Serum selenium levels, the selenoprotein glutathione peroxidase and cardiovascular function in black South Africans

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To my promoter Prof Carina Mels and co-promoters Prof Alta Schutte and Prof Johannes van Rooyen, thank you for all the guidance, support, patience and effort to help me write this thesis to the best of my abilities. A special thanks for all the extra after-hours you spent, I appreciate that very much. I could not have asked for a better team.

To Dr Wayne Smith - thank you for your time, support and guidance as co-author in the second article (Chapter 3).

I would like to thank all the participants, researchers, and staff who contributed to the SABPA study, the African-PREDICT study and the PURE-study.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged.*

A very special thanks to my mom, brother, Callie, family and friends, thank you for all the support, love and guidance throughout this journey.

I would also like to specially thank God for giving me the talent and opportunity to be able to complete this thesis.

* *Any opinion, findings, and conclusions or recommendations expressed in this thesis are those of the authors, and therefore, the NRF does not accept any liability in this regard.*

PREFACE

This thesis is presented in article format and consists of three peer-reviewed published or submitted manuscripts (presented in Chapters 3, 4 and 5), as approved by the North-West University's guidelines for postgraduate studies. The layout of this thesis is as follows:

Chapter 1: This chapter offers a general overview of the relevant literature. The motivation, aims, objectives and hypotheses are also included in this chapter.

Chapter 2: A detailed description of the methodology of the three different studies used in this Ph.D. thesis is described in this chapter. These studies include the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) study and the Prospective Urban and Rural Epidemiology (PURE) study.

Chapter 3: The first article explores the link between serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection in schoolteachers of the SABPA study. These results were published in the journal "Food Research International" (2018). *Swart R, Schutte AE, van Rooyen JM, Mels CMC (2018) Serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study. Food Res. Int. 104:69-76. doi 10.1016/j.foodres.2017.06.054.*

Chapter 4: The second article investigated the relationship of selenium and glutathione peroxidase (GPx) on the micro- and macro-vasculature of a young bi-ethnic population from the African-PREDICT study. These results are accepted in the Journal of the American College of Nutrition (2019).

Chapter 5: The final article investigated the association between selenium and large artery structure and function over ten years in black adults of the PURE study. These results were published in the European Journal of Nutrition (2018). *Swart R, Schutte AE, van Rooyen JM, Mels CMC (2018) Selenium and large artery structure and function: a 10-year prospective study. Eur J Nutr. https://doi.org/10.1007/s00394-018-1875-y.*

Chapter 6: The final chapter consists of concluding remarks and recommendations on all three articles.

The promoter and co-promoters were included as co-authors in each manuscript, together with a collaborator, Dr Wayne Smith, who provided additional input in the second manuscript. The first author, namely the Ph.D. candidate, was responsible for the planning, writing and compiling of the manuscript, which included literature research about the topic, writing and submitting an ethics application as well as statistical analyses. All co-authors gave their consent that the manuscripts could be included in this thesis. The relevant references are provided at the end of each chapter. Each manuscript was prepared according to the instructions to authors of the respective journals as summarized at the beginning of each manuscript. In order to ensure uniformity throughout the thesis, the Vancouver reference style was used throughout. Permission to use Figure 5 in Chapter 1 was granted by the author of the original article. Permission to use Figures 6 and 7 were granted by the Agricultural Research Council of South Africa. Other images were compiled by the Ph.D. candidate from images obtained from Servier Medical Art (https://smart.servier.com).

SUMMARY

Motivation

In especially black South Africans non-communicable diseases such as hypertension and cardiovascular disease (CVD) have become an increasing burden with increased morbidity and mortality. Currently South Africa is undergoing rapid urbanization which involves a nutrition transition that may be accompanied by micronutrient malnutrition. Selenium, an important micronutrient, is known to exert indirect antioxidant functions and thereby has beneficial effects in lowering inflammation and endothelial dysfunction through the selenoprotein, glutathione peroxidase (GPx). It was previously shown that a black South African black tend to have both lower selenium and GPx levels when compared to whites. Low selenium levels and expression of GPx may therefore increase oxidative stress, inflammation, endothelial dysfunction and consequently lead to the development of increased arterial stiffness, atherosclerosis and hypertension. To our best knowledge, studies investigating the associations of serum selenium and GPx activity, blood pressure, vascular resistance, arterial compliance, arterial stiffness, measures of the microvasculature as well as measures of large artery structure are limited in especially black populations.

Aim

The central aim of this study was to determine whether serum selenium levels and GPx activity relate with estimates of cardiovascular structure and function in different black South African cohorts.

Methods

Three different study populations were used in the thesis, including the SABPA study, the African-PREDICT study and the PURE study. All the data were collected according to standardised methods. The participants completed questionnaires and anthropometric, cardiovascular and biochemical measurements were performed. In the cross-sectional SABPA study, serum selenium, GPx activity, ambulatory blood pressure and arterial stiffness of 200 black and 209 white school teachers (aged 20-65 years) were measured. In the African-PREDICT study, which is also a cross-

sectional study, 394 young healthy adults (20-30 years of age) were included. We determined serum selenium, GPx activity, microvascular measures (central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriolar-to-venular ratio (AVR) and estimated glomerular filtration rate (eGFR)) and macrovascular measures (pulse wave velocity (PWV), 24h pulse pressure (PP) and augmentation index (AIx)). The PURE study was a longitudinal study, with 10-year follow-up data, which included measurements from baseline (N=987) and follow-up (N=718) of black adults from rural and urban areas in the North West province of South Africa. We measured serum selenium and blood pressure at baseline and carotid intima media thickness (IMT), cross-sectional wall area (CSWA), carotid-femoral pulse wave velocity (c-fPWV) and blood pressure at follow-up.

Results and Conclusions

In the first manuscript we found that serum selenium levels were significantly lower in black compared to white school teachers, independent of sex ($p<0.001$), with 10% of black men and 20% of black women being selenium deficient (<8 µg/100 ml). Inverse associations of 24h systolic blood pressure (SBP) ($\beta=-0.19$; $p=0.039$) and 24h diastolic blood pressure (DBP) ($\beta=-0.21$; $p=0.029$) with selenium were found only in white men. An inverse association between carotid-dorsalis pedis pulse wave velocity (c-dPWV) and GPx activity ($\beta=-0.23$; $p=0.017$) were also found in the same group. In this manuscript, we concluded that, lower serum selenium levels in black populations from the same geographical region as their white counterparts may have an impact on the loss of the vascular protective effects of selenium and selenoproteins such as GPx.

In the second manuscript, we found vascular protective associations between serum selenium and a microvascular measure (AVR ($\beta=0.23$; $p=0.036$)) in black women and with a macrovascular measure (24h PP ($\beta=-0.15$; $p=0.048$)) in white women. In turn, GPx activity also showed a protective association with a microvascular measure (eGFR) in white men ($\beta=0.23$; $p=0.035$), as well as with macrovascular measures (AIx, PP) in the black ($\beta=-0.25$; $p=0.027$) and white men ($\beta=-0.22$; $p=0.035$), and black women ($\beta=-0.32$; $p=0.001$). Therefore, our findings suggest a protective role for the micronutrient selenium and GPx on both the micro- and macro-vasculature in young, healthy bi-ethnic men and women.

In the final manuscript we found that c-fPWV was negatively associated with baseline selenium (β=-0.09; p=0.016) after ten years in the normal selenium group. In the normal selenium group baseline (but not ten-year) blood pressure also associated negatively with baseline selenium (β=-0.09; p=0.007). Both IMT (β=0.12; p=0.001) and CSWA (β=0.10; p=0.003) associated positively with baseline selenium in the total group, normal and selenium deficient groups after ten years. In this manuscript we concluded that a long-term vascular protective association of selenium on arterial stiffness and blood pressure in black Africans with normal selenium levels were found, supporting the notion that selenium fulfils a vascular protective role. However, in contrast, we found a potentially detrimental association between selenium and carotid wall thickness, particularly evident in individuals within the highest quartile of serum selenium.

General Conclusions

In three different study populations we found consistently that selenium and GPx played a vascular protective role in these population groups from a healthy population between 20-30 years, before the onset of CVDs, a middle-aged population group (aged between 20-65 years) with >50% prevalence of hypertension and an older population group of >35 years where the onset of CVDs is evident over ten years (>50% prevalence of hypertension). In contrast, we found a possible detrimental association between selenium and carotid wall thickness, particularly evident in individuals within the highest quartile of serum selenium. However, our findings provide vascular protective associations of selenium and GPx. We suggest that it may be beneficial to ensure optimal selenium status to maintain its health effects and delay or avoid vascular deterioration and eventually CVD in later life. This may lead to preventative strategies to combat the high prevalence of CVDs in black South Africans.

Keywords: Selenium, glutathione peroxidase activity, cardiovascular disease, blood pressure, arterial stiffness, microvasculature, atherosclerosis, blacks, adults.

AFFIRMATION BY AUTHORS

The following researchers contributed to making this study possible:

Ms R Swart

Miss R Swart was responsible for the planning, writing and composition of the manuscript. This included literature research about the topic, writing and submitting an ethics application as well as cleaning of data and statistical analyses. She was involved in clinical measurements in research projects including the African-PREDICT study and PURE study.

Prof CMC Mels (promoter), Prof AE Schutte and Prof JM van Rooyen (co-promoters)

Played an important role in giving recommendations for the framework, writing and composition of the manuscript, methodology as well as formulation of the tables and figures. They also played a role in formulating the conclusions, and they also supervised the statistical analyses (Chapters 3-5). Prof Mels played an important part in obtaining funding for this project.

Dr W Smith

Gave recommendations for the writing of the manuscript and formulation of tables and figures as well as conclusions (Chapter 4).

The statement mentioned below is intended for the co-authors to confirm their roles in the study and thus giving their permission that the manuscripts may form part of this thesis.

Hereby, I declare that I approved the aforementioned manuscripts and that my role in this study as stated above is representative of my actual contribution. I also give my consent that these manuscripts may be used as part of the PhD thesis of Ms R Swart.

_____	_____	_____	_____
Prof CMC Mels	Prof AE Schutte	Prof JM van Rooyen	Dr W Smith

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LIST OF ABBREVIATIONS

ABPM	Ambulatory blood pressure monitoring
African-PREDICT	African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension
AIx	Augmentation index
ANCOVA	Analysis of covariance
AVR	Arteriolar-to-venular ratio
BMI	Body mass index
BP	Blood pressure
c-dPWV	Carotid-dorsalis pedis pulse wave velocity
c-fPWV	Carotid-femoral pulse wave velocity
cm	Centimetre
cPP	Central pulse pressure
CRP	C-reactive protein
CRAE	Central retinal artery equivalent
CRVE	Central retinal vein equivalent
CSWA	Cross-sectional wall area
CVD	Cardiovascular disease
Cwk	Windkessel compliance
DBP	Diastolic blood pressure
EDTA	Ethylenediamine-tetra-acetic acid

eGFR	Estimated glomerular filtration rate
Et al.	Et alia (and others)
GGT	Gamma-glutamyltransferase
GPx	Glutathione peroxidase
HbA1c	Glycated haemoglobin
HDL-C	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
IL-6	Interleukin-6
IMT	Intima media thickness
IMTf	Intima-media thickness of the far wall
IMTn	Intima-media thickness of the near wall
kg	Kilogram
L	Litre
LDL-C	Low-density lipoprotein cholesterol
m	Meter
m^2	Meters squared
m/s	Meters per second
MAP	Mean arterial pressure
mmHg	Milli-meters of mercury
mmol/L	Milli-mole per litre
MU	Measuring units

N	Number of
NaF	Sodium fluoride
NCD	Non-communicable disease
NRF	National Research Foundation
PP	Pulse pressure
PURE	Prospective Urban and Rural Epidemiology
PWV	Pulse wave velocity
r	Regression coefficient
R^2	Relative predictive power of a model
RDA	Reference daily intake
ROS	Reactive oxygen species
SABPA	Sympathetic activity and Ambulatory Blood Pressure in Africans
SBP	Systolic blood pressure
SD	Standard deviation
SePP	Selenoprotein P
SNP	Single nucleotide polymorphisms
TPR	Total peripheral resistance
U/l	Units per litre
VIF	Variance inflation factor

CHAPTER 1:
LITERATURE OVERVIEW

1.1 General Introduction

Hypertension is the primary risk factor for the development of cardiovascular disease (CVD) (1). The incidence of hypertension is alarming both globally and in sub-Saharan Africa, where 1.13 billion individuals globally had hypertension in 2015 (2).

It is suggested that the increased incidence in hypertension and CVD in South Africa may be due to rapid urbanisation (3). Urbanisation is accompanied by a nutrition transition, which includes changes from a more traditional diet which is high in fibre and low in fat (4) to high-energy density foods that are more palatable. It was indicated by Vorster *et al*. in 2011 that South Africa is in the nutrition-related non-communicable disease (NCD) phase of the nutrition transition, which may be accompanied by micronutrient malnutrition (5). Selenium, amongst others, is an important micronutrient with antioxidant properties (6).

Selenium helps to maximize biological functions including thyroid hormone metabolism, the immune system and antioxidant defence systems such as glutathione peroxidase (GPx) activity (7). GPx is involved in maintaining cellular redox balance by decreasing the circulating levels of hydrogen peroxide as well as lipid- and phospholipid hydroperoxides (8). Serum levels of selenium are dependent on dietary selenium intake (9), with meat, Brazil nuts, intestines, seafood, cereals, cheese and milk being selenium rich dietary sources (10). Selenium levels in plant foods are dependent on the levels of selenium in the soil (11). In this regard, studies indicated that the soil in regions of Africa may be selenium deficient (12-17).

Evidence from a meta-analysis showed that randomized controlled trials on the association of selenium with CVD are inconclusive. This may be due to selenium supplementation given in combination with other vitamins and minerals as well as using small population groups (18). However, in a recent meta-analysis conducted by Zhang et al. (2016) (19), the results of 16 prospective observational studies indicated a protective role for selenium, in a narrow selenium range (55-145 µg/l), against the development of CVD.

Different physiological mechanisms may explain the association of low selenium levels with the development of CVD. One of these mechanisms may involve oxidative stress, with strong evidence that oxidative stress is involved with the development of CVD (20-23). Oxidative stress is associated with increased inflammation and oxidation of low-density lipoprotein cholesterol (LDL-C) (24-26). This may lead to endothelial dysfunction and the development of atherosclerotic lesions (27, 28). Oxidative stress-induced endothelial dysfunction is also linked to changes in the microvasculature (such as retinal vessel calibres and renal function) and the macrovasculature (arterial stiffness and atherosclerosis), which may contribute to the increased incidence of hypertension and CVD (29, 30).

Since most of the previous studies investigating the relationship between selenium and GPx and vascular protective effects were done in mostly American and European populations, it is unclear whether selenium and GPx play vascular protective roles from an early age before the onset of CVD and in black populations. In this chapter, a broad overview of the literature will be provided, while focusing on the biology of selenium and GPx activity and their effects on the microvasculature, arterial stiffness, atherosclerosis as well as the link with hypertension and CVD.

1.2 Literature Overview

1.2.1 Hypertension and cardiovascular diseases in sub-Saharan Africa

Globally adults with high blood pressure increased from 594 million people in 1975 to 1.13 billion people in 2015 (2). The total number of adults with hypertension in high-income countries decreased from 1975, but in low- and middle-income countries in sub-Saharan Africa, the incidence of NCD increased (2). In 2015, sub-Saharan Africa was among a few other countries (including southern Asia, central and eastern Europe) that had the highest mean blood pressures (2) **(Figure 1-1)**. Hypertension and CVDs are ranked as some of the major causes of death in Africa, and it is estimated that the prevalence of hypertension will be around 216.8 million by the year 2030 (31). In addition to the high prevalence of hypertension it was reported that only around 7% of the hypertensive population in sub-Saharan Africa had controlled hypertension (32).

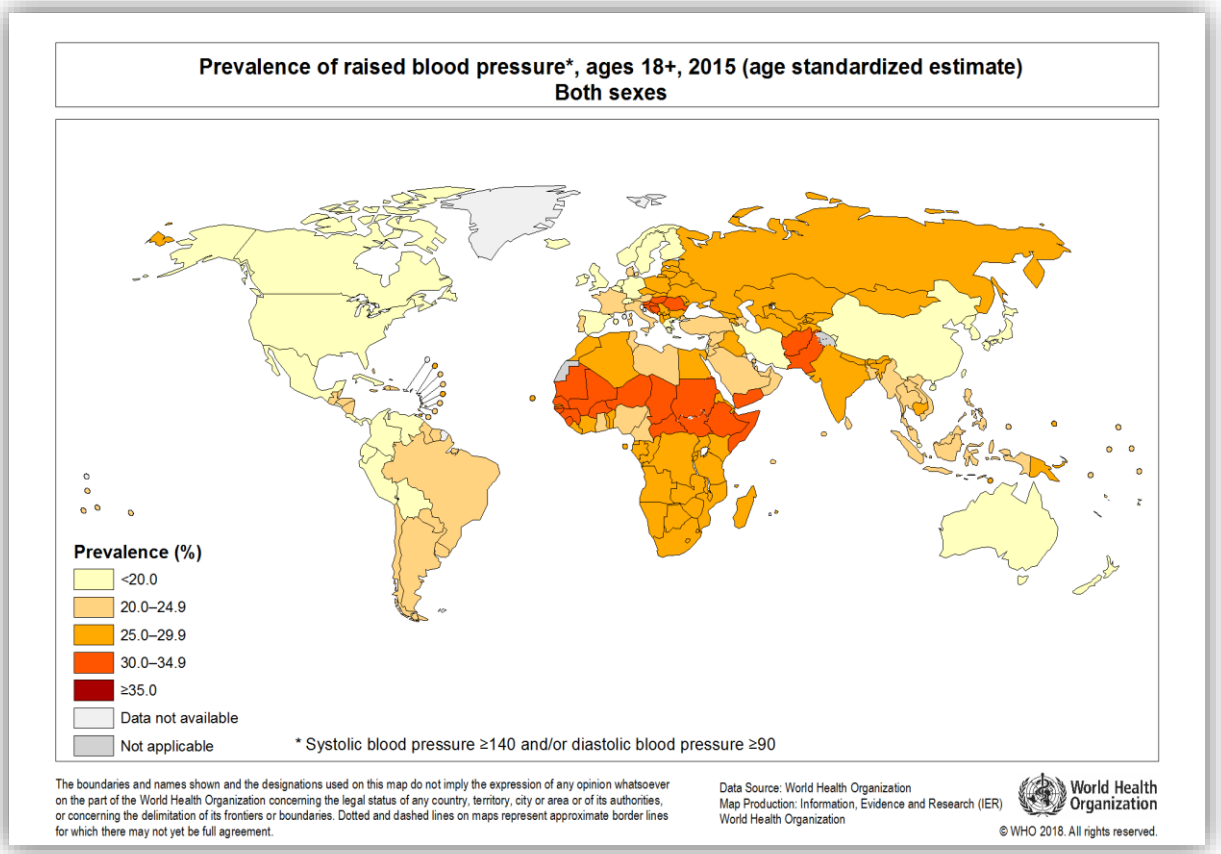

Figure 1-1. ***Prevalence of raised blood pressure in ages higher than 18 years, in 2015 for men and women*** *(Adapted from WHO website) (33)*.

In addition to the CVD burden, various countries in Africa face a double burden of nutrition–related diseases, where under- and over-nutrition are evident in these populations (5). In Africa, there has been an increase in non-communicable diseases, which may be due to the rapid socio-economic development and urbanization, which are related to the nutrition transition experienced in these population groups (3). In South Africa, the nutrition transition involves changes in dietary patterns by making use of more palatable and convenient foods rather than traditional foods that are low in fat and high in fibre (5). This includes more energy-dense foods such as sweetened beverages, sugar, fats and more products of animal origin such as red meat and lower plant protein sources (34, 35). These changes in dietary patterns increase the risk for the development of obesity and NCD in South Africa (34, 36). Deficiencies of important micronutrients such

as selenium, with antioxidant properties, may lead to increased oxidative stress, which is associated with the development of NCD such as CVDs, cancer, diabetes and chronic lung disease (5).

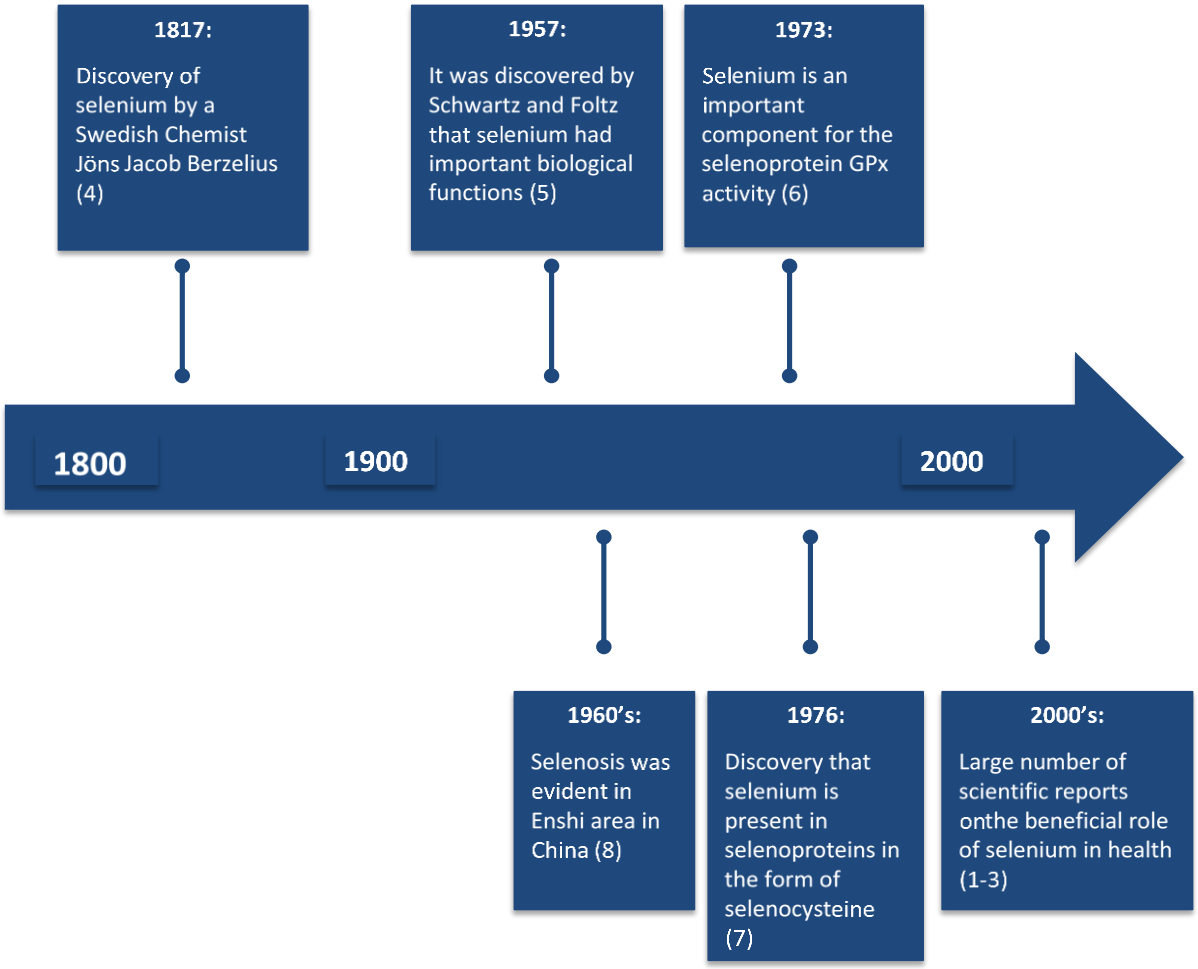

Figure 1-2. ***Timeline of selenium*** *(Adapted from Tan et al. (2018) (37))*

1.2.2 Biology of selenium

Selenium, the 34th element on the periodic table (^{34}Se), is a non-metal trace element (11). In 1817, selenium was discovered by Jöns Jacob Berzelius, a Swedish chemist, and he named selenium after the Goddess of the moon, Selene (38). Selenium was first seen as a toxin; however, in 1957 it was discovered by Schwartz and Foltz that selenium had important biological functions (39). These biological functions include its involvement in the antioxidant defence system, where it was shown in the early 1970s, that selenium is

an important component for the selenoprotein, GPx (40) **(Figure 1-2)**. Selenium also exerts important functions on the immune system, thyroid hormone metabolism and the cardiovascular system (41, 42).**Selenoprotein synthesis**

In **Figure 1-3**, the selenium metabolism of organic and inorganic dietary forms is illustrated (43). There are different chemical forms of dietary selenium including selenite, selenate, selenomethionine and selenocysteine (44-46). The main organic forms of selenium are obtained from animal and plant sources, which then provide selenium as selenomethionine, selenocysteine and selenium-methylselenocysteine (43). The inorganic dietary forms of selenium, selenite and selenate (47), are reduced by thioredoxin and thioredoxin reductase. The inorganic forms can also be converted by glutathione disulphide to selenodiglutathione. It is then reduced to glutathioselenol by glutathione reductase. In a reaction with glutathione disulphide it is converted to hydrogen selenide. Selenoproteins are broken down to hydrogen selenide by lyases and it can then be converted to selenophosphate by selenophosphate synthase. Selenocysteine is converted by selenocysteine synthase for the incorporation into selenoproteins. Hydrogen selenide can be converted by methyltransferase to methylated metabolites that are excreted in urine, faeces and breath (43).

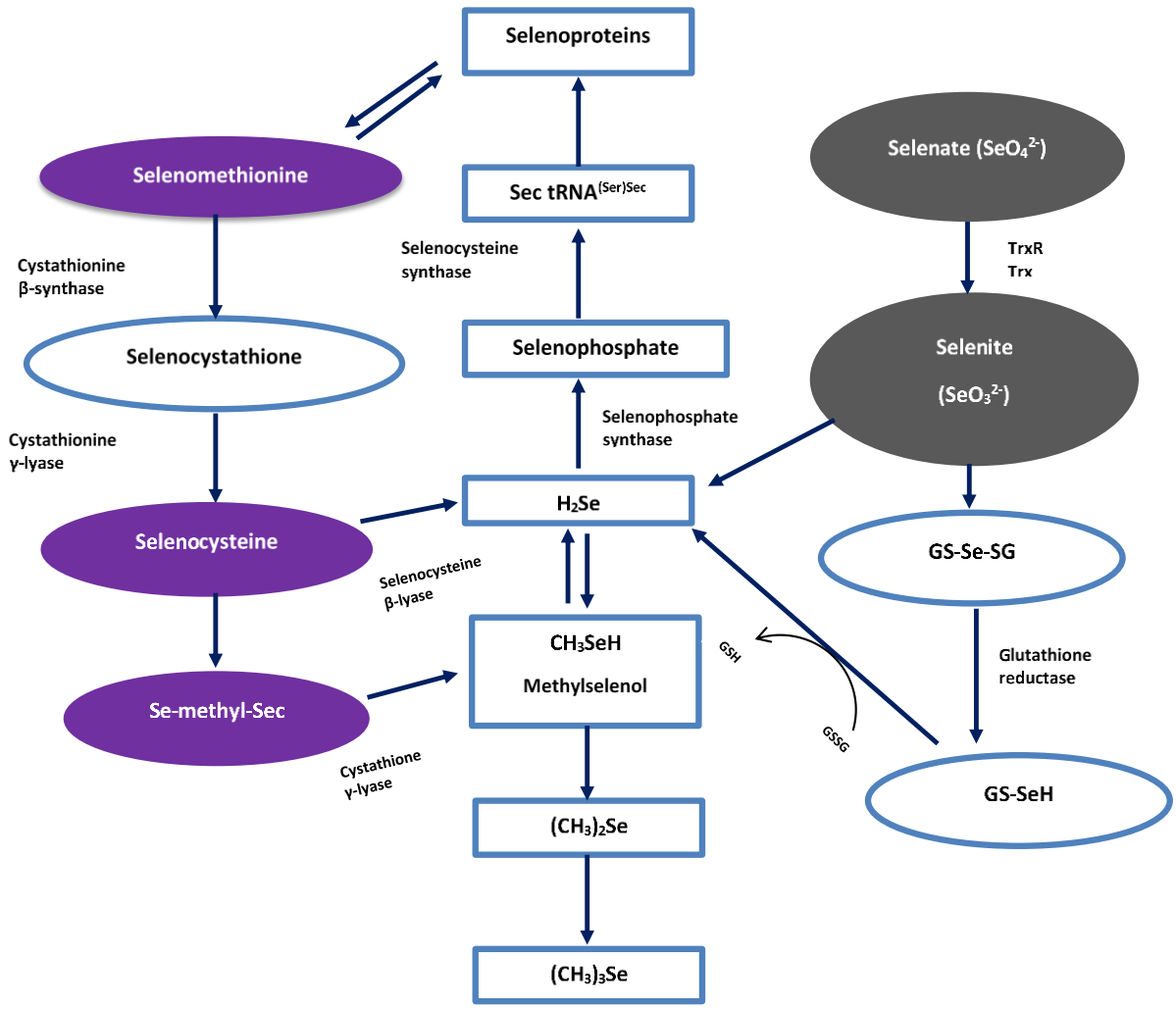

Figure 1-3. ***Selenium metabolism from organic and inorganic dietary forms*** *(Adapted from Matmiller et al. (2013)) (43). Organic dietary forms are indicated in purple and the inorganic forms are indicated in grey. Sec, selenocysteine; Se-methyl-Sec, selenium-methylselenocysteine; TrxR, thioredoxin reductase; Trx, thioredoxin; GS-Se-SG, selenodiglutathione; GSSG, glutathione disulfide; GS-SeH, glutathioselenol; H2Se, hydrogen selenide.*

1.2.4 Selenoproteins

Selenium mediates its functions by being incorporated into several selenoproteins (48). Selenocysteine (Sec) is a 21st amino acid in the genetic code and is a major form of selenium in the cell. Sec is encoded by the UGA codon and proteins which contain Sec and is therefore responsible for the important health benefits of selenium (49). To date

over 25 selenoproteins have been identified in humans (50). Some of the most important selenoproteins include the GPx family of enzymes **(Table 1-1)** which consists of GPx-1, GPx-2, GPx-3, GPx-4 and GPx-6 as well as thioredoxin reductases (TrxR1-3), thyroid hormone deiodinases (DIO1-3) and selenoprotein P (SePP) (51). The family of GPx exerts different functions on the cardiovascular system as illustrated in **Figure 1-4**.

Table 1-1. Glutathione peroxide family of enzymes and their functions (49, 52-56).

Selenoproteins	Localization	Functions
GPx-1 (Cytosolic)	Cytosol	Antioxidant defence Reduces H2O2
GPx-2 (Gastrointestinal)	Cytosol	Reduces H_2O_2 in the gut Antioxidant defence
GPx-3 (Plasma)	Plasma	Reduces H_2O_2 in blood Anti-inflammatory role
GPx-4 (Phospholipid hydroperoxide)	Cytosol Mitochondria Nucleus	Decreases phospholipid peroxide Regulates apoptosis
GPx-6 (Olfactory)	Cytosol	Reduces cellular H_2O_2 (olfactory epithelium)

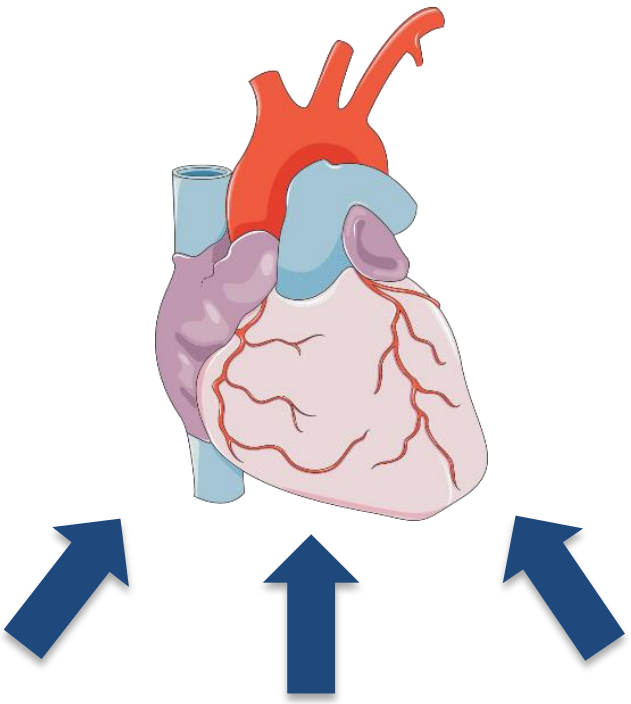

Figure 1-4. ***The protection of the heart by the GPx family of enzymes*** *(Adapted from Benstoem et al. (2015) (57)) (Image obtained from Servier Medical Art).*

GPx is involved in maintaining cellular redox balance by decreasing the circulating levels of hydrogen peroxide as well as lipid and phospholipid hydroperoxides (8). Therefore, GPx can maintain membrane integrity by converting (reducing) peroxides to alcohols and during this reaction, reduced glutathione is oxidized (58, 59). Selenium deficiency may influence GPx activity, which may cause clinical morbidities such as CVD (12-17). It is therefore suggested that indirectly selenium has antioxidant properties which may prevent or delay the development of CVD (60).

1.2.5 Selenium status

The recommended daily intake of selenium for healthy women is 60 µg/day (61). However, higher daily selenium intake (70 µg/day) is recommended for men (61). Dietary sources of selenium include meat, seafood, cereals, bread, fish, eggs, vegetables, grains, dairy products and Brazil nuts (62-64). Selenium deficiency is classified as intake less than 11 µg/day, which may cause diseases such as Keshan disease and Kashin Beck disease (65-68). When selenium status is measured, serum selenium samples are used to analyse short-term selenium intake (69), whereas toenail samples can be used to measure long term selenium intake (70, 71). In contrast elevated selenium levels >800 µg/day are referred to as selenosis (selenium toxicity), which may have harmful biological effects such as hair loss, fatigue, skin rash, abdominal pain, nausea, vomiting, diarrhoea and brittle nails (62, 72-74). If selenium toxicity occurs over a long period of time, it can lead to kidney failure, myocardial infarction, respiratory symptoms as well as other cardiac problems and neuronal lesions (75). Therefore, there is a relatively narrow range for optimal selenium status (41), which ranges between 80-120 µg/L (56). Previous studies indicated a typical U-shaped curve for selenium, where too low (<80 µg/L) or too high (>120 µg/L) serum levels of selenium may have detrimental health effects **(Figure 1-5)** (56, 76). Previously, low selenium levels were linked with increased risk for cardiovascular events (60, 77, 78), whereas high serum selenium levels are also associated with adverse cardiometabolic effects such as increased prevalence of diabetes (79). This may be ascribed to its effects on platelet aggregation, vasoconstriction, oxidative stress as well as shifting of prostaglandin from prostacyclin to thromboxane (11, 13, 80).

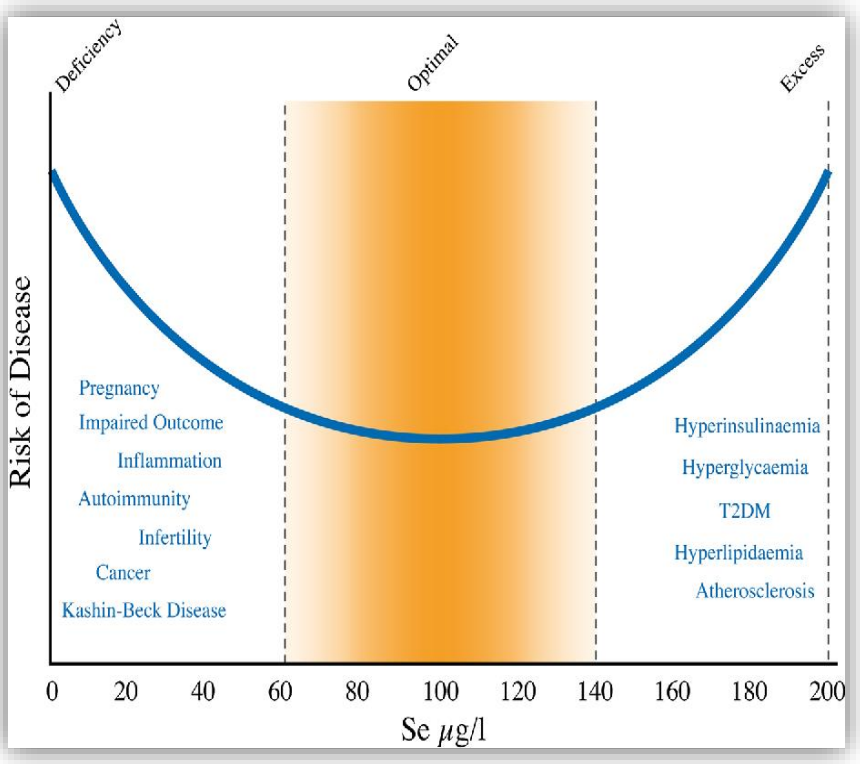

Figure 1-5. ***The U-shaped relationship of selenium levels with the risk of disease*** *(With permission from the author (56)).*

1.2.6 Factors influencing selenium status

Various factors may influence serum selenium levels, including environmental factors, race, sex and genetic factors (11, 81-84).

1.2.6.1 Environmental factors

In this regard, environmental factors refer to the content of selenium in soil, which differs around the world. For example, in some areas of China the soil has very low selenium content, whereas in the western parts of the United States of America, Israel and Ireland the selenium content in the soil is high (11). Most of the world's population as well as certain regions in Africa tend to have sub-optimal selenium levels (85-87). The concentration of selenium in foods is mostly dependent on the amount of selenium present in the soil in which crops are grown and also the specific food sources and the amount of selenium to which animals are exposed to (11, 88, 89). **Figure 1-6**, created in

2003, illustrates that most South African soil did not have adequate selenium levels at that time. In addition to soil selenium content, the pH of the soil also plays a role on selenium uptake in plants, where selenium is more available to plants grown in a high pH environment (90). In South Africa soil pH also differs in various regions (91), where the western part of South Africa seems to have suitable pH levels for selenium uptake by plants, but the selenium levels in the soil are low **(Figure 1-7)**. The diet of the majority of especially low-income South Africans consists of maize products as staple food (92). A previous study conducted by Courtman *et al*. (2012) indicated that 94% of maize tested in silos throughout South Africa was selenium deficient (91). This may contribute to increased selenium deficiency found in especially black South Africans (92, 93).

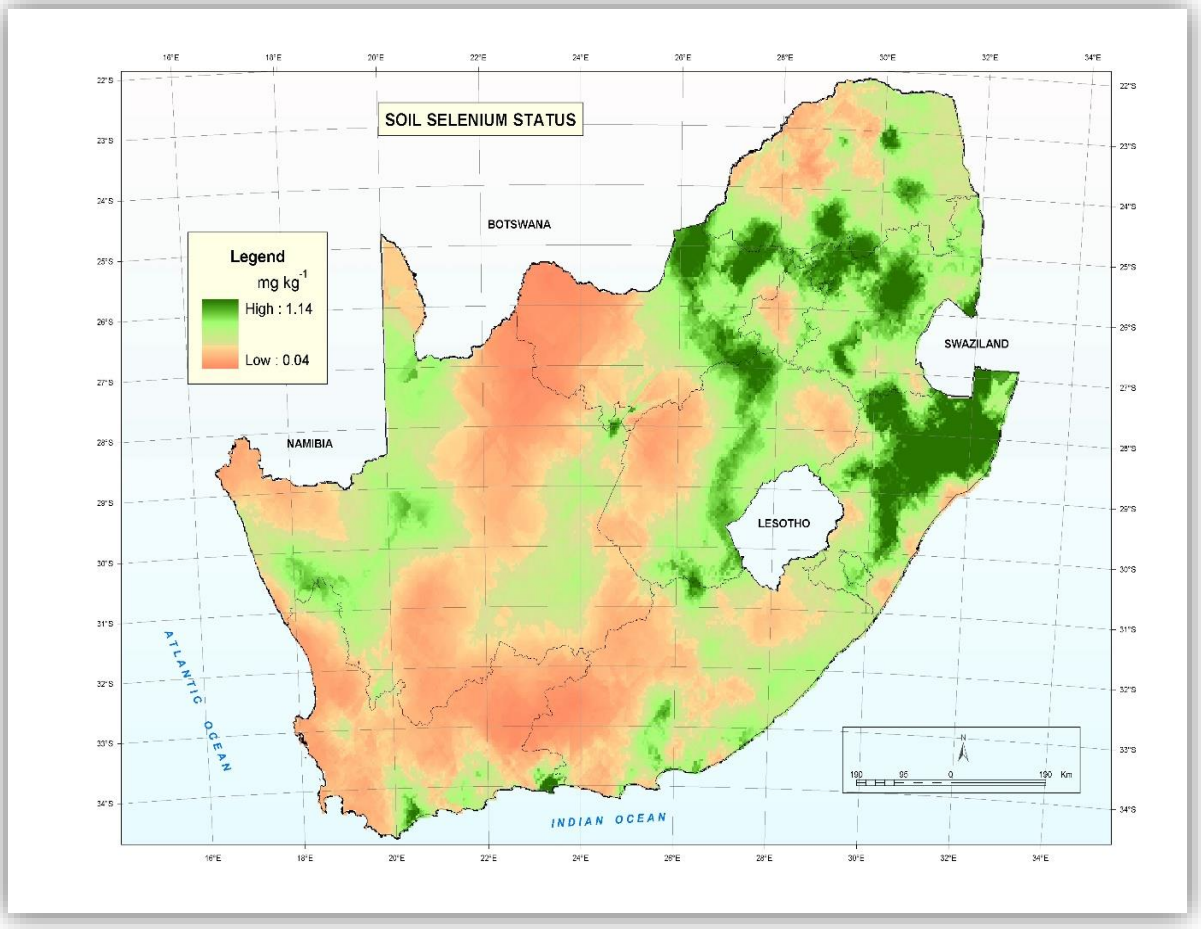

Figure 1-6. ***South African soil selenium status (2003)*** *(With permission of Agricultural Research Council, South Africa) (94).*

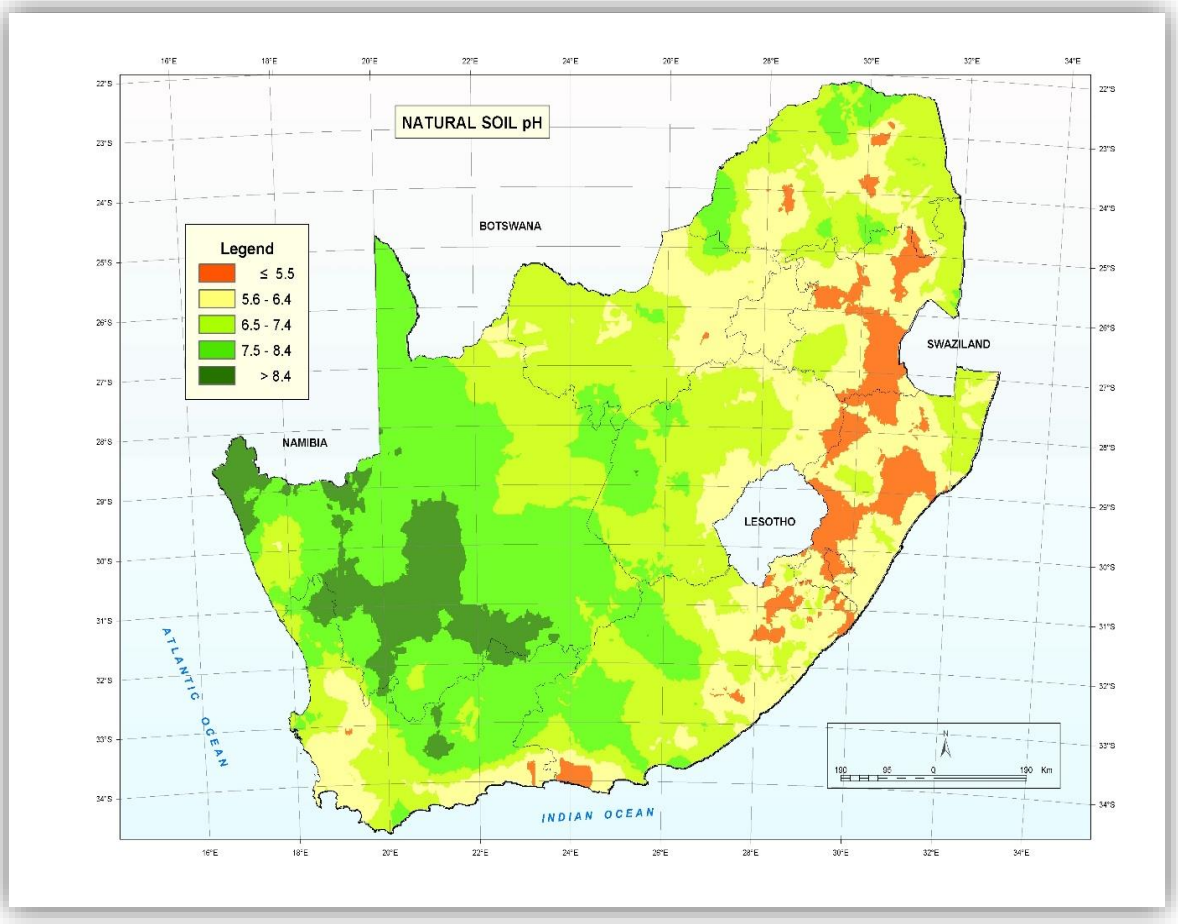

Figure 1-7. ***South African soil pH (2003)*** *(With permission of the Agricultural Research Council, South Africa) (94).*

1.2.6.2 Race

Results from the third National Health and Nutrition Examination Survey in the US, which included 10,779 black and white individuals (aged ≥12 years), indicated that African Americans had lower selenium levels compared to whites (95). Results from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA study) revealed that black South Africans have lower GPx activity when compared to their white counterparts (96, 97). Whether this difference in GPx activity is the result of lower serum selenium levels (and selenium intake) remains to be established. It therefore seems plausible that the effects of a decreased GPx activity in black individuals may be linked to factors such as low selenium status (98).

1.2.6.3 Sex

It was indicated that women retain selenium more efficiently in comparison with men in all organs, except in the gonads (99). There tend to be differences in the expression of selenoproteins between men and women, including GPx in the liver (82), SePP mRNA in the kidney and liver (100) and iodothyronine deiodinase in the liver, kidney and anterior pituitary (82, 100, 101). However, the mechanisms causing these sex-differences of selenoprotein expression in different tissues are unknown (82, 100).

1.2.6.4 Genetics

It was previously found that there are selenoprotein single-nucleotide polymorphisms (SNPs), which may influence selenium utilization and metabolism, including GPx-1 rs1050450, GPx-4 rs713041, selenoprotein P plasma 1 s3877899, selenoprotein 15 rs5845, selenoprotein S rs28665122 and selenoprotein S rs4965373 (102). It was previously found that the GPx-1 rs1050450 C allele is significantly associated with GPx activity, where the presence of the GPx-1 rs1050450 CT genotype translated to the highest correlation between selenium and GPx activity (102). In turn, the presence of the SEPP1 rs3877899 GG genotype translated to the highest correlation between selenium and thioredoxin reductase (102). It was also indicated that individuals with increased selenium levels (116 and 149 ng/ml), who are carriers of the GPx-1 rs1050450 CC and GPx-4 rs713041 TT genotype had lower DNA damage (102). Different selenium requirements for individuals may also be influenced by these different selenoprotein gene polymorphisms (103). Another polymorphism of the GPx-1 gene which is known as Pro198Leu, was previously linked to increased risk for the development of CVD as indicated by increased intima media thickness (IMT) (104) and coronary artery calcification (105). This may suggest that these SNPs may demolish the vascular protective effects of selenium.

1.2.7 Hierarchical distribution of selenium in the human body

When serum selenium levels are inadequate, selenium is not distributed to all tissues and selenoproteins equally (106). Previous studies indicated that selenium deficiency may

lead to increased male infertility (107, 108), and selenium levels and GPx activity decreased in the liver and plasma of rodents fed with a selenium deficient diet (108-110). However, the selenium levels in the brain, testes and endocrine tissues were sustained (107-110) **(Figure 1-8)**. With regard to the selenoproteins GPx-4, GPx-2, TxnRd-1 and TxnR-2 rank higher than GPx-1 and GPx-3 in the hierarchical system of selenoprotein transcription (111, 112). Other selenoproteins namely SePP and the DIO family have an intermediate ranking (106) **(Figure 1-9)**.

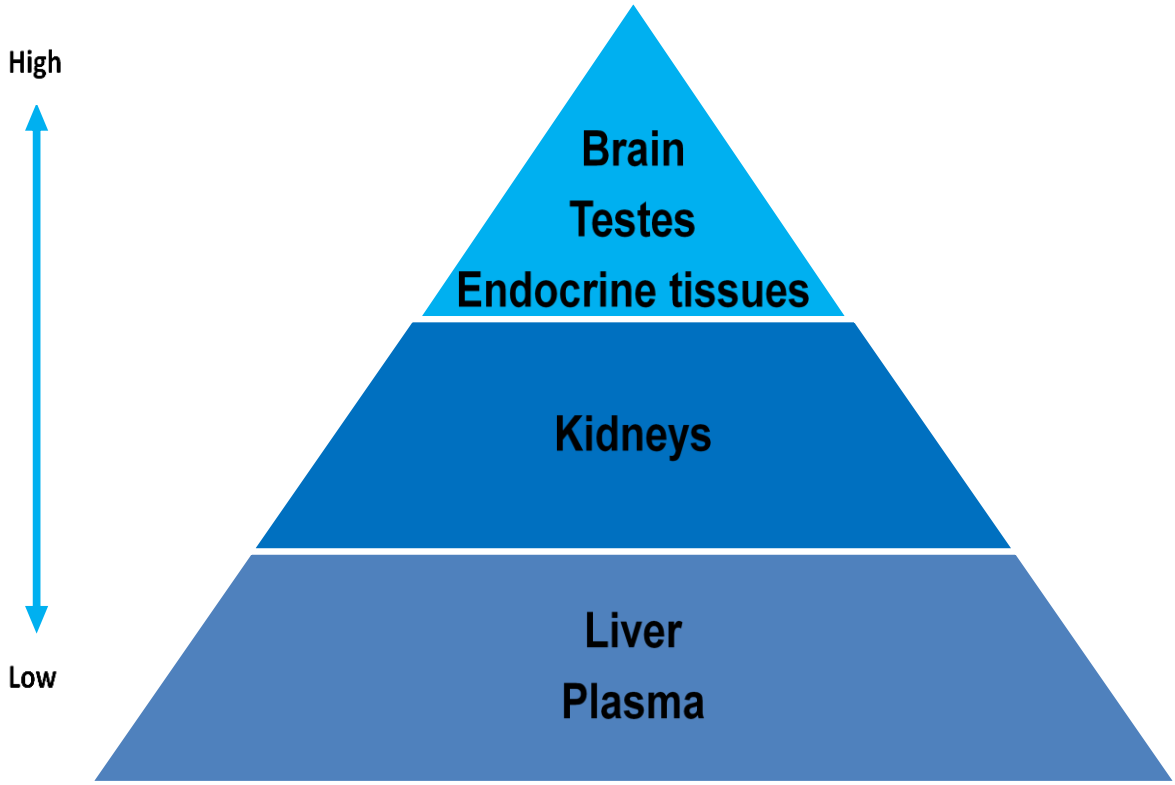

Figure 1-8. Tissue hierarchical distribution of selenium.

1.2.8 Oxidative stress, inflammation and endothelial dysfunction

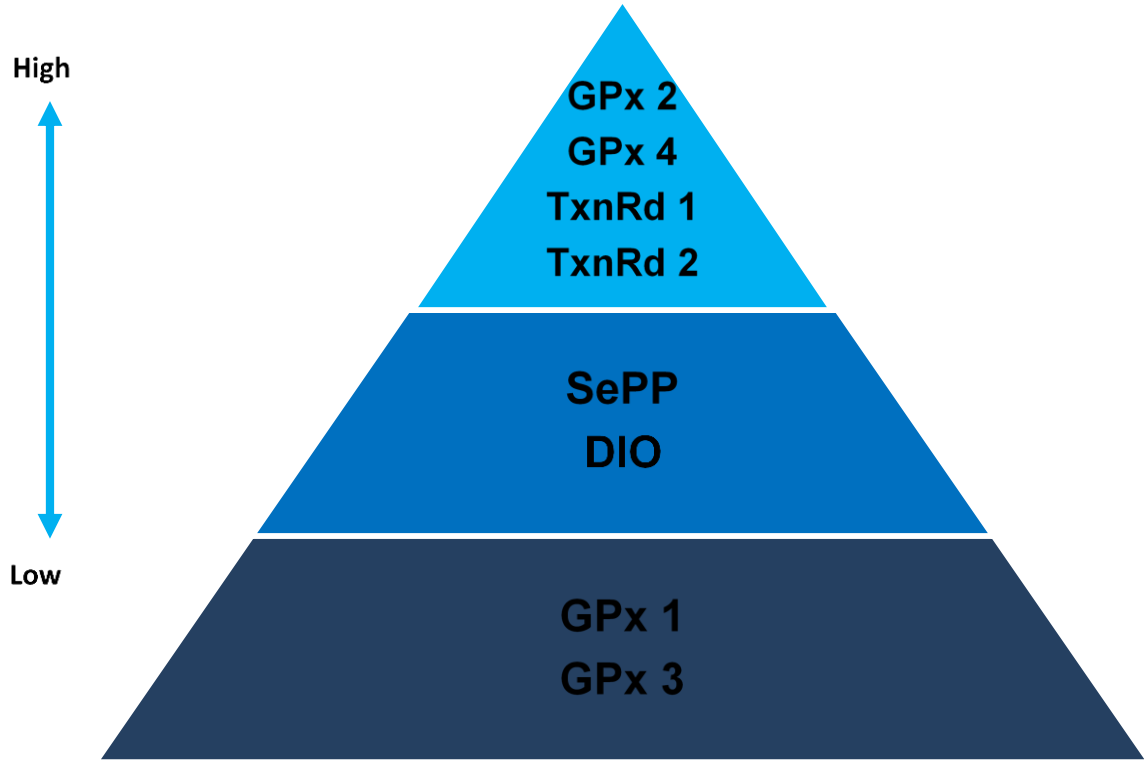

Figure 1-9. ***Hierarchical system of selenoprotein transcription.*** *GPx, glutathione peroxidase; TxnRd, thioredoxin reductase; SePP, selenoprotein P; DIO, iodothyronine deiodinases.*

Oxidative stress is the term used and previously described by Sies et al. (1985) as an imbalance between oxidants and antioxidants **(Figure 1-10)** which can lead to the disruption of redox signalling (113-115). Reactive oxygen species (ROS) also known as oxidants, include free radicals such as hydroxyl radicals, superoxide anion, peroxynitrite and hydrogen peroxide (116). There are different enzymes involved in the production of ROS including endothelial nitric oxide synthase, NADPH oxidase, xanthine oxidase, cyclooxygenases, lipoxygenases and oxidative phosphorylation (27). Increased ROS leads to tissue damage, but if ROS levels are present at the correct amount, it plays a key physiological role in the regulation of vascular tone, immune responses, cellular signalling, regulation of cell growth and differentiation as well as inflammatory responses (117). Oxidative stress and inflammation are closely linked, where oxidative stress may lead to inflammation and vice versa (118, 119) and both oxidative stress and inflammation

lead to endothelial damage. There is a positive feedback system between inflammation and endothelial dysfunction (120-123), where endothelial dysfunction increases a pro-inflammatory state by increasing adhesion molecules (120).

Antioxidant defence systems exist in the body which help to maintain the redox balance (114, 124). The antioxidant defence system includes enzymatic antioxidants such as GPx, catalase and superoxide dismutase and non-enzymatic antioxidants such as vitamin C, vitamin E, carotenoids and glutathione (125). In pathophysiological conditions, tissue damage and alterations may ensue due to the increased ROS production, which exceeds the antioxidant defence system (124, 126-128). A loss of circulating nitric oxide leads to endothelial dysfunction, which may be caused by a decreased expression of endothelial nitric oxide synthase and nitric oxide degradation by ROS. Therefore, oxidative stress, inflammation and endothelial dysfunction are also closely associated with vascular damage and contribute to the development of atherosclerosis and CVDs (129-131).

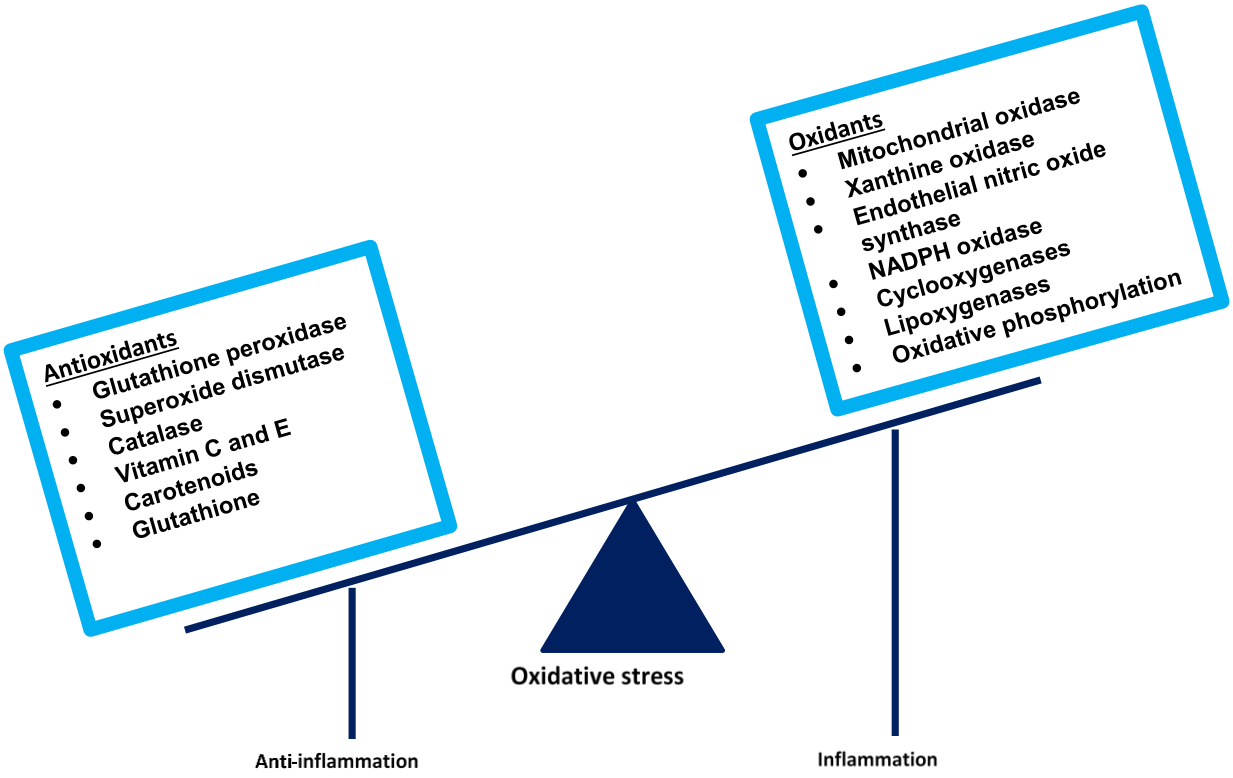

Figure 1-10. The imbalance between oxidants and antioxidants.

1.2.9 Selenium and the microvasculature

Cardiovascular health is determined by the optimal functioning of both the micro- and macrocirculation (30). Structural and functional changes of the microvasculature (as indicated by e.g. retinal vessel calibres and estimated glomerular filtration rate (eGFR)) are predictive of the development of hypertension and CVD (30, 132, 133). The microvasculature of the retina may provide information about the anatomical and physiological characteristics of the brain, kidney and the coronaries (134, 135). Measurements of the retinal microvasculature include the central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE) and arteriolar-to-venular ratio (AVR). It was previously found that changes in the microvascular calibres may precede atherosclerosis and macrovascular dysfunction (136).

From the literature, it is evident that selenium has protective effects on the microvasculature (137-141). It was indicated that increased selenium intake improved the eGFR and therefore it was hypothesized that selenium may be involved in the mechanism regulating blood flow in the kidneys (137). Another study showed that intake of selenium-rich Brazil nuts increased microvascular function, as measured with nailfold video capillaroscopy, in young (15.4 ± 2.0 years) obese girls (138). A decrease in arteriolar-to-venular ratio, was previously associated with an increased risk for hypertension (139, 140), while one study including healthy men (23.4 ± 0.5 years), indicated a protective effect of selenium on the microvascular function as measured in the skin (141).

The mechanism by which selenium exerts a protective effect on the microvasculature, may be through antioxidant properties by decreasing levels of hydrogen peroxide (142), lowering inflammation (143) and preservation of endothelial function (144).

1.2.10 Selenium and arterial stiffness

Arterial stiffness is a strong predictor of cardiovascular morbidity and mortality (145). Pulse wave velocity (PWV) over the carotid femoral segment is the golden standard measurement for large artery stiffness (146) and is defined as the speed that the pulse wave travels from the common carotid artery to the common femoral artery (147, 148). A

normal PWV for young (<30 years) healthy individuals is 6.1 ± 1.4 (m/s) (149), whereas a PWV above ten m/s indicates pathology (150).

Various mechanisms can lead to increased arterial stiffness which is characterized by changes in the structural and cellular elements of the vessel wall (151). Changes in the vasculature may be influenced by hemodynamic forces (152) as well as risk factors such as age, increased blood pressure, obesity, diabetes, African descent, physical inactivity, family history of CVDs (153), salt intake and hormones such as aldosterone (154). The vessel wall consists of three layers namely the intima, media and adventitia. The extracellular matrix, which is a main component of the vessel wall, consists of two prominent proteins namely collagen and elastin and the main types of collagen are type I and type III (155-157). Elastin is the dominant protein in the extracellular matrix and is responsible for the reversible extensibility of the cardiac cyclic loading, whereas collagen is responsible for the prevention of failure at high pressures. However, if there is an imbalance in the elastin to collagen ratio, it may lead to arterial stiffness (158).

Oxidative injury may lead to endothelial dysfunction and lower arterial elasticity (159). An increase in oxidative stress as a consequence of decreased nitric oxide, catabolism of nitric oxide by ROS to peroxynitrate and hydrogen peroxide as well as the inhibition of endothelial nitric oxide synthase, may lead to abnormal vasomotor activity, endothelial pro-coagulant activity, inflammation and eventually arterial stiffness (160, 161).

The potential of antioxidants (selenium and GPx activity) to decrease oxidative stress and inflammation in the vasculature has been previously indicated by several studies (162-166). A previous study which included men and women (aged 44-91 years old), investigated if a broad spectrum of nutrients, including 0.2 mg selenium, will have an effect on arterial stiffness over two months. The results indicated improvements of arterial stiffness (PWV and augmentation index (AIx)) over two months (166). In a randomized placebo-controlled study, it was also found that selenium in conjunction with other antioxidants such as vitamin C, vitamin E and coenzyme Q10, improved large and small artery compliance over six months in participants with cardiovascular risk factors (167). However, another study found no results in apparently healthy participants older than 50 years, which were randomly allocated to receive either a combination of antioxidants

(vitamin C, vitamin E, beta carotene, selenium and zinc) or a placebo daily and were followed over 7.2±0.3 years (168).

In experimental animal work, (male Sprague Dawley rats), it was indicated that low selenium levels were associated with increased oxidative stress, which affected the nitric oxide-mediated vascular response resulting in a negative effect on macrovascular function (rat aortas) (169). In another experimental study, which included spontaneous hypertensive rats, it was demonstrated that selenium has protective effects against degenerative changes of vessel walls (170). Therefore, low selenium levels may lead to endothelial dysfunction and contribute to the development of arterial stiffness (129, 131) and CVDs (11, 13, 17).

1.2.11 Selenium and atherosclerosis

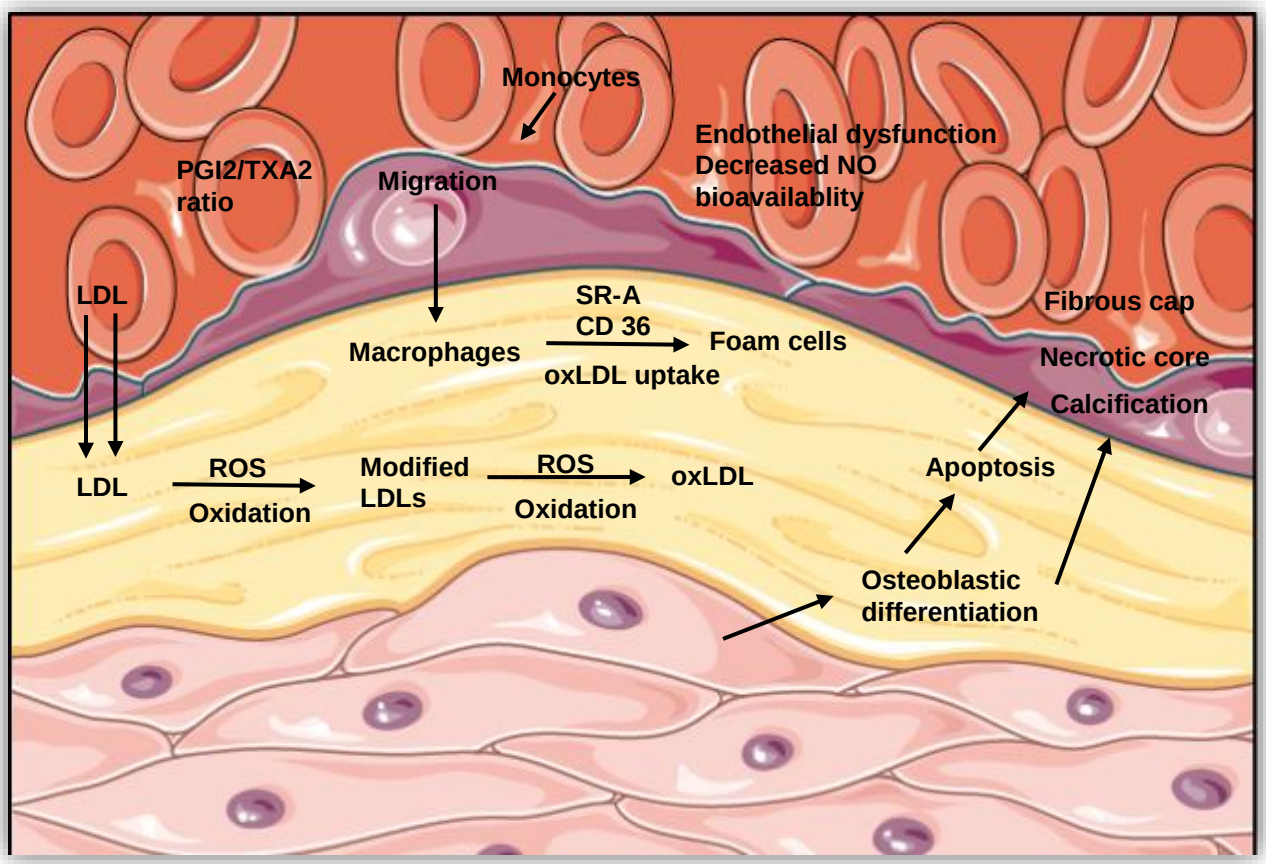

Figure 1-11. ***The atherosclerosis process*** *(Adapted from Liu et al. (2017) (55)) (Image obtained from Servier Medical Art). LDL, low density lipoprotein; ROS, reactive oxygen species; PGI2/TXA2 ratio, prostacyclin/thromboxane ratio; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; SR-A, scavenger receptor class A; CD 36, class B scavenger receptor CD36; oxLDL, oxidized low density lipoproteins.*

Ischaemic heart disease is one of the biggest causes of mortality (171). Atherosclerosis can be defined as a progressive inflammatory vascular disease as a result of the accumulation of leukocytes and smooth muscle cells in the intima which lead to the build-up of cholesterol and fatty tissues, which narrow and harden medium and large arteries. Atherosclerosis develops through structural and endothelial cell dysfunction in the vessel wall over a period of years (172-174). Carotid intima media thickness (IMT) can be used to identify subclinical atherosclerosis (175) and is one of the best methods to

detect early stages of atherosclerosis non-invasively by using the B-mode ultrasonography (176).

In **Figure 1-11**, the atherosclerotic process is illustrated. The atherosclerotic process starts when endothelial cells are damaged by risk factors such as age (177), hypertension (178), smoking (179), diabetes (180) and serum cholesterol (181). This leads to the increased permeability of LDLs through the endothelium and into the intima where the free radicals come in contact with the LDLs which causes oxidized LDLs (oxLDLs) (55). These damaged endothelial cells increase the expression of endothelial adhesion molecules including vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin, which induce the adhesion of monocytes. These adhered monocytes move through the endothelium to the intima by the process of diapedesis, where they differentiate to macrophages (55). The macrophages engulf the lipids, which are derived from oxLDLs by scavenger receptors and is then transformed into foam cells (55, 182, 183). The foam cells increase the accumulation of cholesterol esters, which is known as the fatty streak. The macrophages and T-lymphocytes increase inflammatory markers, which increase the capacity of the vascular smooth muscle cells to migrate from the media to the intima. The vascular smooth muscle cells in the intima thicken the arterial wall and the fatty streak change into a stable atherosclerotic plaque (55). Vascular smooth muscle cells in the plaques proliferate and increase extracellular matrix proteins, which lead to the formation of a fibrous cap and apoptosis of the lipid containing foam cells and form a necrotic core, which consists of dead cells and cholesterol esters. The accumulation of T cells and foam cells leads to increased inflammation which recruits more inflammatory cells and vascular smooth muscle cells in the intima (55). The stable plaque change into unstable plaque by calcification, fibrous cap thinning and injury to the vessel wall, caused by cholesterol crystals in the core. The unstable plaque may rupture and form a blood clot which can move into the blood stream and clog arteries, this may ultimately lead to myocardial infractions or stroke (55, 173, 174, 184).

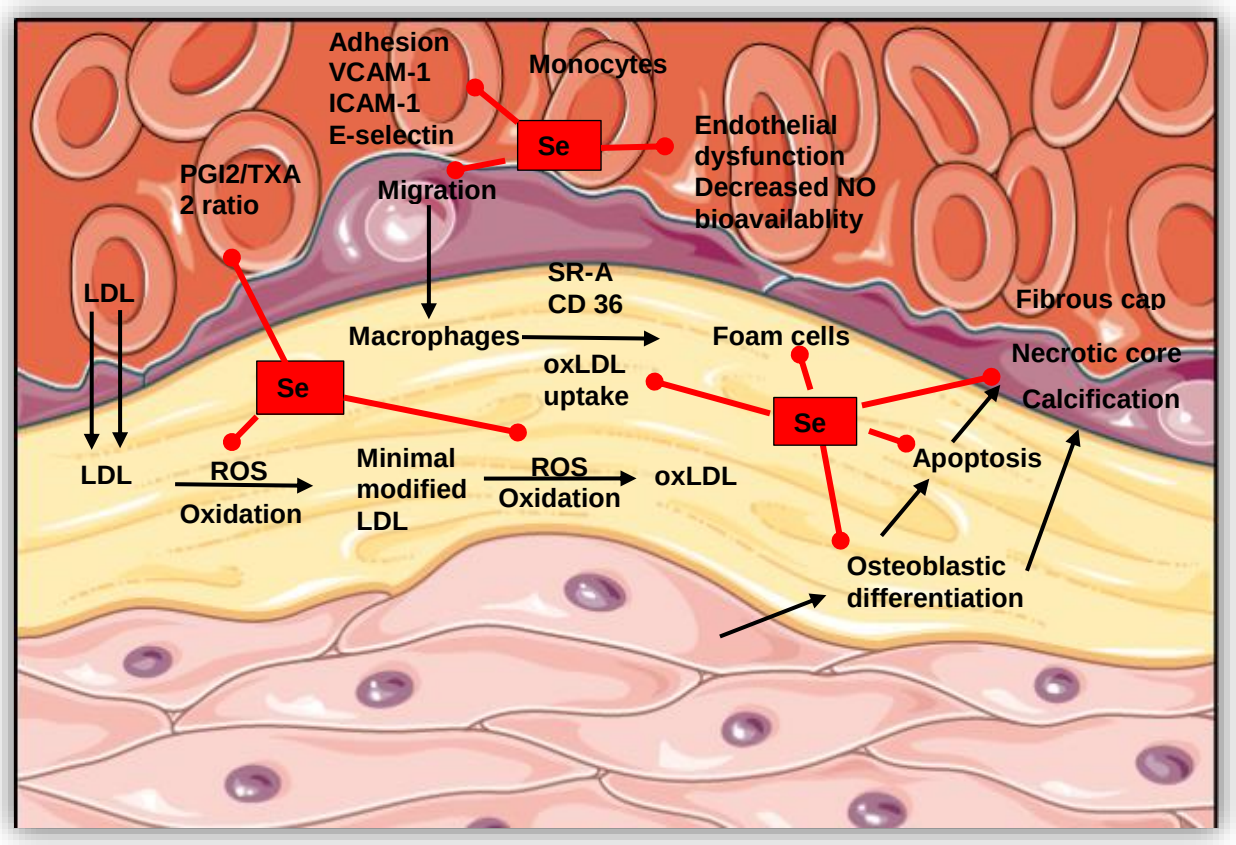

Figure 1-12. ***Selenium's effect on the prevention of the atherosclerosis process*** *(Adapted from Liu et al. (2017) (55)). (Image obtained from Servier Medical Art). LDL, low density lipoprotein; ROS, reactive oxygen species; PGI2/TXA2 ratio, prostacyclin/thromboxane ratio; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; SR-A, scavenger receptor class A; CD 36, class B scavenger receptor CD36; oxLDL, oxidized low density lipoproteins; Se, selenium.*

The development of atherosclerosis that may be prevented by selenium through its antioxidant functions is illustrated in **Figure 1-12**. There are various mechanisms that can be blocked by selenium (55), where selenium can inhibit oxidative stress through an increased appearance of selenoproteins (GPx-1, GPx-4, TrxR-1 and SelPP) which exert antioxidant functions. Therefore, selenium can block oxidative stress, cell damage, LDL oxidation and apoptosis (185-191). Selenium can inhibit inflammation through GPx, by blocking the migration of monocytes (192), the formation of foam cells (193) and eicosanoid metabolism (195). Selenium can also inhibit endothelial dysfunction via GPx by increasing nitric oxide bioavailability, therefore maintaining normal endothelial function (196). It was illustrated that selenium may reduce oxidative stress and intracellular calcium levels and increase GPx and superoxide dismutase, thereby inhibiting vascular smooth muscle cell apoptosis (186, 197). It is also known that selenium may decrease vascular calcification by lowering oxidative stress through increases in antioxidant selenoproteins such as GPx. Selenium blocks the redox sensitive PI3K/AKT and ERK pathways. This results in decreased osteoblastic differentiation of vascular smooth muscle cells (198). Collectively selenium can protect against the development of atherosclerosis, hypertension and CVDs. A study which included middle-aged (ages ranging from 42-60) Finnish men found that low selenium levels are a risk factor for the progression of atherosclerosis (199). In addition, a study conducted on hospitalized patients who were to undergo coronary arteriography found an inverse relationship between low plasma selenium levels and atherosclerosis (200).

Although the above-mentioned mechanisms for selenium and GPx to prevent atherosclerosis are proposed, a few studies showed an adverse relationship between selenium and atherosclerotic markers (201-203). A longitudinal study on young African American and white American participants (aged 20-32 years) does not support the protective mechanism of atherosclerosis in the prevention of CVDs (203). A study which investigated the effect of selenium on histopathological changes in an animal model of cockerel found that optimal selenium levels (0.14 mg) induced atherogenesis via inflammation and smooth muscle proliferation in the media of blood vessels (202). In another cross-sectional study it was suggested that high selenium levels (>160 µg/L) may have led to an increased risk for peripheral artery disease. The U-shaped curve between

selenium and atherosclerosis that was previously described, was again suggested by these authors (201).

1.2.12 Selenium and cardiovascular diseases

Current knowledge gained from prospective studies indicates that selenium deficiency may be a risk factor for the development of CVD. A few studies hypothesized about the positive effects of selenium as treatment for CVD and in this regard various global studies support the beneficial effects of selenium supplementation (204-206). However, there are studies which indicated no correlation between selenium and CVD (207, 208).

Despite some inconclusive results (209), data summarized in two meta-analyses indicate that decreased serum selenium may lead to increased coronary heart disease (18, 210). A prospective study conducted by Blankenberg et al. (2003) which consisted of 636 patients with coronary artery disease, indicated an increase in cardiovascular events with low GPx activity (60). Studies in Finland, known to have low soil selenium content, indicated that the use of selenium-enriched fertilizers since the 1980s improved selenium status. However, even after optimal serum selenium levels were reached, the incidence of CVD remained similar, suggesting that lifestyle factors are stronger determinants of CVDs as compared to selenium status (211).

In intervention studies, the form of selenium supplementation given is also important to consider as some studies used the organic form and others the inorganic form of selenium, which may lead to inconclusive results (212-214). Future studies are needed to shed more light on the potential beneficial cardiovascular effects of selenium, including reduced incidence of CVD and hypertension (41, 57).

1.3 Motivation

When reviewing mean blood pressures throughout all global regions, sub-Saharan Africa was flagged as a region presenting some of the highest mean blood pressures (2). Increased oxidative stress, as a result of increased ROS production or low antioxidant capacity, is associated with the development of hypertension (126, 215). Selenium, a trace element, with indirect antioxidant properties as part of selenoproteins, can be found

in animal and plant-based foods and is associated with decreased oxidative stress and oxidative damage through various pathways (216-218), including the actions of enzymatic antioxidants such as GPx (219). Low selenium levels may therefore lead to the decreased expression of GPx which may lead to increased oxidative stress, inflammation, endothelial dysfunction and eventually to increased arterial stiffness, atherosclerosis and hypertension (57) **(Figure 1-13)**. There are limited data on concentrations of serum selenium or the biological effects of serum selenium levels in people living in sub-Saharan Africa. Furthermore, to the best of our knowledge the associations of serum selenium and GPx activity, blood pressure, vascular resistance, arterial compliance, arterial stiffness, measures of the microvasculature as well as measures of large artery structure have not yet been thoroughly investigated, particularly in black populations – known to be prone to the development of hypertension.

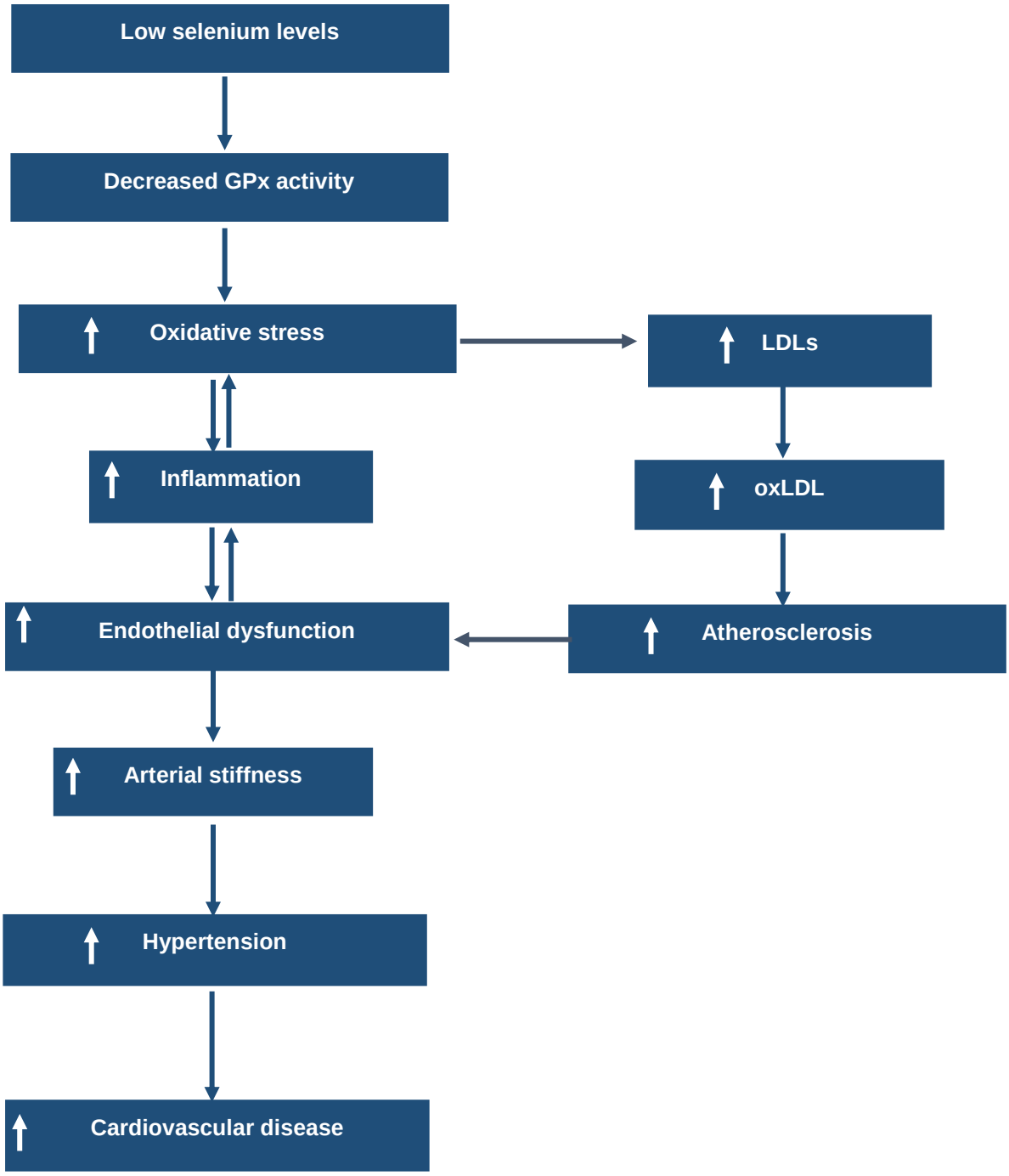

Figure 1-13. ***Mechanisms of selenium deficiency-induced cardiovascular disorders*** *(Adapted from Siti et al. (2015) (130)).*

1.4 Aims, Objectives and Hypotheses

Central aim

The central aim of this study is to investigate the associations of serum selenium levels and GPx activity with estimates of the microvasculature and large artery structure and function in different black South African cohorts.

Article 1: SABPA-study

Aim:

The SABPA-study has been used in order to address these aims: to compare serum selenium levels between black and white adults, as well as to investigate whether serum selenium levels are related to GPx activity, and whether 24h blood pressure, vascular resistance, arterial compliance and arterial stiffness are related to both serum selenium and GPx activity in black and white school teachers (aged 20-65 years) of the SABPA-study.

Objectives:

- To compare serum selenium levels and GPx activity between black and white adults;
- To investigate associations between serum selenium levels and GPx activity in black and white adults;
- To investigate associations of 24h blood pressure, vascular resistance, arterial compliance and arterial stiffness with serum selenium and GPx activity in black and white adults.

Hypotheses:

- Serum selenium levels and GPx activity will be lower in the black participants compared to the white participants;
- Serum selenium levels will correlate positively with GPx activity;

- Blood pressure, vascular resistance and arterial stiffness will associate negatively with serum selenium levels and GPx activity in black participants;
- Arterial compliance will associate positively with serum selenium levels and GPx activity in black participants.

Article 2: African-PREDICT study

Aim:

The African-PREDICT study has been used in order to address these aims: to investigate whether measures of the microvasculature (central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriolar-to-venular ratio (AVR) and estimated glomerular filtration rate (eGFR) and of the macrovasculature (pulse wave velocity (PWV), 24h pulse pressure (24h PP) and augmentation index (AIx)) are related to serum selenium and GPx activity in a young, healthy, black and white cohort.

Objectives:

- To determine whether associations of microvasculature measures (CRAE, CRVE, AVR and eGFR) with serum selenium and GPx activity exist in black and white participants;
- To investigate whether measures of the macrovasculature (PWV, 24h PP and AIx) are associated with serum selenium and GPx activity in black and white participants.

Hypotheses:

- Microvascular measures (CRVE) will associate negatively with selenium and GPx in black participants;
- Microvascular measures (CRAE, AVR and eGFR) will associate positively with selenium and GPx in black participants;
- Macrovascular measures (PWV, 24h PP and AIx) will associate negatively with selenium and GPx in black participants.

Article 3: PURE-study

Aim:

The PURE-study has been used in order to address these aims: to investigate, in the total group and in normal and selenium deficient black adults, whether serum selenium levels are related to measures of large artery structure (carotid intima media thickness (IMT) and cross-sectional wall area (CSWA) and function (blood pressure and arterial stiffness) over ten years in black South African participants from the PURE study (aged >35 years).

Objectives:

- To investigate whether baseline selenium levels are related to follow-up measures of large artery structure (IMT and CSWA) over ten years;
- To determine whether baseline selenium levels are related to follow-up measures of large artery function (blood pressure and arterial stiffness) over ten years.

Hypotheses:

- Baseline selenium levels will associate negatively with follow-up measures of large artery structure (IMT and CSWA) over ten years;
- Baseline selenium levels will associate negatively with follow-up measures of large artery function (blood pressure and arterial stiffness) over ten years.

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CHAPTER 2:
STUDY DESIGN AND METHODOLOGY

Study design

Data from three different studies were used for this thesis. All data were collected in the North West Province **(Figure 2-1 and 2-2)** of South Africa, including the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) and the South African leg of the multi-national Prospective Urban and Rural Epidemiology (PURE) study. The methodology of each of these studies will be discussed below and at the end of the chapter all the consistent measurements across the three studies are summarized in tables.

Figure 2-1. ***A map of South Africa showing the North West province (top) and the Potchefstroom area where the African-PREDICT, SABPA and PURE studies took place along with the Ganyesa/Thlakgameng area where the rural participants of the PURE study reside (bottom).***

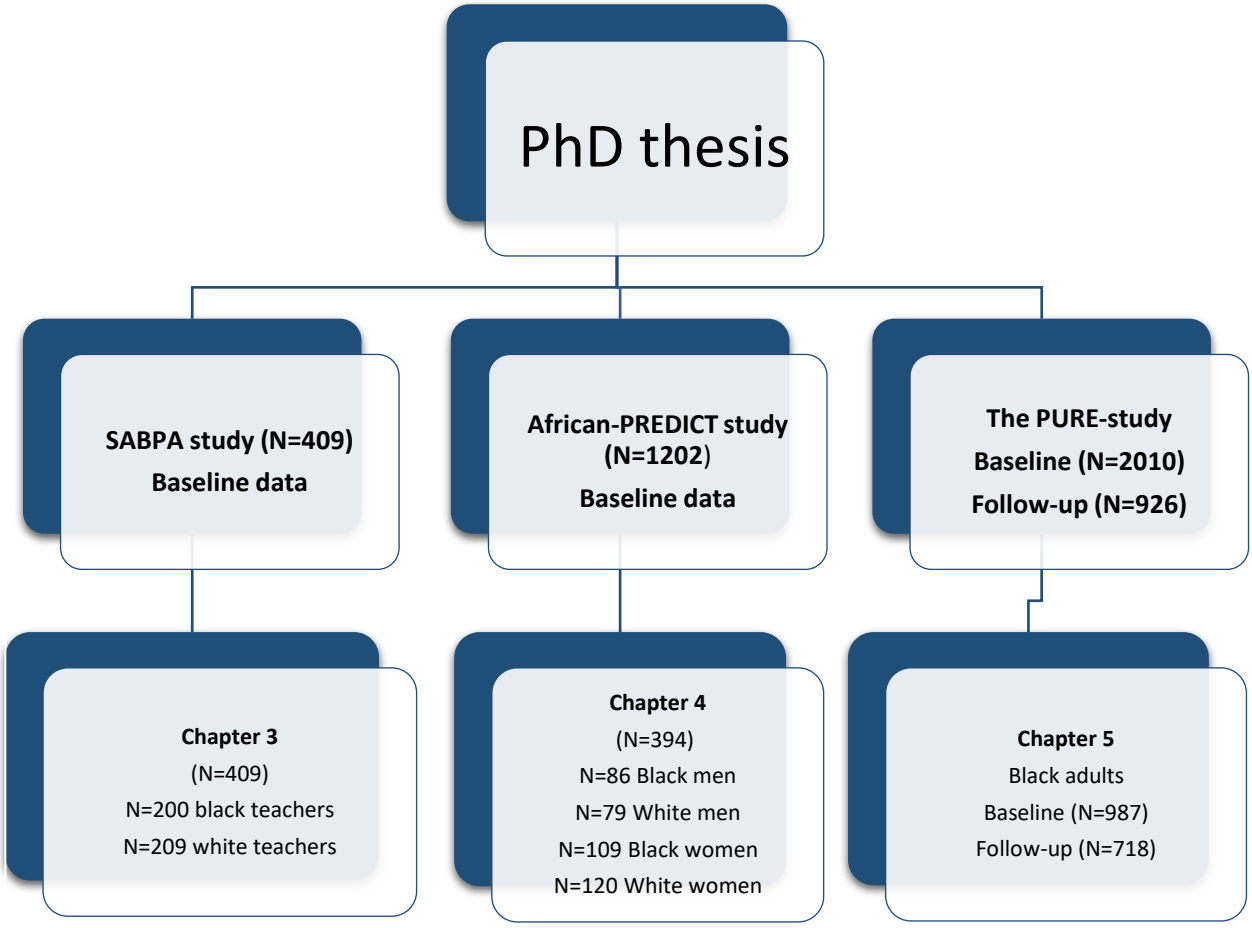

Figure 2-2. Outline of the three different studies used in the thesis.

2.1 The SABPA Study

2.1.1 Study design

A detailed description of the study population and protocol has been published elsewhere (1). The main objective of the SABPA study was to assess the brain-heart link and neural response pathways in a target population group from South Africa (1).

2.1.2 Recruitment process

Headmasters of schools were informed about the SABPA study in September 2007, as their cooperation and support were important in the execution of the study. Recruitment, screening and information sessions with the school teachers as participants were held two months prior to the commencement of the study (November 2007) and informed consent forms were voluntarily signed after these sessions had been held.

2.1.3 Participants

The potential study participants comprised N=2170 urban black and white teachers, enrolled in 43 schools of the Dr Kenneth Kaunda Education District (Klerksdorp and Potchefstroom), North West Province, South Africa. Screening resulted in a population size of 409 eligible and enrolled participants. Phase I (baseline) was conducted between 2008 and 2009 and consisted of 200 black (101 men and 99 women) and 209 white (101 men and 108 men) school teachers aged 20–65 years **(Figure 2-3)**. The reason for this selection was to obtain a homogenous sample from participants with a similar socio-economic status. The exclusion criteria of the SABPA study are illustrated in **Figure 2-3**. For this cross-sectional sub-study we only used baseline data (collected in 2008/9) for data analyses.

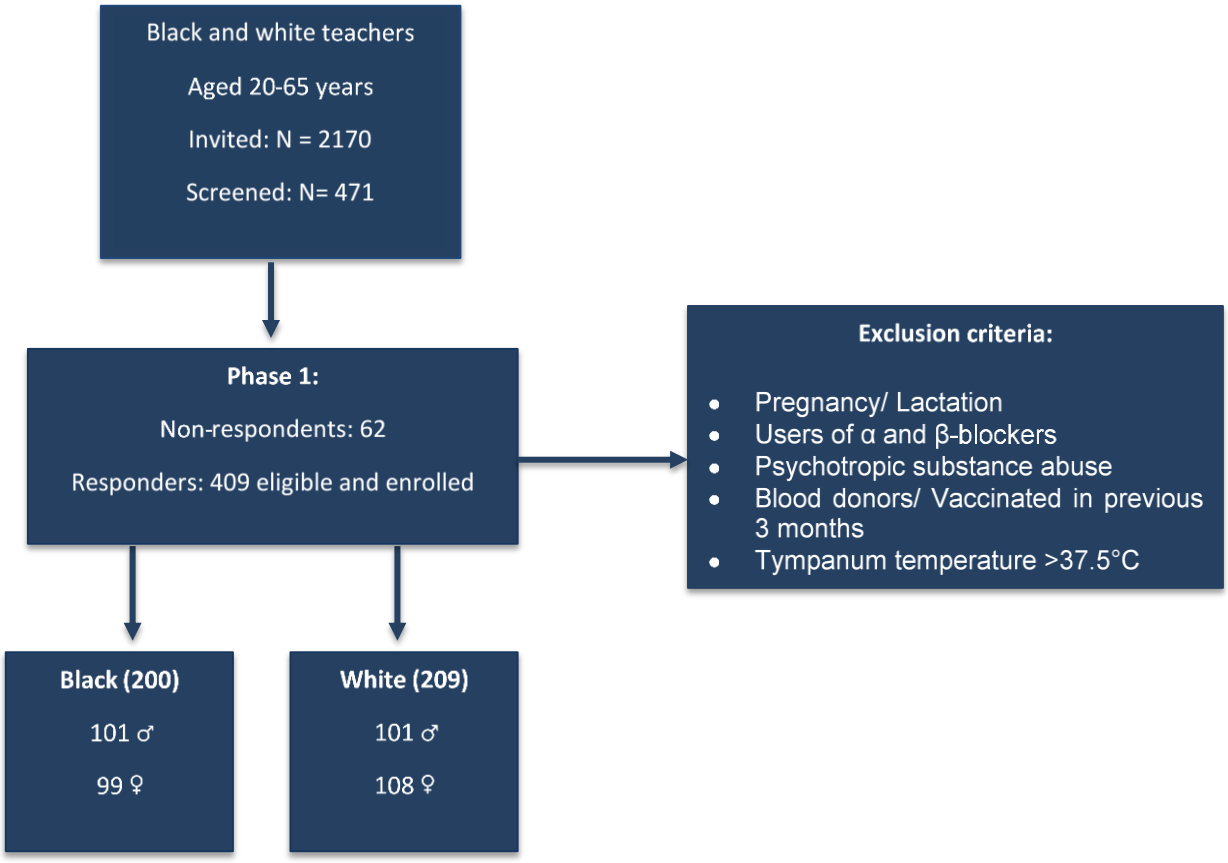

Figure 2-3. The SABPA prospective cohort study population *(1)*.

2.1.4 Organisational procedures

From Monday to Thursday between 07h00 and 08h00, an ambulatory blood pressure monitoring (ABPM) device (Meditech CE120® Cardiotens, Budapest, Hungary) was fitted to the participant's non-dominant arm with an appropriately sized cuff. The ABPM device was programmed to measure blood pressure in 30 min intervals during the day (08h00-22h00) and 60 min intervals during the night (22h00-06h00). On the same day the ABPM device was fitted, the participants were collected at 16h30 and transported to the Metabolic Research Unit of the North-West University (consisting of ten bedrooms, two bathrooms, a living room and kitchen). The participants were informed of the experimental procedures of the following day and spent the night. All participants were tested for the Human Immunodeficiency Virus (HIV) and each participant received pre- and post-counselling from a registered nurse. Participants received a standardised dinner at 18h00, and had their last beverages and two biscuits at 20h30. They were encouraged

to go to bed at around 22h00, fasting overnight. An overnight spot urine sample was collected in the morning and at 06h00 and the ABPM apparatus was removed after which the remaining measurements commenced **(Figure 2-4)**. Only the methodology appropriate to this sub-study will be discussed.

Figure 2-4. ***Measurements of the SABPA study.*** *A, blood sampling; B, blood pressure and ECG (Finometer device); C, anthropometry and ABPM; D, carotid-dorsalis pedis pulse wave velocity.*

2.1.5 Questionnaires

The questionnaires were given out and explained by trained researchers/fieldworkers in the participant's home language. Structured demographic, general health and socio-economic questionnaires were given to participants to gain information regarding age, smoking, alcohol intake, socio-economic status and the use of medication.

2.1.6 Cardiovascular measurements

Finometer

The validated (2, 3) Finometer device® was used to measure continuous blood pressure of the participant in a semi-Fowler's position with their arm at heart level. A finger cuff was placed on the middle phalanx of the middle finger of the left hand and an inflatable cuff was connected on the upper arm. After a ten min resting period, a five min continuous measurement of baseline cardiovascular variables was recorded. The average of the recordings of the last minute was used for further analyses. During the measurement a return-to-flow systolic calibration was performed which provides an individual subject-level adjustment of the finger arterial pressure to the brachial artery pressure level (4). The Finometer is an accurate and sensitive device which can record changes in cardiovascular function (5). Finometer measurements were processed with Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) to determine total peripheral resistance (TPR) and Windkessel compliance (Cwk) (3) which provides information on the dynamics of the cardiovascular system (2, 3). Total peripheral resistance is known to be an important co-determinant of blood pressure, where increased blood pressure due to increased TPR may lead to increased risk of cardiovascular mortality (6). Arterial compliance reflects the buffering capacity of an artery and can be described as the change in volume per unit of pressure. Therefore, a decrease in compliance and/or an increase in vascular resistance may lead to cardiac hypertrophy as a result of increased cardiac afterload (7, 8).

2.1.7 PhD student participation in the SABPA study

Although I did not participate in the research measurements of the SABPA study, I did perform relevant measurements in other research projects in the Hypertension Clinic including:

- Anthropometric measurements;
- HIV testing and counselling;
- ABPM (Explaining and connecting of the ABPM devices to the participant's non-dominant arm to measure 24h SBP and 24h DBP at 30 min intervals during the day and every hour during the night and capturing of ABPM data).
- Laboratory assistance – Centrifuging of blood samples and aliquoting of plasma, serum and urine samples into cryovials. Safe packaging and storage in -80°C biofreezers.

2.2 The African-PREDICT Study

2.2.1 Study design

The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) was undertaken in the North West Province of South Africa. The aim of the African-PREDICT study is to identify early markers or predictors for the development of cardiovascular disease in black South Africans. By identifying these important markers, successful prevention programmes can be implemented among young South Africans.

2.2.2 Recruitment

Participants were recruited from Potchefstroom and surrounding areas via various routes including trained field workers, advertisements on the radio or in the newspapers, or at their workplaces. Potential participants were then invited to attend the screening phase to determine whether they met the inclusion criteria for participation in the African-PREDICT study. All the participants were fully informed about the protocol of the study

before enrolment and if the participants agreed to take part in the study, they had to sign an informed consent form.

2.2.3 Participants

The baseline cohort of the African-PREDICT study included N=1202 apparently healthy, young (aged between 20-30 years) black and white men and women. For the purpose of this sub-study, data from the first 394 participants with complete data were analysed cross-sectionally. The reason for this is that I started in 2016 with my PhD thesis, and therefore only the first 394 participants had complete data. The methodology appropriate to this sub-study will be discussed.

2.2.4 Organisational procedures

Participants who were willing to participate in the study were invited to the Hypertension Research and Training Clinic at the NWU where they were screened by trained researchers, whereas others were screened at their workplace by staff members of the Hypertension Research and Training Clinic. If the participants met the inclusion criteria **(Table 2-1)** they were invited to participate in the research study. They were provided with information leaflets and the trained researchers explained the objectives and measurements of the study in detail to the participants prior to the day on which the study measurements commenced. If they agreed to take part in the African-PREDICT study, the participants were requested to refrain from ingesting food and drinks from 22h00 the previous night.

Table 2-1. Inclusion and exclusion criteria of the African-PREDICT study

Inclusion criteria:	Exclusion criteria:
• Apparently healthy. • Black and White individuals. • Men and Women (equally distributed). • Aged between 20-30 years. • Normotensive during screening phase (Clinic SBP<140 and DBP<90mmHg). • HIV uninfected.	• Type 1 or 2 Diabetes Mellitus. • HIV infection. • Fever (internal ear temperature > 37.5°C). • Medication use • Self-reported previous diagnoses with any chronic disease (such as type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis or renal disease). • Recent surgery or trauma (within the past three months). • Pregnant or lactating women.

African-PREDICT data collection (Figure 2-5)

Figure 2-5. ***Data collection of the African-PREDICT study.*** *A, transportation of participants; B, blood sample collection; C, questionnaires; [1]D, pulse wave velocity.*

During the week, on Tuesdays to Thursdays, participants were transported to and from the Hypertension Clinic and arrived at approximately 08h00. The participants where then familiarised with the research environment and all the measurements were again explained in detail. The participants had the opportunity to ask questions and participation in the study was voluntary and they were able to withdraw any time during the study. The procedures then commenced and the measurements were taken under supervision of a registered nurse, who is head of the Hypertension Research and Training Clinic. The

[1] ECG tracing not visible on picture.

participants received a light meal and a grocery voucher at the end of the day as token of appreciation for their participation.

2.2.5 Questionnaires

The questionnaires were given and explained by trained researchers in the participant's home language. Structured demographic, general health and socio-economic questionnaires were given to participants to gain information regarding age, smoking, alcohol intake, socio-economic status and the use of medication.

2.2.6 Cardiovascular measurements

Ambulatory blood pressure measurements

Participants were fitted with ambulatory blood pressure monitoring devices (Card(X)plore®, MediTech, Budapest, Hungary) to measure 24h systolic blood pressure and 24h diastolic blood pressure. The 24h pulse pressure (PP) was calculated as the difference between average systolic (SBP) and diastolic blood pressure (DBP). Furthermore, PP arises from the interaction between stroke volume and the characteristics of the arterial circulation and is therefore a marker of arterial stiffness and an independent predictor of cardiovascular mortality (9, 10). Appropriate sized cuffs were used which were attached to the non-dominant arm and the ambulatory blood pressure monitoring devices were fitted at the same time each day. The devices were programmed to measure blood pressure at 30-min intervals during the day and every hour during the night. The participants of this study population had a mean successful cuff inflation rate of 86.2 %.

Retinal vessels calibres

The participants had to be in a non-fasting state an hour before the retinal vessel calibres were measures. A trained registered nurse measured the risk for acute anterior glaucoma before the measurement and a 1% Tropicamide eye drop (Alcon Laboratories, Bryanston, South Africa) was administered in the right eye, 30 minutes prior to the measurement to achieve mydriatic conditions. A Zeiss Fundus Camera FF-450 Plus (Imedos Systems UG,

Jena, Germany) was used to perform the retinal photography mainly on the right eye. With the camera at an angle of 50°, colour and monochrome retinal images were taken using Visualis 2.81 software. The VesselMap2 software (Imedos Systems UG, Jena, Germany) was used to analyse the monochrome images. Colour images were used when the monochrome image did not have sufficient quality. All vessels that were within 0.5 – 1.0 optic disc diameters from the outer margin of the optic disc were selected as an artery or vein. The trunk region was primarily selected. The software automatically delineates the vessel margins. The Knudtson formula (computed with the six largest arteries and veins) (11) was used to determine the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE) was measured in measuring units (MU). Where 1 MU is equivalent to 1 µM of the dimensions of the eye are similar to the normal Gullstrand eye. The arteriolar-to-venular ratio (AVR) was calculated as CRAE/CRVE. The reproducibility of this method has been demonstrated in another cohort (1).

Calculation of central pulse pressure

*C*entral pulse pressure (cPP) was previously shown to be more predictive of CVD than peripheral PP (12-14). The SphygmoCor device was used to produce an arterial waveform with the participant in a supine position with a blood pressure cuff around the right upper arm and upper leg to calculate cPP.

2.2.7 PhD student participation in the African-PREDICT study

As PhD student, I formed part of the multidisciplinary research team and participated in the research measurements, which involved the following tasks:

- Eligibility screening of the participants;
- Anthropometric measurements;
- HIV testing and counselling;
- Responsibility for guiding participants to the different research stations where data collection took place;
- Conducting research measurements including:
    - Pulse wave analysis;

- Explaining and connecting of the ABPM devices to measure 24h SBP and 24h DBP at 30 min intervals during the day and every hour during the night. Capturing of ABPM data;
- Questionnaires (General Health Questionnaires and the Berlin Sleep Apnea Questionnaire).
- Laboratory assistance – Centrifuging of blood samples and aliquoting of plasma, serum and urine samples into cryovials. Safe packaging and storage were done in -80°C biofreezers.

2.3 The PURE-Study

2.3.1 Study design and participants

The detailed experimental protocol for data collection was previously described (15). The international Prospective Urban and Rural Epidemiology (PURE) study focuses on the societal determinants of non-communicable diseases in urban and rural areas in low, middle and high-income countries (16).

2.3.2 Recruitment

In 6000 houses (1500 in each community), household census data were gathered regarding the number of people, health profiles and ages. The heads of the households signed an informed consent form to fill out the questionnaire and if no one was home, a non-complier questionnaire was filled out. From the census the possible participants based on the following criteria were selected **(Table 2-2)**:

Table 2-2. Inclusion and exclusion criteria of the PURE-study

Inclusion criteria:	Exclusion criteria:
• Had to show migration stability and also had to be part of the North West province • Apparently healthy • Black ethnicity • Older than 35 years.	• Younger than 35 years • Coloured, Indian and White individuals • Pregnant and lactating women • Intoxicated • Cognitive impairment

2.3.3 Participants

A total of 4000 participants were identified (1000 participants from each community) based on the above-mentioned criteria. The study population consisted of 2010 black volunteers who agreed to participate in the study in the baseline data-collection phase in 2005. The first follow-up assessments were made by the team in 2010, while the second follow-up assessments were completed in 2015 (n=926). These participants were tracked by 16 trained fieldworkers within the communities who visited them at home every three months to ensure retention of participants. In cases of deceased participants, the cause of death was obtained by a family death certificate and verbal autopsy which were coded by a physician according to the International Classification of Diseases codes for underlying causes. Cardiovascular mortality was coded and included cerebrovascular events, myocardial infarctions, cardiac failure and stroke. The methodology appropriate to this sub-study will be discussed.

The sub-study is embedded in the South African leg of the PURE study and includes data collected at baseline (2005) and after ten years (2015). A total of N=1265 participants of the N=2010 who participated at baseline were included, based on the following criteria: i) participants who took part in both the baseline and ten year follow-up phase (N=923); and ii) participants who passed away over the course of ten years (N=342). We excluded N=278 participants with missing baseline and follow-up cardiovascular and/or missing baseline selenium data. A total of N=987 participants were therefore analysed in the

baseline phase and N=718 participants were analysed in the follow-up phase. From the N=718 participants, N=81 participants were randomly selected to determine their serum selenium levels at follow-up **(Figure 2-6)**.

Four different urban and rural areas were identified during baseline data collection in the North West Province and included the following areas:

- Ganyesa, a rural community West of Potchefstroom.
- Thlakgameng, a deep rural community 35 km East of Ganyesa.
- Ikageng township, urban community next to Potchefstroom.
- Informal settlements surrounding Ikageng, urban community

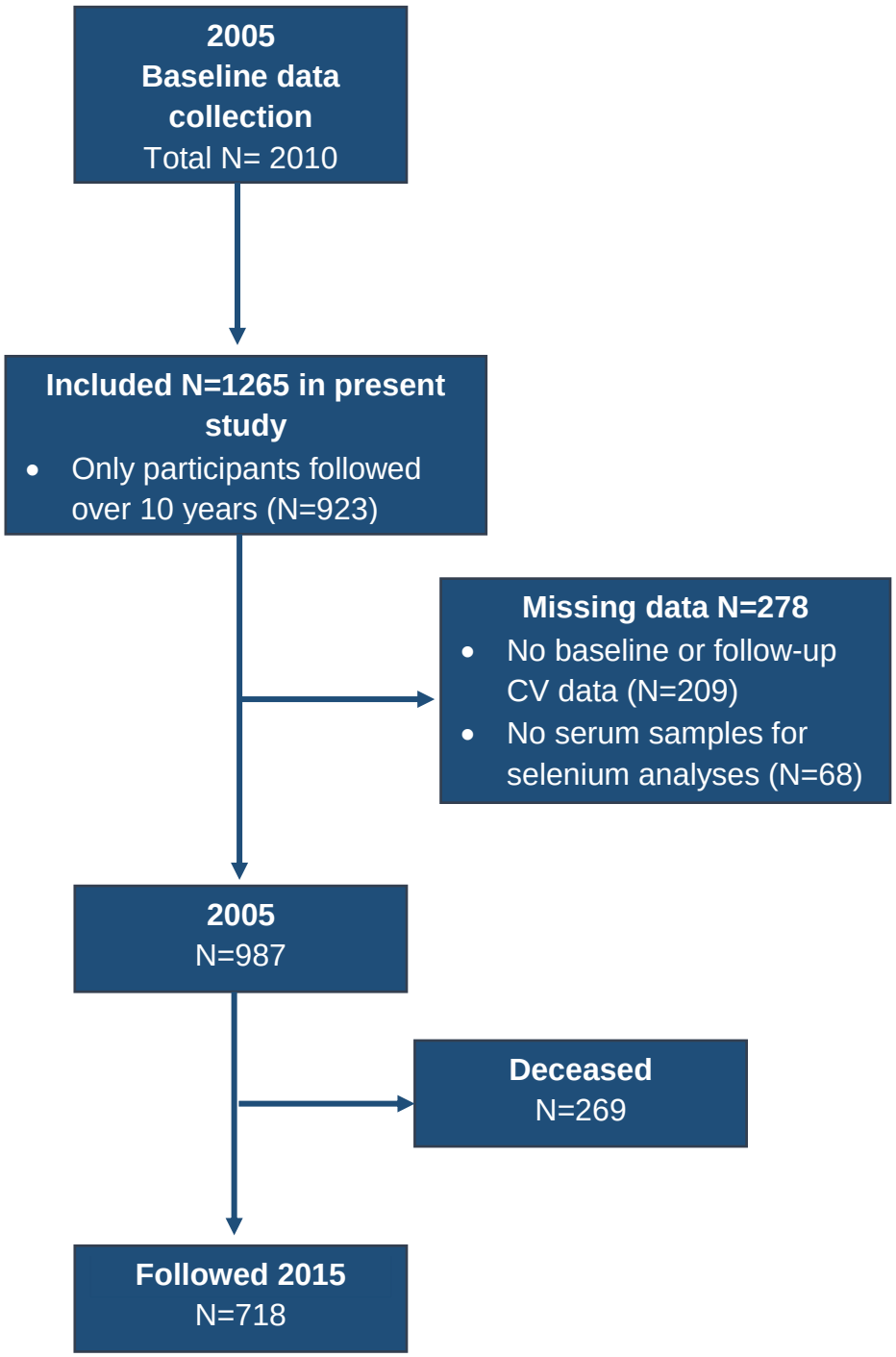

Figure 2-6. Layout of the PURE sub-study.

Figure 2-7. Rural areas of the PURE study

2.3.4 Experimental protocol (Figure 9)

Participants arrived at the rural and urban research facilities at approximately 08h00 after a 10-15 min drive from their communities, and the vehicles were provided by the research team **(Figure 2-7 and Figure 2-8)**. The participants were first introduced to the research setup after which the procedures had been explained by trained African field workers fluent in English and Tswana (participants' home language). All participants gave written informed consent and they were given the opportunity to ask questions concerning the study. Participation was voluntary and participants had the right to withdraw at any stage during the study without fear of victimisation. Participants were asked to fast for at least eight hours and not to smoke or exercise at least 30 min prior to the measurements. After data collection, individual post-counselling was provided to each participant with regards to his or her general health (including HIV status, blood pressure levels and fasting glucose levels) and, where necessary, referrals were made to the local clinic or hospital if irregularities were identified.

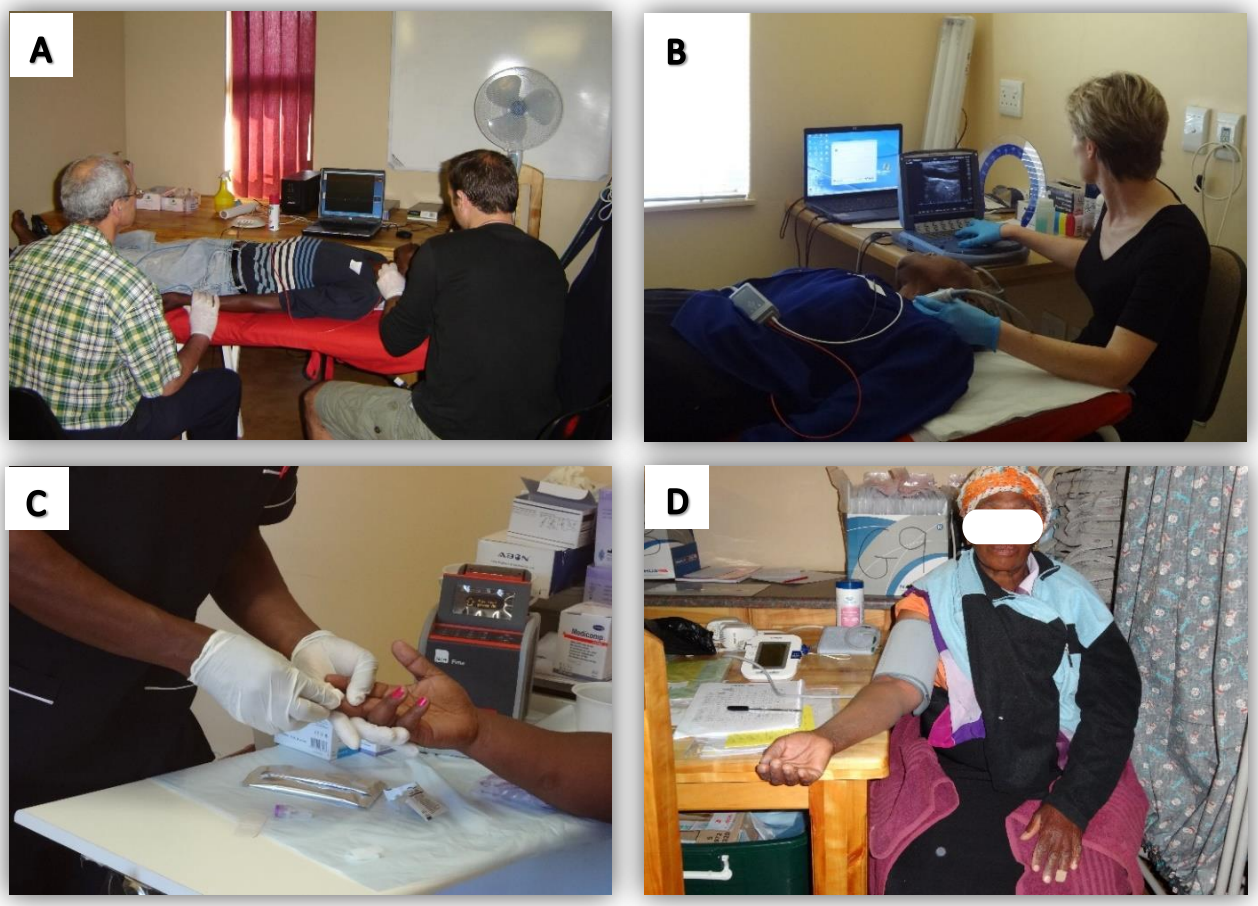

Figure 2-8. ***Data collection for the PURE study.*** *A, pulse wave velocity; B, Intima media thickness measurement; C, HIV testing, D, blood pressure.*

2.3.5 Questionnaires

The questionnaires were given and explained by trained researchers/fieldworkers in the participant's home language. Structured demographic, general health and socio-economic questionnaires were given to participants to gain information regarding age, smoking, alcohol intake, socio-economic status and the use of medication. The adapted BAECKE questionnaire was used to determine the physical activity index (17).

2.3.6 Cardiovascular measurements

Blood pressure

Blood pressure measurements were done while the participants were seated upright with the right arm supported at heart level (in duplicate, five min apart). Brachial SBP and DBP were measured with a validated OMRON device (Omron Healthcare, Kyoto, Japan) at baseline and follow-up. According to International and South African Guidelines (18), hypertension was classified as SBP≥140 and/or DBP≥90 mmHg or use of antihypertensive medication. The brachial PP was calculated as the difference between SBP and DBP. Mean arterial pressure (MAP) was calculated as MAP=DBP+(PP/3).

Intima media thickness

Carotid intima media thickness (IMT) can be seen as a marker of subclinical atherosclerosis (19). The SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothell, WA, USA), with a 6–13 MHz linear array transducer was used to obtain the IMT according to the Mannheim Consensus (20). The IMT is the best, non-invasive and simple method to assess carotid wall thickness in large population studies (19). Images from at least two optimal angles of the left and right common carotid arteries were obtained. The measurements were done on a selected segment of maximum ten mm with good image quality. It was performed by a single reader using a semi-automated program, namely the Artery Measurement Systems (AMS) II v1.139 (Chalmers University of Technology, Gothenburg, Sweden) to determine intima-media thickness of the far wall (IMTf) and near wall (IMTn). The cross-sectional wall area (CSWA) was calculated to confirm structural changes in luminal diameter: $CSWA = \pi(d/2 + CIMT)^2 - \pi(d/2)^2$, where d denotes the luminal diameter.

2.3.7 Student participation in the PURE study

As PhD student, I participated in the collection of the PWV data (SphygmoCor device) of the follow-up PURE study (2015) in both the rural (Ganyesa) and urban areas (Ikageng surrounding Potchefstroom).

2.4 Methodology of the Three Studies

2.4.1 Anthropometric and physical activity measurements (Table 2-3):

In all three studies the measurements were conducted according to standard procedures (21), in a private and temperature-controlled room, to ensure that the privacy of the participants was maintained.

Table 2-3. Anthropometric and physical activity measurements of the three studies

Measurement	Study	Apparatus
Weight (kg)	SABPA and PURE	Precision Health Scale. A & D Company, Tokyo, Japan
	African-PREDICT	SECA electronic scales, SECA, Birmingham, UK
Height (cm)	SABPA and PURE (2005)	Invicta Stadiometer, IP 1465, UK
	African-PREDICT	SECA 213 Portable Stadiometer , SECA, Birmingham, UK
	PURE (2015)	Leicester Stadiometer, SECA, Birmingham, UK
Waist circumference (cm)	African-PREDICT	Lufkin Steel Anthropometric Tape, W606PM, Lufkin, Apex, USA
Body mass index (BMI)	SABPA, African-PREDICT, PURE	Weight (kg)/height (m^2) (22).
Physical activity	SABPA	Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada)
	African-PREDICT	ActiHeart physical activity monitor (CamNtech, Cambridge, UK)
	PURE	Adapted BAECKE questionnaire

2.4.2 Cardiovascular measurements (Table 2-4)

All the cardiovascular measurements were performed in temperature-controlled rooms by trained researchers. We made use of the ABPM devices in the SABPA and African-PREDICT studies, as it is a more accurate measure than office blood pressure, whereas office blood pressure measurements are subjected to mechanical defects, physician error and white coat effects (23). We were unable to make use of the ABPM devices in the PURE study, due to the rural location and limited time to complete the study.

The carotid femoral pulse wave velocity (c-fPWV) is measured between the carotid artery and the femoral artery (24), whereas the carotid-dorsalis pedis pulse wave velocity (c-dPWV) is measured between the carotid artery and the dorsalis pedis artery in the foot (25) **(Figure 2-9)**. For the SABPA study we made use of the c-dPWV (Complior SP device). Although the c-fPWV is seen as the golden-standard measurement for arterial stiffness, as it is easy to use and has the best predictive value for cardiovascular events (26), the c-dPWV which includes both elastic and muscular arteries (27), correlated well with 24h blood pressure (28) and is seen as a readily accessible alternative method for the c-fPWV (29).

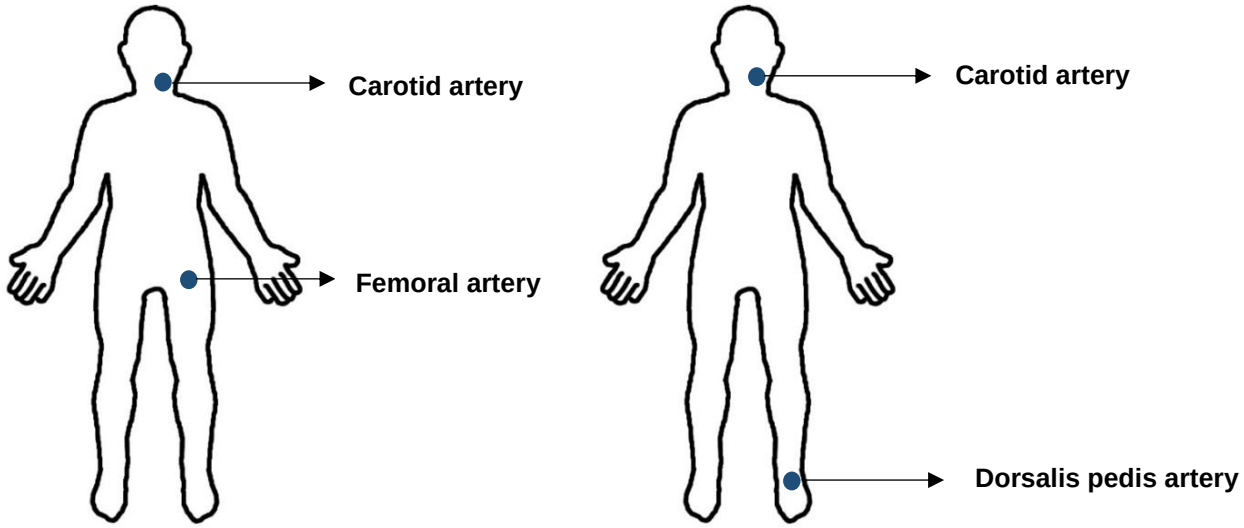

Figure 2-9. Different measuring sites for c-fPWV (left) and c-dPWV (right).

Table 2-4. Cardiovascular measurements of the three studies

Measurement	Study	Apparatus
ABPM devices (24h SBP, 24h DBP, 24h MAP)	SABPA	Cardiotens CE120®, Meditech, Budapest, Hungary
	African-PREDICT	Card(X)plore®, MediTech, Budapest, Hungary
PWV (c-dPWV, c-fPWV)	SABPA	Complior SP device, Artech-Medical, Pantin, France
	African-PREDICT and PURE (2015)	SphygmoCor XCEL device, AtCor Medical, Sydney, Australia
PWV (Augmentation index (AIx))	African-PREDICT	SphygmoCor XCEL device, AtCor Medical, Sydney, Australia

2.4.3 Blood sampling and biochemical measurements (Table 2-5)

Blood samples from fasting participants were obtained from brachial antecubital vein branches with a sterile winged infusion set after a five-min rest in the semi-Fowler's position. Serum and plasma were prepared according to standard procedures. All samples were stored at −80°C until biochemical analyses were performed. In all three studies serum selenium levels were analysed by an external accredited pathology laboratory. The method used inductively coupled plasma mass spectrometry, which can be seen as one of the leading techniques of elemental analysis which includes advantages such as a high sensitivity, a high specimen throughput and the possibility to obtain isotopic information (30, 31). The intra-assay variation was <10% and the inter-assay variation was 13.1% for selenium.

Table 2-5. Biochemical measurements of the three studies

Measurement	Sample	Study	Apparatus
• C-reactive protein (CRP) • Gamma-Glutamyl Transferase (GGT) • Total cholesterol • High density lipoprotein cholesterol (HDL-C) • Triglycerides	Serum	SABPA	Two sequential multiple analysers (Konelab 20i; ThermoScientific, Vantaa, Finland; Unicel DXC 800 Beckman and Coulter, Krefeld, Germany)
		African-PREDICT and PURE 2015	Cobas Integra 400 plus, Roche, Basel, Switzerland
		PURE 2005	Konelab20i™ auto-analyzer, (Thermo Fisher Scientific, Vantaa, Finland)
Low density lipoprotein cholesterol (LDL-C)	Serum	African-PREDICT and PURE 2015	Cobas Integra 400 plus (Roche, Basel, Switzerland)
	-	PURE 2005	Was calculated with the Friedewald formula (32)
Glycated hemoglobin (HbA1c)	Whole blood	SABPA and African-PREDICT	Turbidimetric inhibition immunoassay (Cobas Integra 400, Roche, Basel, Switzerland)
Cotinine (serum)	Serum	SABPA	Homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland)
		African-PREDICT	A chemiluminescence method (Immulite, Siemens, Erlangen, Germany)
Estimated glomerular filtration rate (eGFR)	Serum creatinine	SABPA	The Modification of Diet in Renal Disease Study equation was used to calculate eGFR (33)
		African-PREDICT	The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration Equation (34)
Glutathione peroxidase (GPx) activity	EDTA plasma	SABPA	Assay kits from Cayman Chemical Company (Ann Arbor, MI, USA) on a Bio-Tek FL600 Microplate reader (Winooski, VT, USA.)
	EDTA whole blood	African-PREDICT	Randox assay kits (Randox, Co. Antrim, United Kingdom) on the Cobas Integra 400, Roche, Basel, Switzerland
Selenium	Serum	SABPA, African-PREDICT and PURE	Inductively coupled plasma mass spectrometry method

Measurement	Sample	Study	Apparatus
Glucose	Sodium fluoride (NaF) Plasma	African-PREDICT and PURE 2015	Cobas Integra 400 plus, Roche, Basel, Switzerland
		PURE 2005	Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA)
Creatinine	Serum	African-PREDICT	Cobas Integra 400, Roche, Basel, Switzerland
Albumin and creatinine	Urine	African-PREDICT	Cobas Integra 400, Roche, Basel, Switzerland

2.4.4 HIV testing (Table 2-6)

Table 2-6. HIV testing of the three studies

Study	HIV test	Positive confirmation test
SABPA and	First Response rapid HIV test card (Premier Medical Corporation Limited, Daman, India).	Pareeshak card test (BHAT Bio-tech, India)
African-PREDICT		Abon (Biopharm Corporation Limited Hanyzhou, China)
PURE		2005: Pareeshak card test (BHAT Bio-tech, India) 2010: SD BIOLINE HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea) 2015: Abon (Biopharm Corporation Limited Hanyzhou, China)

2.4.5 Statistical analyses

The statistical analyses are explained in each article (Chapters 3-5) in detail. All the statistics were done with Statistica (TIBCO Software, Palo Alto, California, United States) and figures were done with GraphPad Prism (GraphPad Software Inc., California, USA).

2.4.6 Ethical considerations (Table 2-7)

Table 2-7. Ethical considerations of the three studies

Study	Ethical considerations
• **SABPA** • **African-PREDICT** • **PURE**	The studies fulfilled all the requirements as stated in the Helsinki Declaration (2008) for investigation on human participants and was further approved by the Health Research Ethics Committee of the North-West University (Letter of approval attached as Annexures A)
African-PREDICT	Endorsements by the National Department of Health, under the Section Non-communicable Diseases and the North West Province department of Health
PURE	Department of Health The principal investigator of the PURE-SA study consulted with the mayors of both Potchefstroom and Ganyesa The *inkosi* (tribal chief) of the rural communities in Ganyesa and the community leaders in the urban areas were also approached and verbal permission was granted

2.4.7 Other responsibilities of PhD student.

Apart from direct contributions to data collection and project participants in the African-PREDICT and PURE studies, I also conduct all data cleaning, planning, writing and composition of manuscripts as well as the statistical analyses.

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CHAPTER 3:
SERUM SELENIUM LEVELS, THE SELENOPROTEIN GLUTATHIONE PEROXIDASE AND VASCULAR PROTECTION: THE SABPA STUDY

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Serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study

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Abstract

Selenium is an important co-factor for the optimal functioning of the antioxidant enzyme, glutathione peroxidase (GPx). Studies investigating the association of selenium with blood pressure (BP) and hemodynamic measures are sparse. We therefore investigated whether serum selenium and GPx activity relate with blood pressure and carotid-dorsalis pedis pulse wave velocity (c-dPWV). In this cross sectional study we measured selenium levels, GPx activity, ambulatory blood pressure and arterial stiffness of 200 black and 209 white school teachers from South Africa. Serum selenium levels were significantly lower in black compared to white teachers ($p<0.001$), independent of gender. One in ten black men and one in five black women were selenium deficient (<8µg/100 ml). Only in white men inverse independent associations of systolic blood pressure (SBP) ($\beta= -0.19$; $p= 0.039$) and diastolic blood pressure (DBP) ($\beta=-0.21$; $p=0.029$) with selenium were found. In the same group, an inverse association between c-dPWV and GPx activity ($\beta=-0.23$; $p=0.017$) were also found. To conclude, lower serum selenium levels in black populations from the same geographical region as their white counterparts may impact on the loss of the vasculoprotective effects of selenium and selenoproteins such as GPx.

Keywords: Selenium, glutathione peroxidase, ambulatory blood pressure, arterial stiffness, race

Introduction

South Africa is currently undergoing rapid urbanisation. This involves a nutrition transition which includes changes from a diet high in fibre and low in fat (1) to ultra-processed foods that are more palatable. The nutrition transition may be accompanied by micronutrient malnutrition (2). Serum levels of selenium, an essential micronutrient, are dependent on dietary selenium intake (3). Selenium levels in plant foods are in turn dependent on the amount of selenium present in the soil (4). Meat, cereals, fish, eggs and dairy products are the main food groups which contain selenium (5). White South Africans consume a diet that includes mainly fresh meat products (6), which is a good source of selenium (7). In contrast, the majority of black South Africans consume a diet that includes maize products (8), which may contribute to the aetiology of selenium deficiency found in adults (9) and children (8).

Selenium is an important co-factor for the optimal functioning of the antioxidant enzyme, glutathione peroxidase (GPx) (10-12). Several studies have linked low selenium levels and GPx activity to the development of cardiovascular diseases (13, 14). Partly due to urbanisation, hypertension has become an increasing burden with increased morbidity and mortality, especially in black South Africans (15, 16). During the past four decades, the highest worldwide blood pressure levels have shifted from high-income countries to low-income countries in south Asia and sub-Saharan Africa (17). A systematic review focusing on populations from sub-Saharan Africa indicated a pooled prevalence of hypertension of 30%, whereas only 7% had controlled blood pressure (18). A high prevalence of hypertension was observed in black adults (19), males and particularly older people (17).

Black South Africans tend to have increased arterial stiffness at a younger age when compared to whites (20, 21). Oxidative stress may contribute to increased blood pressure (22, 23), carotid wall thickness (23, 24) and arterial stiffness (22, 25).

Studies investigating the associations of selenium with blood pressure and hemodynamic measures are sparse. Therefore, in this study serum selenium levels were compared between black and white adults. This study also investigated whether serum selenium

levels related to GPx activity; and whether 24h blood pressure, vascular resistance, arterial compliance and arterial stiffness related to both serum selenium and GPx activity.

Materials and Methods

Study population and protocol

This study formed part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. A detailed description of the study population and protocol has been published elsewhere (26). Baseline data (collected in 2008/9) were used in this cross-sectional data analyses. The study population consisted of a homogenous sample of 200 black (101 men and 99 women) and 209 white (108 women and 101 men) school teachers aged 20-65 years from the Dr Kenneth Kaunda Education District in the North West Province of South Africa. Exclusion criteria included the use of α- and β-blockers, an ear temperature >37.5°C, participants who were vaccinated or who had donated blood three months prior to the commencement of the study and pregnant or lactating women. The study fulfilled all the requirements as stated in the Helsinki Declaration (2008) for investigation on human participants and was further approved by the Health Research Ethics Committee of the North-West University (NWU-00036-07-S6). This sub-study was also approved by the Health Research Ethics Committee of the North-West University (NWU-00079-16-S1).

Anthropometric and Physical Activity Measurements

Trained anthropometrists measured weight and height in triplicate with calibrated instruments (Precision Health Scale. A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, UK) (27). Body mass index (BMI) was calculated as weight (kg)/height (m^2). Total energy expenditure was determined with the Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) over a 24h period.

Questionnaires

All the participants completed a general health questionnaire containing questions on lifestyle habits and medication use.

Cardiovascular Measurements

The participants were fitted with ambulatory blood pressure monitoring devices (CardioXplore®, MediTech, Budapest, Hungary) on the participant's non-dominant arm each morning at 08:00 to measure 24h systolic blood pressure (SBP), 24h diastolic blood pressure (DBP) and 24h mean arterial pressure. The ambulatory blood pressure monitoring devices were programmed to measure blood pressure at 30-min intervals during the day (08:00-22:00) and every hour during night time (22:00-06:00) with an overall successful 24h inflation rate of 78.9%.

The validated (28, 29) Finometer device® was used to measure continuous blood pressure. The Finometer device was connected, and after a ten min resting period, a five min continuous measurement of baseline cardiovascular variables was recorded. The average of the recordings of the last minute was used for further analysis. Finometer measurements were processed with Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) to determine total peripheral resistance and Windkessel compliance.

The carotid-dorsalis pedis pulse wave velocity (c-dPWV) was measured as a measure of arterial stiffness. The non-invasive measurement was taken across the carotid-dorsalis pedis region with the participant in a supine position (Complior SP device, Artech-Medical, Pantin, France). The distance was determined by subtracting the carotid artery to suprasternal notch distance from the distal measurement (subtraction method).

Biochemical Analyses

Blood samples from fasting participants were obtained from brachial antecubital vein branches with a sterile winged infusion set after a five min rest in the semi-Fowler's position. Serum and plasma were prepared according to standard procedures. All samples were stored at -80°C until biochemical analyses were performed.

Glycated haemoglobin was measured in EDTA whole blood via a turbidimetric inhibition immunoassay (Integra 400, Roche, Switzerland). Serum C-reactive protein, gamma glutamyl-transferase and the lipid profile including total cholesterol, high density

lipoprotein cholesterol (HDL-C) and triglycerides were determined in serum with two sequential multiple analysers (Konelab 20i; ThermoScientific, Vantaa, Finland; Unicel DXC 800 Beckman and Coulter, Krefeld, Germany). Serum cotinine levels were measured using a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland). Both the intra- and inter-assay coefficients of variation for all the assays were less than 10%. The Modification of Diet in Renal Disease Study equation was used to estimate glomerular filtration rate (30).

Glutathione peroxidase activity was measured in EDTA plasma samples with assay kits from Cayman Chemical Company (Ann Arbor, MI, USA) on a Bio-Tek FL600 Microplate reader (Winooski, VT, USA.) with an intra-assay variability of 5.7% and inter-assay variability of 7.2%. Serum selenium levels were analysed with a inductively coupled plasma mass spectrometry method and serum selenium deficiency was classified as selenium levels <8 µg/100 ml (31). The intra-assay variation was less than 10% and the inter-assay variation was 13.1% for selenium.

Statistical Analyses

Statistical analyses were performed with Statistica 13 (Statsoft Inc., Tulsa, OK, USA). Interactions of sex and ethnicity were tested for the relationship between cardiovascular variables (24h SBP, 24h DBP, total peripheral resistance, arterial compliance and c-dPWV) and both selenium and GPx activity, using multiple regression analyses. Data were expressed as arithmetic mean and standard deviation for normally distributed variables. Variables with a non-Gaussian distribution (glycated haemoglobin, triglycerides, HDL-cholesterol, total cholesterol: HDL-cholesterol, estimated glomerular filtration rate (eGFR), C-reactive protein, selenium, gamma glutamyl-transferase) were logarithmically transformed and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared using independent t-tests and Chi-square tests, respectively. Single regression analyses were done to investigate associations of selenium with GPx activity. Single and partial correlations (while adjusting for age, BMI and cotinine) were performed to investigate associations of 24h blood pressure, c-dPWV, Windkessel compliance and total peripheral resistance with selenium and GPx activity. Multiple regression analyses

were further performed to determine independent associations of cardiovascular variables with selenium and GPx activity. The models were compiled with 24h SBP, 24h DBP, night-time SBP and c-dPWV as main dependent variables and selenium and GPx activity as main independent variables. The following covariates were considered for entry in multiple regression analyses: age, BMI, waist circumference, cotinine, self-reported smoking, gamma glutamyl-transferase, self-reported alcohol use, glucose, glycated haemoglobin, total energy expenditure, C-reactive protein, Interleukin-6, total cholesterol, HDL-cholesterol, triglycerides, total cholesterol: HDL-cholesterol and antihypertensive medication. Based on bivariate correlations between potential covariates and the dependent and main independent variables, the following covariates were entered into the final models: age, BMI, cotinine, total cholesterol, total energy expenditure, antihypertensive medication, gamma glutamyl-transferase, C-reactive protein and glycated haemoglobin. Models with c-dPWV as main dependent variable were additionally adjusted for mean arterial pressure.

Results

Characteristics of the study population

Interactions of ethnicity on the associations of 24h SBP and 24h DBP with selenium (both p= 0.001), and of Windkessel arterial compliance with GPx activity (p= 0.009) were found. Interactions of gender on the associations of 24h SBP (p= 0.040), 24h DBP (p= 0.030) and Windkessel compliance (p= 0.001) with selenium were also found. Groups were stratified according to ethnicity and gender to compare black and white men and women **(Table 3-1)**.

Black men and women had higher 24h SBP, 24h DBP and 24h mean arterial pressure as well as night-time SBP and night time DBP (all p<0.001) when compared to white men and women. In black men Windkessel arterial compliance was lower (p<0.001) while c-dPWV was higher in black women (p= 0.045) when compared to their respective white counterparts. Serum selenium levels were lower in black men and women (p<0.001) whereas GPx was also lower in black women (p= 0.001) when compared to white men and women. Eleven percent of black men and 22% of black women also showed selenium

deficiency (<8 µg/100 ml) – with the incidence of selenium deficiency being higher in blacks (both $p \leq 0.003$) when compared to their white counterparts. Lifestyle factors of black men and women also differed from white participants, including higher gamma glutamyl-transferase in black men and women ($p < 0.001$) – a potential indicator of alcohol use. Total energy expenditure was lower in black men ($p < 0.001$) compared to white men.

Table 3-1: Lifestyle, anthropometric, cardiovascular and biochemical characteristics of black and white men and women.

	Black men	White men	p-values	Black women	White women	p-values
n	101	101		99	108	
Age (years)	43.2 ± 8.17	45.1 ± 11.0	0.17	45.6 ± 7.90	45.0 ± 10.7	0.65
Anthropometric measurements						
Height (cm)	171 ± 6.33	181 ± 6.46	<0.001	159 ± 5.84	167 ± 5.86	<0.001
Body mass (kg)	80.3 ± 17.9	95.7 ± 17.3	<0.001	82.5 ± 19.0	72.8 ± 18.2	<0.001
Body mass index (kg/m²)	27.6 ± 5.77	29.0 ± 5.20	0.059	32.76 ± 7.20	26.26 ± 6.29	<0.001
Cardiovascular measurements						
Ambulatory systolic blood pressure (mmHg)	138 ± 16.0	128 ± 10.4	<0.001	129 ± 15.1	121 ± 12.4	<0.001
Ambulatory diastolic blood pressure (mmHg)	87.9 ± 10.7	79.5 ± 7.44	<0.001	79.0 ± 8.62	73.9 ± 7.66	<0.001
Ambulatory mean arterial pressure (mmHg)	104 ± 12.1	95.6 ± 7.88	<0.001	95.5 ± 10.2	89.4 ± 8.79	<0.001
Night time systolic blood pressure (mmHg)	129 ± 18.0	117 ± 11.6	<0.001	119 ± 14.8	110 ± 14.7	<0.001
Night time diastolic blood pressure (mmHg)	78.7 ± 12.4	68.55 ± 8.28	<0.001	69.5 ± 9.57	64.6 ± 8.65	<0.001
Total peripheral resistance (mmHg/ml/s)	1.07 ± 0.32	1.07 ± 0.68	0.98	0.96 ± 0.42	1.01 ± 0.30	0.30
Arterial compliance (ml/mmHg)	1.88 ± 0.41	2.32 ± 0.52	<0.001	1.86 ± 0.42	1.88 ± 0.44	0.71
cdPulse Wave Velocity (m/s)*	8.93 ± 1.90	8.84 ± 1.90	0.75	8.03 ± 1.26	7.66 ± 1.26	0.045
Biochemical analyses						
Selenium (µg/100ml)	10.1 (7.00; 14.0)	12.7 (8.50; 17.7)	<0.001	9.39 (6.40; 13.0)	13.3 (9.30; 20.8)	<0.001
Selenium deficiency n (%)	11 (10.9)	1 (0.99)	0.003	21 (21.7)	3 (2.78)	<0.001
Glutathione peroxidase (nmol/min/ml)	34.6 ± 13.9	35.1 ± 8.03	0.75	31.9 ± 13.9	37.1 ± 7.82	0.001

	Black men	White men	p-values	Black women	White women	p-values
Glycated haemoglobin (%)	6.14 (5.20; 9.56)	5.64 (5.10; 6.40)	<0.001	5.83 (5.00; 7.60)	5.36 (5.00; 5.90)	<0.001
Triglycerides (mmol/l)	1.46 (0.64; 3.67)	1.30 (0.60; 3.00)	0.18	0.91 (0.40; 2.30)	0.80 (0.40; 2.20)	0.063
Total cholesterol (mmol/l)	4.74 ± 1.17	5.58 ± 1.20	<0.001	4.46 ± 1.20	5.50 ± 1.35	<0.001
High density lipoprotein cholesterol (mmol/l)	0.99 (0.57; 1.64)	0.96 (0.60; 1.40)	0.48	1.16 (0.70; 1.74)	1.34 (0.80; 2.30)	<0.001
Total cholesterol:HDL-cholesterol	4.65 (2.39; 7.78)	5.69 (3.70; 8.80)	<0.001	3.70 (2.30; 6.00)	3.99 (2.70; 6.80)	0.061
Estimated glomerular filtration rate (ml/min/1.73m²)	125 (92.0 ± 175)	93.0 (69.1 ± 122)	<0.001	96.7 (67.7 ± 134)	93.3 (68.9 ± 121)	0.21
C-reactive protein (mg/l)	2.75 (0.27; 14.2)	1.80 (0.99;.8.00)	0.002	7.13 (0.78; 35.7)	2.26 (0.99; 14.3)	<0.001
Lifestyle and comorbidities						
Cotinine (ng/l)	35.5 ± 65.0	30.9 ± 96.7	0.69	18.7 ± 55.4	15.1 ± 53.0	0.63
γ-Glutamyl transferase (U/l)	62.6 (23.7; 280)	27.3 (11.0; 90.0)	<0.001	35.5 (16.7; 117)	13.9 (6.00; 39.0)	<0.001
Total energy expenditure (kcal)/day	2715 ± 800	3674 ± 2059	<0.001	2654 ± 796	2587 ± 644	0.51
Multivitamin intake n (%)	1 (0.99)	7 (6.93)	0.030	0 (0.00)	14 (13.0)	<0.001
Anti-oxidant intake n (%)	0 (0.00)	1 (0.99)	0.32	0 (0.00)	4 (3.70)	0.053
Hypertensive status n (%)	79 (78.2)	56 (55.5)	0.001	53 (53.5)	26 (24.1)	<0.001
Anti-hypertensive medication n (%)	36 (35.6)	14 (13.9)	<0.001	33 (33.3)	13 (12.0)	<0.001
Diabetes n (%)	7 (6.93)	1 (0.99)	0.030	3 (3.03)	1 (0.93)	0.27
HIV infected n (%)	13 (12.9)	0 (0.00)	<0.001	6 (6.06)	0 (0.00)	0.009

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries or n (%). *Adjusted for mean arterial pressure.

Single and Partial Regression Analyses

In **Figure 3-1**, a positive correlation between selenium and GPx activity was evident in the total group (n= 409; r= 0.14; p= 0.004). After exploring the same association separately in black and white men and women, GPx related positively to selenium only in white women (r= 0.26; p= 0.006).

In single regression analyses **(Table A 3-1, Figure 3-2)**, significant associations were predominantly found in white men, where 24h SBP (r= -0.27; p= 0.01), 24h DBP (r= -0.24; p= 0.02), night time SBP (r= -0.27; p= 0.01) and night time DBP (r= -0.20; p= 0.04) associated negatively with selenium. After adjusting for age, BMI and cotinine, the following associations were borderline significant: 24h SBP and selenium (p= 0.052), 24h DBP and selenium (p= 0.080), night-time SBP and selenium (p= 0.056), whereas the association of night time DBP with selenium lost significance (p= 0.19) **(Table A 3-1)**. In single and partial regression analyses, c-dPWV (r= -0.26; p= 0.009) was negatively associated with GPx activity in white men. These associations were absent in black men.

In black women 24h SBP (r= -0.21; p= 0.04) and night time SBP (r= -0.24; p= 0.02) were also negatively associated with GPx activity. After adjusting for age, BMI and cotinine, only the association of night time SBP with GPx remained significant (r= -0.22; p= 0.038) whereas the association of 24h SBP with GPx became borderline significant (p= 0.087). These associations were absent in white women.

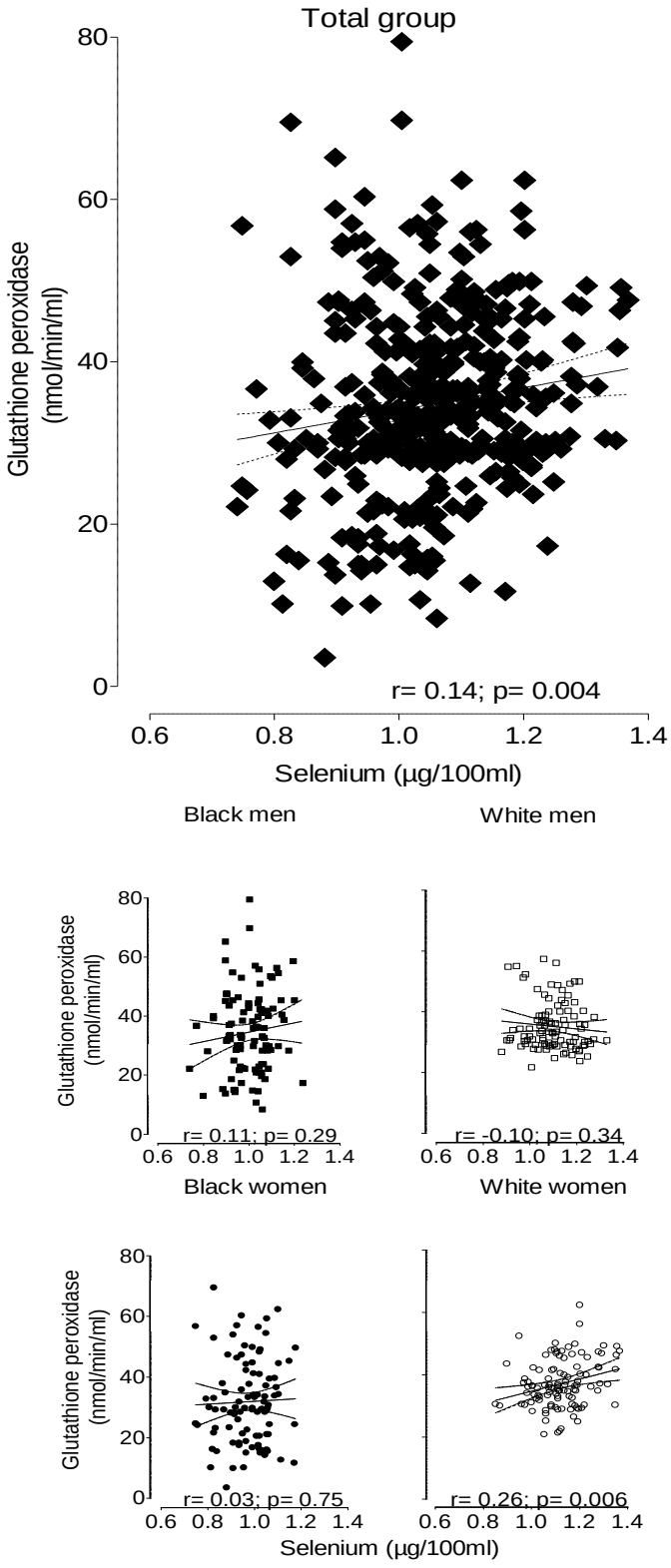

Figure 3-1. ***Single regression analyses between selenium and glutathione peroxidase in the total group as well as in the black and white men and women.***

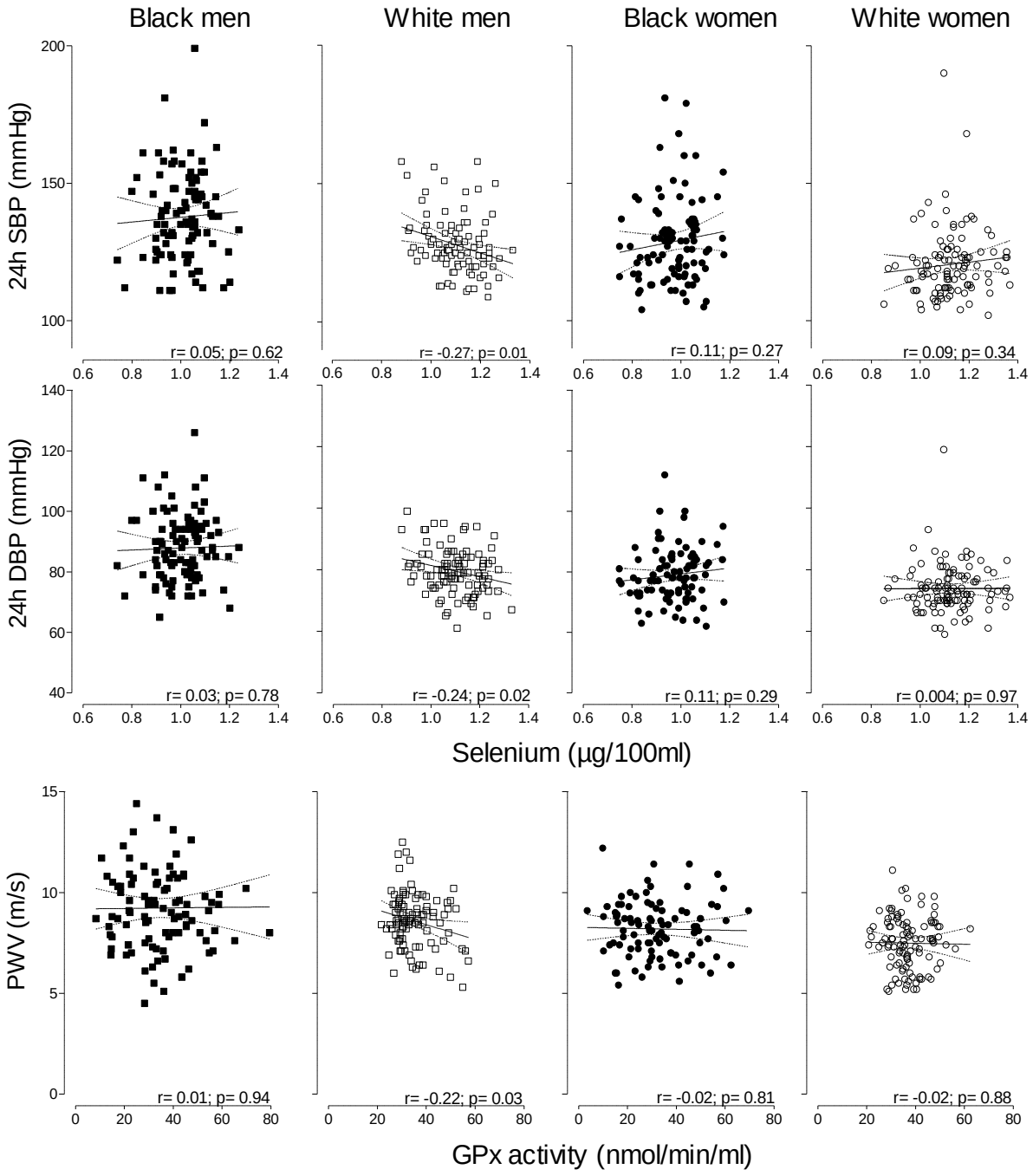

Figure 3-2. Unadjusted correlations of cardiovascular variables with selenium and glutathione peroxidase activity in black and white men and women.

Table A 3-1. Single and partial regression analyses of cardiovascular variables with selenium and glutathione peroxidase activity.

	24h SBP (mmHg)		24h DBP (mmHg)		Night time SBP (mmHg)		Night time DBP (mmHg)		Total peripheral resistance (mmHg/ml/s)		Windkessel arterial compliance (ml/mmHg)		cd-PWV (m/s)	
	Single	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**
Black men (n=101)														
Selenium (µmol/100ml)	r= 0.05 p= 0.62	r= 0.05 p=0.60	r= 0.03 p= 0.78	r= 0.02 p= 0.84	r= 0.01 p= 0.95	r= 0.01 p= 0.89	r= -0.02 p= 0.88	r= -0.01 p= 0.89	r= -0.06 p= 0.56	r= -0.04 p= 0.70	r= 0.15 p= 0.14	r= 0.12 p= 0.26	r= -0.07 p=0.47	r= -0.06 p= 0.55
Glutathione peroxidase (nmol/min/ml)	r= -0.08 p= 0.41	r= -0.002 p=0.99	r= -0.12 p= 0.24	r= -0.07 p= 0.51	r= -0.09 p= 0.39	r= -0.01 p= 0.92	r= -0.15 p= 0.14	r= -0.09 p= 0.37	r= -0.07 p=0.52	r= -0.08 p= 0.45	r= 0.12 p= 0.24	r= 0.02 p= 0.86	r= 0.01 p= 0.94	r= 0.11 p= 0.28
White men (n=101)														
Selenium (µmol/100ml)	r= -0.27 p= 0.01	r= -0.20 p= 0.052	r= -0.24 p= 0.02	r= -0.18 p= 0.080	r= -0.27 p= 0.01	r= -0.19 p= 0.056	r= -0.20 p= 0.04	r= -0.13 p= 0.19	r= 0.01 p= 0.94	r= -0.03 p= 0.77	r= -0.01 p=0.95	r= 0.13 p= 0.21	r= 0.07 p= 0.48	r= 0.12 p= 0.23
Glutathione peroxidase (nmol/min/ml)	r= -0.10 p= 0.32	r= -0.04 p= 0.66	r= -0.04 p= 0.68	r= -0.001 p= 0.995	r= -0.10 p= 0.30	r= -0.05 p= 0.59	r= 0.02 p= 0.82	r= 0.06 p= 0.53	r= -0.03 p=0.74	r= -0.06 p= 0.58	r= -0.08 p= 0.46	r= 0.004 p= 0.97	r= -0.22 p= 0.03	r= -0.26 p= 0.009

	24h SBP (mmHg)		24h DBP (mmHg)		Night time SBP (mmHg)		Night time DBP (mmHg)		Total peripheral resistance (mmHg/ml/s)		Windkessel arterial compliance (ml/mmHg)		cd-PWV (m/s)	
	Single	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**	**Single**	**Partial**
Black women (n=97)														
Selenium (µmol/100ml)	r= 0.11	r= 0.11	r= 0.11	r= 0.13	r= 0.08	r= 0.07	r= 0.06	r= 0.08	r= -0.03	r= 0.02	r= 0.02	r= -0.16	r= 0.10	r= 0.07
	p= 0.27	p= 0.29	p= 0.29	p= 0.20	p= 0.43	p= 0.49	p= 0.57	p= 0.44	p=0.76	p=0.83	p=0.84	p= 0.12	p= 0.33	p= 0.52
Glutathione peroxidase (nmol/min/ml)	r= -0.21	r= -0.18	r= -0.14	r= -0.10	r= -0.24	r= -0.22	r= -0.19	r= -0.17	r= 0.15	r= 0.13	r= 0.09	r= 0.19	r= -0.02	r= 0.07
	p= 0.04	p= 0.087	p= 0.18	p= 0.34	p= 0.02	p= 0.038	p= 0.06	p= 0.11	p= 0.15	p=0.22	p= 0.38	p= 0.066	p=0.81	p=0.53
White women (n=108)														
Selenium (µmol/100ml)	r= 0.09	r= 0.03	r= 0.004	r= -0.05	r= 0.03	r= -0.03	r= -0.02	r= -0.08	r= 0.02	r= -0.10	r= -0.12	r= 0.04	r= 0.02	r= -0.12
	p= 0.34	p= 0.73	p= 0.97	p= 0.60	p= 0.77	p= 0.76	p= 0.82	p= 0.39	p=0.85	p= 0.33	p=0.21	p= 0.70	p=0.88	p= 0.22
Glutathione peroxidase (nmol/min/ml)	r= 0.13	r= 0.06	r= 0.15	r= 0.10	r= 0.14	r= 0.09	r= 0.15	r= 0.10	r= 0.10	r= 0.04	r= -0.10	r= 0.02	r= -0.02	r= -0.19
	p= 0.18	p= 0.56	p= 0.13	p= 0.32	p= 0.15	p= 0.35	p= 0.13	p= 0.30	p=0.31	p= 0.67	p= 0.33	p= 0.80	p=0.88	p= 0.058

SBP, systolic blood pressure; DBP, diastolic blood pressure; cd-PWV, carotid-distal pulse wave velocity. Bold values indicate statistical significance ($p<0.05$).

Multivariate Analyses

In multivariable adjusted regression analyses, the previous results in white men were confirmed, namely negative associations of 24h SBP (β= -0.19; p= 0.039), 24h DBP (β= -0.21; p= 0.029) and night time SBP (β= -0.20; p= 0.040) with selenium, as well as the negative association between c-dPWV and GPx activity (β= -0.23; p= 0.017) **(Table 3-2 and Table A 3-2)**. In black women, the negative association between 24h night time SBP (β= -0.21; p= 0.036) and GPx activity was also confirmed to be independent of various covariates **(Table 3-2 and Table A 3-3)**.

Table 3-2. Summary of forward stepwise regression analyses with carotid-distal pulse wave velocity and 24h blood pressure as dependent variables in the total group as well as in the black and white men and women.

	24h systolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.33	-	-	0.33	-	-
Black men	0.24	-	-	0.24	-	-
White men	0.29	-0.19 (-0.37; -0.01)	0.039	0.26	-	-
Black women	0.14	-	-	0.17	-	-
White women	0.39	-	-	0.40	-	-
	24h diastolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.34	-	-	0.34	-	-
Black men	0.17	-	-	0.18	-	-
White men	0.27	-0.21 (-0.39; -0.02)	0.029	0.23	-	-
Black women	0.13	-	-	0.12	-	-
White women	0.28	-	-	0.28	-	-

	24h night time systolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.30	-	-	0.30	-	-
Black men	0.17	-	-	0.17	-	-
White men	0.22	-0.20 (-0.39; -0.01)	0.040	0.18	-	-
Black women	0.07	-	-	0.11	-0.21 (-0.41; -0.02)	0.036
White women	0.37	-	-	0.38	-	-
	Pulse wave velocity*					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β-value (95% CI)	p-value
Total group	0.31	-	-	0.31	-	-
Black men	0.24	-	-	0.24	-	-
White men	0.16	-	-	0.21	-0.23 (-0.41; -0.04)	0.017
Black women	0.28	-	-	0.28	-	-
White women	0.35	-	-	0.35	-	-

- indicates that the variable did not enter the model. The main independent variables included in the models were selenium and GPx and other covariates included age, body mass index, cotinine, total cholesterol, total energy expenditure, antihypertensive medication usage, y-glutamyl transferase, C-reactive protein, glycated haemoglobin. *Mean arterial pressure additionally added in the model

Table A 3-2. Forward stepwise regression analysis with pulse wave velocity or 24h blood pressure as dependent variables and selenium as main independent variable.

	24 h systolic blood pressure			
	Total group (n=409)			
Adjusted R^2	**0.33**			
	β (95% CI)		**p-value**	
Age (years)	0.10 (0.02; 0.19)		0.020	
Body mass index (kg/m²)	0.28 (0.18; 0.38)		<0.001	
γ-Glutamyl transferase (U/l)	0.15 (0.04; 0.26)		0.008	
Glycated haemoglobin (%)	0.17 (0.08; 0.26)		<0.001	
Race (black/white)	-0.14 (-0.26; -0.01)		0.031	
Gender (men/women)	-0.20 (-0.30; -0.10)		<0.001	
	Black men (n=101)		**White men (n=101)**	
Adjusted R^2	**0.24**		**0.29**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Body mass index (kg/m²)	-	-	0.26 (0.04; 0.48)	0.023
Selenium (µmol/100ml)	-	-	-0.19 (-0.37; -0.01)	0.039
Glycated haemoglobin (%)	-	-	0.26 (0.07; 0.45)	0.009
Age (years)	0.27 (0.07; 0.48)	0.011	-	-
Total energy expenditure (kcal)	0.29 (0.05; 0.53)	0.022	-	-
	Black women (n=99)		**White women (n=108)**	
Adjusted R^2	**0.14**		**0.39**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.25 (0.04; 0.47)	0.021	0.20 (0.01;0.38)	0.037
Total energy expenditure (kcal)	0.33 (0.07; 0.59)	0.014	-	-
Body mass index (kg/m²)	-	-	0.34 (0.04; 0.63)	0.026
	24 h diastolic blood pressure			
	Total group (n=409)			
Adjusted R^2	**0.34**			
	β (95% CI)		**p-value**	
Age (years)	0.10 (0.01; 0.18)		0.023	
Body mass index (kg/m²)	0.14 (0.04; 0.24)		0.007	
γ-Glutamyl transferase (U/l)	0.22 (0.11; 0.33)		<0.001	
Glycated haemoglobin (%)	0.15 (0.06; 0.23)		0.001	
Race (black/white)	-0.15 (-0.27; -0.02)		0.022	
Gender (men/women)	-0.25 (-0.35; -0.15)		<0.001	

	Black men (n=101)		White men (n=101)	
Adjusted R^2	**0.17**		**0.27**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
y-Glutamyl transferase (U/l)	0.24 (0.05; 0.43)	0.015	-	-
Glycated haemoglobin (%)	0.22 (0.04: 0.41)	0.022	-	-
Selenium (µmol/100ml)	-	-	-0.21 (-0.39; -0.02)	0.029
C-reactive protein (mg/l)	-	-	0.23 (0.02; 0.43)	0.031
	Black women (n=99)		**White women (n=108)**	
Adjusted R^2	**0.13**		**0.28**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Cotinine (ng/l)	-0.20 (-0.41; -0.003)	0.050		
Total energy expenditure (kcal)	0.29 (0.03: 0.55)	0.031	0.42 (0.11; 0.73)	0.010
Antihypertensive medication (yes/no)	-0.23 (-0.44; -0.02)	0.032	-	-
C-reactive protein (mg/l)	- 0.23 (-0.44; -0.01)	0.040	-	-
Age (years)			0.20 (0.007; 0.40)	0.046

	24 h night-time systolic blood pressure	
	Total group (n=409)	
Adjusted R^2	**0.30**	
	β (95% CI)	**p-value**
Body mass index (kg/m²)	0.25 (0.15; 0.36)	<0.001
Glycated haemoglobin (%)	0.17 (0.08; 0.27)	<0.001
Race (black/white)	-0.16 (-0.29; -0.03)	0.015
Gender (men/women)	-0.21 (-0.31; -0.11)	<0.001

	Black men (n=101)		White men (n=101)	
Adjusted R^2	**0.17**		**0.22**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.28 (0.06; 0.49)	0.012	**-**	**-**
Glycated haemoglobin (%)	0.22 (0.03; 0.40)	0.026	**-**	**-**
Body mass index (kg/m²)	-	-	0.23 (0.004; 0.46)	0.049
Selenium (µmol/100ml)	-	-	-0.20 (-0.39; -0.01)	0.040
Glycated haemoglobin (%)	-	-	0.20 (0.003; 0.40)	0.049
	Black women (n=99)		**White women (n=108)**	
Adjusted R^2	**0.07**		**0.37**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Total energy expenditure (kcal)	0.31 (0.04; 0.59)	0.026	**-**	**-**
Body mass index (kg/m²)			0.48 (0.18; 0.77)	0.002

	Pulse wave velocity (cd-PWV)*			
	Total group (n=409)			
Adjusted R^2	**0.31**			
	β (95% CI)		**p-value**	
Age (years)	0.26 (0.17; 0.35)		<0.001	
Glycated haemoglobin (%)	0.18 (0.09; 0.27)		<0.001	
Gender (men/women)	-0.19 (-0.30; -0.09)		<0.001	
Mean arterial pressure (mmHg)	0.26 (0.16; 0.36)		<0.001	
	Black men (n=101)		**White men (n=101)**	
Adjusted R^2	**0.24**		**0.16**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.29 (0.07; 0.50)	0.010	0.31 (0.11; 0.50)	0.002
C-reactive protein (mg/l)	-0.24 (-0.47; -0.02)	0.039	0.24 (0.01; 0.46)	0.044
Glycated haemoglobin (%)	0.32 (0.13; 0.51)	0.001	-	-
Mean arterial pressure (mmHg)	0.21 (0.01; 0.42)	0.046	-	-
Body mass index (kg/m²)	-	-	-0.30 (-0.55; -0.06)	0.018
	Black women (n=99)		**White women (n=108)**	
Adjusted R^2	**0.28**		**0.35**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.30 (0.10; 0.50)	0.004	0.36 (0.17; 0.55)	<0.001
Mean arterial pressure (mmHg)	0.41 (0.22; 0.61)	<0.001	0.30 (0.09; 0.50)	0.005
Total cholesterol (mmol/l)	-	-	0.20 (0.03; 0.37)	0.025
Glycated haemoglobin (%)	-	-	0.20 (0.02; 0.38)	0.029

– indicates that the variable did not enter the model. Covariates included in the models were age, body mass index, cotinine, selenium, total cholesterol, total energy expenditure, antihypertensive medication usage, γ-glutamyl transferase, C-reactive protein, glycated haemoglobin. *Mean arterial pressure additionally added in the model.

Table A 3-3. Forward stepwise regression analysis with pulse wave velocity or 24h blood pressure as dependent variables and GPx activity as main independent variable

	24 h systolic blood pressure			
	Total group (n=409)			
Adjusted R^2	0.33			
	β (95% CI)		p-value	
Age (years)	0.10 (0.02; 0.19)		0.021	
Body mass index (kg/m²)	0.28 (0.17; 0.38)		<0.001	
γ-Glutamyl transferase (U/l)	0.15 (0.04; 0.27)		0.007	
Glycated haemoglobin (%)	0.17 (0.08; 0.26)		<0.001	
Race (black/white)	-0.13 (-0.25; -0.02)		0.024	
Gender (men/women)	-0.20 (-0.30; -0.10)		<0.001	
	Black men (n=101)		White men (n=101)	
Adjusted R^2	0.24		0.26	
	β (95% CI)	p-value	β (95% CI)	p-value
Age (years)	0.27 (0.06; 0.48)	0.013	-	-
Total energy expenditure (kcal)	0.28 (0.04; 0.52)	0.022	-	-
Body mass index (kg/m²)	-	-	0.31 (0.09; 0.53)	0.006
Glycated haemoglobin (%)	-	-	0.27 (0.08; 0.46)	0.008
	Black women (n=99)		White women (n=108)	
Adjusted R^2	0.17		0.40	
	β (95% CI)	p-value	β (95% CI)	p-value
Age (years)	0.23 (0.02; 0.43)	0.033	0.18 (0.003; 0.37)	0.050
Total energy expenditure (kcal)	0.32 (0.07; 0.57)	0.015	-	-
Body mass index (kg/m²)	-	-	0.34 (0.05; 0.63)	0.026
	24 h diastolic blood pressure			
	Total group (n=409)			
Adjusted R^2	0.34			
	β (95% CI)		p-value	
Age (years)	0.10 (0.01; 0.18)		0.024	
Body mass index (kg/m²)	0.14 (0.04; 0.24)		0.007	
γ-Glutamyl transferase (U/l)	0.22 (0.11; 0.33)		<0.001	
Glycated haemoglobin (%)	0.14 (0.06; 0.23)		0.001	
Race (black/white)	-0.16 (-0.27; -0.04)		0.007	
Gender (men/women)	-0.25 (-0.35; -0.15)		<0.001	
	Black men (n=101)		White men (n=101)	
Adjusted R^2	0.18		0.23	
	β (95% CI)	p-value	β (95% CI)	p-value
γ-Glutamyl transferase (U/l)	0.24 (0.06; 0.43)	0.013	-	-
Glycated haemoglobin (%)	0.23 (0.04; 0.42)	0.019	-	-
Body mass index (kg/m²)	-	-	0.26 (0.04; 0.49)	0.022

	Black women (n=99)		White women (n=108)	
Adjusted R^2	**0.12**		**0.28**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Total energy expenditure (kcal)	0.28 (0.02: 0.54)	0.039	0.42 (0.11; 0.74)	0.009
Antihypertensive medication (yes/no)	-0.23 (-0.44; -0.02)	0.032	-	-
C-reactive protein (mg/l)	- 0.23 (-0.44; -0.01)	0.039	-	-
	24 h night time systolic blood pressure			
	Total group (n=409)			
Adjusted R^2	**0.30**			
	β (95% CI)		**p-value**	
Body mass index (kg/m²)	0.25 (0.15; 0.36)		<0.001	
Glycated haemoglobin (%)	0.17 (0.08; 0.26)		<0.001	
Race (black/white)	-0.17 (-0.29; -0.05)		0.004	
Gender (men/women)	-0.21 (-0.31; -0.11)		<0.001	
	Black men (n=101)		**White men (n=101)**	
Adjusted R^2	**0.17**		**0.18**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.27 (0.06; 0.49)	0.016	**-**	**-**
Glycated haemoglobin (%)	0.22 (0.03; 0.40)	0.026	0.21 (0.01: 0.41)	0.042
Body mass index (kg/m²)	-	-	0.29 (0.06; 0.52)	0.014
	Black women (n=99)		**White women (n=108)**	
Adjusted R^2	**0.11**		**0.38**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Glutathione peroxidase (nmol/min/ml)	-0.21 (-0.41; -0.02)	0.036	**-**	**-**
Total energy expenditure (kcal)	0.31 (0.05; 0.58)	0.022	-	-
Body mass index (kg/m²)	-	-	0.48 (0.18; 0.77)	0.002
	Pulse wave velocity (cd-PWV)*			
	Total group (n=409)			
Adjusted R^2	**0.31**			
	β (95% CI)		**p-value**	
Age (years)	0.26 (0.17; 0.35)		<0.001	
Glycated haemoglobin (%)	0.18 (0.08; 0.27)		<0.001	
Gender (men/women)	-0.19 (-0.30; -0.09)		<0.001	
Mean arterial pressure (mmHg)	0.26 (0.16; 0.36)		<0.001	
	Black men (n=101)		**White men (n=101)**	
Adjusted R^2	**0.24**		**0.21**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.31 (0.09; 0.52)	0.007	0.32 (0.14; 0.51)	0.001
C-reactive protein (mg/l)	-0.24 (-0.47; -0.02)	0.039	-	-
Glycated haemoglobin (%)	0.3 (0.12; 0.49)	0.002	-	-
Mean arterial pressure (mmHg)	0.22 (0.02; 0.43)	0.038	-	-
Body mass index (kg/m²)	-	-	-0.32 (-0.55; -0.08)	0.009
Glutathione peroxidase (nmol/min/ml)			-0.23 (-0.41; -0.04)	0.017

	Black women (n=99)		White women (n=108)	
Adjusted R^2	**0.35**		**0.29**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Age (years)	0.29 (0.09; 0.48)	0.005	0.37 (0.18; 0.56)	<0.001
Mean arterial pressure (mmHg	0.43 (0.23; 0.63)	<0.001	0.32 (0.11; 0.52)	0.003
Total cholesterol (mmol/l)	-	-	0.18 (0.01: 0.35)	0.038
Glycated haemoglobin (%)	-	-	0.19 (0.01; 0.37)	0.039

Covariates included in the models were age, body mass index, cotinine, glutathione peroxidase, total cholesterol, total energy expenditure, antihypertensive medication usage, γ-glutamyl transferase, C-reactive protein, glycated haemoglobin. *Mean arterial pressure additionally added in the model.

Sensitivity Analyses

When excluding participants taking oral antioxidants and multivitamins (n= 27), the associations of selenium and GPx activity with cardiovascular variables remained significant (all $p<0.05$). Also after excluding Human Immunodeficiency Virus (HIV) infected participants (n= 19) the results remained unchanged.

Discussion

In this study, which included black and white school teachers from the North West Province of South Africa, serum selenium levels were significantly lower in black compared to white teachers, independent of sex. It was also found that one in ten black men, and one in five black women were selenium deficient (<8 µg/100 ml) (31). Only 1% of the white men and 3% of the white women were selenium deficient. Selenium and GPx activity were protectively associated with blood pressure and arterial stiffness, respectively, in white men only.

The lower selenium levels and higher prevalence of selenium deficiency in the black groups indicated in the present study are in line with previous studies from South Africa which indicated that children (8) and black women (9) had inadequate selenium intake. The same results were also found when serum selenium levels were compared between African Americans and white Americans (32). In the present study, all the participants were teachers from the North West Province of South Africa and therefore shared the same geographic location and socio-economic status. However, there may have been dietary and cultural differences (33).

Selenium plays a vascular-protective role (34) as it is involved in the synthesis of selenoproteins such as GPx (35) and thioredoxin reductase (36, 37). It is therefore expected that serum selenium will relate positively to GPx activity. Indeed, a positive association of serum selenium levels with GPx activity in the total group of 409 teachers was found. However, when this link was investigated in the individual groups, the association of serum selenium levels with GPx was only evident in the white women. Although a positive association between these factors was expected, previous studies investigated the link between serum selenium levels and GPx activity and found controversial results. In a previous study which consisted of 27 normal pregnant, 25 pre-eclamptic, and 22 healthy age-matched non-pregnant white women, a positive association of maternal serum selenium with both maternal plasma and placental GPx activity was indicated (36). In another study which included healthy participants from three different geographic areas including New Zealand, Oregon and South Dakota indicated this correlation only in people with very low selenium status (59 ± 11 ng/ml whole blood) (38). These findings contradict the findings of the present study where white women had the highest selenium levels. However, in another study it was found that in 50-69 year old men and women this association was evident only in men and not in women (39), which may indicate gender-specific differences in selenium metabolism and selenoprotein expression (40).

Nutrigenetics, which is the response of genetic variations on nutrients and the interaction thereof with disease states (41), may also be a reason why there were no associations found between selenium and GPx activity in the other individual groups of the present study. Research from previous studies suggested that effective dietary selenium intake may differ amongst individuals which may be due to genetic variants in selenoproteins (42). In turn, this may influence the way in which the body metabolizes and utilizes selenium (43-45).

The results of the present study are in line with previous findings reported by Nawrot et al. which indicated that blood pressure was inversely associated with blood selenium levels in men only (46). When investigating the associations of both selenium and GPx activity with blood pressure and arterial stiffness, significant findings in the current study were mainly encountered in white men, where only 1% was classified as

being selenium deficient. In the same group 24h systolic and diastolic blood pressure were inversely associated with serum selenium. However, inconsistent results were found among studies which investigated the link between blood pressure and serum selenium (47, 48). One study which included men and women aged 59-71 years (n=1389) with normal selenium levels, indicated a positive association between blood pressure and serum selenium levels in men (47). In another study, no association was found between blood pressure and selenium in a population group which consisted of men aged 55-74 years in Finland (48), in which low selenium levels were found in 16% of the men in Eastern and 42% of the men from South Western Finland. This may suggest that these factors are not associated in conditions or populations with very low selenium levels.

In support of the beneficial associations between blood pressure and selenium in white men, it was also found that arterial stiffness was negatively associated with GPx activity. Previously it was reported that oxidative stress was linked to arterial stiffness (2, 25), and the link between GPx and arterial stiffness in the present study suggests that GPx activity may be protective against arterial stiffening. Low selenium levels may lead to increased oxidative stress (49, 50) and inflammation (51, 52), which may cause endothelial dysfunction and contribute to the development of arterial stiffness and hypertension (53, 54).

Apart from the findings in white men, there was also an independent negative association of night-time SBP with GPx activity found in black women. A similar result was previously reported (23) and it was speculated that selenium deficiency may play a role in lower GPx activity in this group and the consequent link with increased blood pressure. In the present study, it was indicated that selenium deficiency was more prevalent in black women (22%) when compared to their white counterparts (3%). However, no link between serum selenium levels and blood pressure was evident in this group.

The results of this study should be interpreted within the context of its strengths and limitations. The limitations of this study include a lack of dietary data to relate dietary selenium intake with serum selenium levels, GPx activity and cardiovascular measures. While the results from this study were consistent after multiple adjustments,

residual confounding effects cannot be excluded due to unknown factors associated with selenium, GPx activity, PWV and 24h blood pressure. The participants of this study were recruited from urban areas in the North West Province and cannot be seen as representative of the entire South African population. This was a well-controlled study which included urbanized black and white participants from the same geographic location and same socio-economic status, allowing comparison between these groups. This study shed light on the protective roles of selenium and GPx activity on the cardiovascular system. Further research is needed to investigate associations of selenium and GPx activity with 24h blood pressure and arterial stiffness in a healthy, younger population to elucidate whether selenium levels and GPx activity play a role in early vascular changes, before the onset of overt cardiovascular disease.

Conclusions

In conclusion, significantly lower selenium levels were found in black adults when compared to their white counterparts from the same geographic region. This may impact on the loss of vasculoprotective effects of selenium and selenoproteins, such as GPx in the hypertension-prone black population.

Conflict of Interest

The authors declare no conflict of interests

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recommendations expressed in this material are those of the author(s); therefore, funders do not accept any liability with regard to this study.

Authors' Contributions

R. Swart was responsible for the planning, writing and composition of the manuscript as well as the statistical analyses. CMC Mels, AE Schutte and JM van Rooyen gave recommendations for the framework, writing and composition of the manuscript as well as the methodology. They also supervised the statistical analyses and helped with the formulation of the tables and figures. CMC Mels played an important part in gaining funding for this project (National Research Foundation - Thuthuka programme (80643)).

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CHAPTER 4:
THE ROLE OF SELENIUM AND GPX: AN INVESTIGATION OF THE MICRO- AND MACRO-VASCULATURE IN A YOUNG BI-ETHNIC POPULATION FROM THE AFRICAN-PREDICT STUDY.

Summary of Instructions to Authors

Journal details	
Title	Journal of the American College of Nutrition
Impact factor	2.175
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Aim and scope	
The Journal of the American College of Nutrition accepts the following types of submissions: Original and innovative research in nutrition with useful application for researchers, physicians, and other health care professionals; Critical reviews on pertinent nutrition topics that highlight key teaching points and relevance to nutrition; Letters to the editors and commentaries on important issues in the field of nutrition; Abstract clusters on nutritional topics with editorial comments; Book reviews; Abstracts from the annual meeting of the American College of Nutrition in the October issue.	
Journal guidelines	
Author guidelines	https://tandfonline.com/action/authorSubmission?journalCode=uacn20&page=instructions
Article structure	Title page; abstract; keywords; introduction, materials and methods, results, discussion; conclusion
Abstract	No longer than 250 words. A structured abstract into the following sections: (a) Objective (1-2 sentences), (b) Methods (3-4 sentences), this may be divided into Design, Setting, Subjects, Interventions or Measures of Outcome, as appropriate, (d) Results (3-4 sentences), and (e) Conclusions (1-2 sentences). Each section of the abstract should feature an appropriate heading.
Keywords	3 to 5 keywords
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Declaration of interest	Yes
Funding	Yes
Ethical considerations	Any study on humans or animals must contain a statement that the appropriate ethics committee approved the research.

The role of selenium and GPx: An investigation of the micro- and macro-vasculature in a young bi-ethnic population from the African-PREDICT study.

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Running title: Selenium, GPx activity and the vasculature.

Disclosure: The authors declare no conflicts of interest.

Abstract

Objective: Selenium plays an important physiological role as component for antioxidant selenoproteins such as glutathione peroxidase (GPx). Since oxidative stress contributes to hypertension development, it is likely that selenium deficiency may contribute to the burden of cardiovascular disease. To better understand the involvement of selenium and GPx in the early development of cardiovascular disease, we investigated in young, healthy black and white men and women whether measures of the micro- and macro-vasculature are related to selenium and GPx activity.

Methods: In young adults (N=394; aged 20-30 years) we determined serum selenium, GPx activity, microvascular measures (central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriolar-to-venular ratio (AVR) and estimated glomerular filtration rate (eGFR)) and macrovascular measures (pulse wave velocity (PWV), 24 hour pulse pressure (24h PP) and augmentation index (AIx)).

Results: In multivariable-adjusted regression analyses, there were vasculo-protective associations between serum selenium and a micro-vascular measure (AVR (β=0.23; p=0.036)) in black women and with a macro-vascular measure (24h PP (β=-0.15; p=0.048)) in white women. In turn, GPx activity also showed a protective association with a micro-vascular measure (eGFR) in white men (β=0.23; p=0.035), as well as with macro-vascular measures (AIx, PP) in the black (β=-0.25; p=0.027) and white men (β=-0.22; p=0.035), and black women (β=-0.32; p=0.001).

Conclusions: Collectively our findings suggest a protective role for the micronutrient selenium and GPx on both the micro- and macro-vasculature in a young, healthy bi-ethnic population.

Keywords: Selenium; Glutathione peroxidase; Microvasculature; Macrovasculature; Young adults.

Abbreviations: AIx, augmentation index; AVR, arteriolar-to-venular ratio; CRAE, central retinal artery equivalent, CRVE, central retinal vein equivalent; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; GPx, glutathione peroxidase; PP, pulse pressure; PWV, pulse wave velocity.

Introduction

Selenium is an essential micronutrient and its serum concentration is increased by the dietary intake of selenium rich food including meat, Brazil nuts, seafood, breads and cereals (1). One of selenium's important physiological roles is its function as a component for antioxidant selenoproteins such as glutathione peroxidase (GPx) (2). Evidence from randomized controlled trials on the association of selenium with cardiovascular diseases (CVD) are inconclusive (3). However, in a recent meta-analysis conducted by Zhang et al. (4), the results of 16 prospective observational studies indicated a protective role of selenium, in a narrow serum selenium range (55-145 µg/l), against the development of CVD.

Selenium deficiency, which commonly occurs in certain regions of Africa (5), may therefore contribute to the burden of CVD (6) and micronutrient malnutrition (7) in African populations. The selenium concentration in food sources is largely dependent on the amount of selenium in the soil (8). A large proportion of black South Africans' diet consists of maize products (9), where 94% of the maize from all maize silos in South Africa contain <50 µg selenium/kg, indicating selenium deficiency (10). This may in part explain not only selenium deficiency found in South-African populations (11, 12), but may also translate to cardiovascular health.

Cardiovascular health is determined by the optimal functioning of both the micro- and macro-circulation (13). Micro-vascular alterations are known to occur early in the pathogenesis of cardiovascular complications (14). Indeed, structural and functional changes of the microvasculature (retinal vessel calibres and renal function) and the macro-vasculature (central arterial stiffness) are predictive of hypertension and CVD development (13, 15-17).

Little is known regarding the relationships of serum selenium and GPx activity with the functioning of the micro- and macro-circulation in young healthy humans. Regarding the microvasculature, it is evident from literature that increased selenium intake improves glomerular filtration rate (18). An intervention study which included young (mean age ±15 years) obese girls showed that microvascular function improved after the intake of Brazil nuts – a selenium rich food source (19). These results suggest a

beneficial role for selenium in microvascular function. At macrovascular level, large artery stiffness is associated with low selenium levels and GPx activity in older populations (mean age of ±65 years) (20, 21). We previously found a vascular protective role of serum selenium and GPx activity in a white population (mean age: ± 45 years) (22). However, another study including apparently healthy adults aged between 20-60 years, failed to establish this link (23).

It is unclear whether selenium and GPx can play an early vascular protective role, particularly in young healthy humans, years before the onset of CVD. Therefore, in this hypothesis generating study, we investigated whether serum selenium and GPx activity are related to measures of the microvasculature (central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriolar-to-venular ratio (AVR) and estimated glomerular filtration rate (eGFR)) and of the macro-vasculature (pulse wave velocity (PWV), 24-hour pulse pressure (24h PP) and augmentation index (AIx)) in a young healthy, black and white cohort.

Methods

Study population and organisational procedures

This study formed part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT). The baseline cohort of the on-going African-PREDICT study includes apparently healthy, young (aged between 20-30 years) black and white men and women, residing in the Potchefstroom area of the North West Province in South Africa. The exclusion criteria were an office blood pressure >140/90 mmHg, HIV-infection, pregnancy, lactation and previous diagnoses with any chronic disease (such as type 1 or 2 diabetes mellitus, liver disease, cancer, tuberculosis or renal disease). The study fulfilled all the requirements as stated in the Declaration of Helsinki for investigation on human participants, and was approved by the Health Research Ethics Committee of the North-West University. Written informed consent was obtained from all the participants. For the purpose of this sub-study, data from the first 394 participants with complete data were analysed cross-sectionally.

Prior to the day of participation, the participants received information leaflets and the measurements were explained. At approximately 08:00 the participants arrived at the Hypertension Clinic at the North-West University in Potchefstroom where they were familiarised with the research environment and procedures commenced. Participants were requested to refrain from food and drinks from 22:00 the previous night.

Questionnaires

Each participant completed a demographic and general health questionnaire to obtain information regarding age, smoking, alcohol intake, socio-economic status and the use of hormonal contraception.

Anthropometric and Physical Activity Measurements

Trained anthropometrists measured weight (SECA electronic scales, SECA, Birmingham, UK), height (SECA 213 Portable Stadiometer, SECA, Birmingham, UK) and waist circumference (Lufkin Steel Anthropometric Tape, W606PM, Lufkin, Apex, USA) in triplicate with calibrated instruments. Body mass index (BMI) was calculated. Mean total energy expenditure was determined with the ActiHeart physical activity monitor (n=369) (CamNtech, Cambridge, UK) over a maximum of seven days.

Cardiovascular Measurements

Participants were fitted with ambulatory blood pressure monitoring devices (CardioXplore®, MediTech, Budapest, Hungary) to measure 24h systolic blood pressure and 24h diastolic blood pressure and 24h PP were calculated as the difference between average systolic blood pressure and diastolic blood pressure. Furthermore, PP arises from the interaction between stroke volume and the characteristics of the arterial circulation and is therefore also a marker of arterial stiffness. Appropriate sized cuffs were used which were attached to the non-dominant arm and the ambulatory blood pressure monitoring devices were fitted at the same time each day. The devices were programmed to measure blood pressure at 30 min intervals during the day and every hour during night time. The participants of this study population had a mean successful cuff inflation rate of 86.2 %.

Assessment of large artery stiffness included AIx and carotid femoral PWV (cfPWV). These were taken with the participant in a supine position using the SphygmoCor XCEL device (SphygmoCor XCEL, AtCor Medical, Sydney, Australia). The AIx was measured using pulse wave analysis with a cuff at the brachial artery. A central arterial waveform was produced that provided a central systolic blood pressure reading, obtained from the peripheral arterial waveform via the built-in generalised transfer function. Central pulse pressure (cPP) was also calculated. For cfPWV assessment, the carotid arterial waveform was measured via applanation tonometry at the same time as the femoral artery waveform (with a cuff placed around the thigh).

Retinal vessel calibres were measured after administering a 1% Tropicamide eye drop (Alcon Laboratories, Bryanston, South Africa) in the right eye, 30 minutes prior to the measurement to achieve mydriatic conditions. Retinal photography was performed using a Zeiss Fundus Camera FF-450 Plus (Imedos Systems UG, Jena, Germany). Colour and monochrome retinal images were taken using Visualis 2.81 software with the camera at a set angle of 50°. Analyses of the monochrome images were performed using VesselMap2 software (Imedos Systems UG, Jena, Germany). All vessels within 0.5 – 1.0 optic disc diameters from the outer margin of the optic disc were selected as either an artery or vein. The trunk region was primarily selected. The software automatically delineates the vessel margins. All vessel calibres were processed via the Knudtson formula (computed with the six largest arteries and veins) (24) to obtain CRAE and CRVE. Both CRAE and CRVE are measured in measuring units (MU) where 1 MU is equivalent to 1 µM if the dimensions of the eye are similar to the normal Gullstrand eye. The AVR was calculated as CRAE/CRVE.

Blood Sampling and Biochemical Analyses

After an overnight fast (8-10 hours), blood samples were collected from brachial antecubital vein branches with a sterile winged infusion set. Serum and plasma were prepared according to standard procedures and stored at -80°C until biochemical analyses were performed. For the preparation of serum samples the blood samples without any anticoagulant were allowed to clot on the bench for 30 min after which it were centrifuged at 1600g at room temperature for 30 min. For the preparation of EDTA plasma samples the blood tubes were centrifuged on 2500g at 4°C for 10 min,

while sodium fluoride plasma samples were centrifuged at 2300g at 4°C for 10 min. Calibration curves were constructed for each new lot of reagents or when requested by the autoanalyser used (Cobas Integra 400 plus). The prescribed quality control material as indicated in the order information section of each test, were used to assess accuracy of results. The quality assurance procedures as programmed by the supplier of the autoanalyser used also consider other factors (e.g. bias) which may affect the quality of the results.

Sodium fluoride plasma samples were used to measure glucose levels (enzymatic reference method with hexokinase), while gamma glutamyl-transferase (enzymatic colorimetric assay), C-reactive protein (particle enhanced turbidimetric assay), creatinine (kinetic colorimetric assay) and the lipid profile (low-density lipoprotein cholesterol, high density lipoprotein cholesterol (homogeneous enzymatic colorimetric assay), total cholesterol and triglycerides (enzymatic, colorimetric method)) were determined in serum (Cobas Integra 400 plus, Roche, Basel, Switzerland). Urinary albumin (immunoturbidimetric assay) and creatinine (kinetic colorimetric assay based on the Jaffé method) were measured (Cobas Integra 400, Roche, Basel, Switzerland) and the urinary albumin-to-creatinine ratio calculated. Glycated haemoglobin (turbidimetric inhibition immunoassay) was measured in EDTA whole blood (Cobas Integra 400, Roche, Basel, Switzerland). Both the intra- and inter-assay coefficients of variation for all the assays were <3.6%. A chemiluminescence method on the Immulite (Siemans, Erglangen, Germany) was used to measure cotinine levels in serum with both the intra- and inter-assay variability <10.8%. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration Equation (25). The GPx activity was measured using Randox assay kits (Randox, Co. Antrim, United Kingdom) for the quantitative *in vitro* determination of GPx in whole blood (EDTA) on the Cobas Integra 400 plus (Roche, Basel Switzerland) with an intra-assay variability of 4.5% and an inter-assay variability of 7.3%. Serum selenium levels were analysed with an inductively coupled plasma mass spectrometry (ICP-MS) method, with a 9.6% intra-assay variation and inter-assay variation of 13.1%. Serum selenium deficiency was classified as selenium levels <8 µg/100 ml (26).

Statistical Analyses

Statistical analyses were performed with Statistica 13.2 (Dell, TX, USA). Interactions of sex and ethnicity were tested and subsequently stratified models were conducted for the relationship between cardiovascular variables (24h PP, cPP, AIx, cfPWV, eGFR, CRAE, CRVE and AVR) with selenium and GPx activity, using multiple regression analyses. Data were expressed as arithmetic mean and standard deviation for normally distributed variables. Variables with a non-Gaussian distribution (glycated haemoglobin, triglycerides, high density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, albumin-to-creatinine ratio, C-reactive protein, gamma glutamyl-transferase) were logarithmically transformed (log10) and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared using independent t-tests (two-sided) and Chi-square tests, respectively.

We further performed multiple regression analyses to determine independent associations of cardiovascular variables with selenium and GPx activity. The models were compiled with either 24h PP, AIx, eGFR, CRAE, CRVE or AVR as dependent variables, and selenium and GPx activity as main independent variables. The following covariates were considered for entry into the models: age, BMI, waist circumference, cotinine, self-reported smoking, gamma glutamyl-transferase, self-reported alcohol use, glucose, glycated haemoglobin, total energy expenditure, C-reactive protein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides. Based on bivariate correlations between potential covariates and the dependent and main independent variables, the following covariates were entered into the final models based on the most significant p-values ($p<0.05$): age, waist circumference, self-reported smoking, total energy expenditure, gamma glutamyl-transferase, C-reactive protein and glucose. In modes where CRAE was the dependent variable, we additionally entered CRVE as a covariate and vice versa (27). We tested for multicollinearity using the variance inflation factor (VIF) and the tolerance value was above 0.1, thus illustrating that there was no multicollinearity. Since we tested multiple hypothesis, we determined at which false discovery rate our findings would still be significant using the Benjamini-Hochberg procedure.

Sensitivity Analyses

When the multiple regression analyses were repeated by additionally adjusting for hormonal contraceptive use in women, the above-mentioned associations remained unchanged. To test whether associations of the micro- and macrovasculature with serum selenium levels are independent of GPx activity, both selenium and GPx were added to the applicable models including eGFR in white men, 24h PP in white women. These associations indicated that selenium is associated with 24h PP in white women, independent of GPx activity. Furthermore, GPx activity was associated with eGFR in white men, independent of selenium. By also additionally adjusting for the socio-economic status all the main results remained unchanged. However, the association of 24h PP and selenium in white women became borderline significant (p=0.051).

Results

Characteristics of the study population

Interactions of gender on the associations of 24h PP (p=0.013), cPP (p=0.012), eGFR (p=0.006), CRVE (p=0.046) with selenium, and of cPP (p=0.020), AIx (p<0.001), CRAE (p=0.003) with GPx activity were found. An interaction of ethnicity on the association between eGFR with GPx activity (p=0.007) was also found. Therefore groups were stratified according to ethnicity and sex to compare black and white men and women.

In **Table 4-1** the characteristics of black and white men and women are shown. Black men had lower 24h systolic blood pressure (p=0.002), 24h mean arterial pressure (p=0.042) and 24h PP (p=0.003) when compared to white men. Both selenium levels (p=0.037) and GPx activity were lower (p<0.001) in the black men when compared to white men. Black men had smaller CRAE (p=0.044) and higher eGFR (p<0.001) than their white counterparts.

No differences in the cardiovascular profile were observed when comparing black and white women, except for the smaller CRAE and AVR (all $p<0.001$) observed in black women. The activity of GPx was lower ($p<0.001$) in black men and women when compared to their white counterparts. Of the total study population only two (1.69%) white women were selenium deficient (<8 µg/100 ml) (26).

Table 4-1. Characteristics of black and white men and women.

	Black men	White men	p-values	Black women	White women	p-values
N	86	79		109	120	
Age (years)	24.3 ± 3.21	25.6 ± 2.94	0.011*	24.4 ± 3.48	25.6 ± 2.83	0.007*
Anthropometric measurements						
Height (cm)	170 ± 6.02	179 ± 6.11	<0.001*	159 ± 6.49	167 ± 6.15	<0.001*
Body mass (kg)	63.1 ± 10.7	89.9 ± 18.9	<0.001*	67.4 ± 14.8	68.8 ± 15.9	0.510
Body mass index (kg/m²)	21.8 ± 3.41	28.0 ± 5.67	<0.001*	26.7 ± 5.75	24.6 ± 5.47	0.005*
Waist circumference (cm)	74.3 ± 8.78	91.6 ± 14.2	<0.001*	79.6 ± 11.8	76.2 ± 12.8	0.035*
Lifestyle						
Cotinine (ng/l)	118 ± 145	53.0 ± 110	0.002*	20.8 ± 54.9	20.1 ± 62.5	0.931
Total energy expenditure (kcal/day)	2230 ± 276	2682 ± 426	<0.001*	2146 ± 419	2186 ± 451	0.505
Smoking n (%)	47 (54.7)	18 (22.8)	<0.001*	12 (11.0)	13 (10.8)	0.966
Hormonal contraception n (%)	-	-	-	56 (53.3)	52 (43.7)	0.150
γ-Glutamyl transferase (U/l)	28.6 (13.0; 101)	24.8 (10.8; 65.4)	0.143	23.0 (10.5; 57.4)	13.9 (6.08; 40.6)	<0.001*
Socio economic status			<0.001*			<0.001*
Low n (%)	54 (62.8)	9 (11.4)		65 (59.6)	9 (7.50)	
Middle n (%)	18 (20.9)	17 (21.5)		30 (27.5)	29 (24.2)	
High n (%)	14 (16.3)	53 (67.1)		14 (12.8)	82 (68.3)	

	Black men	White men	p-values	Black women	White women	p-values
Cardiovascular measurements						
24h systolic blood pressure (mmHg)	121 ± 8.89	124 ± 6.70	0.002*	114 ± 8.09	113 ± 8.44	0.795
24h diastolic blood pressure (mmHg)	70.1 ± 6.36	70.8 ± 6.13	0.504	68.8 ± 5.52	68.3 ± 5.69	0.465
24h mean arterial pressure (mmHg)	90.3 ± 6.66	92.2 ± 5.38	0.042*	86.7 ± 6.09	86.3 ± 6.32	0.593
24h pulse pressure (mmHg)	50.4 ± 6.94	53.6 ± 6.96	0.003*	44.7 ± 5.54	45.0 ± 5.76	0.732
Central pulse pressure (mmHg)	35.7 ± 5.89	37.4 ± 6.29	0.086	31.9 ± 5.55	32.1 ± 4.65	0.677
cfPulse Wave Velocity (m/s)[a]	6.84 ± 0.83	6.65 ± 0.83	0.970	6.00 ± 0.71	5.98 ± 0.82	0.163
Augmentation index (%)[b]	2.41 ± 7.98	6.18 ± 7.90	0.936	10.6 ± 7.53	9.98 ± 6.82	0.294
Central retinal artery equivalent (MU)	156 ± 13.7	160 ± 10.4	0.044*	155 ± 10.2	162 ± 12.6	<0.001*
Central retinal vein equivalent (MU)	248 ± 19.6	249 ± 15.5	0.743	251 ± 17.8	248 ± 17.3	0.285
Arteriolar-to-venular ratio	0.63 ± 0.05	0.65 ± 0.05	0.126	0.62 ± 0.05	0.65 ± 0.05	<0.001*
Biochemical analyses						
Selenium (µg/100ml)	16.1 ± 3.37	17.2 ± 3.70	0.037*	16.5 ± 3.02	16.4 ± 3.63	0.870
Selenium deficiency (<8 µg/100ml) n (%)	0 (0)	0 (0)	-	0 (0)	2 (1.69)	0.178
Glutathione peroxidase (nmol/min/ml)	18.4 ± 1.76	19.8 ± 1.37	<0.001*	18.6 ± 1.74	19.9 ± 1.34	<0.001*
Glucose (mmol/l)	3.87 ± 0.87	5.08 ± 0.42	<0.001*	3.95 ± 0.75	4.64 ± 0.51	<0.001*
eGFR (ml/min/1.73m^2)	141 ± 16.4	105 ± 13.5	<0.001*	139 ± 16.2	109 ± 14.2	<0.001*
Glycated haemoglobin (%)	5.46 (5.03; 5.96)	5.28 (4.86; 5.67)	<0.001*	5.49 (4.96; 6.02)	5.28 (4.88; 5.74)	<0.001*
Triglycerides (mmol/l)	0.83 (0.42; 1.66)	1.09 (0.57; 2.18)	<0.001*	0.72 (0.41; 1.33)	0.91 (0.42; 2.16)	<0.001*
Total cholesterol (mmol/l)	3.81 (2.80; 5.54)	4.64 (3.29; 6.08)	<0.001*	3.74 (2.67; 5.20)	4.67 (3.31; 6.63)	<0.001*

	Black men	White men	p-values	Black women	White women	p-values
High density lipoprotein cholesterol (mmol/l)	1.30 (0.84; 1.82)	1.09 (0.71; 1.72)	<0.001*	1.21 (0.79; 1.87)	1.58 (1.03; 2.36)	<0.001*
Low density lipoprotein cholesterol (mmol/l)	2.26 (1.39; 3.87)	3.16 (1.94; 5.09)	<0.001*	2.34 (1.21; 4.05)	2.81 (1.70; 4.53)	<0.001*
C-reactive protein (mg/l)	0.73 (0.11; 4.62)	0.97 (0.17; 8.26)	0.146	2.03 (0.23; 11.9)	1.07 (0.11; 11.9)	0.001*
Albumin-to-creatinine ratio	0.47 (0.18; 1.84)	0.37 (0.17; 1.37)	0.031*	0.57 (0.25; 2.92)	0.53 (0.22; 2.54)	0.438

Data expressed as arithmetic mean ± standard deviation, geometric mean (5th and 95th percentiles) or % of n
eGFR, estimated glomerular filtration rate
[a]Adjusted for mean arterial pressure.
[b]Adjusted for body height and heart rate.
*indicate statistical significance (p<0.05).

Multivariable Regression Analyses

We reviewed in sub-groups of black and white men and women, the associations between micro- and macrovascular measures with selenium and GPx. In multivariable-adjusted analyses (**Table 4-2**) a significant negative association between 24h PP and GPx activity (β=-0.25; p=0.027) was indicated in black men. In white men, a positive association between eGFR and GPx activity (β=0.23; p=0.035) was found. In white men (β=-0.22; p=0.035) and black women (β=-0.32; p=0.001) we found negative associations between AIx and GPx activity. In black women AVR associated positively with selenium (β=0.23; p=0.036). In white women, negative associations of 24h PP with selenium (β=-0.15; p=0.048) were found.

Table 4-2. Summary of multiple regression analyses with micro- and macrovascular measures as dependent variables in black and white men and women.

	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Black men (n=86)						
24h PP (mmHg)	0.11	-0.03 (-0.25; 0.18)	0.763	0.17	-0.25 (-0.46; -0.03)	0.027*
AIx (%)[a]	0.29	-0.02 (-0.22; 0.17)	0.810	0.29	0.01 (-0.19; 0.21)	0.918
eGFR (ml/min/1.73m^2)[b]	0.20	-0.03 (-0.23; 0.18)	0.796	0.20	-0.01 (-0.22; 0.20)	0.925
AVR[b]	0.04	-0.08 (-0.32; 0.16)	0.496	0.04	-0.10 (-0.35; 0.15)	0.426
White men (n=79)						
24h PP (mmHg)	0.10	0.08 (-0.15; 0.31)	0.483	0.09	0.07 (-0.15; 0.29)	0.535
AIx (%)[a]	0.25	-0.09(-0.31; 0.12)	0.402	0.30	-0.22 (-0.42; -0.02)	0.035*
eGFR (ml/min/1.73m^2)[b]	0.14	-0.18 (-0.41; 0.04)	0.109	0.16	0.23 (0.02; 0.45)	0.035*
AVR[b]	0.10	0.02 (-0.21; 0.25)	0.853	0.10	0.02 (-0.21; 0.24)	0.890
Black women (n=109)						
24h PP (mmHg)	0.10	0.005 (-0.19; 0.20)	0.962	0.10	-0.01 (-0.21; 0.18)	0.897
AIx (%)[a]	0.11	0.03 (-0.17; 0.23)	0.739	0.22	-0.32 (-0.50; -0.14)	0.001*
eGFR (m/lmin/1.73m^2)[b]	0.13	-0.08 (-0.28; 0.12)	0.422	0.14	-0.10 (-0.29; 0.09)	0.306
AVR[b]	0.03	0.23 (0.02; 0.44)	0.036*	-0.02	0.04 (-0.17; 0.26)	0.685
White women (n=120)						
24h PP (mmHg)	0.37	-0.15 (-0.30; -0.001)	0.048*	0.34	0.02 (-0.14; 0.18)	0.826
AIx (%)[a]	0.14	0.09 (-0.08; 0.27)	0.298	0.13	0.01 (-0.17; 0.19)	0.902
eGFR (ml/min/1.73m^2)[b]	-0.02	0.06 (-0.13; 0.26)	0.513	-0.03	0.04 (-0.16; 0.24)	0.681
AVR[b]	0.11	0.07 (-0.11; 0.25)	0.419	0.10	0.04 (-0.15; 0.23)	0.657

The main independent variables included in the models were selenium and GPx and other covariates included age, waist circumference, total energy expenditure, C-reactive protein, y-glutamyl transferase, glucose and self-reported smoking.
[a]Body height additionally added in the model.
[b]24h systolic blood pressure additionally added in the model.
*indicate statistical significance ($p<0.05$).

Discussion

In the present study we investigated whether serum selenium and GPx activity are related to measures of the micro- and macro-vasculature in a healthy black and white population (aged 20-30 years). We found serum selenium to have a protective association with micro- and macrovascular measures, but only in women. On the other hand, GPx activity was protectively associated with micro- and macrovascular measures in black and white men and black women. Despite the findings that selenium and GPx were associated with various different micro- and macrovascular measures in different groups, collectively all of our findings suggest a protective role for the micronutrient, selenium, and GPx on both of these vascular beds in a young, healthy bi-ethnic population.

From the outset we considered this study as explorative and hypothesis generating, however, at a false discovery rate of 20% our findings were still significant. We argued that due to the young healthy nature of our population, the confirmation of links with vascular measurements would be highly informative to understand the potential contribution of these biomarkers to the future development of CVD. Our independent protective associations of either selenium, GPx or both with the vasculature were therefore slightly unexpected, since another study which included an apparently healthy population (20-60 years) failed to link selenium levels with measures of the microvasculature (23).

Changes in the microvasculature are thought to precede structural changes in the macro-vasculature (14) and can be seen as an early predictor for future CVD (28). The micro-vasculature of the retina may also provide information about the anatomical and physiological characteristics of the coronary, renal, and cerebral microcirculation (29, 30). We found a sex-specific protective association of AVR (a microvascular measure) with selenium in black women. Although associations with individual vessel calibres may be more informative (31), a reduced AVR has previously been associated with increased risk for hypertension (32, 33). Similar protective effects were found in other studies which investigated the link between the microcirculation and selenium (19, 34). In one study microvascular function improved in obese girls after increased intake of selenium-rich Brazil nuts (19), while another study reported on the protective

association of selenium with measures of microvascular function in the skin of healthy young men (34). These protective associations may suggest that selenium has beneficial effects on inflammatory processes (35) and endothelial function (36) as well as antioxidant properties by decreasing levels of hydrogen peroxide (37). It may therefore be beneficial to ensure optimal selenium status to maintain its health effects and delay or avoid microvascular deterioration and eventually CVD in later life.

The kidney is known to be a main source of the extracellular selenoprotein GPx 3 production (38) and has a protective role against oxidative stress as well as important roles in the regulation of vascular and endothelial function (39). Our result indicated that GPx activity showed a protective association with eGFR (as a microvascular measure) in white men, which confirm previous studies reporting on an adverse association between kidney function (eGFR) and a decrease in extracellular selenoprotein GPx 3 levels in chronic kidney disease patients (40-42). Although the present study includes a healthy population, our results may demonstrate the protective role of GPx activity to maintain kidney function.

When investigating the associations of the macro-vasculature (24h PP) with selenium, protective associations were found in white women. Other studies reported on the link between the macro-vasculature and selenium, and presented contradictory results. A few studies support our findings as it was indicated in patients with a high risk for cardiovascular events, that low selenium intake is accompanied by increased arterial stiffness (20). We also previously reported on the vascular protective effects of serum selenium and GPx activity, however in older white men (22). In contrast, no beneficial effects of selenium supplementation on arterial stiffness in a healthy middle-aged population were found (43).

The vascular protective effect of selenium in women may be the result of the role selenium plays as component for antioxidant enzymes such as GPx (8, 44). However, since we have demonstrated that selenium is associated with the micro- and macro-vasculature, independently of GPx, other roles by which selenium could affect vascular function should also be considered (45). Other selenoproteins such as thioredoxin reductase 1 (TxnRd 1) and thioredoxin reductase 2 (TxnRd 2) are necessary for early life functions, thus GPx activity ranks lower in the hierarchical selenium supply system

(46-48). The thioredoxin enzymes also have a protective role against oxidative stress and pathological conditions such as CVD (49). However, in the present study, oxidative stress levels are low, possibly due to the young age of this population, thus other important roles of selenium including thyroid hormone metabolism, inflammation and immune function are probably prioritised (50).

Protective associations between macro-vascular measures (AIx, 24h PP) and GPx activity were found in black men and women. GPx activity provides protection against reactive oxygen species though antioxidant glutathione pathways (51). These inverse associations may indicate that a decrease in GPx levels may lead to increase in reactive oxygen species and a decrease in nitric oxide which is known to influence endothelial function. This may eventually result in arterial stiffness (52). This is in agreement with an animal study which indicated that GPx deficiency led to structural cardiac and vascular abnormalities (53). This further underline the protective roles of GPx activity on the macro-vasculature. Although our results are found in different groups with different vascular beds, it is suggested that selenium and GPx have vascular protective roles from an early age.

The results of this cross-sectional study should be interpreted within the context of its strengths and limitations. This was a well-controlled study where all measurements were performed at the same time each day by trained researchers in the Hypertension Research and Training Clinic of the NWU. The measurements were performed in temperature controlled rooms using standard operating procedures and according to good clinical practice. Only one research participant was in a room during measurements. This study included young and apparently healthy black and white participants, with a detailed profile of advanced cardiovascular measurements. The limitations include a lack of dietary data to relate dietary selenium intake with serum selenium levels, GPx activity and cardiovascular measures. However, serum selenium is a more accurate estimate of current selenium status than dietary intake data. We could also not investigate thyroid hormone metabolism and further studies on this topic are warranted. Also, it is known that selenoprotein P (SePP) in plasma is the most conclusive marker for determining the optimal supply of selenium (54), further studies are warranted to investigate SePP in young adults. While the results from this study

were consistent after multiple adjustments, residual confounding effects cannot be excluded due to unknown factors associated with selenium, GPx activity, PP, AIx, eGFR and AVR. The healthy participants of this study were recruited from the North West Province and cannot be seen as representative of the entire South African population. This study was cross-sectional and therefore causality cannot be inferred. Due to the hypothesis generating nature of this study, we suggest that these findings should be investigated in future studies.

Conclusion

Despite the findings that selenium and GPx were associated with various different micro- and macrovascular measures in different groups, collectively all of our findings suggest a protective role for the micronutrient, selenium, and GPx on both of these vascular beds in a young, healthy bi-ethnic population. Since our study population included young adults, our results suggest that selenium and GPx activity are actively involved within the vasculature at a young age before the onset of CVD. Although only two women in our study presented with selenium deficiency, it may be beneficial to ensure optimal selenium status to maintain its health effects and to delay or avoid micro- and macrovascular deterioration and eventually CVD in later life. Further studies, including intervention studies, are needed to confirm the potential vascular protective role of GPx and selenium.

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Disclosure

The authors declare no conflicts of interest.

Authorship

R. Swart was responsible for the planning, writing and composition of the manuscript as well as the statistical analyses. CMC Mels, AE Schutte, JM van Rooyen and W Smith gave recommendations for the framework, writing and composition of the manuscript as well as the methodology. The above-mentioned authors also helped with the collection of the data and supervised the statistical analyses as well as helped with the formulation of the tables.

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CHAPTER 5: SELENIUM AND LARGE ARTERY STRUCTURE AND FUNCTION: A TEN-YEAR PROSPECTIVE STUDY

Summary of Instructions to Authors

Journal details	
Title	European Journal of Nutrition
Impact factor	4.423
Publisher	Springer
Aim and scope	
The European Journal of Nutrition publishes original papers, reviews, and short communications in the nutritional sciences. The manuscripts submitted to the European Journal of Nutrition should have their major focus on the impact of nutrients and non-nutrients on • immunology and inflammation, • gene expression, • metabolism, • chronic diseases, or • carcinogenesis, or a major focus on • epidemiology, including intervention studies with healthy subjects and with patients, • food safety, or • biofunctionality of food and food components.	
Journal guidelines	
Author guidelines	https://www.springer.com/food+science/journal/394?detailsPage=pltci_1886857
Abstract	A structured abstract of 150 to 250 words should be divided into the following sections: • Purpose (stating the main purposes and research question) • Methods • Results • Conclusions
Title	Concise and informative
Keywords	4-6 keywords
Manuscript (word count)	50000 characters
Language	English
Font	Use a normal, plain font (e.g., 10-point Times Roman) for text.
Page numbers	Use the automatic page numbering function to number the pages.
Line numbering	Activate the line numbering function for your manuscript.
Tables	• All tables are to be numbered using Arabic numerals. • Tables should always be cited in text in consecutive numerical order. • For each table, supply a table caption (title) explaining the components of the table. • Identify any previously published material by giving the original source in the form of a reference at the end of the table caption. • Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.
Figures	**Figure Lettering** • To add lettering, it is best to use Helvetica or Arial (sans serif fonts). • Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt). • Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label. • Avoid effects such as shading, outline letters, etc. • Do not include titles or captions within your illustrations.

	Figure Numbering • All figures are to be numbered using Arabic numerals. • Figures should always be cited in text in consecutive numerical order. • Figure parts should be denoted by lowercase letters (a, b, c, etc.). • If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, • "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately. **Figure Captions** • Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file. • Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type. • No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption. • Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs. • Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption. **Figure Placement and Size** • Figures should be submitted separately from the text, if possible. • When preparing your figures, size figures to fit in the column width. • For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm. • For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.
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Declaration of interest	Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research. This note should be added in a separate section before the reference list. If no conflict exists, authors should state: The authors declare that they have no conflict of interest.

Selenium and large artery structure and function: A ten year prospective study

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Abstract

Purpose Despite selenium's beneficial effects in counteracting oxidative stress, inflammation and vascular endothelial dysfunction, controversial results exist regarding the long-term associations between selenium and atherosclerosis, arterial stiffness and hypertension. We investigated in normal and selenium deficient groups (and the total group), whether serum selenium relates to measures of large artery structure and function over ten years.

Methods This longitudinal study included black adults from rural and urban areas in South Africa. Serum selenium and blood pressure were measured at baseline (N=987). At follow-up carotid intima media thickness (IMT), cross-sectional wall area (CSWA), carotid-femoral pulse wave velocity (c-fPWV) and blood pressure were measured (N=718). Selenium deficiency was classified as serum levels <8 µg/100 ml.

Results In multivariable-adjusted regression analyses performed in the normal selenium group, c-fPWV after ten years was negatively associated with baseline selenium (β=-0.09; *p*=0.016). In the normal selenium group baseline (but not 10 year) blood pressure also associated negatively with baseline selenium (β=-0.09; *p*=0.007). Both IMT (β=0.12; *p*=0.001) and CSWA (β=0.10; *p*=0.003) after ten years associated positively with baseline selenium in the total group, normal and selenium deficient groups.

Conclusion We found a long-term vascular protective association of selenium on arterial stiffness and blood pressure in Africans with normal selenium levels, supporting the notion that selenium fulfil a vascular protective role. In contrast, we found a potential detrimental association between selenium and carotid wall thickness, particularly evident in individuals within the highest quartile of serum selenium.

Keywords carotid intima media thickness; atherosclerosis; pulse wave velocity; arterial stiffness; blood pressure; micronutrient.

Introduction

Rapid socio-economic development and urbanization as experienced by many black South Africans are associated with a nutrition transition (1). These changes in dietary patterns and nutrient intake reflect the move from traditional foods to more processed and convenient foods and may be accompanied by micronutrient deficiencies (2). This in turn is related to an increased prevalence of non-communicable diseases (2), such as hypertension and cardiovascular disease (CVD). It is well established that black populations have a higher prevalence of hypertension, therefore increased CVD incidence and mortality when compared to their white counterparts (3).

Selenium is an important dietary micronutrient present in various dietary sources including meat, Brazil nuts, intestines, seafood, cereals, cheese and milk (4). Apart from a high prevalence of CVDs, black populations also tend to have a high prevalence of selenium deficiency (5), which may be due to increased consumption of maize products – a poor source of selenium (6). The functions of selenium range from antioxidative (7) to regulatory functions in processes such as the inflammatory response as well as proliferation and differentiation of immune cells (8, 9). Selenium's functions are carried out by selenoproteins, including glutathione peroxidase (GPx) (10), which are present in the arterial wall (11).

Inconsistent results on the protective roles of selenium on CVD were found in randomized controlled trials in a recent review (12). Intervention studies indicated that selenium has a protective effect against the development of CVD (13, 14), including atherosclerotic CVD (12). This was also seen in a recent cross-sectional study in white men, in which vascular protective associations with selenium and GPx were found (5). Others reported that selenium deficiency may lead to increased blood pressure (15), arterial stiffness (5) and atherosclerosis (16). In contrast, no significant effect of selenium on CVD mortality (17) and coronary heart disease (18) were indicated in two meta-analyses which included several randomized controlled trials. However, it was found that selenium lowered inflammation and oxidative stress, thereby having a protective effect on coronary arteries (18).

Collectively it is known that selenium exerts beneficial effects on oxidative stress, inflammation and endothelial dysfunction, but controversial results exist on the long-term associations of selenium on thickening of the carotid wall, the development of arterial stiffness and increased blood pressure. We therefore investigated in normal and selenium deficient adults as well as in the total group, whether serum selenium levels are related to measures of large artery structure (carotid intima media thickness (IMT) and cross-sectional wall area (CSWA)) and function (blood pressure and arterial stiffness) over ten years.

Materials and Methods

Study design and participants

The international Prospective Urban and Rural Epidemiology (PURE) study focus on the societal determinants of non-communicable diseases in urban and rural areas in low-, middle-, and high-income countries (19). This sub-study is embedded in the South African leg of the PURE study and includes data collected at baseline (2005) and after ten years (2015) in the North West Province, South Africa.

A total of N=1265 participants of the N=2010 who participated at baseline were included, based on the following criteria: i) participants who took part in both the baseline and ten year follow-up phase (N=923); and ii) participants who passed away over the course of 10 years (N=342). We excluded N=278 participants with missing baseline and follow-up cardiovascular and/or missing baseline selenium data. A total of N=987 participants **(Figure 5-1)** were therefore analysed in the baseline phase and N=718 participants were analysed in the follow-up phase. From the N=718 participants, N=81 participants were randomly selected to determine their serum selenium levels at follow-up.

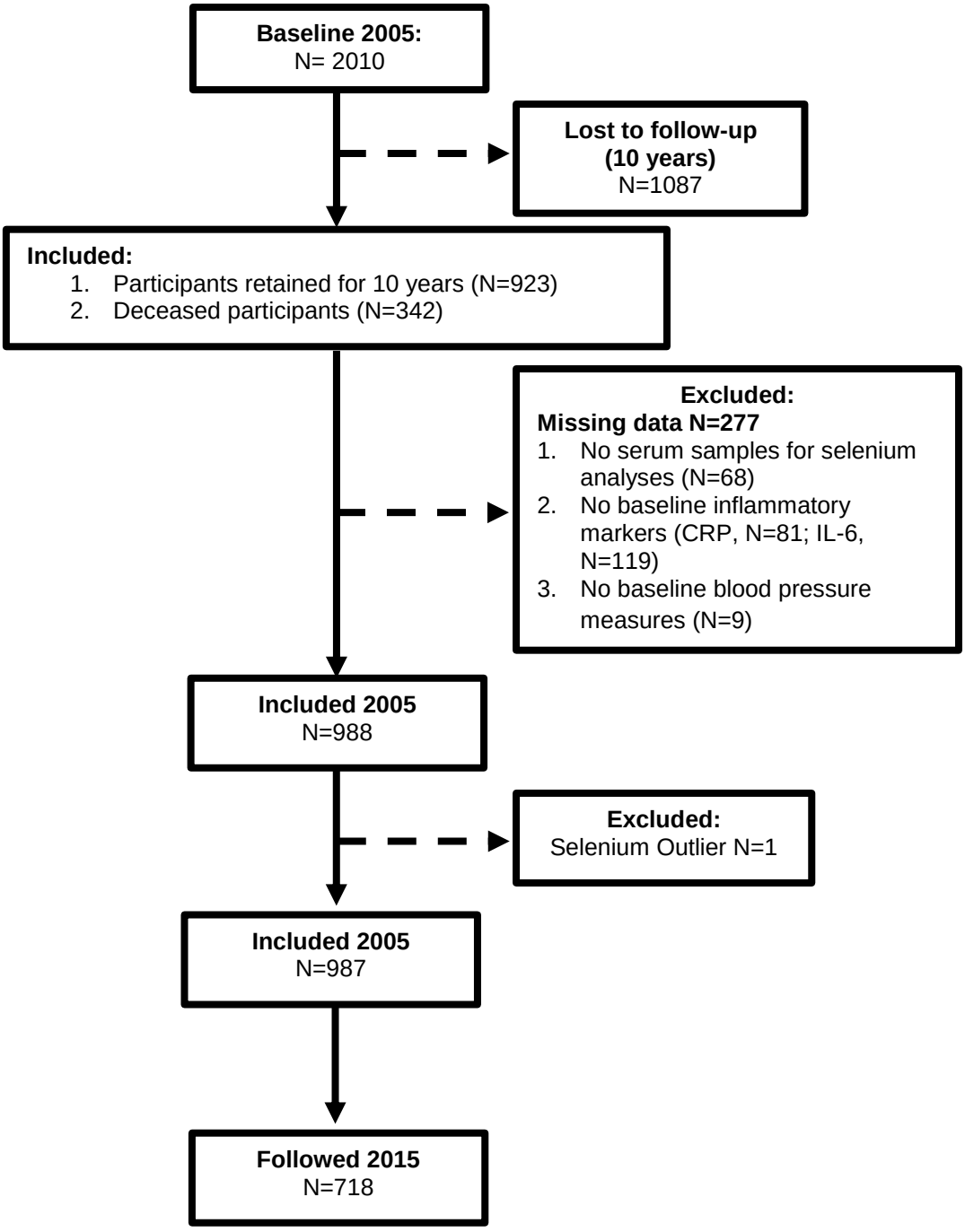

Figure 5-1. Layout of the sub-study.

Experimental Protocol

The detailed experimental protocol for data collection was previously described (20). Briefly, all the participants were fully informed about the protocol of the study, and all gave written informed consent. The study fulfilled the requirements as stated in the

Declaration of Helsinki for investigation in human participants and was approved by the Health Research Ethics Committee of the North-West University.

Anthropometric Measurements

Weight and height were taken in triplicate with calibrated instruments by trained anthropometrists and body mass index (BMI) was calculated.

Questionnaires

Demographic, socio-economic and lifestyle information were obtained by trained field workers. The adapted BAECKE questionnaire was used to determine the physical activity index (21).

Cardiovascular Measurements

Blood pressure measurements were conducted while the participants were seated upright with the right arm supported at heart level (in duplicate, five min apart). Brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a validated OMRON device (Omron Healthcare, Kyoto, Japan) at baseline and follow-up. According to International and South African Guidelines (22), hypertension was classified as SBP≥140 and/or DBP≥90 mmHg or use of antihypertensive medication. Pulse pressure (PP) was calculated as the difference between SBP and DBP. Mean arterial pressure (MAP) was calculated as MAP=DBP+(PP/3).

The SphygmoCor XCEL device (AtCor Medical Pty. Ltd., Sydney, New South Wales, Australia) was used to determine carotid-femoral pulse wave velocity (c-fPWV) at follow-up. The participant was in the supine position, and of the duplicate readings, the second reading was used for analysis. The distances between the pulsated sites were measured using an infantometer, and 80% of these distances were used as the pulse wave travelled distance (23).

The SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothell, WA, USA), with a 6–13 MHz linear array transducer was used to obtain the IMT according to the Mannheim Consensus (24). Images from at least two optimal angles of the left and

right common carotid arteries were obtained. The measurements were done on a selected segment of maximum 10 mm with good image quality. It was performed by a single reader using a semi-automated program, namely the Artery Measurement Systems (AMS) II v1.139 (Chalmers University of Technology, Gothenburg, Sweden) to determine intima-media thickness of the far wall (IMTf) and near wall (IMTn). The CSWA was calculated to confirm structural changes in luminal diameter: CSWA = $\pi(d/2 + CIMT)^2 - \pi(d/2)^2$, where d denotes the luminal diameter (25).

Biochemical Analyses

Blood samples (90 mL in 2005, and 30mL in 2010 and 2015) from fasting participants were obtained from brachial antecubital vein branches with a sterile winged infusion set. Samples were prepared according to standard procedures. Blood collection took place from 7:00 am to 11:00 am. All samples were stored at -80°C until biochemical analyses were performed. In the rural areas, the blood samples were frozen at -18°C for no longer than 5 days and then stored in the laboratory at -80°C until biochemical analyses were performed. Samples were centrifuged at 2,000 x g for 15 minutes at 10°C within 30 minutes after collection. For blood lipids [total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides], gamma glutamyl transferase (GGT) and C-reactive protein (hs-CRP), tubes not containing anticoagulants were used. For plasma glucose, sodium fluoride tubes were used.

Serum selenium levels were analysed with an inductively coupled plasma mass spectrometry (ICP-MS) method and serum selenium deficiency was classified as selenium levels <8 µg/100 ml (26). Glucose levels were determined in sodium fluoride plasma samples with the Vitros DT6011 Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester, New York, USA) in 2005 and a Cobas Integra 400 plus analyser (Roche, Basel, Switzerland) in 2015. Gamma glutamyl-transferase (GGT) and the lipid profile including total cholesterol, low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides as well as C-reactive protein (CRP) were determined in serum with a Konelab20i^{TM} auto-analyser, (Thermo Fisher Scientific, Vantaa, Finland) in 2005 and a Cobas Integra 400 plus (Roche, Basel, Switzerland) in 2015. Both the intra-assay coefficient and inter-assay coefficient of variation were <10%.

Statistical Analyses

Statistical analyses were performed with Statistica 13.3 (TIBCO Software, Palo Alto, California, United States). Aligned with the objective of the study, we divided the population group into selenium deficient and normal selenium groups. Data were expressed as arithmetic mean and standard deviation for normally distributed variables. Variables with a non-Gaussian distribution (glucose, triglycerides, CRP, GGT, c-fPWV, IMTf, IMTn and CSWA) were logarithmically transformed and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared between the normal selenium group and the selenium deficient group, using independent t-tests and Chi-square tests, respectively. We used analyses of covariance (ANCOVAs) to compare cardiovascular measurements (at 10 year follow-up) including SBP, c-fPWV, IMT and CSWA by quartiles of baseline selenium levels, while adjusting for age and sex and SBP were additionally adjusted for baseline SBP. The c-fPWV, IMT and CSWA were additionally adjusted for MAP.

We further performed multi-variable adjusted regression analyses to determine associations of cardiovascular measurements with selenium. The models were compiled with either follow-up SBP, c-fPWV, IMT or CSWA as dependent variables, and baseline selenium as the main independent variable. The following covariates were considered for entry into the models: baseline age, sex, BMI, waist circumference, self-reported smoking, GGT, self-reported alcohol use, glucose, glycated haemoglobin, physical activity index, CRP, total cholesterol, HDL-C, LDL-C and triglycerides. Based on the literature (22, 27) and bivariate correlations between potential covariates and the dependent and main independent variables, we selected the covariates to be included in multivariate regression analyses. We also tested for multicollinearity using the variance inflation factor (VIF) and the tolerance value was above 0.1, indicating no multicollinearity. The following covariates were entered into the final models: age, sex, BMI, self-reported smoking, physical activity index, GGT, CRP, glucose and LDL-C. In models where c-fPWV, IMT and CSWA were the dependent variables, we additionally entered MAP at follow-up as a covariate.

Results

The baseline and follow-up characteristics, stratified according to baseline selenium levels are shown in **Table 5-1**. Participants with selenium deficiency were younger, and had lower BMI but higher GGT (p=0.005) and CRP levels (p<0.001) and reported to use more tobacco (p<0.001) when compared to those with normal selenium levels at baseline. Mean selenium levels of the normal selenium group were 12.7 ± 3.41 µg/100 ml compared to the selenium deficient group with 6.12 ± 1.67 µg/100 ml (p<0.001). After 10 years, the selenium levels (done in a randomly selected subgroup) of the selenium deficient group restored to normal levels and there was no difference between selenium levels of the baseline normal selenium and selenium deficient groups. At follow-up the group with selenium deficiency at baseline reported higher alcohol use (p=0.023), but displayed lower total cholesterol (p=0.025) and LDL-C (p=0.041) compared to the normal selenium group. No differences in the cardiovascular profile were observed at both baseline and follow-up, when comparing the normal selenium group with the selenium deficient group. Deceased participants (N=217) had significantly lower baseline selenium levels (p<0.001) compared to those who survived over ten years **(Supplementary table 5-1)**.

We investigated SBP, c-fPWV, IMT and CSWA at follow-up by quartiles of baseline selenium levels while adjusting for baseline values of age and sex as well as follow-up MAP and baseline SBP **(Figure 5-2)**. A positive trend for IMT (p trend=0.004) and CSWA (p trend=0.033) with increasing baseline selenium quartiles were found. However, we found no trends for SBP (p=0.745) and c-fPWV (p=0.332) with increasing baseline selenium quartiles.

By using multivariable-adjusted regression analyses we reviewed in the total group, associations of 10-year follow-up cardiovascular measurements (SBP, c-fPWV, IMT, CSWA) with baseline selenium **(Table 5-2)**. We found a positive association between IMT and selenium (β=0.12; p=0.001) as well as between CSWA and selenium (β=0.10; p=0.003).

Table 5-1. Characteristics of the study population at baseline and after 10 years, stratified according to normal and deficient baseline selenium levels

	Baseline (N=987)			10 year follow-up (N=718)		
	Normal Se	**Se Deficient[c]**	***p*-values**	**Normal Se**	**Se Deficient**	***p*-values**
N	845	142		637	81	
Sex [women, (%)]	546 (64.6)	82 (57.8)	0.115	438 (68.8)	52 (64.2)	0.406
Age (years)	50.7 ± 10.2	48.8 ± 9.58	**0.042**	59.1 ± 9.15	56.4 ± 8.26	**0.014**
Anthropometric measures						
Height (m)	1.60 ± 0.08	1.61 ± 0.09	0.244	1.59 ± 0.08	1.60 ± 0.09	0.360
Body mass (kg)	62.7 ± 16.2	60.4 ± 18.0	0.137	65.3 ± 17.4	66.2 ± 20.7	0.699
Body mass index (kg/m²)	25.6 ± 6.77	23.4 ± 7.05	0.057	25.9 ± 7.10	25.8 ± 7.76	0.966
Cardiovascular measures						
Systolic blood pressure (mmHg)	133 ± 24.6	132 ± 24.7	0.560	134 ± 25.7	134 ± 25.7	0.989
Diastolic blood pressure (mmHg)	87.7 ± 14.6	87.6 ± 14.3	0.965	85.5 ± 13.6	86.4 ± 13.2	0.613
Mean arterial pressure (mmHg)	103 ± 17.0	102 ± 17.2	0.780	102 ± 16.6	102 ± 16.4	0.793
Pulse pressure (mmHg)	45.7 ± 15.4	44.4 ± 14.4	0.370	48.3 ± 16.7	47.5 ± 17.4	0.700
c-fPulse wave velocity (m/s)[a]	-	-	-	8.61 ± 0.08	8.55 ± 0.08	0.776
Intima media thickness near wall (mm)[a]	-	-	-	0.65 ± 0.09	0.62 ± 0.09	0.071
Intima thickness far wall (mm)[a]	-	-	-	0.65 ± 0.08	0.64 ± 0.08	0.221
Cross-sectional wall area (mm^2)[a]	-	-	-	13.3 ± 0.11	13.0 ± 0.11	0.392

	Baseline (N=987)			10 year follow-up (N=718)		
	Normal Se	**Se Deficient[c]**	***p*-values**	**Normal Se**	**Se Deficient**	***p*-values**
Biochemical variables						
Selenium (µg/100ml)[b]	12.7 ± 3.41	6.12 ± 1.67	**<0.001**	17.5 ± 5.23	18.7 ± 7.31	0.567
Glucose (mmol/l)	4.86 (3.50; 6.70)	4.78 (3.70; 6.30)	0.363	5.32 (4.05; 7.74)	5.21 (3.84; 9.82)	0.523
C-reactive protein (mg/l)	3.16 (0.28; 37.8)	5.06 (0.43; 51.9)	**0.001**	3.44 (0.43; 33.9)	3.65 (0.19; 43.8)	0.712
Total cholesterol (mmol/l)	5.04 ± 1.34	4.85 ± 1.43	0.122	4.62 ± 1.14	4.30 ± 1.17	**0.025**
High density lipoprotein cholesterol (mmol/l)	1.52 ± 0.63	1.47 ± 0.66	0.340	1.39 ± 0.57	1.34 ±0.54	0.484
Low density lipoprotein cholesterol (mmol/l)	2.94 ± 1.15	2.79 ± 1.15	0.162	2.63 ± 1.02	2.37 ± 1.4	**0.041**
Triglycerides (mmol/l)	1.13 (0.54; 2.88)	1.18 (0.58; 2.67)	0.404	1.14 (0.54; 2.77)	1.14 (0.55; 2.72)	0.991
Lifestyle and medication use						
Physical activity index	7.30 ± 1.77	7.15 ± 1.83	0.368	5.08 ± 1.76	4.83 ± 1.65	0.224
γ-Glutamyl transferase (U/l)	56.4 (19.8; 344)	70.6 (21.2; 442)	**0.005**	37.8 (11.6; 212)	41.9 (12.3; 369)	0.354
Alcohol use, n (%)	364 (43.3)	67 (47.9)	0.313	183 (29.2)	33 (41.8)	**0.023**
Tobacco use, n (%)	448 (53.1)	90 (63.8)	**0.018**	237 (37.7)	38 (48.1)	0.075
HIV infected, n (%)	142 (16.8)	19 (13.4)	0.302	118 (18.6)	12 (14.8)	0.411
Hypertension medication, n (%)	149 (17.6)	22 (15.5)	0.533	187 (29.9)	21 (26.9)	0.585
Hypertensive status, n (%)	452 (53.5)	74 (52.1)	0.761	377 (59.2)	55 (67.9)	0.131
Diabetes, n (%)	147 (17.6)	18 (12.8)	0.156	155 (26.4)	19 (25.3)	0.849

Data are expressed as arithmetic mean ± standard deviation, geometric mean (5th and 95th percentile boundaries) or % of N. *P*-values for comparison between groups were obtained with independent t-tests and Chi-square tests. [a]Adjusted for mean arterial pressure. [b]Selenium data in 2015 available for N=81. [c]Selenium deficiency at baseline <8µg/100 ml. Values in bold indicate statistical significance ($p<0.05$).

Supplementary table 5-1. Baseline characteristics of deceased participants compared to those who survived over 10 years

Baseline	Deceased over 10 years (N=270)	Survived over 10 years (N=717)	p-value
Sex [women, (%)]	138 (51.1)	491 (68.4)	**<0.001**
Age (years)	53.2 ± 11.9	49.3 ± 9.14	**<0.001**
Anthropometric measures			
Height (m)	1.61 ± 0.09	1.60 ± 0.08	**0.028**
Body mass (kg)	57.4 ± 14.8	64.2 ± 16.7	**<0.001**
Body mass index (kg/m²)	22.2 ± 6.19	25.2 ± 6.87	**<0.001**
Cardiovascular measures			
Systolic blood pressure (mmHg)	135 ± 27.5	132 ± 23.3	0.113
Diastolic blood pressure (mmHg)	87.9 ± 15.8	87.6 ± 14.0	0.817
Mean arterial pressure (mmHg)	104 ± 18.8	103 ± 16.3	0.376
Pulse pressure (mmHg)	47.3 ± 17.3	44.8 ± 14.4	**0.020**
Biochemical variables			
Selenium (µg/100ml)[b]	10.9 ± 3.91	12.0 ± 3.93	**<0.001**
Glucose (mmol/l)	4.77 (6.50; 1.28)	4.88 (6.80; 1.22)	0.167
C-reactive protein (mg/l)	4.76 (52.8; 4.84)	2.99 (30.3; 4.23)	**<0.001**
Total cholesterol (mmol/l)	4.81 ± 1.39	5.09 ± 1.33	**0.003**
High density lipoprotein cholesterol (mmol/l)	1.49 ± 0.71	1.52 ± 0.61	0.574
Low density lipoprotein cholesterol (mmol/l)	2.71 ± 1.15	2.99 ± 1.14	**0.001**
Triglycerides (mmol/l)	1.15 (2.95; 1.63)	1.14 (2.86; 1.61)	0.795
Lifestyle and medication use			
Physical activity index	6.83 ± 1.83	7.44 ± 1.73	**<0.001**
γ-Glutamyl transferase (U/l)	76.1 (452; 2.58)	52.6 (290; 2.30)	**<0.001**
Alcohol use, n (%)	146 (54.9)	284 (39.8)	**<0.001**
Tobacco use, n (%)	169 (37.2)	369 (51.5)	**0.002**
HIV infected, n (%)	79 (29.3)	83 (11.6)	**<0.001**
Hypertension medication, n (%)	56 (20.7)	115 (16.0)	0.080
Hypertensive status, n (%)	138 (51.1)	335 (46.7)	0.212

Data are expressed as arithmetic mean ± standard deviation, geometric mean (5th and 95th percentile boundaries) or % of N. *P*-values for comparison between groups were obtained with independent t-tests and Chi-square tests. Bold values indicate statistical significance ($p<0.05$).

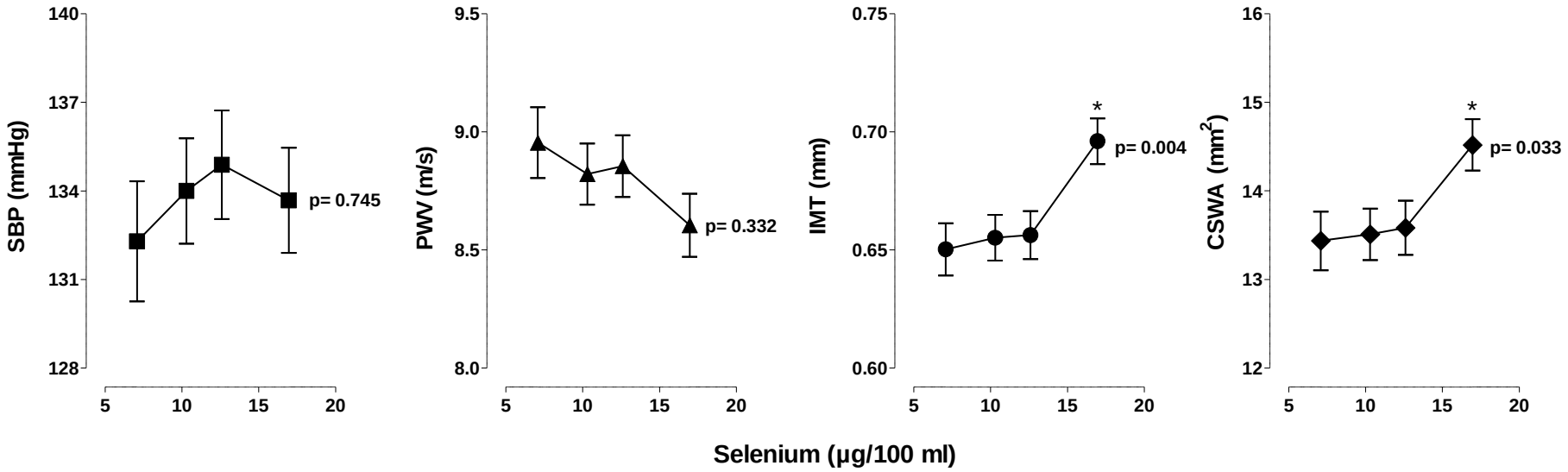

Figure 5-2. ***Quartiles of baseline selenium levels plotted against 10 year follow-up systolic blood pressure (SBP), pulse wave velocity (PWV), carotid intima media thickness (IMT) and cross-sectional wall area (CSWA),*** *adjusted for age and sex. The c-fPWV, IMT and CSWA were additionally adjusted for mean arterial pressure and SBP was additionally adjusted for baseline SBP. *indicates difference between quartile 1 and 4 (p<0.05). Spread represents standard error*

Table 5-2. Multiple regression analyses with 10-year follow-up cardiovascular measures as dependent variables and baseline serum selenium, in the total group (N=690)

	Systolic blood pressure (mmHg)	
	Adjusted R^2=0.09; *p*<0.001	
	β-value (95% CI)	***p*-value**
Selenium (µg/100ml)	-0.03 (-0.10; 0.04)	0.418
Age (years)	0.25 (0.18; 0.33)	**<0.001**
Sex (male/female)	0.11 (0.03; 0.19)	**0.007**
Body mass index (kg/m²)	0.10 (0.01; 0.19)	**0.026**
Physical activity index	0.16 (0.09; 0.24)	**<0.001**
Tobacco use	0.01 (-0.07; 0.08)	0.867
γ-Glutamyl transferase (U/l)	0.10 (0.02; 0.17)	**0.013**
Glucose (mmol/l)	0.05 (-0.02; 0.13)	0.155
C-reactive protein (mg/l)	-0.001 (-0.08; 0.07)	0.969
Low density lipoprotein cholesterol (mmol/l)	-0.06 (-0.13; 0.02)	0.134
	Pulse wave velocity (m/s)[a]	
	Adjusted R^2=0.29; *p*<0.001	
	β-value (95% CI)	***p*-value**
Selenium (µg/100ml)	-0.05 (-0.12; 0.02)	0.163
Age (years)	0.23 (0.16; 0.30)	**<0.001**
Sex (male/female)	0.09 (0.01: 0.17)	**0.024**
Body mass index (kg/m²)	-0.08 (-0.16; 0.01)	0.067
Physical activity index	-0.03 (-0.10; 0.05)	0.461
Tobacco use	0.02 (-0.05; 0.09)	0.562
γ-Glutamyl transferase (U/l)	0.15 (0.08; 0.23)	**<0.001**
Glucose (mmol/l)	0.09 (0.02; 0.16)	**0.015**
C-reactive protein (mg/l)	0.06 (-0.01; 0.14)	0.079
Low density lipoprotein cholesterol (mmol/l)	-0.06 (-0.13; 0.01)	0.112
Mean arterial pressure (mmHg)	0.36 (0.29; 0.43)	**<0.001**

	Intima media thickness (mm)[a]	
	Adjusted R^2=0.19; *p*<0.001	
	β-value (95% CI)	***p*-value**
Selenium (µg/100ml)	0.12 (0.05; 0.19)	**0.001**
Age (years)	0.32 (0.25; 0.40)	**<0.001**
Sex (male/female)	0.06 (-0.02; 0.14)	0.123
Body mass index (kg/m²)	0.01 (-0.08; 0.09)	0.864
Physical activity index	0.12 (0.04; 0.19)	**0.002**
Tobacco use	0.02 (-0.05; 0.10)	0.550
γ-Glutamyl transferase (U/l)	-0.02 (-0.09; 0.05)	0.587
Glucose (mmol/l)	0.01 (-0.06; 0.08)	0.765
C-reactive protein (mg/l)	0.10 (0.03; 0.17)	**0.008**
Low density lipoprotein cholesterol (mmol/l)	0.12 (0.05; 0.20)	**0.001**
Mean arterial pressure (mmHg)	0.13 (0.06; 0.20)	**<0.001**
	Cross-sectional wall area (mm^2)[a]	
	Adjusted R^2=0.23; *p*<0.001	
	β-value (95% CI)	***p*-value**
Selenium (µg/100ml)	0.10 (0.04; 0.17)	**0.003**
Age (years)	0.32 (0.25; 0.39)	**<0.001**
Sex (male/female)	0.14 (0.06; 0.22)	**<0.001**
Body mass index (kg/m²)	0.04 (-0.04; 0.12)	0.319
Physical activity index	0.09 (0.02; 0.17)	**0.010**
Tobacco use	0.06 (-0.01; 0.14)	0.086
γ-Glutamyl transferase (U/l)	0.03 (-0.04; 0.10)	0.410
Glucose (mmol/l)	0.03 (-0.04; 0.10)	0.398
C-reactive protein (mg/l)	0.05 (-0.02; 0.12)	0.199
Low density lipoprotein cholesterol (mmol/l)	0.09 (0.01; 0.16)	**0.018**
Mean arterial pressure (mmHg)	0.22 (0.15; 0.29)	**<0.001**

The main independent variables included in the models were baseline selenium and other baseline covariates included age, sex, body mass index, physical activity index, C-reactive protein, y-glutamyl transferase, glucose, tobacco use and low density lipoprotein cholesterol. [a] Additionally adjusted for follow-up mean arterial pressure. Values in bold indicate statistical significance (*p*<0.05).

When dividing the total group into normal and selenium deficient groups at baseline **(Table 5-3)**, we confirmed the positive associations between IMT and selenium in the normal (β=0.12; *p*=0.003) and selenium deficient groups (β=0.23; *p*=0.034). A positive association was also found between CSWA and selenium in the normal selenium group (β=0.10; *p*=0.006), but not in the selenium deficient group. In addition, a negative association between c-fPWV and selenium (β=-0.09; *p*=0.016) was evident in the normal selenium group, but not in the selenium deficient group (β=0.03; *p*=0.78).

Supplementary Table 5-2 shows the associations between SBP and baseline selenium. We reviewed cross-sectional analyses using baseline SBP, and longitudinal associations between follow-up SBP and baseline selenium. We found a negative association between baseline SBP and selenium only in the normal selenium group (β=-0.08; *p*=0.007), with no associations with SBP (*p*=0.44) at follow-up.

Sensitivity Analyses

When excluding HIV infected participants (N=210) at baseline and follow-up, the results remained mostly the same, except for the positive association between IMT and selenium in the selenium deficient group (N=118) which was now borderline significant (β=0.24; p=0.051). We also excluded participants with diabetes at baseline (N=165) and follow-up (N=174), however our main results were still significant (R^2=0.27; β=-0.09; p=0.029). We also added hypertension status of participants at baseline and follow-up as covariates into the multiple regression model and our main results remained significant (R^2=0.28; β=-0.10; p=0.013).

Table 5-3. Multiple regression analyses in the normal and selenium deficient groups with 10-year follow-up cardiovascular measures as dependent variables

	Normal Se (N=637)		Se deficient (N=81)[b]	
	c-fPulse wave velocity (m/s)[a]			
	Adjusted R^2=0.27; *p*<0.001		Adjusted R^2=0.42; *p*<0.001	
	β-value (95% CI)	*p*-value	β-value (95% CI)	*p*-value
Selenium (µg/100ml)	-0.09 (-0.17; -0.02)	**0.016**	0.03 (-0.17; 0.22)	0.775
Age (years)	0.22 (0.14; 0.30)	**<0.001**	0.28 (0.08; 0.48)	**0.008**
Sex (male/female)	0.09 (0.003; 0.17)	**0.042**	0.09 (-0.14; 0.32)	0.458
Body mass index (kg/m²)	-0.08 (-0.17; 0.01)	0.092	-0.15 (-0.38; 0.08)	0.198
Physical activity index	-0.02 (-0.09; 0.06)	0.693	-0.01 (-0.22; 0.19)	0.891
Tobacco use	0.001 (-0.08; 0.08)	0.986	0.17 (-0.04; 0.37)	0.113
γ-Glutamyl transferase (U/l)	0.16 (0.08; 0.23)	**<0.001**	0.11 (-0.10; 0.32)	0.301
Glucose (mmol/l)	0.09 (0.01; 0.17)	**0.021**	0.13 (-0.07; 0.33)	0.197
C-reactive protein (mg/l)	0.08 (-0.001; 0.15)	0.054	0.05 (-0.14; 0.24)	0.588
Low density lipoprotein cholesterol (mmol/l)	-0.05 (-0.13; 0.03)	0.216	-0.18 (-0.38; 0.03)	0.097
Mean arterial pressure (mmHg)	0.35 (0.27; 0.42)	**<0.001**	0.39 (0.18; 0.60)	**0.001**

	Intima media thickness (mm)[a]			
	Adjusted R^2=0.18; *p*<0.001		Adjusted R^2=0.23; *p*=0.003	
	β-value (95% CI)	***p*-value**	**β-value (95% CI)**	***p*-value**
Selenium (µg/100ml)	0.12 (0.04; 0.19)	**0.003**	0.23 (0.02; 0.44)	**0.034**
Age (years)	0.33 (0.26; 0.41)	**<0.001**	0.21 (-0.01; 0.42)	0.061
Sex (male/female)	0.04 (-0.04; 0.12)	0.314	0.16 (-0.09; 0.41)	0.207
Body mass index (kg/m²)	0.02 (-0.07; 0.11)	0.677	-0.03 (-0.27; 0.22)	0.833
Physical activity index	0.13 (0.05; 0.21)	**0.001**	0.03 (-0.19: 0.25)	0.789
Tobacco use	0.01 (-0.07; 0.09)	0.857	0.15 (-0.06; 0.37)	0.173
γ-Glutamyl transferase (U/l)	-0.02 (-0.10; 0.06)	0.615	-0.11 (-0.33: 0.11)	0.324
Glucose (mmol/l)	0.01 (-0.07; 0.08)	0.889	0.08 (-0.14; 0.29)	0.473
C-reactive protein (mg/l)	0.08 (0.01; 0.16)	**0.034**	0.16 (-0.05; 0.36)	0.137
Low density lipoprotein cholesterol (mmol/l)	0.13 (0.05; 0.20)	**0.001**	0.07 (-0.15; 0.29)	0.518
Mean arterial pressure (mmHg)	0.11 (0.04; 0.19)	**0.004**	0.32 (0.09; 0.54)	**0.007**

	Cross-sectional wall area (mm^2)[a]			
	Adjusted R^2=0.22; p<0.001		Adjusted R^2=0.25; p=0.001	
	β-value (95% CI)	***p*-value**	**β-value (95% CI)**	***p*-value**
Selenium (µg/100ml)	0.10 (0.03; 0.18)	**0.006**	0.21 (0.001; 0.42)	0.053
Age (years)	0.34 (0.26; 0.41)	**<0.001**	0.13 (-0.08; 0.34)	0.232
Sex (male/female)	0.11 (0.03; 0.19)	**0.006**	0.30 (0.05; 0.54)	**0.020**
Body mass index (kg/m^2)	0.04 (-0.05; 0.13)	0.384	0.07 (-0.17; 0.31)	0.580
Physical activity index	0.11 (0.03; 0.18)	**0.007**	0.00 (-0.22; 0.22)	0.997
Tobacco use	0.06 (-0.02; 0.14)	0.133	0.09 (-0.13; 0.30)	0.425
γ-Glutamyl transferase (U/l)	0.02 (-0.05; 0.10)	0.552	-0.004 (-0.22; 0.21)	0.969
Glucose (mmol/l)	0.04 (-0.04; 0.11)	0.345	0.01 (-0.21; 0.22)	0.954
C-reactive protein (mg/l)	0.04 (-0.04; 0.12)	0.304	0.07 (-0.13; 0.27)	0.484
Low density lipoprotein cholesterol (mmol/l)	0.09 (0.02; 0.17)	**0.016**	0.02 (-0.20; 0.23)	0.872
Mean arterial pressure (mmHg)	0.20 (0.13; 0.27)	**<0.001**	0.36 (0.14; 0.58)	**0.002**

The main independent variables included in the models were baseline selenium and other baseline covariates included age, sex, body mass index, physical activity index, C-reactive protein, y-glutamyl transferase, glucose ,tobacco use and low density lipoprotein cholesterol. [a]Additionally adjusted for follow-up mean arterial pressure. Values in bold indicate statistical significance (p<0.05). [b]Selenium deficiency at baseline <8µg/100 ml.

Supplementary table 5-2. Multiple regression analyses in the normal and deficient selenium groups with baseline and follow-up systolic blood pressure as dependent variables

	Normal Se (N=845)		Se deficient (N=142)	
	Baseline systolic blood pressure (mmHg)			
	Adjusted R^2=0.19; *p*<0.001		Adjusted R^2=0.13; *p*=0.002	
	β-value (95% CI)	***p*-value**	**β-value (95% CI)**	***p*-value**
Selenium (µg/100ml)	-0.09 (-0.15; -0.03)	**0.007**	0.11 (-0.06; 0.27)	0.211
Age (years)	0.37 (0.31; 0.44)	**<0.001**	0.26 (0.10; 0.43)	**0.002**
Sex (male/female)	0.05 (-0.02; 0.12)	0.151	0.20 (0.02; 0.38)	**0.034**
Body mass index (kg/m²)	0.19 (0.11; 0.27)	**<0.001**	0.15 (-0.04; 0.35)	0.120
Physical activity index	-0.03 (-0.10; 0.04)	0.358	-0.09 (-0.26; 0.08)	0.296
Tobacco use	-0.005 (-0.07; 0.06)	0.884	-0.04 (-0.21; 0.13)	0.626
γ-Glutamyl transferase (U/l)	0.16 (0.10; 0.23)	**<0.001**	0.08 (-0.09; 0.25)	0.369
Glucose (mmol/l)	0.01 (-0.05; 0.08)	0.715	-0.10 (-0.27; 0.07)	0.245
C-reactive protein (mg/l)	-0.06 (-0.12; 0.01)	0.091	-0.20 (-0.36; -0.04)	**0.016**
Low density lipoprotein cholesterol (mmol/l)	-0.01 (-0.08; 0.05)	0.722	0.18 (0.004; 0.35)	**0.048**

	Follow-up systolic blood pressure (mmHg)			
	Adjusted R^2=0.08; *p*<0.001		Adjusted R^2=0.17; *p*=0.010	
	β-value (95% CI)	***p*-value**	**β-value (95% CI)**	***p*-value**
Selenium (µg/100ml)	-0.03 (-0.11; 0.05)	0.439	0.01 (-0.20; 0.22)	0.929
Age (years)	0.25 (0.17; 0.33)	**<0.001**	0.25 (0.04; 0.47)	**0.024**
Sex (male/female)	0.07 (-0.01; 0.16)	0.099	0.45 (0.21; 0.68)	**<0.001**
Body mass index (kg/m²)	0.09 (-0.001; 0.19)	0.055	0.09 (-0.16; 0.34)	0.481
Physical activity index	0.15 (0.07; 0.24)	**<0.001**	0.22 (-0.003; 0.43)	0.057
Tobacco use	-0.004 (-0.09; 0.08)	0.919	0.05 (-0.17; 0.27)	0.642
γ-Glutamyl transferase (U/l)	0.10 (0.02; 0.18)	**0.012**	0.04 (-0.18; 0.27)	0.699
Glucose (mmol/l)	0.06 (-0.02; 0.14)	0.124	-0.04 (-0.26; 0.18)	0.701
C-reactive protein (mg/l)	0.01 (-0.07; 0.09)	0.861	-0.08 (-0.28; 0.13)	0.462
Low density lipoprotein cholesterol (mmol/l)	-0.08 (-0.16; 0.00)	0.051	0.16 (-0.06; 0.39)	0.150

The main independent variables included in the models were selenium and other covariates included age, body mass index, physical activity index, C-reactive protein, y-glutamyl transferase, glucose ,tobacco use and low density lipoprotein cholesterol. Bold values indicate statistical significance (*p*<0.05).

Discussion

High exposure of selenium has been linked to possible adverse cardiometabolic effects including hyperlipidemia (28) and diabetes (29). From the literature it is evident that “further epidemiological studies and randomized clinical trials across populations with different selenium status should be conducted to determine the causal effect of selenium on cardiovascular disease and risk factors” (30). Therefore, in this longitudinal study, which included a black population from urban and rural areas of the North West Province of South Africa, we investigated whether serum selenium relates to large artery structure (IMT and CSWA) and function (blood pressure and arterial stiffness) over 10 years. We found consistent positive independent associations of selenium with carotid wall thickness. We also found a protective inverse association of selenium with arterial function (blood pressure and c-fPWV), but only in Africans with normal selenium levels – where 14% of our population presented with selenium deficiency.

The positive association between carotid wall thickness and selenium after 10 years, is in contrast with most other studies, which found selenium to be protectively associated against thickening of the intima media (16, 31-33). One study failed to link selenium (measured in toenails) with measures of subclinical atherosclerosis (34). It is noteworthy to mention, that in our multivariable-adjusted regression model, along with serum selenium, other pro-atherogenic factors such as age, inflammation (CRP) and LDL-C (35, 36) were also independently associated with carotid wall thickness – laying credence to our selenium finding. To the best of our knowledge, only one study reported findings which support our findings (37). In this study, the effect of selenium on histopathological changes in an animal model of cockerel was investigated, and it was found that optimal selenium levels induced atherogenesis via inflammation and smooth muscle proliferation in the media of blood vessels (37). In our study, IMT and CSWA values were the highest in the 4th selenium quartile (Figure 2), with a mean selenium level of 16.96 µg/100 ml. However, a narrow selenium range of 5.5-14.5 µg/100 ml is known to have significant protective benefits against CVD (38) and some studies indicated that increased selenium intake in people with adequate-to-high selenium status may lead to adverse effects (39). Selenium supplementation is therefore known to only benefit those with low selenium levels (39). This corresponds

well with previous reviews concluding that there is a U-shaped relationship between selenium and CVD (30, 39, 40).

However, since our study population did not have extremely high selenium levels, it may also be possible that genetic single nucleotide polymorphisms (SNP) of selenoproteins such as GPx may in part explain our finding. It was previously found that specific SNP's may influence selenium's metabolism and utilization and thereby the synthesis of selenoproteins and the responses to environmental stressors (41). A common polymorphism of the GPx1 gene which is known as Pro198Leu, was previously linked to increased risk of CVD including increased IMT (42) and coronary artery calcification (43). This may suggest that these SNPs may demolish the vascular protective effects of selenium; however, the prevalence of this polymorphism in our population is unknown. Nutrigenetics (44) may also have an influence on the selenium distribution in the body, however we were unable to investigate this due to unavailability of data on genetic factors in our population.

Notwithstanding our finding of a potential detrimental association of selenium on carotid wall thickening, we also found a beneficial association of selenium with arterial stiffness over ten years and baseline blood pressure, only in the normal selenium group. This finding is well aligned with previous studies which indicated protective associations of selenium on arterial function (5, 45). Apart from selenium, we also found independent associations of other risk factors for CVD such as age (46), GGT (47) and glucose (48) to be independently associated with arterial function. In spontaneous hypertensive rats, increased selenium intake showed protective effects on degenerative changes and elastin degradation in the vessel walls, suggesting that selenium deficiency may lead to severe degenerative changes in the vessel walls (49). In addition we previously found a protective association between GPx activity and arterial stiffness in white men (aged 20-65 years) (5), although this association was not directly with selenium, selenium performs its roles via the expression of selenoproteins such as GPx (50). We also previously found an independent protective association between 24-hour blood pressure and selenium (5). However, other studies conducted on participants older than 50 years of age found no beneficial effect of antioxidant intake on arterial stiffness, carotid atherosclerosis (51) or blood pressure

(52). Notably, in the 14% of our population studied with selenium deficiency, the beneficial association with arterial stiffness was also absent.

The results of this study should be interpreted within the context of its strengths and limitations. This was a well-controlled 10-year prospective study performed in an understudied black population. The limitations included a lack of dietary data to relate dietary selenium intake with serum selenium levels and cardiovascular measures. From a physiological viewpoint, serum selenium is a more accurate estimate of current selenium status than dietary intake data. Furthermore, c-fPWV and IMT data were not collected at baseline and no data were available on selenoproteins such as glutathione peroxidase, in this study population. While the results from this study were consistent after multiple adjustments, residual confounding cannot be excluded due to unknown factors associated with selenium, c-fPWV, IMT, CSWA and blood pressure. The participants of this study were recruited from the North West Province and cannot be seen as representative of the entire South African population.

Conclusion

We found a potential detrimental association between selenium and carotid wall thickening, particularly evident in individuals within the highest quartile of serum selenium levels. We also found long-term protective associations of serum selenium with arterial stiffness and blood pressure in Africans with normal selenium levels, thereby supporting the notion that selenium fulfils a vascular protective role.

Conflict of Interest

The authors declare that they have no conflict of interests.

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and conclusions are those of the authors and are not necessarily to be attributed to the NRF.

Author Contributions

RS was responsible for the planning, writing and composition of the manuscript as well as the statistical analyses. CMCM, AES and JMvR gave recommendations for the framework, writing and composition of the manuscript as well as the methodology. They also supervised the statistical analyses and helped with the formulation of the tables and figures.

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CHAPTER 6:
CONCLUDING REMARKS

6.1 Introduction

This chapter is a summary of the main findings of the research articles included in the thesis. The results of the three articles are discussed, interpreted, explained and compared to the relevant literature. Conclusions are drawn and recommendations made with regards to the vascular effects of selenium and GPx activity in different cohorts of South Africa.

6.1.1 Summary of main findings

The summary of the main findings of the three research articles (Chapters 3, 4 and 5) of the different studies is as follows:

Article 1: Serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study (Chapter 3) (Figure 6-1).

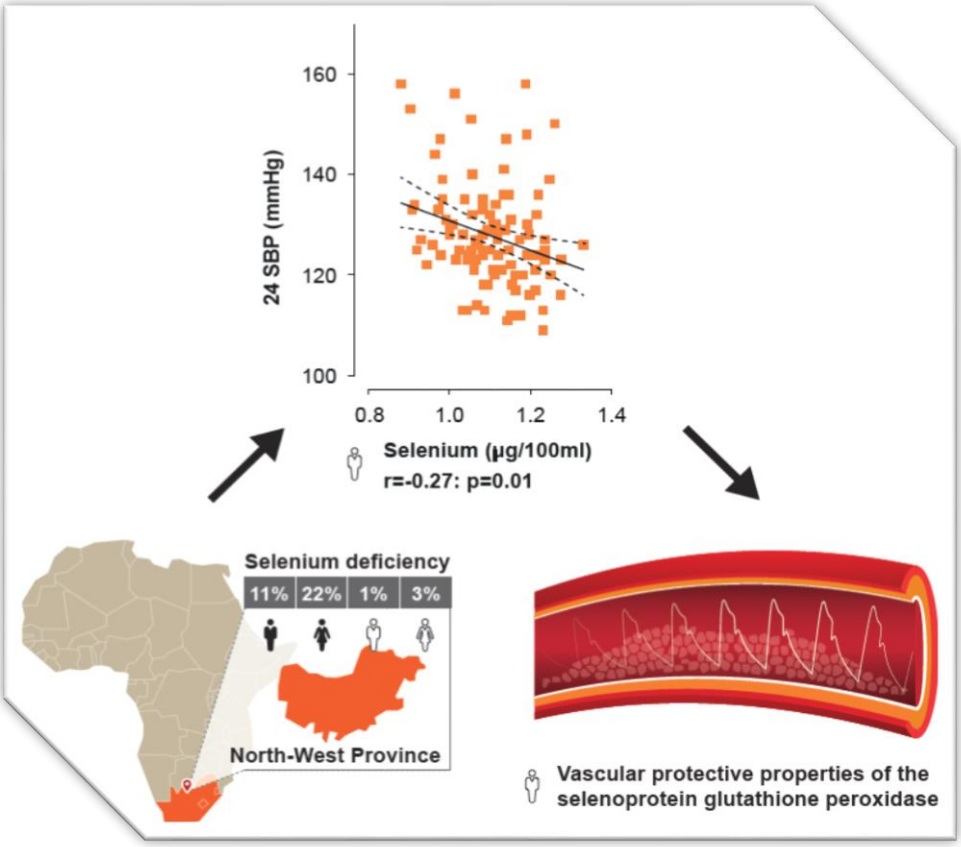


Figure 6-1. ***Selenium's protective associations on blood pressure and the vasculature.***

In the SABPA study, serum selenium levels were compared between black and white adults. We also investigated whether serum selenium levels are related to GPx activity; and whether 24h blood pressure, vascular resistance, arterial compliance and arterial stiffness are related to both serum selenium and GPx activity.

The hypotheses will be reviewed and compared to the results of this study:

Hypothesis 1: Serum selenium levels and GPx activity will be lower in the black participants compared to the white participants.

The results obtained in this cross-sectional study showed that serum selenium levels were significantly lower in black compared to white men and women. Therefore, the first hypothesis is accepted.

Hypothesis 2: Serum selenium levels will correlate positively with GPx activity.

We found a significant positive correlation between selenium and GPx activity in the total group (p=0.004) and in white women (p=0.006) only. We therefore only partially accept the second hypothesis.

Hypothesis 3: Blood pressure, vascular resistance and arterial stiffness will associate negatively with serum selenium levels and GPx activity in black participants.

We found negative associations of 24h systolic blood pressure (SBP) (p=0.039) and 24h diastolic blood pressure (DBP) (p= 0.029) with selenium and of c-dPWV (p=0.017) with GPx activity in white men only. In black women, a negative association between 24h night time SBP and GPx activity was found. No associations of vascular resistance, with selenium or GPx activity were found. We therefore only partially accept the third hypothesis.

Hypothesis 4: Arterial compliance will associate positively with serum selenium levels and GPx activity in black participants.

No association was found between arterial compliance and selenium or GPx activity, we therefore reject the fourth hypothesis.

Article 2: The role of selenium and GPx: An investigation of the micro- and macrovasculature in a young bi-ethnic population from the African-PREDICT study (Chapter 4) (Figure 6-2).

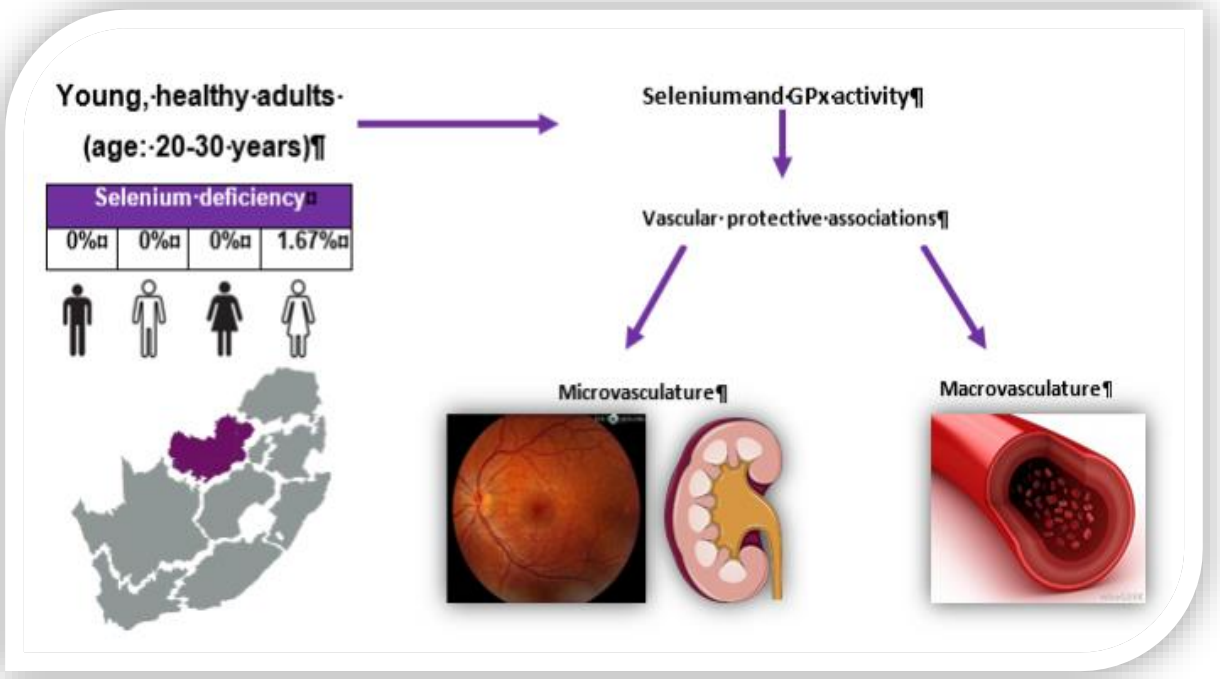


Figure 6-2. ***The vascular protective roles of selenium and GPx activity on the micro- and macrovasculature.***

It is unclear whether selenium and GPx may have a vascular protective role, particularly in young healthy adults, before the onset of CVD. Therefore, we investigated whether measures of the microvasculature (CRAE, CRVE, AVR and eGFR) and of the macrovasculature (PWV, 24h PP, AIx) are related to serum selenium and GPx in a young healthy, black and white cohort.

Hypothesis 1: Microvascular measures (CRVE) will associate negatively with selenium and GPx in black participants.

No association were found between the microvascular measure, CRVE and either selenium or GPx in any of the groups. We therefore reject the first hypotheses.

Hypothesis 2: Microvascular measures (CRAE, AVR and eGFR) will associate positively with selenium and GPx in black participants.

We found positive associations between the microvasculature (AVR) and selenium in black women. We found positive associations between the microvasculature (eGFR) and GPx activity in white men. However, no results were found with the microvascular marker (CRAE) and either selenium or GPx in any of the groups. We therefore only partially accept the second hypotheses.

Hypothesis 3: Macrovascular measures (PWV, 24h PP and AIx) will associate negatively with selenium and GPx in black participants.

We found negative associations between the macrovasculature (24h PP) and GPx activity in black men. In white women, negative associations of 24h PP with selenium were found. In white men and black women we also found a negative association between AIx and GPx activity. No associations were found between PWV and either selenium or GPx and this hypothesis can therefore be partially accepted

Article 3: Selenium and large artery structure and function: A ten year prospective study (Chapter 5) (Figure 6-3).

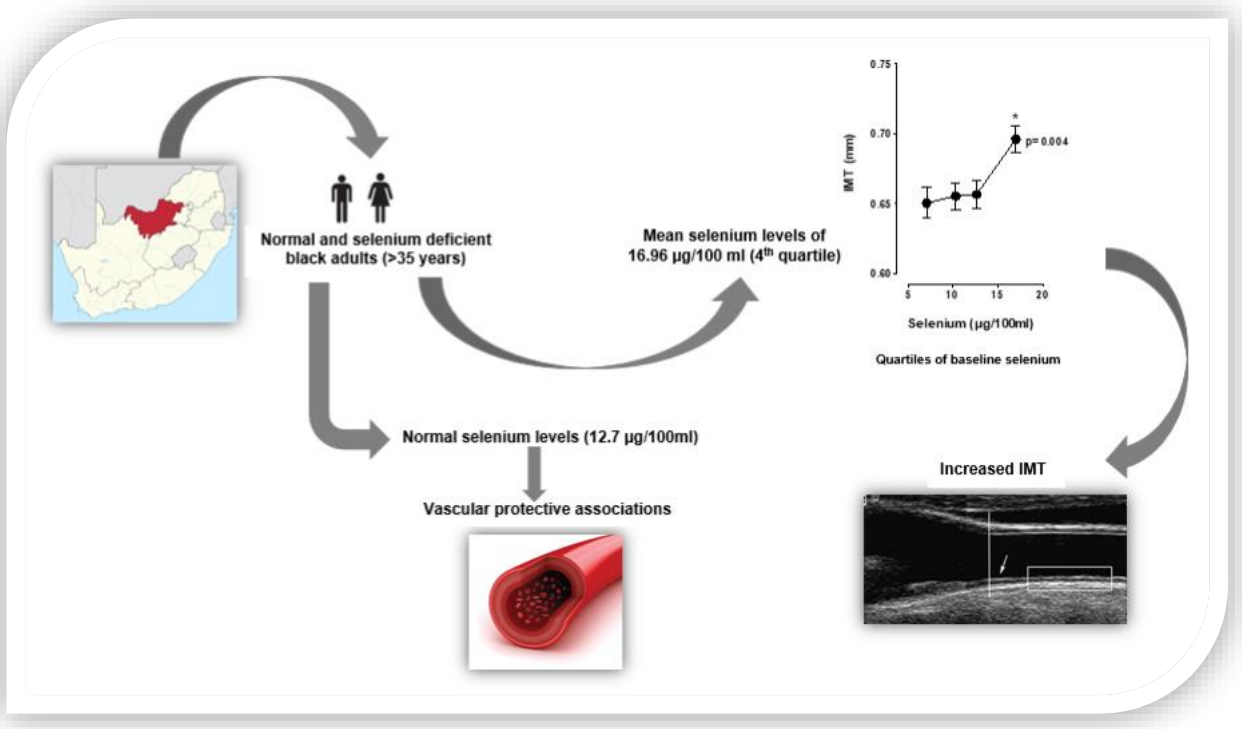


Figure 6-3. ***The associations between selenium and large artery structure and function.***

We investigated in normal and selenium deficient adults as well as in the total group, whether serum selenium levels are related to measures of large artery structure (IMT and CSWA) and function (blood pressure and arterial stiffness) over ten years.

Hypothesis 1: Baseline selenium levels will associate negatively with follow-up measures of large artery structure (IMT and CSWA) over 10 years.

We found positive associations between IMT and selenium in the normal and selenium deficient groups. A positive association was also found between CSWA and selenium in the normal selenium group, but not in the selenium deficient group. The first hypothesis is therefore rejected, as we unexpectedly found positive associations of IMT and CSWA with selenium, which is in contrast with most other studies (1-3).

Hypothesis 2: Baseline selenium levels will associate negatively with follow-up measures of large artery function (blood pressure and arterial stiffness) over 10 years.

We found a negative association between c-fPWV and selenium in the normal selenium group, but not in the selenium deficient group. However, we found no associations between follow-up SBP and baseline selenium, we therefore partially accept the second hypothesis.

6.1.2 Discussion of main findings and comparison with the literature

In this section the findings from this study is interpreted and compared to the literature. Some results confirmed those of previous studies or contributed to existing literature while some were contradictory.

A comparison of selenium and GPx levels between black and white adults

Selenium is known to be a co-factor for GPx activity and is incorporated in the selenoprotein, GPx (4). The optimum activity of GPx will be reached when the recommended daily allowance (RDA) of 55 µg/day for women and 70 µg/day for men for selenium intake is maintained (5).

In the literature there are different cut-off values for selenium status (serum selenium levels), with deficiency classified as serum selenium levels <7 µg/100 ml (6) or <8 µg/100 ml (7). In the thesis we used a cut-off value of <8 µg/100 ml for selenium deficiency (7). Our results of the SABPA and African-PREDICT studies indicated that black South Africans have lower selenium levels as well as lower GPx levels compared to their white counterparts **(Table 6-1)**. In the SABPA study (Chapter 3) it was found that 11% black men and 22% black women were selenium deficient. In the African-PREDICT study (Chapter 4) only two white women were selenium deficient, although the selenium levels and GPx activity differed significantly between the black and white participants **(Table 6-1)**.

Table 6-1. Comparison of selenium and GPx activity among black and white adults of the SABPA and African-PREDICT studies.

SABPA study						
	Black men	**White men**	***p-value***	**Black women**	**White women**	***p-value***
Selenium (µg/100 ml)	10.1 (7.00; 14.0)	12.7 (8.50; 17.7)	<0.001	9.39 (6.40; 13.0)	13.3 (9.30; 20.8)	<0.001
GPx activity (nmol/min/ml)	34.6 ± 13.9	35.1 ± 8.03	0.75	31.9 ± 13.9	37.1 ± 7.82	0.001
African-PREDICT study						
	Black men	**White men**	***p-value***	**Black women**	**White women**	***p-value***
Selenium (µg/100 ml)	16.1 ± 3.37	17.2 ± 3.70	**0.037**	16.5 ± 3.02	16.4 ± 3.63	0.870
GPx activity (nmol/min/ml)	18.4 ± 1.76	19.8 ± 1.37	**<0.001**	18.6 ± 1.74	19.9 ± 1.34	**<0.001**

Our results contribute to the existing literature which indicate that South African children (8) and black women (9) have inadequate selenium intake (< two-thirds of the RDA). However, we only found a small group of participants to be selenium deficient especially in the African-PREDICT study. Previous findings indicated lower selenium levels with an increase in age (10, 11), which may in part explain why we only had two participants with selenium deficiency in the young population of the African-PREDICT study. In addition, it was also found that African Americans had lower serum selenium levels compared to white Americans (12). Previous results based on our study population (SABPA study), indicated that GPx activity was lower in black compared to white adults (13, 14). Results from another South African study population with calcific chronic pancreatitis is also in agreement with our results (15). This pilot study investigated the micronutrient antioxidant profiles of black chronic pancreatitis patients as well as control subjects in Soweto. When comparing the results of the Sowetan controls with reference ranges from Manchester, United Kingdom, they found lower serum selenium levels in these black patients (15).

There are various possible factors which may influence the ethnic differences in selenium levels, including environmental factors (concentration of selenium in the soil and pH of the soil) (16, 17), dietary intake (18), socio-economic status (19) and human genetics (20). Environmental factors refer to the content of selenium and pH levels in soil, which differs in different regions of South Africa and may influence the selenium levels in food (21). Dietary intake is influenced by the socio-economic status, which differs between South African groups, however the SABPA study obtained a sample from a similar socio-economic status. We also adjusted for socio-economic status in the African-PREDICT study. In the PURE study, urban participants had lower selenium levels at baseline ($p<0.001$) and follow-up ($p=0.032$). White South Africans commonly consume a higher selenium content diet, which consists of more meat products compared to their black counterparts (22, 23).

However, all the participants from the different study populations used in this thesis were from the North West Province of South Africa and therefore shared the same geographic location. This suggests that there may have been dietary and cultural preferences food types. Different food preparation methods such as the boiling of food may also play a role. Previous results indicated that 40% of selenium content are lost during boiling of food whereas the addition of salt and vinegar to food may reduce selenium levels by 50% (24, 25). Selenoprotein gene polymorphisms may also have led to different selenium levels (26) and this should be investigated in future studies. It is further unclear whether foods consumed were imported or grown elsewhere, which may have affected the selenium content.

Table 6-2. Comparison of selenium levels after ten years in the original normal and selenium deficient groups of the PURE study.

PURE study						
	Baseline selenium			Follow-up selenium		
	Normal Se	Se deficient	p-value	Normal Se	Se deficient	p-value
N	**845**	**142**		**74**	**7**	
Selenium (µg/100 ml)	12.7 ± 3.41	6.12 ± 1.67	**<0.001**	17.5 ± 5.23	18.7 ± 7.31	0.567

Table 6-3. Comparison of selenium levels between baseline and follow-up in the total group.

PURE study			
	Baseline selenium N=987	Follow-up selenium N=81	p-value
Selenium (µg/100ml)	12.0 ± 3.76	17.6 ± 5.39	**<0.001**

In the PURE study (Chapter 5) we found that the mean selenium levels of the normal selenium group were 12.7 ± 3.41 µg/100 ml compared to the selenium deficient group with 6.12 ± 1.67 µg/100 ml. After 10 years, (in a sub-sample) the selenium levels of the selenium deficient group restored to normal and there was no difference between selenium levels of the normal selenium group and the selenium deficient group **(Table 6-2)**. We also found in the total group that baseline and follow-up selenium levels differed significantly over ten years **(Table 6-3)**. The change in selenium over ten years may be due to various factors. The rural area of Ganyesa, became more urbanized over ten years, which includes more nearby supermarkets and technology. Since 2005 with the baseline study, the community consumed food that was grown in their local area but due to increased urbanization, food products that are high in selenium including, meat, eggs, fish and dairy products became more readily available. The staple food for most poor, underdeveloped urban black communities in South Africa consist of maize products (27) and a previous study which was conducted on all the

maize silos in South Africa showed that 94% of the maize from all maize silos were selenium deficient (21). On the 7th of April 2003 a mandatory food fortification program of maize and wheat flour was introduced in South Africa (28). In October 2003, 4 out of the 18 different maize meal products were fortified with vitamins and minerals including, vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, iron and zinc in South Africa, however not with selenium (29).

However, two popular South African maize meal brands are currently fortified with the selenium including Iwisa and Nyala **(Figure 6-4)**. This fortification of maize with selenium may also be one of the reasons why the selenium levels of the population group of the PURE study became normal over 10 years.

Figure 6-4. Selenium fortified maize meal in South Africa.

6.1.2.1 The link between selenium and the selenoprotein GPx.

Selenium is a component of GPx (4) and the antioxidant effects of selenium is therefore mediated via GPx (30). It is therefore expected that serum selenium will relate positively to GPx activity. Indeed, we found a positive association of serum selenium levels with GPx activity in the total group and only in white women of the SABPA study **(Figure 6-5)** (Chapter 3). We also investigated the link between

selenium and GPx activity in the African-PREDICT study, however we found no associations between selenium and GPx activity.

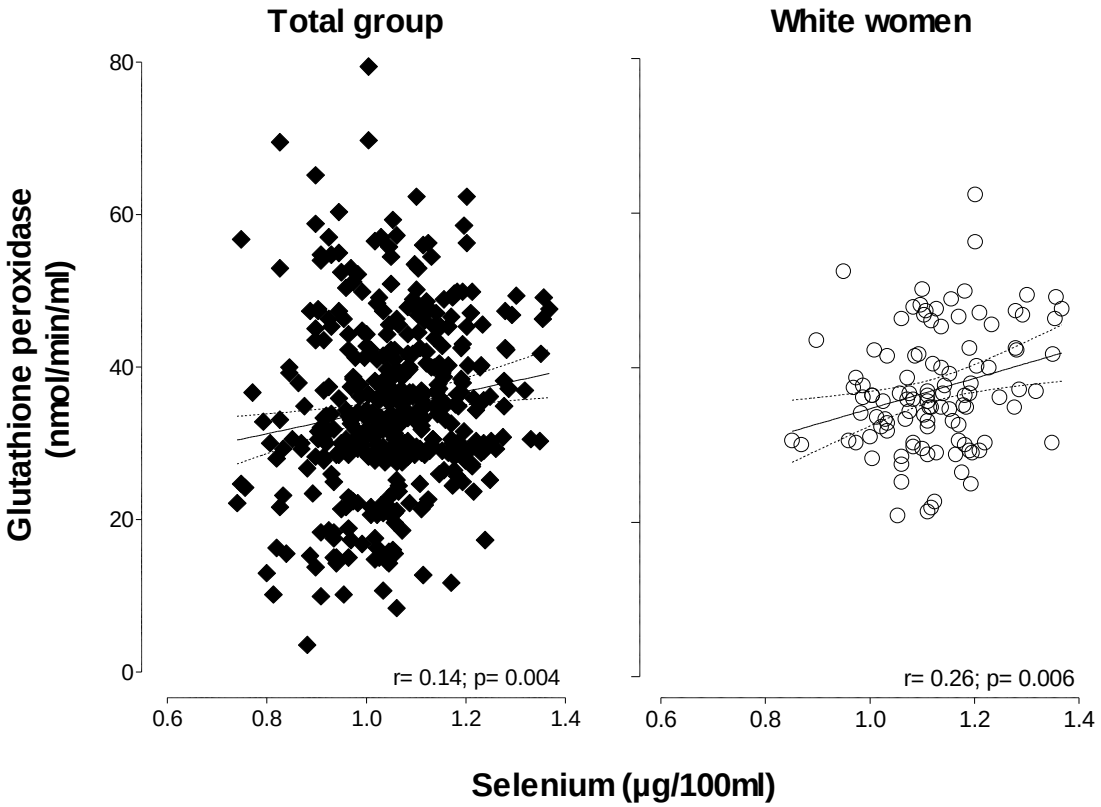


Figure 6-5. A positive association between serum selenium and GPx activity in the total group and white women in the SABPA study.

When comparing our results to the literature, we found controversial results. A study which investigated intensive care patients with critical illnesses were divided into groups. The healthy volunteers were designated as controls (Group 1), the ICU patients were divided into three Groups where Group 2 consisted of patients without systemic inflammatory response syndrome, Group 3 patients with SIRS and Group 4 consisted of patients with systemic inflammatory response syndrome and multiple organ dysfunction syndrome (31). They investigated whether systemic inflammatory response syndrome had an effect on selenium levels and they investigated the association between serum selenium and GPx-3 activity. They found a positive

association between selenium and GPx-3 in the group without systemic inflammatory response syndrome (Group 2) and the group with systemic inflammatory response syndrome and multiple organ dysfunction syndrome (Group 4), which indicate that low serum selenium levels are associated with low GPx-3 (31). In contrast, a previous study showed a positive association between selenium and GPx activity in plasma and erythrocytes in healthy women on a normal selenium diet (32). Another study which also included healthy participants from three different geographic areas including New Zealand, Oregon and South Dakota indicated that this correlation was only evident in people with very low selenium status (5.9 ± 1.1 µg/100 ml in whole blood) (33). These findings contradict the findings of the present study where white women of the SABPA study was the only group except for the total group where we found a positive association between selenium and GPx activity and the white women had the highest selenium levels (13.3 µg/100 ml). However, in another study it was found that in 50–69 year old men and women this association was evident only in men and not in women, which is in contrast with our findings (34). This may indicate sex-specific differences in selenium metabolism and selenoprotein expression (35).

In the family of GPx, GPx-2 (intestinal) and GPx-4 (membrane) rank higher in the hierarchical selenium supply system in comparison with GPx-1 (cytosolic) and GPx-3 (plasma) (36, 37). In this study we measured GPx-1 and this may however be a reason why we only found a positive association between selenium and GPx activity in white women who presented with the highest selenium values. This may indicate that all the other selenoproteins are already adequately supplied with selenium. Whereas, in the other individual groups who presented with inadequate selenium levels, other selenoproteins such as GPx-2 and GPx-4 may have been prioritized.

The reason why we did not find an association between serum selenium and GPx in the African-PREDICT study may be due to only a few participants having selenium deficiency, which may be due to the young age of the population, as it was previously found that selenium decrease with increasing age (10, 11).

Another possible reason why we did not find an association between selenium and GPx in the African-PREDICT study as well as lower serum selenium levels in the participants of the SABPA study may be due to selenoprotein single-nucleotide

polymorphisms (SNPs), which may have an influence on selenium metabolism and utilization. This includes, GPx-1 rs1050450, GPx-4 rs713041, selenoprotein P plasma 1 s3877899, selenoprotein 15 rs5845, selenoprotein S rs28665122 and selenoprotein S rs4965373 (38). Where it was previously found that the GPx-1 rs1050450 C allele associates with GPx activity. Therefore, people with the GPx-1 rs1050450 CT genotype had the highest correlation between selenium and GPx activity (38).

6.1.2.2 Vascular protective associations of selenium and GPx activity

Prospective and randomized controlled studies found inconsistent results, although most of the studies found selenium and GPx activity to be vascular protective (39). However, we mostly found vascular protective associations of selenium and GPx activity throughout all three studies (although a possible detrimental association between selenium and IMT/CSWA was indicated in the PURE study). These vascular protective associations were evident, from a young (mean age 25 years) and middle-aged population (mean age 45 years) to an older, population group with a higher prevalence of hypertension and HIV infection (follow up mean age 58 years).

6.1.2.2.1 Blood pressure and selenium

The protective associations of blood pressure with selenium were mainly evident in white men (Chapter 3) and from the literature, it is evident that blood pressure was inversely associated with blood selenium levels in men only (40). In another study which included older men (55-74 years of age) it was found that low serum selenium levels (<4.5 µg/100 ml) were associated with cardiovascular and all-cause mortality (41). In contrast, one study, which included men and women (59-71 years), found higher selenium levels in men with hypertension and vascular disease (42). These contrasting results, may be as a result of different age groups of participants and the measurement of selenium status in different matrices (serum, plasma, urine, whole blood).

6.1.2.2.2 Arterial stiffness and selenium/GPx activity

Our findings contribute to previous studies which indicated a protective effect of selenium on arterial stiffness in patients with a high risk for cardiovascular events (39).

A study conducted in spontaneously hypertensive rats, suggested that selenium deficiency may lead to severe degenerative changes in the vessel walls (43). In contrast, in a healthy middle-aged human population no beneficial effects of selenium supplementation on arterial stiffness were found (44).

Our results of protective associations of arterial stiffness with selenium and GPx were predominantly evident in whites, who had normal selenium levels and in black participants who were in the normal selenium group (Chapter 3 and 5). These findings contribute to the existing literature, since findings made in the African-PREDICT study suggest that selenium and GPx activity may be beneficial of protection of the vasculature from an early age (25.0 ± 3.12 years), before the onset of cardiovascular diseases through to middle age (44.7 ± 9.44 years) in the SABPA study with 52.8% prevalence of hypertension and even in older age (53.8 ± 9.30 years) where cardiovascular disease is already evident (58.2% prevalence of hypertension).

The mechanism related to the vascular protective effects of selenium and GPx may involve the maintenance of nitric oxide bioavailability and protection against oxidative damage. Therefore, low selenium levels may lead to a decrease in antioxidant function which in turn may lead to increased oxidative stress (45, 46) and inflammation (47, 48). This may cause endothelial dysfunction and may contribute to the development of arterial stiffness and increased blood pressure (49, 50) and eventually increase the risk for the development of various cardiovascular diseases (51-53) **(Figure 6-6)**.

However, due to the cross-sectional nature of the African-PREDICT study, our findings are based purely on associations and future research is needed on the protective role of selenium and GPx on arterial stiffness.

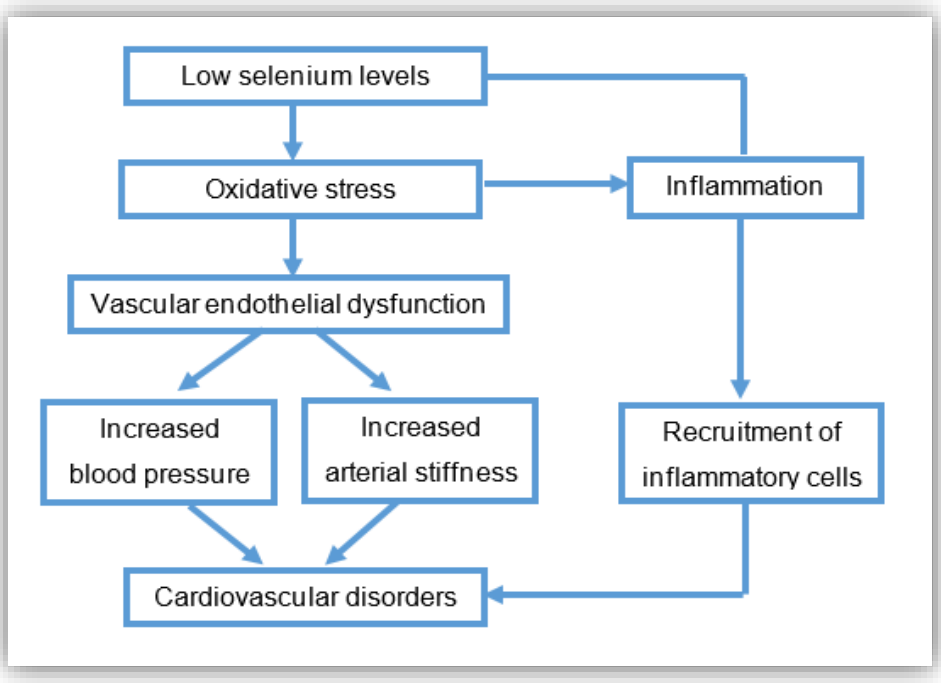


Figure 6-6. Mechanisms of selenium deficiency-induced cardiovascular disorders.

6.1.2.3 The microvasculature and selenium/GPx activity

6.1.2.3.1 Microvascular measures

We found sex-specific protective associations of microvascular measures (AVR and eGFR) with selenium and GPx. However, no significant associations with individual vessel calibres (CRAE, CRVE) (Chapter 4) were found, which is known to be more informative (54). Similar protective effects were found in other studies which investigated the link between the microcirculation and selenium (55, 56). In one study microvascular function improved in obese girls after increased intake of selenium-rich Brazil nuts (55), while another study reported on the protective association of selenium with measures of microvascular function in the skin of healthy young men (56). In contrast previous studies reported on an adverse association between kidney function (eGFR) and a decrease in extracellular selenoprotein GPx 3 levels in chronic kidney disease patients (57-59). Our study's protective associations once again highlight the beneficial effects of selenium on inflammatory processes (60), endothelial function (61) and eventually CVD **(Figure 6-6)**. Our results may demonstrate the protective role

of GPx activity to maintain kidney function. Changes in the microvasculature is known to be an early predictor of future CVD (62), therefore it may be beneficial to ensure optimal selenium status to maintain its health effects and delay or avoid microvascular deterioration and eventually CVD in later life.

Although we found various different associations between selenium/GPx and the micro- and macrovascular measures in different groups, collectively all of our findings suggest a protective role for the micronutrient, selenium, and GPx on both of these vascular beds in young adults.

6.1.2.4 Sub-clinical atherosclerotic markers and selenium

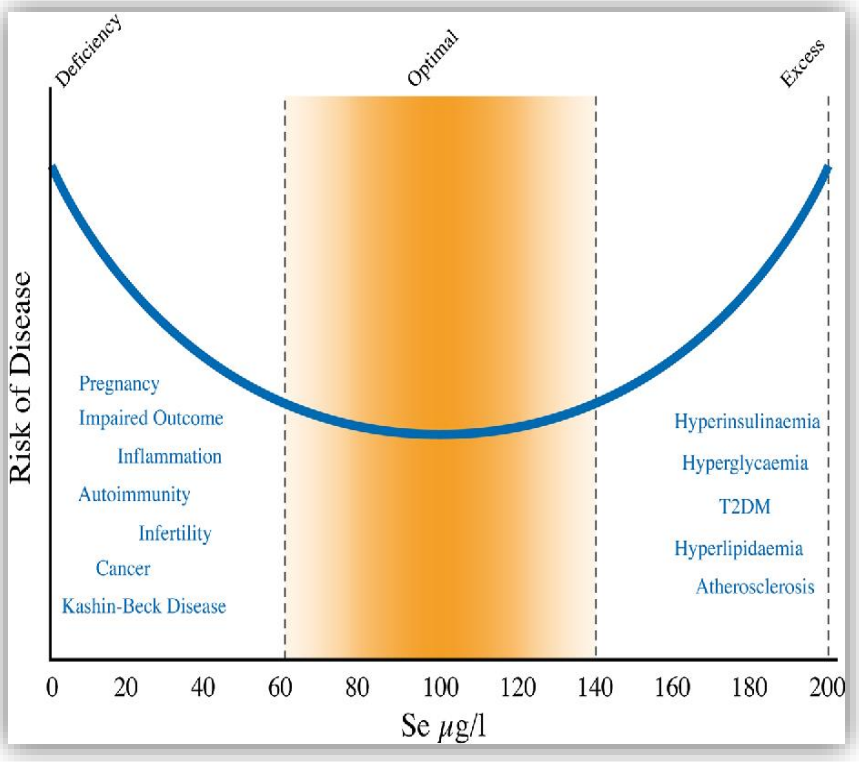
It has been speculated that selenium may prevent atherosclerotic disease (1-3), however we found a surprising positive association between selenium and carotid wall thickness after ten years (Chapter 5). In line with our results, a previous cross-sectional study found a U-shaped relationship between selenium and atherosclerosis. The known beneficial serum selenium range is between 8-12 µg/100 ml (63), however we also found mean selenium levels above 16 µg/100 ml in our population group with the highest IMT and CSWA values. It was also suggested that selenium levels higher than 16 µg/100 ml increased the risk of peripheral artery disease (64). An animal study reported findings which support our findings (65). In this study, the effect of selenium on histopathological changes in an animal model of cockerel was investigated, and it was found that optimal selenium levels (0.14 mg) induced atherogenesis via inflammation and smooth muscle proliferation in the media of blood vessels (65).

Another possible explanation that may have affected our results may be the influence of genetic single nucleotide polymorphisms (SNP) of selenoproteins such as GPx. However, since SNPs were not measured in our population, we are unable to confirm whether this has affected our results.

The U-shaped relationship of selenium with disease states:

As previously mentioned selenium is known to exhibit protective benefit against CVD between a narrow selenium range of 8-12 µg/100 ml (68) . However, as it can be seen in Figure 7 if selenium levels are too low or to high it may have possible adverse

cardiometabolic effects (74). Therefore, selenium supplementation must be used with caution when the baseline selenium levels of the population are unknown. These results indicate that an increase of selenium levels may prevent atherosclerosis, however in populations with low selenium levels (65-67).



The U-shaped relationship of selenium levels with the risk of disease *(With permission from the author (56)).*

Although controversy was found among some of our findings, our results consistently indicated the vascular protective association of selenium and GPx on blood pressure, arterial stiffness and the microcirculation in different age groups of South Africa. Therefore, our findings contribute to current knowledge, indicating that selenium and GPx activity are beneficial from an early age onwards. Intervention studies in South Africa are warranted to investigate this further.

6.2 Limitations, Chance and Confounding

In the thesis, it is crucial to reflect on the limitations and factors that may have confounded our results. The limitations of this thesis include a lack of dietary data to

relate dietary selenium intake with serum selenium levels, GPx activity and cardiovascular measures. However, from a physiological viewpoint, serum selenium is a more accurate estimate of current selenium status than dietary intake data (66).

The selenoprotein P (SePP) in plasma is known to be the most conclusive marker for determining the optimal supply of selenium (67) however, we were unable to investigate SePP. We were also unable to investigate the thyroid hormone metabolism. It is also known that specific genetic SNP's influence selenium's metabolism and utilization, however the prevalence of these polymorphisms in our population is unknown. It is therefore suggested that future studies investigate these topics. Furthermore, in the PURE study, we did not measure baseline c-fPWV and IMT data, therefore we could not investigate the associations between selenium levels with long term changes of arterial structure and we also only measured a small number of follow-up selenium samples.

The results from this thesis were consistent after multiple adjustment, however, residual confounding effects cannot be excluded due to unknown factors associated with selenium, GPx activity, arterial stiffness, blood pressure, renal function, retinal vessel calibres or sub-clinical atherosclerosis. In the African-PREDICT study, the results may have been more consistent in the different groups, if higher statistical power was achieved.

All the participants of the three different studies were recruited from only one province in South Africa namely the North West Province. Therefore, our population group cannot be seen as a representative of the entire South African population. The SABPA and African-PREDICT studies were cross-sectional studies. The PURE study was a longitudinal study, although the results are only based on associations.

6.3 Recommendations

- Further research is needed to investigate associations of dietary selenium with serum selenium levels, GPx activity and cardiovascular measures in population groups ranging from young and healthy to older populations. Dietary selenium levels is an easier and inexpensive method to determine selenium levels and to compare to serum selenium levels.

- We also suggest that the positive association between selenium and IMT as well as the vascular protective associations found in the PURE study should be investigated further.
- Future studies investigating the thyroid hormone metabolism and SePP in plasma are warranted to shed light on these topics.
- A larger population which represents the entire South African population group is needed to clarify if our results are widely applicable.

6.4 Final Conclusions and Perspectives

Collectively, results from this thesis indicated consistently that selenium and GPx showed vascular protective associations in different populations including a healthy population (aged 25.0 ± 3.12 years), a middle-aged population group (aged 44.7 ± 9.44 years) with >50% prevalence of hypertension and an older population group of 53.8 ± 9.30 years, where the onset of CVD are evident over ten years (>50% prevalence of hypertension). These results suggest that even from an early age, with or without CVD, selenium and GPx may be beneficial on the vasculature. An exception was observed where selenium associated positively with carotid wall thickness. We suggest that it may be beneficial to ensure optimal selenium status to maintain its benefits on the vasculature and delay or avoid vascular deterioration and eventually CVD in later life. This may lead to preventative and therapeutic strategies to alleviate the increased burden of CVDs in South Africa.

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ANNEXURE A - ETHICS CERTIFICATE

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Faculty of Health Sciences
Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

5 August 2016

Prof CMC Mels
Physiology

Dear Prof Mels

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00079-16-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: Serum selenium levels, the selenoprotein glutathione peroxidase and cardiovascular function in black South Africans

Study leader/supervisor: Prof CMC Mels

Student: R Swart

Application type: Single study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 13/07/2016 of the HREC, Faculty of Health Sciences, and was approved on 05/08/2016.

The commencement date for this study is 05/07/2016 dependent on fulfilling the conditions indicated below. 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 up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Dr Wayne Towers
HREC Chairperson

Prof Minrie Greeff
Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2016 Applications\Applications 06 - 13 July 2016\NWU-00079-16-S1 (CMC Mels-R Swart)\NWU-00079-16-S1(CMC Mels-R Swart)-AL\NWU-00079-16-S1(CMC Mels-R Swart)-AL.docm
19 August 2016

File reference: 9.1.5.3

ANNEXURE B - LANGUAGE EDITOR STATEMENT

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 thesis by

R Swart

MSc (Physiology)

With the title

Serum selenium levels, the selenoprotein glutathione peroxidase and cardiovascular function in black South Africans

Prof Annette L Combrink

Accredited translator and language editor

South African Translators' Institute

Membership No. 1000356

Date: 8 November 2018

ANNEXURE C – TURNITIN REPORT

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ORIGINALITY REPORT

19%	12%	13%	15%
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PRIMARY SOURCES

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2	repository.nwu.ac.za Internet Source	2%
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7	Michél Strauss, Wayne Smith, Aletta E. Schutte. "Inter-arm Blood Pressure Difference and its Relationship with Retinal Microvascular Calibres in Young Individuals: The African-PREDICT Study", Heart, Lung and Circulation, 2016 Publication	1%

ANNEXURE D - PUBLISHED SABPA STUDY MANUSCRIPT

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Serum selenium levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study

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Glutathione peroxidase
Ambulatory blood pressure
Arterial stiffness
Race

ABSTRACT

Selenium is an important co-factor for the optimal functioning of the antioxidant enzyme, glutathione peroxidase (GPx). Studies investigating the associations of selenium with blood pressure (BP) and hemodynamic measures are sparse. This study investigated whether 24h blood pressure, vascular resistance, arterial compliance and arterial stiffness relate to both serum selenium and GPx activity. In this cross-sectional study selenium levels, GPx activity, ambulatory blood pressure and arterial stiffness of 200 black and 209 white school teachers from South Africa were measured. Serum selenium levels were significantly lower in black compared to white teachers ($p < 0.001$), independent of sex. One in 10 black men and one in five black women were selenium deficient (< 8 μg/100ml). Only in white men inverse independent associations of 24h systolic BP ($\beta = -0.19$; $p = 0.039$) and 24h diastolic BP ($\beta = -0.21$; $p = 0.029$) with selenium were found. In the same group, an inverse association between carotid-dorsalis pedis pulse wave velocity (cd-PWV) and GPx activity ($\beta = -0.23$; $p = 0.017$) were also found. To conclude, lower serum selenium levels in black populations from the same geographical region as their white counterparts may impact on the loss of the vasculoprotective effects of selenium and selenoproteins such as GPx.

1. Introduction

South Africa is currently undergoing rapid urbanisation. This involves a nutrition transition which includes changes from a diet high in fibre and low in fat (Hawkes, 2006) to ultra-processed foods that are more palatable. The nutrition transition may be accompanied by micronutrient malnutrition (Vorster, Kruger, & Margetts, 2011). Serum levels of selenium, an essential micronutrient, are dependent on dietary selenium intake (Fairweather-Tait, Collings, & Hurst, 2010). Selenium levels in plant foods are in turn dependent on the amount of selenium present in the soil (Rayman, 2000). Meat, cereals, fish, eggs and dairy products are the main food groups which contain selenium (Rayman, 2008). White South Africans consume a diet that includes mainly fresh meat products (O'Keefe, Kidd, Espitalier-Noel, & Owira, 1999), which is a good source of selenium (Dumont, Vanhaecke, & Cornelis, 2006). In contrast, the majority of black South Africans consume a diet that includes maize products (Labadarios et al., 2005), which may contribute to the aetiology of selenium deficiency found in adults (Hattingh, Walsh, Bester, & Oguntibeju, 2008) and children (Labadarios et al., 2005).

Selenium is an important co-factor for the optimal functioning of the antioxidant enzyme, glutathione peroxidase (GPx) (Fairweather-Tait et al., 2011; Muth, Oldfield, Remmert, & Schubert, 1958; Rayman, 2012). Several studies have linked low selenium levels and GPx activity to the development of cardiovascular diseases (Alehagen et al., 2015; Zhang, Liu, Guo, & Song, 2016). Partly due to urbanisation, hypertension has become an increasing burden with increased morbidity and mortality, especially in black South Africans (Lloyd-Sherlock, Beard, Minicuci, Ebrahim, & Chatterji, 2014; Sliwa, Stewart, & Gersh, 2011). During the past four decades, the highest worldwide blood pressure levels have shifted from high-income countries to low-income countries in south Asia and sub-Saharan Africa (Collaboration, 2017). A systematic review focusing on populations from sub-Saharan Africa indicated a pooled prevalence of hypertension of 30%, whereas only 7% had controlled blood pressure (Ataklte et al., 2015). A high prevalence of hypertension was observed in black adults (Schutte, Schutte, et al., 2011), males and particularly older people (Collaboration, 2017).

Black South Africans tend to have increased arterial stiffness at a younger age when compared to whites (Mokwatsi, Schutte, & Kruger, 2017; Schutte, Huisman, et al., 2011). Oxidative stress may contribute to increased blood pressure (Kruger et al., 2012; van Zyl, Huisman, & Mels, 2016), carotid wall thickness (Schutte et al., 2009;

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van Zyl et al., 2016) and arterial stiffness (Kruger et al., 2012; Mokhaneli, Fourie, Botha, & Mels, 2016).

Studies investigating the associations of selenium with blood pressure and hemodynamic measures are sparse. Therefore, in this study serum selenium levels were compared between black and white adults. This study also investigated whether serum selenium levels related to GPx activity; and whether 24 h blood pressure, vascular resistance, arterial compliance and arterial stiffness related to both serum selenium and GPx activity.

2. Materials and methods

2.1. Study population and protocol

This study formed part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study. A detailed description of the study population and protocol has been published elsewhere (Malan, Hamer, Frasure-Smith, Steyn, & Malan, 2015). Baseline data (collected in 2008/9) was used in this cross-sectional data analyses. The study population consisted of a homogenous sample of 200 black (101 men and 99 women) and 209 white (108 women and 101 men) school teachers aged 20–65 years from the Dr. Kenneth Kaunda Education District in the North West Province of South Africa. Exclusion criteria included the use of α- and β-blockers, an ear temperature > 37.5 °C, participants who were vaccinated or who donated blood three months prior to the commencement of the study and pregnant or lactating women. The study fulfilled all the requirements as stated in the Helsinki Declaration (2008) for investigation on human participants and was further approved by the Health Research Ethics Committee of the North-West University (NWU-00036-07-S6). This sub-study was also approved by the Health Research Ethics Committee of the North-West University (NWU-00079-16-S1).

2.2. Anthropometric and physical activity measurements

Trained anthropometrists measured weight and height in triplicate with calibrated instruments (Precision Health Scale. A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, UK) (Marfell-Jones, Stewart, & de Ridder, 2012). Body mass index (BMI) was calculated as weight (kg)/height (m^2). Total energy expenditure was determined with the Actical® activity monitor (Mini Mitter Co., Inc., Bend, OR; Montreal, Quebec, Canada) over a 24 h period.

2.3. Questionnaires

All the participants completed a general health questionnaire containing questions on lifestyle habits and medication use.

2.4. Cardiovascular measurements

The participants were fitted with ambulatory blood pressure monitoring devices (CardioXplore®, MediTech, Budapest, Hungary) on the participant's non-dominant arm each morning at 08:00 to measure 24 h systolic blood pressure (SBP), 24 h diastolic blood pressure (DBP) and 24 h mean arterial pressure. The ambulatory blood pressure monitoring devices were programmed to measure blood pressure at 30-min intervals during the day (08:00–22:00) and every hour during night time (22:00–06:00) with an overall successful 24 h inflation rate of 78.9%.

The validated (Schutte, Huisman, Van Rooyen, Malan, & Schutte, 2004; Wesseling, Jansen, Settels, & Schreuder, 1993) Finometer device® was used to measure continuous blood pressure. The Finometer device was connected, and after a 10 min resting period, a 5 min continuous measurement of baseline cardiovascular variables was taken. The average of the recordings of the last minute was used for further analyses. Finometer measurements were processed with Beatscope 1.1 software (Finapres Medical Systems, Amsterdam, the Netherlands) to determine total peripheral resistance and Windkessel arterial compliance.

The carotid-dorsalis pedis pulse wave velocity (cd-PWV) was measured as a measure of arterial stiffness. The non-invasive measurement was taken across the carotid-dorsalis pedis region with the participant in a supine position (Complior SP device, Artech-Medical, Pantin, France). The distance was determined by subtracting the carotid artery to suprasternal notch from the distal measurement.

2.5. Biochemical analyses

Blood samples from fasting participants were obtained from brachial antecubital vein branches with a sterile winged infusion set after a 5 min rest in the semi-Fowler's position. Serum and plasma were prepared according to standard procedures. All samples were stored at − 80 °C until biochemical analyses were performed.

Glycated haemoglobin was measured in EDTA whole blood via a turbidimetric inhibition immunoassay (Integra 400, Roche, Switzerland). Serum C-reactive protein, gamma glutamyl-transferase and the lipid profile including total cholesterol, high density lipoprotein cholesterol (HDL−C) and triglycerides were determined in serum with two sequential multiple analysers (Konelab 20i; ThermoScientific, Vantaa, Finland; Unicel DXC 800 Beckman and Coulter, Krefeld, Germany). Serum cotinine levels were measured using a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland). Both the intra- and inter-assay coefficients of variation for all the assays were < 10%. The Modification of Diet in Renal Disease Study equation was used to estimate glomerular filtration rate (Levey et al., 1999).

Glutathione peroxidase activity was measured in EDTA plasma samples with assay kits from Cayman Chemical Company (Ann Arbor, MI, USA) on a Bio-Tek FL600 Microplate reader (Winooski, VT, USA.) with an intra-assay variability of 5.7% and inter-assay variability of 7.2%. Serum selenium levels were analysed with a inductively coupled plasma mass spectrometry method and serum selenium deficiency was classified as selenium levels < 8 μg/100 ml (Rükgauer, Klein, & Kruse-Jarres, 1997). The intra-assay variation was < 10% and the inter-assay variation was 13.1% for selenium.

2.6. Statistical analyses

Statistical analyses were performed with Statistica 13 (Dell, TX, USA). Interactions of sex and ethnicity were tested for the relationship between cardiovascular variables (24 h SBP, 24 h DBP, total peripheral resistance, Winkessel arterial compliance and cd-PWV) and both selenium and GPx activity, using multiple regression analyses. Data was expressed as arithmetic mean and standard deviation for normally distributed variables. Variables with a non-Gaussian distribution (glycated haemoglobin, triglycerides, HDL-cholesterol, total cholesterol:HDL-cholesterol, estimated glomerular filtration rate (eGFR), C-reactive protein, selenium, gamma glutamyl-transferase) were logarithmically transformed and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Means and proportions were compared using independent *t*-tests and Chi-square tests, respectively. Single regression analyses were done to investigate associations of selenium with GPx activity. Single and partial correlations (while adjusting for age, BMI and cotinine) were performed to investigate associations of 24 h blood pressure, cdPWV, Windkessel arterial compliance and total peripheral resistance with selenium and GPx activity. Multiple regression analyses were further performed to determine independent associations of cardiovascular variables with selenium and GPx activity. The models were compiled with 24 h SBP, 24 h DBP, night time SBP and cd-PWV as main dependent variables and selenium and GPx activity as main independent variables. The following covariates were considered for entry in multiple regression analyses: age, BMI, waist circumference, cotinine, self-reported smoking, gamma glutamyl-transferase, self-reported alcohol use, glucose, glycated haemoglobin, total energy expenditure, C-reactive

protein, Interleukin-6, total cholesterol, HDL-cholesterol, triglycerides, total cholesterol:HDL-cholesterol and anti-hypertensive medication. Based on bivariate correlations between potential covariates and the dependent and main independent variables, the following covariates were entered into the final models: age, BMI, cotinine, total cholesterol, total energy expenditure, anti-hypertensive medication, gamma glutamyl-transferase, C-reactive protein and glycated haemoglobin. Models with cd-PWV as main dependent variable were additionally adjusted for mean arterial pressure.

3. Results

3.1. Characteristics of the study population

Interactions of ethnicity on the associations of 24 h SBP and 24 h DBP with selenium (both $p = 0.001$), and of Windkessel arterial compliance with GPx activity ($p = 0.009$) were found. Interactions of sex on the associations of 24 h SBP ($p = 0.040$), 24 h DBP ($p = 0.030$) and Windkessel arterial compliance ($p = 0.001$) with selenium were also found. Groups were stratified according to ethnicity and sex to compare black and white men and women (Table 1).

Black men and women had higher 24 h SBP, 24 h DBP and 24 h mean arterial pressure as well as night time SBP and night time DBP (all $p < 0.001$) when compared to white men and women. In black men Windkessel arterial compliance was lower ($p < 0.001$) while cd-PWV was higher in black women ($p = 0.045$) when compared to their respective white counterparts. Serum selenium levels were lower in black men and women ($p < 0.001$) whereas GPx was also lower in black women ($p = 0.001$) when compared to white men and women. Eleven percent of black men and 22% of black women also showed selenium deficiency (< 8 μg/100 ml) – with the incidence of selenium deficiency being higher in blacks (both $p \leq 0.003$) when compared to their white counterparts. Lifestyle factors of black men and women also differed from white participants, including higher gamma glutamyl-transferase (a potential indicator of alcohol use) in black men and women ($p < 0.001$). Total energy expenditure was lower in black men ($p < 0.001$) compared to white men.

3.2. Single and partial regression analyses

In Fig. 1, a positive correlation between selenium and GPx activity was evident in the total group ($n = 409$; $r = 0.14$; $p = 0.004$). After exploring the same association separately in black and white men and women, GPx related positively to selenium only in white women ($r = 0.26$; $p = 0.006$).

In single regression analyses (Table A1, Fig. 2), significant associations were predominantly found in white men, where 24 h SBP ($r = -0.27$; $p = 0.01$), 24 h DBP ($r = -0.24$; $p = 0.02$), night time SBP ($r = -0.27$; $p = 0.01$) and night time DBP ($r = -0.20$; $p = 0.04$) associated negatively with selenium. After adjusting for age, BMI and cotinine, the following associations were borderline significant, 24 h SBP and selenium ($p = 0.052$), 24 h DBP and selenium ($p = 0.080$), night time SBP and selenium ($p = 0.056$), whereas the association of night time DBP with selenium lost significance ($p = 0.19$) (Table A1).

Table 1
Lifestyle, anthropometric, cardiovascular and biochemical characteristics of black and white men and women.

	Black men	White men	p-values	Black women	White women	p-values
n	101	101		99	108	
Age (years)	43.2 ± 8.17	45.1 ± 11.0	0.17	45.6 ± 7.90	45.0 ± 10.7	0.65
Anthropometric measurements						
Height (cm)	171 ± 6.33	181 ± 6.46	< 0.001	159 ± 5.84	167 ± 5.86	< 0.001
Body mass (kg)	80.3 ± 17.9	95.7 ± 17.3	< 0.001	82.5 ± 19.0	72.8 ± 18.2	< 0.001
Body mass index (kg/m^2)	27.6 ± 5.77	29.0 ± 5.20	0.059	32.76 ± 7.20	26.26 ± 6.29	< 0.001
Cardiovascular measurements						
24 h Systolic blood pressure (mmHg)	138 ± 16.0	128 ± 10.4	< 0.001	129 ± 15.1	121 ± 12.4	< 0.001
24 h Diastolic blood pressure (mmHg)	87.9 ± 10.7	79.5 ± 7.44	< 0.001	79.0 ± 8.62	73.9 ± 7.66	< 0.001
24 h Mean arterial pressure (mmHg)	104 ± 12.1	95.6 ± 7.88	< 0.001	95.5 ± 10.2	89.4 ± 8.79	< 0.001
Night time systolic blood pressure (mmHg)	129 ± 18.0	117 ± 11.6	< 0.001	119 ± 14.8	110 ± 14.7	< 0.001
Night time diastolic blood pressure (mmHg)	78.7 ± 12.4	68.55 ± 8.28	< 0.001	69.5 ± 9.57	64.6 ± 8.65	< 0.001
Total peripheral resistance (mmHg/ml/s)	1.07 ± 0.32	1.07 ± 0.68	0.98	0.96 ± 0.42	1.01 ± 0.30	0.30
Windkessel arterial compliance (ml/mmHg)	1.88 ± 0.41	2.32 ± 0.52	< 0.001	1.86 ± 0.42	1.88 ± 0.44	0.71
cdPulse wave velocity (m/s)[a]	8.93 ± 1.90	8.84 ± 1.90	0.75	8.03 ± 1.26	7.66 ± 1.26	0.045
Biochemical analyses						
Selenium (μg/100 ml)	10.1 (7.00; 14.0)	12.7 (8.50; 17.7)	< 0.001	9.39 (6.40; 13.0)	13.3 (9.30; 20.8)	< 0.001
Selenium deficiency n (%)	11 (10.9)	1 (0.99)	0.003	21 (21.7)	3 (2.78)	< 0.001
Glutathione peroxidase (nmol/min/ml)	34.6 ± 13.9	35.1 ± 8.03	0.75	31.9 ± 13.9	37.1 ± 7.82	0.001
Glycated haemoglobin (%)	6.14 (5.20; 9.56)	5.64 (5.10; 6.40)	< 0.001	5.83 (5.00; 7.60)	5.36 (5.00; 5.90)	< 0.001
Triglycerides (mmol/l)	1.46 (0.64; 3.67)	1.30 (0.60; 3.00)	0.18	0.91 (0.40; 2.30)	0.80 (0.40; 2.20)	0.063
Total cholesterol (mmol/l)	4.74 ± 1.17	5.58 ± 1.20	< 0.001	4.46 ± 1.20	5.50 ± 1.35	< 0.001
High density lipoprotein cholesterol (mmol/l)	0.99 (0.57; 1.64)	0.96 (0.60; 1.40)	0.48	1.16 (0.70; 1.74)	1.34 (0.80; 2.30)	< 0.001
Total cholesterol:HDL-cholesterol	4.65 (2.39; 7.78)	5.69 (3.70; 8.80)	< 0.001	3.70 (2.30; 6.00)	3.99 (2.70; 6.80)	0.061
Estimated glomerular filtration rate (ml/min/1.73m^2)	125 (92.0 ± 175)	93.0 (69.1 ± 122)	< 0.001	96.7 (67.7 ± 134)	93.3 (68.9 ± 121)	0.21
C-reactive protein (mg/l)	2.75 (0.27; 14.2)	1.80 (0.99;0.8.00)	0.002	7.13 (0.78; 35.7)	2.26 (0.99; 14.3)	< 0.001
Lifestyle and comorbidities						
Cotinine (ng/l)	35.5 ± 65.0	30.9 ± 96.7	0.69	18.7 ± 55.4	15.1 ± 53.0	0.63
γ-Glutamyl transferase (U/l)	62.6 (23.7; 280)	27.3 (11.0; 90.0)	< 0.001	35.5 (16.7; 117)	13.9 (6.00; 39.0)	< 0.001
Total energy expenditure (kcal/day)	2715 ± 800	3674 ± 2059	< 0.001	2654 ± 796	2587 ± 644	0.51
Multivitamin intake n (%)	1 (0.99)	7 (6.93)	0.030	0 (0.00)	14 (13.0)	< 0.001
Anti-oxidant intake n (%)	0 (0.00)	1 (0.99)	0.32	0 (0.00)	4 (3.70)	0.053
Hypertensive status n (%)	79 (78.2)	56 (55.5)	0.001	53 (53.5)	26 (24.1)	< 0.001
Anti-hypertensive medication n (%)	36 (35.6)	14 (13.9)	< 0.001	33 (33.3)	13 (12.0)	< 0.001
Diabetes n (%)	7 (6.93)	1 (0.99)	0.030	3 (3.03)	1 (0.93)	0.27
HIV infected n (%)	13 (12.9)	0 (0.00)	< 0.001	6 (6.06)	0 (0.00)	0.009

Data expressed as arithmetic mean ± standard deviation or geometric mean with 5th and 95th percentile boundaries or n (%).
[a] Adjusted for mean arterial pressure.

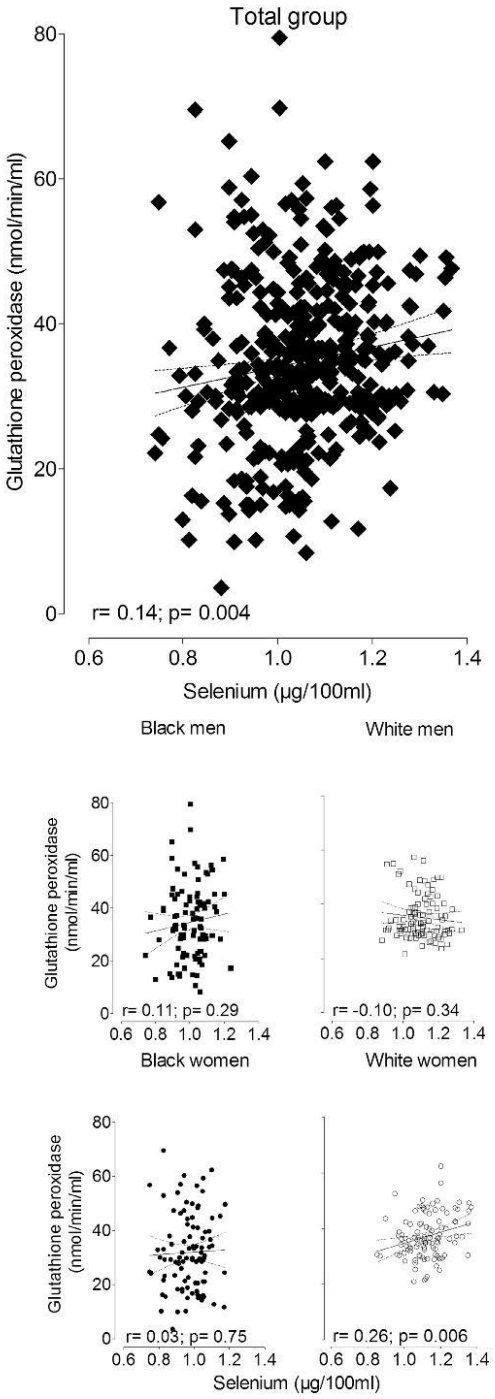

Fig. 1. Single regression analyses between selenium and glutathione peroxidase in the total group as well as in the black and white men and women.

In single and partial regression analyses, cd-PWV ($r = -0.26$; $p = 0.009$) was negatively associated with GPx activity in white men. These associations were absent in black men.

In black women 24 h SBP ($r = -0.21$; $p = 0.04$) and night time SBP ($r = -0.24$; $p = 0.02$) were also negatively associated with GPx activity. After adjusting for age, BMI and cotinine, only the association of night time SBP with GPx remained significant ($r = -0.22$; $p = 0.038$) whereas the association of 24 h SBP with GPx became borderline significant ($p = 0.087$). These associations were absent in white women.

3.3. Multivariate analyses

In multivariable adjusted regression analyses, the previous results in white men were confirmed, namely negative associations of 24 h SBP ($\beta = -0.19$; $p = 0.039$), 24 h DBP ($\beta = -0.21$; $p = 0.029$) and night time SBP ($\beta = -0.20$; $p = 0.040$) with selenium, as well as the negative association between cd-PWV and GPx activity ($\beta = -0.23$; $p = 0.017$) (Table 2 and Table A2). In black women, the negative association between 24 h night time SBP ($\beta = -0.21$; $p = 0.036$) and GPx activity was also confirmed to be independent of various covariates (Table 2 and Table A3).

3.4. Sensitivity analyses

When excluding participants taking oral anti-oxidants and multivitamins (n = 27), the associations of selenium and GPx activity with cardiovascular variables remained significant (all $p < 0.05$). Also after excluding HIV infected participants (n = 19) the results remained unchanged.

4. Discussion

In this study, which included black and white school teachers from the North West Province of South Africa, serum selenium levels were significantly lower in black compared to white teachers, independent of sex. It was also found that one in 10 black men, and one in five black women were selenium deficient (< 8 µg/100 ml) (Rükgauer et al., 1997). Only 1% of the white men and 3% of the white women were selenium deficient. Selenium and GPx activity were protectively associated with blood pressure and arterial stiffness, respectively, in white men only.

The lower selenium levels and higher prevalence of selenium deficiency in the black groups indicated in the present study are in line with previous studies from South Africa which indicated that children (Labadarios et al., 2005) and black women (Hattingh et al., 2008) had inadequate selenium intake. The same results were also found when serum selenium levels were compared between African Americans and white Americans (Vogt, Ziegler, Patterson, & Graubard, 2007). In the present study, all the participants were teachers from the North West Province of South Africa and therefore shared the same geographic location and socio-economic status. However, there may have been dietary and cultural differences (Kant & Graubard, 2007).

Selenium plays a vascular-protective role (Chan et al., 2012) as it is involved in the synthesis of selenoproteins such as GPx (Mousavi et al., 2015) and thioredoxin reductase (Mistry, Wilson, Ramsay, Symonds,- & Pipkin, 2008; Tanguy, Grauzam, De Leiris, & Boucher, 2012). It is therefore expected that serum selenium will relate positively to GPx activity. Indeed, a positive association of serum selenium levels with GPx activity in the total group of 409 teachers was found. However, when this link was investigated in the individual groups, the association of serum selenium levels with GPx was only evident in the white women. Although

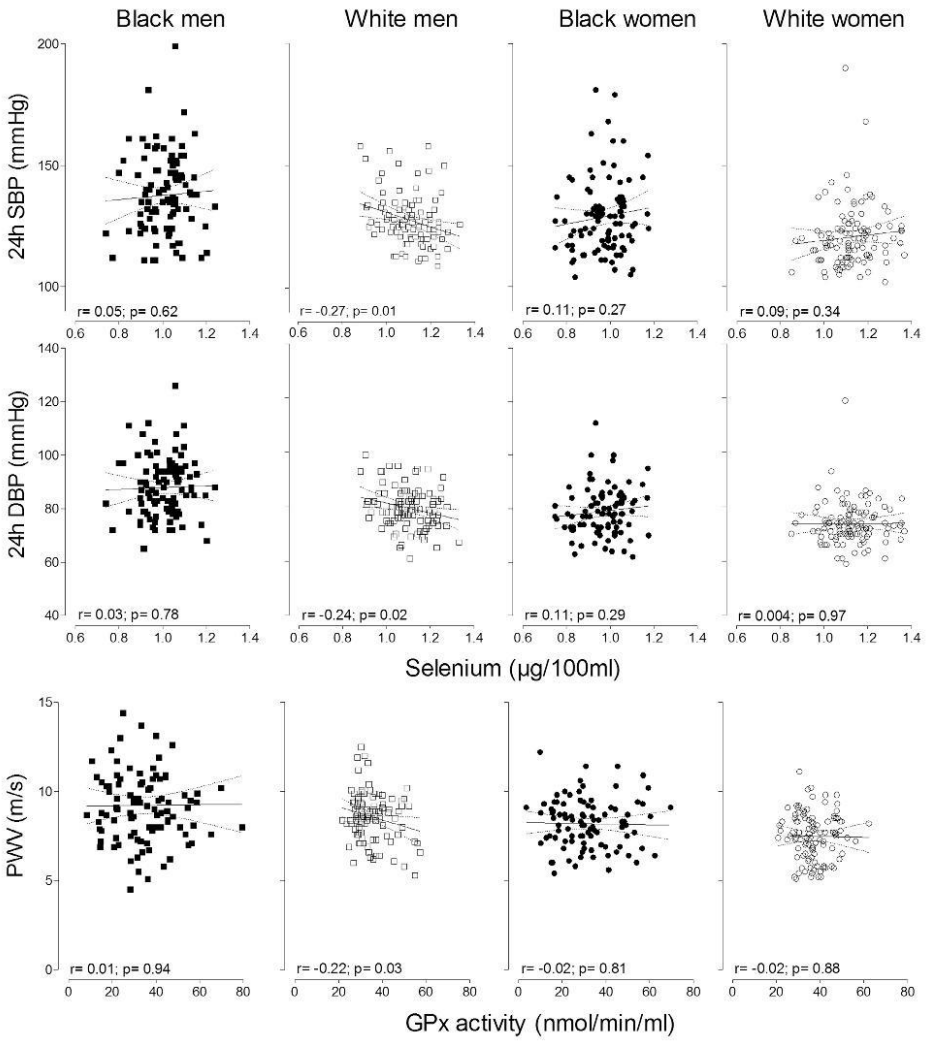

Fig. 2. Unadjusted correlations of cardiovascular variables with selenium and glutathione peroxidase activity in black and white men and women.

a positive association between these factors was expected, previous studies investigated the link between serum selenium levels and GPx activity and found controversial results. In a previous study which consisted of 27 normal pregnant, 25 pre-eclamptic, and 22 healthy age-matched non-pregnant white women, a positive association of maternal serum selenium with both maternal plasma and placental GPx activity was indicated (Mistry et al., 2008). In another study which included healthy participants from three different geographic areas including New Zealand, Oregon and South Dakota it was indicated that this correlation was only evident in people with very low selenium status (59 ± 11 ng/ml in whole blood) (Whanger, Beilstein, Thomson, Robinson, & Howe, 1988). These findings contradict the findings of the present study where white women had the highest selenium levels. However, in another study it was found that in 50–69 year old men and women this association was evident only in men and not in women (Åkesson et al., 1997), which may indicate sex-specific differences in selenium metabolism and selenoprotein expression (Schomburg & Schweizer, 2009).

Nutrigenetics, which is the response of genetic variations on nutrients and the interaction thereof with disease states (Joost et al., 2007), may also be a reason why there were no associations found between selenium and GPx activity in the other individual groups of the present study. Research from previous studies suggested that effective dietary selenium intake may differ among individuals which may be due to genetic variants in selenoproteins (Johnson, 2009). In turn, this may influence the way in which the body metabolizes and utilizes selenium (Mathers & Hesketh, 2007; Ommen et al., 2010; Zeisel, 2010).

The results of the present study are in line with previous findings reported by Nawrot et al. (2007) which indicated that blood pressure

Table 2
Summary of forward stepwise regression analyses with carotid-dorsalis pedis pulse wave velocity and 24 h blood pressure as dependent variables in the total group as well as in the black and white men and women.

	24 h systolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.33	–	–	0.33	–	–
Black men	0.24	–	–	0.24	–	–
White men	0.29	− 0.19 (− 0.37; − 0.01)	0.039	0.26	–	–
Black women	0.14	–	–	0.17	–	–
White women	0.39	–	–	0.40	–	–
	24 h diastolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.34	–	–	0.34	–	–
Black men	0.17	–	–	0.18	–	–
White men	0.27	− 0.21 (− 0.39; − 0.02)	0.029	0.23	–	–
Black women	0.13	–	–	0.12	–	–
White women	0.28	–	–	0.28	–	–
	24 h night time systolic blood pressure					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β–value (95% CI)	p-value
Total group	0.30	–	–	0.30	–	–
Black men	0.17	–	–	0.17	–	–
White men	0.22	− 0.20 (− 0.39; − 0.01)	0.040	0.18	–	–
Black women	0.07	–	–	0.11	− 0.21 (− 0.41; − 0.02)	0.036
White women	0.37	–	–	0.38	–	–
	Pulse wave velocity[a]					
	Selenium			Glutathione peroxidase		
	Adjusted R^2	β-value (95% CI)	p-value	Adjusted R^2	β-value (95% CI)	p-value
Total group	0.31	–	–	0.31	–	–
Black men	0.24	–	–	0.24	–	–
White men	0.16	–	–	0.21	− 0.23 (− 0.41; − 0.04)	0.017
Black women	0.28	–	–	0.28	–	–
White women	0.35	–	–	0.35	–	–

– Indicates that the variable did not enter the model. The main independent variables included in the models were selenium and GPx and other covariates included age, body mass index, cotinine, total cholesterol, total energy expenditure, anti-hypertensive medication usage, gamma glutamyl transferase, C-reactive protein, glycated haemoglobin.

[a] Mean arterial pressure additionally added in the model.

was inversely associated with blood selenium levels in men only. When investigating the associations of both selenium and GPx activity with blood pressure and arterial stiffness, significant findings in the current study were mainly encountered in white men, where only 1% was classified as being selenium deficient. In the same group 24 h systolic and diastolic blood pressure were inversely associated with serum selenium. However, inconsistent results were found among studies which investigated the link between blood pressure and serum selenium (Coudray, Roussel, Mainard, Arnaud, & Favier, 1997; Virtamo et al., 1985). One study which included men and women aged 59–71 years (n = 1389) with normal selenium levels, indicated a positive association between blood pressure and serum selenium levels in men (Coudray et al., 1997). In another study, no association was found between blood pressure and selenium in a population group which consisted of men aged 55–74 years in Finland (Virtamo et al., 1985), in which low selenium levels were found in 16% of the men in Eastern and 42% of the men from South Western Finland. This may suggest that these factors are not associated in conditions or populations with very low selenium levels.

In support of the beneficial associations between blood pressure and selenium in white men, it was also found that arterial stiffness was negatively associated with GPx activity. Previously it was reported that oxidative stress was linked to arterial stiffness (Kruger et al., 2012; Mokhaneli et al., 2016), and the link between GPx and arterial stiffness in the present study suggest that GPx activity may be protective against arterial stiffening. Low selenium levels may lead to increased oxidative stress (Majzunova, Dovinova, Barancik, & Chan, 2013; Vaziri, 2008) and inflammation (Ishibashi, 2013; Touyz, 2005), which may cause endothelial dysfunction and contribute to the development of arterial stiffness and hypertension (Leong, Najib, Das, Mustafa, & Jaarin, 2009; Savoia et al., 2011).

Apart from the findings in white men, there was also an

independent negative association of night time SBP with GPx activity found in black women. A similar result was previously reported (van Zyl et al., 2016) and it was speculated that selenium deficiency may play a role in lower GPx activity in this group and the consequent link with increased blood pressure. In the present study, it was indicated that selenium deficiency was more prevalent in black women (22%) when compared to their white counterparts (3%). However, no link between serum selenium levels and blood pressure was evident in this group.

The results of this study should be interpreted within the context of its strengths and limitations. The limitations of this study include a lack of dietary data to relate dietary selenium intake with serum selenium levels, GPx activity and cardiovascular measures. While the results from this study were consistent after multiple adjustments, residual confounding effects cannot be excluded due to unknown factors associated with selenium, GPx activity, PWV and 24 h blood pressure. The participants of this study were recruited from urban areas in the North West Province and cannot be seen as representative of the entire South African population. This was a well-controlled study which included urbanized black and white participants from the same geographic location and same socio-economic status, allowing comparison between these groups. This study shed light on the protective roles of selenium and GPx activity on the cardiovascular system. Further research is needed to investigate associations of selenium and GPx activity with 24 h blood pressure and arterial stiffness in a healthy, younger population to elucidate whether selenium levels and GPx activity play a role in early vascular changes, before the onset of overt cardiovascular disease.

5. Conclusions

In conclusion, significantly lower selenium levels were found in black adults when compared to their white counterparts from the same geographic region. This may impact on the loss of vasculoprotective effects of selenium and selenoproteins, such as GPx in the hypertension-prone black population.

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Conflict of interest

The authors declare no conflict of interests.

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Authors' contributions

R. Swart was responsible for the planning, writing and composition of the manuscript as well as the statistical analyses. CMC Mels, AE Schutte and JM van Rooyen gave recommendations for the framework, writing and composition of the manuscript as well as the methodology. They also supervised the statistical analyses and helped with the formulation of the tables and figures. CMC Mels played an important part in gaining funding for this project (National Research Foundation - Thuthuka programme (80643)).

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