.32	0.13 (-0.02; 0.29)	0.10
Monocytes (x103µl)	0.40	0.001 (-0.15; 0.15)	0.37	0.37	0.06 (-0.24; 0.08)	0.34	0.37	-0.04 (-0.20; 0.11)	0.58	0.31	0.09 (-0.07; 0.25)	0.28
CRP (mg/l)	0.40	-0.03 (-0.22; 0.16)	0.37	0.37	-0.01 (-0.22; 0.19)	0.89	0.37	-0.02 (-0.22; 0.17)	0.82	0.30	-0.03 (-0.24; 0.17)	0.75
IL-6 (pg/ml)	0.40	-0.02 (-0.19; 0.15)	0.37	0.37	0.37 (-0.18; 0.20)	0.90	0.37	-0.003 (-0.18; 0.18)	0.98	0.30	-0.05 (-0.24; 0.13)	0.57

Models were adjusted for race, sex, age, waist circumference, cotinine, gamma-glutamyltransferase, glycated haemoglobin, cholesterol, and estimated glomerular filtration rate.

N, number of participants; 24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure; Adj, adjusted; TBARS, thiobarbituric acid reactive substances; tGSH, total glutathione, GR, glutathione reductase; GPx, glutathione peroxidase; L-homoarg, L-homoarginine; SDMA, symmetric dimethylarginine; NLR, Neutrophil/Lymphocyte ratio; CRP, C-reactive protein, and IL-6, interleukin-6. *P, P<0.05; **P, P<0.01; ***P, P<0.001.

Supplementary Table 3.4. Multiple regression analysis of cardiovascular markers and markers of oxidative stress, with an additional adjustment for inflammation in physically active and –inactive groups, respectively.

Physically active (n=84)												
	24h SBP (mmHg)			24h DBP (mmHg)			24h MAP (mmHg)			24h PP (mmHg)		
	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value
TBARS (mg/g Crn)	0.52	0.01 (-0.16; 0.18)	0.90	0.46	-0.006 (-0.19; 0.18)	0.95	0.50	0.001 (-0.18; 0.18)	0.99	0.35	0.04 (-0.16; 0.24)	0.72
tGSH (µM)	0.51	0.09 (-0.07; 0.26)	0.27	0.48	0.19 (0.02; 0.36)	0.034	0.52	0.15 (-0.01; 0.32)	0.075	0.31	-0.06 (-0.26; 0.14)	0.56
GR (nmol/min/ml)	0.53	-0.01 (-0.18; 0.15)	0.87	0.47	0.10 (-0.08; 0.27)	0.28	0.51	0.05 (-0.12; 0.22)	0.56	0.38	-0.16 (-0.36; 0.03)	0.098
GPx (nmol/min/ml)	0.53	0.06 (-0.12; 0.23)	0.52	0.47	0.09 (-0.09; 0.28)	0.33	0.52	0.08 (-0.10; 0.25)	0.38	0.35	0.0005 (-0.20; 0.20)	1.00
L-homoarg (µmol/l)	0.55	0.18 (0.01; 0.36)	0.039	0.50	0.21 (0.03; 0.39)	0.026	0.55	0.21 (0.03; 0.38)	0.023	0.35	0.08 (-0.13; 0.29)	0.45
SDMA (µmol/l)	0.53	0.09 (-0.09; 0.27)	0.32	0.47	0.09 (-0.10; 0.28)	0.36	0.52	0.09 (-0.09; 0.27)	0.32	0.35	0.07 (-0.14; 0.28)	0.53
Physically inactive (n=132)												
	24h SBP (mmHg)			24h DBP (mmHg)			24h MAP (mmHg)			24h PP (mmHg)		
	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value	Adj R^2	b* (95%CI)	p-value
TBARS (mg/g Crn)	0.40	-0.02 (-0.16; 0.13)	0.84	0.30	0.03 (-0.14; 0.19)	0.75	0.36	0.008 (-0.15; 0.16)	0.92	0.30	-0.06 (-0.22; 0.11)	0.50
tGSH (µM)	0.40	0.09 (-0.06; 0.24)	0.25	0.30	0.14 (-0.02; 0.30)	0.092	0.37	0.12 (-0.03; 0.27)	0.13	0.30	0.007 (-0.15; 0.17)	0.93
GR (nmol/min/ml)	0.39	0.05 (-0.09; 0.18)	0.51	0.30	0.05 (-0.09; 0.20)	0.47	0.36	0.05 (-0.10; 0.20)	0.47	0.28	0.02 (-0.13; 0.17)	0.82
GPx (nmol/min/ml)	0.40	0.09 (-0.05; 0.23)	0.22	0.30	0.05 (-0.10; 0.21)	0.49	0.36	0.07 (-0.07; 0.22)	0.33	0.30	0.10 (-0.05; 0.25)	0.19
L-homoarg (µmol/l)	0.39	-0.06 (-0.22; 0.11)	0.48	0.32	-0.10 (-0.27; 0.07)	0.27	0.28	0.01 (-0.25; 0.08)	0.91	0.37	-0.08 (-0.17; 0.19)	0.33
SDMA (µmol/l)	0.44	0.25 (0.10; 0.39)	0.001	0.35	0.20 (0.05; 0.36)	0.013	0.42	0.23 (0.08; 0.38)	0.003	0.32	0.21 (0.04; 0.37)	0.014

Models were adjusted for race, sex, age, waist circumference, cotinine, gamma-glutamyltransferase, glycated haemoglobin, cholesterol, estimated glomerular filtration rate, and C-reactive protein.

N, number of participants; 24h SBP, twenty-four hour systolic blood pressure; 24h DBP, twenty-four hour diastolic blood pressure; 24h MAP, twenty-four hour mean arterial pressure; 24h PP, twenty-four hour pulse pressure; Adj, adjusted; TBARS, thiobarbituric acid reactive substances; tGSH, total glutathione, GR, glutathione reductase; GPx, glutathione peroxidase; L-homoarg, L-homoarginine; and SDMA, symmetric dimethylarginine.

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SUMMARY AND CONCLUSIONS

1. Introduction and aim

Cardiovascular disease (CVD) incidence is on the rise [1] and is more prevalent in low- and middle income countries, such as South Africa, where individuals often remain untreated for non-communicable diseases [2]. Data from the 2011-2012South African National Health and Nutrition Examination Survey (SANHANES) showed that 52% of hypertensive adults in the United States of America were controlled, compared to only 8.5% controlled hypertensive adults in South Africa [3]. The authors stated that this may partly be due to cost of travel to healthcare services, poor adherence to medication use, and possibly ineffective medication, among other factors [3]. It is recommended that physical activity deserves top priority as it has been demonstrated in high-income countries to be effective at lowering the prevalence of CVD [1]. High intensity physical activity is needed to obtain an anti-inflammatory and anti-oxidant effect, resulting in increased nitric oxide synthesis capacity [4,5,6]. However, moderate physical activity seems more practical and easy to adhere to, especially in older populations. This is especially important as CVD prevalence increases with age [7]. Physical activity may be an affordable and effective manner by which CVD can be prevented and/or treated in conjunction with dietary and pharmacological regimens. In our study, only 3.57% of physically active participants achieved high intensity physical activity levels. Therefore, the results of this study are attributed to the advantages of moderate physical activity.

This study aimed to investigate the interplay of vascular function measures with oxidative stress, inflammation, and nitric oxide synthesis capacity in physically active and inactive South Africans. This chapter summarises the main findings of the study and reflects on the hypotheses. Results will be interpreted, discussed and compared with related literature, followed by conclusions and recommendations for future researchers investigating similar topics.

2. Hypotheses and main findings

We firstly hypothesised that: "Physically inactive South Africans will display worse vascular function (lower Windkessel compliance (Cwk), higher total peripheral resistance (TPR), twenty-four hour (24h) systolic blood pressure (SBP), 24h diastolic blood pressure (DBP), 24h mean arterial pressure (MAP), and 24h pulse pressure (PP)), oxidative stress (higher thiobarbituric acid reactive substances (TBARS), as well as lower total glutathione (tGSH), glutathione peroxidase (GPx), and glutathione reductase (GR)), inflammation (higher C-reactive protein (CRP), interleukin-6 (IL-6), neutrophil-lymphocyte ratio (NLR), and monocytes), and nitric oxide synthesis capacity (higher L-homoarginine, as well as lower asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)) profiles when compared to physically active individuals".

As illustrated in **Figure 4.1**, we found improved vascular function (Cwk) and nitric oxide synthesis capacity (L-homoarginine) in the physically active group compared to the physically inactive group. However, this hypothesis is only partially accepted, as inflammatory profiles were similar between groups and oxidative stress (TBARS) was higher in the physically active group compared to the physically inactive group.

We secondly hypothesised that "Markers of vascular function (Cwk, TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP) will adversely associate with markers of oxidative stress (TBARS, tGSH, GPx, and GR), inflammation (CRP, IL-6, NLR, and monocytes), and nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) in the physically inactive group. Furthermore, markers of vascular function (Cwk, TPR, 24h SBP, 24h DBP, 24h MAP, and 24h PP) will beneficially associate with markers of oxidative stress (TBARS, total glutathione, GPx, and GR), inflammation (CRP, interleukin-6, neutrophil-lymphocyte ratio, and monocytes), and nitric oxide synthesis capacity (L-homoarginine, ADMA, and SDMA) in the physically active group."

As hypothesised, we found beneficial associations between vascular function (24h SBP, 24h DBP, and 24h MAP) and nitric oxide synthesis capacity markers (L-homoarginine) in the physically active group with multiple regression analyses. In the same analyses, the physically inactive group showed that vascular function (24h SBP, 24h DBP, 24h MAP, and 24h PP) adversely associated with nitric oxide synthesis capacity (SDMA), as illustrated in **Figure 4.2**. However, the hypothesis can only be partially accepted. There were significant correlations of TPR and Cwk with oxidative stress and inflammation in partial regression analyses. However, significance was lost in multiple regression analyses. Unexpectedly, vascular function (24h DBP) inversely correlated with an endogenous anti-oxidant (tGSH) in the physically active group after full adjustments were made. Furthermore, adjustments for inflammation (CRP) in multiple regression indicated no change in results and proved that our findings are independent of inflammation.

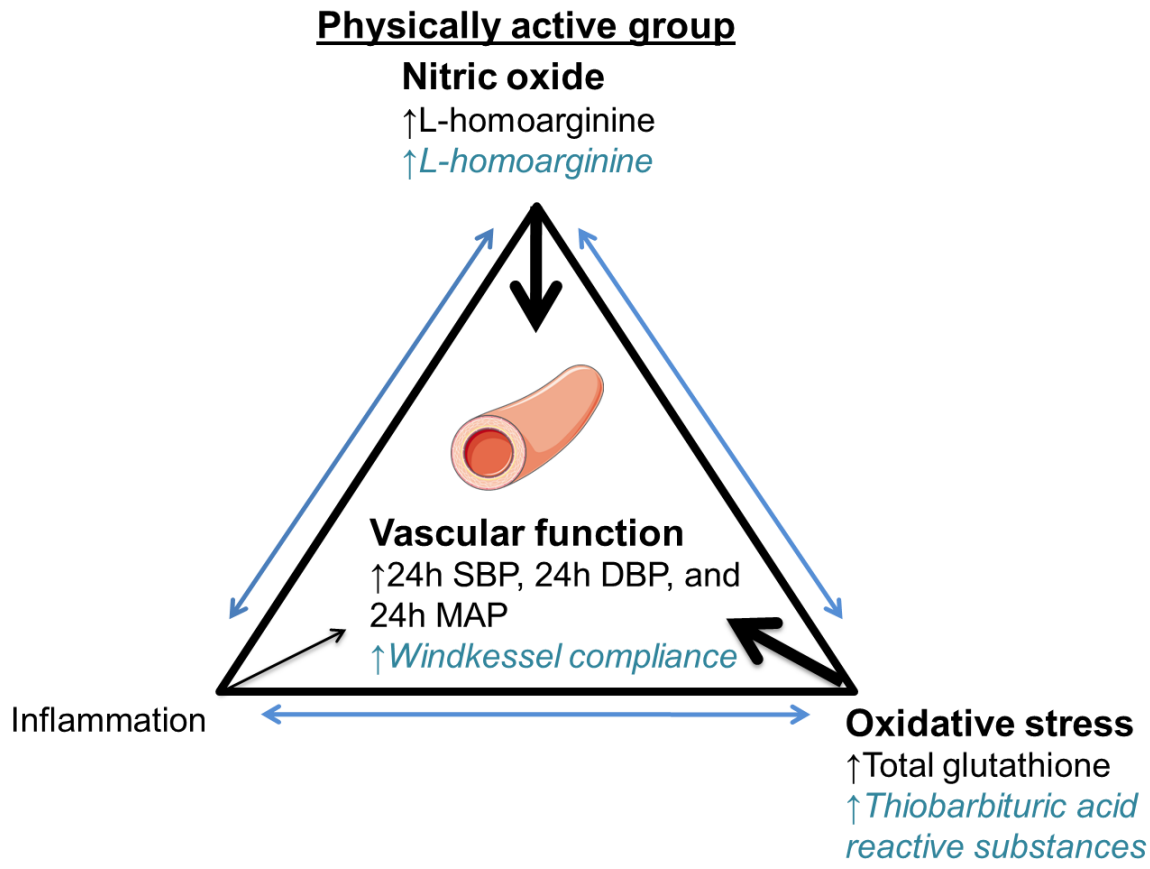

Figure 4.1: Associations of blood pressure with L-homoarginine and total glutathione as well as increased L-homoarginine and thiobarbituric acid reactive substances in the physically active group

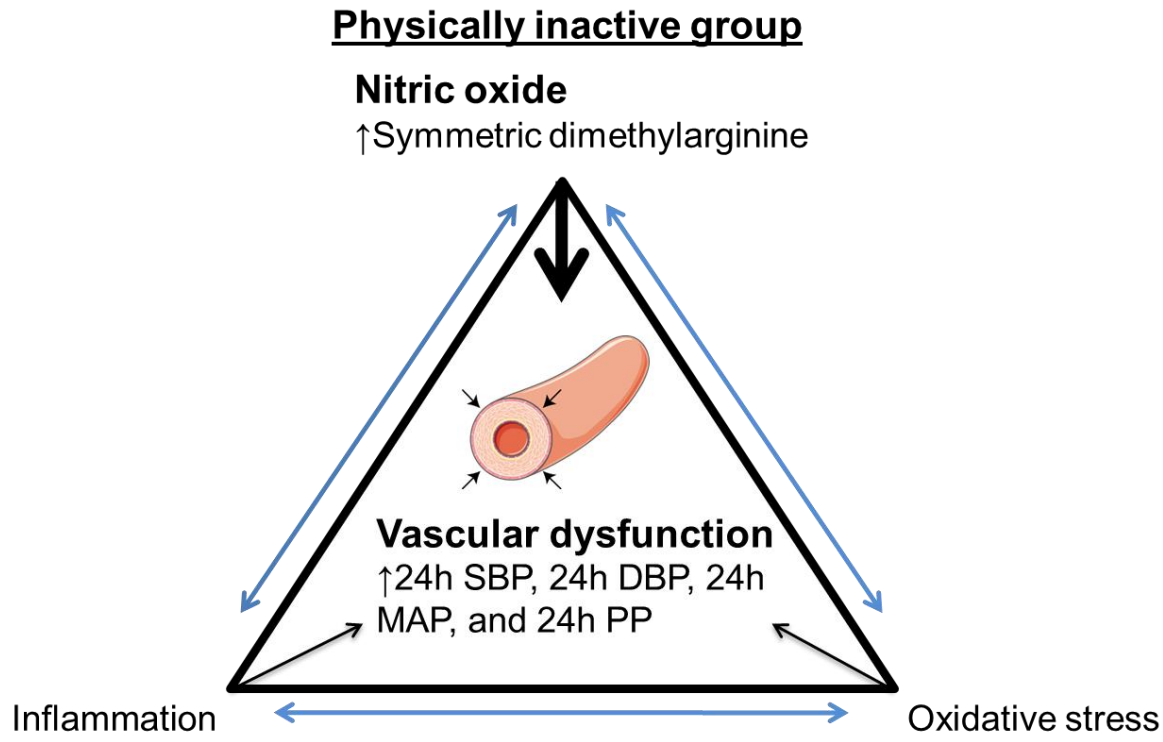

Figure 4.2: Associations of blood pressure with symmetric dimethylarginine in the physically inactive group

3. Reflection on hypotheses and comparison of main findings with relevant literature

3.1. Vascular function compared between physically active and inactive groups

When comparing the findings of the current study with the literature, there are both similarities and controversies. Higher Cwk found in the physically active group is in agreement with another study which demonstrated that master endurance athletes have superior vascular function determinants, such as increased Cwk and decreased TPR, when compared to their sedentary peers [8]. In our study, the majority of participants only achieved moderate and not high intensity physical activity levels as would be expected with endurance athletes. Nevertheless, we still indicated that even moderate physical activity resulted in higher arterial compliance. This may suggest that high levels of physical activity may not be a requirement for cardiovascular benefits. However, TPR was similar when comparing the physically active and inactive groups. According to Lee *et al.* [7], after 50 years of age, arterial stiffness adversely affects Windkessel compliance to a greater extent than TPR. In our study the mean age of the total group was 49.7 years and this may in part explain why Windkessel compliance rather than TPR was different between the groups.

We did not find any significant differences in 24h blood pressures between the physically active and inactive groups. A study on the same population found that hypertensive participants (blood pressure 140±17 / 93±10 mmHg) had significantly more (10.7%) sedentary hours per day compared to the normotensive group (blood pressure 117±10 / 79±8 mmHg) [9]. This also shows that it may possibly be more effective to focus on minimising sedentary hours than increasing the intensity of physical activity. Again, this may be more practical for older populations.

3.2 Oxidative stress and inflammation compared between physically active and inactive groups

In other studies, high intensity physical activity resulted in decreased oxidative stress and inflammation [4,6]. As presented in **Figure 4.1**, our study found increased levels of TBARS in the physically active group. This contradicts other research [10], but may be explained by the Hormesis theory [11]. The physically active participants may have increased oxidative stress (TBARS) from physical activity; however, oxidative stress levels are not yet high enough for the body to adapt by up-regulating endogenous anti-oxidant enzymes [11]. Additionally, increased TBARS in the physically active group could be explained by other factors, such as diet [12].

Similar waist circumferences between groups may also partly explain similarities in inflammatory profiles between groups, as abdominal adipose tissue distribution has an inflammatory effect on the vasculature [13]. It may also be that high intensity physical activity is needed to improve the inflammatory profile [4,5].

3.3 Nitric oxide synthesis capacity compared between physically active and inactive groups

We found that the physically active group has increased L-homoarginine, which is an analogue of L-arginine [14]. It is argued that homoarginine-mediated arginase inhibition will lead to increased levels of arginine, and a subsequent increase in nitric oxide synthesis [14,15]. The reference range for L-homoarginine in healthy humans is 2 to 3 mmol/l. However, other studies presented 1.41 to 5.00 mmol/l for men and 1.20 to 5.53 mmol/l for women [15]. Elevated levels of L-homoarginine are needed to obtain an arginase inhibiting effect which indicates that other mechanisms may also play a cardioprotective role [15]. L-homoarginine may also lower blood pressure by facilitating nitrate excretion or by acting as an alternative substrate for nitric oxide synthase [14]. Decreased nitric oxide plays a key role in vascular dysfunction and from our results it seems that even moderate physical activity addresses this problem, as illustrated in **Figure 4.1** and **Figure 4.2**. Similar to our study,

other studies have found that physical activity upregulates the L-arginine-nitric oxide pathway [16]. However, controversial results indicate that L-homoarginine is a weak substrate for nitric oxide synthase and may have a hypertensive effect by competing with arginine [15]. Limited studies could be found regarding increased L-homoarginine and physical activity.

3.4 Additional findings when comparing physically active and inactive groups

The physically active group included 18.3% less black participants than the inactive group. Similar results were found in another African cohort which demonstrated that black men did not only have more sedentary time, but also had a higher prevalence of hypertension when compared to white men [17]. In addition to these findings, a higher body mass index in the physically active group may be a result of increased muscle mass from physical activity, as a similar waist circumference between groups indicates similar amounts of abdominal adipose tissue.

The physically active group had lower GGT, which is a cardiovascular risk marker associated with oxidative stress and inflammation [18]. In agreement with our study, other research also shows that physically active individuals may have lower GGT levels [18]. Gamma-glutamyltransferase is further associated with alcohol abuse and increased levels of abdominal adipose tissue [18]. However, there were no differences in alcohol use or waist circumference between the groups in our study.

3.5 Associations of blood pressure with oxidative stress and inflammation

Instead of tGSH having a hypotensive effect as an anti-oxidant [19], **Figure 4.1** illustrated a positive association of 24h DBP with tGSH in the physically active group. Another SABPA study showed that black men have increased tGSH and L-arginine, despite having higher blood pressure, when compared to white men [20]. It was suggested that increased oxidative stress resulted in a subsequent upregulated redox status in black men [20]. Therefore, we did adjust for race in our study. In addition, the physically active group showed lower levels of GGT, which has the ability to degrade glutathione [18].

Cardiovascular variables did not associate with markers of inflammation in multiple regression analyses, therefore we repeated the multiple regression analyses, with an additional inclusion of CRP to the models. However, all associations between variables remained significant as with the previous multiple regression which showed that our findings are independent of inflammation. Other studies proved that high intensity physical activity

resulted in decreased inflammation [4,5]. In our study, moderate intensity physical activity was not efficient to achieve lower levels of inflammation.

3.6 Associations of blood pressure with nitric oxide synthesis capacity

The physically active group indicated that 24h SBP, 24h DBP, and 24h MAP correlated with L-homoarginine. However, with our first hypothesis we explained that L-homoarginine may have a hypotensive effect. The association between blood pressure and L-homoarginine may be a result of an upregulated nitric oxide synthesis system, as with the association between 24 DBP and tGSH. Research regarding L-homoarginine and its association with blood pressure are controversial [15]. The possibility exists that normal levels may have a hypotensive effect and that abnormal levels of L-homoarginine are associated with vascular dysfunction [15]. Research that found positive associations of blood pressure with L-homoarginine argued that L-homoarginine is a weak substrate for nitric oxide synthase which competes with L-arginine for nitric oxide production [15].

Moreover, in the physically inactive group, **Figure 4.2** showed that 24h SBP, 24h DBP, 24h MAP, and 24h PP related to SDMA. Research also revealed that SDMA is an indirect inhibitor of nitric oxide synthase by increasing the production of reactive oxygen species and thereby decreasing L-arginine transport from plasma into endothelial cells [21,22]. These results suggest that moderate physical activity may alter vascular function through modifications in nitric oxide synthesis capacity.

4. Chance and confounding

Various factors could have confounded the results of this study including the methodology, statistical analyses, and interpretation of results. Controversy exists over the mechanism and function of L-homoarginine [14,15,23]. However, in conjunction with other nitric oxide synthesis capacity markers such as ADMA and SDMA, and vascular function measures, the mechanism and function of L-homoarginine may become clearer. This sample group does not represent the entire South African population as all participants are from the Potchefstroom district in the North-West Province of South Africa. However, this study included black and white South African men and women with similar socio-economic status.

Regarding the results, the possibility of chance should be taken into consideration. We investigated all the statistical results from a physiological perspective, as all statistical significance does not necessarily indicate physiological significance. Therefore, possible physiological mechanisms were used to interpret the associations shown in this study. Several adjustments were made for possible confounders in regression analyses. Adjustments for confounders may have caused over- or underestimation of the associations

found between markers of vascular function with oxidative stress, inflammation, and nitric oxide synthesis capacity.

5. Recommendations for future research

- Future studies should aim to discover an optimal physical activity level reference range to improve vascular function, oxidative stress, inflammation, and nitric oxide synthesis capacity.
- The reference ranges and mechanisms by which physical activity improves vascular function, oxidative stress, inflammation, and nitric oxide synthesis should be investigated in black and white, male and female, as well as young and older populations, as these factors all affect the normal cardiovascular physiology.
- Future studies should include prospective and intervention studies to investigate the effect of habitual physical activity on vascular function, oxidative stress, inflammation, and nitric oxide synthesis capacity.
- Further studies need to include more nitric oxide synthesis capacity markers to explain the nitric oxide pathway regarding moderate physical activity. For instance, L-arginine and L-homoarginine should be included for more clarity regarding L-homoarginine and its role in exercise.
- From our study it is evident that nitric oxide is a mechanism by which moderate physical activity improves vascular function. Future studies may investigate whether L-arginine supplementation has additional vascular benefits in moderate physically active individuals.

6. Conclusions

In comparison to other studies, this study is unique as literature regarding the interplay between vascular function with oxidative stress, inflammation, and nitric oxide synthesis capacity in South Africans is limited. It is also evident from the literature that high intensity physical activity is needed to improve vascular function, oxidative stress, inflammation, and nitric oxide synthesis capacity [4,5,6]. However, we demonstrated that even moderate physical activity may alter vascular function (Cwk), oxidative stress (GGT), and nitric oxide synthesis capacity (L-homoarginine). In the physically active group, correlations of vascular function (24 SBP, 24h DBP, and 24 MAP) with nitric oxide synthesis capacity (L-homoarginine) were present, along with correlations between vascular function (24h DBP) with oxidative stress (tGSH). In the physically inactive group, vascular function (24h PP, 24h SBP, 24h DBP, and 24h MAP) negatively correlated with nitric oxide synthesis capacity (SDMA). This indicates a possible mechanism by which moderate physical activity mitigate the development of cardiovascular disease in a black and white South African population.

Government interventions should therefore aim to improve physical activity, even at moderate intensities, as a cost-effective manner to improve cardiovascular health in South Africa.

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Appendix A: Ethics approval

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ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)** on **02/11/2017**, the North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby **approves** your study as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Vascular function, oxidative stress and inflammation in South Africans with an active- and inactive lifestyle: The SABPA study

Study Leader/Supervisor: Prof CMC Mels
Student: E van Niekerk-20979134

Ethics number:

N	W	U	-	0	0	1	0	6	-	1	7	-	A	1
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study

Commencement date: 02/11/2017 **Risk:** Minimal

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

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 study leader (principle investigator) must report in the prescribed format to the NWU-RERC via HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-RERC and HREC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *HREC can be contacted for further information or any report templates via Ethics-HRECApply@nwu.ac.za or 018 299 1206.*

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

Yours sincerely,

Prof. Refilwe Phaswana-Mafuya
Chair NWU Research Ethics Regulatory Committee (RERC)

Appendix B: Socio-demographic questionnaire

General Health and Sociodemographic Questionnaire

PARTICIPANT NAME (initials , surname)

SEX ☐

RACE White ☐ Black ☐ Indian ☐ Coloured ☐

Date of BIRTH ☐☐ ☐☐ ☐☐☐☐

HOUSE N: P.BOX N: .....

STREET: .....

Post Code: .....TOWN.....

MOBILE phone number.....

P_DUR ☐☐☐ **Number of years staying in Potchefstroom.**

Question 1: Marital status.

MS_SI ☐ Unmarried

MS_SIP ☐ Unmarried, living with partner

MS_MA ☐ Married, living with “legal” wife/husband

MS_MAP ☐ Married, partner other than “legal” husband/wife

MS_DI ☐ Divorced, not living with new partner

MS_DIP ☐ Divorced, living with new partner

MS_WW ☐ Widow or widower, not living with new partner

MS_WWP ☐ Widow or widower, living with new partner

Question 2: Education

Still attending school?

SC_NOW ☐ Now ?

SC_LOC ☐☐☐☐☐ School or institution

EDU DI ☐ Completed DIPLOMA

EDU DE ☐ Completed DEGREE

Question 3: Past occupation.

P_HINS ☐ Long-lasting health problems

P_DUR ☐☐☐ Number of years

P_P_LOC ☐☐☐☐☐☐ Address

Question 4:

SALARY ☐ Employee receiving salary

S_FULL ☐ Full-time basis

S_PART ☐ Part-time basis

S_SUBE ☐☐☐ Persons subordinated to you

Question 5:

EDU DI ☐ DIPLOMA

EDU DE ☐ DEGREE

EDU WW ☐ Hours of work per week

Question 6: **(**Family members alive)

FH_F ☐ Father

FH_GFf ☐ Grandfather (father's side)

FH_GMf ☐ Grandmother (father's side)

FH_M ☐ Mother

FH_GFm ☐ Grandfather (mother's side)

FH_GMm ☐ Grandmother (mother's side)

FH_Ch ☐ Children

FH_GCh ☐ Grandchildren

FH_BSf ☐ Brothers or sisters of your father

FH_BSm ☐ Brothers or sisters of your mother

FH_BS ☐ Own brothers or sisters

Family history: Hypertension, Diabetes, Stroke, Heart attack?

.....

.....

.....

.....

.....

Question 7:

C_DIS ☐ Disease affecting your heart or blood vessels

C_COD1 Disease ☐☐ ☐☐☐☐ C_BMY1 Starting date ☐☐☐☐

C_EMY1 Date of cure ☐☐☐☐ C_NAGP1 Treating physician .....…*…….

C_COD2 Disease ☐☐ ☐☐☐☐ C_BMY2 Starting date ☐☐☐☐

C_EMY2 Date of cure ☐☐☐☐ C_NAGP2 Treating physician .....…*…….

C_COD3 Disease ☐☐ ☐☐☐☐ C_BMY3 Starting date ☐☐☐☐

C_EMY3 Date of cure ☐☐☐☐ C_NAGP3 Treating physician .....…*……..

C_COD4 Disease ☐☐ ☐☐☐☐ C_BMY4 Starting date ☐☐☐☐

C_EMY4 Date of cure ☐☐☐☐ C_NAGP4 Treating physician .....…*……..

Question 8:

K_DIS ☐ Diseases affecting your kidneys or urinary tract

K_COD1 Disease ☐☐ ☐☐☐☐ K_BMY1 Starting date ☐☐☐☐

K_EMY1 Date of cure ☐☐☐☐ K_NAGP1 Treating physician .....…*……

K_COD2 Disease ☐☐ ☐☐☐☐ K_BMY2 Starting date ☐☐☐☐

K_EMY2 Date of cure ☐☐☐☐ K_NAGP2 Treating physician .....…*……..

K_COD3 Disease ☐☐ ☐☐☐☐ K_BMY3 Starting date ☐☐☐☐

K_EMY3 Date of cure ☐☐☐☐ K_NAGP3 Treating physician .....…*……..

K_COD4 Disease ☐☐ ☐☐☐☐ K_BMY4 Starting date ☐☐☐☐

K_EMY4 Date of cure ☐☐☐☐ K_NAGP4 Treating physician .....…*……..

Question 9:

L_DIS ☐ Kidney stones or stones in you urinary tract

L_REPC ☐ Repeated pain attacks

L_EVAC ☐ Passed a stone with urine

L_OPER ☐ Surgical treatment

L_NOW ☐ Still suffering from kidney stones or stones in urinary tract

Question 10:

DIABET ☐ Diabetes

D_DIET ☐ Diet and avoiding sweet foodstuffs

D_ORAL ☐ Pills

D_INS ☐ Insulin

Question 11:

HYPERT ☐ Hypertension

HY_MY ☐☐☐☐ When ?

HY_Th ☐ Treatment

Question 12:

DISEAS ☐ Currently in good health

DS_CD1 Disease ☐☐ ☐☐☐☐ DS_BMY1 Starting date ☐☐☐☐

DS_EMY1 Date of cure ☐☐☐☐ DS_NAGP1.....……*…….. Treating physician

DS_CD2 Disease ☐☐ ☐☐☐☐ DS_BMY2 Starting date ☐☐☐☐

DS_EMY2 Date of cure ☐☐☐☐ DS_NAGP2 .....……*……. Treating physician

DS_CD3 Disease ☐☐ ☐☐☐☐ DS_BMY3 Starting date ☐☐☐☐

DS_EMY3 Date of cure ☐☐☐☐ DS_NAGP3 .....……*…….. Treating physician

DS_CD4 Disease ☐☐ ☐☐☐☐ DS_BMY4 Starting date ☐☐☐☐

DS_EMY4 Date of cure ☐☐☐☐ DS_NAGP4 .....……*……. Treating physician

DS_CD5 Disease ☐☐ ☐☐☐☐ DS_BMY5 Starting date ☐☐☐☐

DS_EMY5 Date of cure ☐☐☐☐ DS_NAGP5.....……*……. Treating physician

DS_CD6 Disease ☐☐ ☐☐☐☐ DS_BMY6 Starting date ☐☐☐☐

DS_EMY6 Date of cure ☐☐☐☐ DS_NAGP6.....……*…….. Treating physician

Question 13:

D_HYPT ☐ Drugs to lower blood pressure

DH_NOW ☐ Now ?

	Name of drug		Tablets/day/dosage
DH_CD1	☐☐☐☐☐☐	DH_DO1	☐☐ ☐
DH_CD2	☐☐☐☐☐☐	DH_DO2	☐☐ ☐
DH_CD3	☐☐☐☐☐☐	DH_DO3	☐☐ ☐
DH_CD4	☐☐☐☐☐☐	DH_DO4	☐☐ ☐

Question 14:

D_DIUR ☐ Diuretics

DD_NOW ☐ Now ?

	Name of drug		Tabets/day/dosage
DD_CD1	☐☐☐☐☐☐	DD_DO1	☐☐ ☐
DD_CD2	☐☐☐☐☐☐	DD_DO2	☐☐ ☐
DD_CD3	☐☐☐☐☐☐	DD_DO3	☐☐ ☐
DD_CD4	☐☐☐☐☐☐	DD_DO4	☐☐ ☐

Question 15:

D_ANAL ☐ Taking pain-killers

DA_YE ☐☐ How many years ?

DA_SAL ☐ Salicylic acid (Disprin)

DA_PAR ☐ Paracetemol (Grand-Pa)

DA_OTH ☐ Analgesic drugs for arthritis (Brufen)

	Name of drug		Units/week
DA_CD1	☐☐☐☐☐☐	DA_DO1	☐☐
DA_CD2	☐☐☐☐☐☐	DA_DO2	☐☐
DA_CD3	☐☐☐☐☐☐	DA_DO3	☐☐
DA_CD4	☐☐☐☐☐☐	DA_DO4	☐☐

Question 16:

DR_2WK ☐ Medication during last 2 weeks

	Name of medicine		Units/day
DR_CD1	☐☐☐☐☐☐☐☐	DR_DO1	☐☐
DR_CD2	☐☐☐☐☐☐☐☐	DR_DO2	☐☐
DR_CD3	☐☐☐☐☐☐☐☐	DR_DO3	☐☐
	Name of medicine		Units/day

DR_CD4 ☐☐ ☐☐ ☐☐☐☐ DR_DO4 ☐☐

Name of medicine Units/day

DR_CD5 ☐☐ ☐☐ ☐☐☐☐ DR_DO5 ☐☐

Name of medicine Units/day

DR_CD6 ☐☐ ☐☐ ☐☐☐☐ DR_DO6 ☐☐

Question 17:

T_NOW ☐ Currently smoking

T_CTf ☐☐ Cigarettes with filter per day

T_CT ☐☐ Cigarettes without filter per day

T_CTgt ☐☐☐ Grams of tobacco per day

T_PIgt ☐☐☐ Grams of tobacco per week for pipe

T_SCgr ☐☐ Small cigars per week

T_Cgar ☐☐ Cigars per week

T_AGE ☐☐ When started smoking ? (age)

T_INHA ☐ Inhalation

Question 18:

T_P_PAST ☐ Smoking in the past

T_P_1CDY ☐ At least one cigarette per day during one year

T_P_AGE ☐☐ Age at which participant quitted smoking

T_P_CTf ☐☐ Cigarettes with filter per day

T_P_CT ☐☐ Cigarettes without filter per day

T_P_CTgt ☐☐☐ Grams of tobacco per day

T_P_PIgt ☐☐☐ Grams of tobacco per week for pipe

T_P_SCgr ☐☐ Small cigars per week

T_P_Cgar ☐☐ Cigars per week

T_P_WHY ☐☐ Reason to stop smoking

Question 19:

E_NOW ☐ Current consumption alcoholic beverages

E_BEER ☐☐ Glasses of beer per day

E_TBEER ☐☐ Glasses of traditional beer per day

E_WINE ☐☐ Bottles of wine per week

E_THLOKd ☐☐ Boxes of Thlokwe per day

E_THLOKw ☐☐ Boxes of Thlokwe per week

E_SPIRITt ☐☐ Tot Spirits per day

E_SPIRITb ☐☐ Bottle of Spirits per week

E_LIQR ☐☐ Bottle of Liquor per week

E_AGE ☐☐ When started drinking alcohol regularly ? (age)

Question 20:

E_P_PAST ☐ Consumption of alcoholic beverages in the past

E_P_AGE ☐☐ When stopped ? (age)

E_BEER ☐☐ Glasses of beer per day

E_TBEER ☐☐ Glasses of traditional beer per day

E_WINE ☐☐ Bottles of wine per week

E_THLOKd ☐☐ Boxes of Thlokwe per day

E_THLOKw ☐☐ Boxes of Thlokwe per week

E_SPIRITt ☐☐ Tot Spirits per day

E_SPIRITb ☐☐ Bottle of Spirits per week

E_LIQR ☐☐ Bottle of Liquor per week

E_P_WHY ☐☐ Why stopped consuming alcoholic beverages ?

Question 21:

C_NOW ☐ Now consumption of caffeine-containing beverages

C_REG ☐☐ Cups of coffee

C_COKE ☐☐ Glasses of Coca-cola

C_OTH ☐☐ Other

C_TEA ☐ Tea

C_DECAF ☐ Decaffeinated coffee

C_DECA_N ☐☐ Number of cups of decaffeinated coffee per day

Question 22:

M-NOW ☐ Periods

Question 23:

DCCET ☐ Ever taken "the pill" ?

DC_NOW ☐ “The pill” now ?

DC_COD ☐☐☐☐☐☐ Name of "the pill"

DC_YE ☐☐☐☐ How long ? (years, months)

Question 24:

PR_PST ☐ Pregnant before

PR_N ☐☐ Number of pregnancies

PR_ABO ☐ Number of miscarriages

PR_LIB ☐ Children born alive

PR_STB ☐ Children stillborn

Question 25:

M_NOW ☐ Still periods

M_IRYE ☐☐☐☐ Since when irregular periods ?

M_DISYE ☐☐☐☐ Since when periods completely disappaeared ?

M_P_SPON ☐ Spontaneous disappearance

M_P_HYST ☐ Removal of only womb

M_HYSTYE ☐☐☐☐ Date (month/year)

M_P_OVRR ☐ Removal of only right ovary

M_OVRRYE ☐☐☐☐ Date (month/year)

M_P_OVRL ☐ Removal of only left ovary

M_P_OVR2 ☐ Removal of both ovaries

M_OVR2YE ☐☐☐☐ Date (month/year)

M_P_ORHR ☐ Removal of right ovary together with womb

M_ORHRYE ☐☐☐☐ Date (month/year)

M_P_OLHR ☐ Removal of left ovary together with womb

M_OLHRYE ☐☐☐☐ Date (month/year)

M_P_HRT ☐ Removal of both ovaries and womb

M_HRTstart ☐☐☐☐ Date (month/year)

MS_COD1 ☐☐☐☐ Underlying disease 1

MS_COD2 ☐☐☐☐ Underlying disease 2

MS_COD3 ☐☐☐☐ Underlying disease 3

M_P_DRUG ☐ Periods suppressed by taking "the pill"

MD_P_COD ☐☐ ☐☐☐☐ Name of "the pill"

MD_P_MN ☐☐☐ Number of months

Question 26:

E_EXCS ☐ Results sent only to yourself

R-EXGP ☐ Results sent only to your family doctor

E_S_GP ☐ Results sent to yourself and your family doctor

Question 27:

C-GP ☐ Consent to contact the subject's physician(s)

Appendix C: Instruction for Authors

(*European Journal of Preventive Cardiology*)

1. What do we publish?

1.1 Aims & Scope

Before submitting your manuscript to *European Journal of Preventive Cardiology*, please ensure you have read the Aims & Scope.

1.2 Article Types

European Journal of Preventive Cardiology publishes papers on all aspects of clinical and public health disciplines that address the causes and prevention of cardiovascular disease, as well as cardiovascular rehabilitation and exercise physiology.

The following are the various article types which the journal publishes. Authors must adhere to the page/word counts given here. The word counts cover all sections of a manuscript, including title, author names/affiliation, abstract, keywords, figure/table legends and references.

Full Research Articles
These should not exceed 5,000 words (including references, tables and figures, with each table or figure reducing that word count by 250). They may include up to a maximum of 6 figures and/or tables and up to 40 references. Research articles should be divided into the following sections: (1) Title page, (2) Abstract and up to six Keywords, (3) Introduction, (4) Methods, (5) Results, (6) Discussion, (7) Acknowledgements, (8) Funding, (9) Conflict of interest, (10) Authors' Contributions, (11) References, (12) Figure legends, (13) Appendices, (14) Tables, (15) Figures. The Abstract should be divided into the following sections 'Aims', 'Methods', 'Results' and 'Conclusion'; it should not exceed 250 words.

Clinical Practice/Education
Reviews and Issues for Debate articles, which focus on education in the clinical practice environment, are welcomed. These should not exceed 5,000 words (including references, tables and figures, with each table or figure reducing that word count by 250). They may include up to a maximum of 6 figures and/or tables and up to 30 references. A formal structured division is not required, but an Abstract (less than 250 words length) is necessary.

Review/Consensus Document/Position Papers:
The European Journal of Preventive Cardiology publishes a limited number of scholarly, comprehensive **Review Papers, Position Papers and Consensus Documents**, which may be invited or not.
Consensus Documents / Position Papers should be preferably endorsed by a Scientific Association, or Section or Working Group of an Association and therefore the Authors should acknowledge that they are writing on behalf of it. **Position Papers** are prepared by a single scientific body, while the Consensus Documents by more than one. They should summarise and critically evaluate research in the subject area, and should discuss implications for the future. These papers are expected to be around 10 journal pages long (ie. around 8,700 words, with each table or figure reducing that word count by 250), with an unstructured abstract (with no headings) which should not exceed 250 words, and they may include up to 45–50 references.

N.B. **Systematic reviews** should follow the format of full research articles and should be submitted as a Full Research article during the submission process.

Editorials
All editorials should be limited to 1,300 words (excluding references), with a maximum of 10 references. They do not require an abstract and may include one table and/or figure. In particular, the addition of one figure would be welcome and could add to the understanding and attractiveness of the article. The following different categories of editorials may be considered:
- **Invited Editorial.** Written upon invitation by the Editor, it is a comment to a research article and should discuss its results, compare them with the current literature and give a personal interpretation of the study.
- **Commentary.** This is a commentary on a topical item. It may be invited or not. When we receive more commentaries regarding a similar topic they may be gathered under the category of "Different viewpoints" in the index page. However, their labelling will remain "Commentary" in the title page so that they may be considered alone.
- **In the News.** This is a comment on recent events, congress or trials underway.

Short Reports
These reports should not exceed 1300 words and should comprise a Background section (≈100 words), Aims (≈50 words), Methods (≈300 words), Results (300 words) and Conclusion (250 words). The editorial team reserves the right to decide which of the tables/figures submitted are necessary. No abstract is required.

Research Letters
Research Letters based on original research findings are also allowed. They are less formal than a Short Report, i.e. not structured, and with no Abstract. The letter may include up to 1000 words, including a maximum of 10 references, and one figure and/or Table. While there should be no sub-headings, however, a short description of methods, results and conclusions is required.

Letters to the Editor
Letters to the Editor may regard comments to an article published in our journal in a previous issue. These letters should have a maximum of 3 authors, should not exceed 700 words and have a maximum of 5 references, including one reference to the article that they are about. We may ask for a reply from the authors of the original article and the letter and its reply will be published together.

Type of paper	Maximum number of pages	Maximum number of words	References
Full Research Paper	6 journal pages	5,000 words (with each table or figure reducing that word count by 250. Maximum of 6 figures and/or tables)	Up to 40 references
Clinical Practice/Education	6 journal pages	5,000 words (with each table or figure reducing	Up to 30 references

		that word count by 250. Maximum of 6 figures and/or tables)	
Review paper	10 journal pages	8,700 words (with each table or figure reducing that word count by 250)	Up to 45–50 references
Short report	2 journal pages	1,300 words (with each table or figure reducing that word count by 250)	
Commentary	2 journal pages	1,300 words (may include 1 figure and/or table)	Up to 10 references
Editorial	2 journal pages	1,300 words (may include 1 figure and/or table)	Up to 10 references
Research Letters	2 journal pages	1,000 words (may include 1 figure and/or table)	Up to 10 references
Letter to the Editor	1 journal page	700 words (with each table or figure reducing that word count by 250)	Up to 5 references

1.2.2 Special issues and sponsored supplements

If you have an idea for a special issue, or are interested in publishing a sponsored supplement, please contact the editorial office directly to discuss: ejpceditorialoffice@sagepub.com.

1.3 Writing your paper

Number pages consecutively, beginning with the title page. All Research papers must be arranged in sections under the headings and in the order indicated below (begin each on a separate page):

Title page

- The title page should carry the full title of the paper, consisting of no more than 20 words (only common abbreviations should be used if absolutely necessary); titles should be clear and brief, conveying the message of the paper.
- All authors' names: the full first name, middle name/initial (optional) and last name of each author should appear; if the work is to be attributed to a department or institution, its full name and location should be included. Persons listed as authors should be those who substantially contributed to the study's conception, design, and performance as per the guidance in section above.
- The affiliations of all the authors; when authors are affiliated to more than one institution, their names should be connected using a,b,c. These letters should follow the surname but precede the address; they should be used only for the second and subsequent addresses.
- Information about previous presentations of the whole or part of the work presented in the article.
- The sources of any support, for all authors, for the work in the form of grants, equipment, drugs, or any combination of these.
- Disclaimers, if any.

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- The peer review process as well as publication will be delayed if you do not provide up to date e-mail address, telephone and fax numbers.
- Word count: please list full word count (including references).

Structured abstract

The second page should carry an abstract not exceeding 250 words and should include sections on Background, Design, Methods, Results and Conclusions. Please list the abstract word count at the end of the abstract. Abstracts are required for Full Research Papers, Reviews and Clinical Practice/Education papers only (they are not required for Letters, Editorials and Commentaries). For Reviews, an abstract is required, which may be structured or unstructured.

Keywords

The abstract should be followed by a list of 3-10 keywords which will assist the cross-indexing of the article and will be published. The terms used should be from the Medical Subject Headings list of the Index Medicus.

Main text

Full papers of an experimental or observational nature should be divided into sections headed Introduction, Methods, Results and Discussion.

Use of abbreviations should be kept to an absolute minimum; abbreviations and abbreviated phrases should be written out at first mention followed by the abbreviation in parentheses. Avoid those not accepted by international bodies. Systéme International (SI) units should be used where appropriate.

Units: The Systéme International (SI)

The *European Journal of Preventive Cardiology* employs SI Units (see Quantities, Units, and Symbols, 2nd edn. London: The Royal Society of Medicine; 1975). All submitted papers should use this system, which should only be departed from where long-established clinical usage demands it (e.g. the measurement of blood pressure in mmHg). Where helpful, other units of measurement may be included in parentheses. Whenever possible, renin should be expressed in terms of the International Standard Renin Unit [Bangham et al.: Clin Sci 1975, 48(suppl):135s-159s]. Derived SI units may also be used, and for basic and derived units prefixes to denote multiples and submultiples may be used.

The SAGE Author Gateway has some general advice on how to get published, plus links to further resources.

1.3.1 Make your article discoverable

When writing up your paper, think about how you can make it discoverable. The title, keywords and abstract are key to ensuring readers find your article through search engines such as Google. For information and guidance on how best to title your article, write your abstract and select your keywords, have a look at this page on the Gateway: How to Help Readers Find Your Article Online.

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2. Editorial policies

2.1 Peer review policy

Submitted papers will undergo full peer review, and written comments, when available, will be returned with all refereed manuscripts. Reports for provisionally accepted papers will include a review by a statistician which authors will be required to follow when revising their manuscript. The final decision on the acceptance or rejection of a manuscript will be made by the Editors.

As part of the submission process you will be asked to provide the names of two peers who could be called upon to review your manuscript. Recommended reviewers should be experts in their fields and should be able to provide an objective assessment of the manuscript. Please be aware of any conflicts of interest when recommending reviewers. Examples of conflicts of interest include (but are not limited to) the below:

- The reviewer should have no prior knowledge of your submission,
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Please note that the Editors are not obliged to invite any recommended/opposed reviewers to assess your manuscript.

The Editor or members of the Editorial Board may occasionally submit their own manuscripts for possible publication in the journal. In these cases, the peer review process will be managed by alternative members of the Board and the submitting Editor/Board member will have no involvement in the decision-making process.

2.2 Authorship

Papers should only be submitted for consideration once consent is given by all contributing authors. Those submitting papers should carefully check that all those whose work contributed to the paper are acknowledged as contributing authors.
The list of authors should include all those who can legitimately claim authorship. This is all those who:

(i) Made a substantial contribution to the concept or design of the work; or acquisition, analysis or interpretation of data,
(ii) Drafted the article or revised it critically for important intellectual content,
(iii) Approved the version to be published,

(iv) Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Authors should meet the conditions of all of the points above. When a large, multicentre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship, although all contributors who do not meet the criteria for authorship should be listed in the Acknowledgments section. Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship guidelines for more information on authorship.

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It is necessary to complete an Author Responsibility Form as part of the submission process to stipulate the roles and responsibilities of each individual author who contributes to the manuscript. This information is required for submission – your manuscript will be returned if this is not completed.

Authors are also required to declare their contribution to the manuscript under a separate heading within the manuscript, as per categories specified in the Authorship Responsibility Form. This should appear after any other declarations and before your references. Please state author names as initials. For example:

Author contributions
AB and CD contributed to the conception or design of the work. EF contributed to the acquisition, analysis, or interpretation of data for the work. AB and CD drafted the manuscript. EF critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

2.3 Acknowledgements

All contributors who do not meet the criteria for authorship should be listed in an Acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, or a department chair who provided only general support.

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Individuals who provided writing assistance, e.g. from a specialist communications company, do not qualify as authors and so should be included in the Acknowledgements section. Authors must disclose any writing assistance – including the individual's name, company and level of input – and identify the entity that paid for this assistance. It is not necessary to disclose use of language polishing services.

Any acknowledgements should appear first at the end of your article prior to your Declaration of Conflicting Interests (if applicable), any notes and your References.

2.4 Funding

European Journal of Preventive Cardiology requires all authors to acknowledge their funding in a consistent fashion under a separate heading. Please visit the Funding Acknowledgements page on the SAGE Journal Author Gateway to confirm the format of the acknowledgment text in the event of funding, or state that: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

2.5 Declaration of conflicting interests

It is the policy of *European Journal of Preventive Cardiology* to require a declaration of conflicting interests from all authors enabling a statement to be carried within the paginated pages of all published articles.

Please ensure that a 'Declaration of Conflicting Interests' statement is included at the end of your manuscript, after any acknowledgements and prior to the references. If no conflict exists, please state that 'The Author(s) declare(s) that there is no conflict of interest'. For guidance on conflict of interest statements, please see the ICMJE recommendations here.

2.6 Research ethics and patient consent

Medical research involving human subjects must be conducted according to the World Medical Association Declaration of Helsinki.

Submitted manuscripts should conform to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, and all papers reporting animal and/or human studies must state in the methods section that the relevant Ethics Committee or Institutional Review Board provided (or waived) approval. Please ensure that you have provided the full name and institution of the review committee, in addition to the approval number.

For research articles, authors are also required to state in the methods section whether participants provided informed consent and whether the consent was written or verbal.

Information on informed consent to report individual cases or case series should be included in the manuscript text. A statement is required regarding whether written informed consent for patient information and images to be published was provided by the patient(s) or a legally authorized representative.

Please also refer to the ICMJE Recommendations for the Protection of Research Participants

2.7 Clinical trials

European Journal of Preventive Cardiology conforms to the ICMJE requirement that clinical trials are registered in a WHO-approved public trials registry at or before the time of first patient enrolment as a condition of consideration for publication. The trial registry name and URL, and registration number must be included at the end of the abstract.

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The relevant EQUATOR Network reporting guidelines should be followed depending on the type of study. For example, all randomized controlled trials submitted for publication should include a completed CONSORT flow chart as a cited figure and the completed CONSORT checklist should be uploaded with your submission as a supplementary file. Systematic reviews and meta-analyses should include the completed PRISMA flow chart as a cited figure and the completed PRISMA checklist should be uploaded with your submission as a supplementary file. The EQUATOR wizard can help you identify the appropriate guideline.

Other resources can be found at NLM's Research Reporting Guidelines and Initiatives.

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3. Publishing Policies

3.1 Publication ethics

SAGE is committed to upholding the integrity of the academic record. We encourage authors to refer to the Committee on Publication Ethics' International Standards for Authors and view the Publication Ethics page on the SAGE Author Gateway.

3.1.1 Plagiarism

European Journal of Preventive Cardiology and SAGE take issues of copyright infringement, plagiarism or other breaches of best practice in publication very seriously. We seek to protect the rights of our authors and we always investigate claims of plagiarism or misuse of published articles. Equally, we seek to protect the reputation of the journal against malpractice. Submitted articles may be checked with duplication-checking software. Where an article, for example, is found to have plagiarised other work or included third-party copyright material without permission or with insufficient acknowledgement, or where the authorship of the article is contested, we reserve the right to take action including, but not limited to: publishing an erratum or corrigendum (correction); retracting the article; taking up the matter with the head of department or dean of the author's institution and/or relevant academic bodies or societies; or taking appropriate legal action.

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4. Preparing your manuscript for submission

4.1 Formatting

The preferred format for your manuscript is Word. Please include all text and tables in a Word document when submitting your manuscript.

4.2 Artwork, figures and other graphics

Figures supplied in colour will appear in colour online and in the print issue. There is no charge for reproducing figures in colour in the printed version.

4.2.1 Illustrations

Size and presentation

When preparing illustrations, it should be kept in mind that they will be printed in the Journal either at column width (about 84mm wide) or at page width (about 170 mm wide). Figures should be professionally drawn and photographed; freehand or typewritten lettering is unacceptable. Photomicrographs must have internal scale markers. If photographs of people are used, their identities must be obscured or the picture must be accompanied by written permission to use the photograph. Photographs may be cropped or deleted at the discretion of the Editor.

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All illustrations must have legends. These should be typed using double spacing, beginning on a separate page, each with an Arabic numeral corresponding to the illustration to which it refers. All abbreviations used in the illustration must be defined in the legend. Internal scales should be explained, and staining methods for photomicrographs identified.

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All illustrations should have a label pasted on the back bearing the figure number, the title of the paper, the author's name and an arrow indicating the top of the figure. Avoid writing directly on the back of prints. Do not mount illustrations.

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Supply halftone illustrations (photographs) as sharp, glossy, black-and-white prints, preferably to a width of 84mm or, when the illustration demands it, to a width of 170mm.

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Artwork should be submitted either as glossy prints or as high-quality laser prints; dot-matrix printers do not produce artwork suitable for publication.

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Each table should be typed on a separate sheet in double spacing. Tables should not be submitted as photographs. Each table MUST have a title and should be assigned an arabic numeral, e.g. (Table 3). Vertical rules should not be used. Tables should not duplicate the content of the text. Each table should consist of at least two columns.

Table headings

If applicable, table headings should indicate whether the figures used represent percentages, by (%) after the figure, or units. Columns should always have headings.

Table footnotes

Information should be listed in the following order:

- Abbreviations and symbols should be defined in the order in which they appear in the table (reading across each line rather than down columns); spell out ALL abbreviations and symbols used in the table, even if they have already been listed in previous tables or the text itself when giving a key, use a comma rather than =, e.g. H, hypertensive NOT H=hypertensive.
- Any additional comments should follow the explanation of abbreviations and symbols.
- Keys to the P values should be listed in the following order (note the use of asterisks for probability): *P<0.05, **P<0.01, ***P<0.001; asterisks are the only symbols that should be used with P values; DO NOT use @ or #.

Checklist for data in tables

- the data are consistent with those cited in the relevant parts in the text,
- totals add up correctly,
- percentages have been calculated correctly.

For guidance on the preparation of illustrations, pictures and graphs in electronic format, please visit SAGE's Manuscript Submission Guidelines.

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This journal is able to host additional materials online (e.g. datasets, podcasts, videos, images etc) alongside the full-text of the article. For more information please refer to our guidelines on submitting supplementary files.

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Corresponding author:
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5. Submitting your manuscript

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6. On acceptance and publication

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7. Further information

Any correspondence, queries or additional requests for information on the manuscript submission process should be sent to the European Journal of Preventive Cardiology editorial office as follows:

Rosemary Allpress, Editorial Assistant: EJPCEditorialoffice@sagepub.com

Appendix D: Language editor certificate

Declaration

This is to declare that I, Annette L Combrink, accredited language editor and translator of the South African Translators' Institute, have language-edited the dissertation

by

E van Niekerk

with the title

Vascular function, oxidative stress and inflammation in South Africans with an active and inactive lifestyle: The SABPA study

Prof Annette L Combrink
Accredited translator and language editor
South African Translators' Institute
Membership No. 1000356
Date: 23 October 2018

Appendix E: Turn-it-in report

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Publication

8	Mels, Catharina M. C., Ilisma Loots, Edzard Schwedhelm, Dorothee Atzler, Rainer H. Böger, and Aletta E. Schutte. "Nitric oxide synthesis capacity, ambulatory blood pressure and end organ damage in a black and white population: the SABPA study", Amino Acids, 2015. Publication	1%
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