THE IMPACT OF A VEGETARIAN DIET AND FASTING ON CARDIOVASCULAR PARAMETERS

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Declaration

I, Edgar Matome Phukubje, declare that this dissertation, except where acknowledged on and referenced accordingly, is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree to the current or any other institution.

I certify that the assessments contained in this dissertation have the approval of the Committee for Research in Human Subjects (Medical) of the University of the Witwatersrand, Johannesburg. The ethics approval number is **M170214**.

Edgar Matome Phukubje

Signed on the 21st June 2018

We certify that the above statement is correct:

Dr MJ Maseko	Ms L Mokotedi
(Supervisor)	(Supervisor)

Signed on the 21st June 2018

Dedication

This dissertation is dedicated to my mother, Raynett Morubula and grandmother Asnatt Ngoakwana Phukubje. Thank your continued support and understanding even when situations were not permissible. Moreover, to the memory of my late grandfather Jan Phuti Phukubje, 1958–2008.

Ke Phukubje, ya lla Moletjie la Matlala la falala; ya tshela moedi ke mpyana!

Abstract

It is widely known that a fruit and vegetable based diet is associated with a reduction in blood pressure (BP) in European/ Western countries. However, information about the effects of a short-term fruit and vegetable fasting diet on BP and arterial stiffness in sub-Saharan countries such as South Africa remains uncertain. Hence the focus of this study was to investigate the impact of a fruit and vegetable fasting diet on BP and pulse wave velocity (PWV); a measure of arterial stiffness. Our study was divided into two parts. For the first part of the study, 39 participants of African ancestry who were on a religious fruit and vegetable fasting diet for three weeks were recruited. The impact of a fruit and vegetable fasting diet on serum and urinary electrolytes on BP and PWV were assessed. For the second part of the study, 625 participants from the same population group were recruited to look at the cross-sectional relationship between urinary electrolytes on BP and arterial stiffness. The 21-day fruit and vegetable based fast did not produce any significant changes in serum sodium (Na^+), potassium (K^+) and magnesium (Mg^{2+}) concentrations (all $P>0.05$). However, serum calcium (Ca^{2+}) concentrations were significantly increased after the fruit and vegetable fasting diet ($p=0.0299$). Furthermore, total cholesterol (TCHOL) and low-density lipoproteins (LDL) were also significantly decreased after the fruit and vegetable fasting diet ($p=0.0148$ and $p=0.0423$ respectively). The 21-day fruit and vegetable based fast did not produce any significant changes in bodyweight, BMI, waist circumference and WHR; all $P>0.05$). Urinary Na^+ excretion concentrations of twenty-four hours ($p<0.0001$), daytime ($p=0.0277$) and night-time ($p=0.0212$) were significantly reduced after the fruit and vegetable fasting diet. Twenty-four hour urinary Ca^{2+} ($p=0.0168$) and Ca^{2+}/Mg^{2+} ($p=0.0096$) were also significantly reduced after the fruit and vegetable fasting diet. The fruit and vegetable fasting diet had a significant effect on systolic blood pressure (SBP) and PWV as both were reduced ($p=0.0150$ and $p=0.0419$ respectively). In the large cross-sectional study, both systolic BP (C_SBP) and diastolic BP (C_DBP) were significantly associated with arterial stiffness (both $P<0.0001$). A multivariate regression analysis showed that C_SBP was significantly associated with dietary K^+, Mg^{2+} and Na^+ ($r^2= -0.13$, $p=0.0464$; $r^2= -0.14$, $p=0.0336$ and $r^2= 0.16$, $p=0.0125$ respectively). C_DBP was only associated with K^+ ($r^2= -0.10$, $p=0.0222$). Both Na^+ and Mg^{2+} were independently associated with arterial stiffness ($r^2= 0.24$, $p=0.0005$

and r^2= -0.18, p=0.0080 respectively). Since both BP and arterial stiffness are determined by nutrients found mainly in fruits and vegetables, the findings of this study indicate the significant role of a fruit and vegetable fasting diet in the management and control of HT and in preventing large artery pathology.

Acknowledgements

I would like to extent my fervent gratitude to my supervisors, Dr Muzi Maseko and Ms Lebogang Mokotedi. Your support throughout this journey was tremendous and I highly appreciate it. This milestone would not have been possible without your guidance. Above all I am grateful for your patience. To the head of the School of Physiology, Professor William Daniels, a special thank you for your support in all endeavours throughout this study. Additionally, I would like to Mrs Mokgadi Maseko for opening your home to me and being a mother to me. I would also like to further thank you and Ms Delene Nciweni for the technical support during data collection. To colleagues at the South African Hypertension and Diet Study (SAHDS), thank you for the guidance, support and motivation. I would like to extend my gratitude for the continued spiritual support I received from Moriana Kgare and my cousin Tshepiso Ngoetsana. I would like to also acknowledge the constant encouragement and inspiration received from Thamsanqa Nyundu, Makgomo Mogashoa and Excellent Mahlo. Finally, a big thank you to the participants of the APOGH and SAHDS study for your voluntary participation in my study. Furthermore, my extended gratitude goes to the Ernst & Ethel Eriksen Trust, the University of the Witwatersrand's post-graduate office and the Faculty of Health Sciences' FRC for the financial support.

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ACEI	Angiotensin-converting enzyme inhibitors
ADF	Alternate day fasting
APOGH	African Project on Genes in Hypertension
ARB	Angiotensin receptor blockers
Atg	Autophagy-related proteins
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
C_DBP	Conventional diastolic blood pressure
C_SBP	Conventional systolic blood pressure
Ca^{2+}	Calcium
CAD	Coronary artery disease
CBPM	Conventional blood pressure monitoring
CCB	Calcium channel blockers
CHD	Coronary heart disease
CKD	Chronic kidney disease
CLS	Clinical Laboratory Services
CR	Caloric restriction
Crt	Creatinine
CVDs	Cardiovascular diseases
DASH	Dietary Approach to Stop Hypertension
DBP	Diastolic blood pressure
FSH	Follicle stimulating hormone
HbA1c	Glycated haemoglobin
HDLs	High-density lipoprotein cholesterol
HT	Hypertension
K^{+}	Potassium
LDLs	Low-density lipoprotein cholesterol
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
mg/dL	Milligrams per decilitre
Mg^{2+}	Magnesium

mm Hg	Millimetre mercury
mmol/d	Millimoles per day
mmol/l	Millimoles per litre
mTOR	Mammalian target of rapamycin
Na^+	Sodium
ng/mL	Nanogram per millilitre
pg/mL	Picogram per millilitre
PWV	Pulse wave velocity
ROC	Receiver Operating Characteristic
SA	South Africa
SAHDS	South African Hypertension and Diet Study
SBP	Systolic blood pressure
SOWETO	South Western Townships
TCHOL	Total cholesterol
TOD	Target organ damage
TRGL	Triglycerides
UK	United Kingdom
WHO	World Health Organisation
WHR	Waist to hip ratio

Preface

Elevated blood pressure (BP) contributes to the burden of cardiovascular diseases. The progression of elevated BP into hypertension (HT) resultantly increases mortality and morbidity rates. The prevalence of HT in sub-Saharan regions including South Africa is steadily rising closer to the world's prevalence. Although HT was previously regarded as a disease of affluence, the transition brought about by westernisation and urbanisation has since changed the HT trend. The pathophysiological mechanisms behind HT have not been definitively elucidated; however, lifestyle and environmental factors hugely contribute to its manifestation. More importantly, members of African descent are reported to be salt-sensitive; therefore, the combination salt-sensitivity with an increased risk for the development of HT predisposes them to severe end organ damage due to salt retention. In addition, socioeconomic disparities and noncompliance with pharmacological treatment within SA have further been shown to be barriers to effective HT control.

The control and management of HT in black South Africans may require an ethnic specific approach as most of members of African ancestry often present with resistant HT. It is for this reason that non-pharmacological interventions may present with an affordable approach. The South African black population's daily dietary intake of electrolytes does not reflect the WHO's recommendations. In black South African's, studies have reported that daily electrolytes intakes are below the recommended levels for calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+}); whereas sodium (Na^{+}) intake is higher than recommended <100 mmol per day. Increasing the daily intake of Ca^{2+}, K^{+} and Mg^{2+} and simultaneously reducing Na^{+} potentiates significant BP and cardiovascular improvements. Presumably a diet high in fruits and vegetables may result in a decreased dietary Na^{+} intake whilst increasing the intake of cardio-protective nutrients like K^{+} and Mg^{2+}. This dietary change could result in improved BP control and reduced arterial stiffness at a population level.

Due to the increased incidence of uncontrolled HT in the black South African communities, it has become of paramount importance to investigate the impact of a fruit and vegetable diet on cardiovascular parameters. For this reason, the current

study aimed to determine the effects of a fruit and vegetable diet on two main cardiovascular parameters, BP and arterial stiffness. Furthermore, the second part of the study sought to study the relationship between dietary electrolytes, BP and arterial stiffness. The first chapter of this study introduces BP and discusses its mechanism and control relating to HT and end organ damage. The second chapter outlines the methodological procedures employed in the study. Chapter three presents the results obtained in the current study. In the fourth chapter these findings are discussed in the context of the current literature. Finally, Chapter 5 highlights the conclusion and limitations of the current study.

CHAPTER 1

INTRODUCTION

1.1. Introduction

Cardiovascular diseases (CVDs) are disorders affecting the heart and circulation. They include coronary artery disease (CAD), cardiac arrest, peripheral vascular disease, stroke and hypertension (HT) (Cappuccio, 1997). CVDs are recognised as one of the leading causes of mortality worldwide, attributable to about 30% of the reported global deaths (WHO, 2016). The high mortality presents both a health and economic burden.

There exist disparities in the distribution of the burden of CVDs among developed and developing countries. Developed countries present with increased CVDs prevalence as well as awareness (Smith *et al*., 2012; Hennekens, 1998). Furthermore, such countries have improved health care systems and as a result this permits early diagnosis of CVDs and consequently they initiate treatment regimens early. This is followed by a slight decline in CVDs mortality and improved survival. In the United Kingdom (UK), the 2012/3 National Health reported an increased CVD treatment expenditure of £68 Billion (± One Trillion rands). Consequently, the mortality rate was reported at 8% compared to other cases such as cancer mortality reported at 29% (Smolina *et al*., 2012). In this regard, developed countries such as the UK have experienced an increase in treated cases of CVDs (Bhatnagar *et al*., 2015).

Developing countries on the other hand, are still challenged with latent awareness because they are still burdened by infectious diseases and malnutrition (Gaziano, 2007). Sub-Saharan regions experiencing the epidemiological transition, including South Africa (SA), are showing patterns of increased prevalence and will probably still experience increases in the prevalence in the coming decades. Urbanisation and the adoption of a westernised culture is a major role player in the resultant increased prevalence in sub-Saharan regions (Moran *et al*., 2013). The transition therefore presents with an increased health burden in these regions, more especially a shift in risk factors for CVDs (Mbewu, 2009).

There are several risk factors for CVDs including modifiable and non-modifiable risk factors. Non-modifiable risk factors include family history, gender, age and ethnicity

(Payne, 2012). Indeed, age is a strong predictor of cardiovascular outcome as studies have reported that advanced age is related with the development of CVDs (Rapsomaniki *et al*., 2014). However, this notion is steadily declining with younger populations presenting with CVDs at earlier ages (Lakatta, 2002). Ethnicity and gender are the most challenging with regards to the management of CVDs (Yusuf *et al*., 2001). Attempts to understand the mechanisms behind this presentation have included physiological and behavioural risk factors (Forouhi and Sattar, 2006).

Modifiable or behavioural risk factors include dietary habits, sedentary lifestyle and substance abuse (Piepoli *et al*., 2016). The negative effects of these factors have been observed to increase with urbanisation and the transition from rural to more western lifestyles (Payne, 2012). In combination, these risk factors exacerbate the rate at which CVDs present and the severity thereof. These risk factors are crucial because they affect physiological markers such as lipid profiles and evaluated blood pressure (BP) (Mufunda *et al*., 2006; Long and Dagogo-Jack, 2011). The resultant manifestations owing to the interaction of these factors are leading on the global and local scale with regards to the reported morbidity and mortality rates (Payne, 2012).

1.2. Hypertension

Hypertension (HT) is diagnosed at systolic blood pressure (SBP) of ≥140 mm Hg and diastolic blood pressure (DBP) of ≥90 mm Hg (Roger *et al*., 2012). There exist great differences in the progression, awareness, treatment and control of HT among developing and developed countries (Mills *et al*., 2016). Developed countries have experienced a decreased prevalence of HT over the past decade. European countries present with a 60% HT prevalence compared to the United States and Canada at 28% and 27% respectively (Wolf-Maier *et al*., 2003). In contrast, the prevalence has increased by 7% in developing countries (Hogerzeil *et al*., 2013). South Africa is fast transitioning from the traditional African lifestyle to a more westernised lifestyle, including dietary changes and the adoption of sedentary lifestyles. This fast-paced transition to a westernised lifestyle has therefore predisposed the South African populace to the early development of HT (Thorogood *et al*., 2007). The prevalence of HT is also strongly related to the migration from rural to urban areas, with a direct association between duration of living in urban areas

and the development of HT (Steyn *et al*., 2008). Regardless, the rural populace also presents with increased reports of HT (Koma and Lebelo, 2017). Some studies even report that in these settings there is poor awareness and thus HT may be underreported; leading to poor management and control in rural areas (Ntuli *et al*., 2015).

Members of African descent have among one of the highest prevalence of HT compared to other ethnicities (Cooper *et al*., 2015) and the resultant outcome is complicated by target organ damage (TOD) (Ogah and Rayner, 2013). The first survey conducted more than a decade ago with over 13000 participants revealed a 21% HT prevalence in the South African population aged between 15 and older of which three quarters of that sample size included black South Africans (Steyn *et al*., 2008). This supports the notion of the differences in the prevalence of HT among South Africans. It can be considered that the development of HT is more allied to environmental and lifestyle factors than genetic factors (Rayner, 2010). Increased dietary salt and decreased potassium (K^+) intakes have been reported as important dietary factors in the development of HT (Newson *et al*., 2013). A salt-oriented approach seems to better provide some justification to the noted differences in how HT presents among members of African descent as compared to their European counterparts (Sanders, 2009).

In a country battling with a burden of chronic diseases of lifestyle, deviations exist in the management and control of HT compared to other ailments (Mayosi *et al*., 2009). Barring from the burdened health care system, patient education and understanding of HT also plays a pivotal role (Dennison *et al*., 2007). Although the consequences, all together with the prescribed treatment and control regimens are all the same universally, the development of HT in members of African descent justifies a differential approach to treatment and control (Rayner and Spence, 2017). Members of African descent seem to be resistant to HT treatment. Even after early diagnosis and initiating treatment, this populace struggles to effectively control HT (Howard *et al*., 2006). Hence, considering the substantial burden of HT in this population, focusing on the treatment and management of HT in this population is of great importance.

1.3. Control of Hypertension

Reports have outlined that the poor management of HT with its translation into cardiovascular morbidity and mortality negatively affects the health care system of SA (Tchialeu *et al*., 2016). Hence appropriate treatment strategies are needed to overturn the observed HT burden. In this regard, SA lacks sufficient large-scale randomised trials focusing on the black community and how they respond and/or adhere to HT treatment (Rayner, 2010). The management of HT depends on the time of diagnosis and the type of HT treated. Furthermore, recommending pharmacological treatment and lifestyle modifications are at times largely determined by the presence or absence of other cardiovascular risk factors (Seedat *et al*., 2014a). The goal in HT management also aims at simultaneously reducing the manifestation of other cardiovascular complications (Weber *et al*., 2014). In this regard, there is a need for more studies that will add to the current knowledge and practice which may help recognise that individualised treatment regimens may be a possibility in black South African hypertensives (Brewster *et al*., 2016).

1.3.1. Pharmacological intervention

Various antihypertensive drugs are prescribed for the treatment and management of HT (Thomopoulos *et al*., 2015). The first line of treatment is the use of diuretics, prescribed as low-dose thiazide (Seedat *et al*., 1984). Additionally, depending on the type and severity of the HT, other antihypertensive drugs such as calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), reserpine, enalapril and spironolactone may also be prescribed (Sareli *et al*., 2001). Nonetheless, there still exist complications with black South African hypertensives (Brewster *et al*., 2016). The administration of β-blockers and ACEI enervates the response in groups of African descent; whereas CCB and diuretics show a better response (Brewster and Seedat, 2013). Perhaps the differing responses may be attributable to the threshold at which pharmacological interventions are initiated. However, some effectiveness has been reported with combination therapies (Weber *et al*., 2014). It has been postulated that a combination regimen may lead to better adherence and improved HT control (Gupta *et al*., 2010).

The definition and classification of increased BP and the resultant diagnosis of HT is established and well accepted (Mancia *et al*., 2013a); however, it is the different BP limits at which patients are prescribed antihypertensive treatment that further attributes to poor HT management (Seedat *et al*., 2014b). SBP profile between 130–139 mm Hg and a DBP profile between 85–89 mm Hg is optimal but not high enough to recommend pharmacological treatment (Tirosh *et al*., 2010). The accepted BP profile of 140/90 mm Hg is the standard for pharmacological treatment. What poses a challenge to this cut-off range is that HT in members of African descent manifests differently to other counterparts. Furthermore, age alongside ethnicity challenges the cut-off range because HT in young patients will manifest differently when compared to patients above 60 years of age (Tirosh *et al*., 2010). Moreover, Seedat *et al*. (2014b) recommends that combination therapy should be prescribed at BP ≥160/100 mm Hg. Therefore, emphasis is still placed on the need for specific treatment plans for members of African descent presenting with different forms of HT because data on the BP cut-off points for mono- or combination therapy and age is limited amidst the controversy regarding when and how to initiate pharmacological treatment.

The South African HT guidelines further recommend both pharmacological and lifestyle modifications in the management of HT although there are some objections regarding proper implementation and compliance (Rayner, 2010). Nonetheless, the combination of pharmacological treatment and lifestyle modifications exhibit sufficiently effective results. Undoubtedly modifying lifestyle behaviour in the prevention and management of HT is of key importance (Perk *et al*., 2012). Depending on the manifestation and severity of HT, lifestyle interventions may decrease the mortality rate associated with HT (Elmer *et al*., 2006). What is noteworthy regarding lifestyle modifications is that sometimes these are recommended irrespective of the type of CVDs treated because generally they improve the entire vasculature including the heart and kidney function. The mostly recommended lifestyle interventions include decreased animal fat and protein, physical exercise, weight reduction, reducing alcohol intake, increased consumption of fruits and vegetables and dietary salt reduction (Dickinson *et al*., 2006).

1.3.2. Dietary salt reduction

The South African National Department of Health has embarked on several initiatives targeted at reducing dietary salt intake to below 5 g per day (Hofman and Tollman, 2013). The South African population's sodium (Na^+) intake is at an estimated 6-11 g of salt per day (Wentzel-Viljoen *et al*., 2013). This figure is above the WHO recommendation of <5 g per day. Urbanisation affects dietary patterns across various communities and thus the observation by Wentzel-Viljoen may have increased since it was last reported. Swanepoel *et al*. (2016) reported Na^+ excretion rates at 7 g per day, thus justifying that SA's salt consumption is above the recommended limits.

The effects of dietary Na^+ intake on BP increase plays an integral role in the progression and severity of HT in members of African descent (Tchialeu *et al*., 2016). The association between increased dietary Na^+ intake and BP is strong. Excess dietary Na^+ intake has been reported to adversely affect BP regulation in hypertensives and salt-sensitive populations (Sookram *et al*., 2015). Incorporating salt reduction interventions in the management of HT at population levels introduces a cheaper prevention method (Bloom *et al*., 2011).

The effect of dietary Na^+ reduction on BP seems to be modest when observed in normotensives than hypertensives. Although there are positive baseline reports, the minimal change might bring into question the duration and quantity of the intervention. Graudal *et al*. (2015) observed that dietary Na^+ reduction decreases BP. Albeit, the notion that members of African descent may retain Na^+ plays a role in this regard (Graudal *et al*., 2011). At the hand of isolating susceptible populations and other confounding factors during dietary Na^+ reduction interventions, there is an opportunity to determine whether lifelong reductions could significantly lower BP and reduce mortality rates in members of African descent (Sanders, 2009).

1.3.2.1. Sodium-to-potassium ratio

Increased dietary Na^+ intake exacerbates the development of HT. Such is more overwhelming when there is also a deficiency in K^+ (O'Donnell *et al*., 2011). The observed BP elevation due to increased dietary Na^+ intake has been shown to be

intertwined with decreased K^+ (He and MacGregor, 2001). Resultantly, to elicit any benefits in reducing dietary Na^+, one cannot ignore that the sodium to potassium (Na^+/K^+) ratio plays a pivotal role in this regard. As integral as reducing dietary Na^+ is, there is a converse relationship between decreased Na^+ and K^+, because K^+ deficiency negates the impact of decreased dietary Na^+ (Figure 1.1) (Adrogue and Madias, 2014). Accordingly, there exist a direct relationship between BP and Na^+, and an inverse correlation between K^+ and BP (He and MacGregor, 2001). At variance with the above, increasing K^+ intake has been independently reported to reduce BP (Lind *et al*., 1991). Consequently, the cheaper lifestyle intervention of reducing Na^+ intake introduces yet another nutritional challenge when this adoption should be supplemented with increased K^+ intake in poverty challenged black communities of SA (Swanepoel *et al*., 2016).

Notwithstanding, the additive effects of increasing K^+ and reducing Na^+ are noteworthy (Sacks *et al*., 2001). Efforts that are directed towards increasing K^+ intake through fruits and vegetables while decreasing Na^+ will have positive effects in reducing BP. Decreasing dietary Na^+ and increasing K^+ intake through this intervention may help balance the Na^+/K^+ ratio (Adrogue and Madias, 2007). Reducing dietary Na^+ intake can be a realistic and effective intervention; for this reason, the overall outlook on dietary intervention is also important in the relationship between K^+ excretion and Na^+ intake (Charlton *et al*., 2008).

1.3.3. Other lifestyle modifications

1.3.3.1 Reducing alcohol intake

There exists a relationship between increased alcohol intake and increased BP. Drinking more than 30 g of ethanol per day may elevate BP (Husain *et al*., 2014; Strogatz *et al*., 1991). Moreover, studies have reported that moderate reductions in alcohol intake may reduce SBP by 3 mm Hg and DBP by 2 mm Hg (Xin *et al*., 2001). Studies further report that a dose-relationship may exist between alcohol intake and BP reduction (Beilin *et al*., 1996). However, there is still some controversy on the threshold for alcohol intake and subsequent BP increase (Roerecke *et al*., 2017). Furthermore, no consensus has been reached between trials on whether the short-

term or long-term alcohol reduction may help prevent HT to the same magnitude (Fuchs *et al*., 2001).

1.3.3.2 Decreased animal fat and protein

High intake of animal protein and unprocessed fat has a direct association with CVDs including HT (Micha *et al*., 2010; McAfee *et al*., 2010). It is for this reason that decreased animal protein may reduce BP and help prevent HT (Prescott *et al*., 1988). However, the notion that decreasing meat intake may have a positive effect on BP is mostly reported in feeding trials like the DASH diet which administer fruit and vegetables, carbohydrates and decreased dairy products (Beilin and Burke, 1995; Rouse *et al*., 1983). Without any proper isolation of the effects of meat products on BP in these studies, it therefore leads to the extrapolation that the observed BP reduction may be a result of the interplay of the minerals, nutrients and electrolytes embedded in these diets (Hodgson *et al*., 2006). Therefore, owing to the variability that exist in these diets that are recommended in HT management, the effects of animal fat and protein will affect the progression and presentation of CVDs including HT differently (Sayer *et al*., 2015). Hence decreasing animal fat and protein as a lifestyle modification to lower BP still needs to be further elucidated in isolation.

1.3.3.3 Weight reduction

Excess bodyweight that is further associated with increased adiposity accounts for more than 70% risk of developing essential HT (Hall *et al*., 2015). Resultantly, weight loss is recommended in the control and prevention of HT (Stevens *et al*., 2001). A weight loss regiment that may decrease bodyweight by about 5 kg is associated with a 4 mm Hg SBP reduction and 3 mm Hg DBP reduction (Corrigan *et al*., 1991; Staessen *et al*., 1988). Consequently, recommending weight reduction may be an effective measure in the prevention and control of HT at population level. It is reported that notable body weight reduction may occur at six weeks of the intervention (Neter *et al*., 2003). Moreover, the effects further decrease BP when the regimen is combined with antihypertensive treatment (Ebrahim *et al*., 1998). However, the exact mechanism between HT, obesity and the effects of weight loss on BP is still unknown (Rocchini, 2002). The long-term effects of weight reduction on

BP still need to be investigated; furthermore, there is still a need to report on whether weight reduction will persistently reduce BP post the intervention (Stevens *et al*., 2001).

1.3.3.4 Physical exercise

The effects of exercise on BP reduction have been reported (Whelton *et al*., 2002; Fagard, 2001; Kelley and Kelley, 2000). Exercise treatment is defined as the adoption of a training regimen to prevent or treat chronic conditions (Wallace, 2003). Cardiovascular exercise is the most commonly preferred exercise training and shows some effectiveness in BP reduction (Nicholls, 1990). A 20–60 minute exercise duration for at least 3 days a week is recommended as a lifestyle modifying behaviour to lower BP (Brown *et al*., 2013). Exercise may reduce SBP by 15 mm Hg and DBP by 3 mm Hg (Tipton, 1991). Moreover, BP reduction may also occur in the absence of bodyweight reduction or body fat (Pescatello *et al*., 1991). There exist some differences in the effects of high-intensity exercise (Cornelissen and Smart, 2013) and low-intensity exercise (Cornelissen *et al*., 2011) in reducing BP; however, their differences are not so significant. Furthermore, there is still a need to determine the extent to which the duration of each regimen may significantly reduce BP (Fletcher *et al*., 1996).

Increased BP and the progression into HT affect arterial functioning and has consequently been associated with stiffening arteries (Vlachopoulos *et al*., 2006). Since there exists an intertwined relationship between HT and arterial stiffness, as measured by pulse pressure and pulse wave velocity (PWV), regulating any indices associated with HT such as modulating endothelial function resultantly may reduce arterial stiffness (Pase *et al*., 2010; He and McGregor, 2002).

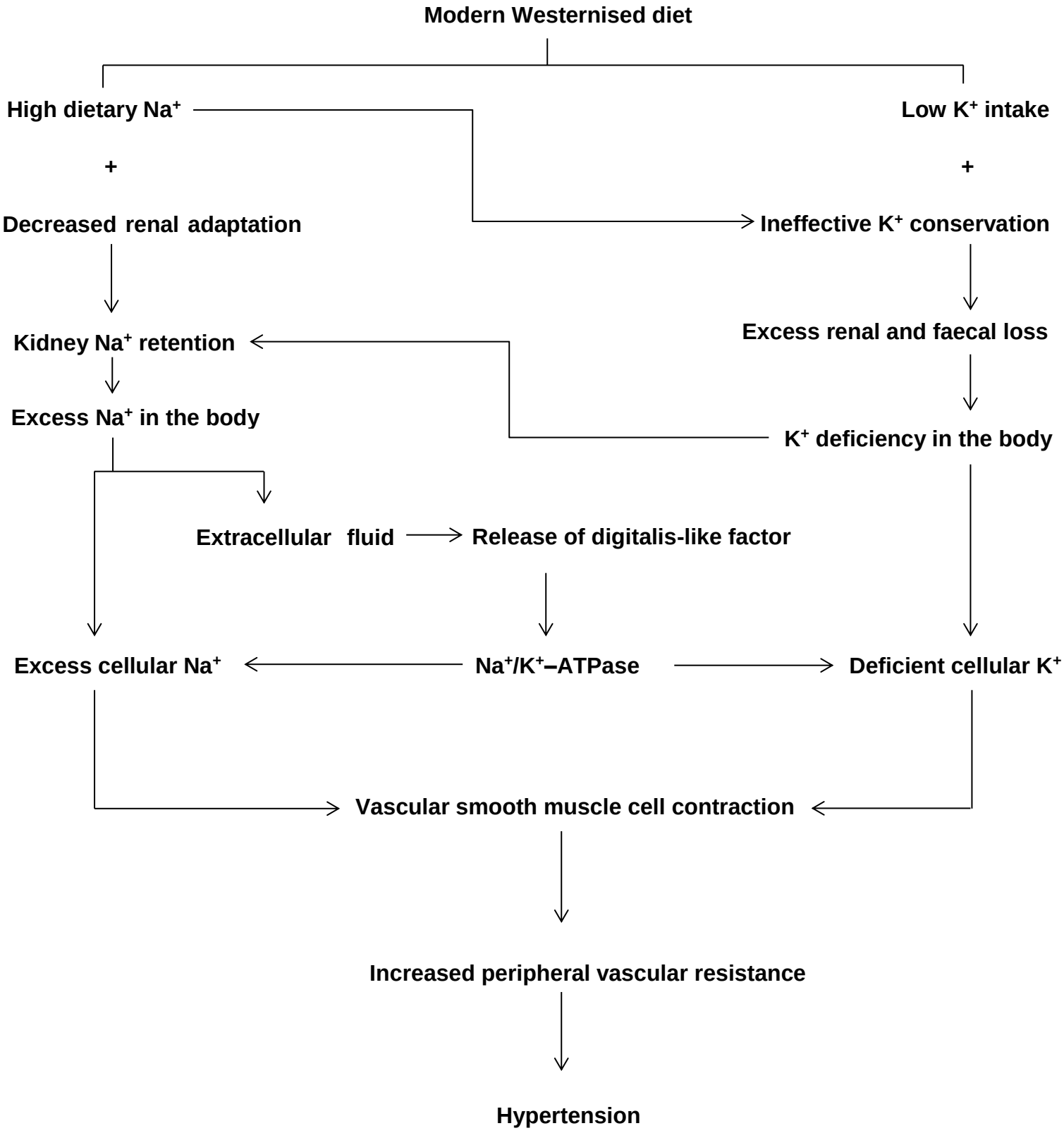


Figure 1. 1 The pathogenesis of HT because of dietary intake. Excess Na^+ from westernised diets, deficient of K^+, imbalances the Na^+/K^+ ratio in the body; consequently, increasing peripheral vascular resistance. Adopted from Adrogue and Madias, 2007.

1.4. Arterial stiffness

Arterial stiffness is defined within parameters such as reduced arterial wall distensibility, increased PWV, increased pulse pressure and reduced wave reflections (O'Rourke *et al*., 2002). Arterial stiffness expresses the relationship between change in pressure and change in the circulating blood volume (Townsend *et al*., 2015). Arterial stiffness has been widely reported with increased age and the presence of disease including diabetes mellitus, renal failure, dyslipidaemia and HT (Mitchell, 2015). Changes to arterial wall properties affect its load-bearing components and thus forces the heart to increase its working capacity and further predisposes the left ventricle to hypertrophy (Safar and London, 2000; Hodes *et al*., 1995). This is brought about when arterial distension is decreased, and with this decrease, is the capacity to buffer the effects of increased systolic pressure during contraction. Changes in larger artery distensibility and elasticity reduce the storage capacity of the arteries and there exist a risk of reduced perfusion (Zieman *et al*., 2005). Consequently, a counter measure to maintain stroke volume puts pressure on the pumping heart and hence the predisposition to left ventricular hypertrophy (LVH) (O'Rourke and Hashimoto, 2007). Moreover, pulse pressure increases because the reduced buffering effect of the arteries leads to premature arrival of the reflected pressure wave back to the heart; therefore, augmenting the central aortic pressure wave form, a further predisposition to TOD resulting from stiffening arteries (Avolio, 2013). Moreover, plaque and lesions may accumulate and lead to obstruction of the artery and thus supporting the reported synonymous relationship between atherosclerotic lipid accumulation and calcium deposit with arterial stiffness (Wilkinson *et al*., 2009). Vascular obstruction and arterial stiffness negatively affects cardiovascular function and disease risk factors and they have an increased incidence with TOD related to kidney function, the brain and the heart (Leoncini *et al*., 2006) Figure 1.2 illustrates changes that lead to end organ damage resulting from arterial stiffness.

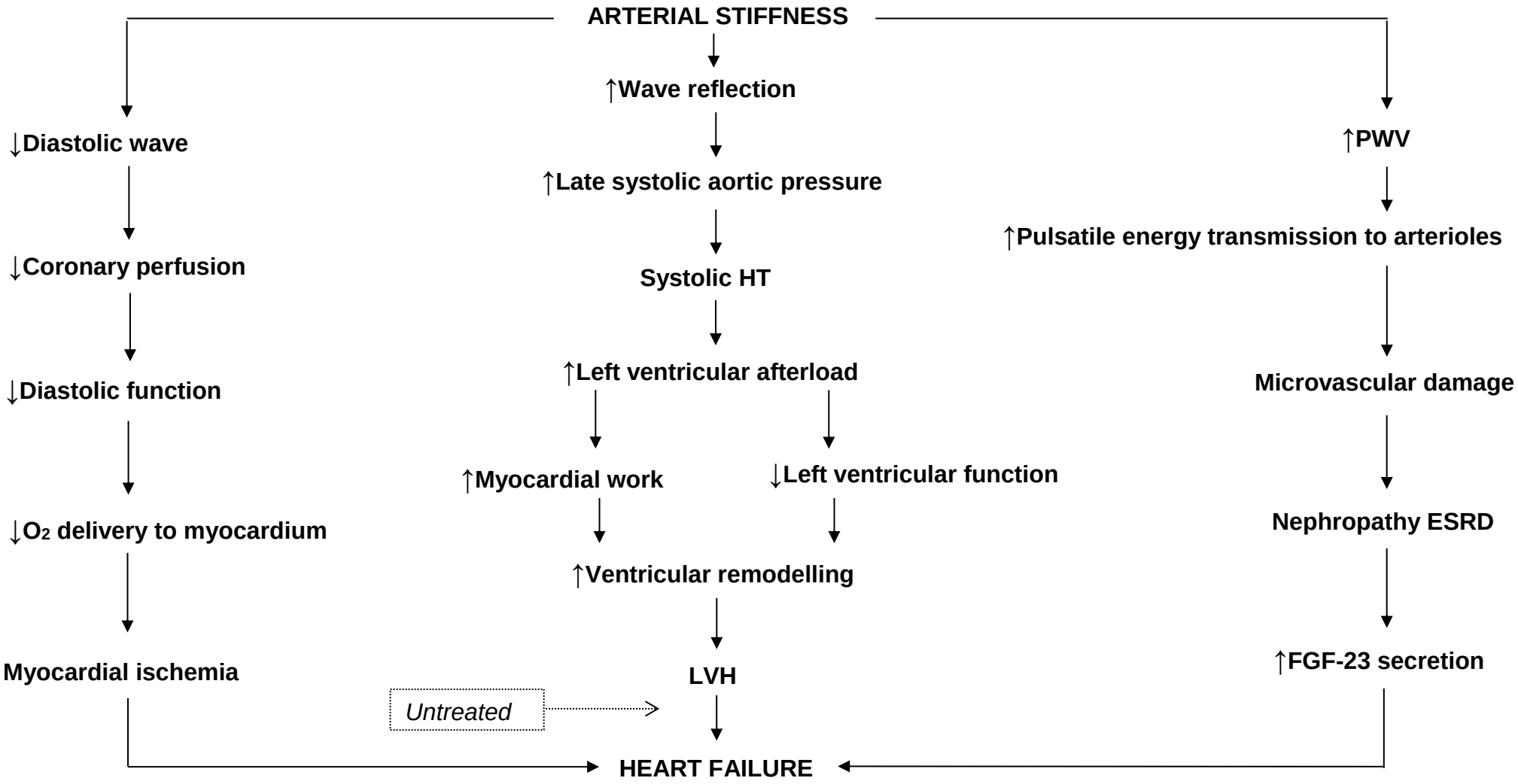


Figure 1. 2 A schematic representation of the haemodynamic links between arterial stiffness and target organ damage. Modified from Vervloet *et al* (2017); Georgianos *et al*. (2016); Spartano *et al*. (2014). Abbreviations: PWV, Pulse wave velocity; HT, Hypertension; O_2, Oxygen; ESRD, End-stage renal disease; LVH, Left ventricular hypertrophy; FGF-23, Fibroblast growth factor-23; ↑, Increased; ↓, Decreased.

1.4.1. Serum lipids and arterial stiffness

Adverse changes in serum lipid profile elicit negative effects in vascular function. Any change in the concentration of the circulating lipids, whether in an elevated or reduced concentration may damage arterial functioning and elasticity; this shows their relevancy in the early stages of arterial stiffness (Zhao *et al*., 2014; Wang *et al*., 2011; Wilkinson and Cockcroft, 2007; Ferrier *et al*, 2002; Dart and Chin-Dusting, 1999). Hyperlipidaemia is characterised by increased low-density lipoproteins (LDLs), increased triglycerides (TRGLs) and decreased high-density lipoproteins (HDLs) (Zhu *et al*., 2018). In assessing the relationship between serum lipids and arterial stiffness, there is a need to understand that serum lipid profiles are associated with atherosclerosis (Li *et al*., 2014). Atherosclerosis is a disease marked by intima thickening, and it affects blood vessels. It is mainly characterised by stiffening of the arteries owing to many risk factors, including hyperlipidaemia (Vlachopoulos *et al*., 2015; Mancia *et al*., 2013b). These conditions may co-exist in an individual and they may appear to be similar, nonetheless, atherosclerosis can cause arterial stiffness; however, the converse may not be true (Mulè *et al*., 2017).

Genetic predisposition to atherosclerosis suggests early reduction of arterial wall characteristics owing to the prolonged effects of hyperlipidaemia and this exacerbates the progression of arterial stiffness (Pitsavos *et al*., 1998). Studies that have investigated the association between serum lipids and arterial stiffness have fallen short of reaching consensus. Only about 10% of the literature suggests a positive correlation (Cecelja and Chowienczyk, 2009; Wilkinson *et al*., 2002; Cameron *et al*., 1995; Lehmann *et al*., 1992).

1.4.1.1. Total cholesterol and arterial stiffness

Cholesterol is a principal sterol, and is typically produced by the hepatic cells. It is a precursor for biochemical pathways including the synthesis of vitamin D and steroids hormones. It is mostly transported in plasma through lipoproteins and is susceptible to oxidation (Martini *et al*., 2012; Fox, 2009). Total cholesterol (TCHOL) is defined as the sum of circulating lipids, but mostly measured by total HDLs, LDLs and TRGLs. Elevated plasma TCHOL is treated with dietary constrictions pertaining reduced

animal fat because it may contain high concentrations of cholesterol. Hypercholesterolemia is associated with increased CVD related mortality (Wang *et al*., 2009).

In individuals that present with asymptomatic atherosclerosis, arterial stiffness and TCHOL are confounded by age, mean arterial pressure (MAP) and heart rate (Dart *et al*., 2004). The perpetual effect of TCHOL on large artery function may occur gradually with increased MAP and perhaps not mainly due to the circulating lipids (Stewart *et al*., 2003). Therefore, when there is no association between TCHOL and arterial stiffness the effects of TCHOL may just be too minute to isolate from the overall effect of the serum lipids (Dart and Chin-Dusting, 1999). Secondly, atheroma may also exacerbate the effects of the circulating lipids on an already impending arterial stiffness; thus, leading to the reported positive correlation between arterial stiffness and carotid artery disease (Dart *et al*., 2004). At the same time, when no association exist in populations such as the elderly and hypertensives, it leads to the implication that the predictive power of arterial stiffness in these population occurs without the effects of TCHOL, this may then allow the premise of arterial stiffness as an independent risk factor coronary heart disease (Toikka *et al*., 1999; Kupari *et al*., 1994). The inconclusive findings on the association should still permit separate analysis of their risk to CVDs. In addition, the role of TCHOL in the pathogenesis of atheroma and arterial stiffness should not be ignored (Ferrier *et al*., 2002).

1.4.1.2. Low-density lipoproteins and arterial stiffness

Low-density lipoproteins are plasma proteins that deliver cholesterol to peripheral tissues. Increased plasma LDL concentrations is common in people who eat diets rich in cholesterol and saturated fats, furthermore familial hypercholesteremia is attributable to the liver's low LDL receptors that would otherwise remove LDLs from the blood (Fox, 2009). LDLs are synonymous with atherosclerosis because they may end up in arterial plaques.

Low-density lipoproteins are atherogenic and elevated concentrations are associated with CHD (Coronary heart disease) (Onat *et al*., 2010). LDLs also show a strong association with arterial stiffness (Johansen *et al*., 2012). The risk increases further

when LDLs circulate in their oxidised form (Freedman *et al*., 1998). A possible explanation may be embedded in that oxidised LDLs abet oxidative stress and inflammation. Additionally, this may cause further calcium deposit within the arterial wall and promote elastin damage (Wilkinson and Cockcroft, 2007). Individuals who have been exposed to elevated concentrations of LDLs since childhood are mostly at risk of presenting with premature atherosclerosis and therefore may be susceptible to early arterial stiffness (Yuan *et al*., 2006). There is an apparent co-existence between elevated LDLs and TRGLs in some studies (Aznaouridis *et al*., 2007). An attempt to describe their effects on arterial function is through the reactive oxygen species that may be generated by TRGLs. They also interfere with pro-atherosclerotic signalling pathway, thereby inducing abnormal smooth muscle and endothelial vasodilatory responses (Lupattelli *et al*., 2000).

1.4.1.3. High-density lipoproteins and arterial stiffness

High-density lipoproteins (HDLs) are heterogeneous in form and carry multiple functions. They function primarily in transporting excess cholesterol from peripheral tissues back to the liver for storage or excretion in the bile. HDLs have anti-inflammatory and anti-thrombotic properties; more of the beneficial role stems from their reverse cholesterol transport, stimulating nitric oxide production and repair (Campbell and Genest, 2013). HDL concentrations are largely determined by genetics, although lifestyle behaviour affects their concentrations (Fox, 2009). In the progression of atherosclerosis, HDLs suppress the development of atherosclerotic lesion by carrying the cholesterol through the blood to the liver for metabolism. Increased HDL concentrations are associated with decreased incidences of atherosclerosis and CVDs (Gordon *et al*., 2013; Martini *et al*., 2012).

HDLs may also independently reduce the risk of arterial stiffness (Wang *et al*., 2018). They show their cardio-protective capabilities through the transport of cholesterol away from the arterial wall. Some trials revealed that a reduction of 1 mg/dL of HDLs decreased the risk for complicated CHD by 2-3% (Rubins *et al*., 1999). Notwithstanding, the association between HDLs and arterial stiffness is somewhat controversial. In a cohort of apparently healthy males, an inverse relationship was noted between arterial stiffness and HDLs (Wang *et al*., 2013). On the contrary, a

negative correlation exists in the middle-aged cohort and those above 50 years (Kozakova *et al*., 2015; Zhao *et al*., 2013). There might be a role that gender plays in the protection by HDLs against arterial stiffness; nonetheless, no clear evidence has been reported. A study by de Oliveira Alvim *et al*. (2017) reported that HDLs reduce the risk of arterial stiffness in post-menopausal females, but the results could not be reproducible in pre-menopausal females and in men. In any event that alters HDLs (biological or not), such leads to the risk of arterial stiffness because the change in HDLs removes along with it the endothelial protective actions. However, the exact mechanism through which HDLs may protect against arterial stiffness is not clearly defined (Sorrentino *et al*., 2010).

1.4.1.4. Triglycerides and arterial stiffness

Triglycerides are described as neutral fats. They are the most important and abundant form of dietary lipids and primarily consists of fatty acids. They are stored by adipocytes and liver cells and serve as blood-borne energy carriers used by the liver and other organs (Martini *et al*., 2012). TRGLs storage within cells absorbs and accumulates lipid soluble particles. This accumulation has both negative and positive side effects. High blood TRGL concentrations predisposes to early development of atherosclerosis (Sherwood, 2015).

The predisposition by TRGLs to arterial stiffness exists independent of the other lipids. Furthermore, when TRGLs remains elevated in the presence of increased HDL and decreased LDL it still shows a positive correlation to the risk of arterial stiffness (Johansen *et al*., 2012). It is for this reason that decreased TRGLs concentrations are associated with decreased risk of CHD (McBride, 2008). Although not well recognised, TRGLs have an independent association with CHD, a disease marked by arterial stiffness (Mattace-Raso *et al*., 2006). Therefore, to some degree, increased TRGLs may lead to arterial stiffness even after adjusting for traditional risk factors (Gottsäter *et al*., 2015).

Much as the exact mechanism regarding the pathogenesis of arterial stiffness in the wake of atherosclerosis or rather hyperlipidaemia is still unclear, studies reporting that elevated LDLs and TRGLs are risk factors and that increased HDLs are

cardioprotective hold some relevance (Turner *et al*., 1998). However, another challenge ensues when the ratio of TRGLs to HDLs is not symmetrical. Drexel *et al*. (2005) observed that the HDL/ TRGL ratio shows a strong correlation with arteriosclerosis even after adjusting for traditional risk factors. On the assumption that increasing HDLs has beneficial effects on endothelial function, this effect is negated in the presence of elevated TRGLs. The implication being that the HDL/ TRGL ratio carries some weight in the development of arterial stiffness (Asia Pacific Cohort Studies Collaboration, 2005).

1.4.2. The relationship between arterial stiffness and blood pressure

Elevated BP variability that persists for extended periods may lead to arterial modelling, which may gradually lead to the progression of arterial stiffness (Tedla *et al*., 2016). Wood (1998) reported that HT accelerates arterial stiffness. He further postulated that sustained elevated MAP leads to the stiffening of arteries, and that the consequent structural changes are a risk factor for TOD. Furthermore, elevated SBP acts on smooth muscles and leads to smooth muscle cell proliferation, causing vascular damage that manifests as arterial stiffness (Prospective studies, 2002; Schmieder, 2010).

Both arterial stiffness and HT readily present with increasing age (Ferreira *et al*., 2011). Although the aetiology has not been definitively proven, the growing knowledge that its prevalence increases with age is well accepted (AlGhatrif *et al*., 2013). Nevertheless, arterial stiffness and HT are gradually becoming prevalent in younger individuals (Sun, 2015). Regardless of the underlying cause, the effects of elevated BP and arterial stiffness are reported with decreasing aorta elasticity and increased PWV, this effect in turn increases SBP and pulse pressure (Vlachopoulos *et al*., 2011).

Arterial stiffness is an independent risk factor for CVDs, because decreased arterial distensibility and increased augmentation index are associated with increased risk of developing HT (Karras *et al*., 2012). For the longest of times, researchers had supported the notion that arterial stiffness was a consequence of HT; however, the growing consensus is that the stiffening of arteries may be contributing to the

pathogenesis of HT (Weisbrod *et al*., 2013). Galis *et al*. (2013) observed changes in vasculature at 2 months in their animal study and at six months BP was elevated; thus, evidently arterial stiffness preceded HT. The resolution of their study was that arterial stiffness has a causal role in HT because it precedes HT; and it contributes to its development and progression (Mitchell, 2014; Galis *et al*., 2013).

Laurent *et al*. (2003) reported that arterial stiffness may be an early marker of vascular dysfunction, and that this may be the perpetuation of increased BP and progression into HT. Arterial stiffness that co-exists with HT exhibits some characteristics that exacerbate arterial wall degeneration, initiating a feedback mechanism wherein HT leads to further arterial damage (Wood, 1998). The pathological process leading to arterial stiffness still warrants investigation. Owing to the inter-play between arterial stiffness and HT, the American Heart Association has recommended that both arterial stiffness and BP should be measured and their implications should be considered (Townsend *et al*., 2015).

Studies observing that BP depends on arterial stiffness base these findings on basal BP, therefore in the presence of arterial stiffness it is not clear at what stage does reducing BP also lead to improved arterial function (Wohlfahrt *et al*., 2013; Shirai *et al*., 2006). Lim *et al*. (2015) inferred that the simplest change in arterial stiffness would be attributable to changes in BP; however, these changes may be independent of each other. They further postulated that changes in arterial stiffness may not be a secondary effect but may be occurring simultaneously and yet still not be directly related to the BP change. Furthermore, Kaess *et al*. (2012) reported that during lifestyle interventions the observed BP decrease may be due to the modulation of the intervention on the overall vasculature which leads to the BP decrease. On another contradicting point, Laurent *et al*. (2006) found that changes in BP profile attenuates arterial elasticity and that it is the reduction in BP that may improve arterial function.

Conventionally, arterial stiffness plays a role in the initiation and progression of HT and the same with HT in the initiation and progression of arterial stiffness (AlGhatrif and Lakatta, 2015). Studies suggest a bi-directional relationship. Although the association still begs the questions, is arterial stiffness a true risk factor for HT or is it

just a risk marker for its development and progression? Secondly, does arterial stiffness cause HT or is it only just related to its development with a causal role in its pathogenesis? Despite all the inconclusive reports regarding the cause and effect between HT and arterial stiffness, one cannot dispute that imminent elevated BP negatively affects the functioning of blood vessels and resultantly may lead to arterial stiffness (Franklin, 2005). Considering the bi-directional relationship between BP and arterial stiffness, it is therefore worth exploring whether non-pharmacological interventions aimed at reducing BP will elicit the same effects on arterial stiffness both in normotensives and hypertensives. Dietary intervention such as the DASH diet offer health benefits in both normotensives and hypertensives (Appel *et al*., 1997) and thus may also influence arterial compliance.

1.5. Fruit and vegetable diet

Attempts to lower CVD risk factors have been focusing on various behavioural and dietary factors. In both normotensives and hypertensives, diet modulates BP (Steffen *et al*., 2005). Adopting a vegetarian diet generally lowers BP profile (Sacks *et al*., 1981). There is a general agreement that increasing fruits and vegetables and decreasing dietary salt and animal fat is important (Baker *et al*., 2016). Vegetarian diets typically comprise of plant products, exclude meat and are associated with a lower prevalence of HT (Yokoyama *et al*., 2014). Their reduced energy, lower fat content and increased fibre merits their associated BP-lowering effects (Berkow and Barnard, 2005). Adopting a fruit and vegetable diet between six and eight weeks reduces BP by 6 mm Hg SBP and 4 mm Hg DBP (Graudal *et al*., 2012). Individuals who maintain a fruit and vegetable diet confers some protection against BP abnormalities and CVD related outcomes (Margetts *et al*., 1986). A six-month intervention study reported that 5 servings of fruits and vegetables per day substantially reduced BP (John *et al*., 2002). Miura *et al*. (2004) postulated that long term ingestion of fruits or vegetables can independently suppress BP increase. The inverse relationship between BP and eating fruits and vegetables has been established (Wang *et al*., 2012). However, there is still a need to elucidate the associated benefits of the fruit and vegetable diet individually. Table 1.1 gives

examples of common fruit and vegetables and some phytochemicals that are within them.

Dietary guidelines have also included the DASH (Dietary Approaches to Stop Hypertension) diet, owing to its emphasis of a healthy eating plan (Krauss *et al*., 2001). The DASH diet is a combination diet of fruits and vegetables, low-fat dairy products, low saturated fats, poultry and fish (Appel *et al*., 1997; Svetkey, 1999). The diet recommends five to nine servings of fruits and vegetables per day. Findings from the DASH trail demonstrated the efficacy of plant foods and dairy products in both normotensives and hypertensives over an eight-week observation period. Thus, postulating that the benefits of the DASH diet may be the combined effects of the entire diet.

The DASH diet in its entirety is not a reduced sodium diet (Tyson *et al*., 2012). Furthermore, guidelines support reduced dietary Na^+ to the DASH diet; otherwise called the DASH-sodium diet (Feyh *et al.,* 2016). It is this combination DASH diet that may yield even more beneficial results (Forman *et al*., 2009). The DASH-sodium study reported reduced BP over a four-week sodium reduction observation (Sacks *et al*., 2001).

The DASH diet is deemed as the golden standard for reducing and managing BP considering it was stimulated by the notion that BP profiles are ethnically different. Although it is generally assumed that dietary habits would be helpful on a population standard, another aspect highlighted from the DASH finding was the increased efficacy in the black population. They reported a 13 and 6 mm Hg SBP and DBP reduction in the black population respectively. Accepting reports that members of African descent exhibit some susceptibility to salt-sensitivity, the DASH findings also support that ethnic differences along with habitual dietary patterns are also important (Langford, 1983). Consequently, the benefits of this diet would greatly reduce the prevalence of resistant HT and perhaps improve the response to management in this populace (Agyemang and Bhopal, 2002).

Table 1.1 Examples of common fruit and vegetables and phytochemicals

Vegetables	Fruit	Phytochemicals
Carrots	Apples and Apricots	Allium sulfides
Corn	Avocado	Anthocyanins
Cucumber	Bananas	Carotenoids
Eggplant	Berries	Fibre
Ginger root	Citrus fruits	Flavonoids
Mushrooms	Fig	Isothiocyanates
Peppers	Grape	Phenols
Potatoes	Honeydew melon	Phytosterols
Summer squash	Kiwifruit	Protease inhibitors
Sweet potatoes	Mango	Saponins
Allium vegetables	Nectarine and Peach	Tannins
Cruciferous vegetables	Pineapple	Terpenes
Green leafy vegetables	Watermelon	Vitamins and Minerals

Modified form: Beecher, 1999; Bucobo, 1998; Steinmetz and Potter, 1996; Mangels *et al*.,1993; Nutrient Data Bank, 1991.

1.6. Fasting

Al-Regaiey (2016) defined fasting as the reduction of calorie intake without malnutrition. It is postulated that fasting slows aging and prevents or delays the progression of CVD risk factors (Longo and Fontana, 2010). There exist both biochemical and physiological mechanisms regarding fasting and its effects. Physiological changes that affect CVD development comprise of improved insulin sensitivity and reductions in BP (Weiss et al., 2014; Fontana et al., 2004). This is compelling because CVD risk factors increase with increasing BP, glucose and insulin levels (Roberts et al., 2001). A 10% to 40% reduction in calorie intake demonstrates significant outcomes on BP, body weight and glucose metabolism (Wan et al., 2003). Studies have reported that reduced calorie intake decreases bodyweight, which simultaneously improves glucose tolerance (Barnard et al., 2015).

It introduces an association between fasting, glucose regulation and bodyweight attenuation which result in improved BP profile (Carlson et al., 2007).

Fasting confers effective improvements in health, maintaining cardiovascular function and increasing lifespan. Fasting was noted to optimise the health and function of cardiovascular function (Weiss and Fontana, 2011). In the interest of its effects on the heart and blood vessels, fasting proposes the possibility of longevity without severe CVD risk factors and slow deterioration of structural functionality in organs that would normally be susceptible (Fontana et al., 2010). Fasting may also have a beneficial effect on ageing, albeit the mechanism is still debated (Heilbronn and Ravussin, 2003; Weindruch, 1996). It is suggested that these effects are elicited because fasting affects other biological pathways relating to defence and aging. Ageing has deteriorating effects on the heart and vasculature (Lakatta, 2003). It is associated with increased arterial stiffness (Tanaka et al., 2000), diminished left ventricular functioning (Lakatta, 1999) and impaired endothelial functioning (Gerhard et al., 1996). However, the beneficial effects of fasting may be the alleviation and prevention of the above anomalies (Castello et al., 2005). Even with noted cardiovascular protection fasting generally improves health outcomes; it confers protection independent of any disease processes (Weiss and Fontana, 2011).

Investigations reporting low fasting plasma glucose and insulin concentrations also published significant changes in serum TRGL (Stote et al., 2007). Serum cholesterol, in its elevated concentrations, predisposes to the progression of atherosclerosis, a risk factor associated with elevated BP (Bales and Kraus, 2013). Excepting the magnitude and duration of fasting, its protective sustained benign effects include reducing serum lipid profiles such as TCHOL and TRGL. The reduction thereupon reduces the risk of developing atherosclerotic CVDs. Such is corroborated by the observation in the absence of carotid artery intima-media thickening and the absence of atherosclerotic plaques (Fontana et al., 2004).

Fasting shows better arterial compliance (Zuo et al., 2016). Animal studies have showed reduced arterial stiffness observed through slower pulse wave velocity and preservation of vascular smooth muscle (Ahmet et al., 2011). Although its impact is not clear, fasting implemented for a considerable period may reduce arterial

stiffness. However, the reduced stiffness may not necessarily result from fasting alone (Figueroa et al., 2012; Dengel et al., 2006). Meanwhile when fasting is combined with interventions such as physical activity, there is observed enhancement in arterial elasticity; such is induced from the reduced BP and body composition parameters (Rossow et al., 2014). Therefore, this indicates that the effects on vasculature during fasting probably ensue from interactions between various biological components. Human studies on fasting are still at a developmental stage. Most human investigations study biomarkers of aging and cardiovascular risk. Nonetheless, there are still some limitations in elucidating the molecular basis for its mechanism and long-term effects (Holloszy and Fontana, 2007).

There are various forms of fasting that exist, below are four commonly reported types of fasting.

1.6.1. Alternate day fasting

Alternate day fasting (ADF) is a practice that involves withholding food for 24 hours. Food intake, frequency and quantity is reduced or altered during this period (Varady *et al*., 2007). The effects of the fast on metabolic variable may only be notable after a few weeks of consistent fasting (Heilbronn *et al*., 2005).

1.6.2. Intermittent fast

Intermittent fasting refers to severe calorie restriction once a week. It involves about 80% restriction towards energy needs for once or twice a week (Mattson, and Wan, 2005). It has reported benefits in reducing body weight and cholesterol levels; however, participants need about 24 weeks consistent intermittent fasting for notable changes to occur (Klempel *et al*., 2012).

1.6.3. Ramadan fasting

This is an Islamic fast practice; during the ninth month of the Islamic calendar, the observation takes place every day from dawn till sunset and lasts between 28 and 30 days (Benaji *et al*., 2006). The fast aims at teaching self-discipline and restraint; furthermore, it is aimed at reminding Muslims how the less-fortunate live their lives (Aziz, 1996).

1.6.4. Daniel fast

The Daniel fast is a 21-days fasting regiment that is restricted to a plant-based dietary plan. During the fast participants are not permitted to eat animal products, preservatives or drink alcohol based beverages (Trepanowski et al., 2012). The changes observed following a 21-day Daniel fast result from the reduced total fat, saturated fat, and dietary cholesterol (Ferdowsian and Barnard, 2009). Moreover, the fruit and vegetable-based diet also has increased dietary fibre and is rich in micronutrients (Jiang et al., 2011; Liu et al., 2000). The diet improves cardiovascular and glycaemic parameters. Adhering to the fast shows improvements in blood glucose and insulin concentrations (Barnard et al., 2005), circulating LDL-C and improved BP profile (Bloomer et al., 2011). Furthermore, the diet may decrease C-reactive protein, a marker of systemic inflammation (Bloomer et al., 2010). BP changes following the fast may result from the variety of the fruit and vegetables in the diet because they possess surrogate markers for nitric oxide NOx; an important effector in BP elevation (Bloomer et al., 2011; Lundberg et al., 2010).

1.7. Study rationale

There is an increased prevalence of HT in the black population and the situation is compounded by the limited resources in the health care sector (Peltzer and Phaswana-Mafuya, 2013). Non-pharmacological interventions like fasting and a fruit and vegetable diet could be beneficial in this population because they may present an effective means of controlling HT. The reported beneficial effects of such interventions on the cardiovascular system denote some importance in the management of cardiovascular complications. However, studies that report on fasting and its impact on cardiovascular pathology are mostly animal-based, and thus there is a need to determine if the same effects can be observed in human studies (Weiss and Fontana, 2011). Animal studies report that fasting elicit cardio-protection; therefore, there is a need to translate these findings to human studies to quantify the long-term effects of fasting (Marzetti et al., 2009). Moreover, there is limited evidence on the lifelong effects of fasting on age-related diseases. Furthermore, the combined effects of fasting and a fruit and vegetable diet have not been reported in members of African descent. To the best of our knowledge no study

has investigated the cardiovascular benefits of fasting and a fruit and vegetable diet and the relationship between BP and target organ changes in members of African descent.

1.8. Purpose of the study

1.8.1. Aim

The following study was divided into two aims:

I. The aim of the first study was determine changes in cardiovascular parameters following the adoption of a vegetarian fasting diet in a population residing in the metropolitan area of the South Western Township (SOWETO), South Africa.

II. The aim of the second study was to determine whether there is a relationship between dietary electrolytes BP profile and PWV.

1.8.2. Study objectives

A. The objectives of the first study were to:

1. Determine the impact of a fruit and vegetable fasting diet on serum electrolytes (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+} and Crt) and lipid profile (TCHOL, HDL, LDL and TRGL) concentrations.
2. Determine the differences between 24-hour, daytime and night-time urinary excretion rates of Ca^{2+}, K^{+}, Mg^{2+}, Na^{+} and Crt before and after the fruit and vegetable fasting dietary intervention.
3. Determine the impact of the fruit and vegetable fasting diet on blood pressure and arterial stiffness.

B. The objectives of the first study were to:

1. Determine if blood pressure and arterial stiffness are associated with urinary Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} concentrations.
2. Determine the relationship between blood pressure and arterial stiffness.

CHAPTER 2

METHODS AND MATERIALS

2.1 Study population

This research project was approved by the University of the Witwatersrand's ethics committee for research in human subjects (Medical) was conducted according to the principles outlined in the declaration of Helsinki. Approval number for the intervention study **M170214** (Appendix 1). Study participants were recruited from Soweto, a township in the south west of Johannesburg (Figure 2.1). The minimum age of the participants was 18 years and there was no upper age limit.

This study was divided into two components to achieve the set objectives. The first component of the study was the fruit and vegetable fasting diet study which had a total sample of 39 participants. The first study investigated the impact of a fruit and vegetable based fasting diet on serum electrolyte concentrations and urinary excretion rates of calcium (Ca^{2+}), potassium (K^{+}), magnesium (Mg^{2+}) and sodium (Na^{+}). These participants were specifically recruited because they were partaking in a voluntary 21 days religious fast. During this three-week period the participants restricted themselves to a strict fruit and vegetable diet. The second part of the study was part of an existing African Project on Genes in Hypertension (APOGH) study. For this study 278 participants were recruited to add to an existing 347 sample of participants who had 24-hour urine samples to make up a total sample of 625 participants to investigate the relationship between these nutrients and cardiovascular target organ changes. There was no dietary intervention for the 625 participants and therefore this component of the study was cross-sectional.

Figure 2.1 An example of dwelling settlements where participants were recruited from.

2.2 Participants demographics

The details of the study were explained to the participants following which they gave informed written consent and a standardised questionnaire was administered (Appendix 2). The questionnaire was explained and assistance was provided when requested. The questionnaire helped document demographic data, level of education, current or past occupational information. Furthermore, the questionnaire helped to document information regarding past and/or current medical history. These included the presence of hypertension, diabetes or kidney disease. The use of any medication prior or at the time of the clinical measurements was documented. Substance use whether in the past or of late such as smoking tobacco or alcohol consumption was also documented. Menstrual history, pregnancies and any contraceptive use was documented in female participants. The fasting participants filled in a dietary questionnaire that documented their food intake during the three-week fasting as well.

2.3 Blood sampling

Participants were asked to fast overnight prior to clinical visit. Following an overnight fast, venous blood of about 18 ml was drawn from the median cubital vein of the participants' arm. The samples were taken to Central Laboratory Services (CLS) for analysis which included creatinine, electrolyte concentrations and serum lipid profiles. The lipid profiles included triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol concentrations. For dietary intake serum concentrations of Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} were investigated. Blood glucose and glycated haemoglobin were also assessed. For endocrine testing serum concentrations of insulin, renin, leptin and aldosterone were taken. The cut-off values for the diagnosis of any disorders arising from the above parameters were defined.

2.4 Anthropometric measurements

Anthropometric measurements were taken according to the international standards for anthropometric assessments (Marfell-Jones *et al*., 2012). During all the measurements, the participants were asked to take their shoes off and minimal clothing was worn which allowed access to areas of measurements. Weight measurements were recorded using an electronic scale (Healthometer Professional, McCook, IL, USA) to the nearest 0.1 kg. Height was measured using a stadiometer (Seca portable stadiometer, Hamburg, Deutschland, Germany) to the nearest 0.1 cm. When the measurement was taken the head of the participant was placed in the Frankfort plane with the body standing upright and a sliding head board was lowered to the vertex of the head. Using body weight and height measurements, the participants' body mass indices (BMI) were determined as weight in kilograms divided by height in metres squared (kg/m^2). These findings were used to categorise the participants as underweight, normal, overweight or obese using the cut-off values in Table 2.1.

Waist and hip circumferences were determined using a tape measure with participants wearing minimal clothing on the abdominal and thigh regions. Waist circumference was determined at the narrowest width midway between the iliac crest

and the lowest rib to the nearest 0.1 cm at the end of a gentle expiration. The hip circumference was measured at the widest part of the hips and largest part of the buttocks to the nearest 0.1 cm. Subsequently, the waist to hip ratio (WHR) was calculated from the waist circumference and the hip circumference to determine central adiposity as guided by the cut-off values in Table 2.2.

Skinfold thickness was measured using a Harpenden skin-fold calliper at the triceps (located on the posterior midline of the upper arm that is measured halfway between the acromion and olecranon processes), biceps (located on the anterior midline of the upper arm over the belly of the biceps muscle, a little higher than the level used to mark the triceps site), subscapular region (located below the inferior angle of the scapula at the bottom of the shoulder blade) and suprailiac region (located above the anterior superior iliac crest at the top of the hip bone); to whose mean values were obtained to the nearest 0.1 mm to determine sub-cutaneous obesity.

Table 2. 1 Body mass index (BMI) indices threshold values

BMI (kg/m^2)	**Weight Classification**	**Relative Risk of disease**
<18.5	Underweight	
18.5 – 24.9	Normal	No risk
25.0 – 29.9	Overweight	Increased risk
30.0 – 34.9	Obese (Class I)	High risk
35.0 – 39.9	Obese (Class II)	Very high risk
≥40.0	Morbidly Obese	Extremely High risk

Adapted from WHO, 2000. Obesity: Preventing and managing the global epidemic.

Table 2. 2 Waist: Hip ratio classification

		Relative Risk of disease			
Gender		No risk	Average	At risk	High risk
Males	<0.85	0.85 – 0.89	0.90 – 0.95	0.95 – 1.0	≥ 1.0
Females	<0.75	075 – 0.79	0.80 – 0.85	0.85 – 0.90	≥ 0.9

Adapted from Heyward and Wagner, 2004. Applied body composition assessment.

2.5 Conventional blood pressure measurements

Brachial blood pressure of the participants was measured using an automated sphygmomanometer (Omron, Kyoto, Japan) (Figure 2.2) with the participants seated on a chair with a back and arm rest. Participants were advised not to cross their legs and they rested the arm at which the BP was measured on a table that was set beside the chair at an elevation close to the heart level. Caution was taken by using a cuff suitable to the arm circumference of each participant when the BP measurements were taken. A standard cuff with inflatable bladder measuring at 22 x 12 cm was used, and participants with slightly bigger arm circumferences were measured using bigger cuff sizes with bladders at 31 x 14 cm and higher. Without interruptions, participants rested for five minutes before taking the first BP measurement. Five consecutive measurements were taken at one-minute intervals. The five measurements were averaged to determine the participants' systolic and diastolic BP in mm Hg and resting heart rate. Blood pressure measurements were measured as previously described by O'Brien *et al*. (2003). Hypertension was defined according to published guidelines. Auscultatory measurements at ≥140 mm Hg systolic and 90 mm Hg diastolic were defined as hypertensive states. Furthermore, participants who received anti-hypertensive treatment or had elevated BP as noted during clinical measurements were classified as hypertensives. Table 2.3 depicts the thresholds for conventional BP.

Table 2. 3 Conventional BP indices threshold values

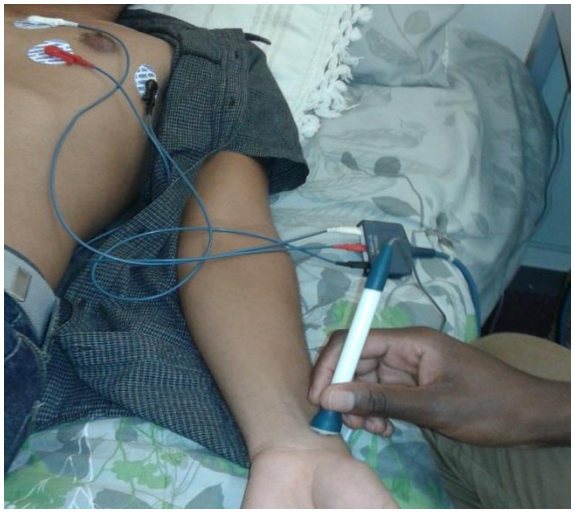
Definitions and classification of conventional BP (mm Hg)		
Stage	**Systolic BP**	**Diastolic**
Normal	< 120	< 80
Elevated	120–129	80–84
High normal	130–139	85–89
Grade 1	140–159	90–99
Grade 2	160–179	100–109
Grade 3	≥ 180	≥ 110
Isolated systolic	≥ 140	< 90

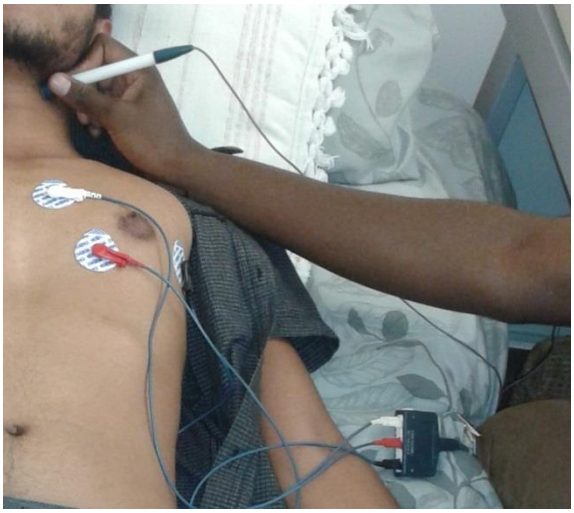
Adapted from Mancia *et al*. (2013)

Figure 2.2 Conventional BP measurements using an automated sphygmomanometer.

2.6 Measurements of arterial stiffening

Measurements of arterial stiffness were performed as described by Shiburi *et al*. (2006). Arterial stiffening was measured using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas), interfaced with a computer employing SphygmoCor software version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The participants were allowed to rest in the supine position for 15 minutes; then the carotid, femoral and brachial waveforms were recorded by applanation tonometry at an 8-s period. The pulse was calibrated by manual measurements of BP before the measurements were recorded. The aortic pulse wave was calculated using a validated generalised transfer function. Before measuring pulse wave velocity, distances from the suprasternal notch to the carotid sampling site (labelled as distance A), and from the suprasternal notch to the femoral artery (labelled as distance B) were measured. Thus, pulse wave velocity was calculated as the difference between distance B and A (femoral and carotid). The pulse transmit time was determined from the average of 10 consecutive beats (the mean time difference between sites A and B). Lastly, aortic pulse wave velocity was determined as the ratio of the distance in metres to the transit time in seconds.





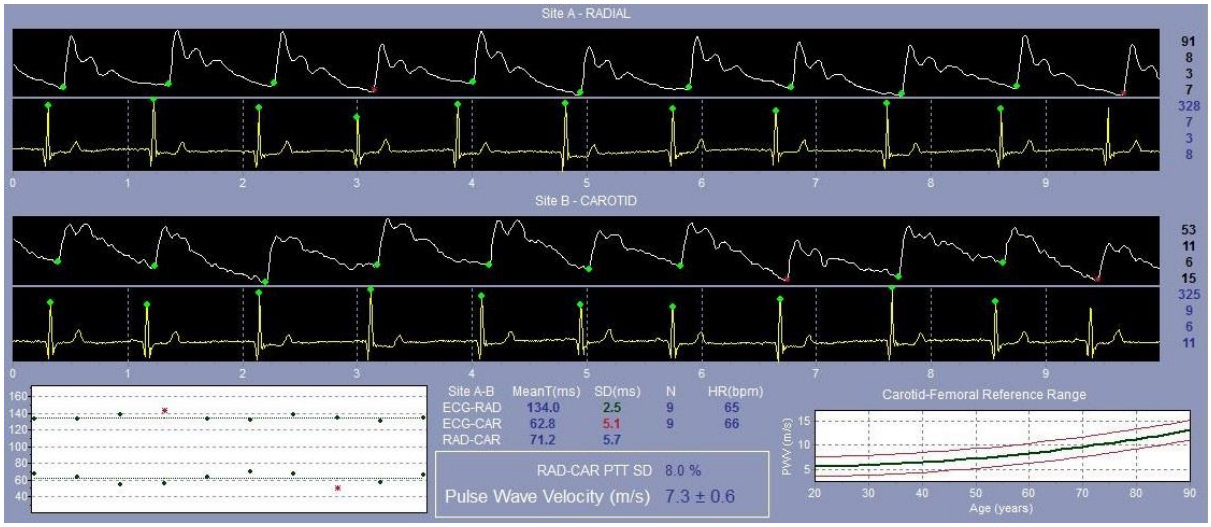


Figure 2. 3 Demonstration of the arterial stiffness analysis measured through PWV using two sites (Radial and Carotid pulse waves); the femoral region is not shown in the picture and the resultant outcome.

2.7 Urinary electrolyte excretion

2.7.1 Fresh urine samples

During clinical visits and upon arrival at the clinic the participants were asked for fresh urine samples before commencing with other measurements. Standard urinalysis was done using routine urine test strips (Siemens Multistix® 10 SG, Siemens Healthcare (Pty) Ltd, Midrand, South Africa). The participants' pH, specific gravity and the presence or absence of protein and blood were determined to screen

for renal pathology and any clinical conditions. Further analysis of the electrolyte concentrations and excretion rates was done at CLS.

2.7.2 24-hour Urine samples

After all measurements were performed, participants were issued specimen bottles to collect 24-hour urine samples. For anatomical reasons, female participants were issued beakers to use and then transfer the urine into the specimen bottles. Subsequently, these specimen bottles were collected from the participants after 24-hours. The APOGH participants were only issued one specimen bottle to collect a single 24-Hour urine sample; whereas, the fasting diet study participants were issued two specimen bottles. These urine bottles were labelled as daytime and night-time sample to separate and differentiate between the two collected samples. This was done to allow separate determination of the electrolyte excretion rates and ratios between daytime and night-time. Participants were given careful instruction on how to differentiate between the times for the sample collection. The urine samples were taken to CLS for determination of 24-hour, daytime and night-time urinary excretion rates of Ca^{2+}, K^{+}, Na^{+}, Mg^{2+} and creatinine.

2.8 Daily dietary intake recall

Daily dietary recall was done by the participants who were taking part in the fasting study. To record their nutrient intake, participants were issued a dietary recall form to document their daily oral intake for three weeks. The questionnaire recorded the frequency of each meal, the type of meal that was eaten, the time at which it was eaten and how the food was also prepared. Different types of food models and house hold measures were used for the quantification of food portion sizes. The dietary recall questionnaire is included as appendix 3.

2.9 Inclusion criteria

The participants who were recruited for the 21-days fast were selected because they routinely observed fasting for religious purposes. This was not a feeding study and therefore no participant was asked to fast. Only members who were, at the time of clinical measurements, beginning their fast were allowed in the study. Therefore,

clinical measurements were taken before and after the fast. Nonetheless, because the second measurements were only taken after the 21 days, participants who had not fasted for 21 days, and/ or those who had consumed meats and animal products during the fast were excluded for analysis.

For the cross-sectional study, community members who had completed 24-hour urine collections were included for analysis. Based upon the 95% confidence intervals for each group, a 24-hour urine sample was considered acceptable if 24-hour urine creatinine (mmol) was >3.5 and <35 for males and >3.5 and <30 for females. Samples with urine volumes <500 ml/day were also assumed to be incomplete urine collections. These approaches are standard approaches and have been published on numerous occasions by other groups (Maseko *et al*., 2006).

2.10 Statistical analysis

Data management and statistical analysis was performed using the SAS software version 9.4 (The SAS Institute Inc., Cary, North Carolina, USA). Continuous data and characteristics of participants was expressed as means ± SD or as frequencies (%). Paired t-tests were performed to describe the difference in the studied parameters before and after fasting. The predictors of BP were identified and confounders included: smoking, alcohol use, diabetes, body height and weight, age, sex, and heart rate, hypertension treatment, indices of adiposity, 24-hour electrolyte excretion rates, Na^+/K^+ ratio, along with use of medication. These variables may contribute to cardiovascular damage. Sex differences may also have an effect on the manifestation of cardiovascular pathologies; however, for the purposes of our current study data was not analysed according to gender differences because the focus was not sex-specific. Mixed procedures were also employed for further adjustment of non-independence in non-linear models. Urinary electrolyte excretion rates were assessed in models separate from those used for Na^+/K^+ ratios; subsequently analysis for BMI, waist circumference and WHR was also assessed separately for probability values. $P < 0.05$ was considered to be statistically significant.

CHAPTER 3

RESULTS

PART A:

Dietary intervention study

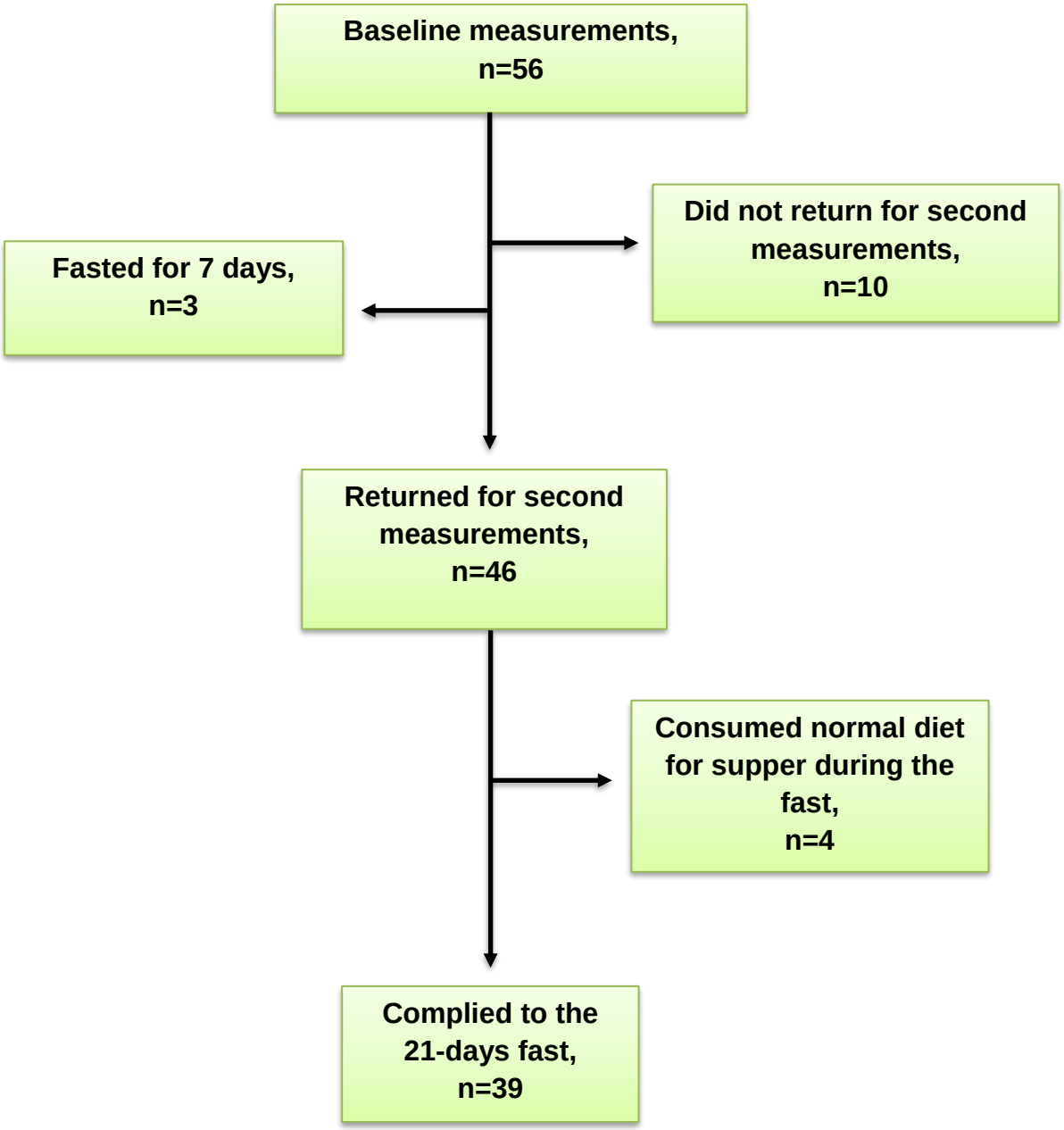


Figure 3.1 Schematic representation of the participants who were measured before and after the fast. n= the number of participants.

Table 3.1 illustrates the general characteristics of the participants. The average age was 34.9±13.2 years. There were more female participants than male participants in the study 61.5%. The participants were overweight and had an average BMI 27.8±16.0 kg/m^2. The cohort had only 7.7% hypertensive participants; 5.2% of the participants were diabetic. Regular smokers were recorded at 12.8%. Participants who regularly consumed alcohol were 12.8%.

Table 3.1 General characteristics of the dietary intervention study population

Sample Number	**39**
Age (years)	34.9±13.2
Female %	61.5
BMI (kg/m^2)	27.8±16.0
% Hypertensive	7.7
% Diabetic	5.2
Regular alcohol (% subjects)	12.8
Regular tobacco (% subjects)	12.8

BMI, body mass index; ± values are the standard deviation.

Table 3.2 shows the different types of fruits and vegetables consumed by the participants during the 21-day fast. The participants were given a daily dietary recall form and this information was self-recorded over the 3 weeks. The study was not a controlled feeding study and therefore participants were not told what food types to eat; however, they were required to list every item that they consumed. Commonly consumed fruits and vegetables were Apples, Bananas, vegetable soup and frozen mixed vegetables. Furthermore, the table list meal frequencies and if the food was eaten raw or cooked. Most of the participants consumed one meal a day, and had restricted themselves to only drinking water during the day. Other beverages like tea and juice were also included in the diet. Fresh or raw fruits and vegetables were

mostly consumed as a snack during the day, after supper or mostly in the form of salads. There was a challenge regarding cooking methods, salt and other food flavourings were added to the discretion of the participants and thus might have influenced the salt content when they prepared their supper.

Table 3.2 The eating pattern of the participants and type of fruits and vegetables consumed

Eating plan					
Meal frequencies (%)		**Meal preparation (%)**		**Beverages (%)**	
Breakfast	22.2	Cooked	82.8	Fruit juice	35.2
Lunch	34.4	Fresh/ Raw	27.6	Rooibos tea	48.2
Snacking	17.2				
Supper	100				
The types of fruit and vegetables consumed (%)					
Apple	65.1	Cauliflower	27.2	Pineapple	43.8
Avocado	27.2	Corn	40.7	Plums	41
Banana	58.3	Grapes	47.9	Potato	64.5
Beets	33.7	Salad	51	Pumpkin	47.2
Berries	23.7	Litchi	23.8	Spinach	47.5
Broccoli	51	Mango	41.4	Pear	40.7
Brown rice	27.2	Mushroom	33.8	Sweet potato	40.7
Butternut	44.1	Nectarine	37.2	Tomato	47.2
Cabbage	44.1	Peach	41	Vegetable soup	50.7
Carrot	44.1	Pawpaw	27.2	Watermelon	34.1
Peanuts & Raisins	24.1	Frozen mixed vegetables	67.6		

Table 3.3 shows the blood pressure, lipid profiles and serum electrolyte concentrations of the participants before and after the fruit and vegetable fasting dietary intervention. SBP was lower after the dietary intervention as compared to before the fruit and vegetable fasting dietary intervention (117.5±14.0 and 114.2±14.5 respectively, p=0.0150). Likewise, DBP was also lower after the fruit and vegetable fasting dietary intervention, however the change was not significant (73.9±9.5 and 71.4±9.5 respectively, p=0.1181). TCHOL concentrations was significantly reduced after the dietary intervention as compared to before the dietary intervention (4.08±0.81 mmol/l and 4.40±1.03 mmol/l respectively, p=0.0148). No significant changes were observed in HDL concentrations after the dietary intervention (1.34±0.36 and 1.24±0.32 respectively, p=0.2728). A 0.18 mmol/l difference was observed in LDL concentrations after the intervention (2.55±0.88 before and 2.37±0.86 after, p=0.0423). TRGL concentrations decreased from 1.13±0.55 mmol/l to 1.05±0.54 mmol/l, however the change was not significant (p=0.6814). At a difference of 0.06 mmol/l, serum Ca^{2+} concentration was significantly increased after the intervention (p=0.0299). Serum Crt before and after the fast had a 0.06 mmol/l difference, however the change was not significant (p=0.4351). Changes in serum K^{+} concentrations (4.28±0.30 before and 4.33±0.24 after) and Na^{+} concentrations (139.21±2.38 before and 140.28±2.14 after) were not significant (p>0.05). Serum Mg^{2+} was not significantly different after the dietary intervention as compared to before the dietary intervention (0.78±0.07 and 0.80±0.04 respectively, P>0.05). Anthropometric measurements did not show any significant changes after the dietary intervention (P>0.05). Bodyweight decreased by 3.18 kg after the dietary intervention. BMI decreased by 0.51 kg/m^2 after the intervention. Waist circumference decreased from 90.84±16.73 cm to 88.80±18.01 cm. WHR was relatively the same before and after the dietary intervention.

Table 3.3 Blood pressure, lipid profiles and serum electrolytes of the population before and after the fruit and vegetable fasting dietary intervention

	Before Fasting	After Fasting	P value
Blood Pressure (mm Hg)			
C_SBP	117.5±14.0	114.2±14.5	0.0150*****
C_DBP	73.9±9.5	71.4±9.5	0.1181
Lipid Profile (mmol/l)			
TCHOL	4.40±1.03	4.08±0.81	0.0148*****
HDL	1.34±0.36	1.24±0.32	0.2728
LDL	2.55±0.88	2.37±0.86	0.0423*****
TRGL	1.13±0.55	1.05±0.54	0.6814
Serum Electrolytes (mmol/l)			
Ca^{2+}	2.26±0.41	2.32±0.41	0.0299*
Crt	73.30±15.90	73.24±13.72	0.4351
K^{+}	4.28±0.30	4.33±0.24	0.6247
Mg^{2+}	0.78±0.07	0.80±0.04	0.4498
Na^{+}	139.21±2.38	140.28±2.14	0.0706
Anthropometric indices			
Weight (kg)	76.11±19.47	72.93±19.58	0.1908
BMI (kg/m^2)	27.77±6.22	27.26±6.39	0.7149
Waist circumference (cm)	90.84±16.73	88.80±18.01	0.9189
WHR	0.83±0.08	0.83±0.09	0.1588

C_SBP, conventional systolic blood pressure; C_DBP, conventional diastolic blood pressure; TCHOL, Total cholesterol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; TRGL, Triglycerides; Ca^{2+}, Calcium; Crt, Creatinine; K^{+}, Potassium; Mg^{2+}, Magnesium; Na^{+}, Sodium; WHR, Waist/ Hip ratio; ± values are the standard deviations.

Table 3.4 shows the urinary electrolyte excretions (index of dietary intake). The 24-hour Ca^{2+} excretion concentration was significantly lower after compared to before the dietary intervention (p=0.0168). However, there was no significant change in the Ca^{2+} daytime and night-time excretion (p=0.3946 and p=0.0626 respectively). There was no significant change in Crt in the 24-hour, daytime and night-time excretions (p=0.1185, p=0.4060 and p=0.4429 respectively). K^{+} excretion was also not significant for the 24-hour (p=0.6839), daytime (p=0.3545) and night-time (p=0.8627) excretions respectively. There was no significant change in Mg^{2+} in the daytime and night-time excretions (p=0.1274 and p=0.7400 respectively), however the 24-hour Mg^{2+} concentration was significantly increased after the intervention (p=0.0448). Na^{+} excretions were significantly lower across all the time intervals after the dietary intervention (24-hour; p<0.0001, daytime; p=0.0277 and night-time; p=0.0212, respectively). The Na^{+}/K^{+} showed no significant change in the 24-hour, daytime and night-time excretions (p=0.8035, p=0.9646 and p=0.7008 respectively). The 24-hour Ca^{2+}/Mg^{2+} ratio showed a significant change (p=0.0096); however, the daytime and night-time excretion ratios were not significant (p=0.2815 and p=0.0940 respectively).

Table 3.4 Urine electrolyte excretions (mmol/d) before and after the fruit and vegetable fasting dietary intervention

	Before Fasting			After Fasting			P Values		
	24-hour Urine	**Daytime Urine**	**Night-time Urine**	**24-hour Urine**	**Daytime Urine**	**Night-time Urine**	**P^{24} value**	**P^{D} value**	**P^{N} value**
Ca^{2+}	2.27±2.16	2.01±2.01	2.39±2.09	1.82±1.47	1.73±1.76	2.29±2.16	0.0168*****	0.3946	0.0626
Crt	8.63±6.66	8.72±6.64	10.02±8.79	9.65±7.18	9.43±6.43	10.17±8.00	0.1185	0.4060	0.4429
K^{+}	32.19±22.12	35.76±23.91	31.14±21.44	35.11±19.27	39.64±25.17	33.61±22.29	0.6839	0.3545	0.8627
Mg^{2+}	2.25±1.41	2.05±1.01	2.59±1.61	2.80±1.24	2.33±1.53	3.09±1.72	0.0448*	0.1274	0.7400
Na^{+}	97.61±55.03	99.63±54.60	97.79±51.29	90.64±52.11	84.96±64.27	92.79±58.37	<0.0001*****	0.0277*****	0.0212*****
Na^{+}/K^{+}	3.46±1.67	3.50±2.46	3.99±2.67	2.68±1.26	2.29±1.35	3.06±1.76	0.8035	0.9646	0.7008
Ca^{2+}/Mg^{2+}	1.00±0.65	1.03±0.99	0.93±0.55	0.77±0.63	0.98±0.71	0.77±0.62	0.0096*****	0.2815	0.0940

Ca^{2+}, Calcium; Crt, Creatinine; K^{+}, Potassium; Mg^{2+}, Magnesium; Na^{+}, Sodium; Na^{+}/K^{+}, Sodium Potassium Ratio; Ca^{2+}/Mg^{2+}, Calcium Magnesium Ratio; P^{24}, p value for 24-hour Urine; P^{D}, p value for daytime urine; P^{N}, p value for night-time urine; ± values are the standard deviations.

PART B:

Cross-sectional study

Table 3.5 illustrates the general characteristics of the participants. The average age of the cohort was 45.2±18.5 years. There were more female participants than male participants in the study 63%. The participants were overweight and had an average BMI of 29.1±7.8 kg/m^2. Hypertensive participants contributed 34% of the study population and 12% were diabetic. Participants who regularly consumed alcohol were 23% and regular smokers were recorded at 17%.

Table 3. 5 General characteristics of the non-intervention study population

Sample Number	625
Age (years)	45.2±18.5
Female %	63
BMI (kg/m^2)	29.1±7.8
% Hypertensive	34
% Diabetic	12
Regular alcohol (%)	23
Regular tobacco (%)	17

BMI, body mass index; ± values are the standard deviations.

Table 3.6 shows the blood pressure, lipid profiles and serum electrolyte concentrations of the participants that were in the non-intervention study. SBP and DBP were 129.6±21.4 and 84.1±11.9 mm Hg respectively. The lipid profile of the participants, i.e. TCHOL, HDL, LDL and TRGL concentrations were reported at 4.6±1.1 mmol/l, 1.4±0.4 mmol/l, 2.6±0.9 mmol/l and 1.2±1.2 mmol/l respectively.

Serum Ca^{2+} concentration was 1.6±1.9 mmol/l, lower than the expected range of 2.05 to 2.56 mmol/l. Urinary K^{+} excretion was lower than 120 mmol/d (28.5±21.8 mmol/d). Serum Mg^{2+} was 2.3±1.9 mmol/l, above recommended concentrations of 0.65–1.10 mmol/l. Serum Na^{+} concentrations were within normal limits at 137.4±2.6 mmol/d (135–147 mmol/l). The average urinary Na^{+} excretion of this population was higher than the recommended Na^{+} values of 100 mmol/d (105.6±78.4). Hormones studied were aldosterone (200.7±167.4 pg/mL), insulin (14.4±16.6 mmol/l), leptin (24.2±25.3 ng/mL) and renin (36.4±73.8 ng/mL). Furthermore, the ratio between aldosterone and renin was 27.4±53.1.

Table 3.7 presents the regression analysis between urine electrolytes, blood pressure and pulse wave velocity. The relationship between Ca^{2+} with C_SBP, C_DBP and PWV was not significant (p=0.2809, p=0.2592 and p=0.3487 respectively) and the proportion of the variance was low (r^2= -0.07, r^2= -0.04 and r^2= -0.05 respectively). There was a positive relationship between K^{+} with C_SBP (r^2= -0.13; p=0.0464) and C_DBP (r^2= -0.10; p=0.0222). However, no significant association was observed between K^{+} and PWV (r^2= -0.10; p=0.1633). Dietary Mg^{2+} was also significantly associated with C_SBP (r^2= -0.14; p=0.0336) and PWV (r^2= -0.18; p=0.0080); however, no significant relationship was noted with C_DBP (r^2= -0.04; p=0.2823). There was a significant relationship between Na^{+} with C_SBP (r^2= 0.16; p=0.0125) and PWV (r^2= 0.24; p=0.0005). No significant relationship was observed between Na^{+} and C_DBP (r^2= 0.03; p=0.4598). The association between Ca^{2+}/Mg^{2+} with C_SBP, C_DBP and PWV was not significant (p=0.9204, p=0.7998 and p=0.3849 respectively) and the proportion of the variance was low (r^2= 0.01, r^2= 0.01 and r^2= 0.03 respectively). There was a significant relationship between Na^{+}/K^{+} with C_SBP, C_DBP and PWV (p=0.0245, p=0.0232 and p=0.0013 respectively) and the proportion of the variance was moderate (r^2= 0.10, r^2= 0.10 and r^2= 0.13 respectively).

Table 3.6 Blood pressure, lipid profiles and serum electrolyte concentrations of the non-intervention study participants

Measured variables		Normal Ranges
Blood Pressure (mm Hg)		
C_SBP	129.6±21.4	120–129
C_DBP	84.1±11.9	80–84
Lipid Profile (mmol/l)		
TCHOL	4.6±1.1	≤5.2–6.2
HDL	1.4±0.4	1.0–1.3
LDL	2.6±0.9	<2.59–3.34
TRGL	1.2±1.2	<1.7–2.2
Serum and urinary electrolytes		
Serum Ca^{2+} (mmol/l)	1.6±1.9	2.1–2.6
Urinary K^{+} (mmol/d)	28.5±21.8	25–125
Serum Mg^{2+} (mmol/l)	2.3±1.9	0.85–1.10
Serum Na^{+} (mmol/l)	137.4±2.6	135–145
Urinary Na^{+} (mmol/d)	105.6±78.4	40–220
GHB (%)	6.2±1.5	4–5.6
Hormones concentrations		
Aldosterone (pg/mL)	200.7±167.4	10–210
Insulin (mmol/l)	14.4±16.6	≤5.5–≥11.1
Leptin (ng/mL)	24.2±25.3	0.3–23.7
Renin (ng/mL)	36.4±73.8	≥27
A/R	27.4±53.1	

C_SBP, conventional systolic blood pressure; C_DBP, conventional diastolic blood pressure; TCHOL, Total cholesterol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; TRGL, Triglycerides; Ca^{2+}, Calcium; K^{+}, Potassium; Mg^{2+}, Magnesium; Na^{+}, Sodium; GHB, glycated haemoglobin; A/R, aldosterone renin ratio; ± values are the standard deviations.

Table 3.7 Relationship between blood pressure and pulse wave velocity with serum electrolytes in the non-intervention study

	r^2	Confidence intervals			P value
C_SBP (mm Hg) vs					
Ca^{2+}	-0.07	-0.20	to	0.06	0.2809
K^{+}	-0.13	-0.26	to	0.01	0.0464*****
Mg^{2+}	-0.14	-0.27	to	0.01	0.0336*****
Na^{+}	0.16	0.03	to	0.29	0.0125*****
Ca^{2+}/Mg^{2+}	0.01	-0.07	to	0.10	0.9204
Na^{+}/K^{+}	0.10	-0.10	to	0.16	0.0245*
C_DBP (mm Hg) vs					
Ca^{2+}	-0.04	0.12	to	0.03	0.2592
K^{+}	-0.10	-0.16	to	0.01	0.0222*****
Mg^{2+}	-0.04	-0.12	to	0.03	0.2823
Na^{+}	0.03	0.04	to	0.10	0.4598
Ca^{2+}/Mg^{2+}	0.01	0.06	to	0.10	0.7998
Na^{+}/K^{+}	0.10	0.10	to	0.16	0.0232*
PWV (m/s) vs					
Ca^{2+}	-0.05	-0.33	to	0.01	0.3487
K^{+}	-0.10	-0.23	to	0.04	0.1633
Mg^{2+}	-0.18	-0.6	to	0.10	0.0080*****
Na^{+}	0.24	0.11	to	0.36	0.0005*****
Ca^{2+}/Mg^{2+}	0.03	-0.04	to	0.12	0.3849
Na^{+}/K^{+}	0.13	-0.05	to	0.21	0.0013*****

C_SBP, conventional systolic blood pressure; C_DBP, conventional diastolic blood pressure; PWV, pulse wave velocity; Ca^{2+}, Calcium; K^{+}, Potassium; Mg^{2+}, Magnesium; Na^{+}, Sodium; ± values are the standard deviations.

Table 3.8 shows the relationship between blood pressure and pulse wave velocity with serum lipids. TCHOL was significantly associated with C_SBP (r^2= 0.11; p=0.0062). There was also a significant relationship between LDL and C_SBP (r^2= 0.10; p=0.0159). Nonetheless, HDL and TRGL did not show any significant association with C_SBP (r^2= -0.01; p=0.8112 and r^2= 0.05; p=0.2047 respectively). There was also no significant relationship between all the lipid profiles with C_DBP (p=0.5946 TCHOL, p=0.6415 HDL, p=0.8544 LDL and p=0.2823 TRGL); and the proportion of the variance was also low (r^2= 0.02, r^2= -0.02, r^2= 0.01 and r^2= 0.04 respectively). There was a significant relationship between TCHOL and PWV (r^2= 0.18; p<0.0001). Decreasing HDL also showed a significant association with PWV (r^2= -0.10; p=0.0194). Moreover, LDL had a significant association with PWV (r^2= 0.12; p=0.0030). However, TRGL did not show any significant relationship with PWV (r^2= 0.04; p=0.3037).

Table 3.8 Relationship between blood pressure and pulse wave velocity with serum lipids in the non-intervention study

	r^2	Confidence interval			P value
C_SBP (mm Hg) vs					
TCHOL	0.11	0.03	to	0.18	0.0062*****
HDL	-0.01	-0.08	to	0.07	0.8112
LDL	0.10	0.02	to	0.17	0.0159*****
TRGL	0.05	-0.03	to	0.13	0.2074
C_DBP (mm Hg) vs					
TCHOL	0.02	-0.06	to	0.09	0.5946
HDL	-0.02	-0.09	to	0.06	0.6415
LDL	0.01	-0.07	to	0.08	0.8544
TRGL	0.04	0.03	to	0.12	0.2823
PWV (m/s) vs					
TCHOL	0.18	0.10	to	0.26	<0.0001*****
HDL	-0.10	-0.01	to	0.18	0.0194*****
LDL	0.12	0.04	to	0.20	0.0030*****
TRGL	0.04	-0.04	to	0.12	0.3037

C_SBP, conventional systolic blood pressure; C_DBP, conventional diastolic blood pressure; PWV, pulse wave velocity; TCHOL, Total cholesterol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; TRGL, Triglycerides; ± values are the standard deviations.

Figure 3.2 shows the effects of the three-week dietary intervention on pulse wave velocity (PWV). PWV was significantly reduced after compared to before the dietary intervention (6.11 ± 0.26 and 5.34 ± 0.23 respectively; p=0.0419).

Figures 3.3 and 3.4 show the relationship between BP and PWV. A positive relationship was observed between elevated C_SBP (Figure 3.2) and elevated C_DBP (Figure 3.2) with PWV (r^2=0.30; p<0.0001 and r^2=0.09; p<0.0001 respectively).

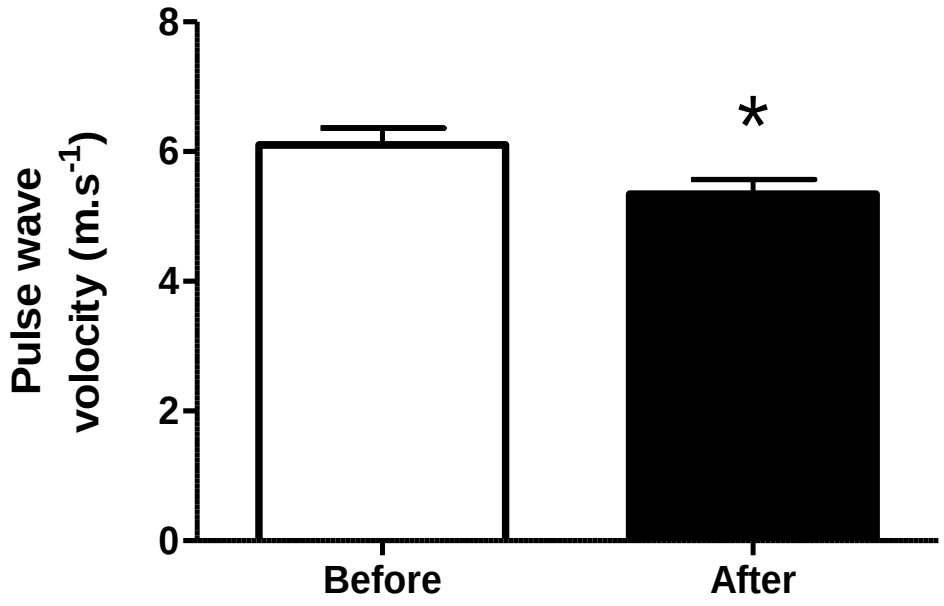


Figure 3.2 Changes in pulse wave velocity before and after the fruit and vegetable fasting dietary intervention. * P<0.05 versus before intervention.

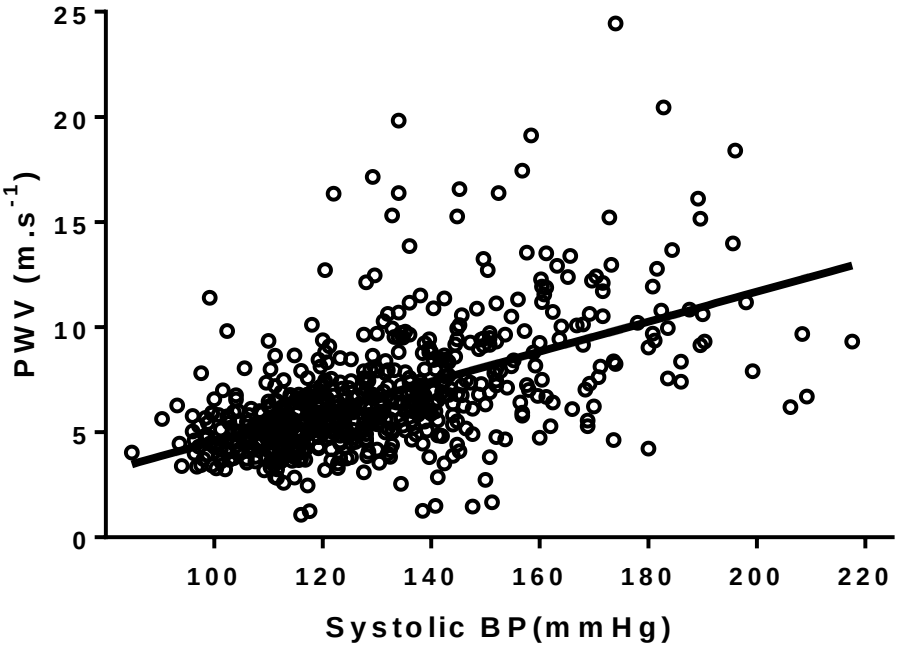


Figure 3.3 Normal fit plot for the relationship between C_SBP and PWV. There was a positive relationship between C_SBP and PWV (r^2=0.304; p<0.0001).

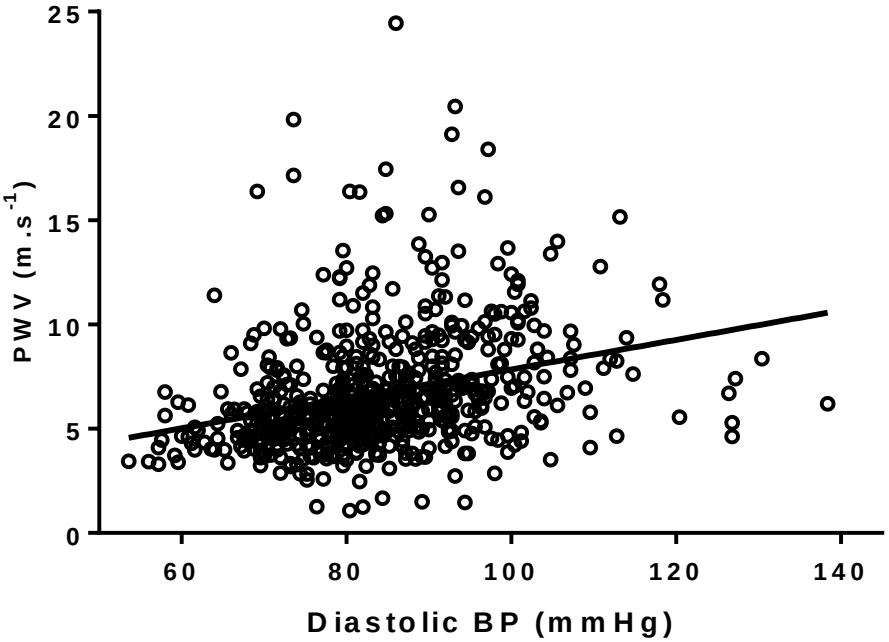


Figure 3.4 Normal fit plot for the association between C_DBP and PWV. There was a positive relationship between C_DBP and PWV (r^2=0.088; p<0.0001).

CHAPTER 4

DISCUSSION

4.1 Introduction

In the current study we investigated the relationship between micro-nutrients, serum lipids and cardiovascular parameters. For this purpose, we divided the study into two parts: a dietary intervention study consisting of 39 participants and a larger cross-sectional study consisting of 625 participants. In the dietary intervention study, we measured micro-nutrients, serum lipids, PWV and conventional BP before and after the fast. In the larger cross-sectional study, we investigated the relationship between cardiovascular parameters and micro-nutrients. We also determined the association between cardiovascular parameters and serum lipids. The main findings of the current study are that a 3-week fruit and vegetable fasting diet decreases SBP and PWV. In addition, serum Ca^{2+} increased significantly after the fruit and vegetable fasting diet. Furthermore, lipid profiles including TCHOL and LDL were also significantly decreased after the fruit and vegetable fasting diet. The current study is the first study to show decreased concentrations of urinary Na^{+} excretion after urinary concentrations of Na^{+} excretions were stratified into 24-hour, night-time and daytime urine. By confirming that a fruit and vegetable fasting diet decreases BP and has beneficial effects on the pathological changes in the vasculature, it remains of paramount importance to reinforce a fruit and vegetable diet as a strategy for the treatment of HT in groups of African ancestry.

4.2 The impact of the fruit and vegetable fasting diet on blood pressure

Our study shows that the 3-week fruit and vegetable fasting diet resulted in the reduction in SBP. After 3 weeks of a fruit and vegetable fasting diet, SBP was significantly reduced. In a similar study, Alonso *et al*. (2004) reported an average 2.2 mm Hg decrease in SBP in a population who consumed a fruit and vegetable diet. In addition, Appel *et al* (2003) showed a decreased in SBP in participants who maintained the fruit and vegetable diet for 8 weeks which was significantly longer than our 3-week period. The study by Appel *et al*. (2003) highlights another important aspect of a fruit and vegetable diet. The investigators had a relatively large population sample of 459 and hence were able to compare differences between hypertensives and normotensives. Their findings showed that the fruit and vegetable diet was more effective in reducing BP in hypertensives compared to normotensive

participants. Even though we were not able to compare different groups because of our relatively small sample size, we can deduce that our results would be similar if we had a larger sample size. This has serious implications to our study population which has a high prevalence of HT (Cooper *et al*., 2015; Steyn *et al*., 2008) because it confirms that a fruit and vegetable diet is important in the control of HT.

Since BP does not have a threshold for cardiovascular events, BP-related deaths are not only markedly reported in hypertensives but there is an increased prevalence in BP related mortality in normotensives (Stamler, 1991). This highlights the importance of the adoption of a fruit and vegetable diet at a population level because it can provide protection even to those who are not classified as hypertensive according to the current guidelines. Current recommendations for HT control rely mostly on the guidelines that initiate treatment based on elevated BP; however, the lack of threshold implies that the majority of the population may be at risk of BP-related CVDs and yet still fall below the required criteria to commence with pharmacological interventions (Appel, 2003). Therefore, even the 3 mm Hg decrease in SBP observed in this study could have significant preclinical cardiovascular benefits to this population. Moreover, if BP was reduced by 3 mm Hg over a 3-week period, it is possible that prolonged consumption over a longer period would yield even further reduction in BP. The most important finding of our study is that a fruit and vegetable fasting diet may play an integral role in the control and management of HT in a population of African descent.

4.3 Micro-nutrient-mediated cardiovascular benefits

In this study both serum concentrations and urinary electrolytes excretion rates were measured before and after the fruit and vegetable fasting dietary intervention. There were no significant changes in serum electrolyte concentrations of Crt, K^+ and Na^+ after the fast. This is due to the efficient physiological mechanisms that regulate serum electrolyte concentrations (Touyz, 2003). For example, an increase in plasma Na^+ concentration increases plasma osmolarity resulting in increased antidiuretic hormone secretion and increased water reabsorption (Riphagen *et al*., 2016). The excess water dilutes the plasma normalising plasma osmolarity. Similarly, by different mechanisms, plasma concentrations of Ca^{2+}, K^+ and Mg^{2+} are tightly

regulated (Touyz, 2003). Nevertheless, urinary electrolytes excretion, despite its limitations, is still a widely accepted method of observing changes in dietary electrolytes intake. Hence the 24-hour urinary excretion rate is still accepted as a 'gold standard' for assessing dietary electrolytes intake (Land *et al*., 2014). Dietary interventions owing to Na^+ reduction and subsequent increases in K^+ and Mg^{2+} intakes are reported in literature (Sookram *et al*., 2015; O'Donnell *et al*., 2011; Sacks *et al*., 2001). However, few studies seldom report on the impact of these electrolytes as single entities in regulating BP.

4.3.1 Calcium

In the current study, serum Ca^{2+} concentrations increased and 24-hour urinary Ca^{2+} concentrations were reduced after the fruit and vegetable fasting dietary intervention. This may be due to abstention from any animal products including dairy products. Since dairy products are the richest source of Ca^{2+}, removal of dairy products from the diet could have resulted in the observed differences in the serum and 24-hour urinary Ca^{2+} concentrations. Furthermore, there is an association between Vitamin D and Ca^{2+} absorption in the intestines (Norval *et al*., 2016). Therefore, despite the slight increased serum Ca^{2+} that was noted in our target population the lack of data regarding their vitamin D status does not allow our study to definitively report on the decreased urinary Ca^{2+} excretion rate. Literature reports that vitamin D is available in some food products. However, certain populations may be vitamin D deficient (Green *et al*., 2015). Most regions in Africa, including SA, have food security challenges; therefore, dietary factors may largely contribute to vitamin D deficiency. Consequently, it affects dietary Ca^{2+} and its absorption (Ross *et al*., 2011; Holick *et al*., 2011; Bischoff-Ferrari *et al*., 2006).

Despite the important physiological functions which include muscle contraction and blood coagulation, Ca^{2+} has been associated with a reduction in BP and arterial stiffness (Uemura *et al*., 2014). In the second part of our study with a larger sample size, we investigated the relationship between 24-hour urinary Ca^{2+} excretion rates and both BP and arterial stiffness. After adjusting for covariates such as age, gender, HT, smoking, alcohol intake and BMI, there was no significant relationship between urinary Ca^{2+} excretion and both BP and arterial stiffness. Our findings were

contradictory to the findings conducted in other population groups which found a significant association between Ca^{2+} and both BP (da Silva Ferreira *et al*., 2016) and PWV (Fabris *et al*., 2016). In a study conducted in a Japanese population, the researchers found a significant negative association between dietary Ca^{2+} and PWV (Uemura *et al*., 2014). Similarly, Kesse-Guyton *et al.* (2010) found a strong association between low dietary Ca^{2+} intake and arterial stiffness. Similar observations were made in studies that have investigated the relationship between dietary Ca^{2+} intake and BP. In a cross-sectional study, the investigators found a strong association between dietary Ca^{2+} intake and reduction in SBP (Kim *et al*., 2012). Therefore, the beneficial effects of Ca^{2+} on BP and arterial stiffness may be due to the role of Ca^{2+} in the regulation of smooth muscle contractility and down regulation of the renin-angiotensin-aldosterone system (Mizuno *et al*., 2008). This raises a question concerning why these beneficial effects were not observed in our current study. Since our sample size was relatively large, the most logical explanation is the low dietary Ca^{2+} intake in this population. The low Ca^{2+} intake could have resulted in a regression dilution which might have obliterated the relationship between Ca^{2+} and cardiovascular parameters.

4.3.2 Magnesium

The potential effect of Mg^{2+} on reducing BP has not been extensively reported. A limited number of studies have assessed the relationship between BP and dietary Mg^{2+} from a fruit and vegetable diet. To the amount of existing literature, findings are inconsistent regarding dietary Mg^{2+} and its effects on BP reduction (Touyz, 2003; Jee *et al*., 2002; Resnick, 1997). Kawano *et al*. (1998) reported that Mg^{2+} supplementation reduces conventional BP. They further reported a 2.5 mm Hg reduction in ambulatory SBP and a 1.4 mm Hg reduction in ambulatory DBP (Kawano et a; 1998). However, these reports are from cohorts recruited in Western countries, hence discrepancies may exist in different regions owing to different environmental and socioeconomic factors. According to our knowledge, no study has investigated the effects of a fruit and vegetable-based diet on serum and urinary Mg^{2+} concentrations in an African population. Therefore, the findings of this study

provide meaningful evidence on the importance of dietary Mg^{2+} in the control and probably prevention of HT and arterial stiffness in people of African descent.

In the current study, although the participants consumed fruits and vegetables which are known to be rich sources of Mg^{2+}, no significant changes in serum Mg^{2+} concentrations were observed before and after the fast. The most logical explanation for the absence of significant changes in serum Mg^{2+} after fasting is that measuring serum Mg^{2+} concentration is the least sensitive method of assessing Mg^{2+} status (Volpe, 2013). As Mg^{2+} is stored mostly in soft tissues and bones (Aikawa, 1980), only less than 1% is stored in serum (Rude, 1996). Consequently, no changes in serum Mg^{2+} concentrations were observed in this study even though our participants consumed foods known to be rich sources of Mg^{2+}. On the other hand, 24-hour urinary Mg^{2+} excretion has been shown to be a better index of dietary Mg^{2+} intake compared to serum Mg^{2+} concentrations. Hence this procedure has been used in a number of studies to determine Mg^{2+} status (William *et al*., 2014; Joosten *et al*., 2013; Tzoulaki *et al*., 2011).

Using the more reliable 24-hour urinary Mg^{2+} excretion protocol, we found a significant difference in the Mg^{2+} status of our participants before and after the fruit and vegetable diet. The significance and the uniqueness of our findings provide evidence that Mg^{2+} status can be improved by eating a diet rich in fruit and vegetables. Most studies that have investigated Mg^{2+} status used Mg^{2+} supplements which have a high content of Mg^{2+} that does not represent the Mg^{2+} that is naturally present in the typical diet (Rosanoff, 2010; Jee *et al*., 2002). Therefore, their findings are not presentative of a population's dietary Mg^{2+} intake. Mg^{2+} is the fourth most abundant mineral in the body and plays a very significant role in several physiological processes including cellular energy production, regulation of muscle contraction, maintenance of normal heart rhythm, DNA and RNA synthesis and glucose metabolism (Veronese *et al*., 2016; Barbagallo *et al*., 2010; Pasternak *et al*., 2010; Sonita and Touyz, 2007; Preuss, 1997). Therefore, the findings of this study have far reaching consequences in highlighting the important role of a fruit and vegetable diet in community health.

After observing that a fruit and vegetable diet results in significant changes in the Mg^{2+} status in our population sample, the next step was to investigate whether Mg^{2+} is associated with any cardiovascular benefits. So, using a larger sample size, we investigated the relationship between Mg^{2+} and both BP and PWV. Although a number of studies have investigated the relationship between Mg^{2+} and BP, there are few community based studies that have investigated the impact of dietary Mg^{2+} on arterial stiffness. Previous studies have looked at hypertensive patients (Afsar and Elsurer, 2014), participants with renal disease (Joosten *et al*., 2013) or overweight participants (Joris *et al*., 2016). Therefore, our study is one of a few cross-sectional studies to investigate the impact of Mg^{2+} on arterial stiffness at a population level. We found a negative association between Mg^{2+} and PWV implying that Mg^{2+} reduces arterial stiffness. This was a very significant finding because PWV is an independent predictor of cardiovascular TOD. In an animal model study, Bender and associates were able to show that mice fed a Western diet (high fat) had an increase in arterial stiffness (Bender *et al*., 2015). Similarly, human dietary intervention studies also show that a Western type diet that is rich in processed carbohydrates and sugar causes an increase in arterial stiffness. In a cross-sectional study of 265 participants, Recio-Rodriguez and associates found positive associations between arterial stiffness and dietary fat intake (Recio-Rodriguez *et al*., 2014). In addition, Chan *et al.* (2014) observed a strong positive association between a high carbohydrate diet and arterial stiffness. These findings confirm that a Westernised diet, which is high in fats and carbohydrates, is associated with increased arterial stiffness. A few studies have confirmed that people in urban developing communities like Soweto, where we conducted our study, eat mainly a Western type diet (Pretorius and Sliwa, 2011; Steyn *et al*., 2006; Bourne and Steyn, 2000). Hence our current study observed a significant reduction in arterial stiffness after the 21-day fruit and vegetable diet. Studies based on animal models show that the Western type diet increases the transforming growth factor-β (TGF-β) which has been shown to reduce the elastin/collagen ratio resulting in increased arterial stiffness (Bender *et al*., 2015). On the other hand, Mg^{2+} has an opposite effect to TGF-β and reduces arterial stiffness (Nielsen, 2010). Hence in our study we showed a negative association between Mg^{2+} and arterial stiffness. Mechanisms that govern

Mg^{2+}'s ability to reduce arterial stiffness are based on its ability to act as a Ca^{2+} channel blocker (Barbagallo *et al*., 2010).

4.3.3 Calcium-to-magnesium ratio

In the current study, the 24-hour Ca^{2+}/Mg^{2+} ratio was significantly reduced due to the reduced Ca^{2+} intake and the increased Mg^{2+} intake. This has very significant health implications to our population since the interaction between Ca^{2+} and Mg^{2+} is associated with CVDs (Sato *et al*., 2018; Dai *et al*., 2013). It is possible that the reduction in BP and arterial stiffness observed in this study is due to the reduction in the Ca^{2+}/Mg^{2+} ratio. The mechanisms underlying the cardiovascular changes may be mediated by the actions of Mg^{2+} on vascular smooth muscle cells. Mg^{2+} acts as a Ca^{2+} channel blocker thus reducing the effects of Ca^{2+} on vascular smooth muscle contraction. The decreased vascular smooth muscle tone results in reduced BP and arterial stiffness observed in this study. In a study conducted by Park *et al*. (2017) in a Korean population, they were able to show a positive association between Ca^{2+}/Mg^{2+} ratio and coronary calcification. They attributed the observed changes to Mg^{2+}'s inhibitory role on Ca^{2+} entry into cells. The decrease in the Ca^{2+}/Mg^{2+} in our participants after a 21-day fruit and vegetable diet indicate the importance of dietary interventions in an effort to reduce cardiovascular related morbidity and mortality.

4.3.4 Sodium

In the dietary intervention study, we were able to show that the fruit and vegetable fasting dietary intervention resulted in reduced 24-hour, night-time and daytime Na^{+} excretions. This is the first study to investigate the impact of a fruit and vegetable diet on urinary Na^{+} excretion where Na^{+} excretion was stratified into 24-hour, night-time and daytime urine. Our results show a significant reduction in Na^{+} excretion in all the three categories after the 21-day fruit and vegetable diet fast. These findings highlight the beneficial effects of a fruit and vegetable diet in the reduction of dietary salt intake. The main reason for the reduction in salt intake is that meat and its products have a high salt content compared to vegetable-based products. Besides the high content of salt that is added when preparing meat, salt is also used as a preservative in canned meat products. Processed meat products like biltong (what

international countries would normally call Beef jerky), boerewors (sausages) and bacon have a very high salt content (Temme *et al.,* 2017*;* Pretorious, 2013; Charlton *et al*., 2008). Since the participants abstained from these products during the fasting period, it was not surprising that their dietary salt intake was reduced.

The main reason for separating urine samples into daytime and night-time was to observe if there is a difference in renal salt excretion between daytime and night-time. Before the fast, there were no differences in renal urinary handling but after the fast, significant differences were observed. There was a significant increase in night-time renal Na^+ excretion compared to daytime. This means reduced dietary salt intake could improve kidney function resulting in increased renal Na^+ excretion at night when the body is at rest (Meneton *et al*., 2005). The increased Na^+ excretion could account for the observed beneficial changes as indicated by the reduced arterial stiffness and BP after the fast. However no clear conclusions can be drawn but future studies should be conducted to investigate renal mechanisms responsible for the observed changes.

In the large cross-sectional study, there was a positive association between Na^+ and SBP. The association between dietary salt intake and HT was first described by Dahl in 1960. Since then several studies conducted in different population groups have shown this association (Khaledifar *et al*., 2015; Ha, 2014). However, studies conducted in this population have yielded contradictory results; with most studies showing no association between dietary salt intake and BP. Nonetheless, a recent study was able to show that the contradictory findings are due the high prevalence of obesity in this population (Maseko *et al*., 2018). This incidence of obesity was confirmed in the current study as well. The average BMI of this population sample was 29 kg/m^2. Indeed, studies have shown that increased adiposity results in increased salt retention and the subsequent reduction in urinary Na^+ concentration (Rocchini, 2000). This causes regression dilution which abolishes the relationship between 24-hour urinary Na^+ excretion and BP. However, despite these limitations, we were able to confirm findings that dietary Na^+ intake is associated with an increase in BP in this population. Mechanisms responsible for the dietary salt-induced elevation in BP have been well established (Hall, 2016; Rodriguez-Iturbe

and Vaziri, 2007; He and MacGregor, 2004; Simchon *et al*., 1991). Increased salt intake results in increased plasma osmolarity. This stimulates the thirst mechanism resulting in increased water intake. This normalises plasma osmolarity but it also increases BP (Meneton *et al*., 2005).

The main novel finding in this study regarding dietary salt intake is the association between dietary salt intake and PWV. No previous study conducted in this population was able to show the relationship between Na^+ and PWV independent of BP. Even after adjusting for BP and other confounding factors like age and BMI, the association remained. Mechanisms responsible for this independent associated between Na^+ and PWV are thought to be mediated by reduced nitric oxide production (Fujiwara *et al*., 2000). Animal model studies show that increased dietary salt intake results in endothelial cell damage and reduced nitric oxide secretion (Kanbay *et al*., 2011 Bragulat and Sierra, 2002). Since nitric oxide is a vasodilator, its reduction results in increased vasoconstriction and increased arterial stiffness. The findings of our study indicate that the current recommendations to reduce dietary salt intake may ascribe a double benefit to this population. Since both BP and PWV are independent predictors of cardiovascular target organ damage, salt reduction results in favourable cardiovascular benefits in salt sensitive African populations.

4.3.5 Potassium

The cross-sectional study showed a negative association between K^+ and both SBP and DBP, indicating that dietary K^+ intake (indexed by 24-hour urinary K^+ excretion) is associated with a reduction in both SBP and DBP. This is the first study to show this relationship in this population. These findings imply that K^+ can control BP independent of Na^+. Most studies have focused on Na^+ reduction in an effort to control BP (Pimenta *et al*., 2009; Appel *et al*., 2001; Elmer *et al*., 1991) and K^+ was mostly ignored because its effects on BP were considered to be related to Na^+. The recommendation of a fruit and vegetable diet which is rich in K^+ plays a central role in the management of HT. John *et al*. (2002) noted that increasing the intake of fruits and vegetables to 3-5 servings per day, presents the potential to decrease SBP by at least 4 mm Hg.

There were no significant changes in both serum and urinary K^+ in the dietary intervention study. Our study population's dietary K^+ intake levels are below the recommended levels, 32.19±22.12 mmol/day (Charlton *et al*., 2005). Contrary to the findings of our study, most studies reported an increased urinary K^+ excretion subject to recommending a K^+-rich diet that includes green leafy and root vegetables (Knoops *et al*., 2004; Appel *et al*., 1997). Therefore, according to the study published by Mente *et al*. (2009) the insignificant K^+ changes observed in our study population after the fast may indicate that our study population is consuming a poor-quality diet. Lower K^+ excretion rates are furthermore associated with fast-foods diets and high-energy drinks among other food types (Hu *et al*., 2000). The notion of poor dietary habits is further based on the findings that significant urinary K^+ excretion is positively correlated with intakes of fruit and vegetables (Thompson and Veneman, 2005).

Most interventions that aim at increasing K^+ concentrations recommend dietary intake at least between 60–120 mmol/day (Houston and Harper, 2008; Whelton and He, 1999); recognising that even modest increases in dietary K^+ will reduce BP. It is therefore motivating considering the prospect that even small reductions in BP may evoke positive effects in our study population, amidst their salt-sensitivity and resistant HT. Therefore, a small reduction in BP through increased dietary K^+ provided by a fruit and vegetable diet will greatly impact the current HT prevalence our study population.

Our findings therefore emphasise the need to increase consumption of fruit and vegetables that are rich in dietary electrolytes including K^+; because of its effects on BP (Appel, 2009; Appel *et al*., 2006; Williams *et al*., 2004; Guidelines Committee, 2003; WHO, 2003). Studies have proposed that increased K^+ intake stimulates the Na^+: K^+ pump and causes opening of K^+ channels; resultantly, the efflux of K^+ hyperpolarises the endothelial cells (Dick & Tune, 2010). This hyperpolarisation is then conducted to the vascular smooth muscle cells which then decrease cytosolic Ca^{2+} concentrations; therefore, promoting vasodilatation and ultimately resulting in decreased BP (Dick & Tune, 2010). Increased dietary K^+ intake has the potential to lower BP. This emphasises the importance of incorporating foods rich in K^+ as a

means of preventing HT. Particularly in salt sensitive individuals of African descent who are prone to developing HT.

4.4 Lipid profiles predict systolic blood pressure and arterial stiffness

The association between lipid profiles and cardiovascular risk factors was first demonstrated in the Framingham study in 1959 (Dawber *et al*., 1959). Since then other studies have shown the association between lipid profiles and cardiovascular risk (Langsted *et al*., 2008). Inflammatory factors together with cholesterol and HDL play a significant role in the pathogenesis of atherosclerosis which results in increased arterial stiffness (Manco *et al.*, 2017). These inflammatory markers have been found in high concentrations in overweight or obese individuals (Telle-Hansen *et al*., 2017). In this regard, our population which has a high incidence of obesity is prone to increased arterial stiffness induced by a diet high in cholesterol. However, no studies have been conducted in this population to determine the relationship between lipid profiles and arterial stiffness.

We first investigated the impact of a fruit and vegetable fasting dietary intervention on lipid profiles. We measured lipid profiles (TCHOL, HDL, LDL and TRGL) before and after the fasting dietary intervention. TCHOL and LDL concentrations were significantly reduced after the 21-day fruit and vegetable diet. Observational studies have assessed the effects of dietary intake on lipids concentrations (Jacqmain *et al*., 2003; Thorogood *et al*., 1990); however, limited data is available on the association between the consumption of fruit and vegetables and lipid profiles in a community-based population. In this regard, this is the first study conducted in this population to show that a fruit and vegetable diet reduces TCHOL and LDL concentrations, both of which are cardiovascular risk factors associated with atherosclerotic plaque (Janoudi *et al*., 2015; Onat *et al*., 2010). Djoussé and colleagues reported that eating a fruit and vegetable diet decreased LDL concentrations (Djoussé *et al*., 2004). Furthermore, Fornés and colleagues also reported decreased LDL concentrations owing to the fruit and vegetable diet (Fornés *et al*., 2000). However, Obarzanek *et al*. (2001) did not report any significant changes in LDL concentrations after a fruit and vegetable diet. Contrary to other studies (Morgantini *et al*., 2018; Keshtkaran *et al*., 2017), our current study did not note any significant changes in HDL cholesterol and

TRGLs. In a four-week intervention study, there was a significant increase in HDL cholesterol (Kurowska *et al*., 2000). The insignificant change in HDL cholesterol and TRGLs can be attributable to the duration of the dietary intervention and perhaps even the type of fruit and vegetable diet that our participants consumed because our study was not a controlled feeding dietary intervention. Therefore, future studies should have longer dietary interventions and controlled feeding dietary interventions which could possibly have an effect HDL cholesterol and TRGL concentrations.

In the large cross-sectional study, we set out to determine if these serum lipids are associated with BP and arterial stiffness. After correcting for confounders, there was a positive association between SBP and both TCHOL and LDL concentrations. PWV was positively associated with TCHOL and LDL but negatively associated with HDL concentrations. Our results suggest that TCHOL and LDL concentrations increase both BP and arterial stiffness while HDL concentrations reduce arterial stiffness. Our study is therefore the first to show that in an African population with a high incidence of obesity, plasma lipids are associated with SBP and arterial stiffness. Moreover, we were able to illustrate that a fruit and vegetable diet reduces the serum lipids that mediate atherosclerosis resulting in a reduced SBP and arterial stiffness.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

In conclusion, a three-week fruit and vegetable diet is associated with an improved BP profile and arterial compliance. The electrolytes of interest (Mg^{2+} and K^{+}) provided through the dietary intervention reduce both BP and PWV. Our study population's dietary electrolytes intake is below recommended values. Nonetheless, the effects of these electrolytes on BP regulation cannot be ignored. Therefore, barring from dietary supplements, our study showed that electrolytes provided naturally through the dietary intervention elicits desired effects with regards to BP regulation. Serum K^{+} and Na^{+} did not show any significant change neither did urinary K^{+} excretion; nonetheless, urinary Na^{+} excretion was significantly decreased. Our study supports that urinary electrolyte excretion rates may be used as an index of dietary intake; moreover, they can be used as predictors of cardiovascular outcomes. In the cross-sectional study the significance of the electrolytes' relationship in reducing PWV and leading to a further improvement in arterial compliance adds merit to the negative association between a fruit and vegetable diet and the risk of TOD. What was noteworthy in the non-interventional study was the negative relationship between K^{+} and BP. Potassium is seldom reported independent of Na^{+} in the regulation of BP. Therefore, increasing dietary K^{+}, barring from the effects of Na^{+} carries with it some clinical relevance, especially in members of African descent. Separating the urinary electrolytes excretions into daytime and night-time during the dietary intervention revealed an increase in night-time Na^{+} excretion rates. In a cohort that is reported to retain salt, our study thus proposes that a fruit and vegetable diet may improve Na^{+} handling by the kidneys and further help regulate BP and related TOD. A fruit and vegetable dietary intervention results in a decreased salt intake which may directly affect arterial stiffness. Finally, the dietary intervention decreased concentrations of circulating TCHOL and LDL. In abstaining from these lipids during the dietary intervention, our current study population stands to benefit regarding the risk of atherosclerosis, elevated BP and TOD. More especially given that our population was slightly overweight, a confounding factor to the risk of atherosclerotic plaque and related risk factors for CVD mortality. Although the 21-days fruit and vegetable fasting dietary intervention did not decrease BMI significantly, the BP lowering effects of the dietary electrolytes presented an interesting observation; that in the absence of significant BMI decrease

a fruit and vegetable diet can still decrease BP profile and arterial stiffness. It therefore introduces an independent relation between dietary interventions and BP with or without subsequent body weight decrease. However, this notion needs further elucidation. The significant association between the three-week fruit and vegetable diet and BP carries some clinical importance to both normotensives and hypertensives because elevated BP profile is associated with adverse TOD. To such a degree it will abate the currently burdened health care systems because of the potential to regulate BP profile in both hypertensives and normotensives. More importantly because there is currently no threshold for which BP leads to complicated CVDs. Normotensives may further benefit immensely from this diet because the combined potential to reduce PWV and improve arterial compliance may prevent risk factors for and even delay the progression of HT and associated TOD.

5.2 Possible study limitations and future perspective

Findings of the current study should be interpreted within the context of potential limitations. As noted with most cross-sectional studies, the cohort consisted of more females than male participants. Familial settings and socio-economic statuses impact hugely on gender-based participation in studies of this nature. A balanced gender-based analysis of the current findings would deem useful in stratifying the effects of the diet in both genders. Participants in this study did not consume a controlled diet; therefore, certain factors pertaining to affordability of certain fruits and vegetables may limit the types of fruits and vegetables our population consumed. The dietary recall questionnaire administered in the present study does not account for preparation of the food. All noted intakes were as self-reported by the participants. It therefore begs the question of the validity of self-reported dietary intake because there may be additional omitted information that may have had some effects. They were not given standard meals during the observation period as compared to most trials but rather they consumed different plant-based food types of their choice. It is worthy to note the differences in which these foods were prepared. It is also worth mentioning that dietary behaviour differs among ethnic groups; and factors such as cultural norms also play a role (Liu *et al*., 2001). Moreover, in a

controlled intervention study, the amount of fruits and vegetables or the quantity may be quantified to further extrapolate exact concentrations that can elicit the desired effects on BP and PWV. Additionally, this may provide a way to study the exact mechanisms that a fruit and vegetable diet exerts on BP profile and cardiovascular parameters. A trend that was noted in the current study and it thus underreports on the effects of the intervention. In the indices of urinary electrolytes, spot urine analysis was not performed. Determining if the same results from 24-hour urinalysis could be observed from spot analysis may add further statistical power to studies this nature. The observed effects are reported from a three-week intervention, an extended intervention period is necessary to report on the effects of sustained fruit and vegetable diet owing the reported disparities when participants are on a prolonged fruit and vegetable diet. This is the first time the effects of Mg^{2+} are associated with BP and PWV, thus future studies should build on these findings for reproducibility and in a bigger sample; and perhaps even the inclusion of controlled fruit and vegetable diet. A three-week fruit and vegetable diet positively influence BP and PWV, including such findings into current clinical practices is needed. Education on the correct intervention and including advice of the importance of adherence are also needed at community levels. Effective BP control is seldom achieved in population of African descent; this is attributed to poverty and other factors. Therefore, dietary education at community level may help bridge the gap regarding inaccessible health care to poor communities.

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Appendix 1: Ethical clearance certificate

R14/49 Mr Edgar Matome Phukubje et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170214

NAME: (Principal Investigator)	Mr Edgar Matome Phukubje et al
DEPARTMENT:	School of Physiology Hypertension Clinic, School of Physiology University of the Witwatersrand
PROJECT TITLE:	Impact of Fasting on Cardiovascular Parameters
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Muzi Maseko
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/06/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature — Date: 30/06/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2 Standard questionnaire

South African Hypertension and Diet Study (SAHDS)

STANDARD QUESTIONNAIRE

PARTICIPANT IDENTIFICATION

Surname: _____

Name(s): _____

Identification number: _____

Sex: _____

Date of birth: _____

Residential Address: _____

_____

Street: _____

Town: _____

Postal code (zip code): _____

Cellphone number: _____

HOW TO COMPLETE THE QUESTIONNAIRE?

The participants may complete the questionnaire themselves. However, they may also request the help of the team of fieldworkers. After completion, the questionnaire should be inserted in the envelope which should then be closed and sealed. All information provided will be treated confidentially and will be used anonymously in the statistical analysis.

[***This section will be stored separate from the remainder of questionnaire**.]*

SECTION A

Question 1: Specify your current marital state.

- Unmarried Yes ☐ No ☐
- Unmarried, but living together with partner Yes ☐ No ☐
- Married Yes ☐ No ☐
- Divorced Yes ☐ No ☐
- Widow or widower Yes ☐ No ☐

_

Question 2: Are you still attending school?

Yes ☐ No ☐

- Is taking courses a secondary activity for you? For instance, are you combining further education with an occupational or professional activity? Yes ☐ No ☐

Question 3 (a): Are you currently employed?

Yes ☐ No ☐

Question 3 (b): Are you self-employed or were you self-employed in your previous occupation?

Yes ☐ No ☐

- If yes:
  - What is your present occupation: _____
  - When did you start your current professional activity: _____

Question 4: If you are currently not employed which was the last job you had and for how many years?

- Last occupation: _____

  For how long did you practice your last job?_____

Question 5: If you are presently not working, please answer the below questions.

- Have you ever been employed before Yes ☐ No ☐
- Are you retired? Yes ☐ No ☐
- Other reasons:_____

Question 6: Which is the highest level of education you have successfully completed?

_____

SECTION B

_

Question 8: Please, specify whether some of your relatives currently suffer (or have suffered) from high blood pressure (hypertension).

Please, tick one box for each relative.

- Your father	Yes ☐	No ☐	Unknown ☐
- Your grandfather (father's side)	Yes ☐	No ☐	Unknown ☐
- Your grandmother (father's side)	Yes ☐	No ☐	Unknown ☐
- Your mother	Yes ☐	No ☐	Unknown ☐
- Your grandfather (mother's side)	Yes ☐	No ☐	Unknown ☐
- Your grandmother (mother's side)	Yes ☐	No ☐	Unknown ☐
- Your own children	Yes ☐	No ☐	Unknown ☐
- Your grandchildren	Yes ☐	No ☐	Unknown ☐
- Brothers or sisters of your father	Yes ☐	No ☐	Unknown ☐
- Brothers or sisters of your mother	Yes ☐	No ☐	Unknown ☐
- Your own brothers or sisters	Yes ☐	No ☐	Unknown ☐

Question 9: Are you currently in good health?

Yes ☐ No ☐

- If No: please, specify the diseases you currently have or previously had.

Disease	Diagnosed date (month/year)	Date of cure (month/year)

_

Question 10: Have you ever, or do you now suffer from a disease affecting your heart or blood vessels?

Yes ☐ No ☐

- If yes: please, specify the diseases affecting your heart or blood vessels. Please, provide for each disease the date (month/year) on which the first symptoms occurred, if applicable, the date (month/year) on which you were cured.

Disease	Starting date (month/year)	Date of cure (month/year)

_

Question 11: Have you ever, or do you now suffer from a disease affecting your kidneys or urinary tract?

Yes ☐ No ☐

- if yes: please, specify the diseases of your kidney or urinary tract. Please, provide for each disease the date (month/year) on which the first symptoms occurred, if applicable, the date (month/year) on which you were cured and the name of the doctor who cared for you.

Disease	Starting date (month/year)	Date of cure (month/year)

Question 12: Do you have diabetes?

Yes ☐ No ☐

- If yes:

- Do you follow a diet and avoid eating sweet foodstuffs? Yes ☐ No ☐
- Are you taking pills, which lower blood sugar? Yes ☐ No ☐
- Are you being treated with insulin injections? Yes ☐ No ☐

_

Question 13: Have you ever been told by a health professional that you have an elevated blood pressure?

Yes ☐ No ☐

- If yes:
- When was the diagnosis established for the first time: _____ (month/year)
- Have you ever received treatment for the elevated blood pressure? Yes ☐ No ☐

Question 14: Did you ever take or are you taking now drugs to lower your blood pressure?

Yes ☐ No ☐

- If yes:

- Please, specify the name of the drug(s) and the number of tablets that you are taking each day.

Name of drug	Tablets/day

Question 15: Have you ever taken drugs which eliminate salt and make you pass urine more frequently or in larger amounts?

Yes ☐ No ☐

- If yes:

- Please, specify the name of the drug(s) and the number of tablets that you are taking each day.

Name of drug	Tablets/day

Question 16: Have you ever take pain-killers on a regular basis, for instance against headaches, tooth pain, painful periods, etc.?

Yes ☐ No ☐

- If yes:

- During how many years? _____ years
- Please, specify the name of the tablets which you are currently taking (or were taking) in the past on a regular basis.

Name of pain-killer	Tablets/day

Question 17: Did you take medicines during the past 2 weeks?

Yes ☐ No ☐

- If yes: please, specify the drugs that you have been taken as well as the daily dose of each medicine.

Name of medicine	Amount/day

Question 18: Do you currently smoke?

Yes ☐ No ☐

- If yes: (If Yes: skip question 19)

- At what age did you start smoking regularly: _____
- Do you inhale the smoke? yes ☐ no ☐
- How much do you smoke:
  - ◊ Cigarettes with filter per day _____
  - ◊ Cigarettes without filter per day _____
  - ◊ Grams of tobacco per day for hand-rolled cigarettes _____
  - ◊ Grams of tobacco per week for pipe _____
  - ◊ Cigars per week _____

Question 19: Did you smoke in the past?

Yes ☐ No ☐

- If yes:

- At what age did you smoke for the first time? _____
- How much did you smoke in the past:
  - ◊ Cigarettes with filter per day _____
  - ◊ Cigarettes without filter per day _____
  - ◊ Grams of tobacco per day for hand-rolled cigarettes _____
  - ◊ Grams of tobacco per week for pipe _____
  - ◊ Cigars per week _____

- Please, specify the reason(s) why you stopped smoking.

_____

Question 20: Do you currently consume alcoholic beverages?

Yes ☐ No ☐

- If yes: (If Yes: skip question 21)

- How much alcohol do you currently consume?
  - ◊ Number of glasses of beer per day _____
  - ◊ Number of bottles of wine per week _____
- At what age did you start drinking alcohol regularly? _____

Question 21: Did you regularly consume alcoholic beverages in the past?

Yes ☐ No ☐

- If yes:

- At what age did you stop consuming alcohol regularly: _____
- How much alcohol did you consume in the past?
  - Number of glasses of beer per day _____
  - Number of bottles of wine per week _____

- Please, specify the reason(s) why you stopped consuming alcoholic beverages:

_____

_____

Question 22: Do you drink coffee or caffeine-containing beverages (cola) on a regular base?

Yes ☐ No ☐

- If yes:

- Specify the number of cups of coffee and the number of glasses of cola you consume on average per day.
  - Cups of coffee :_____
  - Glasses of cola:_____
  - Other: _____
- Do you use decaffeinated coffee? Yes ☐ No ☐
  - If yes, specify number of cups of decaffeinated coffee you drink on average per day: _____

_____

Question 23: Please, grade on a scale from 1 to 10 the physical efforts in your daily life, including your job, sports, and activities at leisure time.

_____ Points (please, provide a value ranging from 1 to 10)

A few examples:

- A civil servant who performs his work mainly seated, but who walks to his work place and in his leisure time engage in gardening could rate his physical efforts at 4-5 points.
- An older person who spends the whole day resting in his chair, could rate his physical effort at 1 point.
- A manual worker who each day has to load a truck with sand, just using a spade, cycles 20 km to and from his work place and in his leisure time has an additional job as a construction worker, could rate his physical efforts at 9-10 points.

_____

Question 24: Do you practice any sports activities on a regular basis?

Yes ☐ No ☐

- If yes:

  - Which sport(s) do you practice on a regular basis?_____
  - At what age did you start practicing sports regularly: _____
- At present, how many hours per week do you spend practicing sport:_____

_____

Question 25: Do you walk regularly?

Yes ☐ No ☐

- If yes:

- How many hours do you walk on average per day :_____

• How many kilometers do you walk per day: _____

Question 26: Please, grade on a scale from 1 to 10 the psychological tensions and stress that you are currently facing in your daily life.

_____ Points (please, provide a value from 1 to 10)

A few examples:

- A student, who is still dependent on his parents, maintains friendly relations with his fellow students and does not have to fear exams, because he is very bright, might rate his stress level at 1-2 points.
- A housewife who has to care for many children, who hardly gets her work done and who in addition has marital problems with her husband, could rate her stress level at 9-10 points.
- An employee with a quiet job, without problems in his family, but who is not completely sure that he will keep his job, and therefore feels unsecure, could rate his stress level at 5-6 points.

SECTION C

QUESTIONS 28-32 (FOR WOMEN ONLY)

Question 28: Did you already have your periods?

Yes ☐ No ☐

Question 29: Have you ever taken "The Pill"?

Yes ☐ No ☐

- If yes:

• Do you still take it now? Yes ☐ No ☐
• What is the name of the Pill you are taking: _____
• Overall, how long have you taken the pill for: _____(Years, months, etc.)

Question 30: Are you pregnant at present?

Yes ☐ No ☐

Question 31: Have you been pregnant before?

Yes ☐ No ☐

- If yes:

- How many times have you been pregnant? _____
- How many miscarriages did you have? _____
- How many children were born alive? _____
- How many children were stillborn? _____

Question 32: (Only for women older than 30 years)

Do you still have your periods?

Yes ☐ No ☐

- If no:

- Please, specify since when (month/year) your periods become irregular: _____

At present are your periods suppressed by taking "the pill"? Yes ☐ No ☐

- If yes: Please, specify the name of "the pill" and the number of months that you have been taking it:

Name:_____

Number of months:_____

SECTION D

DECLARATION

Please, provide your signature to attest your approval or disapproval to use this information in our study.

Signature: _____

Date: _____

The End

Appendix 3 Daily dietary intake

South African Hypertension and Diet Study (SAHDS)

Daily dietary intake

General information

Participant code	D	0	0	0	0	0

Date of birth	D	D	M	M	Y	Y	Y	Y	

Gender	M	F1= Male, 2= Female

For Fasting participants only:			
Type of fast			
Start date		End date	
Fasting period			
Day of fast on the date of clinical measurements			
Meals per day			

Please give a detailed description of your dietary information including portion sizes where possible & the time							
Day	**Date**	**Breakfast**	**Snack**	**Lunch**	**Supper**	**Beverages**	**Additional Information**
1							
2							
3							

4							
5							
6							
7							
8							

9							
10							
11							
12							
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14							
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16							
17							
18							

19							
20							
21							