THE ASSOCIATION OF BODY WEIGHT, 25-HYDROXY VITAMIN D, SODIUM INTAKE, PHYSICAL ACTIVITY LEVELS AND GENETIC FACTORS WITH THE PREVALENCE OF HYPERTENSION IN A LOW INCOME, BLACK URBAN COMMUNITY IN MANGAUNG, FREE STATE, SOUTH AFRICA.

RONETTE LATEGAN

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Ronette Lategan

Thesis submitted in fulfilment of the requirements for the PhD Dietetics in the Faculty of Health Sciences, Department of Nutrition and Dietetics, University of the Free State

PROMOTER: DR VL VAN DEN BERG

CO-PROMOTER: PROF CM WALSH

CO-PROMOTER: PROF CD VILJOEN

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I certify that the thesis hereby submitted by me for the Ph.D. (Dietetics) at the University of the Free State is my independent effort and had not previously been submitted for a degree at another university / faculty. I furthermore waive copyright of the thesis in favour of the University of the Free State.

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LIST OF ABBREVIATIONS

American Heart Association	AHA
Antidiuretic hormone	ADH
Angiotensinogen	AGT
Angiotensin converting enzyme	ACE
Angiotensin receptor blockers	ARB
Assuring Health for All in the Free State	AHA-FS
Body adiposity index	BAI
Calories	kcal
Committee on Medical Aspects of Food Policy	COMA
Department of Health	DOH
Dietary Approaches to Stop Hypertension	DASH
Dietary Reference Values	DRV
Dual-energy X-ray absorptiometry	DXA
European Group for the Study of Insulin Resistance	EGIR
Institute of Medicine	IOM
Integrated chip technology	ICT
Fluorescent treponemal antibody	FTA
Genome wide association scan	GWAS
G protein-coupled receptor kinase type 4	GRK4
Highly active antiretroviral therapy	HAART
Human Immunodeficiency virus	HIV
Institute of Medicine	IOM
The International Study of Salt and Blood Pressure	INTERSALT
Mangaung University Community Partnership Programme	MUCPP
National Diet and Nutrition Survey	NDNS
National Health and Nutrition Examination Survey	NHANES
The Third National Health and Nutrition Examination Survey	NHANES III
National Research Foundation	NRF
Non-communicable diseases	NCD
Physical activity level	PAL
Plasma 25-Hydroxy vitamin D	25(OH)D
Polymerase chain reaction	PCR
Potassium	K
Previous Day Physical Activity Recall	PDPAR
Profiles of Obese Women with the Insulin Resistance Syndrome	POWIRS
Reference Nutrient Intake	RNI

Renin-angiotensin-aldosterone system	RAAS
Single nucleotide polymorphism	SNP
South Africa	SA
Sodium	Na
STEPwise approach to Surveillance	STEPS
Tablespoon	Tbsp
Teaspoon	Tsp
Tolerable upper intake level	UL
Transition and Health During Urbanisation of South Africans	THUSA
United States	US
United States Department of Agriculture	USDA
Waist to height ratio	WHtR
World Health Organization	WHO

CHAPTER 1

ORIENTATION TO THE STUDY

1.1 BACKGROUND AND MOTIVATION

Hypertension is a public health problem responsible for a large and increasing proportion of the global disease burden (Couch & Krummel, 2008:865; Norman *et al.,* 2007:692). It is one of the leading causes of morbidity and mortality in middle-income countries, and is increasing in low-income countries (Norman *et al*., 2007:692). Morbidity and mortality related to undiagnosed, untreated, and/or uncontrolled hypertension also places a substantial strain on health care delivery systems (NIH, 2004:Online).

Under-diagnosis and/or inadequate treatment of hypertension may lead to extensive organ damage. Blood pressure is mainly influenced by cardiac output and peripheral resistance, with the sympathetic nervous system and the kidney (through the renin-angiotensin system) as the major role players. Blood pressure is therefore directly, consistently and continuously related to cardiovascular disease, independent of other risk factors; with increasing blood pressure increasing the risk of organ damage (Couch & Krummel, 2008:867). End-stage diseases associated with hypertension-induced organ damage include myocardial infarction, stroke, left ventricular hypertrophy, renal disease and blindness. Despite causing extensive organ damage, hypertension is dubbed the 'silent killer', as a sufferer can be asymptomatic for years and then suddenly experience a fatal stroke or heart attack (Steyn, 2006:80; Couch & Krummel, 2008:865; Ehret *et al*., 2008:1507).

Primary hypertension typically affects 90-95% of hypertensive individuals. Some cases of hypertension may however occur secondary to other, usually endocrine related, diseases. Whereas secondary hypertension may be curable by treating the underlying conditions, no absolute cure is presently available for primary hypertension (Couch & Krummel, 2008:865). Timely detection of primary hypertension, and lifestyle and dietary changes, often combined with medical treatment, are therefore vitally important to avoid the negative impact of this condition on health and quality of life, and/or premature deaths. Studies show that a reduction of just 3 mmHg in systolic blood pressure may lower the mortality risk for stroke and coronary heart disease by 8% and 5% respectively (Couch & Krummel, 2008:869).

The *National Health and Nutrition Examination Survey* (NHANES) reported an age-adjusted prevalence of 32.4%, 23.3%, and 22.6%, for hypertension among the black, white, and Mexican populations in the United States (US) for the period 1988-1991 (Burt *et al*., 1995:Online).

According to the American Heart Association (2008:4), 33.6% of the US population suffered from hypertension in 2005, with the highest prevalence among blacks (42.6% in males and 46.6% in females), while another third of the population was estimated to be pre-hypertensive (Appel, 2009:358). Figures released by the National Centre for Health Statistics (2011:Online) for US residents indicate that the total age adjusted prevalence of hypertension has increased from 25.5% in 1988-1994, to 31.2% in 2007-2008, with the highest prevalence among blacks (41.4% in males and 44.4% in females). A report from the American Heart Association (2010:Online) estimated the direct and indirect cost of hypertension in the US for 2010 to be $76.6 million.

In 1998, the first *Demographic and Health Survey* conducted in South Africa (SA), found that 23.9% of South Africans suffered from hypertension, with the age adjusted prevalence being 25.1% and 25.3% for men and women, respectively (Steyn, 2006:82; Steyn *et al*., 2001:1720). Regrettably the data relating to the prevalence of hypertension from the subsequent *2003 SA Demographic and Health Survey* were later found to be inaccurate (Department of Health, 2007:256). However, the *South African Stroke Risk in General Practice Study* (Connor *et al*., 2005:334) found that hypertension was the most common risk factor for stroke (55%) in all population groups visiting general medical practices, with the highest prevalence among black patients (59%). The *South African National Burden of Disease Study* also estimated that hypertension was responsible for 9% of all deaths (estimated at 46 888) in South Africa in 2000, making it the second leading cause of mortality after sexually transmitted diseases (Norman *et al*., 2007:6950).

The Heart of Soweto Study, undertaken at the Chris Hani Baragwanath Hospital, found that 46% of all black patients, who presented to the Cardiology Unit of the hospital, were hypertensive, with more females than males affected (Stewart *et al.,* 2011:23).

Mollentze *et al.* (1995:94) described the prevalence of hypertension in black adult males and females in Mangaung during 1991-1992, clearly demonstrating a strong age-related increase in blood pressure as indicated in Table 1.1.

Table 1.1 Prevalence of hypertension in Mangaung during 1991-1992 (Mollentze *et al.*, 1995:94, Table V)

Blood pressure category	**25-34 years**	**35-44 years**	**45-54 years**	**55-64 years**	**≥65 years**
≥ 160/95mmHg and/or treatment	12.4%	31.0%	61.2%	52.9%	78.1%
≥ 140/90 but < 160/95	9.5%	18.3%	10.7%	26.5%	12.5%
Total hypertensive	21.9%	49.3%	71.9%	79.4%	90.6%

The *Medical Research Council Report on Chronic Diseases of Lifestyle* concluded that hypertension in South Africa is "inadequately treated and poorly controlled" (Steyn, 2006:93). The detection, management and treatment of primary hypertension in South Africa therefore pose an enormous challenge.

In order to successfully address hypertension as a health problem, the aetiology and risk factors need to be well understood. The current aetiological model of hypertension involves a complex interplay of genetic and environmental factors.

The prevalence of hypertension increases with aging, although the problem occurs at all ages. Several dietary and lifestyle factors play a role in determining the risk for hypertension. A strong relationship exists between body weight and the risk for hypertension, with the prevalence of hypertension being two to six times higher in overweight than normal weight individuals. It is estimated that thirty percent and more of hypertensive cases can be ascribed to obesity. Furthermore, the increase in blood pressure associated with aging also correlates to the age-related increases in body weight, while weight loss results in lowered blood pressure. The mechanism of obesity-induced hypertension may be attributed to over-activation of the sympathic nervous system, the renin-angiotensin system, and elevated inflammatory pathways (Couch & Krummel, 2008:870-871; Steyn, 2006:84).

Various studies have shown an inverse relationship, often age related, between vitamin D status, measured as serum 25-hydroxy vitamin D (25(OH)D) levels, and systolic blood pressure. Lower circulating levels of 25(OH)D are associated with an increased risk of hypertension (Judd *et al.,* 2008:140; Forman *et al*., 2007:1068; Li *et al*., 2004:388). The mechanism involves the role of vitamin D as a negative regulator of the renin gene (Rammos *et al.,* 2008:Online). Vitamin D deficiency seems to increase blood pressure by increasing the expression of renin, which in turn increases activiation of angiotensin II, and subsequently causes vasoconstriction and retention of sodium and water in the kidney (Rammos *et al.,* 2008:Online; Li *et al*., 2004:387).

Reducing dietary sodium intake is regarded as an effective way to lower blood pressure. Salt (sodium) -sensitive hypertension refers to blood pressure that rises or falls with corresponding changes in dietary sodium intake (Couch & Krummel, 2008:865). Population studies have shown a positive association between sodium intake and blood pressure over a wide range of sodium intakes (Couch & Krummel, 2008:872; Norat *et al.*, 2008:395; Appel, 2009:360). Various studies point to a greater effect of sodium intake on blood pressure in black populations, as well as in middle- and older-aged individuals, and indicate that genetic and other dietary factors (such as potassium intake) may influence blood pressure response to sodium intake (Appel, 2009:360). On the other hand, higher intakesrenin of potassium are associated with lower blood pressure levels, and dietary potassium has been shown to have a powerful, dose-dependent inhibitory effect on

sodium sensitivity (Adrogué & Madias, 2007: 1968). Potassium intakes can be increased by increased intakes of fruits and vegetables (He & MacGregor, 2001: 497).

It is widely recognized that less active people are 30-50% more likely to develop hypertension than people who are active (Couch & Krummel, 2008:872; Lambert *et al.*, 2001:S14). Increasing activity levels, especially from being inactive to being moderately active, has been shown to be a valuable approach to the prevention and treatment of hypertension (Lambert, *et al.*, 2001: S13).

Essential hypertension, like many other diseases, is influenced by a variety of genes, with the risk of the disease being determined by small quantitative changes in the expression of various different genes, combined with environmental factors (Sookoian *et al.*, 2007:5). A considerable number of gene variants have been studied as candidates to determine the risk of hypertension. The majority of these genes influence blood pressure by controlling the amount of sodium and water reabsorbed in the kidney (Cummings, 2006:110).

The *angiotensinogen (AGT)* gene is responsible for manufacturing the protein AGT in the liver, which on activation to angiotensin by renin in the kidney, controls sodium and water retention to raise blood pressure (Cummings, 2006:110). An A/G nucleotide polymorphisms at the -217 and -793 promotor region of the *AGT* gene has been found to be associated with the prevalence of hypertension, especially in the male African American population (Jain *et al.,* 2002: 36889; Markovic *et al*., 2005:92). The polymorphism caused by a substitution of threonine to methionine at amino acid chain position 235 (M235T) is also associated with an increase in risk for hypertension. A meta analysis by Staessen *et al.* (1999:9), which included 69 studies and a total of 27 906 subjects, confirmed the presence of the T allele as a marker for hypertension in Caucasians - with individuals homozygous for TT having a 31% (p=0.001) greater risk, and TM heterozygotes having 11% (p=0.03) greater risk, than MM homozygotes.

The hormone, dopamine, influences blood pressure by increasing sodium excretion in the kidney (Prasad *et al*., 2008:2) and a defect in the signaling of the renal dopamine receptor has been shown to play a role in hypertension (Sen *et al*., 2005:1206). Polymorphisms of the gene, *G protein-coupled receptor kinase type 4* (*GRK4*), are associated with salt-sensitivity and a type of hypertension marked by low renin levels. The *GRK4* variants, R65L (G448T), A142V (C679T) and A486V (C1711T), have been shown to predict salt sensitivity correctly in 94.4% of cases in a Japanese population (Sanada *et al*., 2006:356). These three *GRK4* alleles are thought to increase the expression of *GRK4* protein in the kidney, which disrupts the function of dopamine receptors, leading to impaired renal dopamine-induced sodium excretion, even in the absence of hypertension (Sanada *et al.*, 2006:358; Winstead, 2002:Online). In the same Japanese population, the *GRK4* A142V genotype as a single indicator was 78.4% predictive of salt sensitivity, and the 2-

locus model of *GRK4* A142V and *CYP11B2* C-344T was 77.8% predictive of low-renin hypertension (Sanada *et al.*, 2006:356).

Aldosterone is synthesized in the adrenal glands by the enzyme aldosterone synthase which is encoded by the *CYP11B2* gene (Rajan *et al.*, 2010:379). The C-344T single nucleotide polymorphism (SNP) in the promoter of this gene is associated with an increased risk for hypertension, and the -344C allele with a decreased risk for hypertension (Sookoian *et al*., 2007:7; Rajan *et al.*, 2010:382).

In the future, the ability to genetically screen communities for alleles associated with essential hypertension may allow the early detection of salt sensitive individuals. However, although genetic composition plays an important role in determining the pre-disposition for hypertension, environmental factors, including the dietary and lifestyle factors discussed above are equally important to determine the expression of these genes (Cummings, 2006:110). Lifestyle changes that are effective in the prevention and treatment of hypertension include weight-loss in overweight subjects, decreased alcohol intake, increased consumption of fruit, vegetables and low fat dairy products, reduced intakes of fat (especially saturated fat and cholesterol), reduced intake of dietary sodium, increased physical activity, and cessation of smoking (WHO/ISH, 2003:1987; NIH, 2004:Online; Seedat *et al*., 2006:343; Couch & Krummel, 2008:869).

Studies have shown that hypertension is not just more prevalent, but also more severe in black populations compared to whites, and is associated with a greater degree of target-organ damage for any given blood pressure level (Lindhorst *et al*., 2007:244). Four specific issues were raised in a recent consensus statement on the treatment of hypertension in blacks, namely the high prevalence of hypertension; the occurrence of severe hypertension (>180/110 mm Hg); poor blood pressure control over time; and the high prevalence of co-morbid conditions in this population group (Flack *et al*., 2010:781). In light of various lines of evidence which suggest that blacks may tend to retain more sodium in the kidney than whites (making them more salt sensitive), Lindhorst *et al.* (2007:245) pointed out in a narrative review on the issue, that it would be “reasonable to conclude that an acquired or inherited predisposition toward salt retention provides a basis for differences in blood pressure between blacks and whites”. Similarly Opie and Seedat (2005:3562) concluded that more studies are needed on black Africans as they might be genetically and environmentally different from black Americans.

1.2 PROBLEM STATEMENT

The Assuring Health for All in the Free State (AHA-FS) study is a prospective and longitudinal epidemiological study, conducted by the Department of Nutrition and Dietetics, University of the Free State, South Africa, which was launched in 2007 with funding from the National Research

Foundation (NRF). The aim of AHA-FS is to determine how living in urban and rural areas influences the lifestyles of populations in ways that may predispose them to both chronic diseases (such as obesity, diabetes and cardiovascular disease) as well as under nutrition. During 2007, baseline data for the rural area were collected in Springfontein, Trompsburg and Philippolis. (Walsh *et al*., 2006:2,7). During 2009 the baseline data for the urban area were collected in the Mangaung area of Bloemfontein, specifically in the township areas of Freedom Square, Turflaagte, Namibia, Kagisanong, Chris Hani and the Rocklands Buffer area.

The urban survey included 431 adults, with ages between 25 and 63 years and a mean age of 44.4 years ±10.7 (SD). Hypertension (≥140/90mmHg) was diagnosed in more than half (56.87%) of this population (51% of the males and 58.7% of the females) with the mean systolic and diastolic blood pressure measurements being 135.5 ±23.9 (SD) mmHg and 89.8 ±17.4 (SD) mmHg, respectively. This high prevalence of hypertension, often in the presence of medical treatment, motivated the current study which aimed to investigate selective factors that could affect blood pressure levels and which, if addressed, could assist in the prevention of hypertension in this community.

1.3 AIM AND OBJECTIVES

The main aim of the study was to determine the association of body weight, serum 25-hydroxy vitamin D, sodium and potassium intakes, physical activity levels and genetic factors, to the prevalence of hypertension in a low income, black urban community in Mangaung, Free State, South Africa.

1.3.1 Objectives

In order to achieve this aim the objectives were to determine:

1.3.1.1 the association between blood pressure and

i. body weight;
ii. serum 25-hydroxy vitamin D levels;
iii. sodium and potassium intake;
iv. levels of physical activity; and

1.3.1.2 the presence of specific gene variants linked to hypertension in this community.

1.4 STRUCTURE OF THIS THESIS

This thesis is structured as a series of articles organised according to the aims and objectives of the research study. Chapter 2 provides a general literature overview of variables researched in the

study, as well as other relevant information. Chapter 3 describes the methodology followed in the study. Chapters 4 to 8 consist of five articles describing in turn the effects of body weight, 25(OH) D, sodium and potassium intakes, activity level and genetics, on blood pressure, according to the objectives of this study. Chapter 9 summarises the conclusions and the recommendations for interventions based on the findings of this study.

1.5 BIBLIOGRAPHY

Adrogué HJ and Madias NE. 2007. Sodium and potassium in the pathogenesis of hypertension. The New England Journal of Medicine, 356:1966-1978.

American Heart Association (AHA). 2008. American Heart Association. Heart Disease and Stroke Statistics – 2008 Update. [Online]
Available from: http://www.americanheart.org/statistics [Accessed January 19th, 2009].

American Heart Association (AHA). 2010. American Heart Association. Heart Disease and Stroke Statistics – 2010 Update: A report from the American Heart Association. [Online]
Available from: http://circ.ahajournals.org/cgi/reprint/121/7/e46 [Accessed June 21st, 2010].

Appel LJ. 2009. DASH Position paper: Dietary approaches to lower blood pressure. The Journal Of Clinical Hypertension, 11 (7): 358-368.

Burt VL; Whelton P; Roccella EJ; Brown C; Cutler JA; Higgins M; Horan MJ and Labarthe D. 1995. Prevalence of Hypertension in the US Adult Population: Results From the Third National Health and Nutrition Examination Survey, 1988-1991. Hypertension, 25:305-313.

Connor M, Rheeder P, Bryer A, Meredith M, Beukes M, Dubb A and Fritz V. 2005. The South African stroke risk in general practice study. South African Medical Journal, 95 (5): 334-339.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Cummings MR. 2006. Human heredity – principles and issues. 7th edition. Belmont: Thomson Brooks/Cole.

Department of Health. 2007. South Africa Demographic and Health Survey 2003, Full Report. Pretoria: Department of Health.

Ehret GB, Morrison AC, O'Connor AA, Grove ML, Baird L, Schwander K, Weder A, Cooper RS, Rao DC, Hunt SC, Boerwinkle E and Chakravarti A. 2008. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. European Journal of Human Genetics, 16: 1507–1511.

Flack JM, Sica DA, Bakris G, Brown AL, Ferdinand KC, Grimm RH(Jr), Hall WD, Jones WE, Kountz DS, Lea JP, Nasser S, Nesbitt SD, Saunders E, Scisney-Matlock M, Jamerson KA, on behalf of the International Society on Hypertension in Blacks. 2010. Management of High Blood Pressure in Blacks: An Update of the International Society on Hypertension in Blacks Consensus Statement. Hypertension, 56: 780-800. [Online]
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/780 [Accessed November 9th, 2010].

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC and Curhan GC. 2007. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension, 49: 1063-1069.

He FJ and MacGregor GA. 2001. Beneficial effects of potassium. British Medical Journal, 323: 497–501.

Jain S, Tang X, Narayanan CS, Agarwal Y, Peterson SM, Brown CD, Ott J and Kumar A. 2002. Angiotensinogen gene polymorphism at _217 affects basal promoter activity and is associated with hypertension in African-Americans. The Journal of Biological Chemistry, 277, 39: 36889–36896.

Judd SE, Nanes MS, Ziegler TR, Wilson PWF and Tangpricha V. 2008. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. American Journal of Clinical Nutrition, 87: 136-141.

Lambert EV, Bohlmann I and Kolbe-Alexander T. 2001. Be active – physical activity for health in South Africa. South African Journal of Clinical Nutrition, 14 (3): S12-S16.

Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W and Kong J. 2004. Vitamin D: a negative endocrine regulator of the rennin-angiotensin system and blood pressure. The Journal of Steroid Biochemistry and Molecular Biology, 89-90: 387-392.

Lindhorst J, Alexander N, Blignaut J and Rayner B. 2007. Differences in hypertension between blacks and whites: an overview. Cardiovascular Journal of Africa, 18 (1): 241-247.

Markovic D, Tang X, Guruju M, Levenstien MA, Hoh J, Kumar A and Ott J. 2005. Association of angiotensinogen gene polymorphisms with essential hypertension in African-Americans and Caucasians. Human Heredity, 60: 89-96.

Mollentze WF, Moore AJ, Steyn AF, Joubert G, Steyn K, Oosthuizen GM and Weich DJV. 1995. Coronary heart disease risk factors in a rural and urban Orange Free State black population. South African Medical Journal; 85: 90-96.

National Center for Health Statistics. 2011. Health, United States, 2010: With Special Feature on Death and Dying. [Online].
Available from: http://www.cdc.gov/nchs/data/hus/hus10.pdf#066 [Accessed March 4th , 2011].

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009]

Norat T, Bowman R, Luben R, Welch A, Khaw KT, Wareham N and Bingham S. 2008. Blood pressure and interactions between the angiotensin polymorphism AGT M235T and sodium intake: a cross-sectional population study. American Journal of Clinical Nutrition, 88: 392-397.

Norman R, Gaziano T, Laubscher R, Steyn K, Bradshaw D and the South African Comparative Risk Assessment Collaborating Group. 2007. Estimating the burden of disease attributable to high blood pressure in South Africa in 2000. South African Medical Journal, 97 (8): 692-698.

Opie LH and Seedat YK. 2005. Hypertension in Sub-Saharan African populations. Circulation, 112: 3562-3568.

Prasad P, Kumar KMP, Ammini AC, Gupta A, Gupta R and Thelma BK. 2008. Association of dopaminergic pathway gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. BMC Genetics [Online], 9:26
Available from: http://www.biomedcentral.com/1471-2156/9/26 [Accessed January 20th, 2009].

Rammos G, Tseke P and Ziakka S. 2008. Vitamin D, the renin-angiotensin system, and insulin resistance. International Urology and Nephrology [Online].
Available from: http://www.springerlink.com.proxy.lib.fsu.edu/content/ap8281748l28474p/fulltext.pdf [Accessed May 1st , 2011].

Rajan S, Ramu P, Umamaheswaran G and Adithan C. 2010. Association of aldosterone synthase (CYP11B2 C-344T) gene polymorphism and susceptibility to essential hypertension in a south Indian Tamil population. Indian Journal of Medical Research, 132: 379-385.

Sanada H, Yatabe J, Midorikawa S, Hashimoto S, Watanabe T, Moore JH. Ritchie MD, Williams SM, Pezzullo JC, Sasaki M, Eisner GM, Jose PA and Felder RA. 2006. Single-nucleotide polymorphisms for diagnosis of salt-sensitive hypertension. Clinical Chemistry, 52 (3): 352-360.

Seedat YK, Croasdale MA, Milne FJ, Opie LH, Pinkney-Atkinson VJ, Rayner BL and Veriava Y. 2006. South African hypertension guideline 2006. South African Medical Journal, 96 (4): 337-362.

Sen S, Nesse R, Sheng L, Stoltenberg SF, Gleiberman L, Burmeister M and Weder AB. 2005. Association between a dopamine-4 receptor polymorphism and blood pressure. American Journal of Hypertension, 18 (9): 1206-1210.

Sookoian S, Gianotti TF, González CD and Pirola CJ. 2007. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. Journal of Hypertension, 25:5–13.

Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R and Fagard R. 1999. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. Journal of Hypertension, 17: 9–17.

Stewart S, Libhaber E, Carrington M, Damasceno A, Abbasi H, Hansen C, Wilkinson D and Sliwa K. 2011. The clinical consequences and challenges of hypertension in urban-dwelling black Africans: Insights from the Heart of Soweto Study. International Journal of Cardiology, 146: 22-27.

Steyn K. 2006. Hypertension in South Africa, In Chronic diseases of lifestyle: 1995-2005. Technical report. Ed. by Steyn K, Fourie J and Temple N. Cape Town: South African Medical Research Council: 80-96.

Steyn K, Gaziano TA, Bradshaw D, Laubscher R and Fourie J. 2001. Hypertension in South African adults: results from the Demographic and Health Survey, 1998. Journal of Hypertension, 19: 1717-1725.

Walsh C, Van der Merwe L, Van Zyl S, Van Wyk H, Groenewald D and Joubert G. 2006. Protocol: Assuring Health for All (AHA) in the Free State. Bloemfontein: University of the Free State.

Winstead ER. 2002. Gene variants linked to inherited forms of hypertension. Genome News Network [Online].
Available from: http://www.genomenewsnetwork.org [Accessed March 13th , 2008].

World Health Organization; International Society of Hypertension Writing Group (WHO/ISH). 2003. WHO/ISH statement on management of hypertension. Journal of Hypertension, 21: 1983–1992.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Hypertension is defined as a persistent high arterial blood pressure, with systolic pressure (blood pressure during cardiac contraction) 140mmHg or higher and/or diastolic pressure (blood pressure during the relaxation phase) 90mmHg or higher (Couch & Krummel, 2008:866; Seedat *et al*., 2006:337; WHO/ISH, 2003:1983, AHA, 2011:Online). The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (NIH, 2004:Online) classifies hypertension as normal, pre-hypertension, stage 1 hypertension and stage 2 hypertension, in order to guide management at various blood pressure levels, as set out in Table 2.1. For classification, the mean of two or more measurements should be taken in a seated position on each of two or more occasions.

Table 2.1 Classification of blood pressure in adults aged 18 years and older (NIH, 2004:Online, Table 3)

Blood pressure classification	**Systolic blood pressure mm Hg**	**Diastolic blood pressure mm Hg**
Normal	<120	and <80
Pre-hypertension	120-139	or 80-89
Stage 1 hypertension	140-159	or 90-99
Stage 2 hypertension	≥160	or ≥100

The use of prescription medication to manage hypertension is often also included as a diagnostic criterion for hypertension, even if the blood pressure is normal as a result of the medication (Wallace *et al.*, 2007:51; Grundy *et al.*, 2005:2739-2741; Nelms *et al.,* 2011:288).

From the Framingham Heart Study, Vasan *et al*. (2002:1006) reports a residual lifetime risk of 90% for developing hypertension in adults that were non-hypertensive at the age of 55 or 65 and surviving to age 80-85. This predicts a growing public health burden for health authorities in countries with aging populations.

The World Health Report of 2008 rates elevated blood pressure as one of the top ten risk factors of overall disease burden and projects an increase in non-communicable diseases and accidents as causes of death in the future (WHO, 2008:8) as indicated in Figure 2.1.

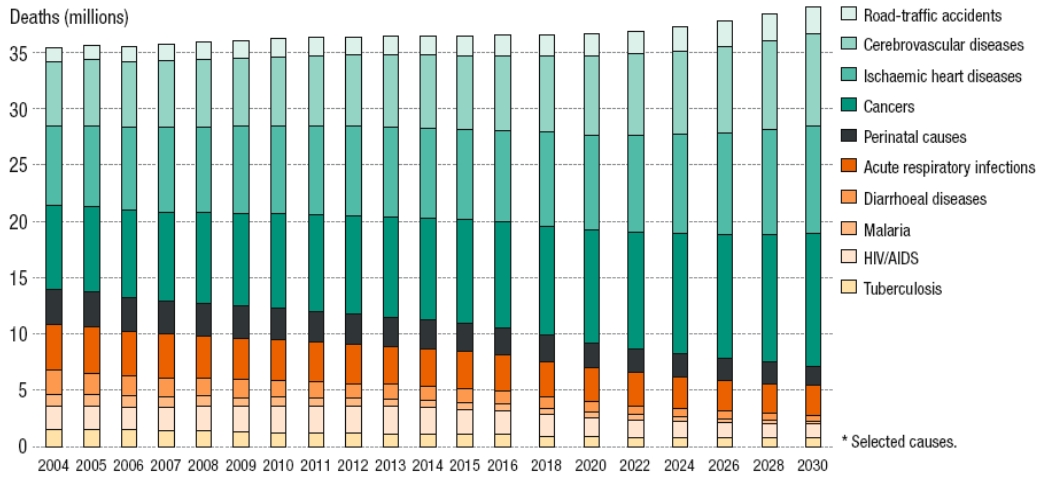

Figure 2.1 The projected shift towards non-communicable diseases and accidents as causes of death (WHO, 2008:8, Figure 1.8)

Left ventricular hypertrophy is one of the structural consequences of hypertension, and second only to advancing age, also the strongest predictor known for cardiovascular morbidity and mortality (Bacon *et al.*, 2004:307; Meijs *et al.*, 2007:295). With treatment however, hypertrophy of the ventricle is reversible, with an accompanying reduction in cardiovascular risk (Okin *et al.*, 2004:2347; Devereux *et al.*, 2004:2353).

In a meta-analysis of 61 studies with data for one million adults, it was found that an increase of 20mmHg systolic blood pressure or 10mmgHg usual diastolic blood pressure, was associated with more than a twofold increase in death rate from stroke and a twofold increase in death from ischemic heart disease and other vascular causes (Prospective Studies Collaboration, 2002:1903). Usual blood pressure was found to be strongly and directly related to vascular and overall mortality, without evidence of a threshold, at least to 115/75 mmHg (Prospective Studies Collaboration, 2002:1903). When pooling data from randomized control trials, He and Whelton (1999: S219) found that an average reduction of 12-13 mmHg in systolic blood pressure over a 4 year period was associated with a 21% reduction in coronary heart disease, 37% reduction in stroke, 25% reduction in total cardiovascular mortality and a 13% reduction in all-cause mortality rates, indicating that systolic blood pressure is an independent and strong predictor of cardiovascular disease risk.

Hypertension is associated with coronary heart disease, heart failure, chronic kidney disease, stroke or transient ischemic attacks, peripheral arterial disease and advanced retinopathy (NIH, 2004:Online; Seedat *et al*., 2006:337). However, any increase in blood pressure above the normal range of 120/80 mmHg is associated with an increase in the incidence of cardiovascular disease and renal disease, emphasising the importance of early prevention and effective treatment (Couch

& Krummel, 2008:866). Populations of African descent have significantly higher rates of hypertension than European populations, with an increased risk for stroke (Agyemang *et al.*, 2009:5). Whelton *et al*. (2002:1884) reported the considerable effect that the lowering of blood pressure levels has on mortality rates, as indicated in Table 2.2.

Table 2.2 The effect of a reduction in blood pressure on mortality rate (Whelton *et al*., 2002:1884)

Reduction in blood pressure (mmHg)	Reduction in mortality rate (%)		
	Stroke	Coronary Heart Disease	Total
2	-6	-4	-3
3	-8	-5	-4
5	-14	-9	-7

2.2 MECHANISMS OF BLOOD PRESSURE CONTROL IN THE BODY

Cardiac output and the resistance of peripheral blood vessels to the flow of blood determine blood pressure in the human body. When the diameter of blood vessels is decreased, blood pressure increases and when the diameter is increased, resistance is lower, resulting in a lower blood pressure. Blood pressure needs to be regulated cautiously to ensure that it is high enough to force blood through the systemic circulation, but not too high as to cause vascular damage (Nelms *et al.,* 2011:287; Couch & Krummel, 2008:869). Blood pressure control is regulated by the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS) and renal function, all three affecting cardiac output and therefore blood pressure (Nelms *et al.,* 2011:287). The sympathetic nervous system is responsible for short-term blood pressure control and the kidney for long-term control. A drop in blood pressure and/or blood volume is registered by baroreceptors in the jugular arteries which send sympathetic nerve messages to the adrenal glands to secrete norepinephrine. Norepinephrine is a potent vasoconstrictor, leading to constriction of small arteries and arterioles resulting in an increase in blood pressure (Couch & Krummel, 2008:869). The parasympathetic nervous system on the other hand decreases heart rate through the indirect release of acetylcholine (Nelms *et al.,* 2011:287).

Simultaneously a drop in blood pressure or blood volume or an increase in extracellular fluid osmolality is registered by baroreceptors in blood vessels, as well as by osmoreceptors in the hypothalamus. Both these mechanisms trigger a hypothalamic reaction which controls thirst and signals the posterior pituitary gland to release the hormone arginine vasopressin, previously known

as antidiuretic hormone (ADH). Vasopressin stimulates the kidney to reabsorb water without stimulating sodium retention as well. Water retention increases blood volume and thus blood pressure while decreasing the osmolality of the extra cellular fluid. Vasopressin also acts as a vasoconstrictor causing increased blood pressure (Nelms *et al*, 2011:124;288).

Blood pressure is however, mainly regulated by the kidney. The juxtaglomerular cells register a drop in arterial blood pressure or blood volume and reacts by secreting renin into the circulation. Renin activates the renin-angiotensin system by converting circulating angiotensinogen, which is mainly produced in the liver and in adipose tissue (Cooper *et al.*, 1998:571), with smaller quantities produced in the kidney, brain, heart, adrenal gland, and vascular walls (Jain *et al.*, 2002, 36889), to the decapeptide angiotensin I. Angiotensin I in turn is converted to the active hormone angiotensin II, by angiotensin converting enzyme (ACE) which removes a C-terminal dipeptide (Jain *et al.,* 2002:36889). This step takes place in the pulmonary circulation since ACE concentrations are high in the lungs (Nelms *et al*., 2011: 288). Angiotensin II then increases blood pressure by means of two mechanisms. The first is by direct vasoconstriction of arterioles, which increases peripheral resistance, and results in increased blood pressure. The second mechanism is by acting on the adrenal cortex to facilitate aldosterone secretion, which increases sodium and chloride reabsorption, causing water retention in the kidneys, resulting in an increase in blood volume, and therefore increased blood pressure (Jain *et al.,* 2002:36889; Couch & Krummel, 2008:869; Nelms *et al.,* 2011:287). The increase in blood pressure in return inhibits renin and aldosterone release, preventing blood pressure to increase further. Figure 2.2 summarises basic blood pressure control through the RAAS.

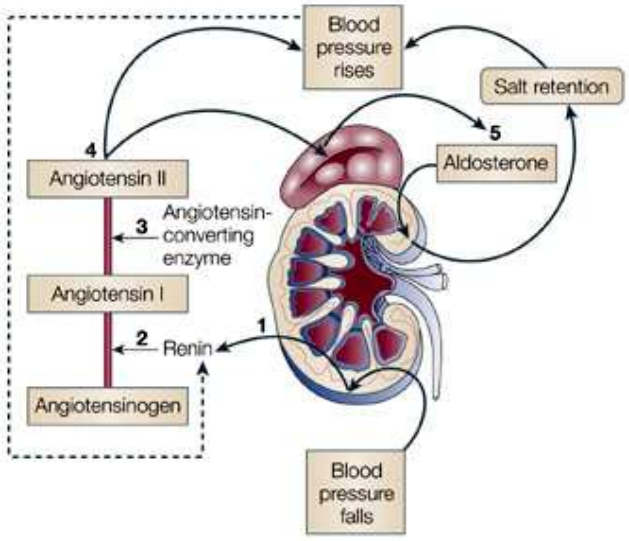

Figure 2.2 The renin-angiotensin-aldosterone system (RAAS) (Nature, 2009:Online)

2.3 ETIOLOGY

Hypertension is a complex disorder with many risk factors that influence its pathogenesis. Blood pressure levels are determined by an interaction between internal disorders, primarily involving the kidney, several genetic influences, and a variety of environmental factors (Wallace *et al.*, 2007:49; Adrogué & Madias, 2007:1966). Hypertension can be classified as primary or essential hypertension, where the cause is not known, and secondary hypertension, where the cause is identifiable and may be diagnosed biochemically (Gaw *et al*., 2008:134).

2.3.1 Secondary Hypertension

Various clearly identifiable causes of secondary hypertension and the appropriate diagnostic tests in each case are summarised by the National Institutes of Health, National Heart, Lung and Blood Institute (NIH, 2004:Online) and Caw *et al.* (2008:134), as portrayed in Table 2.3.

Table 2.3 Identifiable causes of secondary hypertension and appropriate diagnostic tests (NIH, 2004:Online, Table 8; Caw *et al.*, 2008:134)

Identifiable causes of hypertension	**Diagnostic test**
Chronic kidney disease	Reduced estimated glomerular filtration rate and/or proteinuria
Coarctation of the aorta	Computed tomography angiography
Renal artery stenosis	Magnetic resonance angiography. Associated with elevated renin concentrations.
Cushing's syndrome and other glucocorticoid excess states including chronic steroid therapy	History; dexamethasone suppression test
Drug induced / related	History; drug screening
Pheochromocytoma	24-hour urinary metanephrine and normetanephrine
Primary aldosteronism and other mineralocorticoid excess states	24-hour urinary aldosterone level or specific measurements of other mineralocorticoids
Renovascular hypertension	Dopper flow study; magnetic resonance angiography
Obesity / Sleep apnea	Sleep study with O_2 saturation and increased neck circumference
Thyroid / parathyroid disease	Blood levels of thyroid-stimulating hormone / parathyroid hormone

Among these secondary causes of hypertension, primary aldosteronism may be highlighted. The sodium and water retaining hormone, aldosterone, is secreted in higher than required amounts in reaction to the current blood volume and sodium status, independent of the renin-angiotensin system. This often results not only in an increase in blood pressure, but also in other cardiovascular risks. The availability and wider use of plasma aldosterone/renin ratio as a diagnostic test for the prevalence of primary aldosteronism has shown that this treatable cause of hypertension has a higher prevalence (5-13%) and should be considered when treating patients with hypertension (Stowasser *et al*., 2010:39). A message and warning to health care providers from the study of Du Cailar *et al*. (2010: 868) however, is to be aware that organ damage can still occur, despite reasonably good blood pressure control through pharmacological blockade of the renin-angiotensin system, because of the combined adverse effects of a high dietary sodium intake and breakthrough of aldosterone.

2.3.2 Primary (Essential) Hypertension

The etiology of primary hypertension is not completely understood, but seems to be related to a complex interplay of genetic factors and environmental and lifestyle factors which contribute to intra-uterine adaptations, the development of insulin resistance, and inflammatory changes in the kidney.

2.4 Pathophysiology of hypertension

2.4.1 Genetic influence

Although members of the human species is genetically closely linked, there are important differences in the individual genome. Mutations, deletions and additions in certain genes that cause the absence or dysfunction of the proteins manufactured by them, can lead to specific disease conditions. There are also other site specific differences throughout the genome, called single-nucleotide polymorphisms (SNPs), which might not be expressed by causing changes in the amino acid that is produced from the codon. SNPs typically lead to a change in function of a protein, rather than severe impairment or total loss of function and can therefore be quite common in the genetic profile of a community (Barnes, 2008: 1890).

Essential hypertension has been estimated to be about 30-50% heritable (Felder *et al*., 2002:3872). In a study of European American and African American twins, systolic blood pressure was estimated to be 57% heritable for both groups and diastolic blood pressure 45% and 58% heritable in the European and African groups, respectively (Snieder *et al*., 2003:1199). Jain *et al*. (2002:36889) estimates that about 45% of the differences in blood pressure between people can be accounted for by genetic differences and Bengra *et al. (2002:2132)* estimates the genetic origin

of essential hypertension at 30-50%. The influence of genetics in addressing the problem of hypertension in a community can therefore not be ignored. Stowasser *et al.* (2010:39) supports the value of genetic testing, especially for the 11 beta-hydroxylase/aldosterone synthase gene to detect familial hyperaldosteronism.

Wallace *et al. (*2007:50) recommends that when research is done to determine the genetic impact on hypertension, individuals with known causes of hypertension, like diabetics, older- and obese individuals, be excluded, to ensure that the hypertensive individuals being studied are more likely to carry genetic variants causing hypertension rather than other non-genetic factors that could contribute to hypertension.

Chronic diseases such as hypertension are likely the result of more than one gene and multiple variants of each gene that interacts with different environmental factors, with each combination making a small contribution to overall homeostasis, function, and therefore health (DeBusk *et al*., 2005:591; Ehret *et al.,* 2008: 1508). In their study amongst 1017 African American adults, Adeyemo *et al*. (2009:Online) found that a genome wide association scan (GWAS), using more than 800 000 genetic markers, found a significant correlation with systolic blood pressure for only five genes. Of the five genes, only two were linked to known pathways having an influence on hypertension. The low success rate of using GWAS to identify contributing genes in the etiology of hypertension may be evidence to the possibility that hypertension might rather be modulated by a larger number of low-risk variants, each with a small effect and low penetrance in comparison to genes causing other diseases (Adeyemo *et al.,* 2009:Online).

Other research shows that multiple genes are implicated in the prevalence of hypertension and although all of these genes have not yet been identified, evidence suggests that they are distributed amongst many chromosomes (Wallace *et al.*, 2007:49). To be relevant, Teo, Small and Kwiatkowski (2010: 150) recommend that GWAS should be done in specific regions, not only to ensure relevance to the local health problems, but also to take into account the specific environmental conditions, such as rural or urban habitat, sanitation, diet, activity and other lifestyle factors as well as exposure to infections.

Although it is recognized that an array of genetic factors are responsible for the onset and development of hypertension, polymorphisms of the *Angiotensinogen* (*AGT*), *Aldosterone synthase* (*CYP11B2*) and *G protein-coupled receptor kinase type 4* (*GRK4*) genes were investigated for the purpose of this study, and will be further discussed.

2.4.1.1 Polymorphism of the *angiotensinogen* (*AGT*) gene

As discussed in the pathophysiology of hypertension, angiotensin I is produced after interaction of renin with angiotensinogen, which in return is converted to angiotensin II that plays an important role in blood pressure regulation. Genetic variations of the *AGT* gene impact on the plasma concentration of angiotensinogen, which in turn influences blood pressure (Zafarmand *et al*., 2008:e2533). The *angiotensinogen* gene is among several genes shown to be linked to hypertension, with strong support for its role in the pathogenesis of essential hypertension (Norat *et al.*, 2008:392). In humans the *angiotensinogen* gene is found in the chromosomal region 1q42-43 and includes five exons and four introns (Staessen *et al.*, 1999: 9). Research by Markovic *et al.* (2005:94) favours the hypothesis that the promoter region of *AGT* is associated with essential hypertension although the exact location and nature is not clear. Various molecular variants exist, with typical variants at base positions -6,-20,-217,-793 and -776 often researched (Markovic *et al*., 2005:89). The haplotype AAAAT for base positions -6,-20,-217,-793 and -776 for the AGT gene seems to indicate an increased risk for essential hypertension in black males and females as well as white females (Markovic *et al.,* 2005:94). A study conducted by Jain *et al*. (2002:36889) also shows that an *angiotensinogen* polymorphism at -217 with nucleoside A affects basal promoter activity and is significantly associated with hypertension in African-Americans, but not in Caucasians. Tiago and co-workers (2002:1484) failed to find an association between the -20A→C variant of the *AGT* gene and hypertension in a South African study that included 521 black subjects, but found that the presence of the -20A→C allele influenced body size to blood pressure relationship in hypertensive individuals (Tiago *et al.*, 2002:1486).

Identification of the *AGT* G-6A polymorphism offers challenges and the tightly linked M235T polymorphism is often effectively assessed as surrogate with the T allele corresponding to the A allele of *AGT* G-6A (Norat *et al.*, 2008:392-393, 396). The *AGT* polymorphism that encodes threonine instead of methionine (M235T) caused by a T→C single-nucleotide polymorphism (SNP) at codon 235 in the proximal promoter has therefore been extensively studied to investigate possible relationships with hypertension (Staessen *et al.*, 1999:9; Norat *et al.*, 2008:392). Pratt *et al.* (1998:878) describes the significant effect of the T235 gene haplotype on serum AGT levels, even when correcting for race, gender, age and BMI. In a meta-analysis including a total sample size of 27 907, the prevalence of the T allele was 52.1%, distributed in a genotype frequency of 30.6%TT, 42.9% TM and 26.5% MM. The T allele was also related to race with 77% in blacks, 78% in Asians and 42.2% in whites (Staessen *et al.*, 1999:10). A prevalence of 35% for the MM variant of the *AGT* M235T genotype and 16% for the TT variant was found in a white older adult population consisting of 11 384 participants (Norat *et al.*, 2008:394).

In the meta-analysis by Staessen *et al.* (1999:13) the presence of a T allele was associated with an increased risk for hypertension. This increased risk was 31% in TT homozygotes and 11% in TM

heterozygotes when compared to the MM reference group (Staessen *et al*., 1999:10), with an unexpected association present only in whites and not in blacks (Staessen *et al*., 1999:10). Staessen *et al.* (1999:13) furthermore found a significant increase of 11% in the circulating angiotensinogen levels in TT subjects and 7% in TM subjects when compared to subjects with the MM allele. Norat *et al.* (2008:395) confirmed the effect by describing a significant increase in the mean systolic and diastolic blood pressure in females with the presence of the TT allele compared to the MT or MM genotypes, but failed to show this relation in males. Although a relation between sodium intake and blood pressure was found in all subjects of this study, the effect found was greater in persons with the T allele than in those not carrying the T allele (Norat *et al.*, 2008:396). Tiago *et al.* (2002:1484) however could not find the same association between the presence of the M235T variant of the AGT gene and hypertension in a large South African study with black participants.

2.4.1.2 Polymorphisms of the *G protein-coupled Receptor Kinase type 4* (*GRK4*) gene

The seven G protein-coupled receptor kinases (GRK's) can be divided into three sub families, with GRK4, GRK5 and GRK6 belonging to the GRK4 subfamily (Felder *et al*., 2002:3872). Although GRK4 was previously thought to be expressed mainly in the brain and testes, Felder *et al*. (2002:3875) have reported the presence of mRNA of all isoforms in renal proximal tubules.

The neurotransmitter dopamine causes natriuresis in the kidney and has a vasodilator effect, facilitating an antihypertensive role in the kidney (Felder *et al*., 2002:3872). A defect in the functioning of the dopamine receptor leads to hypertension (Sen *et al*., 2005:1206). Dopamine via D1-type receptors is responsible for half of the increased sodium excretion when sodium intake is increased (Felder *et al*., 2002:3872). Malfunctioning of dopamine D1 receptors is often not a primary defect, but rather a result of uncoupling from its G Protein / effector enzyme complex (Sanada *et al.*, 2006:353; Felder *et al*., 2002:3872). Activation of variants of the G protein-coupled receptor kinase type 4 (GRK4) gene, has been shown to inhibit the dopamine D1 receptor, leading to decreased sodium excretion (Sanada *et al.*, 2006:353: Lohmueller *et al*., 2006:27).

Bengra *et al.* (2002:2132) has described three GRK4 polymorphisms, 448G3T (R65L), 679C3T (A142V), and 1711C3T (A486V), that are located in the binding and membrane targeting domains of the GRK4 gene, that can on their own or by interaction with other genes involved in the renin-angiotensinogen system, be the cause of essential hypertension. They were however only able to demonstrate the significant presence of one SNP (1711C3T (A486V)) in the hypertensive group of their study when screening for six hypertension related SNP's in an Italian population. In a study of *GRK4* gene polymorphisms by Lohmueller *et al*. (2006:27) different allele frequencies as well as different haplotype structure between different populations (African, Caucasian, Hispanic and Asian) were shown. Gender differences were shown by Bhatnagar *et al*. (2009:332) who found an

association between *GRK4* A142V and blood pressure response to metoprolol in males, but not in females.

Salt sensitivity is defined as a 10% or more increase in mean arterial pressure (determined as diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure)) when sodium intake is increased from low to high (Sanada *et al.*, 2006:353). Sanada *et al.* (2006:356) reported that the genetic model that best predicted salt sensitivity in their study undertaken in Japan, included the R65L, A142V and A486V variants of the *GRK4* gene, which predicted salt sensitivity correctly in 94.4% of cases. They found that this model had a sensitivity of 83% and 100% specificity in their Japanese study population. They also identified the *GRK4* A142V genotype to be 78.4% predictive of salt sensitivity as a single indicator and the 2-locus model of *GRK4* A142V and *CYP11B2* C-344T as 77.8% predictive of low-renin hypertension in their study group (Sanada *et al*., 2006:356).

2.4.1.3 Polymorphism of the *aldosterone synthase* (*CYP11B2*) gene

Aldosterone is synthesized by aldosterone synthase in the adrenal cortex and is encoded by the *CYP11B2* gene which is located on chromosome 8q22 (Rajan *et al.*, 2010:379). The role of the *CYP11B2* gene has been extensively researched to evaluate its role in hypertension and cardiovascular disease. Particular attention is paid to the C-344T single nucleotide polymorphism in the 5' distal promoter region of the gene (Sookoian *et al*., 2007:5). This polymorphism is implicated in the increased aldosterone to renin ratio in individuals with essential hypertension with a 94% chance of a normal aldosterone/renin ratio in subjects with a C/C genotype (Nicod *et al*., 2003:2499). In a meta-analysis by Sookoian *et al*. (2007:7) of 19 studies, including a total of 11 225 subjects, the -344T allele was associated with an increased risk for hypertension and the -344C allele with a decreased risk for arterial hypertension. In an Indian population, a significant association between the C-344T polymorphism and essential hypertension was shown in male subjects, but not females (Rajan *et al*., 2010:382). Chen *et al.* (2011:Online) found amongst a Han Chinese study population that the rs3802230 C allele of the *CYP11B2* gene could be used as a risk indicator for essential hypertension and that the AAGC haplotype might indicate genetic proneness to essential hypertension. Cheng and Xu (2010: 301) however, could not find any association between essential hypertension and the 344 C/T polymorphism of *CYP11B2* in a meta-analysis including almost 7500 participants from the same population group.

When focussing on a black population, Henderson, Haiman and Mack (2004:270) found that the presence of the T allele was associated with an increased risk for hypertension in African American males and females, but not in Latin-Americans living in the same environmental circumstances. This association was also found in a black South African population from Johannesburg (n=231)

where the presence of a TT allele was significantly related with systolic blood pressure amongst newly diagnosed hypertensive individuals (Tiago *et al.*, 2003:1007).

2.4.2 Intra-uterine adaptations

Poor intra-uterine growth in early life seems to enhance the process of arterial aging, resulting in a higher prevalence of hypertension described in adults born with a low birth weight (Aviv, 2001:1061). Intra-uterine malnutrition also influences kidney function. This is hypothesized to occur because nephrogenesis exclusively takes place before birth, and intrauterine growth restriction, which results in a low birth weight, impairs the formation of nephrons, resulting in a reduced number of nephrons in people who were small at birth. The number of nefrons depends on the size of the body at 34 weeks of gestation, the critical period for nephrogenesis. Fewer nephrons to carry the hemodynamic burden, thus leads to an increased risk for hypertension later in life (Schreuder & Nauta, 2007:265; Barker *et al.*, 2009:447; Reyes & Manalich, 2005:S107).

Individuals with a low birth weight also seem to be more susceptible to a hostile environment later in life, with increased responses to stress and greater vulnerability to the effects of inactivity and a higher risk to develop glucose intolerance if they do not exercise (Barker *et al.*, 2009:448).

Babies born with a low birth weight and who are underweight or short during infancy, often have lower muscle mass, which will persist into later life as muscle cell replication mostly occur before the age of one. Rapid weight gain later in life, may lead to a low muscle mass in relation to fat mass, leading to insulin resistance later in life (Barker *et al.*, 2009:451).

Slow pre- and post natal growth also influences the development of the liver. As the liver regulates lipid metabolism, an atherogenic lipid profile is established with malnutrition, with altered set-points for non-HDL cholesterol metabolism. Set-points for HDL cholesterol metabolism however are only determined by feeding practices during infancy (Barker *et al.*, 2009:451-452).

Another concept how intra-uterine growth impairment influence blood pressure levels later in life, is by affecting vascular development. It is especially vascular development in the brain that is influenced during the rapid periods of growth, before and after birth (Barker *et al*., 2009:452). Protein metabolism is often impaired in the case of intra-uterine growth impairment and because protein metabolism is established during the period of weaning, low rates of protein synthesis and reduced linear growth can be expected as a result of lower protein synthesis if malnutrition is not corrected early enough (Barker *et al*., 2009:452).

Intra-uterine under nutrition also influences the setting of hormones and metabolism. A hierarchy exists for growth and development of organs. Because the mother performs some of the bodily

functions for the baby, the kidneys, muscles and lungs are lower on the hierarchy, thereby protecting the development of organs such as the brain in the case of under nutrition. Insulin resistance, associated with a low birth weight, are often seen as continuation of the foetal response to glucose being prioritised for use by the brain at the expense of glucose transport to the muscles for growth (Barker *et al.,* 2009:448). Insulin resistance has also been linked to be responsible for the higher risk for diabetes associated with reduced foetal growth (Lithell *et al.,* 1996:406).

2.4.3 Insulin resistance

Insulin resistance and hyperinsulinaemia is often implicated in the development of hypertension and between 25-40% of non-obese, non-diabetic individuals with hypertension are insulin-resistant (Lind, Berne & Lithell, 1995:1457). Insulin and glucose levels are risk factors for hypertension, especially in the presence of a strong family history of the disease. Obesity, especially central obesity, however seems to be a confounding factor in the relationship between insulin and blood pressure (Fagot-Campagna *et al.*, 1997:542). Insulin has an anti-natriuretic effect, causing increased extracellular and intravascular volume, and high insulin levels seems to exert a direct trophic effect on the smooth muscle cells of arterioles, resulting in a chronic hyperactive sympathetic nervous system, leading to increased blood pressure levels (Astrup, 2005:383). A number of mechanisms have been proposed how insulin resistance influence blood pressure and Figure 2.3 by Kotchen (1999:Online) illustrates possible associations of hypertension with insulin resistance.

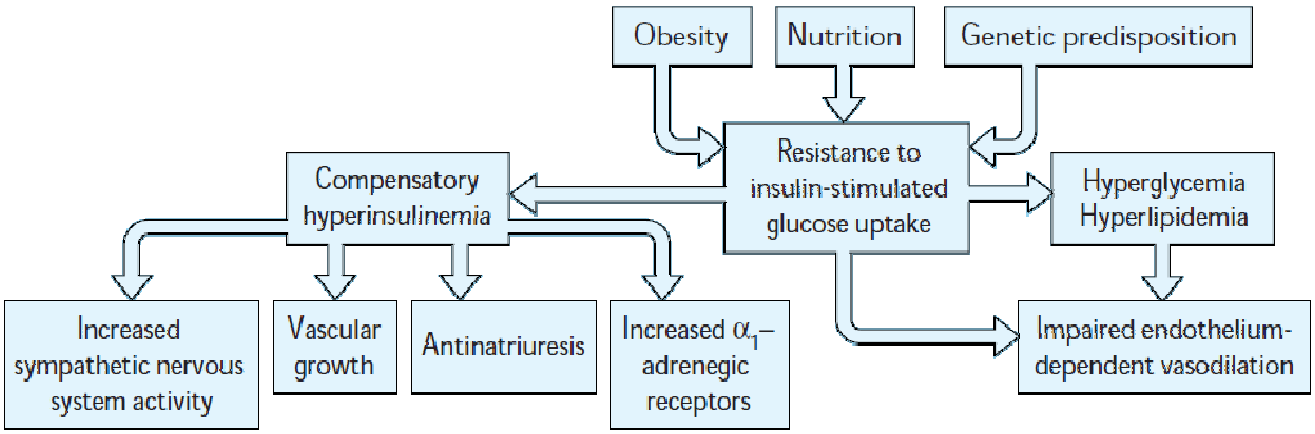

Figure 2.3 Hypertension and insulin resistance (Kotchen, 1999:Online, Figure 5-7)

Chronic inflammation seems to play a role in the development of hypertension, with higher levels of inflammatory markers, particularly C-reactive protein, found in hypertensive individuals. C-reactive protein inhibits the formation of nitric oxide by endothelial cells, leading to vasoconstriction, adherence of leukocytes, platelet activation and clotting (Couch & Krummel, 2008:869).

2.4.4 Acquired subtle renal injury

Acute renal vasoconstriction is in most cases the cause of renal injury (microvascular and tubulointerstitial), leading to an increase in blood pressure with normalisation of blood pressure that takes place after adaptation. Although blood pressure may restore, subtle renal injury has developed causing an increase in blood pressure with increased sodium intake (Johnson *et al.,* 2002:920). The mechanisms are explained in Figure 2.4.

With renal disease, blood flow through the kidney is decreased, which can be the result of atherosclerosis in the lumen of a renal artery, compression of a vessel by a tumour, gene mutations or renal endothelial cell dysfunction. Angiotensin II is released to improve blood flow, causing vasoconstriction and retention of sodium, chloride and water, which result in an increased blood volume and cause an increase in entire body arterial pressure. Renal endothelial cell dysfunction may also cause a decrease in nitric oxide and prostacyclin, both responsible for vasodilation (Nelms *et al.,* 2011:289).

2.4.5 Aging

A steady but continuous rise in systolic blood pressure of approximately 0.6 mmHg per year is normally seen with aging up to the age of 60 years (Burt *et al.,* 1995:Online) and an increase in blood pressure with aging is therefore expected. The National Health and Nutrition Examination Survey (NHANES) (Burt *et al.,* 1995:Online; AHA, 2011:Online) reports a slower age related increase in blood pressure amongst females compared to males up to the age of 44. From 45 to 65 years the prevalence of hypertension between the genders is similar, with females catching up, because of a sharper rate of increase in blood pressure, resulting in the same or even higher blood pressure values in females compared to males after the seventh decade of life. Similarly, Aviv (2001:1061) has described an increase in systolic blood pressure with aging and a gender difference in blood pressure increase with aging, and suggests that this may be due to differences in biologic aging and chronological aging between men and women. Stiffening of the arteries seems to play a major role in the rise in blood pressure with aging (Aviv, 2001:1061).

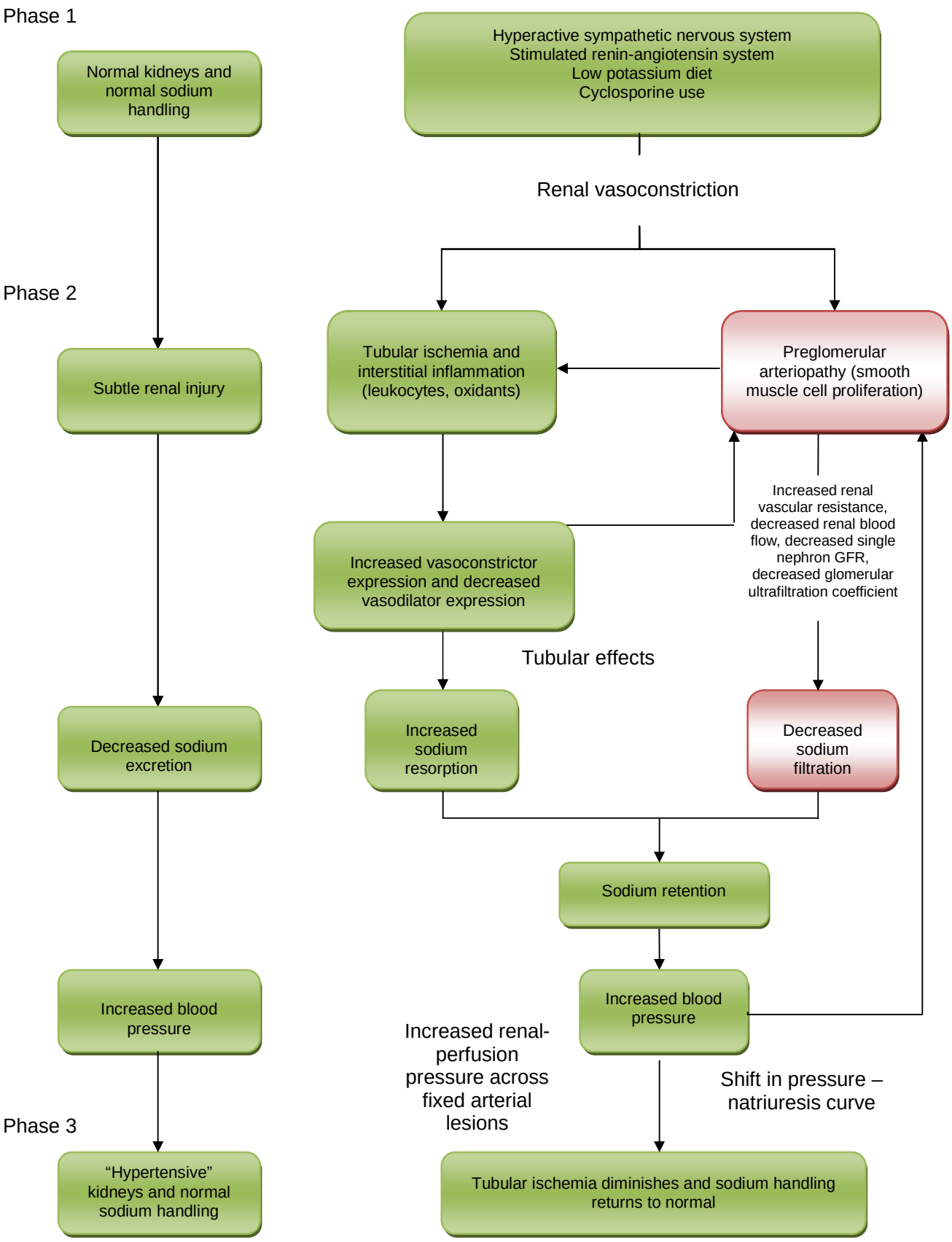

Figure 2.4 **Physiology of the development of hypertension (Johnson *et al.*, 2002:921, Figure 3)**

2.4.6 Human Immunodeficiency Virus (HIV) infection

During 2009, the incidence of HIV infection in South Africa in the 15-49 years age group was estimated at 17.8% (UNICEF, 2010:Online). Groenewald *et al.* (2011:Online) reports a similar HIV infection rate of 17.1% in the rural segment of the AHA-FS study in the Southern Free State. As in the general population, hypertension seems to be common among HIV infected individuals. Research has shown that the prevalence of hypertension does not seem to be directly influenced by HIV status or by the use of highly active antiretroviral therapy (HAART). The only factors found to be associated with hypertension where the role of HIV status and HAART were investigated in multivariate models, included increasing age, higher body mass index (BMI), longer duration of HIV and diabetes (Medina-Torne, 2011:Online; Bloomfield *et al.,* 2011:Online; Baekken *et al.*, 2008:2131; Jung *et al*., 2004:2250; Bergersen *et al.,* 2003:731).

2.5 PREVENTION AND TREATMENT OF HYPERTENSION

With an understanding of the genetic origins of disease, prevention and treatment should be tailored to suit each individual's specific needs (Ferguson, 2009:452) and various treatment options for hypertension will therefore be discussed in the following section.

In essential hypertension, the prevention of left ventricular hypertrophy is an important goal of treatment (Du Cailar *et al.*, 2010: 865) which can be achieved by reducing blood pressure levels. In cases of primary aldosteronism, surgical treatment in the form of a unilateral laparoscopic adrenalectomy, medication which antagonises aldosterone action and low dose glucocorticoids are generally considered as medical treatment options (Stowasser *et al*.,2010:50). In the following section, pharmacologic treatment will briefly be discussed and dietary and lifestyle changes will be further discussed as prevention and treatment options for hypertension.

2.5.1 Pharmacologic treatment of hypertension

Blood pressure in hypertensive individuals can be lowered effectively with pharmacological treatment. Several classes of drugs have been shown to be effective in the treatment of hypertension, with most patients often requiring two or more anti-hypertensive medications to achieve blood pressure goals. It is recommended that a second drug with a different pharmacologic action be added when use of a single drug in adequate dosage is not able to achieve the blood pressure goal (NIH, 2004:Online). Table 2.4 provides a list of commonly used oral antihypertensive drugs.

Table 2.4 Oral antihypertensive drugs (NIH, 2004:Online; Gaw *et al*., 2008:135; Nelms *et al*., 2011:291)

Class of oral antihypertensive drugs	Mechanism
Diuretics	Decrease blood volume by increasing urinary output; inhibit renal sodium and water reabsorption. Thiazide diuretics are commonly used and enhance the efficacy of other drugs. Loop diuretics results in more powerful natriuresis than thiazide diuretics.
Aldosterone-receptor blockers	Interrupt aldosterone, which increases sodium and water excretion.
Beta-1-Blockers	Block beta-adrenergic receptors in the heart, kidneys and brain, causing a reduction in cardiac output and renin and noradrenaline release.
Alpha Adrenergic Blockers	Blocks vascular muscle response to sympathetic stimulation; reduces stroke volume.
ACE inhibitors	ACE inhibitors inhibit angiotensin-converting enzyme, thereby reducing production of angiotensin (vasoconstrictor) and aldosterone.
Angiotensin II receptor blockers (ARB's)	ARB's interferes with the renin-angiotensin system by blocking angiotensin receptors.
Calcium channel blockers	Reduce entry of calcium into vascular smooth muscle, thereby causing a reduction in vascular tone and peripheral arterial resistance

Drugs used in the treatment of hypertension are costly, not effective for everyone and may have adverse effects that impair quality of life and reduce adherence. Certain anti-hypertensive drugs may even exacerbate other conditions, generally associated with hypertension, such as insulin resistance and hyperlipidaemia (Bacon *et al*., 2004:307).

Due to the possible adverse effects of pharmacological treatment and poor response of some patients to drug treatment, the focus of interest is shifting more towards the development and implementation of lifestyle and behavioural interventions in the prevention and management of high blood pressure (Bacon *et al.,* 2004:307).

2.5.2 Dietary and lifestyle intervention in the prevention and treatment of hypertension

Various lifestyle and dietary factors have been shown to play a role in the development and management of hypertension. Controllable risk factors contributing to hypertension include stress, salt and potassium consumption, overall diet, alcohol consumption, body weight and activity level; with age, family history and ethnicity constituting non-controllable contributing factors (Wallace *et al.*, 2007:49). The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure recommends specific lifestyle changes to prevent and manage hypertension (Table 2.5). In a literature review by Bacon *et al.* (2004:313), the authors concluded that although pharmacological treatment is effective in many patients, it might be inconvenient, expensive and accompanied with adverse effects in many others. They recommend behavioural change in the form of exercise, diet (less alcohol, sodium and saturated fats, adequate potassium, more fruit, vegetables, nuts and low fat dairy) and weight loss to complement or even replace anti-hypertensive medication. The authors ascribe the effect that lifestyle changes have on favourable blood pressure changes to beneficial adaptations in the cardiovascular system, with improvement in left ventricular hypertrophy, a decrease in arterial stiffness and better endothelial function (Bacon *et al.*, 2004:313).

Table 2.5 Lifestyle modifications to manage hypertension (NIH, 2004:Online, Table 9; Appel, 2009:359; Helms *et al.,* 2011:293, Table 13.4)

Modification	Recommendation	Average systolic blood pressure recommendation
Weight reduction	Maintain normal body weight (BMI 18.5-24.9).	5-20 mmHg/10kg
Adopt the DASH eating plan	Consume a diet rich in fruits and vegetables (8-10 servings/day), with enough low-fat dairy products (2-3 servings/day) and a reduced intake of saturated and total fat. Aim for 4.7 g of potassium/day.	8-14 mmHg
Dietary sodium reduction	Reduce dietary sodium intake to no more than 100 mEq/L (2.4 g sodium or ≈6 gram sodium chloride) and reduce even further to 1.5 g in Black population groups and older people or where organ damage is evident.	2-8 mmHg
Physical activity	Engage in regular, aerobic physical activity such as brisk walking (at least 30 minutes per day, most days of the week).	4 - 9 mmHg
Moderation of alcohol consumption	Limit consumption to no more than 2 drinks per day in males and no more than 1 drink per day in females and lighter-weight persons.	2 – 4 mmHg

According to Welton *et al.* (2002,:1884) lifestyle interventions are more likely to be successful and the reductions in risk of hypertension are likely to be greater when persons who are older and

individuals with a higher risk of developing hypertension are targeted in comparison with younger individuals or those with a lower risk. Prevention strategies early in life however provide the greatest long-term potential to avoid hypertension and to reduce the burden of blood pressure-related complications in a community.

Lifestyle and dietary modifications, influencing blood pressure, will be described further.

2.5.2.1 Weight reduction

In the third National Health and Nutrition Examination Survey (NHANES III), the association between all-cause mortality and elevated BMI was found to be weak, with 1,2% of deaths associated with a BMI in the obese category. When limiting mortality figures to those linked in the literature to obesity, this association was stronger, with 5.5% of deaths related to a BMI in the obesity range over the study period (Flegal & Graubard, 2009:1217-1218). However, a strong relation exists between body weight and hypertension with the risk of developing hypertension being two to six times higher in overweight than normal weight persons, with almost all clinical trials recommending weight loss as treatment for lowering blood pressure (Couch & Krummel, 2008:870-871).

The main objectives to facilitate weight reduction is firstly to prevent age-related weight gain by increasing activity, decreasing sedentary leisure practices and decreasing energy intake (NIH, 2004:Online: Appel, 2009:360). A meta-analysis by Neter *et al.* (2003:881) of 25 randomized, controlled trails that included 4874 participants, showed an average blood pressure reduction of -4.4 / -3.6 mmHg for every 5kg of weight lost, equalling a reduction of ≈ 1mmHg for every kilogram of weight loss (Neter *et al*., 2003:883). This blood pressure lowering effect of weight loss was found to be even more pronounced in individuals using antihypertensive medication (Neter *et al.*, 2003:882-883). In his position statement, Appel (2009:360) also highlights the importance of public programs to prevent overweight and obesity in order to address the growing problem of hypertension. Appel (2009: 359) further describes that a 20% prevention rate for hypertension is possible amongst pre-hypertensives with modest weight reduction, with or without sodium reduction and that weight reduction also offers the prospect of medication reduction or even total drug withdrawal. In a literature review Bacon *et al. (*2004:311) also describe strong scientific support for using weight loss as an effective intervention to reduce blood pressure in both normotensive and hypertensive overweight individuals.

The exact mechanism of how obesity causes hypertension and the effect of weight loss on blood pressure is not clear, but Neter *et al.* (2003:882-883) suggest the following possible mechanisms:

- An over active renin-angiotensin-aldosterone system in obese individuals resulting in higher renin activity and aldosterone levels than in lean individuals;

- Increased sympathetic nervous system activity in obese hypertensive individuals;
- Inhibition of the natriuretic peptides system leading to vasodilation and natriuresis; and
- The effect of insulin resistance and hyperinsulinemia.

Other proposed mechanisms how obesity causes hypertension include that the excess amounts of adipose tissue results in increased networks of capillaries responsible for blood circulation (Smolin & Grosvenor, 2008:419), which can increase flow resistance, as well as the presence of chronic inflammation (Couch & Krummel, 2008:871).

A study by the research group of Rossi *et al.* (2008:2570) showed that BMI correlated with plasma aldosterone concentration, independent of age, sex, and sodium intake in patients with primary hypertension, but not in those with arterial hypertension due to primary aldosteronism. This association was mostly seen in patients within the overweight-obese category and suggests a link between visceral adiposity and aldosterone secretion.

Although body size and level of obesity is closely related to blood pressure, not all obese persons are hypertensive and even in cases of severe obesity, blood pressure might be normal. Blood pressure response to obesity may therefore also be influenced by genetic factors (Tiago *et al.,* 2002:1483).

A study by Katzmarzyk *et al.* (2011:1272) determined an optimal threshold for BMI and waist circumference for cardio-metabolic risk in African American males and females, as most reference values are based on European and Asian studies. These cut-off values are indicated in Table 2.6.

Table 2.6 Optimal threshold values for BMI and waist circumference in the identification of cardio-metabolic risk of African Americans (Katzmarzyk *et al*., 2011:1272).

Indicator	Males	Females
BMI (kg/m^2)	30.4	32.9
Waist circumference (cm)	99.1	96.8

A meta-analysis by Lee *et al*. (2008:646) showed that indices of abdominal obesity are better predictors of cardiovascular disease risk than BMI and that waist to height ratio (WHtR) shows the best association with hypertension, while BMI showed the weakest association with hypertension of the four indicators compared (Lee *et al.*, 2008:647). WHtR is considered to be more sensitive than BMI as an early warning indicator of health risks and a better obesity indicator to predict hypertension; it is cheaper and easier to measure and calculate than BMI; a WHtR of 0.5 and more indicates an increased risk in males and females for people in different ethnic groups, and it can be used for adults as well as children (Ashwell & Hsieh, 2005:304; Sayeed *et al.*, 2003:1).

Body fat distribution, especially a high proportion of fat distributed around the waist, is an important diagnostic indicator of the metabolic syndrome, which is also associated with the prevalence of hypertension (Eckel, Grundy & Zimmet, 2005:1420; Alberti *et al*., 2009:1641). Although cut-off values of 88cm for females and 102cm for males are generally used, the health consequences of abdominal fat distribution have resulted in lowering of these recommended waist circumference thresholds to 80cm and 94cm respectively for women and men in Sub-Saharan Africa (Alberti *et al.,* 2009:1642). This lower cut-off point is also recommended by the European Group for the Study of Insulin Resistance (EGIR) (Balkau & Charles, 1999:443).

South African reference values to evaluate adiposity do not exist. Kim *et al*. (2011:38) have used the body fat percentage cut-off points as predictors of obesity-related cardiovascular disease in Koreans as shown in Table 2.7:

Table 2.7 Classification of fat percentage in Koreans to predict cardiovascular disease (Kim *et al*., 2011:38).

Classification	Males	Females
Normal	<17%	<32%
Overweight	17-20.9%	32-36.9%
Obese	≥ 21%	≥ 37%

Possible factors that may contribute to hypertension in overweight /obese individuals, include increased sympathetic nervous system activity, insulin resistance and hyperinsulinemia, sodium retention, and enhanced vascular reactivity (Hsueh &, Buchanan, 1994: 405). Eckel, Grundy and Zimmet (2005:1420) have explained the effect of insulin resistance and the metabolic syndrome on blood pressure by summarizing various authors' research. The authors conclude that although insulin has a vasodilatory effect, it also has a secondary effect of sodium reabsorption in the kidney. With insulin resistance, the vasodilatory effect of insulin seems to be lost, but the effect on sodium reabsorption is preserved. This effect of higher sodium reabsorption has been found to be true in white individuals, but not necessarily in African populations with the metabolic syndrome (Eckel, Grundy & Zimmet, 2005:1420). Insulin has also been shown to increase sympathetic nervous system activity and high levels of circulating fatty acids associated with the metabolic syndrome and central obesity seems to mediate vasoconstriction (Eckel, Grundy & Zimmet, 2005:1420).

Hydrogen sulphide has an endogenous, endothelium-dependent vasodilatory effect, with animal studies showing that low plasma hydrogen sulphide levels are associated with endothelial dysfunction and insulin resistance. Whiteman *et al.* (2010: 1722) have confirmed a similar effect in

humans in a study that included men from the United Kingdom, indicating that increased adiposity was the major determinant of plasma hydrogen sulphide levels and that the levels are reduced in overweight individuals and those with type 2 diabetes.

2.5.2.2 Physical activity

Exercise training decreases blood pressure in approximately 75% of individuals with hypertension (Hagberg *et al.*, 2000:193) and increased physical activity of 30 minutes per day for most days of the week is widely recognized as effective treatment for lowering blood pressure (Whelton *et al,* 2002:1885; WHO/ISH, 2003: 1987; NIH, 2004:Online; Seedat *et al,* 2006:343; Miyashita *et al,* 2008: 1225; Couch & Krummel, 2008:871; Pescatello *et al.*, 2004:535; Bacon *et al.*, 2004:308). The position statement of the American College of Sports Medicine (Pescatello *et al.*, 2004:535) provides evidence that abnormal or exaggerated blood pressure responses during exercise in normotensive individuals can predict hypertension in future.

Multiple short (3 minutes) bouts of brisk walking (in total 30 minutes) has been shown to be equally effective in lowering resting systolic blood pressure as one continuous 30 minute brisk walk (Hagberg *et al.*, 2000: 205; Miyashita *et al,* 2008:1229). A sustained reduction in resting systolic blood pressure was found for up to 24 hours after 30 minutes of brisk walking performed either continuously or intermittently (Miyashita *et al,* 2008:1229). A study by Cornelissen *et al.* (2010:179) showed no difference in the effect of exercise intensity (33% or 66% of heart rate reserve) in the ability to significantly reduce systolic blood pressure in sedentary adults older than 55 years. A review by Hagberg *et al.* (2000:205) supports this by concluding that low to moderate intensity training reduced blood pressure to the same extent or even more than higher intensity training, with the lowering effect being evident early in the training programme, and greater reductions being associated with prolonged training. The lowering effect has been shown to be more pronounced in people with hypertension than in individuals with a normal blood pressure (Pescatello *et al.*, 2004:537; Cornelissen & Fagard, 2005: 5). It therefore seems that although individual differences in blood pressure reduction occur in studies and that this can be attributed to the frequency, intensity, time and type of exercise (Pescatello *et al.*, 2004:537), exercise requirements to lower blood pressure can include lower intensity exercise for shorter periods of time, adding up to 30 minutes a day, or even resistance or static training (Pescatello *et al.*, 2004:538). This make recommendations easier for population wide implementation. The blood pressure lowering effect of endurance training seems to be similar in males and females (Pescatello *et al.*, 2004:539).

In a meta-analysis of 54 trials, the blood pressure lowering effect of exercise was 3.84mmHg for systolic blood pressure and 2.58mmHg for diastolic blood pressure, with black participants showing a significantly greater reduction in systolic blood pressure in comparison with white participants (-10.96 mmHg vs -3.44 mmHg) (Whelton *et al.*, 2002:500). In contrast, Pescatello *et al.* (2004:540)

states that no convincing evidence exists to support that ethnic differences exist in blood pressure response to chronic or acute exercise. In the review by Cornelissen and Fagard (2005:4) the authors reported a -3.0/-2.4 mmHg reduction in blood pressure with aerobic endurance training. A review by Hagberg *et al. (*2000:204) averaged the beneficial effect of exercise on systolic and diastolic blood pressure much higher at approximately 11 and 8 mmHg respectively.

Although this reduction might sound insignificant, Pescatello *et al.* (2004:545) have reported that a reduction of 2 mmHg in systolic and diastolic blood pressure can reduce the risk of stroke by 14% and 17% and the risk of coronary artery disease by 9% and 6% respectively in the general population.

Whelton *et al.* (2002:500) and Hagberg *et al.* (2000:198) document that aerobic exercise reduces blood pressure in normal weight individuals and significantly reduces blood pressure even in participants that did not lose weight, agreeing with the results of Neter *et al.* (2003:882) and suggesting that the effects of aerobic exercise on blood pressure may be independent of even a substantial change in body weight (Whelton *et al.*, 2002:500). Evidence also indicates that the blood pressure lowering effects of exercise training and dietary induced weight loss are not additive (Hagberg *et al.*, 2000:199), but that females (compared with males) and the middle-aged (compared with young and older patients with hypertension) may obtain greater benefit with exercise training (Hagberg *et al.*, 2000: 204-205).

Adherence to an exercise programme is essential to successfully achieve and maintain the maximum benefit of exercise on blood pressure. In a meta-analysis Whelton *et al.* (2002:500) found that trials with a larger sample size which were longer in duration showed the smallest reduction in blood pressure, possibly indicating poorer compliance as a result of limited supervision and support over the longer intervention period.

It seems that all forms of exercise are effective in reducing blood pressure (Whelton *et al.*, 2002:501) and that the effect is more pronounced in hypertensive individuals (Cornelissen & Fagard, 2005:5) confirming that aerobic exercise is an important strategy for prevention and treatment of high blood pressure. Evidence for specific exercise guidelines regarding frequency, intensity and time is limited, but the American College of Sports Medicine's position statement recommends an exercise program that is primarily aerobic-based combined with resistance training and concludes that the antihypertensive effect of exercise seems to occur at relative low intensity and short duration (Pescatello *et al.*, 2004:542).

The mechanism of hypertension reduction by means of exercise is not very clear because of the multifactorial mechanisms and systems involved (Pescatello *et al.*, 2004:545), but seems to be based on a decrease in systemic vascular resistance (a decrease of 7.1%), involving the sympatic

nervous system and the renin-angiotensin system (Cornelissen & Fagard: 2005:5; Pescatello *et al.*, 2004:545). Exercise training has shown to reduce plasma norepinephrine levels with 29% and plasma renin activity with 20% (Cornelissen & Fagard: 2005: 5). Except for the blood pressure lowering effects, exercise training for $\geq$ 4 weeks has also been shown to have a significant positive effect on other cardiovascular risk factors, such as lowering body weight (1.2kg), reducing waist circumference (by 2.8cm), decreasing body fat percentage (by 1.4%), lowering the homeostasis model assessment index of insulin resistance (by 0.31U) and increasing HDL-cholesterol (by 0.032mmol/L) (Cornelissen & Fagard: 2005:3).

Vigorous exercise has even shown to buffer against the genetic influence on BMI and can assist in protecting against obesity for those with a high genetic risk for obesity (McCaffery *et al*., 2009:1016-1017).

The exercise prescription made by the American College of Sports Medicine for individuals with hypertension is:
Frequency – Most, but preferably all days of the week;
Intensity – Moderately (40- <60% of VO_2R);
Duration – 30 minutes or more of continuous or accumulated physical activity per day; and
Type – Endurance physical activity supplemented with resistance exercise (Pescatello *et al.*, 2004:546).

2.5.2.3 Smoking cessation

Smoking causes an acute and chronic increase in blood pressure, which may be explained by the fact that cigarette smoking interferes with the production of nitrous oxide, which results in impaired endothelial relaxation and vasodilation (Helms *et al*., 2011:289). Nitric oxide is produced from L-arginine, and production of nitric oxide is negatively affected when L-arginine is bound and lowered by components in cigarette smoke (Argacha *et al.*, 2008:1506).

2.5.2.4 Stress management

Mashele *et al.* (2010:210) conclude from their study in a black urbanized South African population that depression was a prominent contributor to hypertension. The World Health Report of 2008 (WHO, 2008:70), also supports the relationship between stress and high blood pressure by listing elevated blood pressure as an adverse effect of unemployment. Stress increases blood pressure by stimulating the nervous system to produce large amounts of vasoconstricting hormones (Kulkarni *et al.*, 1998:34).

2.5.2.5 Dietary interventions

i) Sodium reduction

Sodium and chloride are primarily consumed in the diet in the form of sodium chloride or salt. Absorption of both sodium and chloride occurs mainly in the small intestine and most of what is consumed, is absorbed in the body. Because sodium is mainly excreted in the urine (except in cases of excessive sweating or diarrhea), urinary sodium excretion provides a convenient indication of sodium intake, (IOM 2004:Online). Spot urine samples have been shown to correlate closely with 24-hour urine excretion values and provide an accurate indication of various elements, including sodium and potassium in European and Japanese males and females (Ilich *et al.*, 2009:220; Tanaka *et al.*, 2002:99). Although not regarded as accurate for individual analysis, spot urine sodium and potassium values provides a valuable tool to compare populations (Tanaka *et al.*, 2002: 102).

The recommended adequate intake for sodium is 1.5g (65mmol)/ day, which equals salt intake of 3.8g. The tolerable upper intake level (UL) is 2.3g (100mmol), which equals 5.8g salt /day (IOM 2004:Online). In the UK, the panel on Dietary Reference Values (DRV) from the *Committee on Medical Aspects of Food Policy* (COMA) advised that sodium intakes should be below 3.2g, which is the equivalent of 8g of salt per day. This panel set the Reference Nutrient Intake (RNI) at 1.6g of sodium (4g salt) per day, with COMA's Cardiovascular Review Group recommending that salt intake should gradually be reduced to 6g per day (Food Standards Agency, 2008:Online), which is in closer agreement with the IOM's recommendations.

To convert mmol values to mg of these ions, the molecular weights of sodium (23), chloride (35.5), and sodium chloride (58.5) are used to multiply with (IOM 2004:Online). An easy conversion is thus that 1 gram of salt equals 17.1 mmol of sodium (Food Standards Agency, 2008:Online).

Population studies have shown a positive association between sodium intake and blood pressure over a wide range of sodium intakes and various studies recommend a reduction in dietary sodium intake as an effective way to lower blood pressure or even prevent hypertension in future (Couch & Krummel, 2008:872; Norat *et al.*, 2008:395; Appel, 2009:360; Food Standards Agency, 2008:Online). In their position paper Appel, on behalf of the American Society of Hypertension Writing Group (2009:360), concludes that the effect of sodium on blood pressure seems to be greater in black populations and middle- and older-aged individuals and that genetic and other dietary factors (such as potassium intake) may influence blood pressure response to sodium intake. In this position paper an upper limit of 2.3g sodium per day is recommended for the general population and a maximum of 1.5g sodium per day is recommended for blacks, middle- and older-

aged persons, as well as individuals with hypertension, diabetes, or chronic kidney disease, which makes up the majority of the American population (Appel, 2009:360).

Studies described by Acelajado *et al*. (2010:805) show that a diet low in sodium can minimize the effect of high levels of aldosterone on heart health; and conversely that when a diet high in salt is followed in the presence of high aldosterone levels that left ventricular hypertrophy, proteinuria and therefore disease progression is worsened. These researchers also describe how the harmful effects of a diet high in sodium will manifest increasingly as levels of aldosterone increase. Du Cailar *et al. (*2010: 865) support this by concluding that aldosterone requires the presence of a high sodium diet in order to express its unfavourable effect on the heart. Because of this interaction between dietary sodium intake and aldosterone levels, treatment success in hyperaldosteronism can be increased by either lowering salt intake or by lowering aldosterone levels by treatment with aldosterone blockers (Acelajado *et al.,*2010:805).

In the International Study of Salt and Blood Pressure (INTERSALT), information was collected at 52 centres around the world from 10 079 adults between the ages of 20 and 59. Sodium excretion ranged between the lowest of 0-2 mmol/day in Brazil to the highest of 242 mmol/day in North China, and blood pressure was significantly related to sodium excretion (INTERSALT, 1988:319).

In a study by Norat *et al.* (2008:395) no significant association between potassium intake and blood pressure was found, but highly significant associations between blood pressure and sodium intake were observed. These associations between sodium and blood pressure were the weakest for the MM homozygous AGTM235T genotype, and although all genotypes showed highly significant associations between salt and blood pressure (Norat *et al.*, 2008:397), the researchers were able to show the most evident associations in the highest quintile of sodium intake (Norat *et al.*, 2008:395).

Humans are programmed to function on very low sodium intakes, with a physiological ability to conserve sodium in the body by reducing excretion through urine and sweat to almost zero. Modern diets however have raised the level of sodium intake at which humans must function genetically to almost 40 times higher than what it used to be, providing physiological challenges to the excretory system (He & MacGregor, 2007:21).

ii) Increased potassium intake

A reduction in dietary sodium intake is regarded as an effective way to lower blood pressure, with researchers supporting an overall reduction in sodium content of food in populations as a whole (Couch & Krummel, 2008:872; Norat *et al.*, 2008:395,397; Appel, 2009:360, USDA, 2010:Online). An adequate potassium intake on the other hand has been shown to be protective against

hypertension (Smith *et al.,* 1988:330.) Higher levels of potassium are associated with lower blood pressure levels, with insufficient potassium intake linked to increased blood pressure levels (USDA, 2010:Online) and dietary potassium has been shown to have a powerful, dose-dependent inhibitory effect on sodium sensitivity (Adrogué & Madias, 2007: 1968).

Although sodium is implicated to be responsible for increased blood pressure in most studies, Adrogué and Madias (2007:1966) have provided evidence that it may rather be the interaction between sodium and potassium, than an excess of one and a deficiency of the other that plays an important role in the pathogenesis of hypertension. In the INTERSALT study, the potassium to sodium ratio showed a significant inverse relation with blood pressure, which was greater than that of sodium or potassium alone (INTERSALT, 1988:319). Based on the results of various epidemiological studies, it is speculated that the racial differences in blood pressure between black and white individuals are not due to the lower potassium intakes (as measured by excretion) amongst blacks, as sodium intake is often similar in the two racial groups (Adrogué & Madias, 2007:1968).

Potassium is the major intracellular cation in the body and concentrations are maintained at 145 mmol/L in intracellular fluid, and 3.8 - 5 mmol/L in extracellular fluid. Small changes in extracellular potassium affect neural transmission, muscle contraction and vascular tone (IOM, 2004:Online). Potassium levels are regulated tightly in the body. When potassium levels increase in the body, cellular uptake is increased as a short-term solution to prevent lethally high potassium levels in the extracellular fluid. Aldosterone release is involved in the long-term regulation of potassium balance, causing the kidney to excrete potassium while retaining sodium (Smolin & Grosvenor, 2008:417). Higher potassium intakes are associated with lower blood pressure levels as described by many researchers (IOM, 2004:Online) and form the basis for the inclusion of a high fruit and vegetable intake in the DASH diet.

A meta-analysis described by Adrogué and Madias (2007:1968), concluded that potassium supplementation equal to or exceeding 60mmol/day lowered systolic and diastolic blood pressure in hypertensive and normotensive individuals and that the effect was independent of a baseline deficiency and greater in cases where there were higher levels of sodium excretion. In another study, increased dietary potassium intake led to 81% of participants halving their antihypertensive medication and 38% stopping treatment (Adrogué & Madias, 2007:1968). A diet high in potassium has been shown to lower or even stop sodium sensitivity in both hypertensive and normotensive individuals (Adrogué & Madias, 2007:1968). However, in a large free-living population studied by Norat *et al.* (2008:395), no association could be found between blood pressure and potassium intake as reflected by urinary potassium excretion.

As in the case of sodium, urinary potassium excretion also reflects dietary potassium intake, and between 77 - 90 percent of potassium consumed, is excreted in the urine (IOM, 2004:Online). Racial differences in potassium excretion seem to exist, with black individuals having lower excretion levels than white individuals (Turban *et al.*, 2008:1396). A potassium intake of 4.7 g per day (120 mmol/ d) is set as Adequate Intake level and is recommended in the form of fresh fruit and vegetables to also obtain the other advantages of whole food intake (Appel, 2009:361; IOM, 2004:Online). To convert millimoles (mmol) of potassium to milligrams (mg), the molecular weight of potassium (39.1) is used to multiply with (IOM, 2004:Online).

In general, individuals from industrialised countries on a vegetarian diet have lower blood pressure levels than those following an omnivorous diet; these lower levels are also accompanied with a slower increase in blood pressure with aging. There are however dietary and non-dietary effects of a vegetarian lifestyle other than high potassium intakes, that may contribute to this positive effect on blood pressure levels (Appel, 2009:361). The anti-oxidant contribution of fruit and vegetables (Johnson *et al*., 2002:921) may offer a protective effect. Although an increased fibre intake is associated with lowering blood pressure levels, insufficient evidence exists to recommend increased fibre intake as a way to reduce blood pressure (Appel, 2009:362).

iii) Adopting the Dietary Approaches to Stop Hypertension (DASH) eating plan

The DASH eating plan emphasizes the inclusion of large amounts of fruits, vegetables and low-fat dairy foods and includes whole grains, poultry, fish, and nuts. The DASH diet is lower in fats, red meat, sweets, and sugar-containing beverages and the diet provides potassium, magnesium and calcium close to the 75th percentile of consumption in the USA as well as high amounts of fibre and protein. The sodium content of the DASH eating plan is approximately 3 g per day and the target for macronutrient distribution is 27% fat, 55% carbohydrates and 18% protein (Appel *et al.*, 1997:1118-1119; Appel, 2009:361-362).

The DASH trial showed that adopting certain dietary practices could favourably affect blood pressure levels, even in normotensive individuals. With introduction of the DASH eating plan, a reduction in blood pressure began within two weeks and was maintained for six weeks of participating in an intervention. In the group of hypertensive participants, the DASH combination diet lowered systolic blood pressure by 11.4 mm Hg and diastolic blood pressure by 5.5 mm Hg more than the control diet (Appel *et al.*, 1997:1117, 1122). The blood pressure lowering effect of the DASH eating plan seems to show more profound results in black populations and in individuals with higher blood pressure values (Appel, 2009:361).

In the Dietary Guidelines for Americans 2010 (USDA, 2010:Online), specific provision is made for individuals with hypertension with the number of daily servings recommended from each food group indicated according to different energy intake levels (Table 2.8).

Table 2.8 The DASH eating plan with the number of recommended daily servings from each food group, at various energy intake levels (USDA, 2010:Online, appendix 10).

Food Group	1200 kcal	1400 kcal	1600 kcal	1800 kcal	2000 kcal	2600 kcal	3100 kcal	Serving Sizes
Grains	4–5	5–6	6	6	6–8	10–11	12–13	1 Slice Bread; 1 Oz Dry Cereal; ½ Cup Cooked Rice, Pasta, or Cereal
Vegetables	3–4	3–4	3–4	4–5	4–5	5–6	6	1 Cup Raw Leafy Vegetable; ½ Cup Cut-Up Raw or Cooked Vegetable; ½ Cup Vegetable Juice
Fruits	3–4	4	4	4–5	4–5	5–6	6	1 Medium Fruit; ¼ Cup Dried Fruit; ½ Cup Fresh, Frozen, or Canned Fruit; ½ Cup Fruit Juice
Fat-Free or Low-Fat Milk and Milk Products	2–3	2–3	2–3	2–3	2–3	3	3–4	1 Cup Milk or Yogurt; 1½ Oz Cheese
Lean Meats, Poultry and Fish	3 or less	3–4 or less	3–4 or less	6 or less	6 or less	6 or less	6–9	1 Oz Cooked Meats, Poultry, or Fish 1 Egg
Nuts, Seeds and Legumes	3 /week	3 /week	3–4 / week	4 /week	4–5 / week	1	1	⅓ Cup or 1½ Oz Nuts; 2 Tbsp Peanut Butter; 2 Tbsp or ½ Oz Seeds; ½ Cup Cooked Legumes (Dried Beans, Peas)
Fats and Oils	1	1	2	2–3	2–3	3	4	1 Tsp Soft Margarine; 1 Tsp Vegetable Oil ;1 Tbsp Mayonnaise; 1 Tbsp Salad Dressing
Sweets and added Sugars	3 or less /week	3 or less /week	3 or less /week	5 or less /week	5 or less /week	< 2	< 2	1 Tbsp Sugar; 1 Tbsp Jelly Or Jam; ½ Cup Sorbet, Gelatine Dessert; 1 Cup Lemonade
Maximum Sodium limit	2300 mg/day	2300 mg/day	2300 mg/day	2300 mg/day	2300 mg/day	2300 mg/day	2300 mg/day	

iv) Vitamin D

Vitamin D status depends on how much vitamin D is produced in the skin and vitamin D intake, either through food (high in vitamin D or enriched with vitamin D) or vitamin D supplements. Vitamin D is obtained from dietary sources in the form of ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3), but is also produced photochemically in the skin as vitamin D3 from 7-

dehydrocholesterol in the cell membranes upon UVB (280-315nm) exposure (Webb & Engelsen, 2006:1697; Wang, 2009:188). In the liver all these forms of vitamin D are converted to 25(OH)D, which in return are converted to 1,25dihydroxyvitamin D, the active hormone which acts together with parathyroid hormone and calcitonin to maintain plasma calcium concentrations. 25(OH)D is the major circulating metabolite of vitamin D, has a half-life of about three weeks and is considered a valid indicator of vitamin D status. Although vitamin D can be stored in fatty tissue for a period of time (Webb & Engelsen, 2006:1697), prolonged deficiency of vitamin D causes osteomalacia in adults and rickets in children (Lanham-New *et al.*,2011:145).

As dietary sources of vitamin D are limited to oily fish as richest source, and eggs, meat and fortified foods as other sources, many populations rely on sunlight for vitamin D production. In the United Kingdom, a serum 25(OH)D level of lower than 25nmol/l (≈10ng/ml) has traditionally been considered as the level associated with an increased risk of bone disease and classified as vitamin D deficiency. This level has also been agreed upon as a desirable population minimum (Lanham-New *et al.*,2011:145,151). Until recently, a serum level of lower than 30ng/ml 25(OH)D was regarded as the cut-off point in the United States of America to indicate a deficiency (Gallagher, 2008:77) with other researchers using various other cut-off points to indicate insufficient 25(OH)D levels (Wang, 2009:196; Lips, 2010:297). However, the Institute of Medicine of the National Academies recently lowered the reference value for adequate vitamin D status to higher than 20ng/ml, with a value of 12-20ng/ml indicating inadequacy, and a level lower than 12ng/ml, deficiency (IOM, 2010:Online).

An overview of current research on vitamin D and hypertension by Vaidya and Forman (2010:777), concluded that lower levels of vitamin D may be associated with higher blood pressure, with the accompanying higher risk of developing hypertension. It was further suggested that vitamin D is related to blood pressure through the renin-angiotensin system, but also concluded that supplementation with vitamin D has failed to lower blood pressure levels and should therefore be investigated before recommended as a treatment option. A review by Wang (2009:198) concluded that strong scientific evidence exists that maintaining a good vitamin D status is beneficial to cardiovascular and cerebrovascular diseases and that in many studies higher levels of 25(OH)D were inversely associated with the risk of hypertension. Sun *et al.* (2011: 534) also found in the Nurses' Health Study and Health Professionals Follow-Up Study in the US, that a higher dietary intake of vitamin D was associated with a lower risk of cardiovascular disease in men, but failed to find the same effect in women.

Sun exposure time required to obtain adequate UVB exposure for adequate production of vitamin D3 in human skin, depends on the solar elevation angle (time of day and latitude), as well as surface and atmospheric conditions (Webb & Engelsen, 2006:1698). For a black skin, the need for sun exposure to obtain adequate vitamin D levels can be up to 40 times longer than for a fairer

skin with recommended exposure times indicated in Table 2.9 (Webb & Engelsen, 2006:1698). This is due to the higher amount of cutaneous melanin that blocks conversion of 7-dehidrocholestrol to previtamin D in the black skin (Reis *et al*., 2010:1470). Recommended sun exposure times are typically based on a ¼ of body surface exposed (arms, hands and face) (Webb & Engelsen, 2006:1698).

Table 2.9 Recommended sun exposure times for individuals with black skins to ensure adequate vitamin D status (Webb & Engelsen, 2006:1700, Table 3)

Season	**09:00**	**10:30**	**12:00**
Winter	136 min	86 min	85 min
Autumn / Spring	56 min	29 min	23 min
Summer	33 min	19 min	16 min

In an overview of the worldwide status of vitamin D nutrition, Lips (2010:300) concludes that there is a high prevalence of inadequate vitamin D status (<20ng/ml) and that males in general have a better vitamin D status than females, with latitude and socio-economic status as important determinants of vitamin D status. He recommends moderate sun exposure and vitamin D fortification as a population intervention to address poor vitamin D status.

In a comparison between the results of the NHANES III and NHANES 2000-2004, a decrease in 25(OH)D levels in the American population over the years is described, which is linked to increases in BMI, better sun protection and a lower milk consumption (Looker *et al.,* 2008:1526).

An inverse relationship between BMI and vitamin D status is widely described in the literature (Brock *et al*., 2010:465). Wortsman *et al.* (2000:692-693) explains this by showing that the skins of both obese and normal weight individuals produce the same amount of vitamin D under the same conditions, but that 57% less vitamin D is absorbed into the circulation of obese individuals, due to the higher amount of subcutaneous fat that traps the cholecalciferol. Increasing sun exposure in obese individuals with a low vitamin D status therefore does not seem to be a viable way to correct vitamin D levels, but rather oral supplementation, where vitamin D is available in the system before it is stored in the adipose tissue (Wortsman *et al.*,2000:693).

Vitamin D status does not seem to be affected by HIV status, and similar mean plasma 25(OH)D levels were found in HIV positive and negative individuals, in a study consisting mostly of black participants (72%) (Stephensen *et al.*, 2006:1135).

v) Vitamin C

Although some studies showed a positive effect of vitamin C supplementation on lowering blood pressure levels, the effects of increased vitamin C intake are not clear and certain, and supplementation are currently not included in population strategies (Appel, 2009:362).

vi) Omega-3 polyunsaturated fatty acid supplementation

Omega-3 polyunsaturated fatty acid supplements lower blood pressure in hypertensive individuals in a dose-dependent manner, starting at doses of 3 g per day and more. Because of the possible side effects and high doses required to obtain a blood pressure lowering effect, omega-3 fatty acid supplementation is not routinely recommended in the treatment of hypertension (Appel, 2009:362). The antagonistic effect of omega−3 polyunsaturated fatty acids on the angiotensin II receptor, renin secretion and angiotensin converting enzyme activity may be responsible for lowering blood pressure (Poudyal *et al*., 2011:377-378).

vii) Moderation of alcohol consumption

A meta-analysis of randomized controlled studies by Xin *et al.* (2001:1112) found that blood pressure increases with increased alcohol consumption. A significant reduction in mean systolic and diastolic blood pressures of -3.31 mm Hg (-2.52 to -4.10 mm Hg) and -2.04 mm Hg (-1.49 to -2.58 mm Hg), respectively was found with a self-reported reduction of alcohol intake of between 16% and 100% (Xin *et al.,* 2001:1114). The INTERSALT study also found a strong significant, independent relationship between heavy alcohol consumption and blood pressure levels (INTERSALT, 1988:319). Although moderate alcohol intake is known to protect against heart disease, alcohol consumption above two portions per day is associated with increases in blood pressure levels in a dose related manner. It is therefore recommended that alcohol consumption should be limited to no more than one alcoholic drink per day in females and lighter-weight persons and no more than two alcoholic drinks per day for most males (Appel, 2009:361). In general one alcoholic drink equals 360 ml (12 oz.) of regular beer, 150 ml (5 oz.) of wine (12% alcohol), or 45 ml (1.5 oz.) of distilled spirits. Alcohol exerts both a vasopressor and vasodilatory effect and affects blood pressure further by decreasing sodium excretion and lowering serum potassium levels (Kawano *et al.*, 2004:167-168).

viii) Coffee

In a systematic review and meta-analyses of long term prospective studies, investigating coffee consumption with the risk of hypertension, Zhang *et al*. (2011:1212) found that habitual coffee

consumption of more than three cups a day was not associated with an increased risk of hypertension, when compared with consumption of less than one cup of coffee per day.

ix) Calcium and magnesium

Although some studies have shown inverse relationships, between blood pressure and calcium and magnesium intakes, evidence is not sufficient to recommend either calcium or magnesium supplementation to lower blood pressure (Appel, 2009:362; IOM, 2010:Online).

x) Macronutrient composition of the diet

Some studies indicate that a higher protein intake, especially from plant sources can result in lower blood pressure levels. Evidence also exists that a diet with a low glycaemic index can lower blood pressure more than a diet with a high glycaemic index and that a diet high in sugar can increase blood pressure levels. Increased mono-unsaturated fat intake (resulting in a decrease in carbohydrate intake) has also been associated with lower blood pressure levels. Current evidence linking macronutrient composition of the diet to hypertension is inconsistent and this, together with the indirect impact of other dietary factors on blood pressure make it difficult to make specific recommendations with regard to dietary macronutrient composition to reduce blood pressure (Appel, 2009:362).

2.6 Conclusion

Genetic predisposition, aging and gender are some of the non-modifiable factors that contribute to the prevalence of hypertension. Dietary and lifestyle factors however also play a significant role in blood pressure control and can be modified to reduce blood pressure levels as well as the amount of medication required to maintain blood pressure levels within normal ranges (Bacon *et al.*, 2004:313). Diet and lifestyle however is difficult to change, even if it results in improvement of health, longevity and better quality of life. Individualised approaches are therefore seldom as successful as hoped for and the majority of hypertensive patients prefer the convenience and "easy way out" of using medication to obtain lower and healthier blood pressure levels. Public health strategies that support better food choices and dietary practices as well as increasing physical activity can however make an important impact on addressing the public health problem of hypertension.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

In this chapter, the study sample, inclusion and exclusion criteria, variables and operational definitions, techniques, procedures, statistical analysis as well as ethical considerations of this study which formed part of the Assuring Health for All in the Free State (AHA-FS) study are described.

3.2 STUDY DESIGN

AHA-FS is a prospective and longitudinal epidemiological study, collecting baseline and follow-up data from a rural and an urban population. For the purpose of this study, data from the urban baseline phase were used to describe and interpret associations and relations between sets of data. The urban baseline phase, that encloses this study, is regarded as a cross-sectional study.

3.3 POPULATION

The population of the AHA-FS urban baseline study included the urban areas serviced by the Mangaung University Community Partnership Programme (MUCPP) and included black households in the Freedom Square, Turflaagte, Namibia, Kagisanong, Chris Hani and Rocklands Buffer area of Mangaung, Bloemfontein, South Africa.

3.4 SAMPLE

The sample for the urban baseline phase of the AHA-FS study was drawn from the Mangaung township area. The number of plots in Freedom Square, Turflaagte, Namibia, Kagisanong, Chris Hani and Rocklands Buffer area were counted on a municipal map and an estimate was made of additional informal households in the open areas. A stratified proportional cluster sample was selected, stratified by area and formal plot or squatter households in the open areas. Using randomly selected X and Y coordinates, 100 starting points were selected in this area. From each starting point, five adjacent households were approached and invited to participate in the study. In each area, the following number of starting points were used:

Freedom Square:	40 / 4791 formal houses and 3 / 300 informal houses;
Turflaagte:	18 / 2216 formal houses and 3 / 300 informal houses;
Namibia:	16 / 2011 formal houses and 1 / 60 informal houses;
Kagisanong:	6 / 651 formal houses and 0 / 50 informal houses;
Chris Hani:	9 / 1126 formal houses and 1 / 70 informal houses;
Rocklands Buffer area:	3 / 345 formal houses.

3.5 INCLUSION CRITERIA

The urban AHA-FS study included all adult participants in the sample, 25-64 years of age, who provided informed consent. A total of 391 households were included, and data were collected for 328 adult females, 103 adult males (431 adults in total) and 112 pre-school children. From this sample, all adults with complete data sets for age, gender, blood pressure, body weight, height, activity level, as well as available blood samples for genetic testing and 25(OH)D analysis, were selected. The final sample for the current study thus consisted of 339 adults (76 males and 263 females).

3.6 EXCLUSION CRITERIA

Participants with incomplete data sets for age, gender, blood pressure, body weight, height, activity level or where blood samples for genetic testing and 25(OH)D analysis were not available, were excluded from the current study.

3.7 PROCEDURES AND INFORMATION COLLECTED DURING THE BASELINE SURVEY

Fieldworkers visited the households identified by the sampling process to explain the study to the adult members of the households, using an information letter to the communities (Appendix A), and to invite them to participate. If the household was willing to participate, an information document, which included more information on the project, was given to each participant and an informed consent form (Appendix B) was completed. Each adult participant who gave written, informed consent, was issued with a participation letter (Appendix C), stating and explaining all the relevant arrangements regarding the study.

Over a period of two weeks (2-13 March 2009), on specifically scheduled days, participants gathered at the MUCPP clinic, which is the local municipal health clinic in the area. Participants were required to arrive in a fasting state. On arrival all participants were registered and received an identification checklist with a reference number. Participants were informed about the procedures of the day in the language of their choice, making use of translators, where necessary. Participants

then rotated through different research stations in the venue, where they completed various questionnaires during structured interviews conducted in Afrikaans, English or Sesotho; underwent medical examination and anthropometrical evaluation; and provided blood and urine samples. The stations were indicated on each participant's checklist and each station was signed or checked off after completion. During the interviews or examinations, the following data were collected:

3.7.1 Household questionnaires

A socio-demographic questionnaire, which recorded data on basic demographics of household members, structure of the house; household income, household amenities, and access to water and sanitation, was completed for each household (Appendix D).
A household food security questionnaire, which collected data on agriculture practices, crop and livestock ownership and a hunger scale, was also completed for each household.

3.7.2 Individual Questionnaires

Dietary intake was collected with an adapted 24-hour recall, designed to record usual daily intake as well as a short food frequency questionnaire (Appendix E). Food intake was classified as below, within or above the recommendations of the United States Department of Agriculture's (USDA) Food Guide Pyramid (Shaw *et al*., [n.d.]:Online). The dietary questionnaire was completed for all participating adults and all pre-school children in each household.

Physical activity was calculated with a 24-hour recall of physical activity (Appendix E), from which energy expended was calculated and activity levels categorized as sedentary, low active, active and very active. This questionnaire was completed for all participating adults in each household.

A health questionnaire (Appendix F) was completed for all adults participants in each household and included information on social support (group membership, network of friends, family structure), tobacco and alcohol consumption patterns, medical history and medications, family medical history, alternative medical practices; levels of stress, and behaviours related to stress control. A questionnaire on knowledge, practices and attitudes about nutrition was completed for one adult in each household.

Individual physical and clinical measurements included a medical examination that comprised of the assessment of blood pressure, heart rate, visible signs and symptoms, cardiovascular abnormalities, respiratory abnormalities, abdominal pathology, nervous system abnormalities and skin pathology, as well as Human Immunodeficiency Virus (HIV) pre-test counselling. The medical examination was performed on all participating adults and all pre-school children in each

household and was recorded on a medical examination form (Appendix G). If necessary, a referral letter was issued to participants in need of further medical support (Appendix H).

Anthropometric measurements on adults included height, weight, waist, hip and mid-arm circumferences, and skin fold measurements. In children, height, weight, head and mid-arm circumferences and skin folds were measured. Measurements were taken on all participating adults and all pre-school children in each household and were recorded on an anthropometry form (Appendix I).

For all participating adults in each household, blood samples were taken for a full blood count; HbA1c, glucose and fibrinogen levels; HIV testing and CD4 counts (all performed on fresh samples); and samples were taken and stored for later analysis of 25-OH-D levels and genetic testing. A spot urine sample was also collected from each participating adult.

3.8 OPERATIONAL DEFINITIONS

For the purpose of this study, variables that were measured on the adult participants from the AHA Free State urban baseline study were used. Measures included blood pressure, HIV status, anthropometry (body mass index, waist to height ratio and body adiposity index), serum 25-hydroxy vitamin D levels, sodium and potassium intakes, physical activity levels and hypertension-related genetic factors.

3.8.1 Blood pressure

Blood pressure is the force exerted per unit area on the walls of arteries. Hypertension is defined as persistent high arterial blood pressure, with the systolic blood pressure (blood pressure during the contraction phase of the cardiac cycle) at 140mmHg or higher and/or the diastolic blood pressure (the pressure during the relaxation phase of the cardiac cycle) at 90 mm Hg or higher (Couch & Krummel, 2008:866; Seedat *et al.*, 2006:341). Participants using prescription medication for the management of hypertension at the time of the interview were also regarded as hypertensive for the purpose of this study (Wallace *et al.*, 2007:51).

Mean arterial pressure was calculated by the following formula (Sanada *et al.*, 2006:353):

Mean arterial pressure = diastolic blood pressure + ⅓ (systolic blood pressure – diastolic blood pressure).

3.8.2 HIV status

Hypertension is as common among HIV infected individuals as among the general public, and similar rates are found among those on highly active antiretroviral therapy (HAART) and those not (Medina-Torne *et al.,* 2011:Online). HIV status was confirmed using two fourth-generation serum assays and was categorized as positive or negative according to the presence of the HIV. Serum CD4 counts were only measured for HIV positive participants.

3.8.3 Anthropometry

For the purpose of this study, anthropometric measurements included body weight, height, hip circumference and waist circumference. Weight and height were used to calculate BMI, waist circumference and height were used to calculate waist to height ratio (WHtR) and height and hip circumference were used to calculate body adiposity index (BAI).

3.8.3.1 Body mass index

BMI was used to interpret body weight and describe the degree of adiposity. BMI is the relationship of weight to height and is calculated by dividing weight (kg) by height squared (m^2) (WHO, 2011:Online).

Although ethnic specific, BMI cut-off levels were identified to indicate the optimal threshold for cardio metabolic risk amongst African-Americans (Katzmarzyk *et al.*, 2011:1272), cut-off points as indicated in Table 3.1 were used to interpret BMI (WHO, 2011:Online).

Table 3.1 Classification of body mass index (WHO, 2011:Online)

Classification	**BMI (kg/m^2)**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obesity, class I	30.0-34.9
Obesity, class II	35.0-39.9
Extreme obesity, class III	≥40

3.8.3.2 Waist to height ratio

WHtR was used as an indicator to predict cardiovascular disease risk and is determined by dividing waist circumference (cm) by height (cm). A WHtR of 0.5 or more was used to indicate increased risk (Ashwell & Hsieh, 2005:303).

3.8.3.3 Body adiposity index

Hip circumference and height were used to calculate the level of body adiposity according to the formula described by Bergman *et al.* (2011:Online). The latter authors found that using a calculation that includes height and hip circumference, provides an accurate measure of body adiposity when compared to the golden standard of dual-energy X-ray absorptiometry (DXA). Body adiposity index (BAI) estimates body fat percentage directly (Bergman *et al.*, 2011:Online) and is calculated as follows:

BAI (% body fat) = Hip circumference (cm) / Height $(m)^{1.5}$ -18 (Bergman *et al.*, 2011:Online).

BAI was interpreted using the classification described in the research of Ricciardi *et al.* (2009:4) as indicated in Table 3.2.

Table 3.2 Classification of body adiposity index (Ricciardi *et al.*, 2009:4)

Classification	Fat %	
	Male	Female
Low body fat	10-15	14-20
Average body fat	16-18	21-25
Above average body fat	19-20	26-29
Overweight body fat	21-25	30-35
Obese body fat	$\geq$ 26	$\geq$ 36

3.8.3.4 Waist circumference

Waist circumference was interpreted according to the WHO guidelines as indicated in Table 3.3 (WHO, 2008:Online):

Table 3.3 Classification of waist circumference (WHO, 2008:Online):

Classification	Waist circumference for males	Waist circumference for females
Increased risk of metabolic complications	>94 cm	>80 cm
Substantially increased risk of metabolic complications	>102 cm	>88 cm

Waist circumference, even within the higher parameters of the normal cut-off point (80–88 cm for females and 94–102 cm for males (indicated in Table 3.3), are associated with a higher prevalence of hypertension in middle-aged and older adults, independent of BMI and various other hypertension risk factors (Levine *et al*., 2011:Online). The lower cut-off values of >94cm for males and >80cm for females are also the ethnic specific values agreed upon for Sub-Saharan Africans to indicate risk for metabolic complications (Alberti *et al.,*2009:1642), and are also recognised as the cut-off points to indicate cardio-metabolic risk amongst African-Americans (Katzmarzyk *et al.*, 2011:1272).

3.8.4 Serum 25-hydroxy Vitamin D (25(OH)D) levels

Serum 25-hydroxy vitamin D is the major circulating metabolite of vitamin D and is considered a valid indicator of vitamin D status (Webb & Engelsen, 2006:1697). Low vitamin D levels are associated with an increase in blood pressure (Forman, *et al.,* 2007:1063). A serum level of <30ng/ml 25(OH)D was previously used to indicate a deficiency (Gallagher, 2008:77), but the Institute of Medicine (IOM) of the National Academies (US) recently lowered the cut-off value for adequate vitamin D status to 20ng/ml (IOM, 2010:Online). For the purpose of this study a serum 25(OH) D level of >20ng/ml was categorized as an acceptable vitamin D status, a value of 12-20ng/ml as inadequacy, and a level <12ng/ml as a deficiency (IOM, 2010:Online).

3.8.5 Sodium and potassium intakes

For the purpose of this study sodium and potassium intakes were biochemically quantified and the diet was evaluated for sources of these electrolytes.

3.8.5.1 Biochemical assessment of sodium and potassium intakes

Since food records and dietary recall methods are not sensitive enough to accurately capture electrolyte intakes, determining dietary sodium and potassium intakes in a community setting present various challenges. Leiba *et al*. (2005:462) found that patients underestimated sodium intake by about 30-50% when self-reporting their dietary intakes.

As the balance of ingested sodium is mostly excreted in the urine, urinary sodium levels provide a good indication of sodium intake, except in cases of diarrhea and excessive sweating (IOM 2004:Online). However, 24-hour urine collection is not always practical (Tanaka *et al.,* 2002:97-98), particularly not in the community setting of this study. Therefore, for the purpose of this study, spot urine samples were used to estimate sodium and potassium intakes, as these have been found to correlate closely with 24-hour urine excretion values to provide accurate indications of body levels of various elements, including sodium and potassium (Ilich *et al.*, 2009:220; Tanaka *et al.*, 2002:99).

Sodium intakes were compared with the recommendations by the IOM of an adequate intake (AI) of 1.5g (65mmol) sodium per day, which equals 3.8g of table salt (sodium chloride) per day, and a tolerable upper intake level (UL) of 2.3g (100mmol) sodium per day, which is equal to 5.8g table salt (sodium chloride) per day (IOM, 2004:Online).

Similarly, as between 77 - 90% of potassium consumed, is excreted in the urine, urinary potassium excretion also reflects dietary potassium intake (IOM, 2004:Online). Potassium intakes were compared to the recommendations of the IOM of 4.7g per day (120 mmol per day), which is recommended to be met by consumption of fresh fruit and vegetables, in order to obtain the other advantages of whole foods as well (Appel, 2009:361; IOM, 2004:Online).

3.8.5.2 Dietary sources of sodium and potassium

For the purpose of this study usual dietary intake of sodium-rich foods (salt, stock and soup powders) and fruit and vegetables as sources of potassium were assessed. Intakes in this study were compared with the South African Food Based Dietary Guideline which recommends a combined intake of 5 portions or 400g of fruit and/or vegetables, per day (Love & Sayed, 2001:S24). Frequency of intake was thus categorized as <1 portion per day, 1-4 portions per day and 5 or more portions per day. Although the DASH eating plan, developed to prevent and treat hypertension and published as part of the Dietary Guidelines for Americans (USDA, 2010:Online), recommends an intake of 3-4 portions of vegetables per day and 3-4 portions of fruit per day (thus a combined intake of 6-8 portions of vegetables and/or fruits per day) the South African Food

Based Dietary Guidelines were chosen for the purpose of this study as these guidelines are more relevant to the current study population.

3.8.6 Physical activity level

For the purpose of this study self-reported physical activity levels physical activity levels (PAL), which refers to those activities structured around the household, as well as extra mural activities, were assessed and categorized as indicated in Table 3.4 (Weston, Petosa & Pate, 1997:138).

Table 3.4 Classification of physical activity levels

Classification	PAL
Sedentary	1-1.39
Low active	1.4-1.59
Active	1.6-1.89
Very active	1.9-2.5

For determining relative risk, sedentary and low active values were binned as “inactive”; and active and very active values were binned as “active”.

3.8.7 Genetic factors

Genotype describes the unique genetic composition of an organism (Cummings, 2006:47; DeBusk, 2008:365). Many genes occur in slight variations of the most common version, which is referred to as polymorphisms (DeBusk, 2008:371). Various different genes have been implicated in the aetiology of hypertension and have been identified as candidate genes for screening to determine hypertension risk. However, for the purpose of this study, the genotype of the study population was described by the presence of the following polymorphisms:

- A/G polymorphism at the -217 and C/T polymorphism at the -235 locus of the *angiotensinogen gene* (*AGT*);
- The presence of three polymorphisms (R65L (G448T), A142V (C679T) and A486V (C1711T) of the *G protein-coupled receptor kinase type 4 (GRK4)* gene; and
- The presence of a T/C polymorphism of the *aldosterone synthase* gene -344 *CYP11B2*.

3.9 TECHNIQUES

In the following section, the techniques used in this study to measure blood pressure, anthropometry (BMI and body adiposity index), serum 25-hydroxy vitamin D levels, sodium and

potassium intakes, physical activity levels and hypertension-related genetic factors, are described:

3.9.1 Blood pressure

Blood pressure was measured by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated in a chair for at least five minutes with their feet on the floor and arm supported at heart level. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements was recorded.

3.9.2 HIV status

Fasting venous blood samples were obtained and used to determine HIV status. Primary screening for HIV was performed using the Enzygnost® HIV Integral II Ag/Ab test, which was confirmed by performing the Vironostica® HIV Uni-Form II Ag/Ab test.

3.9.3 Anthropometry

Anthropometric measurements were taken by trained fourth year BSc Dietetics students under supervision of the researchers.

3.9.3.1 Body weight

Body weight was determined using the WHO STEPwise approach to Surveillance (STEPS) method (WHO, 2008:Online). A floor type, Seca 770 digital scale (Medical Scales and Measuring Systems seca kk., Japan) with graduation accurate to 100g and a maximum capacity of 200kg was used on a firm, flat surface. Subjects were weighed in minimal clothing after an overnight fast and after voiding (subjects were weighed after providing an urine sample). Researchers ensured that the subject stood on the centre of the scale without support and with his/her weight distributed evenly between both feet while weight was recorded to the nearest 0.1kg.

3.9.3.2 Height

Height was measured using the WHO STEPS method (WHO, 2008:Online), with a Seca stadiometer (Medical Scales and Measuring Systems seca kk., Japan), accurate to the nearest 5mm. Subjects were requested to stand with knees straight, feet together and the heels, buttocks

and upper part of the back touching the stadiometer. Measures were taken with the head placed in the Frankfort plane and the subject was requested to take a deep breath and stand tall, while their height was being measured.

3.9.3.3 Waist circumference

Waist circumference was measured at the end of several consecutive natural breaths, at a level parallel to the floor, with a non-stretch measuring tape that fitted snugly. Subjects were requested to stand with their arms hanging on the sides of their bodies and measurements were taken accurately to the nearest 5mm midway between the top of the superior iliac crest and the lowest rib in the mid axillary line (WHO, 2008:Online).

3.9.3.4 Hip circumference

Hip circumference was measured at the widest portion of the buttocks, at a level parallel to the floor, with a non-stretch measuring tape that fitted snugly. Subjects were requested to stand with their arms hanging on the sides of their bodies while measurements were taken accurately to the nearest 5mm (WHO, 2008:Online).

3.9.4 Serum 25-hydroxy vitamin D (25(OH)D) levels

Blood samples were obtained through venous puncture after an overnight fast. Active vitamin D levels were estimated from the serum 25(OH)D levels. A chemiluminescent immunoassay from the *Liaison 25(OH) vitamin D total assay kit* from *DiaSorin* (DiaSorin, Stillwater, MN, USA) was used to measure 25(OH) D levels.

3.9.5 Sodium and potassium intake

Neither a 24-hour recall nor a food frequency method is regarded as an accurate measure of dietary intake (Schatzkin *et al*., 2003:1054), but in this study a food frequency questionnaire was utilized to assess the frequency with which sodium and potassium rich foods were consumed in this community (Appendix E).

To calculate sodium and potassium intakes, a spot urine specimen was collected and stored at -60 degrees Celsius. These specimens were thawed for analysis of sodium, potassium and creatinine levels in order to calculate the estimated 24 hour excretion of sodium and potassium. The following

formula developed by Tanaka *et al.* (2002:99) was used to estimate 24-hour sodium and potassium excretion, namely:

24-hour Urine Na (mEq/day) = 21.98 X $XNa^{0.392}$,
where XNa = Spot Na / Spot Cr X (-2.04 X age + 14.89 X weight (kg) + 16.14 X height (cm) – 2244.45);

and

24-hour Urine K (mEq/day) = 7.59 X $XK^{0.431}$,
where XK = Spot K / Spot Cr X (-2.04 X age + 14.89 X weight (kg) + 16.14 X height (cm) – 2244.45).

Urinary sodium, potassium and creatinine levels were determined by an accredited laboratory. Sodium and potassium levels were determined using integrated chip technology (ICT), based on the principle of indirect ion selective electrodes on an *Abbott Architect C4000* machine (Seago *et al.*, 2010:A74#B71). Creatinine levels were assessed by photometrically measuring the chemical reaction between creatinine and sodium picrate (Jaffe reaction). The laboratory results were expressed as mmol/L and was converted to mg/dL by first converting mmol/L to µmol/L by multiplying with 1000 and then by dividing by 88.4 to obtain the conventional unit (Rowlett, 2005:Online):

$$mg/dL = \frac{Mmol/L \times 1000}{88.4}$$

A food frequency questionnaire (Appendix E) was used to evaluate the frequency with which sodium and potassium rich food sources were consumed. Data were collected during a structured interview and questions were included on the frequency of potato chips intake, on the use of salt or salt containing flavourings, as well as on the intake of fruit and vegetables.

3.9.6 Physical activity level

Various methods are used to access physical activity in free living communities, including self-reporting questionnaires, motion sensors (pedometers and accelerometers) and heart rate monitors (Loney *et al.*, 2011:62), with each method having its own strengths and limitations. To accurately determine physical activity in individuals and communities poses a challenge. When choosing an assessment tool, level of accuracy, cost-effectiveness, practicality and degree of invasiveness are factors to bear in mind (Loney *et al.*, 2011:68-69). Accurate physiologic assessments are invasive and can often only be performed in a laboratory or in a controlled environment, and are thus impractical for large population groups (Weston, Petosa & Pate, 1997:138). Instead various activity questionnaires which are inexpensive, easy to administer, have a low participant burden have been developed and validated. Although these questionnaires are

incapable of precise measurements, they are useful tools to describe individual activity levels in study populations (Loney *et al.*, 2011:68-69; Corder *et al.,* 2009:866).

Physical activity levels in the AHA-FS study were described by self-reporting of activity during the previous 24 hours. The Previous Day Physical Activity Recall (PDPAR), as validated by Weston, Petosa and Pate (1997:138,) was used. Their research indicated that the PDPAR provides valid and reliable data with regard to physical activity. Participants were asked to list all activities performed during the previous day and from this data the researchers calculated a physical activity level (PAL) for each participant (Appendix E). The PAL value corresponds to lifestyle activity and represents the energy spent for the described activities, in addition to the energy needs of daily living.

3.9.7 Genetic factors

Blood samples for genetic testing were obtained through venous puncture. Plasma was removed and the cells containing the genetic material stored at -80 degrees Celsius. Genetic analysis was done by the Department of Haematology and Cell Biology, Faculty of Health Sciences from the University of the Free State in a laboratory operating according to ISO 17025 standards.

After thawing the blood cells, 200µL per sample was blotted on fluorescent treponemal antibody (FTA) paper (Whatman, New York), designed to bind and stabilize nucleic acids (Vitha & Yoder, 2005:Online). Each FTA paper sample was labeled, allowed to dry, and stored at room temperature.

FTA disks of 1.2mm in diameter were punched and washed twice with FTA purification reagent, followed by three washes with 0.1X TE buffer (1mM Tris-HCL, pH 8 and 0.1mM EDTA, pH8). The use of FTA paper is considered an efficient method for clinical application with results comparable to traditional DNA extraction procedures (Pezzoli *et al.*, 2007:1182). A multiplex real-time polymerase chain reaction (PCR) assay was performed on the *Stratagene Mx3005P* thermal cycler. VIC and FAM labelled probes allowed separate allele detection using the following PCR conditions: 1 cycle of 10 minutes at 95^{o}C, followed by 50 cycles of 15 seconds at 95^{o}C, and 1 minute at 60^{o}C for T842C (*AGT*), -344C/T (*CYP11B2*) and G448T, C679T and C1711T (*GRK4*), and 1 minute at 62^{o}C for -217 A/G (*AGT*).

3.10 VALIDITY, RELIABILITY AND LIMITATIONS OF THE STUDY

Validity of a measuring tool or research instrument refers to the extent to which the instrument measures what it is supposed to measure (Leedy & Ormrod 2005:28). For this study, content

validity was enhanced by ensuring that all data collected and used was directly related to the aim and objectives of the study.

Reliability refers to the consistency with which a research instrument / investigator yields a certain result when the measure is repeated (Leedy & Ormrod 2005:28). In this study reliability was enhanced by ensuring that all data was collected by trained researchers, using standardised techniques.

3.10.1 Body weight, height, waist and hip circumference

Validity

Validity was ensured by using calibrated measuring equipment. Scales were moved to the zero point before each measurement. The weight recorded by the scale was compared with a known weight and the scale gave the same reading each time when measuring the standard weight.

Reliability

Reliability was improved by using four researchers trained in anthropometrical techniques and measurements taken according to standardised WHO procedures.

3.10.2 Serum 25-Hydroxy vitamin D levels

Validity

25-Hydroxy vitamin D serum levels are described in the literature as a valid indication of circulating vitamin D levels (Gallagher 2008:74).

Reliability

Reliability of 25(OH)D values was ensured by following standardised techniques for sample collection and storage, and by using standard laboratory techniques performed by trained laboratory staff to measure the serum values.

3.10.3 Sodium and potassium intakes

Validity

The gold standard for measuring sodium and potassium intake is considered to be a urine sample collected over 24-hours, but collecting these samples are impractical in population studies. The use of spot urine samples to reflect sodium and potassium intakes has been proven accurate and offers a more practical alternative (El-Bokl *et al.*, 2009:3633-3644; Tanaka *et al.*, 2002:99-101; Ilich *et al.*, 2009:220).

Reliability

Reliability of sodium and potassium intakes were ensured by following standardised techniques for collection and storage of urine samples and by using standard laboratory techniques performed by trained laboratory staff to measure these electrolytes in spot urine samples. Reliability of sodium and potassium intake measurements in this study are somewhat limited by the need to use formulae to calculate 24 hour excretion from spot urine samples (Tanaka *et al.*, 2002:99-101).

Another possible limitation of this study was that urine samples were stored at -60 degrees Celsius for longer than six months, which could possibly have influenced the stability of creatinine in the samples (WHO, 2002:Online). Reliability was however enhanced by also collecting data on dietary intake markers for sodium and potassium to cross-check against the laboratory measures. However, self-reported dietary intakes, although convenient, is not necessarily completely reliable (Schatzkin *et al*., 2003:1054; Schoeller, 1995:18).

3.10.4 Physical activity

Validity

A validated instrument, namely the PDPAR, was used to assess physical activity levels (Weston, Petosa & Pate, 1997:138).

Reliability

In order to enhance reliability researchers trained in using the PDPAR according to a standardised technique administered the instrument. Furthermore, 10% of the sample was repeated at the end of each day, to verify reliability. A limitation in the study is the fact that activity was self-reported and not directly measured. The possibility for repeated over or under reporting therefore exists.

3.10.5 Genetic factors

Validity

Validity was assured by testing for specific gene polymorphisms related to hypertension expression as described in literature. Hypertension is a multifactorial trait, influenced by more than one gene with multiple variants that interact with environmental factors (DeBusk *et al*., 2005:591; Ehret *et al.,* 2008:1508). Limited data is available on the prevalence of these polymorphisms in this population group and therefore specific gene polymorphisms commonly published in similar studies, were selected and tested for.

Reliability

Reliability was ensured by following standardised techniques for sample collections and storage, and validated laboratory techniques, with the inclusion of controls for each assay.

3.11 PROCEDURES

Procedures followed during the baseline of the larger AHA survey are described in section 3.7. Figure 3.1 outlines the procedures that were followed for the current study after baseline data collection:

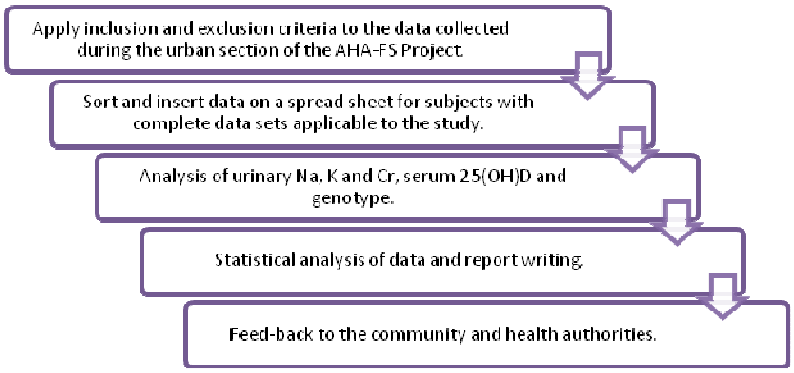

Figure 3.1 Procedures of the current study after baseline data collection

3.12 STATISTICAL ANALYSIS

The researcher performed the statistical analysis by using the Predictive Analytics SoftWare (PASW) Statistics Student Version 18.0 software by *SPSS: An IBM Company*. For the majority of measurements, data were categorized according to the participants' blood pressure into a hypertensive and a normotensive group. Frequencies and percentages were used to summarize

categorical data. Means and standard deviations, or percentiles where appropriate, were used to summarize quantitative variables. Comparisons of means were done using t tests, and chi-square test, two tailed Pearson correlations and multivariate logistic regressions were used as appropriate to describe and test associations between variables.

3.13 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee of the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07C). This study forms part of and is an extension of the previously approved AHA-FS study (ethic approval number ETOVS: 21/07). Approval and permission to conduct the study was also obtained from the Department of Health, local municipalities and community leaders. Written informed consent (Appendix B) was obtained from all participants, using an information letter in which procedures were explained to them in detail in the language of their choice (Sotho, English, Afrikaans). Participation was voluntary and participants were free to withdraw from the study at any time. Confidentiality was retained during all stages of the research by ensuring that no names were disclosed, or written down in questionnaires. Codes were used in all data analysis and results. All subjects received a light snack after the fasting blood samples were drawn and received transportation money to refund them for any travel costs to and from the venue. Feed-back and medical results were provided to participants who wanted to receive feed-back, and a final report of findings will be made available to the Free State Department of Health and to community leaders.

3.14 SUMMARY

A sample of 339 male and female participants between the ages of 25 and 64 provided informed consent and were included in this study. A wide array of data was collected at a central research location as part of the urban phase of the AHA-FS study. For the purpose of this study, blood pressure, HIV status, anthropometry, serum 25-hydroxy vitamin D levels, sodium and potassium intakes, physical activity, and hypertension related genetic factors were determined using valid and reliable techniques and measurements.

3.15 BIBLIOGRAPHY

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J-C, James WPT, Loria CM and Smith SC. 2009. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation,120:1640-1645. [Online].

Available from: http://circ.ahajournals.org/cgi/content/full/120/16/1640 [Accessed 3 June 3rd 2011].

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal of Clinical Hypertension, 11 (7): 358-368.

Ashwell M and Hsieh SD. 2005. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. Journal of Food Sciences and Nutrition, 56 (5):303-307.

Bergman RN, Stefanovsk D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, Xiang AH and Watanabe RM. 2011. A better index of body adiposity. Obesity, 19 (5): 1083-1089.

Corder K, Van Sluijs EMF, Wright A, Whincup P, Wareham NJ and Ekelund U. 2009 Is it possible to assess free-living physical activity and energy expenditure in young people by self-report? American Journal of Clinical Nutrition, 89:862–70.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Cummings MR. 2006. Human heredity – principles and issues. 7th ed. Belmont: Thomson Brooks/Cole.

DeBusk R. 2008. Assessment: nutritional genomics, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 364-379.

DeBusk RM, Fogarty CP, Ordovas JM and Kornman KS. 2005. Nutritional genomics in practice: Where do we begin? Journal of the American Dietetic Association, 105: 589-598.

Ehret GB, Morrison AC, O'Connor AA, Grove ML, Baird L, Schwander K, Weder A, Cooper RS, Rao DC, Hunt SC, Boerwinkle E and Chakravarti A. 2008. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. European Journal of Human Genetics, 16: 1507–1511.

El-Bokl MA, Senousy BE, El-Karmouty KZ, Mohammed IE, Mohammed SM, Shabana SS and Shalaby H. 2009. Spot urinary sodium for assessing dietary sodium restriction in cirrhotic ascites. World Journal of Gastroenterology, 15(29): 3631-3635.

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC and Curhan GC. 2007. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension, 49: 1063-1069.

Gallagher ML. 2008. The nutrients and their metabolism, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 39-143.

Institute of Medicine of the National Academies (IOM). 2004. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. [Online].
Available from: http://iom.edu/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx [Accessed March 4th, 2011].

Institute of Medicine of the National Academies (IOM). 2010. Dietary Reference Intakes for Calcium and Vitamin D. Ed by Ross AC, Taylor L, Yaktine AL and Del Valle HB. [Online].
Available from: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Report-Brief.aspx [Accessed December 15th, 2010].

Ilich JZ, Blanuša M, Orlić ZC, Orct T and Kostial K. 2009. Comparison of calcium, magnesium, sodium, potassium, zinc and creatinine concentration in 24-h and spot urine samples in women. Clinical Chemistry and Laboratory Medicine, 47 (2): 216-221.

Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL, Ravussin E, Ryan DH and Bouchard C. 2011. Ethnic-Specific BMI and Waist Circumference Thresholds. Obesity, 19: 1272–1278.

Kim C-H, Park HS, Park M, Kim H and Kim C. 2011. Optimal cutoffs of percentage body fat for predicting cardiovascular disease risk factors in Korean adults. The American Journal of Clinical Nutrition, 94: 34-39.

Leedy PD and Ormrod JE. 2005. Practical Research. 8th ed. New Jersey: Prentice Hall.

Leiba A, Vald A, Peleg E, Shamiss A, Grossman E. 2005. Does dietary recall adequately assess sodium, potassium, and calcium intake in hypertensive patients? Nutrition, 21(4):462-466.

Levine DA, Calhoun DA, Prineas RJ, Cushman M, Howard VJ and Howard G. 2011. Moderate waist circumference and hypertension prevalence: the REGARDS study. American Journal of Hypertension [Online].
Available from: http://www.nature.com.proxy.lib.fsu.edu/ajh/journal/vaop/ncurrent/pdf/ajh2010258a.pdf

[Accessed February 4th, 2011].

Loney T, Standage M, Thompson D, Sebire SJ and Cumming S. 2011. Self-Report vs. Objectively Assessed Physical Activity: Which Is Right for Public Health? Journal of Physical Activity and Health, 8: 62 -70.

Love P and Sayed N. 2001. Eat plenty of vegetables and fruits every day. The South African Journal of Clinical Nutrition, 14 (3): S24-S32.

Medina-Torne S, Ganesan A, Barahona I and Crum-Cianflone NF. 2011. Hypertension is common among HIV-infected persons, but not associated with HAART. Journal of the International Association of Physicians in AIDS Care [Online].
Available from:
http://jia.sagepub.com.proxy.lib.fsu.edu/content/early/2011/08/26/1545109711418361.full.pdf+html [Accessed November 4th, 2011].

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Pezzoli N, Silvy M, Woronko A, Le Treut T, Lévy-Mozziconnaci A, Reviron D, Gabert J and Picard C. 2007. Quantification of mixed chimerism by real time PCR on whole blood-impregnated FTA cards. Leukemia Research, 31: 1175-1183.

Ricciardi R, Metter EJ, Cavanaugh EW, Ghambaryan A and Talbot LA. 2009. Predicting cardiovascular risk using measures of regional and total body fat. Applied Nursing Research, 22: 2-9.

Rowlett R. 2005. How many? A dictionary of units of measurement. [Online].
Available from: http://www.unc.edu/~rowlett/units/index.html [Accessed June 7th 2011].

Sanada H, Yatabe J, Midorikawa S, Hashimoto S, Watanabe T, Moore JH. Ritchie MD, Williams SM, Pezzullo JC, Sasaki M, Eisner GM, Jose PA and Felder RA. 2006. Single-nucleotide polymorphisms for diagnosis of salt-sensitive hypertension. Clinical Chemistry, 52 (3): 352-360.

Schatzkin A, Kipnis V, Carroll RJ, Midthune D, Subar AF, Bingham S, Schoeller DA, Troiano RP and Freedman LS. 2003. A comparison of a food frequency questionnaire with a 24-hour recall for

use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. International Journal of Epidemiology,32:1054–1062.

Schoeller DA. 1995. Limitations in the assessment of dietary energy intake by self-report. Metabolism, 44(2):18-22.

Seago JL, Li Q and Pistone D. 2010. Abbott ARCHITECT ICT (ISE) Module Use-Life Response Characteristics. Clinical Chemistry, 56:6: A74#B71.

Seedat YK, Croasdale MA, Milne FJ, Opie LH, Pinkney-Atkinson VJ, Rayner BL and Veriava Y. 2006. South African hypertension guideline 2006. South African Medical Journal, 96 (4): 337-362.

Shaw A, Fulton L, Davis C and Hogbin M. [n.d.] Using the food guide pyramid: A resource for nutrition educators. US Department of Agriculture, Food, Nutrition and Consumer services, Centre for Nutrition Policy and Promotion. [Online].
Available from: http://www.nal.usda.gov/fnic/Fpyr/guide.pdf [Accessed June 7th 2011].

Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H and Hashimoto T. 2002. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. Journal of Human Hypertension 16: 97–103.

United States Department of Agriculture (USDA). 2010. Dietary Guidelines for Americans 2010. [Online].
Available from: http://dietary guidelines.gov [Accessed June 21st 2011].

Vitha S and Yoder DW. 2005. High throughput processing of DNA samples on FTA paper for PCR analysis. [Online].
Available from: http://microscopy.tamu.edu/lab-protocols/protocols-FTA_paper_processing.pdf [Accessed September 16th 2011].

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

Webb AR and Engelsen. 2006. Calculated Ultraviolet Exposure Levels for a Healthy Vitamin D Status. Photochemistry and Photobiology, 82(6):1697-1703 [Online].
Available from: http://www.bioone.org/doi/full/10.1562/2006-09-01-RA-670 [Accessed March 1st, 2011].

Weston AT, Petosa R and Pate RR. 1997. Validation of an instrument for measurement of physical activity in youth. Medicine and Science in Sports and Exercise, 29 (1): 138-143.

World Health Organization (WHO). 2002. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. [Online].
Available from: http://whqlibdoc.who.int/hq/2002/WHO_DIL_LAB_99.1_Rev.2.pdf [Accessed July 29th, 2011].

World Health Organization (WHO). 2008. WHO STEPS Surveillance: Guide to physical measurements. [Online].
Available from: http://www.who.int/chp/steps/part3_section3.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2008. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008 [Online].
Available from http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online].
Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

CHAPTER 4

THE ASSOCIATION OF BODY MASS INDEX, WAIST TO HEIGHT RATIO, BODY ADIPOSITY INDEX AND WAIST CIRCUMFERENCE WITH HYPERTENSION IN AN URBAN BLACK COMMUNITY IN MANGAUNG, SOUTH AFRICA

ABSTRACT

Hypertension is responsible for a large and increasing proportion of the global disease burden and is becoming increasingly prevalent in low-income countries. Various genetic and environmental factors have been found to influence blood pressure, with especially body weight showing a strong relationship with hypertension.

Objectives: The objective of this study was to determine the association of various indices of body adiposity, namely body mass index (BMI, kg/m^2), waist to height ratio (WHtR), body adiposity index (BAI) (expressed as % body fat) and waist circumference (WC), with blood pressure in a low-income, black, urban community in Mangaung, South Africa.

Methods: Baseline data from the urban phase of the Assuring Health for All in the Free State (AHA-FS) study were used. Field workers visited households selected in a stratified proportional cluster sample, to encourage participation in the research. At the research centre, body weight, height, waist and hip circumference and blood pressure were measured. The presence of the human immunodeficiency virus (HIV) was determined using calibrated equipment and following standardized techniques. Weight and height were used to calculate BMI, WC and height were used to calculate WHtR, and height and hip circumference were used to calculate BAI.

Results: 339 Adults (76 males and 263 females) were included in the study, with a mean age of 44.3 $\pm$ 10.6 (SD) years, ranging between 25 and 63. More than a third (39.8%) of the study population was HIV positive. In total 63.4% (57.9% of the males and 65.0% of the females) had blood pressure values $\geq$140/90mmHg or were currently using antihypertensive medication, implicating hypertension. Based on BMI, 44.8% of the study population had normal weights or were underweight, 23% were overweight, and 32.1% were obese. The majority (58.6%) had a WHtR equal to or above the cut-off point of 0.5, indicating increased cardiovascular risk. The mean BAI was 34.1%, with 76.3% of participants having an overweight/obese body fat percentage. WC above 88cm was measured in 44.3% (n=116) of females, and WC above 102cm was measured in 3.9% (n=3) of males. Significant positive correlations were found between mean arterial blood pressure and BMI (r=0.261; p<0.001), WHtR (r=0.357; p<0.001) and BAI (r=0.245; p<0.001). Controlling for age, the prevalence of hypertension was significantly and positively (p< 0.001)

correlated with BMI in the group as a whole, and with WC in females (p<0.001). WC was significantly correlated to BMI in males (r=0.893, p<0.001) and females (r= 0.891, p<0.001) in this study population, and WHtR seemed to be a stronger predictor of mean arterial pressure than BMI or BAI in this population. HIV status was inversely correlated with BMI (r=-0.262;p<0.001), WHtR (r=-0.299;p<0.001), BAI (r=-0.226;p<0.001) and WC in females (r=-0.364;p<0.001). HIV status was inversely associated with the prevalence of hypertension (χ^2=20.424, df=1, ρ<0.001), but as as mean age and body adiposity levels both effects blood pressure and differed statistically significant between the HIV positive and negative groups (p<0.001), the inverse association could have resulted from the effect of age and body adiposity levels on blood pressure.

Conclusion: More than half of this study population suffered from hypertension, two out of five were HIV positive and the majority was overweight or obese, increasing their morbidity and premature mortality risk. All indices of abdominal obesity and body fatness, including BMI, WHtR, BAI and WC were significantly related to blood pressure, supporting weight loss as first line intervention for treatment and prevention of hypertension and its accompanying disease burden in this urban black population. Findings also confirm the use of WHtR as a practical measure to screen for hypertension in this population.

Key words: hypertension, body mass index, waist to height ratio, adiposity index, waist circumference, HIV, urban black population, AHA-FS, South Africa.

4.1 INTRODUCTION

Hypertension is a public health problem responsible for a large and increasing proportion of the global disease burden (Couch & Krummel, 2008:865; Norman *et al.,* 2007:692). It is one of the leading causes of disease in middle-income countries, and is becoming increasingly prevalent in low-income countries as well (Norman *et al*., 2007:692). Elevated blood pressure levels pose a risk for myocardial infarction, stroke, left ventricular hypertrophy, renal disease and blindness. Under-diagnosis and/or inadequate treatment lead to extensive organ damage and premature death (Couch & Krummel, 2008:865; Steyn, 2006:80) which affects the economy of a country, making prevention and early intervention important objectives for health authorities worldwide.

The Assuring Health for All in the Free State (AHA FS) study is an epidemiologic study that aims to provide an estimate of the disease burden attributable to infectious diseases and under nutrition on the one hand, and obesity and its comorbidities on the other. In the urban baseline phase of this study, abnormal high blood pressure levels were measured in more than half (56.9%; n=415) of the total population and mean systolic and diastolic blood pressure measurements were 135.5 ±23.9 (SD) mmHg and 89.8 ±17.4 (SD) mmHg respectively. This high prevalence of hypertension, often in the presence of medical treatment, was the main motivation for further investigation of the primary causes of hypertension in this population. The aim of this sub-study was to determine the

association and impact of adiposity status as indicated by body mass index (BMI), waist to height ratio (WHtR), body adiposity index (BAI) and waist circumference (WC) with hypertension in this low-income, black, urban community in Mangaung, South Africa.

Various genetic and environmental factors influence blood pressure. A strong relationship exists between body weight and the prevalence of hypertension, with the risk of developing hypertension being two to six times higher in overweight than normal weight persons (Couch & Krummel, 2008:870-871). In population surveys that were conducted in Finland and included almost 60 000 participants, overweight and general adiposity on its own were associated with an increased risk for heart failure (Hu *et al.*, 2010:237). A strong relationship also exists between the degree of obesity as measured by BMI, WC or waist hip ratio and the prevalence of ischemic stroke, regardless of gender or race (Yatsuya *et al*., 2010:417). Furthermore, elevated blood pressure is also closely linked to increased WC as a marker of abdominal obesity, and both these parameters are included in the diagnostic criteria for the diagnosis of the metabolic syndrome which refers to a cluster of diseases, including cardiovascular diseases, which are associated with insulin resistance (Eckel, Grundy & Zimmet, 2005:1415;1419). Almost all clinical trials therefore recommend weight loss as first-line treatment for lowering blood pressure (Steyn, 2006:84; Couch & Krummel, 2008:870-871; Appel, 2009:359; Neter *et al.*, 2003: 878). In a meta-analysis of 4874 participants, 5.1 kg of weight lost was calculated to affect a reduction of 4.44mmHg in systolic pressure and a reduction of 3.57mmHg in diastolic pressure, which translates to a reduction of 1.05 mmHg and 0.92 mmHg in systolic and diastolic blood pressure respectively, per kilogram of weight lost (Neter *et al.*, 2003: 878).

Various studies found that markers of general obesity and abdominal obesity are good predictors of cardiovascular risk. Nordstrand *et al.* (2011:Online) reported that most of the general and abdominal obesity markers (WC, WHtR, BMI and visceral fat percentage) measured in their study on female morbidly obese patients, were good predictors of arterial stiffness. Similarly, Levine et al (2011:Online), reported that a WC even within the higher parameters of the normal cut-off points (80–88 cm for females and 94–102 cm for males) was associated with a high prevalence of hypertension in middle-aged and older adults, independent of BMI and various other hypertension risk factors.

Over the last decade, studies among various population groups have highlighted the need for ethnic specific anthropometric cut-off points to predict the risk for metabolic complications leading to chronic diseases of lifestyle In 2009 the International Diabetes Federation Task Force on Epidemiology and Prevention; the US National Heart, Lung, and Blood Institute; the American Heart Association; the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity published a joint statement on the diagnosis of the metabolic syndrome which included a list of the most current recommended ethnic specific WC

thresholds for abdominal obesity. According to this list, the cut-off points of 80cm for females and 94cm for males was recommended for Sub-Sahara Africans pending further studies (Alberti *et al,* 2009:1622*).*

The WHO, on the other hand, recognises two thresholds for abdominal obesity depending on the risk of metabolic complications including cardiovascular complications, namely an increased risk at a WC of >80 cm in females and >94 cm in males, and substantially increased risk at a WC of >88cm in females and >102cm in males (WHO, 2008:Online). Katulanda *et al.* (2010:Online) describes the need for ethnic specific anthropometric cut-off points when determining the risk for cardiovascular disease. They found BMI, WC and waist-hip ratio all to be associated with increased cardiovascular risk and propose that a BMI of 21.5 kg/m^2 or more, and a WC of 76 cm or more should be used for males and females to predict cardiovascular risk in a Sri Lankan population (Katulanda *et al.,* 2010:Online). The European Group for the Study of Insulin Resistance (EGIR) supports these lower reference values, by proposing a WC cut-off value of 80cm for females and 94cm for males (Balkau & Charles, 1999:442-443).

WHtR and BAI are other indexes of abdominal obesity, which are associated with cardiovascular risk. Ashwell and Hsieh (2005:303) and Sayeed *et al.* (2003:1) recommends WHtR as a better en more sensitive obesity marker than BMI and waist to hip ratio for predicting hypertension and as an early warning sign of metabolic health risks. An advantage of using WHtR is that the same cut-off points can be used for males and females, even within different ethnic groups (Ashwell & Hsieh, 2005:303). BAI estimates the percentage of body adiposity directly (Bergman *et al.*, 2011:Online), providing an easy screening tool for metabolic health risk.

Despite the strong evidence that exists to support an association between body weight and hypertension, Schutte *et al.* (2008: 534) failed to show a strong relationship between obesity markers (BMI and fat percentage) and blood pressure among black South African females in the Profiles of Obese Women with the Insulin Resistance Syndrome (POWIRS) study in Potchefstroom, South Africa. These authors also refer to a publication by Walker *et al.* from 1990, which reported similar findings. This study therefore aims to investigate the contributing role of overweight/obesity in the etiology of hypertension in black South Africans further.

As the prevalence of HIV is very high in Sub-Saharan Africa, HIV needs to be considered as a confounding factor when studying South African populations. Hypertension however, seems to be as common among HIV infected individuals as it is among the general population. Although multivariate models linked increasing age, higher BMI, longer duration of HIV and diabetes to hypertension in HIV positive populations, research has shown that the prevalence of hypertension does not seem to be influenced by HIV status or by the use of highly active antiretroviral therapy

(HAART) (Medina-Torne *et al.*, 2011:Online; Bloomfield *et al.,* 2011:Online; Baekken *et al.*, 2008:2131; Jung *et al*., 2004:2250; Bergersen *et al.,* 2003:731).

The aim of the current study was thus to determine the association of various indices of general and abdominal adiposity , namely BMI, WHtR, BAI and WC, with blood pressure in a low- income, black, urban community in Mangaung, South Africa.

4.2 METHODOLOGY

Data from the urban baseline phase of the Assuring Health for All in the Free State (AHA-FS) study were used for this study. Trained field workers visited households, selected in a stratified proportional cluster sample to encourage participation in the research, explain the purpose of the study and obtain written informed consent. Participants assembled at the central research centre, where, amongst other measurements, body weight, height, waist and hip circumference, HIV status and blood pressure were measured after an overnight fast. From the original study population, adult participants, 25-64 years of age, from whom written informed consent were obtained and who had complete data sets for age, gender, blood pressure, body weight, height, activity level and available blood samples for genetic testing and 25(OH)D analysis were included in this study.

Hypertension was defined as systolic blood pressure of 140mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher (Couch & Krummel, 2008:866). In this study, participants using prescription medication for the management of hypertension at the time of the interview were also diagnosed as hypertensive (Wallace *et al.*, 2007:51).

Blood pressure was measured by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated for at least five minutes in a chair with their feet on the floor and arm supported at heart level. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements recorded.

Fasting venous blood samples were obtained and used to determine HIV status. Primary screening for HIV was performed using the Enzygnost® HIV Integral II Ag/Ab test which was confirmed by performing the Vironostica® HIV Uni-Form II Ag/Ab test.

Anthropometric measurements were taken by trained fourth year, BSc Dietetics students under supervision of the researchers. Body weight was determined using the WHO STEPwise approach to Surveillance (STEPS) method (WHO, 2008:Online). A floor type, Seca 770 digital scale (Medical

Scales and Measuring Systems seca kk., Japan) with graduation accurate to 100g and a maximum capacity of 200kg was used on a firm flat surface. Participants were weighed in minimal clothing after an overnight fast and after providing a urine sample. Researchers ensured that the participant stood on the centre of the scale without support and with his/her weight distributed evenly between both feet while weight was recorded to the nearest 0.1kg.

Height was measured using the WHO STEPS method (WHO, 2008:Online), with a Seca stadiometer (Medical Scales and Measuring Systems seca kk., Japan) accurate to the nearest 5mm. Participants were requested to stand with their feet together and the heels, buttocks and upper part of the back touching the stadiometer and knees straight. Measures were taken with the head placed in the Frankfort plane and the participants requested to take a deep breath and stand tall, while height was measured.

BMI, the relationship of weight to height, was used to interpret and describe body weight. BMI was calculated by dividing weight (kg) by height squared (m^2) (WHO, 2011:Online).

The cut-off points as indicated in Table 4.1 were used to interpret BMI.

Table 4.1 Classification of body mass index (WHO, 2011:Online)

Classification	**BMI (kg/m^2)**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obesity, class I	30.0-34.9
Obesity, class II	35.0-39.9
Extreme obesity, class III	≥40

WC was measured at the end of several consecutive natural breaths, at a level parallel to the floor, accurate to the nearest 5mm with a non-stretch measuring tape that fitted snugly. Participants were requested to stand with their arms hanging by the sides of their body and measurements were taken midway between the top of the superior iliac crest and the lowest rib in the mid-axillary line (WHO, 2008:Online).

WC was interpreted according to the WHO guidelines as a substantially increased risk of metabolic complications at a WC of more than 88cm in females and more than 102cm in males and an

increased risk of metabolic complications at a cut-off point of more than 80cm in females and more than 94cm in males (WHO, 2008:Online).

WHtR was determined by dividing WC (cm) by height (cm). A WHtR of 0.5 or more indicates an increased risk for disease (Ashwell & Hsieh, 2005:304).

Hip circumference was measured at the widest portion of the buttocks, at a level parallel to the floor, accurate to the nearest 5mm with a non-stretch measuring tape that fitted snugly. Participants were requested to stand with their arms hanging by the sides of their body while measurements were taken (WHO, 2008:Online).

Hip circumference and height were used to calculate BAI according to the formula described by Bergman *et al.* (2011:Online). The authors found that a calculation that includes height and hip circumference provides an accurate measure of body adiposity when compared to dual-energy X-ray absorptiometry (DXA), which is recognized as a gold standard for estimating body fatness. BAI estimates body fat percentage directly (Bergman *et al.*, 2011:Online) and is calculated as follows:

BAI (% body fat) = Hip circumference (cm) / Height $(m)^{1.5}$ -18 (Bergman *et al.*, 2011:Online).

BAI was interpreted according to the classification of Ricciardi *et al.* (2009:4) as indicated in Table 4.2.

Table 4.2 Classification of body adiposity index (Ricciardi *et al.*, 2009:4)

Classification	Fat %	
	Male	Female
Low body fat	10-15	14-20
Average body fat	16-18	21-25
Above average body fat	19-20	26-29
Overweight body fat	21-25	30-35
Obese body fat	$\geq$ 26	$\geq$ 36

4.3 STATISTICAL ANALYSIS

The researcher performed the statistical analysis by using the PASW (Predictive Analytics SoftWare) Statistics Student Version 18.0 software by *SPSS: An IBM Company*. Frequencies and percentages were used to summarize the categorical data. Means and standard deviations, or percentiles as appropriate, summarized quantitative variables. Comparisons of means were done

using t-tests as appropriate; and chi-square, two tailed Pearson correlations, point bi-serial correlations and multivariate logistic regressions were used to describe and test correlations between variables and determine relative risks.

4.4 RESULTS

339 Adults (76 males and 263 females) with complete data sets from the baseline study were included in this study. Waist and hip circumference was not available for one participant. Participants had a mean age of 44.3 ± 10.6 (SD) years, ranging between 25 and 63 years, as described with other general information in Table 4.3.

Table 4.3 General description of the study population in terms of age, blood pressure and anthropometric measurements

Variable	N	Mean	Std Dev	Minimum	Maximum
Age (years)	339	44.3	10.60	25	63
Systolic blood pressure (mmHg)	339	135.5	23.67	72	203
Diastolic blood pressure (mmHg)	339	89.8	17.57	46	188
Height (cm)	339	159.4	7.84	139.8	180
Weight (kg)	339	70.1	21.40	31.9	140
BMI (kg/m^2) – Total	339	27.8	8.79	13.3	55.7
Male	76	21.4	5.55	14.5	49.9
Female	263	29.6	8.72	13.3	55.7
Waist (cm)- Total	338	86.5	16.99	47	143
Male	76	78.1	13.88	47	134
Female	262	88.9	17.06	59	143
Hip (cm)	338	104.3	18.18	73.5	165
WHtR	338	0.55	0.11	0.27	0.91
BAI (%)	338	34.1	10.23	16.8	66.1
Male	76	24.1	5.15	16.8	50.7
Female	262	37.1	9.46	19.2	66.1

More than a third (39.8%) of the study population was HIV positive. In 41.6% of participants, systolic blood pressure was ≥140mmHg and in 46.6% of participants, diastolic blood pressure was ≥90mmHg indicating hypertension. More than a quarter (25.4%) of the study population was using antihypertensive medication at the time of the study. In total 63.4% (57.9% males and 65.0% females) of participants were classified as hypertensive.

Participants were stratified into an older (>44 years) and a younger group (≤ 44 years). Hypertension was significantly more prevalent in the older than in the younger group (χ^2=45.526, df=1, ρ<0.001). A relative risk for hypertension of 1.78 (95% CI 1.48 - 2.13) was calculated, indicating that the older group had a 78% higher risk of being hypertensive than the younger group. When the genders were compared, while controlling for age, the relative risk for hypertension was 2.4 (95% CI 1.47 - 3.91) for males and 1.66 (95% CI 1.37 - 2.01) for females. Together these findings indicate that in this population, older males had the greatest risk of being hypertensive.

A high prevalence of overweight/obesity was found in this study population, with more than half (55.1%) of participants being either overweight/obese as indicated in Table 4.4.

Table 4.4 Body Mass Index distribution of the study population

BMI kg/m²	Classification	Males (n=76) n (%)	Females (n=263) n (%)	Total (N=339) n (%)
< 18.5 kg/m²	Underweight	19 (25.0)	21 (8.0)	40 (11.8)
18.5 – 24.9 kg/m²	Normal	43 (56.6)	69 (26.2)	112 (33.0)
25.0 – 29.9 kg/m²	Overweight	11 (14.5)	67 (25.5)	78 (23.0)
30.0 – 34.9 kg/m²	Obese Class I	0 (0.0)	39 (14.8)	39 (11.5)
35.0 – 39.9 kg/m²	Obese Class 2	2 (2.6)	32 (12.2)	34 (10.0)
≥40.0 kg/m²	Obese Class 3	1 (1.3)	35 (13.3)	36 (10.6)

According to BMI, 44.8% of the study population had a normal/underweight BMI, 23.0% were overweight and 32.1% obese. An at-risk WHtR of 0.5 or more was found in 58.6% of the population (n=338), including 69.8% of females and 19.7% of males.The mean BAI for this population was 34.1% ±10.23 (SD). According to Bergman *et al*. (2011:Online), BAI estimates body fat percentage directly. Therefore, body fat percentages in the current population were categorized according to the cut-off values used by Ricciardi *et al.* (2009:4) to predict cardiovascular risk, as indicated in Table 4.5. Based on BAI, 76.3% of participants were overweight/ obese.

Table 4.5 Body adiposity level distribution of the study population

Classification	Reference value (%)	Males (n = 76) n (%)	Reference value (%)	Females (n=263) n (%)	Total (N=339)
Low body fat	10-15		14-20	2 (0.8)	2 (0.6)
Average body fat	16-18	4 (5.3)	21-25	30 (11.5)	34 (10.1)
Above average body fat	19-20	16 (21.1)	26-29	28 (10.7)	44 (13.0)
Overweight body fat	21-25	39 (51.3)	30-35	72 (27.5)	111 (32.8)
Obese body fat	$\geq$ 26	17 (22.4)	$\geq$ 36	130 (49.6)	147 (43.5)

WC measures are indicated in Table 4.6. Increased cardiovascular risk according to WC was evident in 66.8% of females with WCs above the cut-off value of 80 cm, and in only 6.5% of males with a WC above the cut-off value of 94 cm. Mean arterial pressure correlated with WC in males ($r=0.262$, $p<0.05$) and females ($r=0.382$, $p<0.001$).

Table 4.6 Waist circumference distribution of the study population

Males (n=76)			Females (n=262)		
Low Risk WC (<94cm) n(%)	Increased Risk WC (94-102cm) n (%)	Central obesity (>102cm) n (%)	Low Risk WC (<80cm) n (%)	Increased Risk WC (80-88cm) n (%)	Central obesity (>88cm) n (%)
71 (93.4)	2 (2.6)	3 (3.9)	87 (33.2)	59 (22.5)	116 (44.3)

The distribution in WC for males and females, according to BMI categories, is indicated in Table 4.7. A significant correlation of a higher BMI with larger WCs is evident in both genders (males, $r=0.893$, $p<0.001$); (females, $r= 0.891$, $p<0.001$). Indeed in this population, overweight/obese BMI was significantly associated ($\chi^2=144.533$, $df=1$, $\rho<0.001$) with central obesity, indicated by a WC above the cut-off value of 102 cm in males and above the cut-off value of 88 cm in females.

Table 4.7 Body Mass Index in relation to waist circumference of the study population

BMI kg/m²	Waist circumference					
	Male (n=76)			Female (n=262)		
	Low Risk (<94cm) n(%)	Increased Risk (94-102cm) n(%)	Central obesity (>102cm) n(%)	Low Risk (<80cm) n(%)	Increased Risk (80-88cm) n(%)	Central obesity (>88cm) n(%)
< 18.5 kg/m²	19 (25.0)	0 (0)	0 (0)	21 (8.0)	0 (0)	0 (0)
18.5 – 24.9 kg/m²	42 (55.3)	1 (1.3)	0 (0)	49 (18.7)	19 (7.3)	1 (0.4)
25.0 – 29.9 kg/m²	10 (13.2)	1 (1.3)	0 (0)	14 (5.3)	33 (12.6)	19 (7.3)
30.0-34.9 kg/m²	0 (0)	0 (0)	0 (0)	3 (1.1)	6 (2.3)	30 (11.5)
35.0-39.9 kg/m²	0 (0)	0 (0)	2 (2.6)	0 (0)	1 (0.4)	31 (11.8)
≥40 kg/m²	0 (0)	0 (0)	1 (1.3)	0 (0)	0 (0)	35 (13.4)
Total	71 (93.4)	2 (2.6)	3 (3.9)	87 (33.2)	59 (22.5)	116 (44.3)

The prevalence of hypertension, according to BMI category is indicated in Table 4.8.

Table 4.8 Body Mass Index in relation to the prevalence of hypertension

BMI kg/m²	Normotensive (n=124) n (%)	Hypertensive (n=215) n (%)
< 18.5 kg/m²	23 (18.5)	17 (7.9)
18.5 – 24.9 kg/m²	57 (46.0)	55 (25.6)
25.0 – 29.9 kg/m²	23 (18.5)	55 (25.6)
30.0-34.9 kg/m²	5 (4.0)	34 (15.8)
35.0-39.9 kg/m²	6 (4.8)	28 (13.0)
≥40 kg/m²	10 (8.1)	26 (12.1)

When the participants were stratified according to low to normal BMI (<25 kg/m^2; low cardiovascular risk) and overweight/obese BMI (≥ 25 kg/m^2; high cardiovascular risk) 76.5% of the overweight/obese group was found to be hypertensive and hypertension was significantly more prevalent (χ^2=30.611, df=1, ρ<0.001) in the overweight/obese group. A t-test comparing the mean BMI of the hypertensive group (29.4 kg/m^2) with the normotensive group (24.9kg/m^2) showed a significant difference (p<0.001). After controlling for age, participants >44 years, with an overweight/obese BMI, had a relative risk of 1.25 (95% CI 1.06 - 1.46) of being hypertensive, compared to participants with a normal/underweight BMI. In the younger group (≤44 years) the

relative risk of being hypertensive was 2.69 (95% CI 1.75 - 4.15) when the person was overweight/obese compared to normal/underweight individuals.

When applying a multivariate logistic regression model (with mean arterial pressure as dependent variable and age and BMI as independent variables), BMI and age accounted for 14.8% of the change in arterial pressure. Furthermore for every 1 year increase in age, or 1kg/m^2 increase in BMI, it was calculated that an increase of 0.48 mmHg in mean arterial pressure could be expected ($p<0.001$).

In Table 4.9, WC according to risk level is categorised according to the prevalence of hypertension. A trend towards increased blood pressure with increasing WC is evident for females in this population.

Table 4.9 Waist circumference in relation to the prevalence of hypertension

Waist circumference Category	Males (n=76)		Females (n=262)	
	Normotensive (n=32) n (%)	Hypertensive (n=44) n (%)	Normotensive (n=92) n (%)	Hypertensive (n=169) n (%)
Lower Risk (≤88cm/102cm) (n= 159)	32 (42.1)	39 (51.3)	52 (19.8)	35 (13.4)
Increased Risk (80-88cm/94-102cm) (n=61)	0 (0)	2 (2.6)	21 (8.0)	38 (14.5)
Central Obesity (>88cm/102cm) (n=118)	0 (0)	3 (3.9)	19 (7.3)	97 (37.0)

This trend was confirmed by an independent samples t-test, showing a significant difference in Mean WC between hypertensive and normotensive males (82.3±15.3 SD; 72.17±8.9 SD) and females (93.5±16.4 SD; 80.5±14.9 SD) ($p<0.001$).

When the female study groups was stratified according to WC as >88cm (indicating central obesity) and ≤88cm, and controlled for age (< 44 years and ≤44 years), the group of older females with central obesity had a relative risk of 1.29 (95% CI 1.08 - 1.53) of being hypertensive. The younger group of females with central obesity had a relative risk of 2.22 (95% CI 1.56 - 3.14) of being hypertensive, indicating that the younger participants who had a central waist fat distribution had a greater risk of being hypertensive than the older women with central obesity.

Both WHtR (r=0.357; p<0.001) and BAI (r=0.245; p<0.001) significantly correlated with mean arterial blood pressure. A significant association was found between a WHtR of ≥0.5 (indicating increased cardiovascular risk) and the prevalence of hypertension (χ^2=43.057, df=1, ρ<0.001). WHtR (r= 0.357) also showed a stronger correlation with mean arterial pressure than BMI (r=0.261) or BAI (r=0.245).

HIV status was inversely associated with the prevalence of hypertension (χ^2=20.424, df=1, ρ<0.001) and an independent sample t-test showed a significant difference in mean arterial pressure between HIV-positive (100.2 mmHg) and HIV-negative (108.2 mmHg) participants (p<0.001). However as as age and body adiposity levels both affects blood pressure and mean values differed statistically significant between the HIV positive and negative groups (p<0.001), the inverse association could have resulted from the effect of age and body adiposity levels on blood pressure. Mean BMI was significantly (p<0.001) lower in HIV positive (24.9±7.4kg/m^2) compared to HIV negative participants (29.6±9.2kg/m^2). A point bi-serial correlation, showed an inverse correlation between HIV status and BMI (r=-0.262;p<0.001), WHtR (r=-0.299;p<0.001), BAI (r=-0.226;p<0.001) and WC (r=-0.364;p<0.001) in females.

4.5 DISCUSSION

The high prevalence of hypertension (63.4%) in this population compares with data from the American *National Health and Nutrition Examination Survey* (NHANES) between 2001-2004 (Reis *et al*., 2008:1471) that found a 61.8% prevalence of hypertension among the American black population older than 40 years. The higher prevalence of hypertension, especially in a population that included younger participants as well (25-63 years), is reason for concern.

In the current study population, the mean BMI was 27.8 kg/m^2, with 23% of these black urban adults being overweight, and 32.1% being obese. Thus more than half (55.1%) of the current study population had BMIs above the normal cut-off points, which is even higher than figures reported by the WHO database, which estimates the total South African prevalence of overweight/obesity at 45.1% (WHO, 2011:Online) and Kolbe-Alexander *et al*. (2008:Online) which estimates it at 48%. Compared to American populations, the mean BMI was lower than the mean BMIs reported by NHANES (Reis *et al*., 2008:1471) of 29.8±0.2 kg/m^2 for Americans of combined races, as well as that reported by the Family Blood Pressure Program Study of 30.1 kg/m^2 in 4968 African Americans (Ehret *et al.*, 2008:1509).

When the genders were separated in the current study, 14.5% and 3.9% of males (mean BMI 21.4 kg/m^2) were respectively overweight and obese; and 25.5% and 40.3% of females (mean BMI 29.6

kg/m^2) were respectively overweight and obese. This was generally similar to the national data from the second *South African Demographic and Health Survey,* conducted in 2003 (Department of Health, 2007:275-277), which reported 18.7% of urban black males to be overweight and 8.1% to be obese (mean BMI 23.1 kg/m^2), and 27.1% of urban black females to be overweight and 33.8% to be obese (mean BMI 28.1 kg/m^2). The evident trend of black South African females to have substantially higher BMIs than black South African males, were also supported by various other South African studies. Charlton *et al.* (2005:42) reported a mean BMI of 28 kg/m^2 in black males (with a 37.7% incidence of obesity) and 33.5 kg/m^2 amongst black females (with a 67.9% incidence of obesity) in a Cape Town study. In the Transition and Health During Urbanisation of South Africans (THUSA) study, 28.6% of the participants were obese and 53.8% of females had a BMI > 25 kg/m^2 (Kruger *et al.*, 2002:424). In contrast with the higher BMIs found amongst females in South Africa, NHANES (Flegal & Graubard, 2009:1215) reported an average BMI of 26.4 kg/m^2 for males and 26.2 kg/m^2 for females among Americans.

This high prevalence of overweight and obesity among the South African black population in the current and other studies, is significant in light of the International Study of Salt and Blood Pressure (INTERSALT), which reported a strong, significant, independent association of BMI with blood pressure (INTERSALT, 1988:319). In the current study, BMI was statistically related to the prevalence of hypertension, with the relative risk for hypertension being higher in the group younger than 40 years if they were also overweight/ obese.

BMI in this study was significantly related ($p < 0.001$) to WC. The mean WC of males was 78.1cm and that of females 88.9cm. Black urban women in the current study therefore had very similar WCs to that (88.5cm) reported in NHANES III for American females (Flegal & Graubard, 2009:121). In contrast, the black South African urban males in the current study had substantially lower WCs than the mean reported (95.6cm) for American males in NHANES III. The incidence of central obesity in this study was also higher than the national data for the urban black South African population from the *South African Demographic and Health Survey* (Department of Health, 2007:280-281), which reported increased WCs (>102 cm for males, and >88 cm for females) in 3.1% of males (vs. 3.9% in the current study), and 39.1% of females (vs. 44.1% in the current study). In Cape Town, South Africa, Charlton *et al.* (2005:42) described even higher mean WCs in a black population (92.cm in males and 92.4cm in females).

In the North West province, Mashele *et al.* (2010:208) found that, as in the current study, urbanized black South African hypertensive males and females were typically older, more obese and had a larger WC compared with the normotensive participants. In the current study, increasing WC correlated significantly with the prevalence hypertension among the females. Furthermore, the results showed that a female with a larger WC at a younger age was particularly more at risk for hypertension.

Using WHtR and BAI as obesity indicators also implicated a high percentage of participants as having an increased risk for chronic diseases. WHtR was increased in 58.6% of participants and 76.3% of all participants had a BAI in the overweight/obese category. The results also pointed to WHtR rather than BMI and BAI, as a useful tool to screen for hypertension in this particular community. These findings concurs with the meta-analysis by Lee *et al*. (2008:646) that indicated that measures of abdominal obesity are better predictors of cardiovascular disease risk than BMI.

More than one in three (39.8%) of the current study population was HIV positive, but although the prevalence of hypertension showed an inverse association with HIV status, this association was propably the result of lower body adiposity levels as indicated by BMI, WHtR, BAI and WC, associated with HIV infection. Similar lack of significant associations between hypertension and HIV status have also been reported by other researchers (Medina-Torne *et al.*, 2011:Online; Bloomfield *et al.,* 2011:Online; Baekken *et al.*, 2008:2131; Jung *et al*., 2004:2250; Bergersen *et al.,* 2003:731).

4.6 CONCLUSION

More than half of this study population had abnormally high blood pressure values ($\geq$ 140/90mmHg) and more than half were overweight/ obese based on BMI. Despite the high prevalence of HIV in this study population, the prevalence of hypertension was not influenced significantly by HIV status. The majority of participants had a WHtR of 0.5 or more, indicating health risk; and WHtR was significantly related to mean arterial pressure. WHtR also seemed to be a stronger predictor of mean arterial pressure than BMI or BAI in this population. The mean BAI fell within the overweight category for males and the obese category for females, with more than three quarters of participants presenting with an overweight/obese BAI. Adiposity levels showed a positive correlation with arterial blood pressure levels. Two thirds of the females and less than ten percent of the males had WCs associated with increased metabolic risk (more than 80cm and 94cm respectively), increasing the risk for disease and premature death. In females, hypertension was significantly related to WC and BMI, and WC and BAI significantly related to BMI. These results support weight loss as the first line of intervention for the treatment and prevention of hypertension with its accompanying disease burden in this population. Furthermore, the results suggest the prospect to screen for hypertension in adults in this population by calculating their WHtRs.

4.7 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee from the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07).

4.8 ACKNOWLEDGEMENT

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4.9 BIBLIOGRAPHY

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J-C, James WPT, Loria CM and Smith SC. 2009. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation,120:1640-1645. [Online].
Available from: http://circ.ahajournals.org/cgi/content/full/120/16/1640 [Accessed 3 June 3rd 2011].

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal Of Clinical Hypertension, 11 (7): 358-368.

Ashwell M and Hsieh SD. 2005. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. Journal of Food Sciences and Nutrition, 56 (5):303-307.

Baekken M, Os I, Sandvik L and Oektedalen O. 2008. Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy. Journal of Hypertension, 26:2126–2133.

Balkau B and Charles MA for the European Group for the Study of Insulin Resistance (EGIR). 1999. Comment on the provisional report from the WHO consultation. Diabetic Medicine, 16:442-443.

Bergman RN, Stefanovsk D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, Xiang AH and Watanabe RM. 2011. A better index of body adiposity. Obesity, 19 (5): 1083-1089.

Bergersen BM, Sandvik L, Dunlop O, Birkeland K and Bruun JN. 2003. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve

and HIV-negative controls: results from a Norwegian study of 721 patients. European Journal of Clinical Microbiology & Infectious diseases, 22:731–736.

Bloomfield GS, Hogan JW, Keter A, Sang E, Carter EJ, Velazquez EJ and Kimaiyo S. 2011. Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. PLoS ONE, 6 (7): e22288 [Online].
Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%Fjournal.pone.0022288 [Accessed November 8th, 2011].

Charlton KE, Phil M, Steyn K, Levitt NS, Zulu JV, Jonathan D, Veldman FJ and Nel JH. 2005. Diet and blood pressure in South Africa: intake of foods containing sodium, potassium, calcium, and magnesium in three ethnic groups, Nutrition, 21 (1): 39–50.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Department of Health. 2007. South Africa Demographic and Health Survey 2003, Full Report. Pretoria: Department of Health.

Eckel RH, Grundy SM and Zimmet PZ. 2005. The metabolic syndrome. Lancet, 365 (9468): 1415-1428.

Ehret GB, Morrison AC, O'Connor AA, Grove ML, Baird L, Schwander K, Weder A, Cooper RS, Rao DC, Hunt SC, Boerwinkle E and Chakravarti A. 2008. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. European Journal of Human Genetics, 16: 1507–1511.

Flegal KM and Graubard BI. 2009. Estimates of excess deaths associated with body mass index and other anthropometric variables. American Journal of Clinical Nutrition, 89:1213–1219.

Hu G, Jousilahti P, Antikainen R, Katzmarzyk PT and Tuomilehto J. 2010. Joint effects of physical activity, body mass index, waist circumference, and waist-to-hip ratio on the risk of heart failure, Circulation, 121:237-244.

Intersalt Cooperative Research Group (INTERSALT). 1988. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. British Medical Journal, (297):319-328.

Jung O, Bickel M, Ditting T, Rickerts V, Welk T, Helm EB, Staszewski S and Geiger H. 2004. Hypertension in HIV-1-infected patients and its impact on renal and cardiovascular integrity. Nephrology Dialysis Transplantation, 19: 2250–2258.

Katulanda P, Jayawardena MAR, Sheriff MHR and Matthews DR. 2010. Derivation of anthropometric cut-off levels to define CVD risk in Sri Lankan adults. British Journal of Nutrition, [Online]. Available from: http://journals.cambridge.org.proxy.lib.fsu.edu/download.php?file=%2FBJN%2FS0007114510004563a.pdf&code=0dce0c0f55f90b94a69d566e33aeaf5e [Accessed February 11th, 2011].

Kolbe-Alexander TL, Buckmaster C, Nossel C, Dreyer L, Bull F, Noakes TD and Lambert EV. 2008. Chronic disease risk factors, healthy days and medical claims in South African employees presenting for health risk screening. BMC Public Health, [Online]. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2475536/pdf/1471-2458-8-228.pdf [Accessed February 22nd, 2011].

Kruger HS, Venter CS, Vorster HH and Margetts BM. 2002. Physical inactivity is the major determinant of obesity in black women in the North West province, South Africa: the THUSA study. Nutrition, 18(5): 422-427.

Lee CM, Huxley RR, Wildman RP and Woodward M. 2008. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of Clinical Epidemiology, 61: 646-653.

Levine DA, Calhoun DA, Prineas RJ, Cushman M, Howard VJ and Howard G. 2011. Moderate waist circumference and hypertension prevalence: the REGARDS study. American Journal of Hypertension [Online]. Available from: http://www.nature.com.proxy.lib.fsu.edu/ajh/journal/vaop/ncurrent/pdf/ajh2010258a.pdf [Accessed February 4th, 2011].

Mashele N, Van Rooyen JM, Malan L and Potgieter JC. 2010. Cardiovascular function and psychological distress in urbanized black South Africans: the SABPA study. Cardiovascular Journal of Africa, 21(4): 206-211.

Medina-Torne S, Ganesan A, Barahona I and Crum-Cianflone NF. 2011. Hypertension is common among HIV-infected persons, but not associated with HAART. Journal of the International Association of Physicians in AIDS Care [Online].

Available from:
http://jia.sagepub.com.proxy.lib.fsu.edu/content/early/2011/08/26/1545109711418361.full.pdf+html [Accessed November 4th, 2011].

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Neter JE, Stam BE, Kok FJ, Grobbee DE and Geleijnse JM. 2003. Influence of weight reduction on blood pressure: A meta-analysis of randomized controlled trials. Hypertension, 42: 878-884.

Nordstrand N, Gjevestad E, N-DinhK, Hofso D, Roislien J, Saltvedt E, Os I and Hjelmesaeth J. 2011. The relationship between various measures of obesity and arterial stiffness in morbidly obese patients. BMC Cardiovascular Disorders, 11:7 . [Online].
Available from: http://www.biomedcentral.com/1471-2261/11/7 [Accessed February 11th, 2011].

Norman R, Gaziano T, Laubscher R, Steyn K, Bradshaw D and the South African Comparative Risk Assessment Collaborating Group. 2007. Estimating the burden of disease attributable to high blood pressure in South Africa in 2000. South African Medical Journal, 97 (8): 692-698.

Reis JP, Michos ED, Von Műhlen D and Miller ER. 2008. Differences in vitamin D status as a possible contributor to the racial disparity in peripheral arterial disease. The American Journal of Clinical Nutrition, 88: 1469 –1477.

Ricciardi R, Metter EJ, Cavanaugh EW, Ghambaryan A and Talbot LA. 2009. Predicting cardiovascular risk using measures of regional and total body fat. Applied Nursing Research, 22: 2-9.

Sayeed MA, Mahtab H, Latif ZA, Khanam PA, Ahsan KA, Banu A and Azad Khan AK. 2003. Waist to height ratio is a better obesity index than body mass index and waist-to-hip ratio for predicting diabetes, hypertension and lipidemia. Bangladesh Medical Research Council Bulletin, 29 (1):1-10.

Schutte AE, Huisman HW, Van Rooyen JM, Schutte R, Malan L, Reimann M, De Ridder JH,Van Der Merwe A, Schwarz PEH and Malan NT. 2008. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? Journal of Human Hypertension, 22(8):528-536.

Steyn K. 2006. Hypertension in South Africa, In Chronic diseases of lifestyle: 1995-2005. Technical report. Ed. by Steyn K, Fourie J and Temple N. Cape Town: South African Medical Research Council: 80-96.

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

World Health Organization (WHO). 2008. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008 [Online].
Available from http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2008. WHO STEPS Surveillance: Guide to physical measurements. [Online]
Available from: http://www.who.int/chp/steps/part3_section3.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online].
Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

Yatsuya H, Folsom AR, Yamagishi K, North KE, Brancati FL and Stevens J for the ARIC Study Investigators. 2010. Race- and sex-specific associations of obesity measures with ischemic stroke incidence in the Atherosclerosis Risk in Communities (ARIC) Study. Stroke, 41:417-425.

CHAPTER 5

HYPERTENSION, VITAMIN D STATUS AND BODY MASS INDEX IN AN URBAN BLACK COMMUNITY IN MANGAUNG, SOUTH AFRICA

ABSTRACT

Obesity and hypertension are major health concerns worldwide, affecting morbidity and mortality in many communities. While a strong relationship exists between body weight and hypertension, current research indicates that higher blood pressure levels may also be associated with lower blood levels of vitamin D. Low vitamin D levels has been linked to obesity markers in both females and males. Vitamin D status and the relationship thereof to obesity and hypertension have not been studied in South-African populations.

Objective: To assess the vitamin D status of a low-income, black, urban community in Mangaung, South Africa, and to examine whether vitamin D levels was associated with body mass index (BMI) and the prevalence of hypertension, taking gender differences and human immunodeficiency virus (HIV) status into consideration.

Methods: Data collected from the Assuring Health for All in the Free State (AHA-FS) study were used. Blood pressure was measured by a registered medical practitioner according to standard guidelines. Weight and height were measured by trained professionals, using calibrated equipment and standardized techniques and BMI (kg/m^2) was calculated. Blood samples were obtained through venous puncture after an overnight fast. Serum concentrations of 25-hydroxyvitamin D (25(OH)D) was determined with chemiluminescent immunoassays and HIV status was determined with antibody/antigen tests.

Results: 339 Adults (263 females, 76 males) were included, with a mean age of 44.3±10.6 (SD) years (range, 25-63 years). A total of 39.8% of the study population was HIV positive. In total, 63.4% of the study population either had blood pressure values ≥140/90mmHg or were currently using antihypertensive medication, implicating underlying hypertension. The mean 25(OH)D concentration for the sample was 38.4ng/ml (96nmol/L), ranging from 8.7-82.2ng/ml, indicating adequate status. Mean 25(OH)D concentrations were 37.0±10.6ng/ml for females and 43.5±11.8ng/ml for males. One female was vitamin D deficient with a 25(OH)D concentration <12ng/ml, while 3.8% (12 females and 1 male) had inadequate levels ranging between 12-20ng/ml. HIV status showed no association with vitamin D levels when a point bi-serial correlation, controlling for BMI, was done. More than half of the sample (23%) was overweight and obese (32%). Overweight/obesity was more prevalent among females (65.8%) than males (18.4%). Inverse correlations were found between BMI and vitamin D levels (p=0.01) and between mean arterial blood pressure and vitamin D levels (p=0.05), but no correlation was found between vitamin D and mean arterial blood pressure after controlling for BMI.

Conclusion: More than half of this urban, black, South-African population had abnormally high blood pressure values and more than a third was HIV positive, increasing their risk for disease and premature death. Although the majority was overweight/obese, almost 96% had adequate vitamin D status, despite expected low usual vitamin D intakes. The latitude (29°10´S) and high levels of sun exposure in Mangaung could have been responsible for the favorable vitamin D levels in the participants. Results from this study confirm the inverse relationship between vitamin D levels and hypertension reported by other researchers, but found that this relationship seemed to be dependent on BMI.

Key words: hypertension, vitamin D, BMI, HIV urban black population, AHA-FS, South Africa

5.1 INTRODUCTION

Obesity and hypertension are major health concerns worldwide, affecting morbidity and mortality in many communities. A strong relationship exists between body weight and the prevalence of hypertension. The risk of developing hypertension is two to six times higher in overweight than normal weight persons, and almost all clinical trials recommend weight loss as first-line treatment for lowering blood pressure (Steyn, 2006:84; Couch & Krummel, 2008:870-871; Appel, 2009:359).

Various studies show an inverse relationship, often age related, between vitamin D status, measured as serum 25-hydroxy vitamin D (25(OH)D) levels, and systolic blood pressure - thus lower serum 25(OH)D levels are associated with a higher risk of hypertension (Vaidya & Forman, 2010:777; Judd *et al.,* 2008:140; Forman *et al*., 2007:1068; Li *et al*., 2004:388). Vitamin D seems to act as a negative regulator of the renin gene (Rammos *et al.,* 2008:Online), with vitamin D deficiency increasing blood pressure by increasing the expression of renin. Renin activates angiotensin II which increases blood pressure by direct vasoconstriction and by causing sodium and water retention in the kidney (Rammos *et al.,* 2008:Online; Li *et al*., 2004:387). In a recent review, Wang (2009:198) concluded that strong scientific evidence exists that maintaining a good vitamin D status is beneficial to cardiovascular and cerebrovascular health. However, as Vaidya and Forman (2010:777) warn in their review, supplementation with vitamin D in many studies has failed to lower blood pressure levels, and should be investigated before recommended as a treatment option.

Vitamin D levels depend on how much vitamin D is produced in the skin as well as on vitamin D intake, through food (high in vitamin D or enriched with vitamin D) or vitamin D supplements. Vitamin D is obtained from dietary sources in the form of ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3), but is also photochemically produced in the skin as pre-vitamin D3, from 7-dehydrocholesterol in the cell membranes when skin is exposed to UVB (280-315nm), (Webb & Engelsen, 2006:1697; Wang, 2009:188). All three these forms are converted in the liver

to 25(OH)D, which is the inactive circulating form of the vitamin. When blood concentrations of calcium decreases, parathyroid hormone is released which stimulates the conversion of 25(OH)D to 1,25-dihydroxyvitamin D in the kidneys. 1,25-Dihydroxyvitamin D is the active, hormonal form of vitamin D which acts together with calcitonin to maintain plasma calcium concentrations. 25(OH)D has a half-life of about three weeks and is considered a valid indicator of vitamin D status. Although vitamin D can be stored in fatty tissue for a period of time (Webb & Engelsen, 2006:1697), prolonged deficiency of vitamin D causes osteomalacia in adults and rickets in children (Lanham-New *et al.*, 2011:145).

The length of time that skin needs to be exposed to sun in order to obtain adequate UVB exposure for sufficient production of vitamin D3 in human skin, depends on the solar elevation angle (time of day and latitude), as well as surface and atmospheric conditions (Webb & Engelsen, 2006:1698). For a black skin, the need for sun exposure to produce adequate vitamin D levels can be up to 40 times longer than what is needed by a fairer skin due to the higher amount of cutaneous melanin in black skins which blocks conversion of 7-dehidrocholestrol to pre-vitamin D in the skin. For a black skin, recommended exposure times in South Africa vary between 136 minutes at 09:00 in the winter to 16 minutes at 12:00 in the summer, based on a ¼ of body surface exposed (arms, hands and face) (Webb & Engelsen, 2006:1698; Reis *et al*., 2008:1470).

An inverse relationship between BMI and vitamin D levels has been widely described in the literature (Brock *et al*., 2010:465; Reis *et al*., 2008:1472). Wortsman *et al.* (2000:692-693) have shown that both obese and normal weight persons' skin produce the same amount of vitamin D under the same conditions, but that 57% less vitamin D is absorbed into the circulation of obese persons, due to the higher amount of subcutaneous fat that traps the cholecalciferol. Sun exposure in obese individuals with a low vitamin D status therefore does not seem to be the solution. Vitamin D should rather be supplemented orally, so that vitamin D is released into the system before it is stored in the adipose tissue (Wortsman *et al.*,2000:693).

The rate of HIV infection in South Africa is estimated at 17.1-17.8% (UNICEF, 2010:Online; Groenewald *et al.,* 2011:Online). Although HIV infection influence body weight, which influence vitamin D levels, similar mean serum 25(OH)D levels have been reported in HIV positive and negative individuals, and vitamin D status does not seem to be affected by HIV status (Stephensen *et al.*, 2006:1135).

The aim of this study was to assess the vitamin D status of a low-income, black, urban community in Mangaung, South Africa, to examine whether vitamin D status was associated with BMI and the prevalence of hypertension.

5.2 METHODOLOGY

Data from the urban baseline phase of the Assuring Health for All in the Free State (AHA-FS) study were used in this study. Trained fieldworkers visited households selected in a stratified proportional cluster sample to encourage participation in the research, explain the purpose of the study and obtain written informed consent. Adult participants, 25-64 years of age for whom written consent was obtained and that had the required data sets available were included in the current study. Participants assembled at the central research centre after an overnight fast, where blood pressure, body weight and height were measured, and blood samples were drawn to measure vitamin D status and HIV status.

Hypertension is defined as systolic blood pressure of 140mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher (Couch & Krummel, 2008:866). In this study, participants using prescription medication for the management of hypertension at the time of the interview were also regarded as hypertensive (Wallace *et al.*, 2007:51).

Blood pressure was measured by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated in a chair for at least five minutes with their feet on the floor and arm supported at heart level. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements recorded.

Anthropometric measurements were taken by trained fourth year, BSc Dietetics students under supervision of the researchers. Body weight was determined using the WHO STEPwise approach to Surveillance (STEPS) method (WHO, 2008:Online). A floor type, Seca 770 digital scale (Medical Scales and Measuring Systems seca kk., Japan) with graduation accurate to 100g, and a maximum capacity of 200kg, was used on a firm flat surface. Participants were weighed in minimal clothing after an overnight fast and after providing an urine sample. Researchers ensured that the participant stood on the centre of the scale without support and with his/her weight distributed evenly between both feet while weight was recorded to the nearest 0.1kg.

Height was measured using the WHO STEPS method (WHO, 2008:Online), with a Seca stadiometer (Medical Scales and Measuring Systems seca kk., Japan) accurate to the nearest 5mm. Participants were requested to stand with their feet together and the heels, buttocks and upper part of the back touching the stadiometer and knees straight. Measures were taken with the head placed in the Frankfort plane, the participant was requested to take a deep breath and stand tall, and height was measured.

Body mass index (BMI), the relationship of weight to height, was used to interpret body weight and describe the degree of adiposity. BMI is calculated by dividing weight (kg) by height squared (m^2) (WHO, 2011:Online).

The cut-off points as indicated in Table 5.1 were used to interpret BMI classifications (WHO, 2011:Online).

Table 5.1 Classification of body mass index

Classification	**BMI (kg/m^2)**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obesity, class I	30.0-34.9
Obesity, class II	35.0-39.9
Extreme obesity, class III	≥40

Blood samples were obtained through venous puncture after an overnight fast. Primary screening for HIV was performed using the Enzygnost® HIV Integral II Ag/Ab test, and the results were confirmed with the Vironostica® HIV Uni-Form II Ag/Ab test.

A chemiluminescent immunoassay from the *Liaison 25(OH) vitamin D total assay kit* from *DiaSorin* (DiaSorin, Stillwater, MN, USA) was used to measure serum 25(OH) D levels as a measure of active vitamin D. Although a serum level of lower than 30ng/ml 25(OH)D was previously used to indicate a deficiency (Gallagher, 2008:77), the *Institute of Medicine of the National Academies* recently lowered the cut-off value for adequate vitamin D status to 20 ng/ml (IOM, 2010:Online). For the purpose of this study a serum 25(OH) D level of more than 20 ng/ml indicated an acceptable vitamin D status, while a value of 12-20 ng/ml indicated inadequacy, and a level lower than 12 ng/ml, deficiency.

5.3 STATISTICAL ANALYSIS

The researcher performed the statistical analysis using the PASW (Predictive Analytics SoftWare) Statistics Student Version 18.0 software by *SPSS: An IBM Company*.
Frequencies and percentages were used to summarize the categorical data. Means and standard deviations, or percentiles as appropriate, were used to summarize numerical characteristics. Comparisons of means were done using t-tests as appropriate. Two tailed Pearson correlations,

chi-square, point bi-serial correlations and logistic regressions were used to describe and test associations between variables.

5.4 RESULTS

339 Adults (76 males and 263 females) with complete data sets were included in this study. Participants in this study had a mean age of 44.3 ± 10.6 (SD) years, ranging between 25 and 63 years (Table 5.2).

Table 5.2 General description of the study population in terms of age, blood pressure, BMI and vitamin D levels

Variable	N	Mean	Std Dev	Minimum	Maximum
Age (years)	339	44.3	10.60	25	63
Systolic blood pressure (mmHg)	339	135.5	23.67	72	203
Diastolic blood pressure (mmHg)	339	89.8	17.57	46	188
Height (cm)	339	159.4	7.84	139.8	180.0
Weight (kg)	339	70.1	21.40	31.9	140.0
BMI (kg/m^2) - Total	339	27.8	8.79	13.3	55.7
Male	76	21.4	5.55	14.5	49.9
Female	263	29.6	8.72	13.3	55.7
25(OH) D (ng/ml) - Total	339	38.4	11.24	8.7	82.2
Males	76	43.5	11.84	15.6	82.2
Females	263	37.0	10.64	8.7	64.8

The mean 25(OH)D for the group was 38.4 ng/ml, and was significantly ($p<0.0001$) higher in males than in females (43.5 ng/ml and. 37.0 ng/ml respectively).

In 41.6% of participants, systolic blood pressure was ≥140mmHg and in 46.6% of participants, diastolic blood pressure was ≥90mmHg indicating hypertension. More than a quarter (25.4%) of the study population was using antihypertensive medication at the time of the study. In total 63.4% (57.9% males and 65.0% females) of the study population had blood pressure values ≥140/90mmHg or were using antihypertensive medication at the time of the study, implicating the presence of hypertension.

A high incidence of overweight/ obesity was found in this study population, with more than half (55.1%) of participants being either overweight or obese as indicated in Table 5.3.

Table 5.3 Body Mass Index (BMI) distribution of participants

BMI kg/m^2	Classification	Males (n = 76) n (%)	Females (n=263) n (%)	Total (N=339) n (%)
< 18.5 kg/m²	Underweight	19 (25.0)	21 (8.0)	40 (11.8)
18.5 – 24.9 kg/m²	Normal	43 (56.6)	69 (26.2)	112 (33.0)
25.0 – 29.9 kg/m²	Overweight	11 (14.5)	67 (25.5)	78 (23.0)
30.0 – 34.9 kg/m²	Obese Class I	0 (0.0)	39 (14.8)	39 (11.5)
35.0 – 39.9 kg/m²	Obese Class 2	2 (2.6)	32 (12.2)	34 (10.0)
≥40.0 kg/m²	Obese Class 3	1 (1.3)	35 (13.3)	36 (10.6)

Based on BMI, 44.8% of the study population had a normal/ underweight BMI status, 23.0% were overweight and 32.1% obese. The mean BMI differed significantly ($p<0.001$) between the hypertensive (29.4±8.7 SD) and normotensive groups (24.9±8.2 SD).

The majority of participants in this study had an acceptable vitamin D status as indicated by 25(OH)D levels in Table 5.4.

Table 5.4 Vitamin D status of participants

Males (n=76)			Females (n=263)		
Deficient levels (<12ng/ml) n (%)	Inadequate levels (12-20ng/ml) n (%)	Acceptable levels (>20ng/ml) n (%)	Deficient levels (<12ng/ml) n (%)	Inadequate levels (12-20ng/ml) n (%)	Acceptable levels (>20ng/ml) n (%)
0 (0.0)	1 (0.3)	75 (22.1)	1 (0.3)	12 (3.5)	250 (73.7)

Only one participant (0.3%) in this population had a clinical vitamin D deficiency while almost 96% of the participants had acceptable 25(OH)D levels (>20ng/ml).

Vitamin D status as distributed according to BMI category is indicated in Table 5.5.

Table 5.5 Vitamin D status in relation to Body Mass Index (BMI) category

BMI kg/m²	25-OH Vitamin D					
	Male (n=76)			Female (n=263)		
	Deficient levels (<12ng/ml) n (%)	Inadequate levels (12-20ng/ml) n (%)	Acceptable levels (>20ng/ml) n (%)	Deficient levels (<12ng/ml) n (%)	Inadequate levels (12-20ng/ml) n (%)	Acceptable levels (>20ng/ml) n (%)
< 18.5 kg/m²	0 (0)	0 (0)	19 (25)	0 (0)	1 (0.4)	20 (7.6)
18.5 – 24.9 kg/m²	0 (0)	1 (1.3)	42 (55.3)	0 (0)	3 (1.1)	66 (25.1)
25.0 – 29.9 kg/m²	0 (0)	0 (0)	11 (14.5)	0 (0)	1 (0.4)	66 (25.1)
30.0-34.9 kg/m²	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)	38 (14.4)
35.0-39.9 kg/m²	0 (0)	0 (0)	2 (2.6)	0 (0)	2 (0.8)	30 (11.4)
≥40 kg/m²	0 (0)	0 (0)	1 (1.3)	0 (0)	5 (1.9)	30 (11.4)
Total	0 (0)	1 (1.3)	75 (98.7)	1 (0.4)	12 (4.6)	250 (95.0)

A Pearson correlation showed an inverse correlation (r=-0.303; p<0.001) between BMI and 25(OH)D levels as a measure of vitamin D status.

Table 5.6 indicates vitamin D status in the presence of hypertension.

Table 5.6 Vitamin D status in relation to the presence of hypertension

Vitamin D status	Males (n=76)		Females (n=263)	
	Normotensive (n=32) n (%)	**Hypertensive (n=44) n (%)**	**Normotensive (n=92) n (%)**	**Hypertensive (n=171) n (%)**
Deficiency (<12 ng/ml)	0 (0)	0 (0)	0 (0)	1 (0.4)
Inadequate levels (12-20 ng/ml)	1 (1.3)	0 (0)	4 (1.5)	8 (3.0)
Acceptable levels (>20 ng/ml)	31 (40.8)	44 (57.9)	88 (33.5)	162 (61.6)

No significant difference in mean 25(OH)D levels between the hypertensive and normotensive participants could be found. However, when a Pearson correlation was done on actual mean

arterial blood pressure and vitamin D levels, a weak inverse correlation was found (r=-0.136; p=0.012).

Given the effect of overweight/obesity, indicated by BMI, on both vitamin D levels and blood pressure, the association between vitamin D levels and blood pressure was also determined after controlling for BMI. This produced no statistical significant association between vitamin D status and a diagnosis of hypertension (χ^2=0.597, df=2, ρ=0.742) or significant correlation between serum vitamin D levels and mean arterial blood pressure levels (r=-0.062; p=0.254). A multiple regression model with mean arterial pressure as dependent variable and age, adiposity index and vitamin D as independent variables, also failed to show a significant correlation between vitamin D levels and mean arterial pressure.

More than a third (39.8%) of the study population was HIV positive and the mean 25(OH)D levels differed significantly (p=0.001) between HIV positive (40.8±12.5 ng/ml) and HIV negative participants (36.9±10.1ng/ml). Mean BMI was significantly (p<0.001) lower in HIV positive (24.9±7.4kg/m^2) than in HIV negative participants (29.6±9.2kg/m^2), but HIV status did not show a significant correlation with 25(OH)D (r=0.101; p=0.06) when BMI was controlled for.

5.5 DISCUSSION

In this study, a high prevalence of hypertension (63.4%) as well as overweight/obesity (55.1%) was found, but contrary to expectations based on the low vitamin D intakes and dark skin colour of the participants, almost 96% of the participants had adequate vitamin D status (>20ng/ml). An inverse correlation (p<0.001) was found between BMI and vitamin D levels, as well as between mean arterial blood pressure and vitamin D levels (p<0.05). However, when controlled for BMI, no significant relationship could be found between serum vitamin D and the prevalence of hypertension or mean arterial blood pressure. It therefore seems that the inverse correlation found between serum vitamin D levels and hypertension may be secondary to the opposing effects of increased body weight that lower serum vitamin D levels on the one hand, and raised blood pressure levels on the other.

The mean serum 25(OH)D level of 38.4 ng/ml found in this study is significantly higher (p<.001) than that reported in the *US National Health and Nutrition Examination Survey* (males 25.2 ng/ml; females 24.6 ng/ml; black population 15.7 ng/ml) (Reis *et al*., 2008:1471), or that reported for participants from the *Prostate Lung, Colorectal, and Ovarian cancer Screening Trial* cohort (males = 24.4 ng/ml; females 26.2 ng/ml) (Brock *et al*., 2010:463). As in other studies, higher mean 25(OH)D levels were found in males compared to females (43.5 ng/ml vs. 37.0 ng/ml) (Lips, 2010:300; Reis *et al*., 2008:1472).

Similar to the high proportion of individuals with adequate vitamin D status found in this adult population, Poopedi *et al.*, (2011:334) also reported that 74% of a population of South African children aged 10 years old, living in the urban area of Johannesburg, had adequate vitamin D status (using cut-off values of ≥75 nmol/l or ≥30 ng/ml). The mean serum 25(OH)D levels for the black children in the study were 34.5 ng/ml for females and 40.1 ng/ml for males (Poopedi *et al*., 2011:336), which agrees with the higher levels found in the black adults in the current study.

In the current study, a noticeably higher mean vitamin D level, compared to most other studies, was found. Indeed due to the black skin colour of the study population, low income levels translating to low intakes of vitamin D rich foods, the fact that there is no routine vitamin D fortification in South Africa, and the large incidence of overweight/obesity (55.%) in this study group, a lower vitamin D status was expected. These high levels of vitamin D may probably be ascribed to the latitude (29°10´S) of and general s unny weather experienced in Mangaung, as well as the fact that the living conditions and lifestyles of this community allows high levels of sun exposure.

The high incidence of HIV did not influence vitamin D levels, except for the indirect influence of BMI. This agrees with Stephensen *et al.* (2006:1135) who found no significant difference between the mean serum 25(OH)D levels of HIV-positive and HIV-negative individuals.

5.6 CONCLUSION

In contrast to the high prevalence of inadequate vitamin D status (<20 ng/ml) reported worldwide (Lips, 2010:300), the current study population had a very low prevalence of inadequate or deficient vitamin D status (≈4%) despite having a black skin colour. The favourable latitude, the fact that all blood samples were taken during autumn and the high levels of sun exposure that is expected from the living conditions of this population, could be possible explanations for the general good vitamin D status, since routine vitamin D fortification of staples is not implemented in South Africa.

Although an inverse correlation ($p<0.001$) was found between BMI and vitamin D status and also between mean arterial blood pressure and 25(OH)D ($p=0.012$), no significant association were found between vitamin D status and the prevalence of hypertension, or vitamin D levels and mean arterial blood pressure, when controlling for BMI. The inverse relationship shown between vitamin D and hypertension therefore may have been caused by the effect of increased body weight to lower vitamin D levels and increase blood pressure levels. HIV status also did not influence vitamin D levels, except through BMI.

5.7 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee from the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07).

5.8 ACKNOWLEDGEMENT

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5.9 BIBLIOGRAPHY

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal of Clinical Hypertension, 11 (7): 358-368.

Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, Peters U, Ahn J, Purdue M, Mason RS, McCarty C, Ziegler RG and Graubard B. 2010. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. Journal of Steroid Biochemistry and Molecular Biology, 121:462–466.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC and Curhan GC. 2007. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension, 49: 1063-1069.

Gallagher ML. 2008. The nutrients and their metabolism, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 39-143.

Groenewald AJ, Van Wyk HJ, Walsh CM, Van der Merwe LJ and Van Zyl S. Staging and haematological abnormalities of HIV-infected persons in the rural Free State Province of South Africa. African Journal of Primary Health Care and Family Medicine [Online].
Available from: http://www.phcfm.org/index.php/phcfm/article/view/222/249 [Accessed November 8th , 2011].

Institute of Medicine of the National Academies (IOM). 2010. Dietary Reference Intakes for Calcium and Vitamin D. Ed by Ross AC, Taylor L, Yaktine AL and Del Valle HB. [Online]. Available from: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Report-Brief.aspx [Accessed December 15th, 2010].

Judd SE, Nanes MS, Ziegler TR, Wilson PWF and Tangpricha V. 2008. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. American Journal of Clinical Nutrition, 87: 136-141.

Lanham-New SA, Buttriss JL, Miles LM, Ashwell M, Berry JL, Boucher BJ, Cashman KD, Cooper C, Darling AL, Francis RM, Fraser WD, De Groot CPGM, Hypp□nen E, Kiely M, Lamberg-Allardt C, Macdonald HM, Martineau AR, Masud T, Mavroeidi A, Nowson C, Prentice A, Stone EM, Reddy S, Vieth R and Williams CM. 2011. Workshop Report: Proceedings of the Rank Forum on Vitamin D. British Journal of Nutrition, 105: 144–156.

Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W and Kong J. 2004. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. The Journal of Steroid Biochemistry and Molecular Biology [Online], 89-90: 387-392.

Lips, P. 2010. Worldwide status of vitamin D nutrition. Journal of Steroid Biochemistry and Molecular Biology, 121:297-300.

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online]. Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Poopedi MA, Norris SA and Pettifor JM. 2011. Factors influencing the vitamin D status of 10-year-old urban South African children. Public Health Nutrition: 14(2): 334–339.

Rammos G, Tseke P and Ziakka S. 2008. Vitamin D, the renin-angiotensin system, and insulin resistance. International Urology and Nephrology [Online]. Available from: http://www.springerlink.com.proxy.lib.fsu.edu/content/ap8281748l28474p/fulltext.pdf [Accessed May 1st , 2011].

Reis JP, Michos ED, Von Műhlen D and Miller ER. 2008. Differences in vitamin D status as a possible contributor to the racial disparity in peripheral arterial disease. The American Journal of Clinical Nutrition, 88: 1469 –1477.

Stephensen CB, Marquis GS, Kruzich LA, Douglas SD, Aldrovandi GM and Wilson CM. 2006. Vitamin D status in adolescents and young adults with HIV infection. American Journal of Clinical Nutrition, 83:1135-1141.

Steyn K. 2006. Hypertension in South Africa, In Chronic diseases of lifestyle: 1995-2005. Technical report. Ed. by Steyn K, Fourie J and Temple N. Cape Town: South African Medical Research Council: 80-96.

UNICEF. 2010. Statistics South Africa. [Online].
Available from: http://www.unicef.org/infobycountry/southafrica_statistics.html [Accessed November 8th 2011].

Vaidya A and Forman JP. 2010. Vitamin D and Hypertension: Current Evidence and Future Directions. Hypertension, 56:774-779. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/774 [Accessed November 9th 2010].

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

Wang S. 2009. Epidemiology of vitamin D in health and disease. Nutrition Research Reviews, 22:188-203.

Webb AR and Engelsen. 2006. Calculated Ultraviolet Exposure Levels for a Healthy Vitamin D Status. Photochemistry and Photobiology, 82(6):1697-1703 [Online].
Available from: http://www.bioone.org/doi/full/10.1562/2006-09-01-RA-670 [Accessed March 1st, 2011].

World Health Organization (WHO). 2008. WHO STEPS Surveillance: Guide to physical measurements. [Online].
Available from: http://www.who.int/chp/steps/part3_section3.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

Wortsman J, Matsuoka LY, Chen TC, Lu Z and Holick MF. 2000. Decreased bioavailability of vitamin D in obesity. The American Journal of Clinical Nutrition, 72: 690-693.

CHAPTER 6

THE ASSOCIATION OF SODIUM AND POTASSIUM INTAKES WITH HYPERTENSION IN A BLACK COMMUNITY IN MANGAUNG, SOUTH AFRICA

ABSTRACT

A reduction in sodium intake with an accompanying increase in potassium intake is widely recommended for the prevention and treatment of hypertension. Some researchers describe a more profound blood pressure elevating effect of sodium in black population groups, urging investigation into this possible race-related cause of hypertension.

Objective: To assess the sodium and potassium intakes of a low-income, black, urban community in Mangaung, South Africa, and to examine whether sodium and potassium intakes are associated with blood pressure levels and the prevalence of hypertension.

Methods: Data collected in the Assuring Health for All in the Free State (AHA-FS) study were used. Blood pressure was measured by a registered medical practitioner using standard procedures. Spot-urine samples were collected and analysed for sodium and potassium excretion levels (to indirectly reflect intakes) by an accredited laboratory, using integrated chip technology (ICT).

Results: 339 Adults (263 females, 76 males) were included in the study, with a mean age of 44.3±10.6 (SD) years (range, 25-63 years). Urine samples for calculating daily sodium and potassium excretion were available for 318 participants. A total of 63.4% of the study population (n=339) were hypertensive. The mean calculated daily sodium excretion was 178.0 ± 53.0 mEq/day (SD), and positive correlations were found between urinary sodium levels and systolic (r= 0.125; p=0.026), diastolic (r=0.145; p=0.010) and mean arterial bloodpressure (r=0.149; p=0.008). Most of the participants (94.3%) had urinary sodium excretion levels that reflected sodium intakes above the recommended tolerable upper intake level. Positive associations between sodium and potassium intakes (as reflected by excretion), but no association between potassium intake and the prevalence of hypertension or mean arterial pressure, were found. No association between potassium:sodium ratio and mean arterial pressure could be found.

Conclusion: In this urban, black, South-African population the prevalence of both hypertension and the level of sodium intakes, as reflected by urinary sodium excretion, were high. Associations between sodium intake and systolic, diastolic and mean arterial pressure were found, with higher sodium intakes being associated with elevated blood pressure levels, indicating the need for dietary sodium reduction strategies to be implemented in order to control hypertension in this

population. Despite high sodium and low potassium intakes, no difference in mean intakes of sodium or potassium was found between the hypertensive and normotensive groups.

Key words: hypertension, sodium intake, potassium intake, spot urine, urban black population, AHA-FS, South Africa

6.1 INTRODUCTION

The prevalence of hypertension is increasing worldwide and salt intake is regarded as the primary cause of increased blood pressure, being responsible for the rise in blood pressure that occurs with aging. A reduction in dietary sodium intake together with other dietary and lifestyle changes are therefore regarded as an effective strategy to lower blood pressure (Matyas *et al.*, 2011:826; He & MacGregor, 2007:18). Primary hypertension and increasing blood pressure with aging are very rare in populations with low sodium intakes (less than 50mmol/day). It appears that sodium intakes exceeding 50-100 mmol/day are required, but are not necessarily the only factor needed to develop primary hypertension (Adrogué & Madias, 2007:1966). Researchers therefore support an overall reduction in sodium content of food in populations as a whole (Couch & Krummel, 2008:872; Norat *et al.*, 2008:395,397; Appel, 2009:360).

In the International Study of Salt and Blood Pressure (INTERSALT), information was collected from 10 079 adults between the ages of 20 and 59 at 52 centres around the world. Data from the INTERSALT study, indicate that if a population lowers their usual sodium intake with 100mmol/day, a reduction in systolic pressure of at least 2.2mmHg can be expected and that this reduction will relate to a four percent lower risk for coronary heart disease and a six percent lower risk for stroke in middle age adults (Stamler *et al*., 1989:570).

Higher potassium intakes on the other hand are associated with lower blood pressure levels as described by a number of researchers (IOM, 2004:Online; Adrogué & Madias, 2007:1967; He & MacGregor, 2001:497). This finding formed the basis for the inclusion of large amounts of fruit and vegetables in the DASH (Dietary Approaches to Stop Hypertension) diet. Not all studies are conclusive on the role of potassium intakes in hypertension, however. Norat *et al.* (2008:395) failed to find any association between blood pressure and potassium intake, as reflected by urinary potassium, in a large free-living population consisting of white, older adults in England that participated in the EPIC (European Prospective Investigation of Cancer) Norfolk study. The argument was raised that it may be the interaction between sodium and potassium, rather than an excess of one and a deficiency of the other in the diet that plays a role in the pathogenesis of hypertension (Adrogué & Madias, 2007:1966). In the INTERSALT study for example, the potassium:sodium ratio was significantly related to systolic blood pressure (INTERSALT, 1988:321), with a higher potassium:sodium ratio reducing blood pressure.

Determining dietary sodium and potassium intakes in a community setting poses various challenges as food records and recall methods do not provide accurate intake levels of specific nutrients, and 24-hour urine collection is not always practical (Tanaka *et al.,* 2002:97-98). Excretion values of various elements obtained from spot urine samples have however been shown to correlate closely with values obtained from 24-hour urine collection, and was found to provide an accurate indication of sodium and potassium excretion in Japanese and European males and females (Ilich *et al.*, 2009:220; Tanaka *et al.*, 2002:99). Thus, to evaluate sodium and potassium intakes of a community, spot urine specimens are useful and can be interpreted by using the following formulae developed by Tanaka *et al.* (2002:101) to estimate 24-hour sodium and potassium excretion:

24-hour Urine Na (mEq/day) = 21.98 X XNa$^{0.392}$,

Where: XNa = (Spot Na / Spot Cr X 10) X (-2.04 X age + 14.89 X weight (kg) + 16.14 X height (cm) – 2244.45)

24-hour Urine K (mEq/day) = 7.59 X XK$^{0.431}$,

Where XK = (Spot K / Spot Cr X 10) X (-2.04 X age + 14.89 X weight (kg) + 16.14 X height (cm) – 2244.45)

Sodium in the diet is consumed mostly in the form of salt (sodium chloride), but is also obtained from other food ingredients and over the counter medications (Angus, 2007:3). Absorption of both sodium and chloride occurs mainly in the small intestine and most of what is consumed, is absorbed into the body. Because sodium is mainly excreted in the urine (except in cases of excessive sweating), urinary sodium excretion provides a convenient indication of sodium intake, and thus salt intake (IOM, 2004:Online). To convert salt intake, expressed in grams, to sodium intake expressed as mmol, the molecular weights of sodium (23), chloride (35.5), and sodium chloride (58.5) are taken into account (IOM, 2004:Online). Simplified, this conversion entails that 1 gram of salt represents17.1 mmol of sodium (Food Standards Agency, 2008:Online).

The recommended daily levels of salt intake vary. The adequate intake for sodium recommended for Northern American populations by the IOM is 1.5g (65mmol)/ day, which equals a salt intake of 3.8g per day. The tolerable upper intake level (UL) is 2.3g (100mmol) per day, which equals 5.8g salt per day (IOM, 2004:Online). In the UK, the panel on Dietary Reference Values (DRV) from the *Committee on Medical Aspects of Food Policy* (COMA) recommends sodium intakes below 3.2g per day equalling 8g per day of salt per day, and set the Reference Nutrient Intake (RNI) at 1.6g of sodium (4g salt) per day. COMA's Cardiovascular Review Group recommends that salt intake should be gradually reduced to 6g per day (Food Standards Agency, 2008:Online), which is in closer agreement with the IOM's recommendations.

Population studies have shown a positive association between sodium intake and blood pressure over a wide range of sodium intakes and various studies recommend a reduction in dietary sodium intake as an effective way to lower blood pressure or even prevent future hypertension (Couch & Krummel, 2008:872; Norat *et al.*, 2008:395; Appel, 2009:360; Food Standards Agency, 2008:Online). A position paper by Appel (2009:360), on behalf of the American Society of Hypertension Writing Group, concluded that the effect of sodium on blood pressure tend to be greater in black populations and in middle- and older-aged individuals; and that genetic and other dietary factors (such as potassium intake) may influence blood pressure response to sodium intake. In this position paper an upper limit of 2.3g sodium per day is recommended for the general population and a maximum of 1.5g sodium per day is recommended for blacks, middle- and older-aged persons, as well as for individuals with hypertension, diabetes, or chronic kidney disease, which represents the majority of the American population (Appel, 2009:360).

Studies found that a high sodium diet progressively manifests in increased aldosterone levels; and that a high salt diet in the presence of high aldosterone levels cause left ventricular hypertrophy, proteinuria and therefore worsens disease progression. Conversely, a diet low in sodium can minimize the effect of high levels of aldosterone on heart health (Acelajado *et al*., 2010:805; Du Cailar *et al.,* 2010:865). Because of this interaction between dietary sodium intake and aldosterone levels, hyperaldosteronism can be treated by either lowering salt intake, and/or by lowering aldosterone levels with aldosterone blockers (Acelajado *et al.,*2010:805).

As with sodium, urinary potassium excretion also reflects dietary potassium intake, and between 77 - 90 % of consumed potassium, is excreted in the urine (IOM, 2004:Online). Turban *et al.* (2008:1399) indicated that the estimated amount of dietary potassium that is excreted in urine ranges between 50-74% in blacks and 60-74% in white participants. Potassium is the major intracellular cation in the body and levels is maintained at 145 mmol/L in intracellular fluid, but much lower at 3.8 - 5 mmol/L in extracellular fluid. Small changes in extracellular potassium levels affect neural transmission, muscle contraction and vascular tone (IOM, 2004:Online). A potassium intake of 4.7 g per day (120 mmol/day) is set as Adequate Intake level by the IOM. It is recommended that potassium should be consumed as fresh fruit and vegetables to obtain the other advantages of whole food intake as well (Appel, 2009:361; IOM, 2004:Online). To convert millimoles (mmol) of potassium to milligrams (mg), the molecular weight of potassium (39.1) is used in multiplication (IOM, 2004:Online).

6.2 METHODOLOGY

Data from the urban baseline phase of the Assuring Health for All in the Free State (AHA-FS) study were used for this study. Trained field workers visited households selected in a stratified proportional cluster sample to encourage participation in the research, to explain the purpose of

the study and to obtain written informed consent. Adult participants, 25-64 years of age for whom written consent was obtained and for whom the required data sets were available, were included in this study. Participants assembled at the central research centre in a fasting state, where amongst others, blood pressure, weight and height were measured, personal information and dietary intake recorded during structured interviews using standardised questionnaires, and a spot urine sample for analysis of sodium and potassium collected.

Hypertension is defined as a systolic blood pressure of 140mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher (Couch & Krummel, 2008:866). Participants using prescription medication for the management of hypertension at the time of the interview were also regarded as hypertensive (Wallace *et al.*, 2007:51).

Blood pressure was taken by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated in a chair for at least five minutes with their feet on the floor and arm supported at heart level. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements was recorded.

Dietary intake information, focussing on salty food and fruit and vegetable intakes, was collected from a dietary intake questionnaire that combined a 24-hour recall of usual intakes with a short food frequency questionnaire. Intakes was classified according to frequency of consumption and fruit and vegetables and intakes of fruits and vegetables were also evaluated according to the recommendations from the South African Food Based Dietary Guidelines for fruit and vegetable consumption (Love & Sayed, 2001:S24) which recommends five portions or 400g of fruit and/or vegetables per day.

As food recall methods are not regarded as accurate and specific for sodium and potassium intakes, spot urine samples were collected from each individual to calculate daily urine sodium and potassium excretion. Leiba *et al.* (2005:462) also recommends urinary sodium excretion as more accurate for assessing sodium intake, based on the fact that patients in their Israeli study underestimated sodium intake by about 30-50% with self-reported intake. Urinary sodium, potassium and creatinine levels were determined by an accredited laboratory. Sodium and potassium levels were determined using integrated chip technology (ICT), based on the principle of indirect ion selective electrodes on an Abbott Architect C4000 machine (Seago *et al.*, 2010: A74#B71). The measurement of creatinine was performed photometrically based upon the chemical reaction between creatinine and sodium picrate (Jaffe reaction). Laboratory results were expressed as mmol/L and these were converted to mg/dL by first converting mmol/L to µmol/L by

multiplying with 1000, and then by dividing by 88.4 to obtain the conventional unit (Rowlett, 2005:Online).

Anthropometric measurements were taken by trained fourth year, BSc Dietetics students under supervision of the researchers.

Body weight was determined using the WHO STEPwise approach to Surveillance (STEPS) method (WHO, 2008:Online). A floor type, Seca 770 digital scale (Medical Scales and Measuring Systems seca kk., Japan) with graduation accurate to 100g and a maximum capacity of 200kg was used on a firm flat surface. Participants were weighed in minimal clothing after an overnight fast and after voiding (participants were weighed after providing a urine sample). Researchers ensured that the participant stood on the centre of the scale without support and with his/her weight distributed evenly between both feet while weight was recorded to the nearest 0.1kg.

Height was measured using the WHO STEPS method (WHO, 2008:Online), with a Seca stadiometer (Medical Scales and Measuring Systems seca kk., Japan) accurate to the nearest 5mm. Participants were requested to stand with their feet together and the heels, buttocks and upper part of the back touching the stadiometer, and knees straight. Measures were taken with the head placed in the Frankfort plane and the participants requested to take a deep breath and stand tall, while height was measured.

Body mass index (BMI), the relationship of weight to height was used to interpret and describe body weight. BMI is calculated by dividing weight (kg) by height squared (m^2) (WHO, 2011:Online).

6.3 STATISTICAL ANALYSIS

The researcher performed statistical analysis using the PASW (Predictive Analytics SoftWare) Statistics Student Version 18.0 software by *SPSS: An IBM Company*.
Frequencies and percentages were used to summarize the categorical data. Means and standard deviations, or percentiles as appropriate, were used to summarize numerical characteristics. Comparisons of means were done using t-tests as appropriate. Two tailed Pearson correlations, chi-square, point bi-serial correlations and logistic regressions were used to describe and test associations between variables.

6.4 RESULTS

339 Adults (76 males and 263 females) with complete data sets for age, gender, blood pressure, body weight, height, activity level, as well as available blood samples for genetic testing and 25(OH)D analysis, from the AHA baseline study were included in this study. Urine samples for 21

participants and dietary intake information for 4 participants were not available. Participants had a mean age of 44.3 ± 10.6 (SD) years, ranging between 25 and 63 years (Table 6.1). In 41.6% of participants, systolic blood pressure was ≥140mmHg and in 46.6% of participants, diastolic blood pressure was ≥90mmHg indicating hypertension. More than a quarter (25.4%) of the study population was using antihypertensive medication at the time of the study. In total 63.4% of the study population (58.4% males and 64.9% females) had blood pressure values ≥140/90mmHg or were using antihypertensive medication at the time of the study, implicating the presence of hypertension. In order to provide an estimate of daily intake, the mean daily urinary sodium and potassium excretion levels are indicated in Table 6.1.

Table 6.1 General description of the study population in terms of age, blood pressure, sodium and potassium excretion.

Variable	N	Mean	Std Dev	Minimum	Maximum
Age (years)	339	44.3	10.60	25	63
Systolic blood pressure (mmHg)	339	135.5	23.67	72	203
Diastolic blood pressure (mmHg)	339	89.8	17.57	46	188
Mean arterial blood pressure (mmHg)	339	105.0	17.96	58.7	191.3
Daily sodium excretion (mmol/day) –					
Total	318	178.0	53.0	46.2	323.4
Males	71	158.4	53.0	56.2	293.1
Females	247	183.6	51.7	46.2	323.4
Daily potassium excretion (mmol/day)					
Total	318	43.6	11.8	19.4	126.9
Males	71	39.0	8.9	19.4	65.8
Females	247	44.9	12.2	20.8	126.9
BMI (kg/m^2)	339	27.8	8.79	13.3	55.7

Females had significantly higher ($p<0.001$) mean sodium and potassium intakes estimated from urinary excretion, than males. Although dietary recall methods are not considered accurate, especially not in community studies, in this study data on the frequency of consumption of potato chips and the use of salt and other salty flavourings like soup powders and stock, were collected to obtain a general indication of the frequency of salty food consumption in this community (Table 6.2).

Table 6.2 Frequency of potato crisps and salt and salty foods consumption

Reported frequency of consumption (times /month)	Crisps (N = 335)	% consuming this frequency	Salt and salty foods (N=335)	% consuming this frequency
0-<1	74	22.1	15	4.5
1	34	10.1		
2	9	2.7		
3	5	1.5		
4	75	22.4	4	1.2
5	1	0.3		
8	54	16.1	4	1.2
12	28	8.4	9	2.7
16	9	2.7		
20	2	0.6	1	0.3
30	43	12.8	233	69.6
60	1	0.3	10	3.0
90			58	17.3
120			1	0.3

Almost a third (32.2%) of participants consumed potato chips once a month or less to not at all. A large proportion (43.0%) consumed potato chips more than once per month and up to 8 times per month, and almost a quarter (24.8%) consumed potato chips three or more times per week. With regard to salt and salty flavourings, only 5.7% of participants did not use these at all or used it only once a week. The majority (73.7%) of participants used salt or salty flavourings more than once a week up to once a day, with 20.6% of participants using salt and salty flavourings two to four times a day. No correlation was found between the self-reported frequency of use of salt and salty flavourings, and daily urinary sodium excretion (p=0.781).

Most (94.3%) participants had a sodium intake (as reflected by excretion) above the tolerable upper intake level of 100mmol sodium day, or 5.8g salt /day. The mean salt (sodium chloride) intake for this population (N=318) was 10.4 ± 3.1 (SD) g per day with a minimum of 2.7g and a maximum of 18.9g.

Although mean sodium excretion of the non-hypertensive group was slightly lower than that of the hypertensive group, an independent samples t-test showed no significant difference (p=0.318)

between the mean sodium excretion of the hypertensive and non-hypertensive groups as indicated in Table 6.3).

Table 6.3 Mean sodium intake (as estimated from calculated urine excretion levels) according to hypertensive status (N=318).

Blood pressure status	N	%	Mean sodium excretion (mmol/day)	Std Dev
Non-hypertensive	115	36.2	174.0	52.3
Hypertensive	203	63.8	180.2	53.3

Urinary sodium excretion showed a weak positive correlation with systolic (r=0.125; p= 0.026), diastolic (r = 0.145; p=0.01) and mean arterial pressure (r=0.149; p= 0.008). Partial correlations, controlling for age and BMI, confirmed the weak positive association (r=0.116; p=0.04) with mean arterial pressure. In multiple regression analysis with mean arterial pressure as dependent variable, age, BMI and daily sodium intake, as reflected by excretion had independent effects as indicated in Table 6.4. Urinary sodium excretion did not show a correlation with reported frequency of intake of crisps or salt and salty flavourings.

Table 6.4 Multiple regression model with mean arterial pressure as dependent variable.

Dependent Variable: Mean arterial pressure

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	64.663	5.596		11.554	.000
BMI	.430	.113	.208	3.826	.000
Age	.488	.090	.285	5.448	.000
Daily Sodium	.039	.019	.112	2.062	.040

Analysis of the frequency of fruit and vegetable intakes indicated that the majority of participants in this study had inadequate intakes of fruit and vegetables (Table 6.5).

Table 6.5 Fruit and vegetable intake indicated by food frequency (N=335)

	<1 portion/day		1-4 portions/day		5 or more portions /day	
	n	%	n	%	n	%
Fruit, fruit juice and/or vegetables	109	32.5	217	64.8	9	2.7

Only one participant had urinary potassium excretion levels above 120 mmol per day (mean 43.6 mmol/day), which is set as the Adequate Intake level. Urinary potassium excretion showed a weak positive correlation with reported frequency of fruit and vegetable intakes (r=0.115; p=0.041), but no association could be found between potassium intake (reflected by excretion) and either the prevalence of hypertension or mean arterial pressure. Mean potassium values did not differ significantly between the hypertensive and non-hypertensive groups. The mean values for potassium excretion according to blood pressure status are indicated in Table 6.6.

Table 6.6 Mean calculated urine potassium excretion (as an indirect measure of intake) according to hypertensive status (N=318)

Blood pressure status	n	%	Mean K excretion (mmol/day)	Std Dev
Non-hypertensive	115	36.2	42.6	11.6
Hypertensive	203	63.8	44.1	11.9

Using Pearson correlations, a positive correlation was found (r=0.47; p<0.001) between daily excretion of sodium and that of potassium. No correlation was found between the sodium:potassium ratio (obtained from urinary sodium and potassium excretion), and mean arterial pressure, systolic pressure or diastolic pressure.

6.5 DISCUSSION

In the current study the mean daily urinary sodium excretion was 178.0 (± 53.0) mmol/day which translates to a mean salt (sodium chloride) intake of 10.4 g/day. In a study in Cape Town, South Africa, Charlton *et al.* (2005:41) measured a mean sodium excretion of 135.3 ± 50.1 mmol/day in black participants and 164.8±91.0 mmol/day in white participants in their study of adults between 20 and 65 years. The mean urinary sodium excretion indicated daily salt intakes of 7.8g/day in black participants, 8.5 g/day in participants of mixed ancestry, and 9.5 g/day in white participants.

Using an one sample T-test, to compare the findings of the current study to that of the Cape Town study, the mean salt intake of black urban participants in the current study was found to be significantly higher ($p<0.001$).

In the United Kingdom (UK) mean sodium intake (±SD) between 1984 and 2008, was 150 (±7 mmol/day (8.8 g salt per day) (McCarron *et al*., 2009:1879). In an assessment of sodium intake (based on 24 hour urine excretion) among 294 males and 398 females between the ages of 19 and 64 during 2008 in the UK, the estimated salt intake was 9.7 and 7.7 g per day, respectively, or 8.6 g/day combined (Food Standards Agency, 2008:Online). When compared to the current study, the mean UK salt intake from the latter study was significantly less ($p<0.001$) than what the black urban South African population consumed.

In the current study the mean daily urinary sodium excretion was 178.0 (± 53.0), ranging between 46.2 and 323.4 mmol/day. When data on sodium intake from 62 sites in 33 countries (n=19151) were pooled, urinary sodium excretion ranged between 117mmol/day and 212 mmol/day with a mean of 162.4 (±22.4) mmol/day, which translates to intakes of 6.8 to 12.4 g salt per day, with a mean value of 9.5 g salt per day (McCarron *et al.*, 2009:1880). The INTERSALT study (Japanese participants) reported a mean daily sodium excretion of 187.2 (± 65.8) mmol/day (Tanaka *et al.*, 2002:99).

In the current study sodium intake (computed from spot urine samples), showed no correlation with the prevalence of hypertension, but showed a weak positive correlation with systolic, diastolic and mean arterial pressure. In contrast, Charlton *et al*. (2005:45) found no association between sodium intake (measured from 24 hour urinary excretion) and either systolic or diastolic blood pressure, in a study population where only 23% of the subjects consumed less than the recommended tolerable upper intake level (UL) of 100mmol Na/day (5.8g salt) (Charlton *et al*., 2005:46). On the other hand, a study by Norat *et al.* (2008:392) in a predominantly white population in England, did report significant associations between urinary sodium excretion levels and blood pressure levels, but no significant association between blood pressure and urinary potassium. The positive association of sodium intake with blood pressure described by Norat *et al.* (2008:395) was stronger for systolic blood pressure than for diastolic blood pressure, whereas the opposite was true for black urban participants in the current study.

In the current study the mean potassium excretion for the whole group was 43.6 (± 11.8) mmol per day, ranging between 19.4 and 126.9 mmol per day. In the INTERSALT study, a mean daily potassium excretion of 45.8 (± 16.0) mmol was reported (Tanaka *et al.*, 2002:99). When the genders were considered separately, potassium excretion for males and females, were reported as 72-84 mmol/day and 56-61 mmol/day, respectively, in the US, and as 82-87mmol/day and 62-67 mmol/day, respectively, in Canada (IOM, 2004:Online). These values are significantly higher

(p<0.001) that the measured urinary potassium excretion calculated for males (39.0 mmol/day) and females (44.9 mmol/day) in the current study. The low urinary potassium excretion values were corroborated by the low intakes of fruit and vegetables reported in the food frequency questionnaires administered during structured interviews with the participants.

Unlike in the INTERSALT study, no direct association was found between the potassium:sodium ratio and blood pressure in the current study (Adrogué & Madias, 2007:1967). Furthermore, contrary to various other studies which reported an inverse relationship between sodium and potassium intakes, in this study a positive association was found between dietary sodium and potassium excretion levels.

6.6 CONCLUSION

Potassium excretion in these black, urban participants was low, probably due to low consumption of fruit and vegetables, which are relatively expensive foods in this community with low socio-economic status. The potential detrimental effect of low potassium intakes on blood pressure was exacerbated in this community by mean daily excretion of sodium above the UL of 100mmol/day, corresponding with salt intakes of 5.8g/day, which was probably due to high intakes of salty foods and the use of high amounts of salt during food preparation. Sodium excretion levels also correlated positively with systolic, diastolic and mean arterial blood pressure. Although many other studies report an inverse relationship between sodium and potassium intakes, a positive association was found between dietary sodium and potassium excretion levels in this study, which could possibly be explained by the practice in this community of preparing vegetables with large amounts of salt. Dietary intake of sodium, as represented by the frequency of chips, salt and salty food consumption, did not correlate with sodium excretion levels, but the frequency of fruit and vegetable consumption correlated with potassium excretion levels. Either using frequency of salty food consumption as an indication of dietary sodium intake does not provide a good reflection of dietary sodium intake, or the food frequency questionnaire used in this study was not sensitive enough to accurately capture salt intake. From this study, it can therefore be concluded that reduction in daily sodium intakes and increased fruit and vegetable consumption, should form part of intervention strategies to address the problem of hypertension in this urban population. Increased fruit and vegetable intake will not only increase potassium intake, but also antioxidant nutrients that would offer protection against oxidative damage.

6.7 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee from the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07).

6.8 ACKNOWLEDGEMENT

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6.9 BIBLIOGRAPHY

Acelajado MC, Pimenta E and Calhoun DA. 2010. Salt and Aldosterone: A concert of bad effects. Hypertension, 56:804-805 [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/804 [Accessed November 9th, 2010].

Adrogué HJ and Madias NE. 2007. Sodium and Potassium in the Pathogenesis of Hypertension. The New England Journal of Medicine, 356:1966-1978.

Angus F. 2007. Dietary salt intake: sources and targets for reduction, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited: 3-17.

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal Of Clinical Hypertension, 11 (7): 358-368.

Charlton KE, Phil M, Steyn K, Levitt NS, Zulu JV, Jonathan D, Veldman FJ and Nel JH. 2005. Diet and blood pressure in South Africa: intake of foods containing sodium, potassium, calcium, and magnesium in three ethnic groups, Nutrition, 21 (1): 39–50.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Du Cailar G, Fesler P, Ribstein J and Mimran A. 2010. Dietary Sodium, Aldosterone, and Left Ventricular Mass Changes During Long-Term Inhibition of the Renin-Angiotensin System. Hypertension, 56: 865-870. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/865 [Accessed November 9th, 2010].

Food Standards Agency. 2008. Dietary Sodium Levels Surveys. [Online].
Available from: http://www.food.gov.uk/science/dietarysurveys/urinary [Accessed March 4th, 2011].

He FJ and MacGregor GA. 2001. Beneficial effects of potassium. British Medical Journal, 323: 497–501.

He FJ and MacGregor GA. 2007. Dietary salt, high blood pressure and other harmful effects on health, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited: 18-54.

Institute of Medicine of the National Academies (IOM). 2004. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. [Online].
Available from: http://iom.edu/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx [Accessed March 4th, 2011].

Ilich JZ, Blanuša M, Orlić ZC, Orct T and Kostial K. 2009. Comparison of calcium, magnesium, sodium, potassium, zinc and creatinine concentration in 24-h and spot urine samples in women. Clinical Chemistry and Laboratory Medicine, 47 (2): 216-221.

Intersalt Cooperative Research Group (INTERSALT). 1988. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. British Medical Journal, (297):319-328.

Leiba A, Vald A, Peleg E, Shamiss A, Grossman E. 2005. Does dietary recall adequately assess sodium, potassium, and calcium intake in hypertensive patients? Nutrition, 21(4):462-466.

Love P and Sayed N. 2001. Eat plenty of vegetables and fruits every day. The South African Journal of Clinical Nutrition, 14 (3): S24-S32.

Matyas E, Jeitler K, Horvath K, Semlitsch T, Hemkens LG, Pignitter N and Siebenhofer A. 2011. Benefit assessment of salt reduction in patients with hypertension: systematic overview. Journal of Hypertension, 29: 821–828.

McCarron DA, Geerling JC, Kazaks AG, and Stern JS. 2009. Can Dietary Sodium Intake Be Modified by Public Policy? Clinical Journal of the American Society of Nephrology, 4: 1878–1882 [Online].
Available from: http://cjasn.asnjournals.org/content/4/11/1878.full.pdf+html [Accessed March 4th 2011].

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].

Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Norat T, Bowman R, Luben R, Welch A, Khaw KT, Wareham N and Bingham S. 2008. Blood pressure and interactions between the angiotensin polymorphism AGT M235T and sodium intake: a cross-sectional population study. American Journal of Clinical Nutrition, 88: 392-397.

Rowlett R. 2005. How many? A dictionary of units of measurement. [Online].
Available from: http://www.unc.edu/~rowlett/units/index.html

Seago JL, Li Q and Pistone D. 2010. Abbott ARCHITECT ICT (ISE) Module Use-Life Response Characteristics. Clinical Chemistry, 56:6: A74#B71.

Stamler J, Rose G, Stamler R, Elliott P, Dyer A and Marmot M. 1989. INTERSALT study findings. Public health and medical care implications. Hypertension, 14:570-577.

Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H and Hashimoto T. 2002. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. Journal of Human Hypertension 16: 97–103.

Turban S, Miller ER, Ange B and Appel LJ. 2008. Racial differences in urinary potassium excretion. Journal of the American Society of Nephrology, 19 (7): 1396-1402.

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

World Health Organization (WHO). 2008. WHO STEPS Surveillance: Guide to physical measurements. [Online]
Available from: http://www.who.int/chp/steps/part3_section3.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online].
Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

CHAPTER 7

PHYSICAL ACTIVITY, BODY MASS INDEX AND THE PREVALENCE OF HYPERTENSION IN A BLACK COMMUNITY IN MANGAUNG, SOUTH AFRICA

ABSTRACT

Obesity and hypertension are major health concerns worldwide, affecting morbidity and mortality in many communities. Increased activity is often advocated as first line treatment and prevention of hypertension, and the blood pressure lowering effects of increased activity are seen even when weight loss is not achieved. The greatest effect of increased activity on blood pressure is evident in hypertensive individuals, especially where activity is increased from an inactive state to moderate activity.

Objective: To describe the activity levels of a low-income, black, urban community in Mangaung, South Africa, in order to evaluate the impact of activity levels on body mass index (BMI) and the prevalence of hypertension.

Methods: Baseline data from the urban phase of the Assuring Health for All in the Free State (AHA-FS) study were used. Field workers visited households selected in a stratified proportional cluster sample, to encourage participation in the research. At the research centre, blood pressure was measured by a registered medical practitioner according to standard guidelines. Weight and height were measured by trained professionals, using calibrated equipment and standardized techniques, and BMI (kg/m^2) was calculated. Physical activity levels were described by self-reporting of activity during the previous 24 hours, using the Previous Day Physical Activity Recall questionnaire, which was completed by trained interviewers during structured interviews. Human immunodeficiency virus (HIV) status was determined using standardized techniques.

Results: 339 Adults (263 females, 76 males) were included, with a mean age of 44.3$\pm$10.6 (SD) years (range, 25-63 years). Based on BMI, 45% were normal/underweight, 23% were overweight and 32% were obese. Among females and males, 66% and 20% respectively, were overweight/ obese. The majority (63.4%) of the study population either had blood pressure values $\geq$140/90mmHg or were currently using antihypertensive medication, implicating underlying hypertension. The mean physical activity level for the group was at the higher range of the low active classification, with the majority (45.7%) categorized as low active. No relation between activity level and BMI or activity level and the prevalence of hypertension were found in this study. More than a third (39.8%) of the study population was HIV positive, but no association between HIV status and activity level could be found. A significant difference in the mean BMI between HIV positive (24.9kg/m^2 $\pm$ 7.4 SD) and HIV negative (29.6 $kg/m^2$$\pm$9.2 SD) participants was found.

Conclusion: More than half of this urban, black, South-African population was overweight/ obese and more than half had abnormally high blood pressure values, increasing the risk for disease and premature death. More than a third (35.1%) of participants reported physical activity levels that can be regarded as active to very active, while the majority reported being low active. No significant association could be shown between activity level, defined by PAL category, and the prevalence of hypertension. Although HIV status showed a negative correlation with BMI, no correlation were found between HIV status and activity level.

Key words: hypertension, physical activity, BMI, HIV, urban black population, AHA-FS, South Africa

7.1 INTRODUCTION

It is widely recognized that less active people are 30-50% more likely to develop hypertension than more active people (Couch & Krummel, 2008:872; Lambert, *et al.*, 2001:S14). Exercise has been shown to decrease blood pressure in approximately 75% of individuals with hypertension (Hagberg *et al.*, 2000:193). Increased physical activity of 30 minutes per day on most days of the week is widely recognized as effective treatment for lowering blood pressure (Whelton *et al,* 2002:1885; WHO/ISH, 2003: 1987; NIH, 2004:Online; Seedat *et al,* 2006:343; Miyashita *et al,* 2008: 1225; Couch & Krummel, 2008:871; Pescatello *et al.*, 2004:535). Furthermore, the blood pressure lowering effect of endurance training seems to be equally effective in both genders (Pescatello *et al.*, 2004:539).

In a meta-analysis of 54 trials, the blood pressure lowering effect of exercise was 3.84mmHg for systolic blood pressure and 2.58mmHg for diastolic blood pressure, with black participants showing a significant greater reduction in systolic blood pressure in comparison to white participants (-10.96 mmHg vs -3.44 mmHg) (Whelton *et al.*, 2002:500). Although this reduction may seem insignificant, according to Pescatello *et al.* (2004:545), a reduction of as little as 2 mmHg in systolic and diastolic blood pressure can reduce the risk of stroke by 14% and 17% respectively, and the risk of coronary artery disease by 9% and 6% respectively in the general population. However, Pescatello *et al.* (2004:540) concedes that as yet, the evidence is not convincing for ethnic differences in the response of blood pressure to chronic or acute exercise.

In studies by Whelton *et al.* (2002:500) and Hagberg *et al.* (2000:198) aerobic exercise reduced blood pressure in normal weight individuals, as well as overweight participants. Moreover, aerobic exercise significantly reduced blood pressure even in participants that did not lose weight. This supports the findings of Neter *et al.* (2003:882) that the effects of aerobic exercise on blood pressure may be independent of change in body weight (Whelton *et al.*, 2002:500).

In the Transition and Health During Urbanisation of South Africans (THUSA) study, physical activity showed the strongest association with the prevalence of obesity besides income and age, in black women in the North West Province (Kruger *et al*., 2002, 427).

It seems that all forms of exercise are effective in reducing blood pressure (Whelton *et al.*, 2002:501). The antihypertensive effects of exercise occur at relative low intensity and low duration (Pescatello *et al.*, 2004:542), while hypertensive individuals seem to be more responsive to the blood pressure lowering effects of exercise than nonhypertensive individuals (Cornelissen & Fagard, 2005:5). Most research therefore confirms that aerobic exercise is an important strategy for prevention and treatment of high blood pressure. This protective effect of moderate or high intensity exercise has shown to be consistent at all categories of BMI (Hu *et al*., 2010:237).

The mechanism by which exercise decreases blood pressure is not clear due to the multifactorial mechanisms and systems involved (Pescatello *et al.*, 2004:545), but seems to be based on a decrease in systemic vascular resistance, involving the sympatic nervous system and the renin-angiotensin system (Cornelissen & Fagard, 2005:5; Pescatello *et al.*, 2004:545). Exercise training has been shown to reduce plasma norepinephrine levels with 29% and plasma renin activity with 20% (Cornelissen & Fagard, 2005: 5).

The prevalence of hypertension does not seem to be affected significantly by HIV status, but HIV infection does affect weight status, which in return affects blood pressure levels (Medina-Torne, 2011:Online; Bloomfield *et al.,* 2011:Online; Baekken *et al.*, 2008:2131; Jung *et al*., 2004:2250; Bergersen *et al.,* 2003:731). When describing the effect of exercise on blood pressure levels and the prevalence of hypertension, the effect of HIV infection on BMI needs to be controlled for.

The aim of this study was to investigate the effect of self-reported physical activity on BMI and on the prevalence of hypertension in a black community in Mangaung, South Africa.

7.2 METHODOLOGY

Data from the urban baseline phase of the Assuring Health for All in the Free State (AHA-FS) study were used for this study. Trained field workers visited households selected in a stratified proportional cluster sample, to encourage participation in the research, explain the purpose of the study and obtain written informed consent. Adult participants, 25-64 years of age for whom written consent were obtained and for whom the required data sets were available, were included in this study. Participants assembled at the central research centre, where physical activity level, body weight, height and blood pressure were measured amongst other data collected.

Physical activity levels were described by self-reporting of activity during the previous 24 hours during a structured interview. The Previous Day Physical Activity Recall (PDPAR), as validated by Weston, Petosa and Pate (1997:138,) was used. In the activity questionnaire, participants were asked to recall all the activities that they performed during the previous day, and from this data the researchers calculated a physical activity level (PAL) for each participant. The PAL value provided an indication of lifestyle activity and represents the energy spent on activities described, in addition to the energy needs of daily living. The PAL values were interpreted as indicated in Table 7.1.

Table 7.1 Classification of physical activity level (PAL) (Weston, Petosa & Pate, 1997:138)

Classification	**PAL**
Sedentary	1 - 1.39
Low active	1.4 - 1.59
Active	1.6 - 1.89
Very active	1.9 - 2.5

To determine relative risks, the sedentary and low active PAL categories were combined into the 'inactive' category, and the active and very active PAL categories were combined into the 'active' category. Reliability in these measurements was pursued by using researchers trained in the use of the PDPAR and repeating 10% of the sample. Despite the fact that the tool has been validated, it is recognized that self-reporting questionnaires in general provide fairly poor estimates of physical activity and that individuals often misreport intensity, duration and frequency of activity. Furthermore, self-reported activity levels often reflect perception of physical activity rather than actual activity levels (Loney *et al.,* 2011:68-69).

Anthropometric measurements were taken by trained fourth year, BSc Dietetics students under supervision of the researchers. Body weight was determined using the WHO STEPwise approach to Surveillance (STEPS) method (WHO, 2008:Online). A floor type, Seca 770 digital scale (Medical Scales and Measuring Systems seca kk., Japan) with graduation accurate to 100g and a maximum capacity of 200kg was used on a firm flat surface. Participants were weighed in minimal clothing after an overnight fast and after voiding (participants were weighed after providing an urine sample). Researchers ensured that the participant stood on the centre of the scale without support, with his/her weight distributed evenly between both feet. Weight was recorded to the nearest 0.1kg.

Height was measured using the WHO STEPS method (WHO, 2008:Online), with a Seca stadiometer (Medical Scales and Measuring Systems seca kk., Japan) accurate to the nearest 5mm. Participants were requested to stand with feet together and the heels, buttocks and upper part of the back touching the stadiometer and knees straight. Height was measured with the head

placed in the Frankfort plane and after the participant was requested to take a deep breath and stand tall.

BMI, which is the relationship of weight to height, was used to interpret body weight and describe the degree of adiposity. BMI is calculated by dividing weight (kg) by height squared (m^2) (WHO, 2011:Online).

The cut-off points as indicated in Table 7.2 were used to interpret BMI (WHO, 2011:Online).

Table 7.2 Classification of BMI (WHO, 2011:Online)

Classification	**BMI (kg/m^2)**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obesity, class I	30.0-34.9
Obesity, class II	35.0-39.9
Extreme obesity, class III	≥40

Blood pressure was taken by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated in a chair for at least five minutes with their feet on the floor and arm supported at heart level. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements recorded.

Hypertension is defined as systolic blood pressure 140mmHg or higher and/or diastolic blood pressure 90 mm Hg or higher (Couch & Krummel, 2008:866). In this study, participants using prescription medication for the management of hypertension at the time of the interview were also regarded as hypertensive (Wallace *et al.*, 2007:51).

7.3 STATISTICAL ANALYSIS

The researcher performed the statistical analysis using the PASW (Predictive Analytics SoftWare) Statistics Student Version 18.0 software by *SPSS: An IBM Company*. Frequencies and percentages were used to summarize the categorical data. Means and standard deviations, or percentiles as appropriate, summarized quantitative variables. Comparisons of means were done

using t tests as appropriate. Chi-square test, two tailed Pearson correlations and multivariate logistic regressions were used to describe and test associations between variables.

7.4 RESULTS

339 Adults (76 males and 263 females) with complete data sets from the baseline AHA-FS study were included in this study. Participants in this study had a mean age of 44.3 ± 10.6 (SD) years, ranging between 25 and 63 years (Table 7.3) and 39.8% of the study population was HIV positive.

Table 7.3 General description of the study population in terms of age, blood pressure, BMI and physical activity.

Variable	N	Mean	Std Dev	Minimum	Maximum
Age (years)	339	44.3	10.60	25	63
Systolic blood pressure (mmHg)	339	135.5	23.67	72	203
Diastolic blood pressure (mmHg)	339	89.8	17.57	46	188
Height (cm)	339	159.3	7.84	139.8	180
Weight (kg)	339	70.1	21.40	31.9	140
BMI (kg/m^2)	339	27.8	8.79	13.3	55.7
Physical activity level (PAL): Total	339	1.55	0.22	0.2	2.6
Males	76	1.55	0.33	1.1	2.6
Females	263	1.55	0.18	0.2	2.2

No correlation between HIV status and activity level could be found. The mean physical activity level for the whole group, as well as for males and females separately, were in the higher range of the 'low active' category, with the distribution as indicated in Table 7.4.

Table 7.4 Activity distribution of participants

PAL category	Classification	Males (n = 76)		Females (n=263)		Total (N=339)	
		n	%	n	%	n	%
1 - 1.39	Sedentary	26	34.2	39	14.8	65	19.2
1.4 - 1.59	Low active	23	30.3	132	50.2	155	45.7
1.6 – 1.89	Active	17	22.4	83	31.6	100	29.5
1.9 – 2.5	Very active	10	13.2	9	3.4	19	5.6

The majority of participants (45.7%) were categorized as low active, with a very small percentage (5.6%) being very active. The relative risk for developing hypertension through an inactive lifestyle was not significantly increased in this study. In the younger age group (≤44 years, n=167) the relative risk was 1.1 (95% CI 0.9-1.3); and in the older age group (>44 years, n=172) the relative risk was 1.3 (95% CI 0.9-1.9).

A high incidence of overweight/ obesity was found in this study population, with more than half (55.1%) of respondents being either overweight / obese as indicated in Table 7.5.

Table 7.5 BMI distribution of participants

BMI kg/m^2	Classification	Males (n = 76)		Females (n=263)		Total (N=339)	
		n	%	n	%	n	%
< 18.5 kg/m²	Underweight	19	25.0	21	8.0	40	11.8
18.5 – 24.9 kg/m²	Normal	43	56.6	69	26.2	112	33.0
25.0 – 29.9 kg/m²	Overweight	11	14.5	67	25.5	78	23.0
30.0 – 34.9 kg/m²	Obese Class I	0	0.0	39	14.8	39	11.5
35.0 – 39.9 kg/m²	Obese Class 2	2	2.6	32	12.2	34	10.0
≥40.0 kg/m²	Obese Class 3	1	1.3	35	13.3	36	10.6

According to BMI, 44.8% of the study population was normal/ underweight, 23.0% was overweight and 32.1% was obese. A significant higher prevalence of overweight/ obesity was found in females when compared to the male participants in this study ($p<0.001$).

A significant difference in the mean BMI between HIV positive (24.9kg/m^2 ± 7.4 SD) and HIV negative (29.6 kg/m^2±9.2 SD) participants was found. BMI distribution according to HIV status is indicated in Table 7.6.

Table 7.6 BMI distribution according to HIV status

BMI kg/m^2	Classification	HIV negative (n = 204)		HIV positive (n=135)		Total (N=339)	
		n	%	n	%	N	%
< 18.5 kg/m²	Underweight	20	5.9	20	5.9	40	11.8
18.5 – 24.9 kg/m²	Normal	51	15.0	61	18.0	112	33.0
25.0 – 29.9 kg/m²	Overweight	47	13.9	31	9.1	78	23.0
30.0 – 34.9 kg/m²	Obese Class I	28	8.3	11	3.2	39	11.5
35.0 – 39.9 kg/m²	Obese Class 2	28	8.3	6	1.8	34	10.0
≥40.0 kg/m²	Obese Class 3	30	8.8	6	1.8	36	10.6

In 41.6% of respondents, systolic blood pressure was ≥140mmHg and in 46.6% of respondents, diastolic blood pressure was ≥90mmHg indicating hypertension. More than a quarter (25.4%) of the study population was using antihypertensive medication at the time of the study. In total 63.4% (57.9% males and 65.0% females) of the study population had blood pressure values ≥140/90mmHg or were using antihypertensive medication at the time of the study, implicating the presence of hypertension.

The relation between physical activity levels and weight status according to BMI is depicted in Table 7.7. No correlation could be found between the level of physical activity and BMI in this population.

Table 7.7 Physical activity level in relation to BMI

BMI classification	Physical activity level							
	Male (n=76)				Female (n=263)			
	Active (PAL>1.59)		Inactive (PAL≤1.59)		Active (PAL>1.59)		Inactive (PAL≤1.59)	
	n	%	n	%	n	%	n	%
Underweight/Normal weight <25 kg/m^2	23	85.2	39	79.6	32	35.2	58	33.7
Overweight/Obese ≥25 kg/m^2	4	14.8	10	20.4	59	64.8	114	66.3

When comparing activity level, defined by PAL category, to the prevalence of hypertension, as indicated in Table 7.8, no significant association could be shown between the level of physical activity and the prevalence of hypertension. Using Pearson correlations and a regression analysis, no association was found between actual activity level as measured by PAL, and mean arterial blood pressure.

Table 7.8 Activity level in relation to the presence of hypertension

Physical Activity Level	(N=339)			
	Normotensive (n=124)		Hypertensive (n=215)	
	n	%	N	%
Sedentary	22	17.7	43	20.0
Low active	54	43.5	101	47.0
Active	37	29.8	63	29.3
Very active	11	8.9	8	3.7

Activity level showed no association with HIV status. Activity level according to HIV status is indicated in Table 7.9.

Table 7.9 Activity level in relation to HIV status

Physical Activity Level	(N=339)			
	HIV negative (n=204)		HIV positive (n=135)	
	n	%	n	%
Sedentary	38	11.2	27	8.0
Low active	93	27.4	62	18.3
Active	62	18.3	38	11.2
Very active	11	3.2	8	2.4

7.5 DISCUSSION

The majority of the black urban participants in the current study reported being sedentary (19.2%) or having low activity levels (45.7%); thus 64.9% (64.5% of males and 65% of females) could be considered inactive. Similar high levels of inactivity were also reported in the 2003 South African Health and Demographic Survey (DOH, 2007:293), where 49.4% of urban black adult males and 66.0% of urban black adult females in a nationally representative sample were reportedly inactive (although a different methodology to the current study was used to assess and classify activity levels). The World Health Survey conducted by the WHO in 2003 in 38 developing countries, reported that 43% of men and 49% of women were inactive. Out of the 38 countries included in this survey, South Africa was ranked as having the third highest prevalence of inactivity (DOH, 2007:292).

Almost two thirds of the study population were hypertensive (63.4%) and although physical inactivity is closely linked to the prevalence of hypertension, no association could be found in this study between physical activity and the incidence of hypertension, even when correcting for age. This confirms findings from the *National Health and Nutrition Examination Survey* (NHANES) 2005–2006 which also failed to report any significant association between activity level and blood pressure (Camhi *et al.,* 2011:Online). Canadian adults on the other hand, who self-reported activity levels that meet the national physical activity guidelines, were however also less likely to self-report chronic conditions, hypertension and general poor health (Bryan & Katzmarzyk, 2011:15). Similarly, Lee and Levy (2011:7) described that normotensive older adults engaged in more physical activity in the form of walking and household activities than those with controlled or uncontrolled hypertension.

Despite the high prevalence of overweight and obesity (55.1%) in this population, no association was found between activity levels and the prevalence of overweight/ obesity in the study. HIV infection was negatively correlated with BMI, but no significant association between HIV status and activity level was found. This study relied on self-reporting of physical activity and the possibility of over reporting of activity exists.

7.6 CONCLUSION

In this study among a black urban population, self-reported activity levels were low. Activity level did not show any significant association with the presence of hypertension or with BMI. A significant difference in the mean BMI between HIV positive and HIV negative participants was found, but no association of HIV status with activity level was found.

Although no association could be shown between self-reported activity level and the prevalence of hypertension, increasing activity levels, especially from inactivity to moderate activity, are still a valuable approach in the prevention and treatment of hypertension, as recommended by various researchers. In South Africa, a small, randomised controlled, community-based trial in the Western Cape, run by peer group leaders, showed that twice weekly low-intensity exercise was very effective in older adults to decreased systolic blood pressure by an average of 4mmHg in exercising groups after 20 weeks, compared with no change in the control group. This change in blood pressure occurred without a change in weight or body composition (Kolbe-Alexander *et al.*, 2006:21). A similar type of program may be effective to significantly alleviate the burden of hypertension and disease in this Mangaung population.

7.7 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee from the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07).

7.8 ACKNOWLEDGEMENT

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7.9 BIBLIOGRAPHY

Baekken M, Os I, Sandvik L and Oektedalen O. 2008. Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy. Journal of Hypertension, 26:2126–2133.

Bergersen BM, Sandvik L, Dunlop O, Birkeland K and Bruun JN. 2003. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. European Journal of Clinical Microbiology & Infectious diseases, 22:731–736.
Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%Fjournal.pone.0022288 [Accessed November 8th, 2011].

Bloomfield GS, Hogan JW, Keter A, Sang E, Carter EJ, Velazquez EJ and Kimaiyo S. 2011. Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. PLoS ONE, 6 (7): e22288 [Online].

Bryan SN and Katzmarzyk PT. 2011. The association between meeting physical activity guidelines and chronic diseases among Canadian adults. Journal of Physical Activity and Health, 8: 10 -17.

Camhi SM, Sisson SB, Johnson WD, Katzmarzyk PT and Tudor-Locke C. 2011. Accelerometer-determined moderate intensity lifestyle activity and cardiometabolic health. Preventive Medicine, [Online].

Available from:
http://www.sciencedirect.com.proxy.lib.fsu.edu/science?_ob=MImg&_imagekey=B6WPG-523V37G-1-1&_cdi=6990&_user=2139768&_pii=S0091743511000582&_origin=search&_zone=rslt_list_item&_coverDate=02%2F05%2F2011&_sk=999999999&wchp=dGLbVzW-zSkzV&md5=158438c38a6b209d1348895d9d8e84e6&ie=/sdarticle.pdf [Accessed March 9th, 2011].

Cornelissen VA and Fagard RH. 2005. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. Hypertension, 46: 1-9.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Department of Health (DOH). 2007. South Africa Demographic and Health Survey 2003, Full Report. Pretoria: Department of Health.

Hagberg JM, Park J-J and Brown MD. 2000. The role of exercise training in the treatment of hypertension – an update. Sports Medicine, 30 (3): 193-206.

Hu G, Jousilahti P, Antikainen R, Katzmarzyk PT and Tuomilehto J. 2010. Joint effects of physical activity, body mass index, waist circumference, and waist-to-hip ratio on the risk of heart failure, Circulation, 121:237-244.

Jung O, Bickel M, Ditting T, Rickerts V, Welk T, Helm EB, Staszewski S and Geiger H. 2004. Hypertension in HIV-1-infected patients and its impact on renal and cardiovascular integrity. Nephrology Dialysis Transplantation, 19: 2250–2258.

Kolbe-Alexander TL, Lambert EV and Charlton KE. 2006. Effectiveness of a community based low intensity exercise program for older adults. Journal of Nutrition Health and Aging, 10(1):21-9.

Kruger HS, Venter CS, Vorster HH and Margetts BM. 2002. Physical inactivity is the major determinant of obesity in black women in the North West province, South Africa: the THUSA study. Nutrition, 18(5): 422-427.

Lambert EV, Bohlmann I and Kolbe-Alexander T. 2001. Be active – Physical activity for health in South Africa. South African Journal of Clinical Nutrition, 14 (3): S12-S16.

Lee Y-S and Levy SS. 2011. Gender and income associations in physical activity and blood pressure among older adults. Journal of Physical Activity and Health, 8: 1 -9.

Loney T, Standage M, Thompson D, Sebire SJ and Cumming S. 2011. Self-Report vs. Objectively Assessed Physical Activity: Which Is Right for Public Health? Journal of Physical Activity and Health, 8: 62 -70.

Medina-Torne S, Ganesan A, Barahona I and Crum-Cianflone NF. 2011. Hypertension is common among HIV-infected persons, but not associated with HAART. Journal of the International Association of Physicians in AIDS Care [Online].
Available from:
http://jia.sagepub.com.proxy.lib.fsu.edu/content/early/2011/08/26/1545109711418361.full.pdf+html [Accessed November 4th, 2011].

Miyashita M, Burns SF and Stensel DJ. 2008. Accumulating short bouts of brisk walking reduces postprandial plasma triacylglycerol concentrations and resting blood pressure in healthy young men. American Journal of Clinical Nutrition, 88: 1225-1231.

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Neter JE, Stam BE, Kok FJ, Grobbee DE and Geleijnse JM. 2003. Influence of weight reduction on blood pressure: A meta-analysis of randomized controlled trials. Hypertension, 42: 878-884.

Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA and Ray CA. 2004. Exercise and hypertension. Position stand of the American College of Sports Medicine. Medicine and Science in Sports and Exercise, 36 (3): 533-553.

Seedat YK, Croasdale MA, Milne FJ, Opie LH, Pinkney-Atkinson VJ, Rayner BL and Veriava Y. 2006. South African hypertension guideline 2006. South African Medical Journal, 96 (4): 337-362.

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

Weston AT, Petosa R and Pate RR. 1997. Validation of an instrument for measurement of physical activity in youth. Medicine and Science in Sports and Exercise, 29 (1): 138-143.

Whelton SP, Chin A, Xin X and He J. 2002. Effect of aerobic exercise on blood pressure: A meta-analysis of randomized, controlled trials. Annals of Internal Medicine, 136: 493-503.

World Health Organization; International Society of Hypertension Writing Group (WHO/ISH). 2003. WHO/ISH statement on management of hypertension. Journal of Hypertension, 21: 1983–1992.

World Health Organization (WHO). 2008. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008 [Online].
Available from: http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online].
Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

CHAPTER 8

POLYMORPHISMS OF THE ANGIOTENSINOGEN (AGT); G PROTEIN-COUPLED RECEPTOR KINASE TYPE 4 (GRK4) AND ALDOSTERONE SYNTHASE (CYP11B2) GENES AND THE ASSOCIATION WITH HYPERTENSION IN A BLACK COMMUNITY IN MANGAUNG, SOUTH AFRICA.

ABSTRACT

Chronic diseases such as hypertension are likely the result of more than one gene and multiple variants of each gene that interacts with different environmental factors, with each combination making a small contribution to overall homeostasis, function, and therefore health. Essential hypertension has been estimated to be heritable in about 30-50% of cases and polymorphisms of various genes have been implicated in this complex condition.

Objective: The objective of this study was to determine the prevalence of polymorphisms of the *AGT* (M235T and -217); *GRK4* (R65L, A142V, A486V) and *CYP11B2* genes and the association with hypertension in a black community in Mangaung, South Africa.

Methods: Data were collected in the Assuring Health for All in the Free State (AHA-FS) study. Blood pressure was measured by a registered medical practitioner according to standard guidelines. Blood samples for genetic testing were obtained through venous puncture. After plasma was removed, DNA was stored and stabilized on fluorescent treponemal antibody (FTA) paper. Genetic screening was performed using real-time polymerase chain reaction (PCR) analysis.

Results: The study included 339 adults (263 females, 76 males), with a mean age of 44.3±10.6 (SD) years (range, 25-63). A total of 63.4% of the study population either had blood pressure values ≥140/90mmHg or were currently using antihypertensive medication, implicating underlying hypertension. The hypertension risk C/C allele of the M235T polymorphism was found in 87% of this population. The presence of this polymorphism could however not be associated with the prevalence of hypertension. R65L of *GRK4* was associated with hypertension, with an allele distribution of 12.1% G/G, 32.6% G/T and 55.3% T/T. The majority of participants in this study (92.1%) had the low risk C/C allele for the *GRK 4* (A486V) gene, but the presence of this allele could not be linked to the prevalence of hypertension. Almost all the participants (97.8%) had the low risk C/C allele for *CYP11B2* (-344).

Conclusion: More than half of this population had abnormally high blood pressure values, increasing the risk for disease and premature death. None of the high risk alleles for hypertension

described in literature, including *AGT* (M235T and -217); *GRK4* (A142V and A486V) or *CYP11B2*, seem to play a major genetic role in the high prevalence of hypertension in this population. It is possible that other factors might have contributed more to the problem of hypertension, possibly masking the effect of genetics. Only *GRK4* (R65L) showed an association with the prevalence of hypertension (p=0.033).

Key words: hypertension, *AGT, GRK4, CYP11B2,* urban black population, AHA-FS, South Africa

8.1 INTRODUCTION

Essential hypertension, like many other diseases, is influenced by genotype, with small quantitative changes in the expression of different genes in combination with environmental factors, determining the risk of the disease (Sookoian *et al.*, 2007:5; Freitas *et al*., 2007:309). A considerable number of gene variants have been studied as candidate genes to determine the risk of hypertension. The renin-angiotensin-aldosterone system (RAAS) is one of the key role players in the regulation of blood pressure. The genes that encode the various components of the RAAS are likely to influence the genetic susceptibility to essential hypertension (Freitas *et al*., 2007:310). The majority of these genes influence blood pressure by controlling the amount of sodium and water reabsorbed in the kidney (Cummings, 2006:110).

Essential hypertension has been estimated to be heritable in about 30-50% of cases (Felder *et al*., 2002:3872). In a study of European American and African American twins, systolic blood pressure was estimated to be 57% heritable for both groups and diastolic blood pressure 45% and 58% heritable respectively (Snieder, Harshfield & Treiber, 2003:1199). Jain *et al*. (2002:36889) estimated that about 45% of the differences in blood pressure between people are as a result of genetic differences. Similarly Bengra *et al.* (2002:2132) suggested that the genetic origin of essential hypertension is between 30-50%. The influence of genetics can therefore not be ignored when addressing the problem of hypertension in a community.

Research has shown that multiple genes are implicated in the prevalence of hypertension in various populations, many of which have not yet been identified (Wallace *et al.*, 2007:49). Although it is widely recognized that an array of genetic factors are responsible for the onset and development of hypertension, for the purpose of this study, polymorphisms of the *angiotensinogen (AGT)*, *G protein-coupled receptor kinase type 4* (*GRK4*) and *aldosterone synthase* (*CYP11B2*) genes were investigated and described in a black population in Mangaung, South Africa.

8.1.1 Polymorphism of the *angiotensinogen (AGT)* gene

The *AGT* gene is responsible for manufacturing the protein AGT in the liver, which on activation to angiotensin by renin in the kidney, controls sodium and water retention to raise blood pressure (Cummings, 2006:110). Genetic variations of the *AGT* gene are thought to impact the plasma concentration of angiotensinogen, which influences blood pressure (Zafarmand *et al*., 2008:e2533). It is therefore not surprising that the AGT gene is among several genes linked to essential hypertension (Norat *et al.*, 2008:392). In humans the *AGT* gene is mapped to the chromosomal region 1q42-43 and consists of five exons and four introns (Staessen *et al.*, 1999:9). Research by Markovic *et al.* (2005:94) concluded that the promoter region of *AGT* is associated with essential hypertension although the mode of action is not understood. Various molecular variants have been described in the AGT promoter, with typical variants at the base positions -6,-20,-217,-793 and -776 (Markovic *et al*., 2005:89). The haplotype AAAAT for base positions -6,-20,-217,-793 and -776 seems to indicate an increased risk for essential hypertension in black males and females as well as white females in the United States of America (Markovic *et al.,* 2005:94).
Tiago *et al.* (2002:1484) failed to find an association between the -20A→C variant of the *AGT* gene and hypertension in a South African study that included 521 black participants, but found that the presence of the -20A→C allele influences the body size to blood pressure relationship in hypertensive individuals (Tiago *et al.*, 2002:1486), with the presence of the homozygotic polymorphism noticeably influencing the systolic blood pressure to BMI ratio. The *AGT* M235T polymorphism are often effectively assessed as surrogate for *AGT* G-6A, with the T allele corresponding to the A allele (Norat *et al.*, 2008:392-393, 396). The A/G polymorphisms at -217 and -793 in the promoter of the *AGT* gene have been found to be associated with the prevalence of hypertension, especially in African American populations (Jain *et al.,* 2002:36889; Markovic *et al*., 2005:92) with no association in Caucasian Americans (Jain *et al*., 2002:36889).

The *AGT* polymorphism that encodes threonine instead of methionine (M235T) caused by a T→C single-nucleotide polymorphism (SNP) at codon 235 has been studied extensively and appears to be associated with hypertension (Russ *et al., 1993:609;* Staessen *et al.*, 1999:9; Freitas *et al.,* 2007:310; Norat *et al.*, 2008:392). Pratt *et al.* (1998:878) describes the significant effect of the T235 polymorphism on serum AGT levels, even when correcting for race, gender, age and BMI. In a meta-analysis including a total population of 27 907 individuals, the incidence of the T allele was 52.1%, distributed in a genotype frequency of 30.6%T/T, 42.9% TM and 26.5% MM. The frequency of the T allele was 77% in blacks, 78% in Asians and 42.2% in whites (Staessen *et al.*, 1999:10). A meta analysis by Staessen *et al.* (1999:9), which included 69 studies with a total of 27 906 participants, confirmed the association of the T allele of AGT as a marker for hypertension in Caucasians, with T/T homozygotes associated with 31% (p=0.001) greater risk, and TM heterozygotes with 11% (p=0.03) greater risk, than the reference MM homozygotes. An incidence of 35% for the MM variant of the *AGT* M235T genotype and 16% for the T/T variant was found in a

white older adult population consisting of 11 384 participants in the United Kingdom (Norat *et al.*, 2008:394).

Norat *et al.* (2008:395) confirmed the effect on blood pressure in an American study population by describing a significantly higher mean systolic and diastolic blood pressure in females with the presence of the T/T allele compared to the MT or MM genotypes, but failed to show any relationship in males. An association between sodium intake and blood pressure was found in all participants, but the effect was greater in persons with the T allele than in those not carrying the T allele (Norat *et al.*, 2008:396). Tiago *et al.* (2002:1484) could however not find an association between the presence of the M235T variant of the *AGT* gene and hypertension in a large South African study with black participants, consisting of 62.9% Ngunis, 36.5% Sothos and 1.6% Vendas.

8.1.2 Polymorphisms of the *G protein-coupled Receptor Kinase type 4 (GRK4)* gene

Salt (sodium) sensitive hypertension refers to blood pressure that rises or falls with corresponding changes in dietary sodium intake (Couch & Krummel, 2008:865). The neurotransmitter, dopamine, causes natriuresis in the kidney and has a vasodilator effect, facilitating an antihypertensive effect in the kidney (Felder *et al*., 2002:3872; Prasad *et al*., 2008:2). A defect in signaling of the renal dopamine receptor has been shown to play a role in hypertension (Sen *et al*., 2005:1206). Dopamine, via D1-type receptors, is responsible for half of the increased sodium excretion when sodium intake is increased (Felder *et al*., 2002:3872). Malfunctioning of dopamine D1 receptors is often not a primary defect, but rather a result of uncoupling from its G protein effector enzyme complex (Sanada *et al.*, 2006:353; Felder *et al*., 2002:3872). Activation of variants of the *G protein-coupled receptor kinase type 4 (GRK4)* gene, has been shown to inhibit the dopamine D1 receptor, leading to decreased sodium excretion (Sanada *et al.*, 2006:353; Lohmueller *et al*., 2006:27).

There are seven G protein-coupled receptor kinases (GRK's) which are characterised into three sub families, with GRK4, GRK5 and GRK6 belonging to the GRK4 subfamily (Felder *et al*., 2002:3872). Although *GRK4* was previously thought to be expressed mainly in the brain and testes, Felder *et al*. (2002:3875) found mRNA of all isoforms in renal proximal tubules. Polymorphisms in the *GRK4 gene* are associated with salt-sensitivity and a type of hypertension characterized by low renin levels. Salt sensitivity is defined as a 10% or more increase in mean arterial pressure (determined as diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure)) when sodium intake is increased from low to high (Sanada *et al.*, 2006:353).

Bengra *et al.* (2002:2132) described three *GRK4* polymorphisms, 448G3T (R65L), 679C3T (A142V), and 1711C3T (A486V), that are located in the binding and membrane targeting domains of the gene, that can on their own or by interaction with other genes be involved in the renin-angiotensinogen system, to cause essential hypertension. However only the presence of the

1711C3T (A486V) SNP in the hypertensive group in an Italian population demonstrated significance. In a study of *GRK4* gene polymorphisms by Lohmueller *et al*. (2006:27) different allele frequencies, as well as different haplotype structure between different US population groups (African American, Caucasian, Hispanic and Asian), were shown. Gender differences were reported by Bhatnagar *et al*. (2009:332) who found an association between *GRK4* A142V and blood pressure response to metoprolol (aβ-adrenoreceptor blocker) in African American males, but not in females.

In a study by Sanada *et al.* (2006:356) it was reported that the genetic model that best predicted salt sensitivity in a Japanese population included the R65L, A142V and A486V variants of *GRK4*, which predicted salt sensitivity correctly in 94.4% of individuals. They found that this model had a sensitivity of 83% and a specificity of 100% in a Japanese study population. It is thought that these three *GRK4* variants increase the expression of *GRK4* in the kidney, which distrupts the function of dopamine receptors, leading to impaired renal dopamine-induced sodium excretion, even in the absence of hypertension (Sanada *et al.*, 2006:358; Winstead, 2002:Online). Additionally the *GRK4* A142V genotype was identified to be 78.4% predictive of salt sensitivity as a single indicator, and the 2-locus model of *GRK4* A142V and *CYP11B2* C-344T as 77.8% predictive of low-renin hypertension in this study group (Sanada *et al*., 2006:356).

8.1.3 Polymorphism of the *aldosterone synthase (CYP11B2)* gene

Aldosterone is synthesized by aldosterone synthase in the adrenal cortex and is encoded by the *CYP11B2* gene which is located on chromosome 8q22 (Rajan *et al.*, 2010:379). The role of the *CYP11B2* gene in hypertension and cardiovascular disease has been extensively researched. Particular attention is paid to the C-344T single nucleotide polymorphism in the 5' distal promoter region of the gene (Sookoian *et al*., 2007:5). A substitution at the -344 promoter region (T →C) has been linked to the presence of hypertension (Freitas *et al.*, 2007:310). This polymorphism is implicated to increase the aldosterone :renin ratio in individuals with essential hypertension with a 94% chance of a normal aldosterone :renin ratio in participants with a C/C genotype (Nicod *et al*., 2003:2499). In a meta-analysis by Sookoian *et al*. (2007:7) of 19 studies, including a total of 11 225 participants, the -344T allele was associated with an increased risk for hypertension and the -344C allele with a decreased risk for arterial hypertension. In an Indian population, a significant association between the C-344T polymorphism and essential hypertension was shown in male participants, but not females (Rajan *et al*., 2010:382).

In a study on a black population, Henderson *et al.* (2004:270) found that the presence of the T allele was associated with an increased risk for hypertension in African American males and females, but not in Latinos living in the same environmental circumstances. This association was also found in a black South African population (58% Nguni, 40% Sotho and 2% Venda) from

Johannesburg (n=231) where the presence of a T/T allele was significantly associated with systolic blood pressure amongst newly diagnosed hypertensive individuals (Tiago *et al.*, 2003:1007).

The aim of this study was to test for and describe the presence of polymorphisms of the *AGT*, *GRK4* and *CYP11B2* genes and determine the association of these with hypertension.

8.2 METHODOLOGY

Data from the urban baseline phase of the Assuring Health for All in the Free State (AHA-FS) study were used for this study. The population of the AHA-FS urban baseline study included the urban areas serviced by the MUCPP clinic and included black (South Sotho) households in the Freedom Square, Turflaagte, Namibia, Kagisanong, Chris Hani and Rocklands Buffer area of Mangaung, Bloemfontein, South Africa.

Trained fieldworkers visited households, which were selected in a stratified proportional cluster sample, to encourage participation in the research, explain the purpose of the study and obtain written informed consent. All adult participants, 25-64 years of age in these households, who provided informed consent, were included in the urban AHA-FS study. A total of 391 households were included, and data were collected for 328 adult females and 103 adult males (431 adults in total). From this sample, all adults with complete data sets for age, gender, blood pressure, body weight, height, activity level as well as available blood samples for genetic testing and 25(OH)D analysis, were selected for the current study. Participants assembled at the central research centre, where blood pressure was measured and blood samples obtained for genetic testing (amongst other assessments).

Hypertension is defined as systolic blood pressure of 140mmHg or higher and/or diastolic blood pressure of 90 mm Hg or higher (Couch & Krummel, 2008:866). In this study, participants using prescription medication for the management of hypertension at the time of the interview were also regarded as hypertensive (Wallace *et al.*, 2007:51).

Blood pressure was taken by a registered medical practitioner according to the guidelines provided by the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure* (NIH, 2004:Online) using an electronic blood pressure monitor (DS-175, Nissei Commerce, Ltd., Tokyo, Japan). Patients were seated in a chair with their feet on the floor and arm supported at heart level for at least five minutes. Caffeine, exercise and smoking were avoided for at least 30 minutes prior to measurement. An appropriately sized cuff was used and the average of two measurements recorded. Mean arterial pressure was calculated by the following formula (Sanada *et al.*, 2006:353):

Mean arterial pressure = diastolic blood pressure + ⅓ (systolic blood pressure – diastolic blood pressure).

For the purpose of this study, the following polymorphisms were used to describe the genotype of the study population:

- A/G polymorphism at the -217 and C/T polymorphism at the -235 locus (M235T) of the *angiotensinogen* (*AGT*) gene;
- Three polymorphisms (R65L (G448T), A142V (C679T) and A486V (C1711T)) of the *G protein-coupled receptor kinase type 4 (GRK4)* gene; and
- T/C polymorphism of the *aldosterone synthase gene* -344 *CYP11B2*.

Wallace *et al. (*2007:50) recommended that when research is done to determine the genetic impact of hypertension, individuals with other known causes of hypertension, like diabetes, old age and obesity should be excluded, to ensure that the hypertensive individuals being studied are more likely to carry genetic variants causing hypertension than having other non-genetic factors that could contribute to their hypertension. Due to the older age of the study population and high prevalence of overweight / obesity, these exclusions were not made for this study population.

Blood samples for genetic testing were obtained through venous puncture. Plasma was removed and genetic material stored at -80 degrees Celsius. Genetic analysis was performed by the Department of Haematology and Cell Biology, Faculty of Health Sciences from the University of the Free State in a laboratory operating according to ISO 17025 standards.

After thawing the blood cells, 200µL per sample was blotted on fluorescent treponemal antibody (FTA) paper (Whatman, New York), labelled, allowed to dry, and stored at room temperature. FTA paper is designed to bind and protect nucleic acids (Vitha & Yoder, 2005:Online). Disks of 1.2mm diameter were punched and used in a real-time polymerase chain reaction (PCR) assay. The use of FTA paper is considered as an efficient method for clinical application, which renders results comparable to traditional approaches (Pezzoli *et al.*, 2007:1182). A multiplex real-time PCR assay was performed on a *Stratagene Mx3005P* thermal cycler, after washing twice with FTA purification reagent and three times with 0.1X TE buffer (1 mM Tris-HCL, pH8 and 0.1 mM EDTA, pH8). Allele detection was performed with VIC and FAM labelled probes, under the following PCR conditions: one cycle of 10 minutes at 95^{o}C, followed by 50 cycles of 15 seconds each at 95^{o}C and 1 minute at 60^{o}C for T842C (*AGT*), -344C/T (*CYP11B2*) and G448T, C679T and C1711T (*GRK4*), or 62^{o}C for -217 A/G (*AGT*).

8.3 STATISTICAL ANALYSIS

The researcher conducted statistical analysis of the data, using the PASW (Predictive Analytics SoftWare) Statistics Student Version 18.0 software by *SPSS: An IBM Company*.
Frequencies and percentages were used to summarize the categorical data. Means and standard deviations, or percentiles as appropriate, were used to summarize numerical characteristics. Comparisons of means were done using t-tests as appropriate. Two tailed Pearson correlations, chi-square, point bi-serial correlations and logistic regressions were used to describe and test associations between variables.

8.4 RESULTS

339 Adults (76 males and 263 females) from the baseline study with complete data sets were included in this study. Participants in the current study had a mean age of 44.3 $\pm$ 10.6 (SD) years, ranging between 25 and 63 years. A general description of the population in terms of age, blood pressure and body mass index (BMI) is provided in Table 8.1.

Table 8.1 General description of the study population in terms of age, blood pressure and BMI

Variable	N	Mean	Std Dev	Minimum	Maximum
Age (years)	339	44.3	10.60	25	63
Systolic blood pressure (mmHg)	339	135.5	23.67	72	203
Diastolic blood pressure (mmHg)	339	89.8	17.57	46	188
Mean arterial pressure (mmHg)	339	105.0	17.96	59	191
BMI (kg/m^2)	339	27.8	8.79	13.3	55.7

In 41.6% of participants, systolic blood pressure was $\geq$140mmHg and in 46.6% of participants, diastolic blood pressure was $\geq$90mmHg indicating hypertension. More than a quarter (25.4%) of the study population was using antihypertensive medication at the time of the study, implicating hypertension. In total 63.4% (57.9% males and 65.0% females) of the study population had blood pressure values $\geq$140/90mmHg or were using antihypertensive medication at the time of the study.

The presence of polymorphisms known to be associated with hypertension, which were identified in this study, is indicated in Table 8.2.

Table 8.2 Presence of *AGT*, *GRK4* and *CYP11B2* polymorphisms

Gene	Homozygous (low risk)		Heterozygous		Homozygous (HT risk)		Total*
	n	%	n	%	n	%	
AGT 217	123	43.2	88	30.9	74	26.0	285
AGT M235T	12	4.6	22	8.4	227	87.0	261
GRK4 R65L	39	12.1	105	32.6	178	55.3	322
GRK4 A142V	31	9.7	93	29.1	196	61.3	320
GRK4 A486V	267	92.1	17	5.9	6	2.1	290
CYP11B2	268	97.8	4	1.5	2	0.7	274

* Total number of participants from a total of 339 participants, where positive identification of polymorphisms could be made.

When compiling cross tables for the polymorphisms for each gene, chi-square tests showed a significant association (χ^2=5.15, df=2, ρ=0.033) for only *GRK4* R65L with the presence of hypertension. The genotype frequencies of the various SNP's according to hypertensive status are indicated in Table 8.3.

Table 8.3 Genotype frequencies of SNP's in *AGT*, *GRK4* and *CYP11B2* in hypertensive (HT) and non-hypertensive (NT) individuals

Gene	SNP	Genotype	Genotype frequency				
			HT (n)	%	NT (n)	%	Total
AGT	-217 A/G (HT=A ; NT=G)	A/A	51	68.9	23	31.1	74
		A/G	57	64.8	31	35.2	88
		G/G	74	60.2	49	39.8	123
	T842C (M235T) (HT=C ; NT=T)	C/C	146	64.3	81	35.7	227
		C/T	12	54.5	10	45.5	22
		T/T	9	75.0	3	25.0	12
GRK4	G448T(R65L) (HT=T ; NT=G)	T/T	115	64.6	63	35.4	178
		T/G	57	54.3	48	45.7	105
		G/G	31	79.5	8	20.5	39
	C679T (A142V) (HT=T ; NT=C)	T/T	123	62.8	73	37.2	196
		T/C	61	65.6	32	34.4	93
		C/C	20	64.5	11	35.5	31
	C1711T (A486V) (HT=T ; NT=C)	T/T	4	66.7	2	33.3	6
		T/C	12	70.6	5	29.4	17
		C/C	168	62.9	99	37.1	267
CYP11B2	-344C/T (HT=T ; NT=C)	T/T	0	0.0	2	100.0	2
		T/C	3	75.0	1	25.0	4
		C/C	168	62.7	100	37.3	268

HT = Hypertensive; NT= Normotensive

8.5 DISCUSSION

A high prevalence (87%) of the hypertension risk C/C allele of the *AGT* M235T polymorphisms was found in the study population. In contrast to other published results (Russ *et al., 1993:609;* Staessen *et al.*, 1999:9; Freitas *et al.,* 2007:310; Norat *et al.*, 2008:392), however, this polymorphism could not be linked to the prevalence of hypertension. Freitas *et al.* (2007:313) describes an allele distribution of C/C – 90; C/T – 78 and T/T – 37 amongst Brazilians, representing a 44% incidence of the C/C allele. This highlights the influence of the genetic background of different populations and the complexity of multifactorial traits, such as hypertension.

With regard to the *GRK4* genes, R65L could be linked to hypertension with an allele distribution of 12.1% G/G, 32.6% G/T and 55.3% T/T. The majority (92.1%) of black (mostly South Sotho) participants in the current study had the low risk C/C allele for the *GRK4* C1711T (A486V) gene, but this allele could not be linked to the prevalence of hypertension as in an Italian study (Bengra *et al.*,2002:2132).

In this urban, black population almost all the participants (97.8%) had the low risk for hypertension C/C allele for *CYP11B2* (-344) with 1.5% having the heterozygote C/T allele and only 2 participants the high risk T/T allele, linked to the presence of hypertension in published studies. The results from this study differ from that of Tiago *et al.* (2003:1008) that found a significant association between the presence of these gene polymorphisms and increased systolic blood pressure in black South Africans from Johannesburg comprising of 58% Ngunis, 40% Sothos and 2% Vendas and identified 75 C/C and C/T participants and 156 T/T participants. Freitas *et al.* (2007:313) also found a different allele distribution amongst Brazilians, representing a 21% incidence of the C/C, 51% C/T and 28% T/T allele.

The genetic composition of the current study population differs from those described in other different, as well as similar population groups and does not show the same association with the prevalence of hypertension. It might be that other combinations of genetic influences may play a more important role than those tested for and used in other studies. These findings also agree with a genome-wide association study (GWAS) among African Americans where no variant reached genome wide significance for its association with diastolic blood pressure (Adeyemo *et al*., 2009:Online).

8.6 CONCLUSION

In this urban, black, South Sotho population, neither the presence of the A/A allele at -217 of the *AGT* gene, the C/C allele of the M235T polymorphism, nor the T/T alleles of *GRK4* A142V and A486V or *CYP11B2*, on their own appeared to be major genetic role players in the high prevalence of hypertension in this study population. Only *GRK4* R65L showed an association with the prevalence of hypertension. The allele distribution of the genes described in this study population also differs extensively from other populations described in literature. Although no positive correlations between the polymorphisms and the prevalence of hypertension could be found in this study, it again emphasis the complexity of the disease and the influence that other factors could play in the etiology of hypertension. Future studies should therefore investigate other polymorphisms that could play a role in hypertension.

8.7 ETHICAL CONSIDERATIONS

Ethical approval for this study was obtained from the Ethics Committee from the University of the Free State, Faculty of Health Sciences (ETOVS: 21/07).

8.8 ACKNOWLEDGEMENT

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8.9 BIBLIOGRAPHY

Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, Zhou J, Lashley K, Chen Y, Christman M and Rotimi C. 2009. A Genome-Wide Association Study of Hypertension and Blood Pressure in African Americans. PLoS Genetics 5(7): e1000564 [Online].
Available from: http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000564 [Accessed May 27th 2011].

Bhatnagar V, O'Connor DT, Brophy VH, Schork NJ, Richard E, Salem RM, Nievergelt CM, Bakris GL, Middleton JP, Norris KJ, Wright J, Hiremath L, Contreras G, Appel LJ, Lipkowitz MS and The AASK Study Investigators. 2009. G-Protein-Coupled Receptor Kinase 4 Polymorphisms and Blood Pressure Response to Metoprolol Among African Americans: Sex-Specificity and Interactions. American Journal of Hypertension, 22 (3): 332-338.

Bengra C, Mifflin TE, Khripin Y, Manunta P, Williams SM, Jose PA and Felder RA. 2002. Genotyping of essential hypertension single-nucleotide polymorphisms by a homogeneous PCR method with universal energy transfer primers. Clinical Chemistry, 48 (12): 2131–2140.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Cummings MR. 2006. Human heredity – principles and issues. 7th ed. Belmont: Thomson Brooks/Cole.

Felder RA, Sanada H, Xu J, Yu P-Y, Wang Z, Watanabe H, Asico LD, Wang W, Zheng S, Yamaguchi I, Williams SM, Gainer J, Brown NJ, Hazen-Martin D, Wong L-JC, Robillard JE, Carey RM, Eisner GM and Jose PA. 2002. G protein-coupled receptor kinase 4 gene variants in human essential hypertension. Proceedings of the National Academy of Sciences of the United States of America, 99:3872-3877.

Freitas SRS, Cabello PH, Moura-Neto RS, Dolinsky LC, Lima AB, Barros M, Bittencourt I and Cordovil IL. 2007. Analysis of renin-angiotensin-aldosterone system gene polymorphisms in resistant hypertension. Brazilian Journal of Medical and Biological Research, 40: 309-316.

Henderson SO, Haiman CA and Mack W. 2004. Multiple polymorphisms in the renin-angiotensin-aldosterone system (ACE,CYP11B2, AGTR1) and their contribution to hypertension in African Americans and Latinos in the multi-ethnic cohort. American Journal of the Medical Sciences, 328(5): 266–273.

Jain S, Tang X, Narayanan CS, Agarwal Y, Peterson SM, Brown CD, Ott J and Kumar A. 2002. Angiotensinogen gene polymorphism at _217 affects basal promoter activity and is associated with hypertension in African-Americans. The Journal of Biological Chemistry, 277, 39: 36889–36896.

Lohmueller KE, Wong LJ, Mauney MM, Jiang L, Felder RA, Jose PA and Williams SM. 2006. Patterns of genetic variation in the hypertension candidate gene *GRK4*: ethnic variation and haplotype structure. Annals of Human Genetics, 70:27-41.

Markovic D, Tang X, Guruju M, Levenstien MA, Hoh J, Kumar A and Ott J. 2005. Association of angiotensinogen gene polymorphisms with essential hypertension in African-Americans and Caucasians. Human Heredity, 60: 89-96.

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Nicod J, Bruhin D, Auer L, Vogt B, Frey FJ and Ferrari P. 2003. A biallelic gene polymorphism of *CYP11B2* predicts increased aldosterone to renin ratio in selected hypertensive patients. The Journal of Clinical Endocrinology and Metabolism, 88: 2495-2500.

Norat T, Bowman R, Luben R, Welch A, Khaw KT, Wareham N and Bingham S. 2008. Blood pressure and interactions between the angiotensin polymorphism *AGT* M235T and sodium intake: a cross-sectional population study. American Journal of Clinical Nutrition, 88: 392-397.

Pezzoli N, Silvy M, Woronko A, Le Treut T, Lévy-Mozziconnaci A, Reviron D, Gabert J and Picard C. 2007. Quantification of mixed chimerism by real time PCR on whole blood-impregnated FTA cards. Leukemia Research, 31: 1175-1183.

Prasad P, Kumar KMP, Ammini AC, Gupta A, Gupta R and Thelma BK. 2008. Association of dopaminergic pathway gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. BMC Genetics [Online], 9:26.
Available from: http://www.biomedcentral.com/1471-2156/9/26 [Accessed January 20th, 2009].

Pratt JH, Ambrosius WT, Tewksbury DA, Wagner MA, Zhou L and Hanna MP. 1998. Serum angiotensinogen concentration in relation to gonadal hormones, body size, and genotype in growing young people. Hypertension, 32: 875-879.

Rajan S, Ramu P, Umamaheswaran G and Adithan C. 2010. Association of aldosterone synthase (*CYP11B2* C-344T) gene polymorphism and susceptibility to essential hypertension in a south Indian Tamil population. Indian Journal of Medical Research, 132: 379-385.

Russ AP, Maerz W, Ruzicka V, Stein U and GroB W. 1993. Rapid detection of the hypertension-associated Met235—Thr allele of the human angiotensinogen gene. Human Molecular Genetics, 2 (5): 609-610.

Sanada H, Yatabe J, Midorikawa S, Hashimoto S, Watanabe T, Moore JH. Ritchie MD, Williams SM, Pezzullo JC, Sasaki M, Eisner GM, Jose PA and Felder RA. 2006. Single-nucleotide polymorphisms for diagnosis of salt-sensitive hypertension. Clinical Chemistry, 52 (3): 352-360.

Sen S, Nesse R, Sheng L, Stoltenberg SF, Gleiberman L, Burmeister M and Weder AB. 2005. Association between a dopamine-4 receptor polymorphism and blood pressure. American Journal of Hypertension, 18 (9): 1206-1210.

Snieder H, Harshfield GA and Treiber FA. 2003. Heritability of blood pressure and hemodynamics in African- and European-American Youth. Hypertension, 41:1196-1201.

Sookoian S, Gianotti TF, González CD and Pirola CJ. 2007. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. Journal of Hypertension, 25:5–13.

Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R and Fagard R. 1999. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. Journal of Hypertension, 17: 9–17

Tiago AD, Samani NJ, Candy GP, Brooksbank R, Libhaber EN, Sareli P, Woodiwiss AJ and Norton GR. 2002. Angiotensinogen gene promoter region variant modifies body size–ambulatory blood pressure relations in hypertension. Circulation, 106:1483-1487.

Tiago AD, Badenhorst D, Nkeh B, Candy GP, Brooksbank R, Sareli P, Libhaber E, Samani NJ, Woodiwiss AJ and Norton GR. 2003. Impact of renin-angiotensin-aldosterone system gene variants on the severity of hypertension in patients with newly diagnosed hypertension. American Journal of Hypertension, 16:1006–1010.

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

Winstead ER. 2002. Gene variants linked to inherited forms of hypertension. Genome News Network [Online].
Available from: http://www.genomenewsnetwork.org [Accessed March 13th , 2008].

Zafarmand MH, Van der Schouw YT, Grobee DE, De Leeuw PW and Bots ML. 2008. The M235T Polymorphism in the *AGT* Gene and CHD Risk: Evidence of a Hardy-Weinberg Equilibrium Violation and Publication Bias in a Meta-Analysis. PLoS ONE, 3 (6): e2533 [Online].
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2432037/pdf/pone.0002533.pdf [Accessed January 28th, 2011].

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

This research study investigated some of the potential factors that could contribute to hypertension in a black, urban population in Mangaung, Bloemfontein, in order to construct and implement appropriate interventions. The true motivation for this quest to find solutions to the problem of hypertension is, as Aviv (2001:1060) summarised it, to extend a healthy life-span and to maintain or improve physical well-being; and to do this by developing and implementing appropriate lifestyle changes relevant to the community, according to the causes of hypertension, rather than simply to continue with expensive, often ineffective hypertension treatment regimens. The conclusions and recommendations from this study will henceforth be discussed according to the five main objectives of the study, namely to determine the association between blood pressure and body weight; serum 25-hydroxy vitamin D levels; sodium and potassium intakes; levels of physical activity; and the presence of specific gene polymorphisms linked to hypertension in this community.

9.1 BODY WEIGHT

Obesity is a major risk factor for non-communicable diseases and it is especially countries in transition from undeveloped to developed that are affected by the accelerated rate of obesity in all income levels and age groups (Popkin, 1994: 285). More than half of this study population had abnormally high blood pressure values ($\geq$140/90mmHg) and 55.1% were overweight/obese according to BMI. The majority of participants had a WHtR of 0.5 or more, indicating health risk; and WHtR was significantly related to mean arterial pressure. WHtR also seemed to be a stronger predictor of mean arterial pressure than BMI or BAI in this population. The mean BAI of this population was 34.1%, with more than three quarters of respondents presenting with an overweight/obese BAI. Adiposity levels showed a positive correlation with arterial blood pressure levels. Two thirds of the females and 6.6% of the males had waist circumferences indicating an increased metabolic risk (more than 80cm and 94cm respectively), increasing the risk for disease and premature death. In females, hypertension was significantly related to waist circumference and BMI, and waist circumference and BAI were significantly related to BMI. Despite the high prevalence of HIV in this study population, the prevalence of hypertension was not significantly affected by HIV status. These results support weight loss as first line intervention for the treatment and prevention of hypertension, with its accompanying disease burden. In this study, the relative easy technique of determining WHtR was also identified as an effective tool to screen for hypertension. The recommendation from a review by Kruger et al. (2005: 497) should be kept in mind, that different ethnic groups are not necessarily affected by industrialisation in the same

degree, and that obesity should be treated on an individual, but also on a population level. It is therefore recommended that strategies be put in place to address overweight/obesity at household level, focussing specifically at females. Community commitment should also be obtained, to ensure that an environment supportive of healthier living is created.

9.2 SERUM 25-HYDROXY VITAMIN D

In contrast to the overview of Lips (2010:300) that describes a high prevalence of inadequate vitamin D status (<20 ng/ml) worldwide, the current study population had a very low prevalence of inadequate or deficient vitamin D status (≈4%). The favourable latitude, the fact that blood samples were taken during early autumn and the high levels of sun exposure that is expected from the living conditions of this population, could be possible explanations for the general good vitamin D status, since routine vitamin D fortification of staples has not been implemented in South Africa. Although an inverse relationship was found between BMI and vitamin D levels and also between mean arterial blood pressure and vitamin D, no significant correlation were found between vitamin D levels, systolic bloodpressure, diastolic bloodpressure or mean arterial blood pressure when controlling for BMI. The inverse relationship shown between vitamin D and blood pressure therefore may be the result of an increase in body weight that resulted in lower vitamin D levels and higher blood pressure levels. HIV status also did not influence vitamin D levels, except through influencing BMI. Although vitamin D levels did not show a correlation with the prevalence of hypertension, it is recommended that the effect of vitamin D status be further investigated in this community, especially during winter months and by monitoring the various components that influence blood pressure (e.g. RAAS) and not only changes in blood pressure as the end result.

9.3 SODIUM AND POTASSIUM INTAKES

In this study, no association was found between sodium or potassium intakes (indirectly reflected by urinary excretion levels) and the prevalence of hypertension, but an association between sodium consumption and blood pressure levels, was found. For the majority of participants, mean daily excretion of sodium was above the level corresponding to the UL of 100mmol/day, or 5.8g salt /day. Potassium excretion was low, indicating low potassium intakes from fruit and vegetables in this population. Although an inverse relationship between sodium and potassium intake has been widely published from other studies, a positive association was found between dietary sodium and potassium excretion levels in this study, which could be explained by the practice of preparing vegetables with large amounts of salt. No association between potassium:sodium ratio and blood pressure levels were found. Dietary intake of sodium, as represented by the frequency of chips, salt and salty food consumption, did not correlate with sodium excretion levels, but the frequency of fruit and vegetable consumption correlated with potassium excretion levels. Making use of the frequency of salty food consumption as an indication of dietary sodium intake does not

seem to provide a good reflection of dietary sodium intake. It is recommended that a sensitive food frequency tool be developed (validated by sodium excretion) to indicate sodium intake more accurate in similar population groups. Sodium excretion levels showed a positive correlation with systolic, diastolic and mean arterial blood pressure. It is therefore recommended that a reduction in daily sodium intake should form part of intervention strategies to address the problem of hypertension in this urban population.

The addition of salt during cooking food and at the table, is regarded as smaller contributors to salt intake, and processed food in the diet seems to be the main contributor of up to 80% of total salt intake (Angus, 2007:3). He and MacGregor (2007:19) confirm this by stating that the majority of salt (75%) that are consumed in developed countries, is added by the food industry to processed foods, as well as in canteens, restaurants and by means of fast foods. They estimate that only 15% of sodium intake is added during cooking and that about 5% is naturally present in food (He & MacGregor, 2007:19). Legislation guiding food sodium levels and voluntary sodium reduction by the industry can therefore result in a significant reduction in the sodium intake of a population.

In the National Diet and Nutrition Survey (NDNS) the mayor determinants found to contribute to sodium intake were cereals and cereal products, contributing a third; meat and meat products contributing 26%; and milk and milk products contributing 8% (Angus, 2007:3).

In this current community maize porridge is the traditional staple food and because it is usually prepared with quite a large amount of salt, it can be assumed that maize porridge would contribute a significant amount of sodium to the diet. To reduce salt intake in populations, He and MacGregor (2007:19) recommends campaigns which warn the public about the dangers of salt, and which aims to achieve the use of less salt during food preparation and at table, as well as a long-term reduction of salt in processed and catering foods. It is recommended that the food sources that contribute the highest amounts of sodium in this population's diet be researched and identified, in order to ensure targeted and specific messages during intervention strategies.

Even moderate reductions in mean daily sodium intake (a reduction of 100mmol or 5.8g of salt per day) in a population can lead to reducing the average systolic blood pressure in a community with at least 2.2 mmHg, which in the US and UK is associated with a 4% lower risk for coronary death and a 6% lower risk for stroke (Stamler *et al.,* 1989:570). This effect becomes even more pronounced when potassium intake is increased and alcohol and body fat levels are reduced.

Potassium intake can be increased by increasing fruit and vegetable consumption. To address the challenge of fruit and vegetables that are an expensive and often unaffordable commodity in this community with a lower socio-economic status, promotion of household vegetable gardens and planting of fruit trees in these communities are recommended.

9.4 PHYSICAL ACTIVITY LEVEL

More than a third of participants in this study reported being active to very active, with the majority reporting low activity levels. Although physical inactivity is closely linked to the prevalence of hypertension, no association could be found in this study between physical activity and the incidence of hypertension. Despite the high prevalence of overweight and obesity, no association was found between activity levels and the prevalence of overweight/ obesity in the study. BMI differed significantly between HIV positive and negative groups, but no significant association between HIV status and activity level was found. This study relied on self-reporting of physical activity and the possibility of over reporting of activity exists. Even though no direct association between activity and the prevalence of hypertension were found in this study, it is recommended that one of the objectives in an intervention study should be to increase activity. Practical recommendations should be made to increase physical activity to an accumulative total of 30 minutes per day, each day of the week or even just two times per week. Increased activity would not only facilitate increased energy expenditure and therefore address overweight/obesity to an extent, but would also improve general heart health.

9.5 GENETIC FACTORS

In this urban, South Sotho population, neither the presence of the A/A allele at -217 of the AGT gene, the C/C allele of the AGT M235T polymorphism, nor the T/T alleles of GRK4 A142V and A486V or CYP11B2, on their own seemed to be major genetic role players in the high prevalence of hypertension. Only GRK4 R65L showed an association with the prevalence of hypertension. The allele distribution of the genes described in this population also differs extensively from other populations described in literature. Although no positive associations between the polymorphisms and the prevalence of hypertension could be found in this study, it again emphasis the complexity of the disease and the influence that other factors could play in the etiology of hypertension. Future studies should therefore investigate other polymorphisms that could play a role in hypertension. This study failed to identify specific gene variants that can be used to screen for early detection of salt sensitive individuals on a genetic basis. Although genetic composition plays an important role in determining the pre-disposition for hypertension, the important environmental factors that determine the expression of different genes should be kept in mind and addressed.

After investigating, body weight, vitamin D status, sodium and potassium intakes, activity and genetic polymorphisms, the recommendations from this study for the urban area of Mangaung, Bloemfontein agrees with the recommendations made by Opie and Seedat (2005:3562) that addressing the problem of obesity and a reduction of salt intake ought to be the two most viable lifestyle changes that could address the epidemic of hypertension in Africa.

Appel (2009:365) recommends labelling of energy content at the point of purchase at restaurants and stores, and government initiatives that encourage physical activity to address overweight and obesity. To reduce sodium intake, it is recommended that food manufacturers and restaurants should gradually reduce the salt content of processed foods. It is recognized that individual behaviour change is very important, but government and employer programmes to promote access and consumption of healthy food can also play an important part in addressing the global problem of hypertension.

9.6 LIMITATIONS OF THE STUDY

This study was conducted on weekdays during normal working hours and although a letter was provided to participants that would inform their employers, it is possible that employed individuals could have been excluded from the study, causing a bias in the study towards the unemployed, older retired individuals, and individuals with medical problems. It is also possible that participants with medical problems were more likely to participate in the study; and very ill, bedbound individuals may have been unable to participate in the research. More women than men took part in the study, which could be because more men are employed. It is therefore acknowledged that the study group might not be representative of the general population in the study area.

Self-reported physical activity and the lack of an appropriately validated questionnaire to determine activity levels for this specific target group is a limitation in the study.

Because of the nature and extend of the study, it was not possible to collect 24 hour urine samples in this population for analysis of urinary sodium and potassium excretion. A validated formula developed by Tanaka *et al.* (2002:101) was however used to estimate 24-hour sodium and potassium excretion.

9.7 RESEARCH SIGNIFICANCE

The incidence and impact of hypertension is widely researched and described in literature. The significance of this study was that various contributing factors were investigated and tested with other factors in order to identify the most probable causes of hypertension in this black, urban population. This study identified the important impact of body fat on blood pressure levels, as well as the importance of reducing sodium intake to address the problem of hypertension. The value of WHtR as a quick and easy method to screen for hypertension risk was confirmed and the negligible impact of vitamin D, activity level and selected gene polymorphisms on hypertension in this population, provides valuable data to influence the development of a tailor-made intervention program for this population.

9.8 BIBLIOGRAPHY

Angus F. 2007. Dietary salt intake: sources and targets for reduction, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited:3-17.

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal Of Clinical Hypertension, 11 (7): 358-368.

He FJ and MacGregor GA. 2007. Dietary salt, high blood pressure and other harmful effects on health, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited: 18-54.

Kruger HS, Puoane T, Senekal M and Van der Merwe M-T. 2005. Obesity in South Africa: challenges for government and health professionals. Public Health Nutrition, 8 (5): 491-500

Lips, P. 2010. Worldwide status of vitamin D nutrition. Journal of Steroid Biochemistry and Molecular Biology, 121:297-300.

Opie LH and Seedat YK. 2005. Hypertension in Sub-Saharan African populations. Circulation, 112: 3562-3568.

Popkin BM. 1994. The nutrition transition in low-income countries: an emerging crisis. Nutrition Reviews, 52(9): 285-298.

Stamler J, Rose G, Stamler R, Elliott P, Dyer A and Marmot M. 1989. INTERSALT study findings. Public health and medical care implications. Hypertension, 14:570-577.

Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H and Hashimoto T. 2002. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. Journal of Human Hypertension 16: 97–103.

SUMMARY

Hypertension is responsible for a large and increasing proportion of the global disease burden and is becoming increasingly significant in low-income countries. The aim of this study was to determine the association of body weight, 25-hydroxy vitamin D, sodium and potassium intakes, physical activity levels and genetic factors, with the prevalence of hypertension in a low income, black urban community.

Various factors influence blood pressure, with especially body weight showing a strong relationship with hypertension. More than half of this study population suffered from hypertension and the majority was overweight or obese, increasing the risk for disease and premature death. All indices of abdominal obesity and body fatness, including BMI, WHtR, adiposity index and waist circumference were significantly related to blood pressure, supporting weight loss as first line intervention for treatment and prevention of hypertension and its accompanying disease burden in this population. Findings also suggest the use of WHtR to screen for hypertension in this population.

Higher blood pressure levels are associated with lower levels of vitamin D and low vitamin D levels have been linked to obesity markers. Although the majority of participants in this study were overweight/obese, almost 96% had adequate vitamin D status, despite expected low vitamin D intakes. HIV status did not influence vitamin D status directly, but through BMI. The latitude and high levels of sun exposure could have been responsible for the favorable vitamin D status in the participants. Results confirm the inverse relationship between vitamin D status and hypertension reported by other researchers, but found that this relationship seemed to be dependent on BMI in this study population.

Lower sodium intakes accompanied with increased potassium intakes are recommended for the prevention and treatment of hypertension. The blood pressure elevating effect of sodium have been found to be even more profound in black population groups, urging investigation into this possible race-related cause of hypertension.
Sodium intakes, as reflected by urinary sodium excretion, were high in this study. Association between sodium intakes and systolic, diastolic and mean arterial pressure were found, with higher sodium intakes being associated with elevated blood pressure levels, indicating the need for dietary sodium reduction strategies to control hypertension in this population. Despite high sodium intakes and low potassium intakes, no association was found between sodium or potassium intakes and the prevalence of hypertension.

Increased activity is often advocated as first line treatment in the prevention of hypertension, even when weight loss is not achieved. The majority of participants in this study reported being sedentary or low active. No significant association could be shown between activity level and the prevalence of hypertension. Although HIV status showed a negative correlation with BMI, no correlation could be found between HIV status and activity level.

Chronic diseases such as hypertension are likely the result of more than one gene and multiple variants of each gene that interacts with different environmental factors, with each combination making a small contribution to overall homeostasis, function, and therefore health. The high risk polymorphisms of the *AGT* (M235T and -217); *GRK4* (A142V, A486V) and *CYP11B2* genes did not seem to play a major genetic role in the high prevalence of hypertension in this population. Only GRK4 (R65L) showed an association with the prevalence of hypertension and a weak negative correlation with mean arterial pressure.

Results show that overweight/obesity and excessive sodium intake are the major contributors towards hypertension in this study population. Intervention programmes should focus on preventative strategies that create awareness to promote weight loss and encourage lower salt consumption.

OPSOMMING

Hipertensie is verantwoordelik vir ‘n groot en toenemende aandeel in die siektelas wêreldwyd en speel toenemend ‘n belangriker rol in lae inkomste lande. Die doel van hierdie studie was om die verband tussen liggaamsmassa, 25-hidroksie vitamien D, natrium- en kalium inname, fisieke aktiwiteit en genetiese faktore met die voorkoms van hipertensie in ‘n lae inkomste, swart stedelike gemeenskap te vergelyk.

Verskeie faktore oefen ‘n invloed op bloeddruk uit, met veral liggaamsmassa wat ‘n sterk verband toon met hipertensie. Meer as die helfde van hierdie hierdie studiepopulasie het aan hipertensie gely en die meerderheid was oorgewig of vetsugtig, wat die risiko vir siekte en voortydige dood verhoog. Alle indikatore van abdominale vetsug en liggaamsvet, insluitende liggaamsmassa indeks, vetindeks en middelomtrek het ‘n betekenisvolle verband met bloeddruk getoon, wat massaverlies as eerste vlak van intervensie ondersteun in die behandeling en voorkoming van hipertensie met die gepaartgaande siektelas in hierdie populasie. Bevindinge ondersteun ook die gebruik van die middel tot lengte verhouding as hulpmiddel om vir hipertensie te sif in hierdie populasie.

Hoër bloeddruk word geasossieer met laer vitamien D vlakke en lae vitamien D vlakke word met merkers vir vetsug verbind. Alhoewel die meerderheid deelnemers in hierdie studie oorgewig/vetsugtig was, het bykans 96% voldoende vitamien D status gehad, ten spite van ‘n verwagte lae vitamien D inname. MIV status het nie vitamien D status direk beinvloed nie, maar wel deur liggaamsmassa indeks. Geografiese ligging en hoë vlakke sonblootstelling kon bydra tot die gunstige vitamien D status in die studie se deelnemers. Resultate bevestig die negatiewe verband tussen vitamien D status en hipertensie soos ook deur ander navorsers beskryf, maar hierdie verhouding is waarskynlik van liggaamsmassa indeks afhanklik in hierdie studiegroep.

Laer natrium inname tesame met verhoogde kalium innames word aanbeveel vir die voorkoming en behandeling van hipertensie. Daar word beskryf dat die bloeddruk verhogende effek van natrium selfs meer uitgesproke is in swart bevolkingsgroepe, wat ondersoek na hierdie rasverwante oorsaak van hipertensie noodsaak. Natrium inname soos gereflekteer deur urinêre natrium uitskeiding was hoog in hierdie studie.

‘n Verband tussen natrium inname en sistolies, diastoliese en gemiddelde arteriële bloeddruk is gevind, met hoër natrium inname wat met verhoogde bloeddruk verband hou en die behoefte aan natriumbeperkingstrategieë in hierdie populasie aandui ten einde hipertensie te beheer. Ongeag hoë natrium innames en lae kalium innames, is geen verband gevind tussen natrium en kalium inname en die voorkoms van hipertensie.

Verhoogde aktiwiteit word dikwels aanbeveel as eerste linie behandeling in die voorkoming van hipertensie, selfs al word massaverlies nie bereik nie. Die meerderheid deelnemers aan hierdie studie het aangedui dat hul onaktief of min aktief is. Geen betekenisvolle verband kon aangedui word tussen aktiwiteit en die voorkoms van hipertensie. Alhoewel MIV status ‘n negatiewe invloed op liggaamsmassa indeks het, kon geen verband tussen MIV status en aktiwiteitsvlak gevind word nie.

Chroniese siektes soos hipertensie is waarskynlik die gevolg van meer as een geen en verskeie variante van elke geen se interaksie met verskillende omgewingsfaktore, met elkeen wat ‘n klein bydrae maak tot algemene homeostase, funksie en daarom gesondheid. Die hoë risiko polimorfismes van die AGT (M235T en -217), GRK4 (A142V en A486V) en CYP11B2 gene speel waarskynlik nie ‘n belangrike genetiese rol in die hoë voorkoms van hipertensie in hierdie populasie nie. Slegs GRK4 (R65L) toon ‘n verband met die voorkms van hipertensie en toon ‘n swak negatiewe verband met gemiddelde arteriële bloeddruk.

Resultate toon dat oorgewig/vetsug en oormatige natrium inname die belangrikste bydraende faktore tot hipertensie in hierdie studie populasie is. Intervensieprogramme behoort te fokus op voorkomingstrategieë wat bewuswording skep om massaverlies te bevorder en laer soutinname aan te moedig.

Bibliography

Acelajado MC, Pimenta E and Calhoun DA. 2010. Salt and Aldosterone: A concert of bad effects. Hypertension, 56:804-805 [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/804 [Accessed November 9th 2010].

Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, Zhou J, Lashley K, Chen Y, Christman M and Rotimi C. 2009. A Genome-Wide Association Study of Hypertension and Blood Pressure in African Americans. PLoS Genetics 5(7): e1000564 [Online].
Available from: http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000564 [Accessed May 27th 2011].

Adrogué HJ and Madias NE. 2007. Sodium and potassium in the pathogenesis of hypertension. The New England Journal of Medicine, 356:1966-1978.

Agyemang C, Addo J, Bhopal R, De Graft Aikins A and Stronks K. 2009. Cardiovascular disease, diabetes and established risk factors among populations of sub-Saharan African descent in Europe: a literature review. Globalization and Health, 5:9.

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J-C, James WPT, Loria CM and Smith SC. 2009. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation,120:1640-1645. [Online].
Available from: http://circ.ahajournals.org/cgi/content/full/120/16/1640 [Accessed 3 June 3rd 2011].

American Heart Association (AHA). 2008. American Heart Association. Heart Disease and Stroke Statistics – 2008 Update. [Online].
Available from: http://www.americanheart.org/statistics [Accessed January 19th, 2009].

American Heart Association (AHA). 2011. American Heart Association. Heart Disease and Stroke Statistics – 2011 Update: A report from the American Heart Association. [Online]
Available from: http://circ.ahajournals.org/cgi/content/full/123/4/e18 [Accessed June 21st, 2011].

Angus F. 2007. Dietary salt intake: sources and targets for reduction, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited: 3-17.

Appel LJ. 2009. ASH Position paper: Dietary approaches to lower blood pressure. The Journal Of Clinical Hypertension, 11 (7): 358-368.

Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin P-H and Karanja N. 1997. A clinical trial of the effects of dietary patterns on blood pressure. The New England Journal of Medicine, 336 (16): 1117-1124.

Argacha J-F, Adamopoulos D, Gujic M, Fontaine D, Amyai N, Berkenboom G and Van de Borne P. 2008. Acute effects of passive smoking on peripheral vascular function. Hypertension, 51:1506-1511.

Ashwell M and Hsieh SD. 2005. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. Journal of Food Sciences and Nutrition, 56 (5):303-307.

Astrup, A. 2005. Obesity, In Human nutrition. Ed. by Geissler C and Powers H. 11th ed. Philadelphia: Elsevier Ltd.

Aviv A. 2001. Hypothesis : Pulse pressure and human longevity. Hypertension, 37: 1060-1066. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/37/4/1060 [Accessed November 4th, 2010].

Bacon SL, Sherwod A, Hinderliter A and Blumenthal JA. 2004. Effects of exercise, diet and weight loss on high blood pressure. Sports Medicine, 34 (5): 307-316.

Baekken M, Os I, Sandvik L and Oektedalen O. 2008. Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy. Journal of Hypertension, 26:2126–2133.

Balkau B and Charles MA for the European Group for the Study of Insulin Resistance (EGIR). 1999. Comment on the provisional report from the WHO consultation. Diabetic Medicine, 16:442-443.

Barker DJP, Osmond C, Kajantie E and Eriksson JG. 2009. Growth and chronic disease: findings in the Helsinki Birth Cohort, Annals of Human Biology, 36(5): 445 -458.

Barnes, S. 2008. Nutritional genomics,polyphenols, diets, and their impact on dietetics. Journal of the American Dietetic Association, 108: 1888-1895.

Bergersen BM, Sandvik L, Dunlop O, Birkeland K and Bruun JN. 2003. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. European Journal of Clinical Microbiology & Infectious diseases, 22: 731–736.

Bergman RN, Stefanovsk D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, Xiang AH and Watanabe RM. 2011. A better index of body adiposity. Obesity, 19 (5): 1083-1089.

Bengra C, Mifflin TE, Khripin Y, Manunta P, Williams SM, Jose PA and Felder RA. 2002. Genotyping of essential hypertension single-nucleotide polymorphisms by a homogeneous PCR method with universal energy transfer primers. Clinical Chemistry, 48 (12): 2131–2140.

Bergersen BM, Sandvik L, Dunlop O, Birkeland K and Bruun JN. 2003. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. European Journal of Clinical Microbiology & Infectious diseases, 22:731–736.

Bhatnagar V, O'Connor DT, Brophy VH, Schork NJ, Richard E, Salem RM, Nievergelt CM, Bakris GL, Middleton JP, Norris KJ, Wright J, Hiremath L, Contreras G, Appel LJ, Lipkowitz MS and The AASK Study Investigators. 2009. G-Protein-Coupled Receptor Kinase 4 Polymorphisms and Blood Pressure Response to Metoprolol Among African Americans: Sex-Specificity and Interactions. American Journal of Hypertension, 22 (3): 332-338.

Bloomfield GS, Hogan JW, Keter A, Sang E, Carter EJ, Velazquez EJ and Kimaiyo S. 2011. Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. PLoS ONE, 6 (7): e22288 [Online].
Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%Fjournal.pone.0022288 [Accessed November 8th, 2011].

Bryan SN and Katzmarzyk PT. 2011. The association between meeting physical activity guidelines and chronic diseases among Canadian adults. Journal of Physical Activity and Health, 8: 10 -17.

Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, Peters U, Ahn J, Purdue M, Mason RS, McCarty C, Ziegler RG and Graubard B. 2010. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. Journal of Steroid Biochemistry and Molecular Biology, 121:462–466.

Burt VL; Whelton P; Roccella EJ; Brown C; Cutler JA; Higgins M; Horan MJ and Labarthe D. 1995.Prevalence of Hypertension in the US Adult Population: Results From the Third National Health and Nutrition Examination Survey, 1988-1991. Hypertension, 25:305-313.

Camhi SM, Sisson SB, Johnson WD, Katzmarzyk PT, and Tudor-Locke C. 2011. Accelerometer-determined moderate intensity lifestyle activity and cardiometabolic health. Preventive Medicine, [Online].
Available from: http://www.sciencedirect.com.proxy.lib.fsu.edu/science?_ob=MImg&_imagekey=B6WPG-523V37G-1-1&_cdi=6990&_user=2139768&_pii=S0091743511000582&_origin=search&_zone=rslt_list_item&_coverDate=02%2F05%2F2011&_sk=999999999&wchp=dGLbVzW-zSkzV&md5=158438c38a6b209d1348895d9d8e84e6&ie=/sdarticle.pdf [Accessed March 9th, 2011].

Charlton KE, Phil M, Steyn K, Levitt NS, Zulu JV, Jonathan D, Veldman FJ and Nel JH. 2005. Diet and blood pressure in South Africa: intake of foods containing sodium, potassium, calcium, and magnesium in three ethnic groups, Nutrition, 21 (1): 39–50.

Chen B, Nie S, Luo S, Zhang W, Xiao C. 2011. Association of the human CYP11B2 gene and essential hypertension in Southwest Han Chinese Population: A haplotype-based case-control study. Clinical and Experimental Hypertension, Jan 26. [Epub ahead of print]

Cheng X and Xu G. 2010. A systemic review of the relationship between aldosterone synthase -344 C/T polymorphism and hypertension in Han. Clinical and Experimental Hypertension; 32 (5): 301-307.

Corder K, Van Sluijs EMF, Wright A, Whincup P, Wareham NJ and Ekelund U. 2009 Is it possible to assess free-living physical activity and energy expenditure in young people by self-report? American Journal of Clinical Nutrition, 89:862–70.

Connor M, Rheeder P, Bryer A, Meredith M, Beukes M, Dubb A and Fritz V. 2005. The South African stroke risk in general practice study. South African Medical Journal, 95 (5): 334-339.

Cooper R, Forrester T, Ogunbiyi O and Muffindac J, for the ICSHIB Investigators. 1998. Angiotensinogen levels and obesity in four black populations. Journal of Hypertension, 16(5):571–575.

Cornelissen VA and Fagard RH. 2005. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. Hypertension, 46: 1-9.

Cornelissen VA, Verheyden B, Aubert AE and Fagard RH. 2010. Effects of aerobic training intensity on resting, exercise and post-exercise blood pressure, heart rate and heart-rate variability. Journal of Human Hypertension, 24: 175–182.

Couch SC and Krummel DA. 2008. Medical nutrition therapy for hypertension, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 865-898.

Cummings MR. 2006. Human heredity – principles and issues. 7th ed. Belmont: Thomson Brooks/Cole.

DeBusk RM, Fogarty CP, Ordovas JM and Kornman KS. 2005. Nutritional genomics in practice: Where do we begin? Journal of the American Dietetic Association, 105: 589-598.

DeBusk R. 2008. Assessment: nutritional genomics, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 364-379.

Department of Health. 2007. South Africa Demographic and Health Survey 2003, Full Report. Pretoria: Department of Health.

Devereux RB, Wachtell K, Gerdts E, Boman K, Nieminen MS, Papademetriou V, Rokkedal J, Harris K, Aurup P and Dahlöf B. 2004. Prognostic significance of left ventricular mass change during treatment of hypertension. Journal of the American Medical Association, 292 (19): 2350-2356.

Du Cailar G, Fesler P, Ribstein J and Mimran A. 2010. Dietary Sodium, Aldosterone, and Left Ventricular Mass Changes During Long-Term Inhibition of the Renin-Angiotensin System. Hypertension, 56: 865-870. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/865 [Accessed November 9th, 2010].

Eckel RH, Grundy SM and Zimmet PZ. 2005. The metabolic syndrome. Lancet, 365 (9468): 1415-1428.

Ehret GB, Morrison AC, O'Connor AA, Grove ML, Baird L, Schwander K, Weder A, Cooper RS, Rao DC, Hunt SC, Boerwinkle E and Chakravarti A. 2008. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. European Journal of Human Genetics, 16: 1507–1511.

El-Bokl MA, Senousy BE, El-Karmouty KZ, Mohammed IE, Mohammed SM, Shabana SS and Shalaby H. 2009. Spot urinary sodium for assessing dietary sodium restriction in cirrhotic ascites. World Journal of Gastroenterology, 15(29): 3631-3635.

Fagot-Campagna A, Balkau B, Simon D, Ducimetière P and Eschwège E. 1997. Is insulin an independent risk factor for hypertension? The Paris Prospective Study. International Journal of Epidemiology 26 (3): 542–550.

Felder RA, Sanada H, Xu J, Yu P-Y, Wang Z, Watanabe H, Asico LD, Wang W, Zheng S, Yamaguchi I, Williams SM, Gainer J, Brown NJ, Hazen-Martin D, Wong L-JC, Robillard JE, Carey RM, Eisner GM and Jose PA. 2002. G protein-coupled receptor kinase 4 gene variants in human essential hypertension. Proceedings of the National Academy of Sciences of the United States of America, 99:3872-3877.

Ferguson LR. 2009. Nutrigenomics approaches to functional foods. Journal of the American Dietetic Association, 109: 452-458.

Flack JM, Sica DA, Bakris G, Brown AL, Ferdinand KC, Grimm RH(Jr), Hall WD, Jones WE, Kountz DS, Lea JP, Nasser S, Nesbitt SD, Saunders E, Scisney-Matlock M, Jamerson KA, on behalf of the International Society on Hypertension in Blacks. 2010. Management of High Blood Pressure in Blacks An Update of the International Society on Hypertension in Blacks Consensus Statement. Hypertension, 56: 780-800. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/780 [Accessed November 9th, 2010].

Flegal KM and Graubard BI. 2009. Estimates of excess deaths associated with body mass index and other anthropometric variables. American Journal of Clinical Nutrition, 89:1213–1219.

Food Standards Agency. 2008. Dietary Sodium Levels Surveys. [Online].
Available from: http://www.food.gov.uk/science/dietarysurveys/urinary [Accessed March 4th, 2011].

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC and Curhan GC. 2007. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension, 49: 1063-1069.

Freitas SRS, Cabello PH, Moura-Neto RS, Dolinsky LC, Lima AB, Barros M, Bittencourt I and Cordovil IL. 2007. Analysis of renin-angiotensin-aldosterone system gene polymorphisms in resistant hypertension. Brazilian Journal of Medical and Biological Research, 40: 309-316.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute. 2005. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation, 112: 2735-2752

Gallagher ML. 2008. The nutrients and their metabolism, In Krause's food and nutrition therapy. Ed. by Mahan KL and Escott-Stump S. 12th ed. Philadelphia: W.B. Saunders Company: 39-143.

Gaw A, Murphy MJ, Cowan RA, O'Reilly DStJ, Steward MJ and Shepherd J. 2008. Clinical Biochemistry. 4th ed. Edinburgh: Churchill Livingstone Elsevier.

Goedecke JH, Jennings CL and Lambert EV. 2006. Obesity in South Africa, In Chronic diseases of lifestyle: 1995-2005. Technical report. Ed. by Steyn K, Fourie J and Temple N. Cape Town: South African Medical Research Council: 65-73.

Groenewald AJ, Van Wyk HJ, Walsh CM, Van der Merwe LJ and Van Zyl S. Staging and haematological abnormalities of HIV-infected persons in the rural Free State Province of South Africa. African Journal of Primary Health Care and Family Medicine [Online].
Available from: http://www.phcfm.org/index.php/phcfm/article/view/222/249 [Accessed November 8th , 2011].

Hagberg JM, Park J-J and Brown MD. 2000. The role of exercise training in the treatment of hypertension – an update. Sports Medicine, 30 (3): 193-206.

He FJ and MacGregor GA. 2001. Beneficial effects of potassium. British Medical Journal, 323: 497–501.

He FJ and MacGregor GA. 2007. Dietary salt, high blood pressure and other harmful effects on health, In Reducing salt in foods. Ed. by Kilcast D and Angus F. Cambridge: Woodhead Publishing Limited: 18-54.

He J and Whelton PK. 1999. Elevated systolic blood pressure and risk of cardiovascular and renal disease: Overview of evidence from observational epidemiologic studies and randomized controlled trials. American Heart Journal, 138: S211-S219.

Henderson SO, Haiman CA and Mack W. 2004. Multiple polymorphisms in the renin-angiotensin-aldosterone system (ACE,CYP11B2, AGTR1) and their contribution to hypertension in African Americans and Latinos in the multi-ethnic cohort. American Journal of the Medical Sciences, 328(5): 266–273.

Hollis BW. 2008. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. The American Journal of Clinical Nutrition, 88 (2): 507S-510S.

Hsueh WA, Buchanan TA. 1994. Obesity and hypertension. Endocrinology and Metabolism Clinics of North America, 23:405–427

Hu G, Jousilahti P, Antikainen R, Katzmarzyk PT and Tuomilehto J. 2010. Joint effects of physical activity, body mass index, waist circumference, and waist-to-hip ratio on the risk of heart failure, Circulation, 121:237-244.

Institute of Medicine of the National Academies (IOM). 2010. Dietary Reference Intakes for Calcium and Vitamin D. Ed by Ross AC, Taylor L, Yaktine AL and Del Valle HB. [Online]. Available from: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Report-Brief.aspx [Accessed December 15th, 2010].

Institute of Medicine of the National Academies (IOM). 2004. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. [Online]. Available from: http://iom.edu/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx [Accessed March 4th, 2011].

Intersalt Cooperative Research Group (INTERSALT). 1988. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. British Medical Journal, (297):319-328.

Ilich JZ, Blanuša M, Orlić ZC, Orct T and Kostial K. 2009. Comparison of calcium, magnesium, sodium, potassium, zinc and creatinine concentration in 24-h and spot urine samples in women. Clinical Chemistry and Laboratory Medicine, 47 (2): 216-221.

Jain S, Tang X, Narayanan CS, Agarwal Y, Peterson SM, Brown CD, Ott J and Kumar A. 2002. Angiotensinogen gene polymorphism at _217 affects basal promoter activity and is associated with hypertension in African-Americans. The Journal of Biological Chemistry, 277, 39: 36889–36896.

Johnson RJ, Herrera-Acosta J, Schreiner GF and Rodriquez-Iturbe B. 2002. Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. The New England Journal of Medicine, 346 (12): 913-923.

Judd SE, Nanes MS, Ziegler TR, Wilson PWF and Tangpricha V. 2008. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. American Journal of Clinical Nutrition, 87: 136-141.

Jung O, Bickel M, Ditting T, Rickerts V, Welk T, Helm EB, Staszewski S and Geiger H. 2004. Hypertension in HIV-1-infected patients and its impact on renal and cardiovascular integrity. Nephrology Dialysis Transplantation, 19: 2250–2258.

Katulanda P, Jayawardena MAR, Sheriff MHR and Matthews DR. 2010. Derivation of anthropometric cut-off levels to define CVD risk in Sri Lankan adults. British Journal of Nutrition, [Online].
Available from: http://journals.cambridge.org.proxy.lib.fsu.edu/download.php?file=%2FBJN%2FS0007114510004563a.pdf&code=0dce0c0f55f90b94a69d566e33aeaf5e [Accessed February 11th, 2011].

Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL, Ravussin E, Ryan DH and Bouchard C. 2011. Ethnic-Specific BMI and Waist Circumference Thresholds. Obesity, 19: 1272–1278.

Kawano Y, Abe H, Kojima S, Takishita S and Matsuoka H. 2004. Effects of repeated alcohol intake on blood pressure and sodium balance in Japanese males with hypertension. Hypertension Research, 27: 167-172.

Kim C-H, Park HS, Park M, Kim H and Kim C. 2011. Optimal cutoffs of percentage body fat for predicting cardiovascular disease risk factors in Korean adults. The American Journal of Clinical Nutrition, 94: 34-39.

Kolbe-Alexander TL, Buckmaster C, Nossel C, Dreyer L, Bull F, Noakes TD and Lambert EV. 2008. Chronic disease risk factors, healthy days and medical claims in South African employees presenting for health risk screening. BMC Public Health, [Online].
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2475536/pdf/1471-2458-8-228.pdf [Accessed February 22nd, 2011].

Kolbe-Alexander TL, Lambert EV and Charlton KE. 2006. Effectiveness of a community based low intensity exercise program for older adults. Journal of Nutrition Health and Aging, 10(1):21-9.

Kotchen TA. Insulin resistance and hypertension. 1999. Insulin resistance and hypertension, In Hypertension and the kidney, Ed. by Wilcox CS, In Atlas of diseases of the kidney, vol. 3. Ed. by Schrier RW, [Online].
Available from: http://www.kidneyatlas.org/book3/adk3-05.QXD.pdf [Accessed November 20th,2011].

Kruger HS, Puoane T, Senekal M and Van der Merwe M-T. 2005. Obesity in South Africa: challenges for government and health professionals. Public Health Nutrition, 8 (5): 491-500

Kruger HS, Venter CS, Vorster HH and Margetts BM. 2002. Physical inactivity is the major determinant of obesity in black women in the North West province, South Africa: the THUSA study. Nutrition, 18(5): 422-427.

Kulkarni S, O'Farrell I, Erasi M and Kochar MS. 1998. Stress and hypertension. Wisconsin Medical Journal, 11: 34-38.

Lambert EV, Bohlmann I and Kolbe-Alexander T. 2001. Be active – Physical activity for health in South Africa. South African Journal of Clinical Nutrition, 14 (3): S12-S16.

Lanham-New SA, Buttriss JL, Miles LM, Ashwell M, Berry JL, Boucher BJ, Cashman KD, Cooper C, Darling AL, Francis RM, Fraser WD, De Groot CPGM, Hypp□nen E, Kiely M, Lamberg-Allardt C, Macdonald HM, Martineau AR, Masud T, Mavroeidi A, Nowson C, Prentice A, Stone EM, Reddy S, Vieth R and Williams CM. 2011. Workshop Report: Proceedings of the Rank Forum on Vitamin D. British Journal of Nutrition, 105: 144–156.

Lee Y-S and Levy SS. 2011. Gender and income associations in physical activity and blood pressure among older adults. Journal of Physical Activity and Health, 8: 1 -9.

Lee CM, Huxley RR, Wildman RP and Woodward M. 2008. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of Clinical Epidemiology, 61: 646-653.

Leedy PD and Ormrod JE. 2005. Practical Research. 8th ed. New Jersey: Prentice Hall.

Leiba A, Vald A, Peleg E, Shamiss A, Grossman E. 2005. Does dietary recall adequately assess sodium, potassium, and calcium intake in hypertensive patients? Nutrition, 21(4):462-466.

Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W and Kong J. 2004. Vitamin D: a negative endocrine regulator of the rennin-angiotensin system and blood pressure. The Journal of Steroid Biochemistry and Molecular Biology [Online], 89-90: 387-392.

Lind L, Berne C and Lithell H. 1995. Prevalence of insulin resistance in essential hypertension. Journal of Hypertension, 13(12):1457-1462.

Lindhorst J, Alexander N, Blignaut J and Rayner B. 2007. Differences in hypertension between blacks and whites: an overview. Cardiovascular Journal of Africa, 18 (1): 241-247.

Lips, P. 2010. Worldwide status of vitamin D nutrition. Journal of Steroid Biochemistry and Molecular Biology, 121:297-300.

Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell U-B and Leon DA. 1996. Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. British Medical Journal, 312:406-410.

Levine DA, Calhoun DA, Prineas RJ, Cushman M, Howard VJ and Howard G. 2011. Moderate waist circumference and hypertension prevalence: the REGARDS study. American Journal of Hypertension [Online]. Available from: http://www.nature.com.proxy.lib.fsu.edu/ajh/journal/vaop/ncurrent/pdf/ajh2010258a.pdf
[Accessed February 4th, 2011].

Lohmueller KE, Wong LJ, Mauney MM, Jiang L, Felder RA, Jose PA and Williams SM. 2006. Patterns of genetic variation in the hypertension candidate gene GRK4: ethnic variation and haplotype structure. Annals of Human Genetics, 70:27-41.

Loney T, Standage M, Thompson D, Sebire SJ and Cumming S. 2011. Self-Report vs. Objectively Assessed Physical Activity: Which Is Right for Public Health? Journal of Physical Activity and Health, 8: 62 -70.

Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF and Yetley EA. 2008. Serum 25-hydroxyvitamin D status of the US population: 1988–1994 compared with 2000–2004. The American Journal of Clinical Nutrition, 88: 1519 - 1527.

Love P and Sayed N. 2001. Eat plenty of vegetables and fruits every day. The South African Journal of Clinical Nutrition, 14 (3): S24-S32.

Markovic D, Tang X, Guruju M, Levenstien MA, Hoh J, Kumar A and Ott J. 2005. Association of angiotensinogen gene polymorphisms with essential hypertension in African-Americans and Caucasians. Human Heredity, 60: 89-96.

Mashele N, Van Rooyen JM, Malan L and Potgieter JC. 2010. Cardiovascular function and psychological distress in urbanized black South Africans: the SABPA study. Cardiovascular Journal of Africa, 21(4): 206-211.

McCaffery JM, Papandonatos GD, Bond DS, Lyons MJ and Wing RR. 2009. Gene X environment interaction of vigorous exercise and body mass index among male Vietnam-era twins. American Journal of Clinical Nutrition, 89: 1011-1018.

McCarron DA, Geerling JC, Kazaks AG, and Stern JS. 2009. Can Dietary Sodium Intake Be Modified by Public Policy? Clinical Journal of the American Society of Nephrology, 4: 1878–1882 [Online].
Available from: http://cjasn.asnjournals.org/content/4/11/1878.full.pdf+html [Accessed March 4th 2011].

Medina-Torne S, Ganesan A, Barahona I and Crum-Cianflone NF. 2011. Hypertension is common among HIV-infected persons, but not associated with HAART. Journal of the International Association of Physicians in AIDS Care [Online].
Available from:
http://jia.sagepub.com.proxy.lib.fsu.edu/content/early/2011/08/26/1545109711418361.full.pdf+html [Accessed November 4th, 2011].

Meijs MFL, Bots ML, Vonken E-JA, Cramer M-JM, Melman PG, Velthuis BK, van der Graaf Y, Mali WPThM and Doevendans PA. 2007. Rationale and design of the SMART Heart study. Netherlands Heart Journal, 15 (9): 295-298).

Miyashita M, Burns SF and Stensel DJ. 2008. Accumulating short bouts of brisk walking reduces postprandial plasma triacylglycerol concentrations and resting blood pressure in healthy young men. American Journal of Clinical Nutrition, 88: 1225-1231.

Mollentze WF, Moore AJ, Steyn AF, Joubert G, Steyn K, Oosthuizen GM and Weich DJV. 1995. Coronary heart disease risk factors in a rural and urban Orange Free State black population. South African Medical Journal; 85: 90-96.

National Center for Health Statistics. 2011. Health, United States, 2010: With Special Feature on Death and Dying. [Online].
Available from: http://www.cdc.gov/nchs/data/hus/hus10.pdf#066 [Accessed March 4th , 2011].

National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program (NIH). 2004. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. [Online].
Available from: http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf [Accessed February 27th, 2009].

Nature [Online].
Available from: www.nature.com [Accessed June 9th, 2009].

Nelms M, Sucher K, Lacey K and Roth SL. 2011. Nutrition therapy and pathophysiology. 2nd ed. United States of America: Wadsworth Cengage Learning.

Neter JE, Stam BE, Kok FJ, Grobbee DE and Geleijnse JM. 2003. Influence of weight reduction on blood pressure: A meta-analysis of randomized controlled trials. Hypertension, 42: 878-884.

Nicod J, Bruhin D, Auer L, Vogt B, Frey FJ and Ferrari P. 2003. A biallelic gene polymorphism of CYP11B2 predicts increased aldosterone to renin ratio in selected hypertensive patients. The Journal of Clinical Endocrinology and Metabolism, 88: 2495-2500.

Norat T, Bowman R, Luben R, Welch A, Khaw KT, Wareham N and Bingham S. 2008. Blood pressure and interactions between the angiotensin polymorphism AGT M235T and sodium intake: a cross-sectional population study. American Journal of Clinical Nutrition, 88: 392-397.

Nordstrand N, Gjevestad E, N-DinhK, Hofso D, Roislien J, Saltvedt E, Os I and Hjelmesaeth J. 2011. The relationship between various measures of obesity and arterial stiffness in morbidly obese patients. BMC Cardiovascular Disorders, 11:7 . [Online].
Available from: http://www.biomedcentral.com/1471-2261/11/7 [Accessed February 11th, 2011].

Norman R, Gaziano T, Laubscher R, Steyn K, Bradshaw D and the South African Comparative Risk Assessment Collaborating Group. 2007. Estimating the burden of disease attributable to high blood pressure in South Africa in 2000. South African Medical Journal, 97 (8): 692-698.

Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, Snapinn S, Harris KE, Aurup P, Edelman JM, Wedel H, Lindholm LH and Dahlöf B. 2004. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. Journal of the American Medical Association, 292(19): 2343-2349.

Opie LH and Seedat YK. 2005. Hypertension in Sub-Saharan African populations. Circulation, 112: 3562-3568.

Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA and Ray CA. 2004. Exercise and hypertension. Position stand of the American College of Sports Medicine. Medicine and Science in Sports and Exercise, 36 (3): 533-553.

Pezzoli N, Silvy M, Woronko A, Le Treut T, Lévy-Mozziconnaci A, Reviron D, Gabert J and Picard C. 2007. Quantification of mixed chimerism by real time PCR on whole blood-impregnated FTA cards. Leukemia Research, 31: 1175-1183.

Poopedi MA, Norris SA and Pettifor JM. 2011. Factors influencing the vitamin D status of 10-year-old urban South African children. Public Health Nutrition: 14(2): 334–339.

Popkin BM. 1994. The nutrition transition in low-income countries: an emerging crisis. Nutrition Reviews, 52(9): 285-298.

Poudyala H, Panchalb SK, Diwana V and Brown L. 2011. Omega-3 fatty acids and metabolic syndrome: Effects and emerging mechanisms of action. Progress in Lipid Research, 50(4): 372-387.

Prasad P, Kumar KMP, Ammini AC, Gupta A, Gupta R and Thelma BK. 2008. Association of dopaminergic pathway gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. BMC Genetics [Online], 9:26.
Available from: http://www.biomedcentral.com/1471-2156/9/26 [Accessed January 20th, 2009].

Pratt JH, Ambrosius WT, Tewksbury DA, Wagner MA, Zhou L and Hanna MP. 1998. Serum angiotensinogen concentration in relation to gonadal hormones, body size, and genotype in growing young people. Hypertension, 32: 875-879.

Prospective Studies Collaboration. 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. The Lancet, 360: 1903-1913.

Rammos G, Tseke P and Ziakka S. 2008. Vitamin D, the rennin-angiotensin system, and insulin resistance. International Urology and Nephrology [Online].
Available from: http://www.springerlink.com.proxy.lib.fsu.edu/content/ap8281748l28474p/fulltext.pdf [Accessed May 1st , 2011].

Rajan S, Ramu P, Umamaheswaran G and Adithan C. 2010. Association of aldosterone synthase (CYP11B2 C-344T) gene polymorphism and susceptibility to essential hypertension in a south Indian Tamil population. Indian Journal of Medical Research, 132: 379-385.

Reis JP, Michos ED, Von Műhlen D and Miller ER. 2008. Differences in vitamin D status as a possible contributor to the racial disparity in peripheral arterial disease. The American Journal of Clinical Nutrition, 88: 1469 –1477.

Reyes L and Manalich R. 2005. Long-term consequences of low birth weight. Kidney International, 68 (Suppl 97): S107–S111.

Ricciardi R, Metter EJ, Cavanaugh EW, Ghambaryan A and Talbot LA. 2009. Predicting cardiovascular risk using measures of regional and total body fat. Applied Nursing Research, 22: 2-9.

Rowlett R. 2005. How many? A dictionary of units of measurement. [Online].
Available from: http://www.unc.edu/~rowlett/units/index.html

Rossi GP, Belfiore A, Bernini G, Fabris B, Caridi G, Ferri C, Giacchetti G, Letizia C, Maccario M, Mannelli M, Palumbo G, Patalano A, Rizzoni D, Rossi E, Pessina AC, Mantero F and the Primary Aldosteronism Prevalence in Hypertension Study Investigators. 2008. Body mass index predicts plasma aldosterone concentrations in overweight-obese primary hypertensive patients. The Journal of Clinical Endocrinology and Metabolism, 93:2566 –2571.

Russ AP, Maerz W, Ruzicka V, Stein U and GroB W. 1993. Rapid detection of the hypertension-associated Met235—Thr allele of the human angiotensinogen gene. Human Molecular Genetics, 2 (5): 609-610.

Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N and Lin P-H. 2001. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. The New England Journal of Medicine, 344 (1): 3-10.

Sanada H, Yatabe J, Midorikawa S, Hashimoto S, Watanabe T, Moore JH. Ritchie MD, Williams SM, Pezzullo JC, Sasaki M, Eisner GM, Jose PA and Felder RA. 2006. Single-nucleotide polymorphisms for diagnosis of salt-sensitive hypertension. Clinical Chemistry, 52 (3): 352-360.

Sayeed MA, Mahtab H, Latif ZA, Khanam PA, Ahsan KA, Banu A and Azad Khan AK. 2003. Waist to height ratio is a better obesity index than body mass index and waist-to-hip ratio for predicting diabetes, hypertension and lipidemia. Bangladesh Medical Research Council Bulletin, 29 (1):1-10.

Schatzkin A, Kipnis V, Carroll RJ, Midthune D, Subar AF, Bingham S, Schoeller DA, Troiano RP and Freedman LS. 2003. A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. International Journal of Epidemiology,32:1054–1062.

Schreuder MF and Nauta J. 2007. Prenatal programming of nephron number and blood pressure. Kidney International, 72: 265-268.

Schutte AE, Huisman HW, Van Rooyen JM, Schutte R, Malan L, Reimann M, De Ridder JH,Van der Merwe A, Schwarz PEH and Malan NT. 2008. Should obesity be blamed for the high prevalence rates of hypertension in black South African women? Journal of Human Hypertension, 22(8):528-536.

Seedat YK, Croasdale MA, Milne FJ, Opie LH, Pinkney-Atkinson VJ, Rayner BL and Veriava Y. 2006. South African hypertension guideline 2006. South African Medical Journal, 96 (4): 337-362.

Sen S, Nesse R, Sheng L, Stoltenberg SF, Gleiberman L, Burmeister M and Weder AB. 2005. Association between a dopamine-4 receptor polymorphism and blood pressure. American Journal of Hypertension, 18 (9): 1206-1210.

Shaw A, Fulton L, Davis C and Hogbin M. [n.d.] Using the food guide pyramid: A resource for nutrition educators. US Department of Agriculture, Food, Nutrition and Consumer services, Centre for Nutrition Policy and Promotion. [Online].

Available from: http://www.nal.usda.gov/fnic/Fpyr/guide.pdf [Accessed June 7th 2011].

Smith WCS, Crombie IK, Tavendale RT, Gulland SK and Tunstall-Pedoe HD. 1988. Urinary electrolyte excretion, alcohol consumption, and blood pressure in the Scottish heart health study. British Medical Journal, 297: 329-330.

Smolin LA and Grosvenor MB. 2008. Nutrition: Science and Applications. New Jersey: John Wiley & Sons, Inc.

Snieder H, Harshfield GA and Treiber FA. 2003. Heritability of blood pressure and hemodynamics in African- and European-American Youth. Hypertension, 41:1196-1201.

Sookoian S, Gianotti TF, González CD and Pirola CJ. 2007. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. Journal of Hypertension, 25:5–13.

Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R and Fagard R. 1999. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. Journal of Hypertension, 17: 9–17.

Stamler J, Rose G, Stamler R, Elliott P, Dyer A and Marmot M. 1989. INTERSALT study findings. Public health and medical care implications. Hypertension, 14:570-577.

Stephensen CB, Marquis GS, Kruzich LA, Douglas SD, Aldrovandi GM and Wilson CM. 2006. Vitamin D status in adolescents and young adults with HIV infection. American Journal of Clinical Nutrition, 83:1135-1141.

Stewart S, Libhaber E, Carrington M, Damasceno A, Abbasi H, Hansen C, Wilkinson D and Sliwa K. 2011. The clinical consequences and challenges of hypertension in urban-dwelling black Africans: Insights from the Heart of Soweto Study. International Journal of Cardiology, 146: 22-27.

Steyn K. 2006. Hypertension in South Africa, In Chronic diseases of lifestyle: 1995-2005. Technical report. Ed. by Steyn K, Fourie J and Temple N. Cape Town: South African Medical Research Council: 80-96.

Steyn K, Gaziano TA, Bradshaw D, Laubscher R and Fourie, J. 2001. Hypertension in South African adults: results from the Demographic and Health Survey, 1998. Journal of Hypertension, 19: 1717-1725.

Steyn NP, Labadarios D, Maunder E, Nel, J and Lombard C. 2005. Secondary anthropometric data analysis of the national food consumption survey in South Africa: The double burden. Nutrition, 21: 4–13.

Stowasser M, Taylor PJ, Pimenta E, Ahmed AHA and Gordon RD. 2010. Laboratory Investigation of Primary Aldosteronism. Clinical Biochemist Reviews, 31(2): 39–56 [Online].
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874431/ [Accessed November 4th , 2010].

Sun Q, Shi L, Rimm EB, Giovannucci EL, Hu FB, Manson JE and Rexrode KM. 2011. Vitamin D intake and risk of cardiovascular disease in US men and women. The American Journal of Clinical Nutrition, 94:534-542.

Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H and Hashimoto T. 2002. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. Journal of Human Hypertension 16: 97–103.

Tiago AD, Badenhorst D, Nkeh B, Candy GP, Brooksbank R, Sareli P, Libhaber E, Samani NJ, Woodiwiss AJ and Norton GR. 2003. Impact of renin-angiotensin-aldosterone system gene variants on the severity of hypertension in patients with newly diagnosed hypertension. American Journal of Hypertension, 16:1006–1010.

Tiago AD, Samani NJ, Candy GP, Brooksbank R, Libhaber EN, Sareli P, Woodiwiss AJ and Norton GR. 2002. Angiotensinogen gene promoter region variant modifies body size–ambulatory blood pressure relations in hypertension. Circulation, 106:1483-1487.

Teo Y-Y, Small KS and Kwiatkowski DP. 2010. Methodological challenges of genome-wide association analysis in Africa. Nature Reviews: Genetics, 11: 149-160.

Turban S, Miller ER, Ange B and Appel LJ. 2008. Racial differences in urinary potassium excretion. Journal of the American Society of Nephrology, 19 (7): 1396-1402.

UNICEF. 2010. Statistics South Africa. [Online].
Available from: http://www.unicef.org/infobycountry/southafrica_statistics.html [Accessed November 8th 2011].

United States Department of Agriculture (USDA). 2010. Dietary Guidelines for Americans 2010. [Online].
Available from: http://www.health.gov/dietaryguidelines/dga2010/DietaryGuidelines2010.pdf [Accessed June 21st 2011].

Vaidya A and Forman JP. 2010. Vitamin D and Hypertension: Current Evidence and Future Directions. Hypertension, 56:774-779. [Online].
Available from: http://hyper.ahajournals.org/cgi/content/full/56/5/774 [Accessed November 9th 2010].

Van der Merwe LJ, Van Zyl S, Walsh CM and Van Rooyen FC. 2007. A profile of findings on medical examination of participants in the AHA-study in three rural Free State towns. Oral presentation: Faculty of Health Sciences Forum 2007, Bloemfontein: University of the Free State.

Vasan RS, Beiser A, Seshadri S, Larson MG, Kannel WB, D'Agostino RB and Levy D. 2002. Residual lifetime risk for developing hypertension in middle-aged women and men. Journal of the American Medical Association, 287 (8): 1003-1010

Wallace C, Xue MZ, Caulfield M and Munroe PB. 2007. Genome scans in hypertension, In Handbook of Hypertension, vol. 24 Genetics of hypertension. Ed. by Dominiczak AF and Connell JMC. Edinburgh: Elsevier: 49-66.

Walsh C; Dannhauser A; Van der Merwe L; Van Zyl S; Van Wyk H; Groenewald D; Joubert G and Sebotsa, MLD. 2006. Protocol: Assuring Health for All (AHA) in the Free State. Bloemfontein: University of the Free State.

Walsh CM and Van Rooyen FC. 2008. Obesity and undernutrition – Africa's double burden of disease. Oral presentation: Faculty of Health Sciences Forum 2008, Bloemfontein: University of the Free State

Wang S. 2009. Epedemiology of Vitamin D in health and disease. Nutrition Research Reviews, 22:188-203.

Webb AR and Engelsen. 2006. Calculated Ultraviolet Exposure Levels for a Healthy Vitamin D Status. Photochemistry and Photobiology, 82(6):1697-1703 [Online].
Available from: http://www.bioone.org/doi/full/10.1562/2006-09-01-RA-670 [Accessed March 1st, 2011].

Weston AT, Petosa R and Pate RR. 1997. Validation of an instrument for measurement of physical activity in youth. Medicine and Science in Sports and Exercise, 29 (1): 138-143.

Whelton PK, Je J, Appel LJ, Cutler JA, Havas S, Kotchen TA, Roccella EJ, Stout R, Vallbona C, Winston MC and Karimbakas J. 2002. Primary prevention of Hypertension: Clinical and public health advisory from the National High Blood Pressure Education Program. Journal of the American Medical Association, 288 (15): 1882-1888.

Whelton SP, Chin A, Xin X and He J. 2002. Effect of aerobic exercise on blood pressure: A meta-analysis of randomized, controlled trials. Annals of Internal Medicine, 136: 493-503.

Whiteman M, Gooding KM, Whatmore JL, Ball CI, Mawson D, Skinner K, Tooke JE and Shore AC. 2010. Adiposity is a major determinant of plasma levels of the novel vasodilator hydrogen sulphide. Diabetologia, 53(8):1722-1726.

Winstead ER. 2002. Gene variants linked to inherited forms of hypertension. Genome News Network [Online].
Available from: http://www.genomenewsnetwork.org [Accessed March 13th , 2008].

World Health Organization; International Society of Hypertension Writing Group (WHO/ISH). 2003. WHO/ISH statement on management of hypertension. Journal of Hypertension, 21: 1983–1992.

World Health Organization (WHO). 2002. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. [Online].
Available from: http://whqlibdoc.who.int/hq/2002/WHO_DIL_LAB_99.1_Rev.2.pdf [Accessed July 29th, 2011].

World Health Organization (WHO). 2008. The World Health report 2008. [Online].
Available from: http://www.who.int/whr/2008/whr08_en.pdf [Accessed July 15th, 2009].

World Health Organization (WHO). 2008. WHO STEPS Surveillance: Guide to physical measurements. [Online].
Available from: http://www.who.int/chp/steps/part3_section3.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2008. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008 [Online].
Available from: http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf [Accessed June 7th, 2011].

World Health Organization (WHO). 2011. Global database on body mass index. [Online].
Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed February 21st , 2011].

Wortsman J, Matsuoka LY, Chen TC, Lu Z and Holick MF. 2000. Decreased bioavailability of vitamin D in obesity. The American Journal of Clinical Nutrition, 72: 690 693.

Xin X, He J, Frontini MG, Ogden LG, Motsamai OI and Whelton PK. 2001. Effects of alcohol reduction on blood pressure: A meta-analysis of randomized controlled trials. Hypertension, 38: 1112-1117.

Yatsuya H, Folsom AR, Yamagishi K, North KE, Brancati FL and Stevens J for the ARIC Study Investigators. 2010. Race- and sex-specific associations of obesity measures with ischemic stroke incidence in the Atherosclerosis Risk in Communities (ARIC) Study. Stroke, 41:417-425.

Zafarmand MH, Van der Schouw YT, Grobee DE, De Leeuw PW and Bots ML. 2008. The M235T Polymorphism in the AGT Gene and CHD Risk: Evidence of a Hardy-Weinberg Equilibrium Violation and Publication Bias in a Meta-Analysis. PLoS ONE, 3 (6): e2533 [Online].
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2432037/pdf/pone.0002533.pdf [Accessed January 28th, 2011].

Zhang Z, Hu G, Caballero B, Appel L and Chen L. 2011. Habitual coffee consumption and risk of hypertension: a systematic review and meta-analysis of prospective observational studies. American Journal of Clinical Nutrition, 93: 1212-1219.

APPENDIX A

INFORMATION LETTER TO COMMUNITIES

Assuring Health for All (AHA) in the Free State

In South Africa a large percentage of people do not have enough to eat or do not eat the right foods. This results in diseases such as poor growth and diseases such as overweight, diabetes, heart disease and high blood pressure.

This study will look at the way that people live and what diseases they have in order to help the communities to develop interventions to address these problems and to build (AHA) their communities.

Where and when will the study be conducted?

The study will be conducted in Mangaung at MUCPP during March 2009.

Which population will be included in the study?

All families in the towns will be included. Everyone will be able to choose whether they would like to participate and no one is obliged to take part. In each household that agrees to participate one to two adults and all pre-school children will be included.

What method will be used?

Questionnaires will be used to collect information from household members in an interview with a student or staff member from the University of the Free State. Where participants cannot understand English or Afrikaans, a Sotho-speaking interviewer will be used. Questionnaires will include information about the Household (e.g. how many people and who live in the household), Food Security (e.g. how much food is available in the household), Dietary Intake (what people eat), Physical Activity (how much exercise people do), Health (what illnesses people have) and Knowledge, practices and attitudes about nutrition (what people know about food).

Weight, height and other measurements such as waist and hip circumference will be measured.

A short medical examination will be performed by a medical doctor (blood pressure, temperature etc.).

Blood and urine samples will be collected by medical doctors (including an HIV test). All results will be completely confidential and no one (except one medical doctor) will know the HIV results of individual persons. All persons who would like to know the results of the HIV test will be seen by the one doctor who will give the result to them and refer them for the relevant management if necessary.

Advantages of the project

The Sesotho word "AHA" means "build" and this is the main focus of the project. AHA FS aims to address and develop healthier lifestyles and diets within the communities involved and in contributing to a well-informed, well-nourished healthy and empowered community.

Individual benefits will include an assessment of health and nutritional status of participants. All participants will have access to the results and assessments will be done free of charge. Should health problems be identified, participants will be referred for relevant management thereof and a nutrition education and counselling service will be offered to these participants by the Department of Nutrition and Dietetics at the University of the Free State during the ongoing intervention planned in these areas.

Community benefits will include the identification of community health problems that need to be addressed and will include the initiation of interventions such as vegetable gardening which will benefit the whole community.

BOPHELO BO BOTLE HO BOHLE FREISTATA (FREE STATE)

Batho ba bangata ba South Africa ha bana dijo tse lekaneng ho ja ka lapeng mme ba bang ba bona ha ba je dijo tse nepahetseng. Hona ho etsa hore batho ba qetelle ba tshwerwe ke mafu a kang hose hole hantle le mafu a kang botenya bo boholo, lefu la tswekere, mafu a pelo le phallo e phahameng ea madi.

Diphuputso tsena ditla shebana haholo le mokgwa oo batho ba phelang ka oona le mafu ao ba nang le ona ele ho etsa meralo ya ho fedisa mathata ana le ho haha sechaba se phelang metseng ena.

Diphuputso tsee ditla tshwarelwa kae? Neng?

Diphuputso ditla etswa Mangaung at MUCPP Hlakubele (March) selemong sa 2009.

Ke bo mang batla ameha nakong ya diphuputso?

Malapa ohle toropong tsena tse boletsweng ka hodimo a tla ameha. Motho e mong le emong o tla ba le kgetho ya hore na o rata ho ba karolo ya diphuputso kapa tjhe mme ha ho motho ya tla qobellwa ho nka karolo diphuputsong tsena. Lapeng le leng le le leng le dumelang ho nka karolo, bana ba eso kene sekolo sa mathomo (ba pre school), bahlankana le barwetsana hammoho le batho ba baholo ba tla kenyeletsoa diphuputsong.

Mokgwa o tla sebedisoa ke ofe?

Ho tla ba le dipotso tse tla botswa ho ba lelapa ke baithuti kapa basebetsi ba univesithi ya Foreistata (University of the Free State) ka mokgwa wa dipuisano. Ha motho a sa bue kapa a sa utlwisise Sekgowa kapa se Afrikaans motho ya botsang dipotso e tla ba ya tsebang Sesotho. Dipotso ditla kenyeletsa dipatlisiso ka lelapa (mohlala: ke batho ba bakae ba phelang ka lapeng?), kanetso ya dijo (mohlala: dijo tse teng ka lapeng di kae?), boikwetliso (motho o ikwetlisa hakae?), bophelo (batho bana le mafu afe?) tsebo ka phepo e nepahetseng (batho ba tsebang ka dijo).

Boima ba mele le bolelele le ntho tse kang bophara ba letheka le dithopola (hips) di tla nkuwa ho sebeliswa sekala le ditheipi.
Ngaka e tla ba teng ho hlahloba mafu a kang phallo ya madi, motjheso le amang.

Madi le metshetshe e tla nkuwa ke dingaka bakeng sa ho tla hlahloba mafu a kenyetsang HIV. Sephetho e tla ba lekunutu mme ha ho motho le a mong ntle ho ngaka ya tla tseba sephetho sa tlhahlobo ya HIV sa motho e mong. Motho e mong le emong ya ka ratang ho tseba sephetho sa hae sa tlhahlobo ya HIV o tla bonwa ke ngaka e tla mofa sephetho mme e mo romelle moo a tlang ho fumana thuso e hlokahalang.

Melemo ya diphuputso tsena
AHA ke lentswe la Sesotho leo diphuputso tsena di thehilweng hodima lona. Diphuputso tsa AHA Foreistata di reretswe ho aha maphelo a batho bohle ka ho netefatsa bophelo bo botle le dijo tse nepahetseng setjhabeng ele ho ba le setjhaba se nang le tsebo, se phetseng hantle mme se nang le boikarabello bakeng sa maphelo a sona.

Melemo ho motho ka mong e kenyeletsa tlhahlobo ya bophelo le phepo e nepahetseng ho motho. Motho e mong le e mong o tla ba le kgetho ya ho tseba sephetho sa tlhahlobo mme se tla etswa ho se tefo ya letho. Ha eba ho fumaneha hore ho na le bothata ba lefu le itseng ho motho, motho eo o tla romellwa moo aka fumanang thuso hape o tla fumana thuto ka phepo e nepahetseng le tlhabollo tse tla etswa ke lekala la phepo (Department of Nutrition and Dietetics) la Yuniversithing ya Freistata ka meralo ya bona e tswelang pele e etseditsweng ho haha setjhaba se phelang ditoropong tsena tse tharo.

Melemo ya setjhaba ka kakaretso se phelang diteropong tsena tse tharo e kenyeletsa tsebo e pharaletseng ka mathata a bophelo a teng metseng ya bona a hlokang ho fediswa. Hona ho tla thusa hore ho etswe meralo e nepahetseng ea boiphidiso e kang ho lema meroho e tla thusa setjhaba ka kakeretso.

APPENDIX B

INFORMED CONSENT FORM

Assuring Health for All (AHA) in the Free State

CONSENT TO PARTICIPATE IN RESEARCH

You have been asked to participate in a research study.

You have been informed about the study by …… .

You may contact Prof Corinna Walsh at 083 297 6030 at any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.

If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research. You are also giving permission that some of the same blood can be retained in storage for possible future research related to the present research question.

The research study, including the above information has been verbally described to me. I understand what my involvement in the study means and I voluntarily agree to participate.

_____	_____
Signature of Participant	Date

For children:

_____	_____
Name/ Signature of Child	Date
_____	_____
Signature of Parent	Date

Assuring Health for All (AHA) in the Free State
INFORMATION DOCUMENT

Study title: Assuring Health for All (AHA) in the Free State

Thank you for being willing to help us in this very important project. We are sure that the project will contribute to improving the health of all the people of the Free State.
We, the University of the Free State, Faculty of Health Sciences, are doing research on determining the factors involved in causing disease and disability in the Southern Free State. Research is just the process to learn the answer to a question. In this study we want to learn what factors need to be addressed in health programmes in the Free State. The study involves research and is not part of routine medical care.

Invitation to participate: We are asking/inviting you to participate in this research study, or/and asking for your permission to include your child in this research study.

What is involved in the study: The aim of the project is to get enough information regarding the development of chronic diseases like diabetes, stroke, high blood pressure and heart disease as well as HIV/AIDS to plan appropriate health and nutrition intervention strategies for the people of the Free State. Trompsburg, Philippolis and Springfontein have been chosen as the rural areas and Mangaung as the urban area.

For this study we need households whom we can follow for 12 years. The baseline survey will be done during March 2009 in Mangaung. You will be asked to visit the MUCPP clinic for one day to take the necessary measurements and to complete the questionnaires. After the baseline survey has been completed, we will implement a nutrition intervention in your community to address the problems identified in the baseline survey. This intervention will form part of the service learning interventions of the University. In addition to the services that we will render in the community, we will visit your community again after six years to repeat the measurements.

All the questionnaires will be filled out at MUCPP clinic by students from the University of the Free State. Respondents from the chosen households will be asked to complete the following questionnaires in an interview with the students:

- Socio-demographic and household questionnaire,
- Household food security and food procurement questionnaire,
- Health questionnaire,
- Knowledge, practices and attitudes (KPA) about nutrition questionnaire,
- Diet and physical activity questionnaire.

We will also take some measurements such as weight, height, skinfold thicknesses, blood pressure, blood samples and a urine sample. With your permission we will draw 60ml of blood in adults and this will only be done once. In adults blood and urine samples will be used to determine the following: Full blood count; HbA1c; Glucose; Insulin; Lipogram; Homocysteine; Red cell Folate; Serum Vitamin B12; Fibrinogen; Gamma glutermyl transferases (GGT); Carbohydrate-deficient transferrin (CDT); Ferritin; Uric acid; Creatinin; C-reactive protein; Albumin; Pre-albumin; Transferrin; Retinol-binding protein; TSH; Iodine (urine); Leptin; Tumour Necrosis Factor alpha; Interleuken 6; Melatonin; Brain natriuretic peptide; ACTH; Cortisol; Orexin; Urotensin-11; Endothelin 1; Plasminogen Activation Inhibitor (PAI-1); Adiponectin; Micro-albuminuria (urine); Glucose tolerance (sub-sample); FFA (sub-sample).

A short medical examination will be performed on all participants to identify any serious health problems.

We would like to retain some of the same blood in storage for possible future research related to the present research question. Blood samples will be stored anonymously for a period of five years at the Department of Chemical Pathology at the University of the Free State. If you are unhappy to have your blood stored for future research, it will be disposed of at the end of the study, once the sample storage and record-keeping requirements of good research practice have been met.

It is very important that we gather quality data and knowledge. Because HIV/AIDS is a devastating illness and affects almost all aspects of health, it is necessary to know if HIV is absent before we analyse the data. It will be to your benefit as well as the benefit of the research to determine your HIV status. Therefore we will also ask permission to draw blood to determine your HIV status and ask questions about your HIV status which you are allowed not to answer. You will be asked to sign a separate consent form for the HIV test. You will receive pre- and post-testing counselling by a medical practitioner and all results will be kept strictly confidential in accordance with the guidelines of the Health Professions Council of South Africa (HPCSA). You will only be informed of your HIV result if you choose to be. All respondents who choose to be informed of the results will be informed by a medical doctor and referred for relevant management. None of the researchers (other than one doctor) will know the HIV status of any participants.

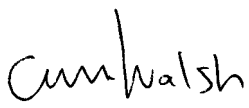
Blood tests will involve an analysis of the genetic composition of red blood cells and are aimed at increasing the understanding of the causes and behaviour of chronic diseases of lifestyle such as obesity, diabetes and heart disease. Genes are what you inherit from your parents. They are found in every part of your body and therefore they will be present in the blood that we draw. The findings may benefit/eventually benefit others in terms of prevention or treatment of diseases. You are free to refuse consent and you do not have to give reasons for doing so. The following arrangements have been made to ensure privacy and confidentiality of your genetic information: All blood samples will be stored anonymously. Your genetic material and information will be used in an identifiable form. The research may reveal information of potential importance to the future health of an identifiable or potentially identifiable participant or the participant's offspring.

Researchers will endeavour to provide information about the outcome of the research. If research generates information about you which may be of relevance to the health of other family members, your consent will be sought before offering to disclose such information to the family members concerned. Your material and information will not be released for other uses without consent, unless required by law.

Risks of being involved in the study: Medical doctors and registered nurses will be responsible for safely drawing blood samples. In the unlikely event that an adverse event results from the procedure, you will be compensated for any expenses.
Benefits of being in the study: By participating in the study you will help us to develop health and nutrition strategies that will benefit the people of the Free State. You will be given pertinent information on the study while involved in the project and after the results are available.

Participation is voluntary, and refusal to participate will involve no penalty or loss of benefits to which you are entitled; you may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled.
Confidentiality: Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research and the Medicines Control Council. If results are published, this may lead to individual/cohort identification.

Kind regards



PROF CORINNA WALSH
Contact details: 083 297 6030 / 051 4013818(W)

Assuring Health for All (AHA) in the Free State

TOESTEMMING TOT DEELNAME AAN NAVORSING

U is versoek om aan ‘n navorsingstudie deel te neem.
U is oor die studie ingelig deur …….. .

U kan Prof Corinna Walsh enige tyd kontak by 083 297 6030 indien u vrae oor die navorsing het of as gevolg van die navorsing beseer is.

U kan die Sekretariaat van die Etiekkomitee van die Fakulteit Gesondheidsweteskappe, UV by telefoonnommer (051) 4052812 kontak indien u enige vrae het oor u regte as ‘n proefpersoon.

U deelname aan hierdie navorsing is vrywillig, en u sal nie gepenaliseer word of voordele verbeur as u weier om deel te neem of besluit om deelname te staak nie.

As u instem om deel te neem, sal ‘n ondertekende kopie van hierdie dokument sowel as die deelnemerinligtingsblad, wat ‘n geskrewe opsomming van die navorsing is, aan u gegee word .

Die navorsingstudie, insluitend die bogenoemde inligting is verbaal aan my beskryf. Ek begryp wat my betrokkenheid by die studie beteken en ek stem vrywillig in om deel te neem.

_____ _____
Handtekening van deelnemer Datum

Vir kinders:

_____ _____
Naam/ Handtekening van kind Datum

_____ _____
Handtekening van ouer Datum

INLIGTINGSDOKUMENT

Studietitel: Assuring Health for All (AHA) in the Free State

Dankie dat u bereid is om ons te help met hierdie baie belangrike projek. Ons is seker dat die projek sal bydra om die gesondheid van alle persone in die Vrystaat te verbeter. Ons, die Universiteit van die Vrystaat, Fakulteit Gesondheidswetenskappe, doen navorsing oor die faktore wat betrokke is by die oorsake van siekte in die Vrystaat. Navorsing is slegs die proses waardeur die antwoord op 'n vraagstuk verkry word. In hierdie studie wil ons leer watter faktore aangespreek moet word in gesondheidsprogramme in die Vrystaat. Die studie behels navorsing en is nie deel van roetine mediese behandeling nie.

Uitnodiging om deel te neem: Ons versoek/nooi u uit om aan 'n navorsingstudie deel te neem of/en vra u toestemming om u kind by die navorsingstudie in te sluit.

Wat behels die studie – Die doelwit van hierdie projek is om genoeg inligting in te samel oor die ontwikkeling van chroniese siektes soos diabetes, beroerte, hoë bloeddruk en hartsiektes sowel as MIV/VIGS om toepaslike gesondheids- en voedingintervensie strategieë te kan beplan vir die mense van die Vrystaat. Trompsburg, Philippolis en Springfontein is as die plattelandse areas gekies en Mangaung as die stedelike area.

Vir die studie benodig ons huishoudings wat ons vir 12 jaar kan opvolg. Die basislyn opname sal in Mangaung gedoen word tydens Maart 2009. U sal gevra word om die MUCPP kliniek vir een dag te besoek waar die nodige metings gedoen sal word en vraelyste voltooi sal word. Nadat die basislynopname voltooi is, sal ons 'n voedingintervensieprogram in u area implementeer om die probleme wat in die basislynopname identifiseer is aan te spreek. Hierdie intervensie vorm deel van die diensleer intervensies van die universiteit. Tesame met die intervensie sal ons ook die gemeenskap elke drie jaar tot ses jaar besoek om die metings te herhaal.

Al die vraelyste sal by MUCPP voltooi word deur studente van die Universiteit van die Vrystaat. Respondente van die gekose huishoudings sal gevra word om die volgende vraelyste te voltooi in 'n onderhoud met die student:

- Sosio-demografiese en huishoudelike vraelys,
- Huisehoudelike voedselsekuriteit en voedselverkrygings vraelys,
- Gesondheids vraelys,
- Kennis, praktyke en houding teenoor voeding vraelys,
- Dieet en fisiese aktiwiteit vraelys.

Ons sal ook sekere metings soos gewig, lengte, velvoudiktes, bloeddruk, bloed monsters en uriene monsters neem. Met u toestemming sal ons in volwassenes 60ml bloed trek en dit sal slegs een keer geskied. In volwassenes sal bloed en uriene monsters gebruik word om die volgende te bepaal: Volbloedtelling; HbA1c; Glukose; Insulien; Lipogram; Homositeïen; Rooisel Folaat; Serum Vitamien B12; Fibrinogeen; Gamma glutermyl transferases (GGT); Carbohydrate-deficient transferrin (CDT); Ferritin; Uriensuur; Kreatinien; C-reactiewe proteïen; Albumien; Pre-albumien; Transferrien; Retinol-binding proteïen; TSH; Jodium (uriene); Leptien; Tumor Nekrosis Faktor alfa; Interleuken 6; Melatonien; Brain natriuretic peptide; ACTH; Kortisol; Orexin; Urotensien-11; Endothelien 1; Plasminogen Activation Inhibitor (PAI-1); Adiponektien; Mikro-albumienuria (uriene); Glukose toleransie (sub-sample); FFA (sub-sample).

'n Kort mediese ondersoek sal ook gedoen word op sekere lede van die huishouding om ernstige gesondheidsprobleme te identifiseer.

Ons wil graag van die bloed bêre vir moontlike toekomstige navorsing wat verband hou met die huidige navorsingsvrae. Bloed monsters sal anoniem gestoor word vir 'n periode van vyf jaar. As u ongelukkig daaroor voel dat u bloed vir toekomstige navorsing geberg word sal daar aan die einde van die studie daarmee weggedoen word sodra die monsterbergings- en aantekeningsvereistes van goeie navorsingspraktyk nagekom is.

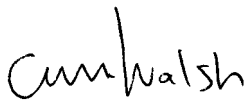
Dit is baie belangrik dat ons inligting van ‘n hoë kwaliteit versamel. Omdat MIV/VIGS ‘n siekte is wat amper alle aspekte van gesondheid beïnvloed, is dit nodig dat ons weet of MIV afwesig is voordat ons die data ontleed. Dit sal tot voordeel van uself en die navorsing strek indien u HIV status bepaal kan word. Dus sal ons toestemming vra om bloed te trek om u MIV status te bepaal en vrae oor HIV vra wat u nie hoef te antwoord indien u nie wil nie. U sal gevra word om ‘n aparte teostemmingsvorm te voltooi vir die HIV toets. U sal voor en na die toets berading ontvang deur ‘n mediese dokter en alle uitslae sal streng vertroulik hanteer word volgens die riglyne van die Health Professions Council of South africa (HPCSA). U sal slegs van u MIV uitslae in kennis gestel word indien u kies om dit te ontvang. Alle respondente wat kies om van hulle uitslae in kennis gestel te word sal deur ‘n mediese dokter in kennis gestel word en verwys word vir die relevante hantering. Die navorsers (met uitsluiting van een dokter) sal nie weet wat u uitslae is nie.

Bloedtoetse sal die analise van die genetiese samestelling van rooibloedselle insluit en is gemik daarop om die oorsake en gevolge van chroniese siektes soos vetsug, diabetes en hartsiektes beter te verstaan. Gene is dit wat u van u ouers erf en word in elke deel van u liggaam aangetref. Daarom sal dit in enige weefsel of bloed wat deur ons verwyder word teenwoordig wees. Die bevindings kan tot ander se voordeel strek met betrekking tot voorkoming en behandeling van die toestand. Dit staan u vry om toestemming te weier en u hoef geen redes daarvoor te verstrek nie. Die volgende reëlings is getref om privaatheid en vertroulikheid van u genetiese inligting te verseker: Alle bloedmonsters sal anoniem geberg word. U genetiese materiaal en inligting sal in ‘n identifiseerbarevorm gebruik word. Die navorsing mag inligting openbaar wat van potensiële belang mag wees vir die toekomstige gesondheid van ‘n identifiseerbare of potensieel identifiseerbare deelnemer of die deelnemer se nakomelinge.

Navorsers sal poog om inligting oor die uitkoms van die navorsing te verskaf. As navorsing inligting aan die lig bring wat van belang mag wees vir die gesondheid van u familielede, sal u toestemming verkry word voordat sodanige inligting aan die betrokke familielede bekend gemaak word. U bloed en inligting sal nie sonder toestemming vir ander gebruike beskikbaar gestel word nie tensy vereis deur die wet.

Risikos van deelname aan die studie: Mediese dokters of geregistreerde verpleegkundiges sal verantwoordelik wees vir die veilige neem van bloedmonsters. In die onwaarskynlike geval dat ‘n negatiewe gevolg ontstaan as gevolg van die prosedure sal u vir enige onkostes vergoed word.
Voordele van deelname aan die studie: Deur aan die studie deel te neem sal u ons help om gesondheids- en voedingstrategieë te ontwikkel wat die mense van die Vrystaat sal baat. Die proefpersoon sal pertinente inligting oor die studie ontvang tydens betrokkenheid by die projek en agterna wanneer die resultate beskikbaar is.
Deelname is vrywillig, en weiering om deel te neem sal geen boete of verlies van voordele waarop die deelnemer andersins geregtig is behels nie; die proefpersoon kan te eniger tyd aan deelname onttrek sonder boete of verlies van voordele waarop die proefpersoon andersins geregtig is.
Vertroulikheid: Daar sal gepoog word om persoonlike inligting vertroulik te hou. Volkome vertroulikheid kan nie gewaarborg word nie. Persoonlike inligting kan bekend gemaak word as die wet dit vereis. Organisasies wat u navorsingsrekords mag ondersoek en/of kopieer vir kwaliteitsversekering en data-analise sluit groepe soos die Etiekkomitee vir Mediese Navorsing en die Medisynebeheerraad in. As resultate gepubliseer word kan dit lei tot individuele/groepsidentifikasie.

Vriendelike groete



Prof CORINNA WALSH
Kontakbesonderhede: 083 297 6030 / 051 4013818(W)

Assuring Health for All (AHA) in the Free State
(Tshepiso ya Bophelo ho bohle ba Foreisitata)

TUMELLO YA HO NKA KAROLO DIPATLISISONG

O kopuwe ho nka karolo dipatlisisong.

O tsebisitswe ka dipatlisiso tsena ke .....

O ka ikopanya le Prof Corinna Walsh ho 083 297 6030 nako e nngwe le e nngwe ha o na le dipotso ka dipatlisiso kapa ha o ka wa lematseha ka lebaka la dipatlisiso.

O ka ikopanya le mongodi wa komiti ya Ethics ho Faculty ya Health Sciences, Yunivesithing ya Foreisitata nomorong ya (051) 4052812 ha o ena le dipotso ka ditokelo tsa hao jwalo ka motho ya nkang karolo dipatlisisong.

O nka ka karolo dipatlisisong ka ho ithaopa, ka hoo o ke ke wa ahlolwa kapa wa lahlehelwa ke di letho ha o ka wa hana ho nka karolo kapa wa tlohella ho nka karolo.

Ha ebe o dumela ho nka karolo, o tla fuwa lengolo le tshwanang le lena le saenuweng hammoho le lengolo le fanang ka tlhahiso leseding, eo e leng tlhaloso e ngotsweng ya dipatlisiso tsena.

Ke hlaloseditswe sepheo sa dipatlisiso, hammoho le tlhahiso leseding ena e ka hodimo ka molomo. Ke utlwisisa ho nka karolo ha ka dipatlisisong mme ke dumela ho nka karolo ka ho ithaopa, ntle le ho qobellwa.

_____	_____
Signature ya ya nkang karolo	Letsatsi

Bakeng sa bana

_____	_____
Signature ea ngoana	Letsatsi
_____	_____
Signature ea motsoali	Letsatsi

Assuring Health for All (AHA) in the Free State
(Tshepiso ya Bophelo ho bohle ba Foreisitata)

LENGOLO LA TLHAHISO LESEDING

Sehloho sa dipatlisiso: Assuring Health for All in the Free State

Re leboha ha o dumetse ho re thusa dipatlisisong tsena tse bohlokwa. Re tshepa ha dipatlisiso di tla re thusa ho ntlafatsa maphelo a batho bohle ba Foreisitata.
Rona, re le Yunivesithi ya Foreisitata, Lefapha la tsa Maphelo, re etsa dipatlisiso ho shebana le dintho tse bakang mafu le boqhwala mona Foreisitata e Borwa.
Dipatlisiso ke mokgwa wa ho ithuta karabo ya potso e itseng. Ka dipatlisiso tsena re batla ho ithuta hore na ke dintho dife tse hlokang hore di amuwe ditsamaisong tsa tsa bophelo mona Foreisitata. Tsena ke dipatlisiso feela, mme ha se karolo ya tshebeletso ya tsa bongaka.

Sememo sa ho nka karolo: Re a o kopa/ re a o mema ho nka karolo dipatlisisong tsena, ebile/ kapa re kopa tumello ya hao ho sebedisa ngwana wa hao dipatlisisong tsena.
Dipatlisiso tsena di kenyeleditse eng: Sepheo sa dipatlisiso tsena ke ho fumana tlhahiso leseding e lekaneng mabapi le tswello pele ya mafu a kang diabetes, stroke, high blood pressure le mafu a pelo hammoho le HIV/AIDS hore ho tle ho thalwe mekgwa ya tsamaiso ya tsa bophelo le phepo bakeng sa batho ba Foreisitata. Trompsburg, Phillippolis le Springfontein di kgethilwe jwalo ka dibaka tsa mahaeng mme Mangaung e kgethilwe e le sebaka sa ditoropong tse tla sebediswang dipatlisisong.
Bakeng sa dipatlisiso tsena re hloka ho sebetsa le malapa ohle motseng wa lona bakeng sa dilemo tse 12. Dipatlisiso tsa pele di tla qala ka Hlakubele (March) 2009. O tla kopuwa ho etela MUCPP letsatsi le le leng hore o tle o tsebe ho tlatsa diforomo le hore o methuwe. Kamora dipatlisiso tsena tsa pele, ho kenywa tshebetsong mekgwa ya tlhokomelo mabapi le tsa phepo sebakeng seo o dulang ho sona hore ho tle ho lokisanwe le mathatha a tla be a hlahelletse dipatlisisong tsa pele. Tlhokomelo ena e tla ba karolo ya ho ithuta ho bile ho fanwa ka ditshebeletso ke baithuti ba Yunivesithi. Hammoho le ditshebetso tseo re tla be re fana ka tsona motseng, re tla etela motse wa hao ka mora dilemo tse tharo hoisa ho tse tseletseng hore ho phethwe ho metha.

Diforomo tsohle di tla tlatswa sebakeng seo liphuputso li tla tswareloa teng ke baithuti ba tswang Yunivesithing ya Foreisitata kapa ke bathusi ba kwetlisitsweng ba tswang motseng wa hao. Batho ba nkang karolo ho tswa malapeng a kgethilweng ba tla kopuwa ho tlatsa diforomo tse latelang ho ya ka dipotso tseo ba tla beng ba di botswa ke moithuti kapa mosebeletsi wa setjhaba:

- Dipotso ka maemo a hao le ka lelapa la hao,
- Theko ya dijo le 'food security',
- Tsebo, tshebetso le mekgwa e amanang le phepo,
- Tsela ya ho ja le boikwetliso.

Re tla o metha boima, botelete, botenya ba momeno wa letlalo (skinfold) le kgatello ya madi (blood pressure), o tla nkuwa madi hammoho le metsi. Ho batho ba baholo re tla hula madi a kana ka dimililitara tse 60 hona ho tla etswa hanngwe feela. Ho tla etswa dihlahlobo tsa bophelo ho batho ba itseng ba lelapa ho shebana le mathata a bophelo a tshosetsang. Ka tumello ea hau re tla ntsa madi a ka bang dimelimetara tse mashome a tseletseng (60ml) ho motho e moholo le tse leshome le metso e mehlano, mme hona ho tla etwa hangoe feela. Madi le metsetse e nkuoeng ho batho ba baholo e tla sebediswa ho hlalhoba matsoai le matsooeana a fumanehang ho tsona a kenyeletsang tse latelang: Full blood count; HbA1c; Glucose; Insulin; Lipogram; Homocysteine; Red cell Folic acid; Serum Vitamin B12; Fibrinogen; Gamma glutermyl transferases (GGT); Carbohydrate-deficient transferrin (CDT); Ferritin; Uric acid; Creatinin; C-reactive protein; Albumin; Pre-albumin; Transferrin; Retinol-binding protein; TSH; Iodine (urine); Leptin; Tumour Necrosis Factor alpha; Interleuken 6; Melatonin; Brain natriuretic peptide; ACTH; Cortisol; Orexin; Urotensin-11; Endothelin 1; Plasminogen Activation Inhibitor (PAI-1); Adiponectin; Micro-albuminuria (urine); Glucose tolerance (sub-sample); FFA (sub-sample). Tse ding tsa matsoai le matsoaeana ana di tla hlahlojoa mading le metsetseng ea bana

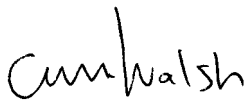
Re ka rata ho boloka a mang a madi hore a tle a tsebe ho sebediswa ka nako e tlang dipatlisisong tse tshwanang le tsena. Madi ana a tla bolokwa kantle le ho ngolwa mabitso bakeng sa dilemo tse hlano. Ha o sa rate ha madi a hao a bolokwa bakeng sa dipatlisiso tse ding, a tla lahlwa ha ho qetwa ka dipatlisiso le hang feela ha a ile a bolokwa hantle e bile dintho tse hlokahalang bakeng sa dipatlisiso di ile tsa fumanwa ka mokgwa o nepahetseng.

Ho bohlokwa haholo hore tlhahiso leseding eo re e fumanang ke e hlwahlwa. Hobane lefu la HIV e le le hlokofatsang haholo, ebile le ama maphelo a rona, ho a hlokahala hore re tsebe hore kokwana-hloko ena e teng kapa tjhe. Ke molemong oa hau le oa mofuputse ho fumana boemo ha hau ba HIV. Ka hona re tla kopa tumello ea ho ntsa madi ho uena ho ea hlahloba boemo ba hau ba HIV mme re tla u botsa lipotso tse ling tse amanang le boemo ba hau ba HIV tseo u sa qobelloeng ho li araba. U ka li araba ka ho rata ha hau. U tla kopuoa ho saena foromo eo u re fang tumello ea ho hlahloba boemo ba hau ba HIV. Ka hoo re tla hula madi le ho o botsa dipotso mabapi le maemo a hao a HIV. O dumelletswe ho se arabe dipotso tsena. Ho tla buisanwa le wena ho tebesitswe maikutlo pele le ka mora dihlahlobo tse tla etswa ke ngaka, mme diphetho tsohle di tla ba sephiri ho ya ka ditaelo tsa Health Professions Council ya South Africa (HPCSA). O tla tsebiswa ka maemo a hao a HIV feela ha o kgetha hore o tsebiswe. Batho bohle ba batlang ho tsebiswa ka sephetho sa dihlahlobo tsa HIV, ba tla tsebiswa ke ngaka, mme ba tla romellwa ho batho ba tla tseba ho ba fa thuso. Babatlisisi ba bang (ka ntle ho dingaka) ba ke ke ba tseba maemo a ba nka karolo a HIV.

Ho hulwa ha madi ho kenyelleditse ho hlahloba ‘genetic composition’ e ho di ‘red blood cells’, mme hona ho tla thusa ho phahamisa kutlwisiso ya dintho tse bakang mafu a kang monono, diabetes le mafu a pelo. Di ‘genes’, ke dintho tseo o di futsang ho tswa ho batswadi ba hao. Di fumanwa dikarolong tsohle tsa mmele kahoo di a fumaneha le ho madi a tla hulwa. Dintlha tse tla fumanwa di tla thusa batho ba bang ka ho thusa ho thibela, kapa ho phekola mafu a fapaneng. O na le tokelo ya ho hana ho nka karolo ebile ha ho hlokahale hore o fane ka lebaka. Ho netefatsa hore dintlha tse tla fumanwang ho wena di dula e le sephiri, ho entswe dintho tsena tse latelang: Madi ohle a tla bolokwa ntle le ho ngolwa mabitso. ‘Genetic material’ ya hao e tla sebediswa ka mokgwa o tsebahalang. Dipatlisiso tsena di ka nna tsa fana ka tlhahiso leseding e bohlokwa bakeng sa bokamoso ba monka karolo kapa ba bana ba hae.

Babatlisisi ba tla leka ho fana ka lesedi mabapi le sephetho sa dipatlisiso. Ha dipatlisiso di ka fana ka dinthla tse leng bohlokwa bakeng sa maphelo a ba bang ba lelapa, tumello e tla kopuwa ho wena pele batho ba amehang ba tsebiswa. Dintlha tsohle tsa hao di ke ke tsa sebediswa nqeng tse ding kantle ho tumello ya hao, kantle le ha ho ka hlokeha hore ho etswe jwalo ho ya ka molao.

Kotsi tse ka bang teng dipatlisisong: Dingaka le baoki ba tla ikarabella ho huleng ha madi ka mokgwa o bolokehileng. Ha ho ka etsahala hore ho be le se o etsahallang se sa lokang o tla lefuwa ditshenyehelo tsa hao tsohle.
Ditholwana tsa ho nka karolo: Ha o nka karolo o tla thusa ho tsweletsa pele metjha ya tsa bophelo le phepo ho thusa batho ba Foreisitata. O tla fuwa tlhahiso leseding e bohlokwa tsamaong ya dipatlisiso le ha sephetho sa dipatlisiso se fumaneha.
O nka karolo ka ho ithaopa, mme ha o hana ho nka karolo o ke ke wa lahlehelwa ke letho; o ka tlohella ho nka karolo nako e nngwe le e nngwe ntle le ho lahlehelwa ke letho.
Sephiri: Ho tla etswa maleba-leba a hore dintlha tsa hao di dule di le lekunutu. O tshepiswa lekunutu ka hohle-hohle. Dintlha tsa hao di phatlalatswa feela ha molao o re jwalo. Mekgatlo e tla hlahloba kapa e tla kopisa dintlha tsa hao ho lekola boleng e kenyeleditse e kang Ethics Committee ya Medical Research hammoho le Medicine Control Council.
Ha diphetho di ka phatlalatswa, hona ho ka lebisa ho tsebiswa ha motho kapa sehlopha.



Ka boikokobetso
Prof Corinna Walsh
Mohala: 083 297 6030 / 051 401 3818

APPENDIX C

PARTICIPATION LETTER

Assuring Health for All (AHA) in the Free State
Participation letter

Dear Participant

Thank you for being willing to help us in this very important project. We are sure that the project will contribute to improving the health of all the people of the Free State.

At the time you receive this letter you would have already been visited by a fieldworker and you have already signed consent to give a blood sample. This letter serves to inform you of the date and time the blood sample and other measurements will be taken at the MUCPP clinic (basement).

IMPORTANT INFORMATION

1. You must be at MUCPP on **……** by 0….h00.
2. You **MUST NOT EAT OR DRINK** anything after ten o'clock of the previous night (10 pm of the night before). This is necessary for the glucose test to be accurate.
3. You **MUST BRING YOUR ID DOCUMENT** with you
4. Bring along a list of people that live in the household with you and their birth dates.
5. You will receive something to eat and drink after the blood sample is taken.
6. After participating in the project, you will receive an amount of R10 to pay for your transport costs.
7. If you are employed, please show this letter to your employer.

Dear Employer

This serves to ask you to give one day's paid leave to…….. in order to allow him/her to attend his appointment with the research team of the Faculty of Health Sciences at the University of the Free State.

Thank you for your cooperation. For any further information please contact Prof Corinna Walsh at 083 297 6030.

Prof C Walsh (project leader)

Assuring Health for All (AHA) in the Free State
Deelname brief

Beste Deelnemer

Dankie dat u bereid is om ons te help met hierdie belangrike projek. Ons is seker dat die projek sal bydra tot die verbetering van gesondheid van al die mense in die Vrystaat.

Teen die tyd wat u hierdie brief ontvang het ‘n veldwerker u al reeds besoek en u het toestemming gegee om deel te neem aan die projek en ‘n bloed monster te gee Met hierdie brief wil ons u graag in kennis stel van die datum en tyd wat die bloed getrek sal word en ander mates geneem sal word by die navorsingseenheid (saal)naaste aan u woning.

BELANGRIKE INLIGTING

1. U moet by MUCPP wees op **……** teen 0….h00.
2. U **MOET NIKS EET OF DRINK** na tien uur die vorige aand (10 pm van die aand voor die toets). Dit is nodig vir die glukose toets om betroubaar te wees.
3. U **MOET U ID DOKUMENT** saam met u kliniek toe bring
4. Bring asseblief ‘n lys van persone wat saam met u in die huis woon saam met hule geboortedatums.
5. U sal ‘n ligte ete en iets om te drink ontvang nadat die bloedtoets voltooi is.
6. Nadat u deelgeneem het , sal u ‘n bedrag van R10 ontvang om te betaal vir u vervoerkostes.
7. Indien u werk, moet u asseblief hierdie brief aan u werkgewer wys.

Geagte Werkgewer

Met hierdie brief vra ons dat u een dag betaalde verlof toestaan aan…….
om dit vir haar/hom moontlik te maak om hierdie afspraak met die navorsingsspan van die Fakulteit Gesondheidswetenskappe by die Universiteit van die Vrystaat by te woon.

Dankie vir u samewerking. Vir verdere inligting kontak asseblief vir Prof Corinna Walsh by 083 297 6030.

Prof C Walsh (projekleier)

Assuring Health for All in the Free State

(Tshepiso ya Bophelo ho bohle ba Foreisitata)

Lengolo la ho nka karolo

Ho ya nkang karolo

Re leboha ha o dumetse ho re thusa mosebetsing ona o bohlokwa. Re tshepa ha mosebetsi ona o tla thusa ho ntlafatsa maphelo a batho bohle ba Foreisitata.

Nako eo o fumanang lengolo lena, o tla be o se o etetswe ke e mong wa bathusi ba rona ebile o tla be o se o saenile ho fana ka tumello ya ho hula madi. Lengolo lena ke le o tsebisang ka letsatsi le nako eo tla methwang le ho hulwa madi tliliniking e haufi le lelapa la hao.

DINTLHA TSA BOHLOKWA

1. O tlamehile ho ba MUCPP ka di ..... nako e le 0.....h00.
2. **O SE KE WA JA KAPA WA NWA** letho ka mora hora ya leshome(10:00) bosiu bo ka pele ho fihlela o hlahlobuwa tliliniking. Hona ho bohlokwa hore dihlahlobo tsa tswekere e be tse nepahetseng.
3. O **TSHWANETSE HO TLA LE BUKANA YA HAO YA BOITSEBISO (ID)** ha o tla
4. O tle o tshwere le wena lenane la batho bohle bao o dulang le bona ka tlung hammoho le matsatsi a bona a tswalo
5. O tla fumana dijo kamora hore o hulwe madi.
6. O tla fuwa tjhelete e e kana ka R10 bakeng sa transport ha o nka karolo dipatlisisong.
7. Ha e be o sebetsa, re kopa o bontshe monga hao lengolo lena.

Monga mosebetsi

Lengolo lena le kopa hore of fane ka letsatsi le leng leng le patallwang ho ..... hore a tle a tsebe ho ba teng ka nako eo a e beetsweng bakeng sa sehlopha se etsang dipatlisiso sa Lefapha la tsa maphelo Yunivesithing ya Foreisitata.

Re lebohela tshebedisano mooho ya hao. Ha ebe o hloka tlhalosetso e fetang mona o ka ikopanya le Dr Corinna Walsh ho 083 297 6030.

Prof C Walsh (moetelledipele)

APPENDIX D

SOCIODEMOGRAPHIC QUESTIONNAIRE

Assuring Health for All (AHA) in the Free State

SOCIO-DEMOGRAPHIC AND HOUSEHOLD QUESTIONNAIRE

(All information in this questionnaire is confidential).

Area in Mangaung:_____ 1

Household number: 2-5

Member number: 6-7

Interviewer: _____ 8-9

Interview Date: D D M M Y Y Y Y 10-17

For each individual living in the household (5-7 days per week), provide the following:

Household Member	Name	Relation to head of household	Sex	Education	Birth date D D M M Y Y Y Y	
0 1	_____					18-31
0 2	_____					32-45
0 3	_____					46-59
0 4	_____					60-73
0 5	_____					1-14
0 6	_____					15-28
0 7	_____					29-42
0 8	_____					43-56
0 9	_____					57-70
1 0	_____					1-14
1 1	_____					15-28
1 2	_____					29-42

For each individual who was living in the house and died within the past 2 years, provide the following:

Name	Relation	Sex	Education	Birth date D D M M Y Y Y Y	
_____					43-54
_____					55-66
_____					67-78
_____					1-12

Household member number of person with whom interview is being conducted: 13-14

How many years have you been living in an urban area _____ 15-16

Encircle the appropriate answer:

First language of household: 17

1. Sotho
2. Tswana
3. English
4. Afrikaans
5. Other, specify_____

Employment status of respondent: 18

1. Housewife by choice
2. Unemployed
3. Self Employed
4. Full time wage earner (receive a salary)
5. Other, specify (part-time, piece job etc.)_____
6. Don't Know

Husband/ partner's employment status: 19

1. Retired by choice
2. Unemployed
3. Self Employed
4. Full time wage earner (receive a salary)
5. Other, specify (part-time, piece job etc.)_____
6. Not Applicable e.g. dead

Type of dwelling: 20

1. Brick, Concrete
2. Traditional mud
3. Tin
4. Plank, wood
5. Other, specify_____

Total number of rooms in house: _____ 21-22

Number of bedrooms: _____ 23

Do you have a bathroom in the house? 1=Yes 2=No 24

Do you have a bathroom outside? 1=Yes 2=No 25

Do you have a kitchen or cooking area inside the house? 1=Yes 2=No 26

Does the household have electricity? 1=Yes 2=No 27

Where do you get drinking water most of the time? 28

1. Own tap
2. Communal tap
3. River, dam
4. Borehole, well
5. Other, specify _____

What type of toilet does this household have? 29

1. Flush
2. Pit
3. Bucket, pot
4. VIP
5. Other, specify _____

What fuel is used for cooking most of the time? ☐ 30

1. Electric
2. Gas
3. Parrafin
4. Wood, Coal
5. Sun
6. Open fire

Do you use a cast iron pot for cooking? ☐ 31

1. Never
2. ≤ Once a week
3. > Once a week
4. Every day

Does the home have a working:

Refrigerator and/or freezer ☐ 32
1.Yes
2.No

Stove (Gas, Coal or electric) or Hot Plate ☐ 33
1. Yes
2. No

Primus or Paraffin Stove ☐ 34
1. Yes
2. No

Microwave ☐ 35
1. Yes
2. No

Radio ☐ 36
1. Yes
2. No

Television ☐ 37
1. Yes
2. No

How many people contribute to the total income? _____ ☐☐ 38-39

Household income per month (including wages, rent, sales of vegs, etc. State grants). ☐ 40
1. None
2. R100-R500
3. R501- R1000
4. R1001-R3000
5. R3001-R5000
6. Over R5000
7. Don't know

Is this more or less the income that you had over the past six months? ☐ 41

1. More
2. Less
3. The same

Race of the family: ☐ 42
1. Black
2. Coloured
3. White
4. Mixed

Codes to be used for coding of questionnaires:

Level of education:

1. None
2. Primary School
3. Std 6-8
4. Std 9-10
5. Tertiary Education
6. Don't Know

Relation to head of the household:

1. Head of the household
2. Spouse
3. Child
4. Sibling
5. Mother/ Father
6. Mother-in-law/ Father-in-law
7. Grandchild
8. Daughter-in-law/ Son-in-law
9. No relation
10. Other

Gender:

1. Male
2. Female

APPENDIX E

DIETARY INTAKE AND ACTIVITY QUESTIONNAIRE

Assuring Health for All (AHA) in the Free State
Dietary intake questionnaire
24-hour recall

Area in Mangaung: _____ 1
Household number: 2-5
Member number (as on socio-demo form): 6-7
Interviewer:_____ 8-9

Food and fluid intake (expressed in exchanges):

Food/Drinks and amounts	Milk and milk products	Meat and meat alternatives	Legumes	Fruit ß-carotene	Vegetables ß-carotene	Fruit Vit C	Vegetables Vit C	Fruit other	Vegetables other	Bread and cereals	Fats and oils	Sweets/Sugar	Alcohol
Breakfast and mid-morning													
Lunch and mid afternoon													
Supper and late night													
Total:													

Evaluation of dietary intake

	Quantity	Energy	Protein	CHO	Fat	Below requirement 1	Within requirement 2	Above requirement 3	
Milk and milk products		530	8	12	5				10
Meat and meat alternatives		315	7		5				11
Legumes		500	7	21	1				12
Soy beans		630	13	8	7				13
Fruit β-carotene		250		15					14
Vegetables β-carotene									15
Fruit vit C		250		15					16
Vegetables vit C									17
Fruit other		250		15					18
Vegetables B		150	2	7					19
Bread and cereal		285	3	15					20
Fats and oils		190			5				21
Sweets/Sugar		170		10					22
Alcohol									23
TOTAL									

Calculated estimated total values for:

Carbohydrate (g):						24-26
Protein (g):						27-29
Fat (g):						30-32
Energy (kJ):						33-37

Food frequency questionnaire

Number of times per day, per week or per month (only use one option)

Food	/day		/week		/month		
Sweets/ chocolates……..							38-43
Chips (crisp)……..							44-49
Cake/ biscuits……							50-55
Cool drinks……							56-61
Cremora……							62-67
Coffee……..							68-73
Tea……..							74-79
Sugar……..							1-6
Full-cream milk……..							7-12
Low fat/ skim milk……..							13-18
Eggs……							19-24
Peanut butter……							25-30
Soya mince/ legumes (baked beans, dried beans/peas, lentils)…..							31-36
Chicken……..							37-42
Red meat……							43-48
Fish……..							49-54
Bread……..							55-60
Porridge, cooked……..							61-66
Cereal (eg. Morevite/ Pronutro)……..							67-72
Samp/ mielie rice……..							73-78
Margarine/ oil/ fat……..							1-6
Fruit juice……							7-12
Fruit……							13-18
Vegetables……							19-24
Salt/ stock/ Royco……							25-30
Alcohol _____.....							31-36

Assuring Health for All (AHA) in the Free State
Dietary intake questionnaire
Children 0-2 years

Area: _____ 1
Household number: 2-5
Member number: 6-7
Interviewer:_____ 8-9

Is the child currently being breast fed? 1 = yes 2 = no 10
Was the child previously breast fed? 1 = yes 2 = no 11
If so for how long (weeks)?_____ 12-13
At what age were solid foods introduced into the child's diet (weeks)?_____ 14-15
How long was the child exclusively breast fed?_____ 16-17
Is the child currently being formula fed? 1 = yes 2 = no 18
If the child is not breast fed, what formula/ milk is used?_____ 19-20
How many bottles does the child receive per day?_____ 21-22
What is the size of the bottle?_____ 23
How many scoops of formula are used to mix one bottle?_____ 24-25
What size tin of formula is bought? 1 = 400g 2 = 900g 26
How long does the tin of formula last (days)?_____ 27-28
Does this fall within recommendations (office use)? 1 = yes 2 = no 29

Where does the mother/ caregiver get the formula milk? 30
1. Buys it at store
2. At clinic

NB: If the child eats solid foods, complete the 24 hour recall.

TYPES OF FORMULAS
1. Pelargon
2. Nan 1
3. Nan 2
4. Nan Alfare'
5. Lactogen
6. S26
7. SMA
8. Infamill
9. Infasoy
10. Isomil
11. Cow's milk
12.
13.
14.

REQUIREMENTS FULFILLED:
Calculate (no of bottles) …… X (size of bottles)……ml = …… ml (total amount/ day)

REQUIREMENTS

Age:	RDA
0-2 mo	360-720ml
3mo	600-1080ml
4mo	900-1050ml
5 mo	900-1200ml
6 mo	720-1050ml (+ solids)
7-8 mo	840-960ml (+ solids)

Assuring Health for All (AHA) in the Free State
24 Hour Physical Activity Recall

Area in Mangaung: _____ | | 1

Household number: | | | | 2-5

Member number: | | 6-7

Interviewer:_____ | | 8-9

Time of day	Activity (Number): Use attached coding list		,			
12:00-12:30 AM			,			
12:30-1:00			,			
1:00-1:30			,			
1:30-2:00			,			
2:00-2:30			,			
2:30-3:00			,			
3:00-3:30			,			
3:30-4:00			,			
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9:00-9:30			,			
9:30-10:00			,			
10:00-10:30			,			
10:30-11:00			,			
11:00-11:30			,			
11:30-12:00 PM			,			

Total PAL Value (A) | | , | | | | 10-14

For activities **not reported in the recall**, complete the frequency of the following:

Activity	Duration	Times/day	Times/week	Average/day	PAL/hr				
Carrying heavy Items						,			
Chopping wood						,			
Driving a car						,			
Food preparation						,			
Gardening (watering)						,			
Gardening (no lifting)						,			
Gardening (mowing)						,			
Housework						,			
Playing soccer						,			
Riding a bicycle						,			
Riding in a car/bus/taxi						,			
Running						,			
Shopping						,			
Skipping rope						,			
Total PAL value B						,			

Total PAL Value (B) | | , | | | | 15-19

Final PAL Value $\frac{(A+B)}{2}$ +1.1 | | , | | | | 20-24

PAL Category (according to table below): | | 25

PAL category	PAL values
1. Sedentary	1-1.39
2. Low active	1.4-1.59
3. Active	1.6-1.89
4. Very active	1.9-2.5

Intensity and Impact of Various activities on Physical Activity Levels in Adults

(derived from Frary & Johnson, 2004, Table 2-4, p. 33)

	PAL/10 min	PAL/30 min
Bath/shower/washing yourself	0.005	0.015
Chopping wood	0.037	0.111
Driving a car	0.005	0.015
Eating	0.005	0.015
Food preparation	0.014	0.042
Gardening - watering plants	0.014	0.042
Gardening – no lifting	0.032	0.096
Gardening – mowing lawn	0.033	0.099
Housework (moderate effort) includes washing laundry	0.024	0.072
Playing soccer	0.088	0.264
Riding a bicycle	0.024	0.072
Riding in a car/bus/taxi	0	0
Running/jogging	0.088	0.264
Sitting with light activity	0.005	0.015
Sitting without activity (e.g. watch TV)	0	0
Sleeping	0	0
Walking (3.2 km/h)	0.014	0.042
Walking (4.8 km/h)	0.022	0.066
Weight lifting	0.061	0.183

Assuring Health for All (AHA) in the Free State
Exchange list calculation form

Area: _____ 1
Household number: 2-5
Member number: 6-7
Interviewer:_____ 8-9

Alcohol exchanges - Krause's Food, Nutrition and Diet Therapy, 2004. Ed. by Mahan KL & Escott S, 11th edition, Appendix 44, p. 1241.

Beverage	servings	Carbs	Protein	Fat	Energy
Beer					
Regular	336	**1 x15g +13**	**0**	**2x (0-1g)**	**x 150 kJ**
Light	336	**0 x 15g +5**	**0**	**2x (0-1g)**	**x 100 kJ**
Ciders	336	**1 x15g +12**	**0**	**0x (0-1g)**	**x 60 kJ**
Distilled spirits					
gin, rum, vodka, whiskey, scotch	42	**0 x15g**	**0**	**2x (0-1g)**	**x 100 kJ**
Dry brandy	28	**0 x15g**	**0**	**1.5 x (0-1g)**	**x 75 kJ**
Table wine					
Dry wine	112	**0 x 15 g+2**	**0**	**2x (0-1g)**	**x 80 kJ**
Red or Rosé	112	**0 x 15g +5**	**0**	**2x (0-1g)**	**x 85 kJ**
Sweet wine	112	**1/3 x 15g +1**	**0**	**2x (0-1g)**	**x 105 kJ**
Light wine	112	**0 x 15g**	**0**	**0x(0-1g)**	**x 50 kJ**
Sparkling wine	112	**1 x 15g+8**	0	**2x (0-1g)**	**x 116 kJ**

The energy contribution (in kilocalories) from an alcoholic beverage can be estimated by multiplying the number of grams of proof and then again by the factor 0.8. For beer and wine, energy can be estimated by multiplying grams by percentage of alcohol (by volume) and then by the factor 1.6

Appendices for dietary intake

Serving recommendations according to the Food Guide Pyramid (USDA, 1992)

	Adults	Children 2- to 6 –year-olds
Bread and cereal	6 – 11 servings /day	6 servings
Fruit	2 – 4 servings / day	2 servings
Vegetables	3 – 5 servings / day	3 servings
Meat and meat alternatives	2 – 3 servings / day	2 servings
Milk and milk products	2 – 3 servings / day	2 servings
Fats and sweets	Use sparingly	Use sparingly

ONE LEGUME PORTION PROVIDES:

21 grams of carbohydrates, 7 grams of protein, 0,7 grams of fat, and 500 kJ.

Split peas (Cooked)……..	½ cup (85g)
Chick peas (Dried & cooked)……	½ cup (85g)
Lentils (Whole; cooked)……	½ cup (90g)
Lentils (Split; cooked)……	½ cup (90g)
Sugar beans (Fried & cooked)……	½ cup (100g)
Kidney beans (White; Dried & cooked)……..	½ cup (90g)
Canned:	
Baked beans in tomato sauce……..	⅓ cup (90g)
Kidney beans (White; solids & liquids)……	⅓ cup (90g)

Soybeans ..... ½ cup (80g)

Provides: 8 grams of carbohydrates, 13 grams of protein, 7 grams of fat, and 630 kJ.

PORTION SIZES –ADULTS

Grain group	**Fruit group**	**Meat group**
1 slice of bread	1 piece of fruit or melon wedge	30g of cooked lean meat, poultry, or fish.
½ cup of cooked rice or pasta	½ cup of juice	1 egg
½ cup of cooked porridge	½ cup of canned fruit	½ cup of cooked dry beans
½ cup of ready-to-eat cereal	½ cup of dried fruit	2 tablespoons of peanut butter (add one fat exchange)
1/3 cup samp		
Vegetable group	**Milk group**	**Fats and sweets**
½ cup of chopped raw or cooked vegetables	1cup of milk or ½ yogurt	2 teaspoons sugar
1 cup of raw leafy vegetables	30g of cheese	2 hardboiled sweets
		10 ml of mayonnaise
		5ml oil, 10ml margarine (medium fat)

PORTION SIZES FOR CHILDREN 2- TO 6-YEAR-OLDS

Grain group	**Fruit group**	**Meat group**
1 slice of bread	1 piece of fruit or melon wedge	30g of cooked lean meat, poultry, or fish.
½ cup of cooked rice or pasta	½ cup of juice	1 egg
½ cup of cooked porridge	½ cup of canned fruit	½ cup of cooked dry beans
½ cup of ready-to-eat cereal	½ cup of dried fruit	2 tablespoons of peanut butter (add one fat exchange)
1/3 cup samp		
Vegetable group	**Milk group**	**Fats and sweets**
½ cup of chopped raw or cooked vegetables	½ cup of milk or ¼ cup of yogurt	2 teaspoons sugar
1 cup of raw leafy vegetables	60g of cheese	2 hardboiled sweets
		10 ml of mayonnaise
		5ml oil, 10ml margarine (medium fat)

PAL calculation

(BEE*+TEF**)+Total of PAL/day=Energy expenditure/day
1.0+10%=1.1+ (PAL/day).

*BEE is always 1 and stands for Basal Energy Expenditure
**TEF=Thermic effect of food and is always 10%

Eg. Walk 20min (0.014*2)=0.028Chopped wood 10 min (0.037*1)=0.037
Played soccer 30min (0.088*3)=0264

Add all the Above totals=0.028 + 0.037 + 0.264 = **0.329= Total Daily PAL**

1+0.1+0.329 **= 1.429**

The answer is then an indication of the amount of physical activity per day.

Due to the fact that not all activities will be mentioned in the 24 hour recall, the physical activity frequency form will be used for cross-referencing and incorporating the information by using the following calculations:

Activity PAL x the frequency x duration of activity = Energy expended per week.

This procedure will be followed for all activities and added to get a total PAL per week. The Total PAL /Week will then be divided by 7 to get the average PAL / day, which will then be used in the following formula:

(BEE+TEF)+Total of PAL/day=Energy expenditure/day
1.0+10%=1.1+ (PAL/day).

EG. chop wood 3 times/ week for 10min each
((0.037*1)*3= 0.111. (The average amount of energy spent on chopping wood per week).
Walk for 30min each day
(0.022*3)*7=0.462

0.111+0.462=0.573=Weekly PAL

1+0.1+0. 573=1.673
1.673/7 =**0.239** = daily energy expenditure

You will now have 2 totals, i.e. (1) the amount of energy expended on average per day, using the Physical Activity Frequency and (2) the amount calculated from the 24 hour recall. These 2 totals will then be added and divided by 2 to get the average energy expended per day. This is done to include activities which are not done on a daily basis:

(1.429+0.239) /2 = 0.834

You then compare your answer in the above formula to the amounts as used by Carroll & Johnson (2004b) to determine daily activity level, which is classified into sedentary, low active, active or very active categories (Carroll & Johnson, 2004b), with the Table below:

PAL category	**PAL values**	**Walking equivalence (miles/day at 3-4 mph)**
Sedentary	1-1.39	
Low active	1.4-1.59	1.5, 2.2, 2.9 for PAL = 1.5
Active	1.6-1.89	3, 4.4, 5.8 for PAL = 1.6 5.3, 7.3, 9.9 for PAL = 1.75
Very active	1.9-2.5	7.5, 10.3, 14 for PAL = 1.9 12.3, 16.7, 22.5 for PAL = 2.2 17, 23, 31 for PAL = 2.5

APPENDIX F

HEALTH QUESTIONNAIRE

Assuring Health for All (AHA) in the Free State

HEALTH QUESTIONNAIRE

(All information in this questionnaire is confidential).

Area in Mangaung:_____ | | 1

Household number: | | | | | 2-5

Member number (as on socio-demographic form): | | 6-7

Interviewer: _____ | | 8-9

Interview Date: | D | D | M | M | Y | Y | Y | Y | 10-17

Marital status: | | 18

1. Child
2. Never married
3. Currently married/ Traditional marriage
4. Living with partner
5. Widowed
6. Separated
7. Divorced
8. Other, specify_____

Which best describes your history of smoking? | | 19

1. Never smoked
2. Currently smoke
3. Formerly smoked

If yes, how many cigarettes per day?_____ | | 20-21

If yes, at what age did you start? _____ | | 22-23

Which best describes your history of snuffing? | | 24

1. Never used snuff
2. Currently use snuff
3. Formerly used snuff

If yes, how many times per day do you snuff_____ | | 25-26

If yes, at what age did you start? _____ | | 27-28

Which best describes your history of alcohol use? | | 29

1. Never used alcohol products
2. Currently use alcohol products
3. Formerly used alcohol products

If currently, what form of alcohol do you use regularly (at least once a week)? 1=yes 2=no

1. Spirits (rum, whisky, gin, vodka etc.) | | 30
2. Wine | | 31
3. Beer | | 32
4. Homemade beer | | 33

At least once a month, do you consume >5 alcoholic drinks per day? 1=yes 2=no | | 34

At what age did you start using alcohol? _____ | | 35-36

On weekends, how many alcohol-containing drinks do you consume?_____ | | | 37-38

Do you feel tired on Monday after heavy alcohol consumption (more than 5 drinks per day) during the weekend? 1=yes 2=no | | 39

Usual sleeping habits:

What time do you usually go to bed at night?_____ | | | : | | | 40-44

What time do you usually wake up in the morning?_____ | | | : | | | 45-49

Do you usually take naps during the day? 1=yes 2=no | | 50

Current disability: 1=yes 2=no

1. Do you have any trouble walking about? | | 51
2. Do you have trouble seeing someone across the room (with glasses worn)? | | 52
3. Do you have trouble reading or seeing individual grains of rice/corn on your plate (with glasses)? | | 53
4. Do you have trouble speaking and being understood? | | 54
5. Do you have trouble hearing? | | 55

Have you experienced any of the following in the last six months? 1=yes 2=no

1. Chest pain or tightness with usual activity | | 56
2. Breathlessness with usual activity | | 57
3. Cough for at least 2 weeks | | 58
4. Wheezing or whistling in the chest | | 59
5. Loose stools/ diarrhoea for at least 3 days | | 60
6. Vomiting | | 61
7. Loss of appetite | | 62
8. Swelling of feet | | 63
9. Blood in urine | | 64
10. Involuntary weight loss of > 3 kg | | 65
11. Skin rash | | 66
12. Joint pain | | 67
13. Sexually transmitted diseases | | 68

Have YOU ever been diagnosed with the following? 1=yes 2=no

1. Diabetes | | 69
2. High blood pressure | | 70
3. Stroke | | 71
4. Heart disease/ Angina/ Heart attack | | 72
5. Heart failure | | 73
6. Cancer | | 74
7. Liver disease/ Hepatitis/ Jaundice | | 75
8. Lung disease e.g. emphysema or asthma | | 76
9. Tuberculosis | | 77
10. HIV/AIDS | | 78
11. Epilepsy | | 79
12. Allergy | | 80

Has a family member (parents, siblings, children) ever been diagnosed with the following? 1=yes 2=no

1. Diabetes		1
2. High blood pressure		2
3. Stroke		3
4. Heart disease/ Angina/ Heart attack		4
5. Heart failure		5
6. Cancer		6
7. Liver disease/ Hepatitis/ Jaundice		7
8. Lung disease e.g. emphysema or asthma		8
9. Tuberculosis		9
10. HIV/AIDS		10
11. Epilepsy		11
12. Allergy		12

Medication

Are you taking medication regularly (ie. at least once per week) 1=yes 2=no | | 13

If yes, list the medication that you are currently using (including traditional medicine).

_____		14-15
_____		16-17
_____		18-19
_____		20-21
_____		22-23
_____		24-25
_____		26-27
_____		28-29

During the past 12 months, have you been hospitalised? 1=yes 2=no		30
If yes, how many times? _____		
If yes, give details (e.g. for specific operation or treatment)_____		23-32
If yes, for how many days?_____		33-34

For women only: 1=yes 2=no

1. Are you currently pregnant?		35
2. Do you still have periods?		36
3. Have you ever used an injectable contraceptive?		37
4. How many live children have you given birth to?_____		38
5. Did you breastfeed any of your children?		39
6. If yes, at what age (months) did you add anything other than breast milk to the diet?_____		40-41

Social situation and stress:

Are you a member of a church?_____ 1=Yes 2=No ☐ 42

Do you attend services at least 2x/month? 1=Yes 2=No ☐ 43

Stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home. How often have you felt stress in the last 2 months? ☐ 44

1. Never
2. A few periods of stress
3. Several periods of stress
4. Permanent stress

Have you experienced any of the following during the past 12 months? 1=yes 2=no

1. Loss of job ☐ 45
2. Retirement ☐ 46
3. Loss of crop/ business failure ☐ 47
4. Household break in ☐ 48
5. Marital separation/ divorce ☐ 49
6. Other major intra-family conflict? If yes, specify _____ ☐ 50
7. Major personal injury or illness ☐ 51
8. Violence ☐ 52
9. Death of a spouse ☐ 53
10 Death or major illness of another family member ☐ 54
11. Wedding of family member ☐ 55
12. New job ☐ 56
13. Birth in the family ☐ 57
14. Separation from family ☐ 58
15. Unavailablity of food/ Food insecurity ☐ 59
16 Other major stress? If yes, specify _____ ☐ 60

During the past 12 months, was there ever a time when you felt sad, blue or depressed for two weeks or more in a row? 1=yes 2=no ☐ 61

Are you willing to answer questions related to HIV/AIDS? 1=yes 2=no ☐ 62

If yes, do you know people who have HIV/AIDS? 1=yes 2=no ☐ 63

If yes, which of these people: 1=yes 2=no

1. Your children ☐ 64
2. Your grandchildren ☐ 65
3. Your spouse ☐ 66
4. Your family members ☐ 67
5. Your friends ☐ 68
6. People in the community ☐ 69

Do you care for orphans in your household? 1=yes 2=no ☐ 70

APPENDIX G

MEDICAL EXAMINATION FORM

Assuring Health for All (AHA) in the Free State

Medical examination

(All information in this questionnaire is confidential).

Area in Mangaung:_____ 1

Household number: 2-5

Member number (as on socio-demographic form): 6-7

Doctor: _____ 8-9

Examination Date: D D M M Y Y Y Y 10-17

Blood pressure: (Right arm supine; systolic / diastolic mmHg) 18-23

Heart rate (bpm) 24-26

1. Are any of the following visible/tangible?

Jaundice	Yes (1)	No (2)	27
Anaemia (Pale)	Yes (1)	No (2)	28
Clubbing	Yes (1)	No (2)	29
Cyanosis	Yes (1)	No (2)	30
Lymphadenopathy	Yes (1)	No (2)	31
Oedema	Yes (1)	No (2)	32

2. Are there any cardiovascular abnormalities? Yes (1) No (2) 33

If yes, specify 1._____ 34-35

_____

2. _____ 36-37

3. Are there any respiratory abnormalities? Yes (1) No (2) 38

If yes, specify 1._____ 39-40

_____

2. _____ 41-42

4. Is there any abdominal pathology? Yes (1) No (2) 43

If yes, specify 1._____ 44-45

_____

2. _____ 46-47

5. Are there any nervous system abnormalities? Yes (1) No (2) 48

If yes, specify 1._____ 49-50

_____

2. _____ 51-52

6. Is there any skin pathology? Yes (1) No (2) 53

If yes, specify 1._____ 54-55

_____

2. _____ 56-57

_____

Comments:

_____
_____
_____
_____

_____ _____
Ondersoeker / Examiner **Datum / Date**

PRE-TEST CONSENT TO HIV TESTING

To be completed by the patient/ client:

Do you know what AIDS is?	Yes	No
Has it been explained to you how the test is done?	Yes	No
Have the advantages of the test been explained?	Yes	No
Have the disadvantages of the test been explained?	Yes	No
Has it been explained to you how a positive result will affect your treatment?	Yes	No
Has it been explained to you what will happen if you are not tested?	Yes	No
Do you want to know the result of your HIV test?	Yes	No

To be completed by the counselor:

Have you explained to the patient/ client:

What AIDS is?	Yes	No
How the test will be done?	Yes	No
What the advantages of testing are?	Yes	No
What the disadvantages of testing are?	Yes	No
Why the information is needed?	Yes	No
How a positive result will affect treatment?	Yes	No
What will happen if the test is not done?	Yes	No
Have you yourself explained the above?	Yes	No
Has a translator been used to explain the above?	Yes	No

_____ _____
Signature of Participant Date

_____ _____
Signature of Counselor Date

For children:

_____ _____
Name/ Signature of Child Date

_____ _____
Signature of Parent Date

APPENDIX H

REFERRAL LETTER

Assuring Health for All (AHA) in the Free State
Referral letter

To whom it may concern

Dear Doctor/Sister

Mr/Ms ……participated in a project of our research group on ……

His/her fasted/random blood glucose was ……..mmol/L.

His/her resting blood pressure was ……..mmHg.

Thank you for attending to this patient.

Regards

……

Prof C Walsh (project leader)

Contact details: 083 297 6030

APPENDIX I

ANTHROPOMETRY FORM

Assuring Health for All (AHA) in the Free State
Anthropometry

Area in Mangaung:_____ [ ] 1

Household number: [ ][ ][ ] 2-4

Member number (as on socio-demographic form): [ ][ ] 5-6

Interview Date: **D D M M Y Y Y Y** [ ][ ][ ][ ][ ][ ][ ][ ] 7-14

Measurer (interviewer):_____ [ ][ ] 15-16

Weight (kg):_____ [ ][ ][ ].[ ] 17-21

Height (cm): _____ [ ][ ][ ].[ ] 22-26

If height cannot be measured:

Knee height (cm):_____ [ ][ ][ ].[ ] 27-31

Demispan (cm):_____ [ ][ ][ ].[ ] 32-36

Circumferences (cm):

Upper-arm (adults and children):_____ [ ][ ].[ ] 37-40

Waist (adults): _____ [ ][ ][ ].[ ] 41-45

Hip (adults): _____ [ ][ ][ ].[ ] 46-50

Wrist (adults):_____ [ ][ ].[ ] 51-54

Head circumference (children):_____ [ ][ ].[ ] 55-58

Bio-impedance fat percentage (adults):_____ [ ][ ].[ ] 59-62

Skinfold thicknesses (mm):

Triceps (adults and children):_____ _____ _____ [ ][ ] 63-64

Biceps (adults): _____ _____ _____ [ ][ ] 65-66

Supra-ileac (adults): _____ _____ _____ [ ][ ] 67-68

Subscapular (adults): _____ _____ _____ [ ][ ] 69-70

Thigh (adults): _____ _____ _____ [ ][ ] 71-72

Calf (adults): _____ _____ _____ [ ][ ] 73-74