Nutrition of ageing black South African women and correlates with anthropometry and cardiometabolic outcomes

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Monday, 6 November 2023

Declaration

Declaration:

Student's contribution to article(s) and agreement of co-author(s)

I, Caroline Kankwende, student number 0402890W, declare that this Thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my Thesis.

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Date…28 September 2023…….

Name of Primary Supervisor…Prof Philippe Gradidge……
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Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of her Thesis. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1: Title: Nutrient Patterns and Body Composition Parameters of Black South African Women.

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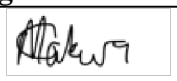
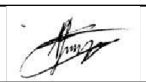
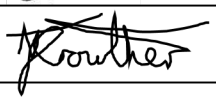
1st author	C. B. T. Makura-Kankwende		27 September 2023
2nd author	P. J.-L. Gradidge		27 September 2023
3rd author	N. J. Crowther		27 September 2023
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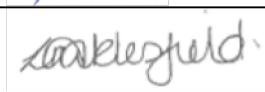
6th author	L. K. Micklesfield		28 September 2023
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This work has been published post-initial examination of the PhD thesis and prior to reexamination. The findings are directly related to the thesis and therefore important to present in the write-up.

Dedications

To Paul, Justin Christian and Aliyah Musau Mbwaya

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I am deeply indebted to my supervisors, Prof Philippe Gradidge, Prof Shane Norris and Dr Tinashe Chikowore for the priceless opportunity to pursue my PhD under their supervision. I appreciate the vast knowledge, skills, mentorship and input that they contributed to my PhD journey. In addition to my supervisors, I would like to thank Nigel Crowther, Lisa Micklesfield, Julia Goedecke and Glory Chidumwa for their contributions to my PhD, which included everything from research conception to data analysis and interpretation to authoring and review of the many articles that resulted in my thesis.

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Lastly, my deepest gratitude and unending appreciation for my husband, kids, family and friends for their continuous encouragement and unwavering faith in my skills.

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Thesis Material and Contributions

Together with my supervisors, I contributed to the development of the study protocol, which included formulating research questions and hypothesis and developing the statistical analysis plan. I assisted with data collection for the food frequency questionnaires and some demographic information collected at follow-up. In addition, I contributed to the project management, data capturing for the baseline nutritional data, quality control and data management for the nutritional database, as well as statistical analysis of the results presented.

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Publications

In the process of completing this PhD thesis, part of the work was reported and published in a series of scientific publications in international, peer-reviewed journals listed below:

Peer Reviewed Publications:

Paper 1 (Chapter 3)

C. B. T. Makura-Kankwende, P. J.-L. Gradidge, N. J. Crowther, S. A. Norris, and T. Chikowore, "Nutrient Patterns and Body Composition Parameters of Black South African Women," *Nutrients*, vol. 13, no. 6, 2021.

Paper 2 (Chapter 4)

C. B. T. Makura-Kankwende, P. J.-L. Gradidge, N. J. Crowther, T. Ratshikombo, J. H. Goedecke, L. K. Micklesfield, S. A. Norris, and T. Chikowore, "Longitudinal association of nutrient patterns with body composition in black middle-aged South African women: a five-year follow-up study", *Int. J. Environ. Res. Public Health,* vol. 19, no. 19, 2022, *19*(19).

Paper 3 (Chapter 5) – draft mode

C. B. T. Makura-Kankwende, P. J.-L. Gradidge, T. Chikowore and S. A. Norris. "Association between nutrient patterns and blood pressure in South African women: a five-year follow-up study"

Nomenclature

1G	Generation 1
2G	Generation 2
3G	Generation 3
ANOVA	Analysis of variance
BMI	Body mass index
Bt20+	Birth to twenty plus
CI	Confidence interval
CV	Coefficient of variation
CVD	Cardiovascular disease
DASH	Dietary apprpoaches to stop hypertension
DXA	Dual-energy X-ray Absorptiometry
EER	Estimated energy requirement
EI	Energy intake
FAO	Food and Agricultural Organization
FBDGs	Food-based dietary guidelines
FFSTM	Fat-free, soft tissue mass
FM	Fat mass
FMI	Fat mass index
GEE	Generalized estimating equations
GPAQ	Global Physical Activity Questionnaire
GSK	GlaxoSmithKline
HDL	High-density lipoprotein
HTN	Hypertension
IHD	Ischemic heart disease
IOM	Institute of Medicine
IQR	Interquartile range
LMI	Lean mass index
LMICS	Low to middle-income countries
MASC	Middle-aged Soweto Cohort
MET	Metabolic equivalents
MetS	Metabolic syndrome
MNET	Media network
MVPA	Moderate–vigorous physical activity
NCDs	Non-communicable diseases
NIH	National Institute of Health
OSA	Obstructive sleep apnoea
PA	Physical activity
PC	Principal components
PCA	Principal component analysis
QFFQ	Quantitative Food Frequency Questionnaire
RMR	Resting metabolic rate

SAMRC	South African Medical Research Council
SANHANES-1	South African National Health and Examination Survey
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SEM	Structural equation modelling
SES	Socio-economic status
SWEET	Study of Women Entering and in Endocrine Transition
T2D	Type 2 diabetes
THUSA	Transition, Health and Urbanization in South Africa
VAT	Visceral adipose tissue
WHO	World Health Organization

Abstract

Background

Obesity is most prevalent among black women who reside in urban areas in South Africa yet the nutrient patterns of this cohort of women has never been investigated, nor have correlates of body composition indices such as adiposity and body mass index (BMI). These body composition indices are important to analyse as they have been shown to be positively associated with hypertension which is prevalent in this cohort of women.

Aim

There were three main goals:

1) To determine the baseline nutrient patterns of middle-aged black South African women residing in Soweto and correlates to body composition indices
2) To evaluate the longitudinal association of nutrient patterns with adiposity in a cohort of middle-aged black South African women over a period of 5.5- yearss
3) To elucidate the longitudinal associations of nutrient patterns and blood pressure and to explore whether this is an indirect effect mediated by body mass index (BMI) using structural equation modelling

Methods

A longitudinal study of children and their families, originally called the Birth to Twenty Plus (Bt20+) cohort and now referred to as the Middle-aged Soweto Cohort (MASC), was used to as the original dataset for this thesis. This study also drew on another embedded study, the Study of Women Entering and in Endocrine Transition (SWEET) study of older women transitioning through menopause. Data on

(i) dietary information;

(ii) body composition and anthropometry measurements;

(iii) blood pressure;

(iv) lifestyle behaviours (physical activity, tobacco use, and alcohol use);

(v) psychosocial factors;

(vi) socioeconomic status; and

(vii) educational status were used. A total of 498 Women aged between 40 and 60 years old were included in the study. Principle component analysis (PCA) was applied on the dietary data both at baseline and at 5- years follow-up. This was conducted to extract nutrient patterns from 25 nutrients derived from the food frequency questionnaire (FFQ) and the resulting nutrient patterns are detailed in results chapter 3 (nutrient patterns derived from the baseline FFQ) and results chapter 4 (comparison between both baseline and follow-up nutrient patterns from the FFQ). Simple and complex body composition were recorded for each participant with complex body measurements taken using (DXA). Chapter 3 details the results of the 3 baseline nutrient patterns and correlates with body composition parameters.

Using generalized estimating equations, associations between both baseline and 5- years follow-up nutrient patterns and adiposity were evaluated. The results are discussed in results chapter 4. Lastly, the results chapter 5 examined associations between both baseline and follow-up nutrient patterns and blood pressure were examined and furthermore,

investigated whether BMI mediates the relationship between repeated measures of nutrient patterns and blood pressure.

Results

The majority of the research participants (88%) were classified as individuals having obese status defined by their BMI. The fat mass index (FMI), lean mass index (LMI), gynoid fat, hip and waist circumference, and visceral and subcutaneous adipose tissue (VAT and SAT respectively) measurements were all substantially larger in the group with predominantly individuals having overweight and obese classification compared to woman in the lean group ($p < 0.001$). Protein consumption was greater in the group with individuals having overweight/obese classification, while fat and carbohydrate consumption were matched. At baseline, the "Plant driven nutrient pattern," characterized by higher factor loadings of plant protein, starch, and B vitamins, explained 25% of the total nutritional variance; the "Animal protein driven nutrient pattern," characterized by animal protein and saturated fat, explained 23% of the variance; and the third pattern was the "Vitamin C, sugar and potassium driven nutrient pattern," which had higher factor loadings of vitamin C, sugar and potassium. At baseline, increased consumption of the animal protein driven nutrient pattern resulted in a 1.19 kg/m2 ($p = 0.002$) increase in BMI, 10.17 cm2 for VAT, 24.43 cm2 for SAT, 0.01 ($p = 0.009$) increase for VAT/SAT ratio, 0.69 kg/m2 ($p = 0.005$) increase for FMI, and 0.48 kg/m2 ($p = 0.002$) increase for LMI. Furthermore at baseline, statistically significant associations were found for the animal protein driven nutrient pattern with all body composition indicators. Subcutaneous adipose tissue increased in the presence of a plant-driven nutrition pattern ($p = 0.045$).

At 5-year follow-up, although the value of the factor loadings of the individual nutrients changed between baseline and follow-up, the nutrients with the highest loadings for each principal component (PC) did not change therefore the overall nutrient patterns remained the same. Only DXA-derived measurements of fat mass, FMI, VAT, and gynoid fat mass (FM) increased with time, while lean mass considerably reduced. Repeated measures of the animal protein driven nutrient pattern was associated with significant increases in FMI, LMI and VAT and repeated measures of the vitamin C, sugar, and potassium driven nutrient pattern was significantly associated with an increase in FMI and LMI. For the purposes of this study, repeated measures of animal-driven nutrient patterns were shown to be significantly related with repeated measures of systolic blood pressure (SBP) only. When structural equation modelling (SEM) was applied, only significant relationships were observed between age and SBP. This relationship was not mediated by BMI but may involve other factors that were not included in this analysis.

Conclusions

This thesis explored the nutrient patterns linked to obesity and cardiometabolic complications, namely blood pressure, in a cohort of black middle-aged African females. It has been previously demonstrated that this cohort has been has a high prevalence of obesity. According to literature reviews, programs focusing on nutritional and behavioural changes could aid African women in their fight against the obesity and hypertension epidemic that we are facing today. The animal-driven nutrient pattern was found to be substantially associated with increases in body fat in this cohort at baseline. At 5-year follow-up, the nutrient patterns remained the same and repeated measurements of the vitamin C, sugar, and potassium-driven nutrient pattern were associated with significant

increases in FMI and LMI and the animal-driven nutrient pattern remained significantly associated with LMI, FMI and VAT, a measure of visceral obesity which is a major risk factor for cardiometabolic conditions. This is problematic in a population that consists predominantly of individuals that are classified as having an obese and overweight status. As a result of a higher BMI, a greater likelihood of developing cardiometabolic multimorbidity exists which is defined as the co-occurrence of two or three cardiometabolic conditions. This may result in reduced quality of life and an increased burden on the already overstretched healthcare system in South Africa. Furthermore, this study found that only the animal protein driven nutrient pattern had a significant relationship with SBP which was significant. When SEM was applied, BMI did not mediate the relationship between blood pressure and any of the nutrient patterns. No other noteworthy direct relationships between blood pressure and the other nutrient patterns were found.

Researchers can apply the findings of this study to improve nutritional policies and guidelines aimed at combating not just obesity, but high blood pressure among black women in Sub-Saharan Africa. It is necessary to conduct further extensive research to verify these findings.

Preface

Inspiration for this PhD came from the obvious gap that exists in South African nutritional guidelines. The current food based dietary guidelines for South Africa and the Roadmap for Nutrition in South Africa 2013-2017, have detailed guidelines for ages 0-24 months, maternal and child nutrition, and persons living with human immunodeficiency virus (HIV) and tuberculosis (TB), however very little nutritional information is available for women transitioning into menopause. This confirms a research gap in available nutritional guidelines thus highlighting the need for targeted research that can contribute to the development of age specific policies aimed at improving nutritional health and health outcomes.

As an Epidemiologist with a keen interest in implementation of healthcare interventions aimed at improving health outcomes, I have worked for some non-profit organizations that supported and delivered prevention, care and treatment services for various healthcare programmes in South Africa. Through my work, I also noted the importance of integrating nutritional guidelines with any healthcare programme to ensure successful healthcare outcomes. Therefore, when the opportunity presented itself to pursue a PhD aimed at nutrition, I was more than excited to get started.

It is known that good nutrition is important throughout life as it contributes towards an improved immune system, good cardiovascular health, bone and teeth strengthening, improved metabolism, weight control and good brain health. In addition to helping prevent chronic disease, good nutrition can also support healthy ageing as well as reduced burden on the overburdened healthcare system.

As we age, the impact of poor nutrition on health increases like chronic disease progression or development. However, by adopting healthy eating habits such as including nutrient-dense foods such as fresh fruits, lean meats and vegetables, we can reduce progression and development of chronic disease. This highlights the need for appropriate nutritional guidelines to alleviate these risks. Furthermore, this population often have almost no knowledge on eating well for their age as they either continue to consume the same energy/calories that they used to when they were younger and/or more active, which leads to an obesity epidemic, or they consume fewer calories resulting in a serious energy deficiency which can lead to a suppressed immune system. It must be noted that as we age, the total energy intake decreases however, there might be an increased need for some key nutrients such as Calcium. These recommendations may not be widely known in a general population and additionally, it is not clear if these recommendations are applicable across all ethnic groups and gender.

I was given an opportunity to investigate the nutrient patterns of ageing women and how their diet correlate to their body composition and hypertension when I was awarded with a PhD fellowship from the Developmental Pathways to Health Research Unit (DPHRU). During the project conceptualization, I examined all available and appropriate literature and confirmed that this indeed was an area under researched. As part of my project, I was granted permission to the 2012/13 historical physical activity and dietary data collected from the Birth-to-Twenty Plus Cohort caregivers. This is a cohort of urban black women residing in Soweto that were not research naive and aged between 40 and 60 years old. This cohort was asked about their food consumption, physical activity, socio-economic status, hypertension

history and body composition assessed in 2012. As part of my PhD, the same cohort of women were invited approximately 5.5- yearss later and the same follow-up data was collected allowing me to document sustainability of nutrient patterns, body composition changes and associations to hypertension. Results from my study, although specific to a population, may generalized and used to inform or assist development of nutrition guidelines for middle-aged women.

The PhD thesis is with publications and is presented in six chapters organised as follows:

Part 1:

Chapter 1 presents the literature review and contextual background

Chapter 2 presents the methodology

Part 2:

Chapters 3-5 is comprised of 3 manuscripts generated from this PhD study and are structured according to the study objectives:

Objective of Paper 1: To establish the baseline nutrient patterns and associations with body composition indicators of middle-aged black South African women residing in Soweto.

Objective of Paper 2: To examine how repeated measures of nutrient patterns correlate with repeated measures of body composition in the same cohort of women over a 5.5- years follow-up period.

Objective of Paper 3: To determine the associations between nutrient patterns and blood pressure, and to identify whether BMI mediates this association.

Part 3:

Chapter 6 integrates the empirical papers and discusses emerging themes, implications and recommendations from the research, details limitations and conclusions from the thesis.

Part 1: Introduction

Good nutrition will prevent 95% of all disease –

Dr Linus Carl Pauling

CHAPTER 1 LITERATURE REVIEW

1.1 INTRODUCTION

Obesity has been linked to a variety of chronic diseases such as cardiovascular disease (CVD), hypertension (HTN), type 2 diabetes (T2D) and stroke (1). Low and middle-income countries (LMICs) countries such as South Africa are reporting higher rates of obesity due to a recent shift in diet as well as urbanization (2). Furthermore, South Africa is currently undergoing a rapid epidemiological transition that is contributing to increased non-communicable diseases (NCDs) risk specifically in the ageing population (3)

Obesity is caused by a number of factors, including increased consumption of ultra-processed foods such, -fat and high sugar foods in as well as behavioural factors such as reduced physical activity (PA) and increased sedentary behaviour (4,5). Little is known about the long-term effects of improper diet on body composition indicators, particularly among black middle-aged African women due to the increased life expectancy over the years.

Body mass index (BMI) is a simple body composition assessment that is frequently used to determine individuals overweight and obesity status (6–8). Overweight and obesity status are generally defined as abnormal or excessive fat accumulations that can be harmful when it comes to health (8). It is calculated by dividing the individual's weight in kilograms divided by the square of their height in metres (kg/m^2). For adults, the World Health Organization (WHO) describes overweight and obesity as a BMI greater than or equal to 25 and a BMI greater than or equal to 30 respectively (9,10).

A health survey conducted in South Africa during 2012 amongst 2254 men and 4170 women showed high obesity rates reported as increased BMI (11). The age disaggregated distribution of BMI from this survey is shown in figure 1 (11).

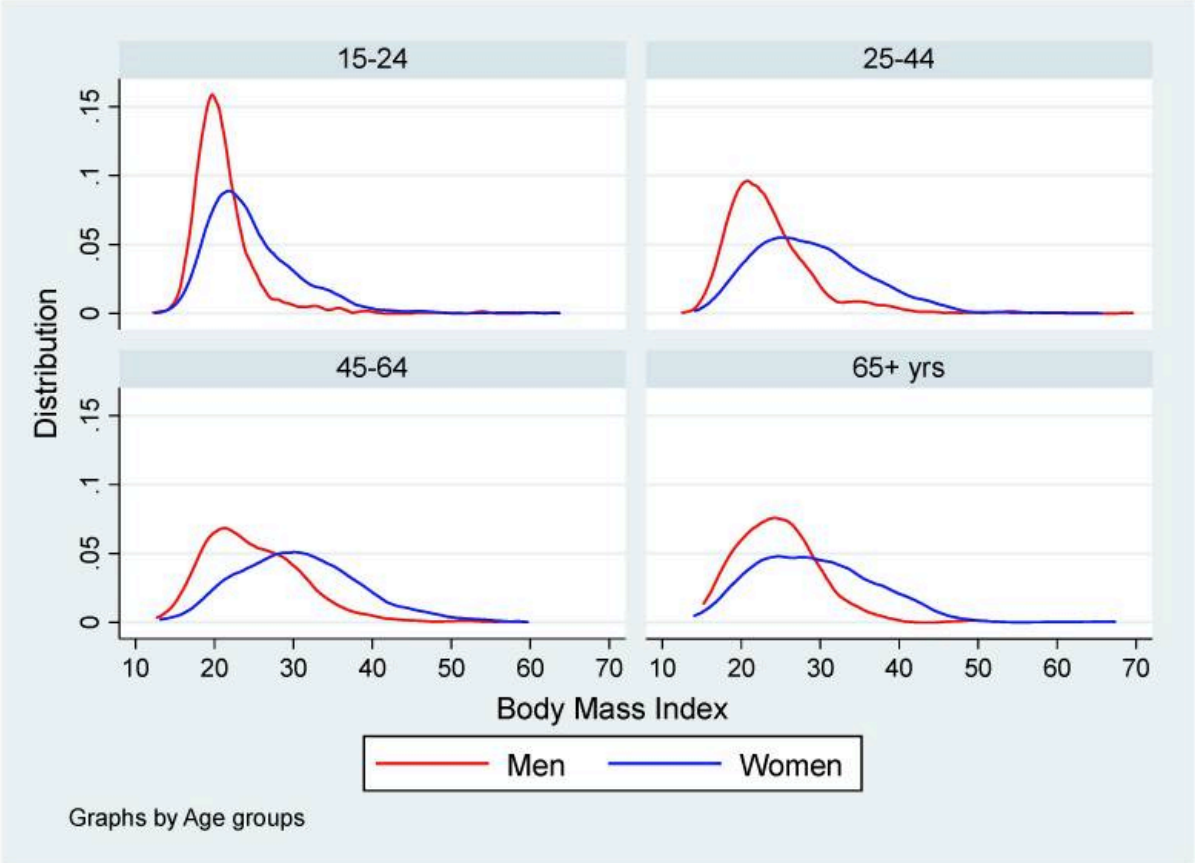

Figure 1. Kernel density showing distribution of BMI (Source: Mchiza et al., 2019).

Figure 1 depicts the BMI distribution by age and the distributions show that the BMI of women was more variable and had a higher median compared to the BMI of men (women had a median BMI of 27.4 kg/m2 compared to men which was 22.0 kg/m2 respectively) (11). BMI distributions were positively skewed, and mean and median BMI significantly increased with age up to age 65 in both men and women., which shows that not only do women have

more fluctuation in BMI, but they also have higher proportions of being overweight and obese when compared with BMI rates in men (11). In conclusion, middle-aged women in South Africa are particularly vulnerable to the country's obesity crisis as previously demonstrated as well as owing to the fact that black African women are predisposed to high obesity rates (12–15).

A systematic review was conducted that examined the dietary patterns of adults and quality of life across 15 countries and results showed that a diet high in saturated fats and ultra-processed foods may result in the onset of obesity and diabetes (16,17). The same review also demonstrated that in an ageing population, a good diet can aid in successful ageing which is as defined as a high physical, psychological, and social functioning in old age without major diseases thus may lead to prolonged happy life (18). Therefore, in a country rated highly on the Global Food Security Index scale, making healthy food choices for sustenance and good health status `may be directly linked to successful ageing (19). In addition, good health may lead to increased engagement in physical activity further increasing and improving health status.

1.2 THE CONTEXT OF THE PROBLEM UNDER STUDY

According to Kiefte-de Jong et. al. 2014, as women age, the risk of developing obesity and related cardiometabolic diseases that include T2D, HTN, CVD and others increases (20). Notably, due to racial and ethnic disparities for the onset of disease, specific epidemiological research studies focusing on ethnic groups, gender and age are needed. Studying the long-term impacts of nutrient patterns in middle-aged black South African women has not been

done in South Africa. This is, to the best of our knowledge, the first research study that examines this.

The first edition of the South African food-based dietary guidelines (FBDGs) were released in 2003 with a revised edition published in 2012 (21). The Nutrition Society of South Africa collaborated with, among others, the Department of Health, the South African Medical Research Council (SAMRC), nutritional scholars, and United Nations agencies to establish these guidelines. The initial FBDGs were intended for a paediatric population (from birth to age 5), however the upgraded FBDGs incorporated additional information for those aged 5 years and older. Nevertheless, there are no specialised FBDGs for middle-aged women, despite the fact that this is an important demographic with diverse dietary needs brought on by menopause. Additionally, the South African food guidelines, unlike those of other countries, lack an extensive list of foods whose intake should be restricted, such as ultra-processed foods and sugary beverages . Instead, it only presents food groupings that are essential for healthy eating (composed of both local and affordable items) (21–25).

My PhD study investigates how nutrient patterns of middle aged black South African women correlate to body composition and blood pressure, and whether their nutrient patterns change over time. Furthermore, this study is able to identify nutrient patterns that should be ingested in moderation for optimal health, which can help with the revising FBDGs for middle-aged black women or designing more appropriate research studies that address limitations highlighted from this study.

1.2.1 Conceptual framework

The conceptual framework by Skelton and Dinan-Young, 2008 below indicates that reduced PA in an ageing female cohort in United Kingdom resulted in increased risk of obesity, and development of cardiometabolic disease (26). This formed the basis of the framework for this PhD study and it can be seen in Figure 2.

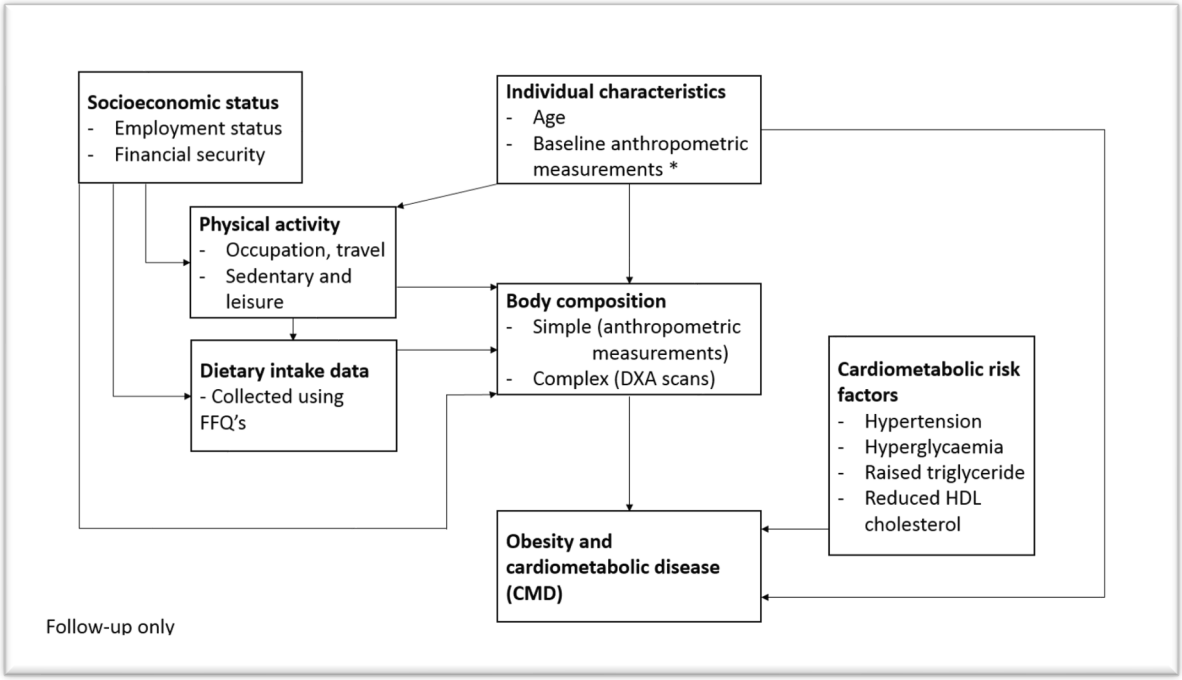

Figure 2. A conceptual framework of Ageing and how lifestyle behaviours affect the development of cardiometabolic risk factors (adapted from Ageing and older people (Skelton and Dinan-Young, 2008)).

Unhealthy food habits, which includes excessive calorie consumption and increased improper dietary behaviours, coupled with inadequate PA affects body composition indicators thus impacting on obesity and cardiometabolic risk. Characteristics such as age, socio-economic status (SES), engagement in PA and baseline anthropometric measurements influence body composition indicators which in turn impact on cardiometabolic risk.

Specifically, in an ageing population, SES directly impacts diet and engagement in PA. Pretorius and Sliwa reported that in Soweto, which is a well-known largely low-income community in Johannesburg, reported that SES largely influences food choices. (27).

1.3 CARDIOMETABOLIC DISEASE RISK FACTORS, CARDIOMETABOLIC DISEASE, AND OBESITY

Cardiometabolic disease refers to the clustering of risk factors that increase the probability of developing NCDs like T2D, CVD or HTN (28,29). Obesity and NCDs are a public health concern because they lead to higher death and morbidity rates, as well as putting a strain on an already overstretched health care system (30). Figure 3 shows that in the Black African population group, hypertensive heart disease had the second deaths per 100, 000 population at 76 deaths per 100,000. Furthermore, the Black African population group also reported the highest deaths per 100,00 when the standardized death rates for only hypertensive heart disease was compared across all the ethnic groups (31,32). Furthermore, Figure 3 shows that NCD risk varies by ethnicity with Black African's having the highest risk for Cardiomyopathy, Cerebrovascular disease and diabetes.

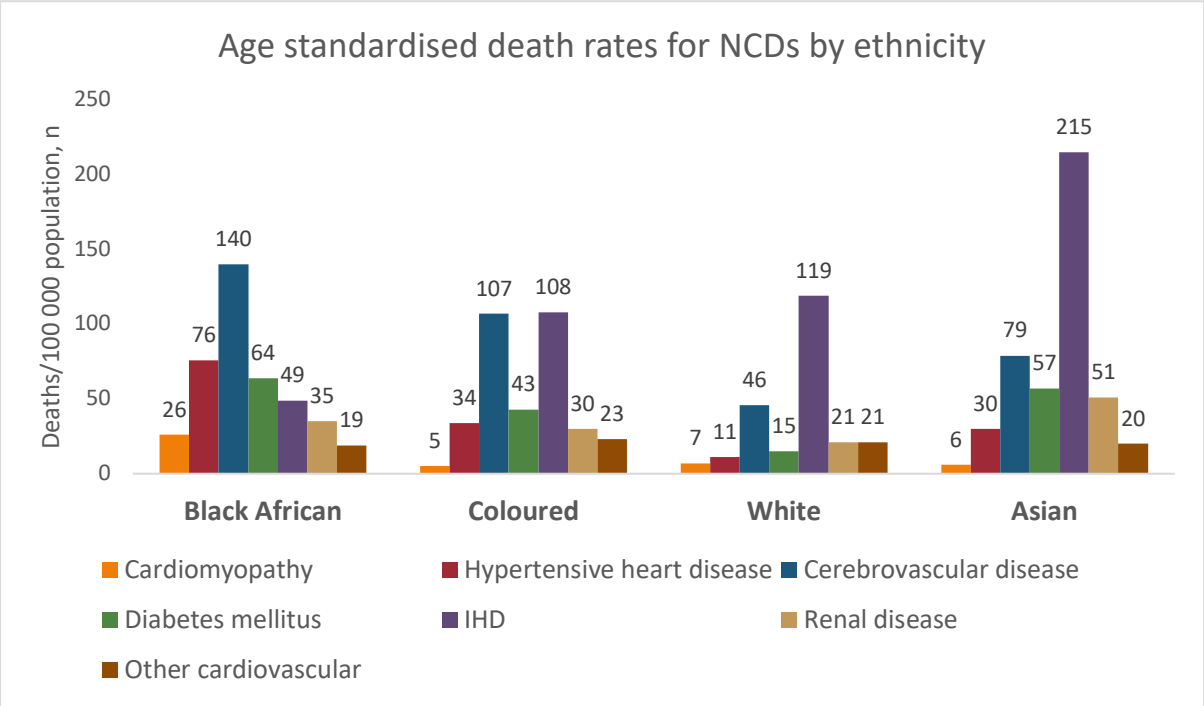

Figure 3 Age standardised death rates for NCDs by ethnicity, South Africa 2010 (Source: Nojilana et al., 2016).

1.3.1 The consequences of cardiometabolic disease, obesity and hypertension

Despite the lack of understanding of the processes, there is strong evidence of a causal relationship between obesity and metabolic illnesses such as insulin resistance, dyslipidemia, and the development of T2D(33–35). Being an individual with overweight status increases your chances of having high triglyceride levels and low high-density lipoprotein (HDL) values by two or more-fold (36,37). Additionally, individuals having obese or overweight status have a four-fold increase in development of diabetes (38–40)

Obesity has economic and social consequences, and obesity-related health problems have a considerable negative economic impact on the South African health-care system, affecting both direct and indirect expenses. Overweight and obese status are an increasing public health concern in South Africa and in 2020, ZAR33,194 million was projected to be the overall cost of this health concern. This amounts to 15.38% of government health expenditure and 0.67% of gross domestic product (GDP) (41).[NO_PRINTED_FORM]

The long-term negative effects of obesity on health and wellbeing include, but not limited to (29,42–44):

1. Sleep and breathing disorders such as sleep apnea and asthma
2. Ischemic heart disease (IHD), HTN and high cholesterol
3. Cancers
4. Vascular disease
5. Surgery complications
6. Type 2 diabetes

In 2010, obesity was responsible for 3.4 million deaths globally (45), and it remains a major risk factor for CVD due to cardiometabolic diseases such HTN and insulin resistance(46). Type 2 Diabetes and CVD are increasingly linked to abdominal fat in the general population, notwithstanding the prevalence of overall obesity (47).

While it is difficult to disentangle the impact of obesity from other risk factors, research has revealed that obesity is linked to endothelial dysfunction independently (48). This is a condition in which the inner lining (endothelial layer) of the small arteries fails to execute all of its essential tasks, leading to a variety of problems in tissues and arteries, including HTN.

Obesity characterized by increased fat, results in increased blood volume, increased oxygen demand, dilatation of the left ventricular chamber and increased cardiac output (49,50) . These are all positive effects on the heart however, with these changes, the heart also undergoes enlargement and thickening (hypertrophy) of the walls which leads to the loss of elasticity and increased pressure to the heart's pumping chamber to when trying to pump blood to the entire body (49,50). Eventually, the heart will be unable to pump with the necessary force, increasing the risk of heart failure. It well documented that prolonged obesity and hypertension, on the other hand, may contribute to hyper-filtration, glomerular hypertension, and renal function loss, as well as increased salt sensitivity thus reinforcing the importance of research studies aimed at targeted intervention to reduce this risk (50)

Obesity causes dyspnoea due to fat deposits reducing diaphragm excursion, and it also causes obstructive sleep apnoea (OSA), which occurs during sleep when the airway in the throat gets obstructed by excess fat and soft tissue (51). As a result, blood pressure rises during night, which is associated with an increased risk of HTN. Approximately 50% of the people with morbid obesity are reported to suffer from OSA and eventually get diagnosed with HTN (51).

Having coronary artery disease and HTN puts individuals with obese status at greater risk of a wide range of medical issues both before and after surgery. Additionally, individuals with obese status who have to undergo surgery, are also more likely to have an infection at the surgical site and deep vein thrombosis (52).

1.3.2 Determinants of cardiometabolic disease and obesity

Examples of obesity contributing factors include socio-demographic variables such as lower educational level, low SES and occupation status, and country food security status(figure 4) (53)

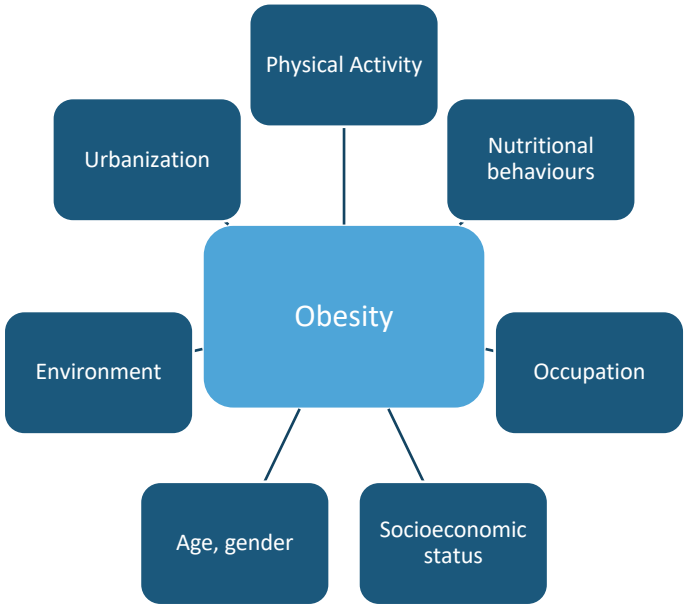

Figure 4 Determinants of obesity in a general population (Source: McNaughton 2012).

1.3.3 Socio-economic factors (e.g., education, occupational status) and demographic influencing factors

Higher rates of obesity are likely to be found in the higher education and income families and this is supported by findings from the Transition, Health and Urbanization in South Africa (THUSA) study, the WHO and a published survey conducted in 103 countries ((54–56).

Secondly, a South African study showed that different population groups and genders were associated differently with the cardio-metabolic disorders of T2D, HTN, and hypercholesterolemia (18). The aforementioned study highlighted the demand for multidisciplinary tailored healthcare management strategies in high-risk populations.

1.3.4 Modifiable risk factors of obesity

Some risk factors for increased risk of progression or development of obese and overweight status genetic and non-modifiable while some are modifiable, which means that changes in these risk factors can lower the risk of obesity onset (57) . These modifiable factors include increased engagement in PA and choosing healthy nutritional choices, both behaviours can delay obesity onset (57).

1.3.4.1 Physical activity (PA)

Urbanization contributes to the adoption of sedentary lifestyles and increased physical inactivity (2,58). By engaging in increased physical activity, the following occurs:

1. Increases individuals overall energy expenditure, which may assist with maintenance of energy balance or even weight lost, provided that individuals do not consume more to compensate up for the extra calories burnt
2. Reduces visceral fat, slowing the development of abdominal obesity.
3. Depression and anxiety are reduced, and this mental improvement may encourage individuals to remain consistent with their exercise routines long-term.

All of the above may eventually lead to decreased progression or risk of development of obesity.

1.3.4.2 Dietary behaviours

While urbanization is associated to improvements in public sanitation, increased access to health care, and improved SES, it is also linked to changes in food as well as PA patterns as mentioned above. Negative modifications in a person's diet and PA habits can have a detrimental influence on their general health and accelerate the development or onset of deficiencies and NCDs (59)

Changes in diet due to nutrition transition may lead to multimorbidity which is defined as a person living with more than one disease and subsequently to the development of chronic nutrition-related diseases. The effects of nutrient transition are illustrated by Hawkes in Figure 5 (60)

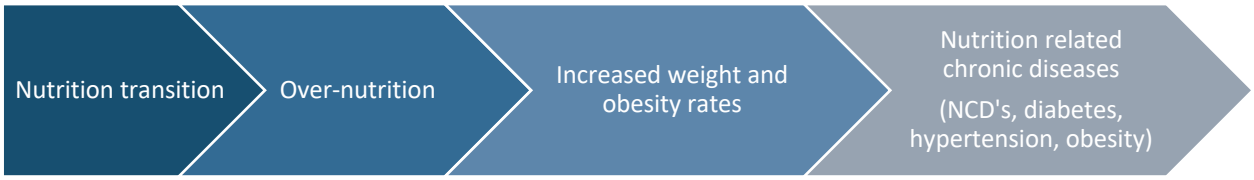

Figure 5 The effects of nutrient transition (Source: Hawkes 2017).

It has been reported that nutrition transition may occur concurrently with urbanization contributing to shifts in a conventional traditional diet which described as a diet that is low in fats and high in fibre, to western or urban diets characterized by saturated fats, high sugar while missing the advantages of unrefined grains such as legumes and homegrown vegetables (61,62). This dietary change contributes to an increased prevalence of NCDs. Currently, NCDs related to diet, such as obesity, T2D, and HTN, make up the majority of adult morbidity and mortality across many high-income and LMICs (63).

It has been shown that when healthy eating dietary habits are followed, cardiometabolic risk factors and the risk of developing cardiometabolic disease are reduced. It is of paramount importance that information on good healthy dietary behaviours is integrated into country health programs to improve the health of the population (55)

It is well known that individual dietary requirements vary depending on ethnicity , age, height, mobility, the existence of chronic or acute illness, metabolism, and the amount of energy necessary for daily activities and lifestyle (34,64) Therefore, dietary guidelines and recommendations are often developed based on age and other factors for individuals to follow and be guided on healthy eating and nutrition to prevent the onset of NCDs (44). Nonetheless, as people age their bodies also change impacting on basic nutritional needs

(26). It is therefore imperative to have age-specific nutritional guidelines per each ethnic group in order to promote healthy ageing.

There is ample proof, notably in LMICs, that cases of food quality decline are on the rise (17). Animal fat consumption has increased significantly in emerging nations, and this change in dietary patterns has been connected to rising incomes (65). The Food and Agricultural Organization (FAO) reports that fat consumption has increased in South Africa, and research has shown that consumption of fat is directly correlated with increasing income. Sugar consumption has increased by 30% globally since 1960, which is another adverse modification in diet (23). Furthermore, there has been increased demand in animal source foods and a decline in intake of cereals across all SES categories (65,66). Figure 6 illustrates the detrimental impact of urbanization on macronutrient intakes by showing the proportion of each macronutrient to total energy consumption for African women from 1975 to 2005 (62). In urban women, mean fat consumption increased by 9% between 1975 and 2005, from 21% to 30%, while mean carbohydrate intake decreased by 8%, from 65% to 57%. The changes in macronutrients were considerably lower among rural women, as the mean fat consumption increased by 5% while the mean carbohydrate intake decreased by 4% during the same investigation period (62).

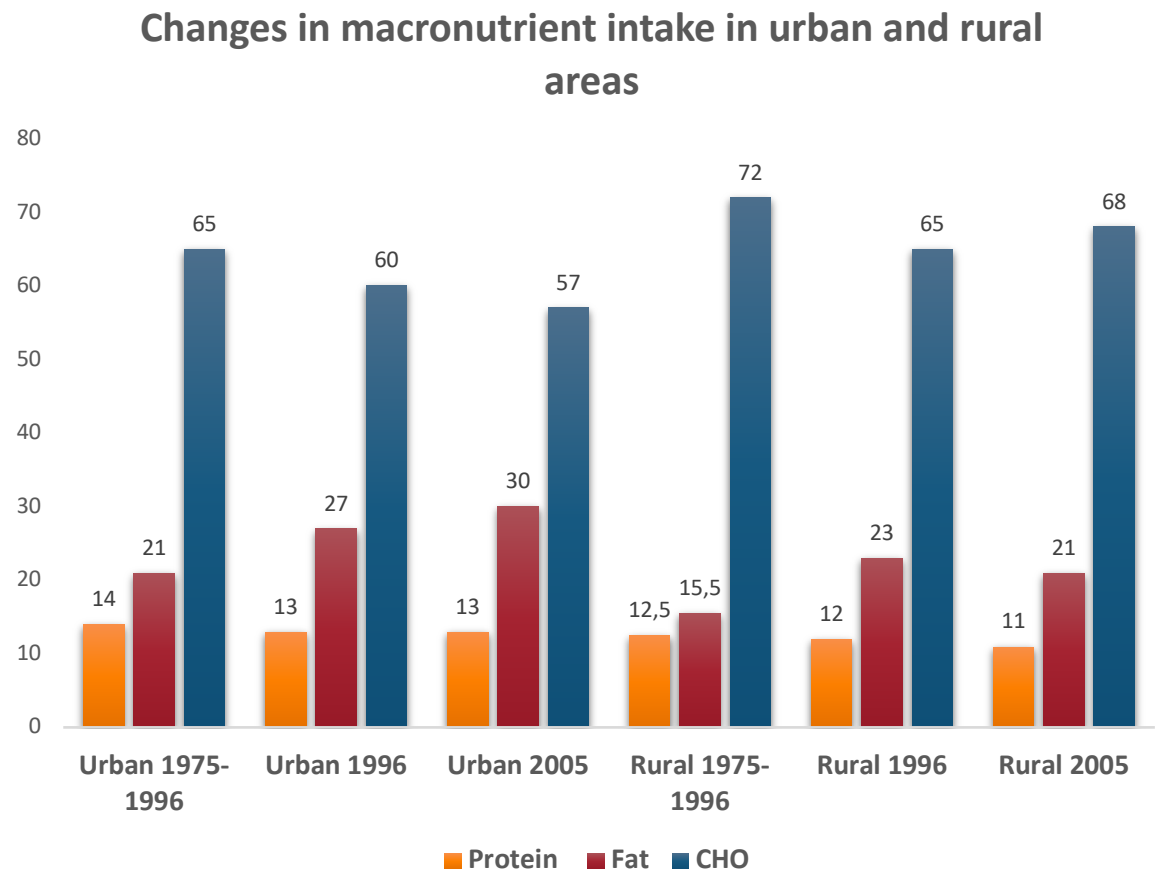

Figure 6 Changes in macronutrient intake as a proportion of total energy consumption in rural and urban areas (Source: Vorster 2011).

Research evidence emphasises that consumption of a poor diet which includes consumption of unhealthy foods such as ultra-processed foods and sugary beverages leads to the development of nutrient-related diseases. By eliminating or reducing consumption of these foods, diet quality is improved which may reduce or prevent the risk of developing nutrient-related diseases such as obesity which has been proven to increase the risk for heart disease, stroke, diabetes, arthritis and some form of cancers (24,25). Hitherto, the majority of dietary guidelines have been aimed at addressing micronutrient deficiency and not diet quality therefore leading to the consumption of unhealthy food which contributes to

development or progression of NCDs in the general population (25). If interventions are implemented that raise awareness on healthy food choices, a reduction in disease burden may occur which could result in decreased demand for healthcare facilities and hospital beds, thus relieving already over-burdened healthcare facilities (45,56).

It should also be noted that a nutritional imbalance leads to malnutrition. Malnutrition has traditionally been defined as a lack of calorie intake or nutrient deficits, with two major disorders highlighted under this umbrella: namely marasmus and kwashiorkor. However, the word malnutrition today encompasses of health disorders induced by either a lack of macronutrients or an excess of micronutrients (67).

Malnutrition is now classified into three distinct categories, according to WHO guidelines:

1. Undernutrition defined as low weight-for-height or age or low height-for-age;
2. An imbalance of micronutrients or macronutrients;
3. Overnutrition defined as overweight or obesity.

In South Africa evidence of the nutrition transition was shown through improved socio-economic growth over time which contributed to improved lifestyles, good health indicators and significant demographic changes (61,62,68,69). Nutrition transition in South Africa was followed by an epidemiological transformation from predominantly infectious diseases to a predominantly chronic degenerative diseases indicating shifts in disease profiles (62). The consequences of dietary changes are further compounded by reduced PA, stressful lifestyles, high intake of alcohol and tobacco use, according to the WHO (9)

There is a plethora of knowledge accentuating rapid nutritional changes in LMICs, including South Africa. Dietary changes and growing obesity and overweight rates (BMI>25.0 kg m^{-2}), have contributed to an increase in NCDs in South Africa since 1980. Consumption of energy went from 2 603 kcal/day to 2 921 kcal/day between 1962 and 2001 according to data from the FAO (21). Furthermore, the amount of fat, protein, and carbohydrate per person increased as well (21).

1.4 NUTRIENT PATTERN ANALYSIS

The complexity of the human diet is demonstrated by nutritional pattern analysis research, a complementary traditional method for assessing the cumulative and interaction impacts of various nutrients consumed (70–72). A nutrition assessment includes anthropometric measures as well as data on an individual's health history, clinical and biochemical traits, dietary patterns, current medication and SES.

It is crucial to analyse nutritional status in order to determine whether an individual has nutritional imbalance as a result of an underlying condition or whether they are vulnerable to acquire a chronic illness as a result of nutritional imbalance. It must be noted that the conventional dietary analysis approach which examined single nutrients and associations to body parameters, has limitations in its ability to demonstrate how nutrient intake affects NCD outcomes because it is difficult to comprehend how different nutrients interact with one another. Furthermore, it is difficult to discern minute effects from different nutrients (72–74).

Principle component analysis is a growing technique in nutritional epidemiology. In recent years made a major shift to the assessment of nutrient patterns, transitioning from the traditional individual food and nutrient technique that was used to determine the possible combined effect of foods and nutrients (5,70–72,75,76). Nutrient patterns can be derived using either an a priori (derived from a hypothesis) or an a posteriori (derived from data) method. The a posteriori technique, is appropriate for presenting an accurate depiction of the variance in nutrition in a particular community. Principal component analysis (PCA), a data-reduction technique based on correlation or covariance matrices of the original variables, is the approach that is most frequently used for these investigations. The outcome results is extraction of a number of nutrient patterns or principal components (PC) that explained the majority of the observed nutrient variance. This is conducted by using the correlation matrix and factor analysis. Nutritional data is first normalized followed by applying the Kaiser-Meyer-Olkin test for reproducibility of the factor analysis (4,70,71).

1.5 ASSESSMENT OF OBESITY

Since 1980, the prevalence of excessive weight gain has more than doubled globally, with almost one-third of the global population categorised as obese or overweight status. This has caused significant public health concerns for policy makers worldwide (77). Obesity rates have skyrocketed in both men and women of all ages, with older people and women bearing a disproportionately higher burden (78,79). While this trend is global, absolute prevalence rates vary by region, country, ethnicity and SES, with higher-income and certain middle-income countries experiencing slower rates of BMI increase compared to LMICs.

It has been well documented that obesity has a negative impact on older individuals morbidity and mortality, as well as their quality of life and increased probability of hospitalisation (16,20,80). Additionally, it has been well documented that increased PA may result in weight-loss leading to decreasing BMI. Interventions such as diet changes, may also have a positive impact on physical functioning in older adults with obesity which may result in increased PA (16,20,81). Translating research findings and incorporating both PA and nutritional recommendations is critical for improving health in an ageing population. This might consequently contribute to improved health in this population and a reduced burden on the healthcare system, releasing funds for other essential healthcare or health systems strengthen projects where appropriate (16,20,80)

Obesity prevalence rates have been increasing and for South Africa, these rates are higher than the global targets as shown in figure 7 (82).

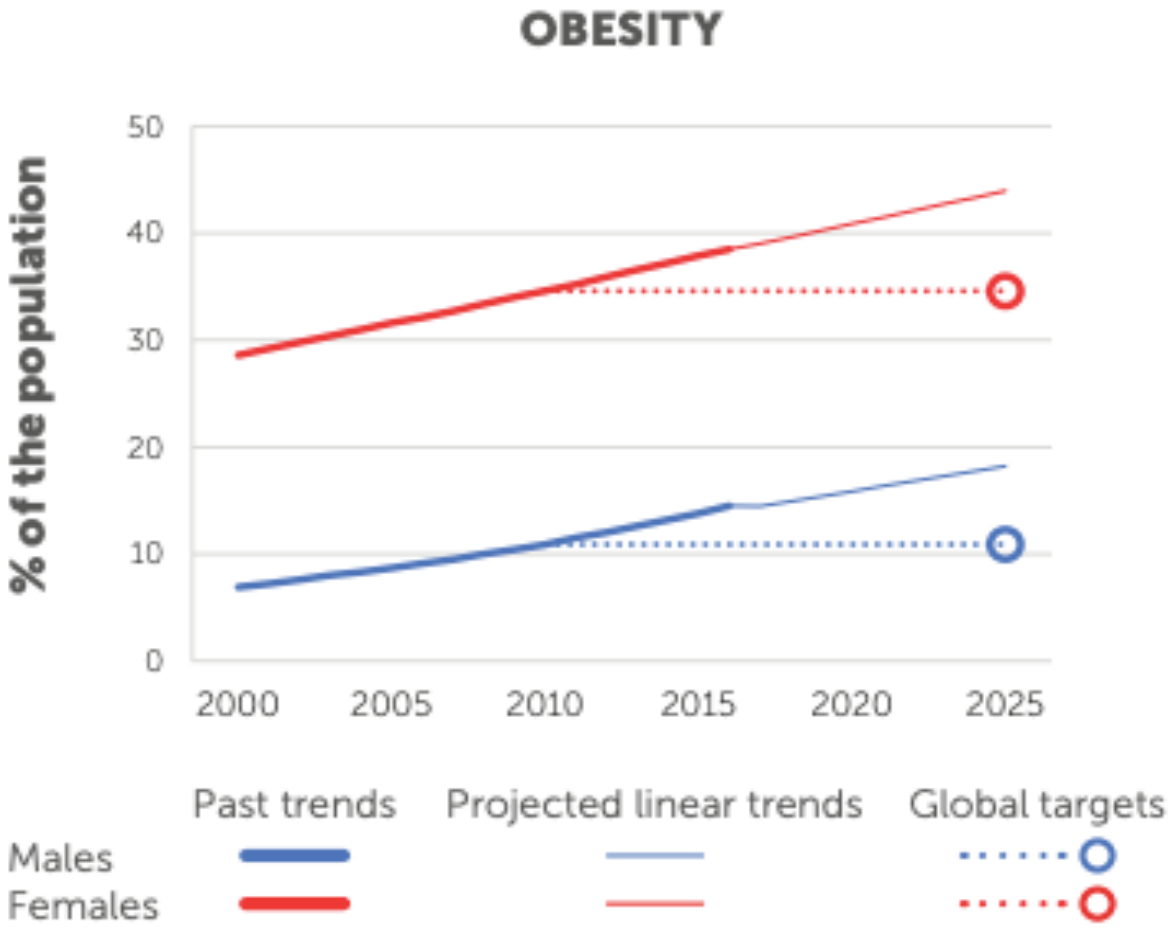

Figure 7 Obesity epidemiological trends in South Africa (Source: WHO, 2018).

According to the 2016 Demographic and Health Survey, in South Africa approximately 30% of women have a normal BMI, 3% are underweight, 27% are overweight (BMI 25.0-29.9), and 41% are obese (BMI 30 or above). (83). When we look at ethnic and gender overweight disaggregation, the black population has the greatest obesity rates when compared to other ethnic groups (figure 8) (84).

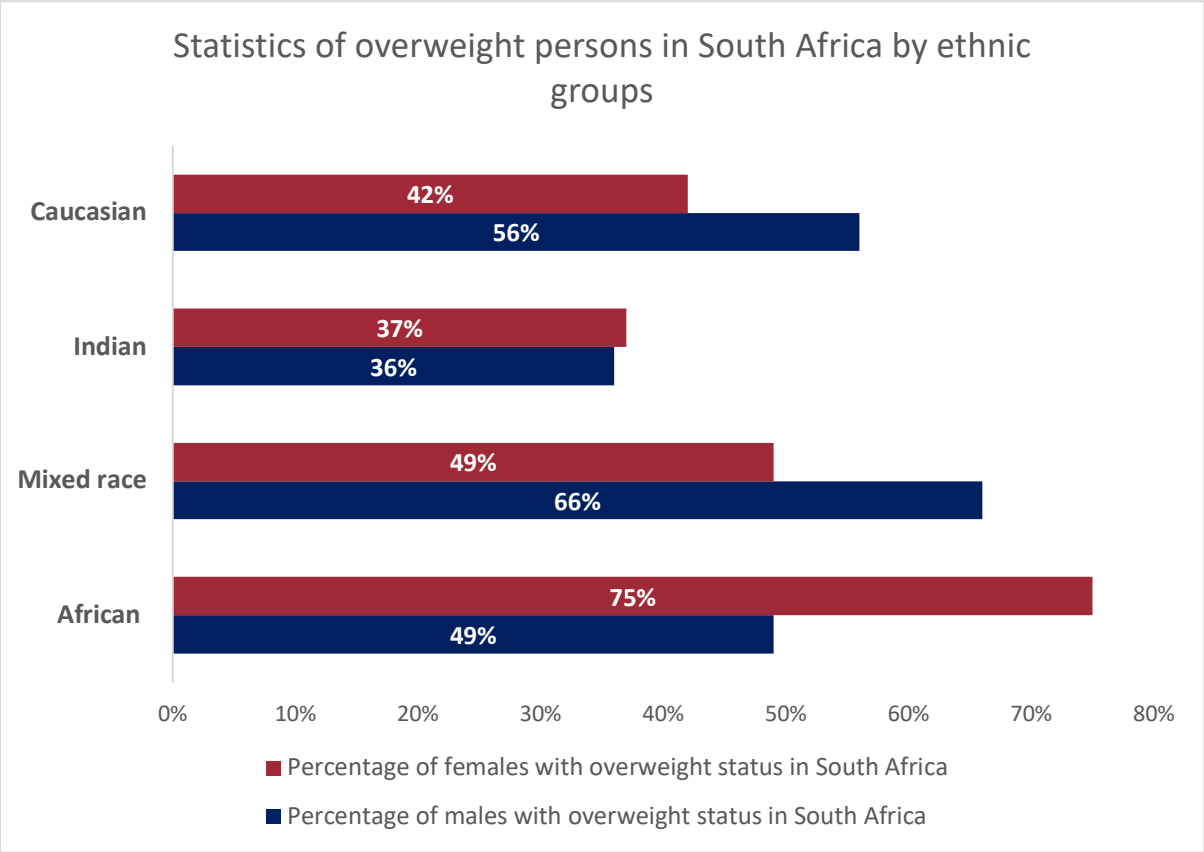

Figure 8 Statistics of overweight persons in South Africa by ethnic groups (Source: Talbot and Pienaar, 2012).

Approximately 75% of the South African population is black (85) with approximately 75% African women considered to be classified as having overweight status (86).

Obesity patterns are changing, according to new studies, and the rising obesity prevalence among the individuals with lower SES has important implications on the healthcare system. Moreover, the challenges confronting the South African health-care system are threefold. First and foremost, the population's health requirements exceed capacity and resources, next, the vast majority of people are unaware of their current state of health

which delays access to care and finally, the manner in which the healthcare system is funded reinforces inequity. With such a large proportion of this vulnerable population group that also reports the largest overweight or obese prevalence rates, results from a PhD study like this will most likely not only benefit this population but relieve the burden in healthcare facilities.

This study is timely as following the Coronavirus pandemic that occurred in 2019, Centres for Disease Control and Prevention reported that not only did the older population have the highest risk of severe illness from the Coronavirus disease, they also reported the most deaths (87). With 35 to 50% of this target population reported to be unvaccinated due to various reasons, efforts must be re-directed to other strategies that can be implemented immediately to improve this populations health (88).

1.5.1 Body mass index (BMI)

This is an inexpensive and fast approach to categorise body composition into the BMI categories based on their weight and height (89). The National Institute of Health (NIH) now employs BMI to categorize people as underweight, normal weight, overweight, or obese, rather than the traditional classification approach which used height versus weight charts (8). Although obesity prevalence rates in some countries, such as the United States of America, are steadily declining or levelling off, South Africa remains one of the countries with the highest obesity prevalence rates which is increasing with a projected increase in obesity among females of 48% by 2025 from 2022. (90,91).

1.5.1.1 Limitations of BMI

It is important to think about the clinical limitations of BMI. Due to the fact that BMI measures excess body weight rather than excess body fat, it serves as a substitute for measuring body fatness. The correlation between BMI and body fat might vary depending on a number of characteristics, including age, sex, ethnicity, and muscle mass. Additionally, calculating BMI does not show how fat is distributed among people or make a distinction between excess fat, muscle mass, or bone mass. For example, on average, older adults tend to have more body fat than younger adults for an equivalent BMI; women have higher total body fat percentage than men with an identical BMI; and individuals with much more muscle mass compared to fat mass may have a higher BMI (8,90). Having a greater proportion of visceral, liver, and muscle fat has also been linked to an increased risk of CVD (34,92). Obesity may be far more pervasive than epidemiological studies have previously shown, and as a result, more immediate action must be taken. Relying solely on BMI to determine obesity prevalence may cause future obesity prevention and control initiatives to be delayed (8).

1.5.2 Whole Body Fat Mass Index (FMI)

With the advent of Dual-energy X-ray Absorptiometry (DXA), the whole body fat mass index (FMI) is one of the most critical factors determining well-being (93,94). FMI is a ratio that looks at fat mass (subtotal whole body less head) to height squared (93). Results showed that while BMI is the most common method used in clinical and epidemiological studies to

assess obesity, it does not directly calculate body fat. It has been shown that FMI provides an accurate assessment of adiposity in addition to being convenient (95,96).

1.5.3 Visceral Adipose Tissue (VAT)

Visceral adipose tissue (VAT) which is fat lining internal organs, found deep in and around the abdomen's inner organs, is good predictor of cardiometabolic health (97,98). Type 2 Diabetes and CVD cause-mortality, and are correlated with excess VAT storage which occurs with growing age. The assessment of the impact of modifiable lifestyle factors such as PA and diet on the VAT deposit can help to establish preventive strategies to combat obesity-related morbidity or mortality.

The key determinant of insulin resistance is visceral fat, and several studies have found associations between excessive visceral fat and an increased inflammatory condition (98). Rather than extra-abdominal (subcutaneous) fat obesity, there is a significant association between increased morbidity and abdominal fat obesity .(97) It has been found that abdominal visceral fat and subcutaneous adipose tissue (SAT) are both associated with cardiovascular risk factors, although VAT has been shown to have the greatest association.

1.5.4 Subcutaneous Adipose Tissue (SAT)

Subcutaneous adipose tissue is the adipose tissue under the skin and this usually increases during development as a child and also varies according to menopausal stage, as SAT storage in pre-menopausal women is higher (97). Subcutaneous adipose tissue, much

like VAT, is closely linked to obesity-related problems, however, the link between SAT and metabolic syndrome (MetS) remains unknown (72).

1.6 CONTRIBUTION OF BODY FAT DISTRIBUTION TO CARDIOMETABOLIC DISEASE

One of the most significant risk factors for cardiometabolic disorders is the distribution of fat in the body. Cardiometabolic risk factors, in particular, appear to be closely associated to VAT (98). Approximately 10% to 30% of individuals having obese status do not have obesity-related cardiometabolic disorders. Although substantial amounts of VAT are associated with an increased risk of having cardiometabolic illness, it should be noted that not all individuals having obese status have cardiometabolic conditions (98).

1.6.1 Changes in body composition over time

Body composition changes are caused by nutritional energy imbalances differences as well as ageing, even without concomitant changes in body weight and BMI (8,90). Body changes due to nutritional energy imbalances occur when either there is a positive energy balance from higher consumption of nutrients resulting in weight gain or weight loss as a result of decreased nutrient consumption. However, in the absence of weight fluctuations body composition changes associated with ageing may still occur (8,89).

As people grow older, body fat increases, while bone mineral density and lean mass decreases (89). Abdominal fat mass (FM) is also on the rise, which is associated with an increased risk of T2D and CVD (93–95). The Florey Adelaide Male Ageing Study's findings showed that an that an increase in general FM resulted in increases in abdominal FM (99).

Moreover, many cross sectional and longitudinal studies have shown that ageing is associated with changes in energy consumption (20,100,101). According to research by Otsuka et al., women over 40 have a 9.1 kcal/year decline in their energy intake (EI) (101)3.

1.6.2 Impact of ageing on energy consumption

Relative to an individual's daily energy consumption, the resting metabolic rate (RMR) is defined as the rate at which one's body utilizes energy while at rest. A previous study, after adjusting for fat-free, soft tissue mass (FFSTM) and gender, showed that RMR was lower in the older population when compared to a younger population (102,103). Cross-sectional studies have also found that RMR decreases with ageing (103).

Most studies explored the associations between ageing, RMR, and body composition parameters and concluded that in older age, RMR decreases relative to a younger age, beyond what changes in body composition may explain (102,104–106). In older adults, the lower RMR may be due in part to slow metabolic rates of the organs, leading to improvements in FM and FFSTM occurring with ageing. As a result, as people age, the required daily energy expenditure also decreases.

1.7 SUMMARY AND RESEARCH GAPS

1.7.1 Summary and gaps in literature

This PhD literature review identified the following research questions:

1. The association of baseline nutrient patterns with body composition indices in middle-aged black African women;;
2. Assess if nutrient patterns of middle-aged black African women change after 5.5-yearss
3. Examine if BMI mediates the relationship between nutrient patterns and blood pressure

Over the last few decades, the steady global rise in obesity prevalence has broadened the epidemiological study horizon. To date, no research in South Africa has investigated longitudinal nutrient behaviours of middle-aged black women and whether BMI mediates the relationship between repeated measures of nutrient patterns and blood pressure. This PhD research is novel and results from this research will contribute to the body of literature and make some recommendations for either public health policy interventions or improvements on research study design.

1.7.2 Relevance and justification

The dramatic rise in the incidence of adult obesity in LMICs is widely recognized as a public health concern. According to current evidence, South Africans are not excluded from the obesity pandemic (12,78,107–111). For these reasons, the need for continuous research into the many complexes and interactive factors in the development and progression of obesity and cardiometabolic disease in adults has been advocated both regionally and globally, with the goal of:

1. Predicting the evolution of epidemics of obesity,

2. Comprehension of its aetiology for development of successful, focused potential interventions in order to slow development of obesity and or cardiometabolic disease,
3. Minimize and avoid the increasing prevalence of NCDs.

South Africa is a well-established country that underwent nutritional transition, and studies have shown that obesity-related risk factors and resulting NCDs are growing (12,107,110). In Western women, there is an abundance of nutritional research and its effects however, there is a paucity of research on longitudinal nutrition consumption in the black South African population and how it contributes to body composition indices and HTN. Such studies include research on how nutrition is correlated to body composition or the risk of MetS and these studies are important as this population group has high prevalence rates of obesity, diabetes and HTN.

Results from this study can identify the course of future research and interventions from the perspective of public health as well as influence policies and nutritional guidelines for black middle-aged women. As the majority of the South African population is black, findings from this PhD research may influence public health policy not only for South Africa, but results may be adopted to other African countries with similar settings.

1.7.3 Aims and objectives

Study aim

This Ph.D. study aimed to investigate how nutrient patterns correlates to body composition at baseline and after a 5.5-year follow-up period. Furthermore, this study aims to examine if nutrient patterns of black middle aged women change over time and if BMI mediates the relationships between repeated measures of nutrient patterns and blood pressure.

1.7.4 Study Objectives

The following objectives will be explored in this study:

Objective 1: An investigation of the nutrient patterns and body composition parameters of middle-aged black South African women in Soweto.

Objective 2: To examine the long-term relationship between nutrient patterns and obesity in a group of middle-aged black South African women over 5.5- yearss.

Objective 3: To elucidate the longitudinal association of nutrient patterns and blood pressure and to explore whether this is an indirect effect mediated by BMI

Due to the nature of this thesis which includes two published peer-reviewed manuscripts with the third in draft format, there may be some duplication of information, particularly in the introduction and discussion sections of the papers as well as the overall thesis.

CHAPTER 2 – METHODOLOGY

2.1 INTRODUCTION

This chapter describes the methods used to collect data for this PhD research. The peer reviewed published articles had similarities in methodology, which are described in more detail in this chapter. If there are any deviations in the methodology, these will be detailed in the respective results chapters. The statistical analysis varies across the three studies and will be discussed in the relevant results chapters. All questionnaires that were used for data collection were conducted in English for standardization and consistency.

2.2 STUDY SETTING

Figure 9 Map of South Africa showing the location of Soweto where the MASC (formally BT20+ cohort) is taken from (Source: Richter, L.M.et. al. (2007).

South Africa is classified as a LMIC with a different range of housing types, rural farms, impoverished peri-urban areas and undeveloped rural areas. Soweto (figure 9) is a peri-urban township in Johannesburg which is located in the Gauteng province of South Africa (112). It was originally established in the 1930s as a district to house the black African miners employed in the surrounding gold mines. Soweto grew to be South Africa's largest city with the highest proportion of black South Africans (approximately 98.5% of residents in Soweto are black South Africans) and 37% are Zulu speaking followed by Sesotho (18%) and Setswana (12%) (85). Although local townships tend to include a mix of wealthy and poorer residents, many portions of Soweto rank among the lowest SES in Johannesburg (107,113). The population of this township increased by 50% between the 2001 Census (population estimated at 860,000) and the 2011 Census (population estimated at 1.3 million) (114).

2.3 MIDDLE-AGED SOWETO COHORT (MASC) STUDY (FORMERLY BT20+)

The MASC study, is Africa's biggest and longest running study of child and adolescent health. Initially, study participants included 3273 children who were born between April and June of 1990, who have been tracked throughout their lives, as well as the caregivers that raised them (112,115). The original study questioned the pregnant women on their overall

demographic features as well as the pregnancy's conditions and data were collected annually which included PA, education, SES and dietary consumption.

Three generations have already joined the MASC cohort, spanning all stages of development. In 1990, Generation 1 birth mothers (1G) gave birth to Generation 2 (2G), and the 2G cohort is now having their own children (Generation 3; 3G). One of the challenges that the project faces is that many of the participants have moved out of Soweto and are no longer able or willing to participate. Nonetheless, the study has continuously collected data with approximately 60% participants still in the study as of 2019.

In 2003and 2013, the BMI categories for the MASC women cohort are classified as demonstrated in figure 11 where less than 25% have been classified as healthy status in 2003 and 2013 (116).

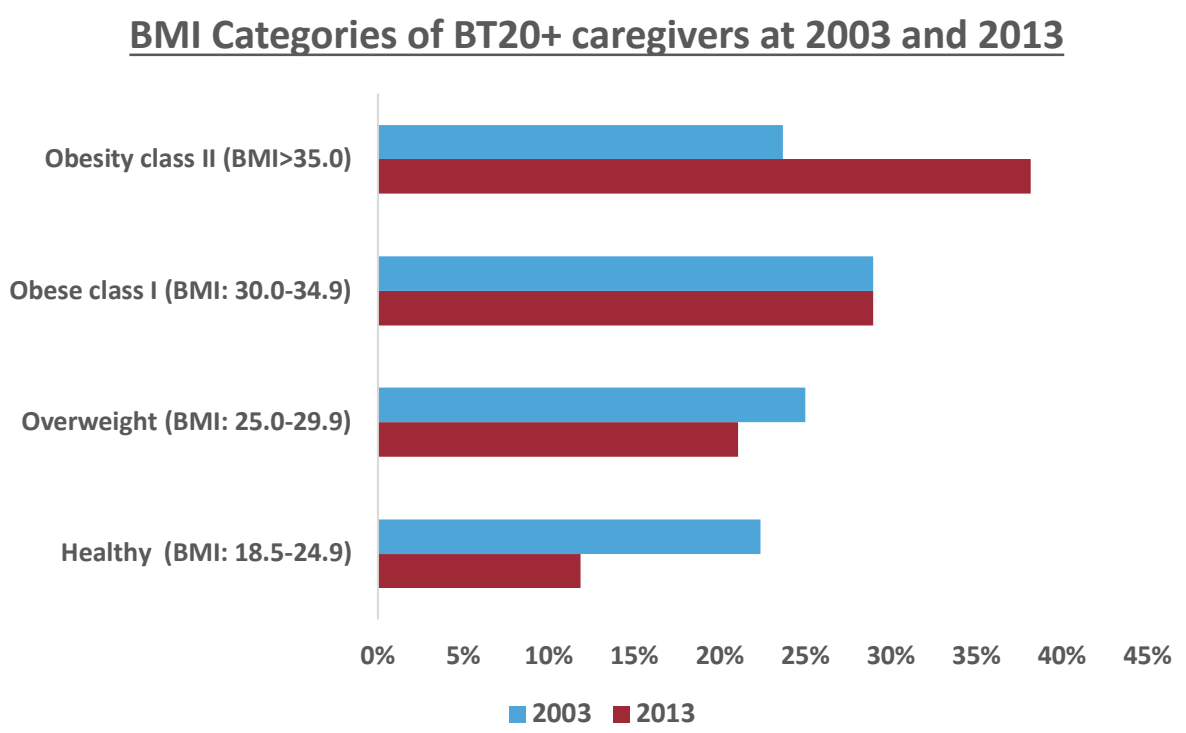

Figure 10 Distribution of BMI in MASC caregivers cohort (Source: Micklesfield no date).

2.4 STUDY OF WOMEN ENTERING AND IN ENDOCRINE TRANSITION (SWEET)

The Study of Women Entering and in Endocrine Transition (SWEET) study, a sub-study nested in the MASC cohort research (117). This study recruited women who were caregivers and biological mothers of the children in the MASC cohort study as participants in this study and as part of the data collection, dietary information was collected from these women and this formed the first wave of nutritional data collection for this PhD study.

These women were invited to participate in a nutritional research study, consented and had baseline nutritional behaviours, anthropometry and BLOOD PRESSURE data collected (Appendix 1). At 5.5-year follow-up, these same women were invited to participate in a new

follow-up study called the GlaxoSmithKline (GSK) study and in that study, participants were also asked for the dietary information using the same tool and this formed the second wave of data collection for this PhD study. Ethical clearance was granted by the University of the Witwatersrand HREC (Medical) committee for Bt20+, SWEET and this PhD with ethics certificate numbers M010556, M090620 and M170718 (Appendix 2, 3 and 4 respectively).

General methods used in all of the publications

2.5 DIETARY DATA COLLECTION: FOOD FREQUENCY QUESTIONNAIRE

A standardised Quantitative Food Frequency Questionnaire (QFFQ), which was designed to collect a seven-day dietary intake, was used to collect data on dietary consumption in the South African population (Appendix 5). This QFFQ was designed from surveys conducted since 1983 (118). The QFFQ includes 214 food items that are indicative of foods eaten by the South African population. This method has been extensively used for the same population as previously reported (4,76,119,120)

Interviewers used high-resolution photographs of foods and beverages during participant interviews to assist with recall of foods and beverages consumed in the seven days prior to data collection. Frequency and quantity of food or beverages consumed during the past seven days, consumed only on rare occasions, and those never eaten were recorded and the process flow for this is depicted in figure 12.

Figure 11 Process flow for QFFQ interviews.

The QFFQ took approximately 40 to 60 minutes to complete. Data was also gathered on how often and how much each participant had eaten over the course of the preceding seven days. The participant was asked to estimate habitual intake by selecting the most accurate representation of their portion size from either two-dimensional actual size drawings of foods, household utensils or three-dimensional validated food models as described and validated by Steyn et. al. (121). The average daily calorie consumption for the previous seven days was calculated by converting these household data into grams. The QFFQ was captured and managed in REDCap, (Research Electronic Data Capture). REDCap is an electronic data capture tool hosted at University of the Witwatersrand and is a secure, web-based application designed to support data capture for research studies, providing (122):

1) an intuitive interface for validated data entry;

2) audit trails for tracking data manipulation and export procedures;

3) automated export procedures for seamless data downloads to common statistical packages; and

4) procedures for importing data from external sources.

Nutrient composition (energy intake, micro- and macronutrients), averaged over seven days, was computed using the program FoodFinder3 offered by the SAMRC, which converts specific food items to nutritional compositions (123). As indicated by Vorster et al., subjects having a total energy intake of less than 3000kJ and more than 30 000kJ were excluded from the study (124,125).

Using the ratio of energy intake (EI) to estimated energy ratio (EER) (EI:EER), we were able to determine the accuracy of reporting energy intake (126). As indicated in Equation 1, the Institute of Medicine (IOM) gender specific equations were used to compute the estimated energy requirement (EER) for the participants. This study used a PA factor of 1.12, derived from the ratio of total energy expenditure and basal energy expenditure, as a measure of PA in view of the moderate PA that has been previously reported for this population (127).

Equation 1 Estimated energy requirements for middle-aged women

$$\text{EER} = 354 - (6.91 * \text{Age (years)}) + \text{physical activity } (9.36 * \text{Weight (kgs)}) + (726 * \text{Height (m)})$$

The EI:EER ratio was used to categorize dietary energy intake reporting. Participants with EI:EER ratio less than 0.7 were classified as under-reporters, 0.7 to 1.42 plausible reporters,

and greater than 1.42 as over-reporters (3). The plausible reporters were utilized as a reference group in multivariate linear regression models.

2.6 ANTHROPOMETRIC BODY MEASUREMENTS

For anthropometric measures, participants were encouraged to dress in minimal clothing and be barefoot and there were no other pre-specified criteria for anthropometric measurements. A wall-mounted stadiometer was used to measure height, which was reported to the closest 0.5cm and weight was measured using a digital scale and quoted to the closest 0.1kg. The participants height and weight measurements were then used to calculate BMI. A flexible and inelastic measuring tape was used to measure the waist and hip circumferences. The narrowest region of the trunk was measured as the waist circumference, and the largest circumference around the hip was measured as the hip circumference, with each participant standing with their feet together and their abdomen relaxed. Data was recorded on the participant questionnaire (Appendix 6).

2.7 DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) SCANS BODY MEASUREMENTS

Dual-energy X-Ray Absorptiometry (DXA scans were conducted to measure Fat-Free Soft Tissue Mass (FFSTM). This was done by taking scans of the whole body minus the head to make provisions for hair extensions worn by many of the participants as study researchers did not ask participants to remove their hair extensions as most times, these could not be removed and were shown to have similar density as soft issue which can affect results. Dual-

energy X-Ray Absorptiometry uses low level x-ray beams that are absorbed by lean muscle, fat, bones and soft tissues and the difference in absorption is used to calculate body composition and bone mineral density.

Dual-energy X-Ray Absorptiometry scans were performed using a Hologic Discovery A (S/N 83145) and with by a single trained technician. Every morning, quality control of the DXA was conducted by scanning a DXA phantom and ensuring that the coefficient of variation (CV) was below 0.5% across all parameters.

Whole body scans (whole body minus head) FM and FFSTM were used to calculate FMI and lean mass index (LMI). Fat mass was used to calculate FMI (FM (kg)/ baseline height (m^2)) and lean mass was used to calculate LMI (whole body lean mass (kg)/ baseline height (m^2)) (128) (CVs for DXA parameters were <2% for total FM and 1% for FFSTM).

2.8 PHYSICAL ACTIVITY (PA)

Self-reports of PA were gathered through the use of the Global Physical Activity Questionnaire (GPAQ). Individuals were divided into two groups at baseline and follow-up based on the GPAQ criteria (129). Physical activity data was recorded on the participant questionnaire (Appendix 6). In order to be considered physically active, participants had to engage in at least 150 minutes of moderate weekly PA (five days per week), 60 minutes of vigorous weekly PA (three days per week), or 600 Metabolic Equivalents (MET) minute weekly moderate-vigorous physical activity (MVPA) (five days per week)(130) Participants who did not meet these requirements were considered inactive.

2.9 SOCIO-ECONOMIC STATUS (SES) AND LIFESTYLE DATA

Details of SES were collected at baseline and at follow-up and questionnaires were administered by research assistants. Household presence of electricity, radio, motor vehicle, fridge, washing machine, telephone, microwave and electronic media network (MNET) were used to determine the SES of households. Other socio-demographic variables collected included age, work status (yes/no), and highest level of education attained.

2.10 METHODOLOGIES UNIQUE TO EACH PUBLICATION

Individual methodology chapters for each publication detail precise information about the unique methods that were used.

2.11 STATISTICAL ANALYSIS

Each article (Chapters 3 to 5) outlines and discusses the statistical approach followed in the methodology chapter of the publication. It must be noted that only Chapters 3 and 4 have been published in a peer reviewed journal and Chapter 5 is in draft mode.

Part 2: Empirical Chapters

Nutrition is vital not only for sustenance but for a healthy life –

McNaughton et al., 2012

CHAPTER 3: PAPER 1

(Appendix 7)

3.1 ABSTRACT

Obesity is more prevalent in black South African women than men. However, little is known about the nutrient patterns associated with body composition indices in black African women. Principle Component Analysis was applied to 25 nutrients derived from QFFQs in 498 middle aged black South African women. Three nutrient patterns, the plant driven, animal protein driven and Vitamin C, sugar and potassium driven nutrient patterns, accounted for 59% of the variance of nutrient intake. Multivariable linear regression using the body composition parameters as outcome variables indicated that a standard deviation increase in the animal protein driven nutrient pattern was significantly associated with increases in BMI (1.29 $kg \cdot m^{-2}$ (95% CI, 0.54-2.04; $p = 0.001$), SAT (26.30 cm^2 (7.97-44.63); $p = 0.005$), (VAT (9.88 cm^2 (5.13-14.63); $p < 0.001$), VAT/SAT ratio (0.01 (0.00-0.02); $p = 0.018$), whole body FMI (0.74 $kg \cdot m^{-2}$ (0.25-1.22); $p = 0.003$), and whole body LMI (0.53 $kg \cdot m^{-2}$ (0.23-0.83); $p = 0.001$). An increase in plant driven nutrient pattern was significantly associated with an increase in SAT of 20.45 cm^2 (0.47-40.43); $p = 0.045$. This study demonstrates that animal protein driven nutrient pattern, characterised by the consumption of more animal protein and fat nutrients, similar to the western diet is associated with increased body fat and lean mass.

Keywords: African women; South Africa; nutrient patterns; obesity.

3.2 INTRODUCTION

The obesity pandemic remains a growing global concern, having a direct impact on NCDs such as HTN, T2D, and CVD (131). The prevalence of obesity is increasing in LMICs such as South Africa, in part due to the ongoing nutritional transition and rapid urbanisation (62,110). In a study conducted in black South African women residing in Soweto, Johannesburg, the prevalence of obesity was found to be 67.8% and the prevalence of morbid obesity (BMI ≥ 40) was 16.8% (28). Similarly, results from the South African National Health and Examination Survey (SANHANES-1) conducted in 2012 reported that 40.1% of women were classified as having obese status and 25.0% were overweight. These rates were higher than those in males, who reported obesity and overweight prevalence rates of 11.6% and 19.6%, respectively (132). It is therefore pivotal to understand the determinants of obesity and body composition parameters that increase NCD risk in black African women as they carry a larger proportion of this burden compared to men.

Obesity is thought to develop from the interaction of genetic, environmental, and modifiable lifestyle factors such as diet and physical activity (133,134). South Africa has experienced a shift from a more active lifestyle to a sedentary lifestyle, associated with increased overweight and obesity rates (62). The adoption of a more Western diet, which mainly consists of a diet high in total and saturated fats and sugars and low consumption of whole grains such as legumes and home grown vegetables, is becoming prevalent (62). More nutrition studies are now being conducted to understand the association of diet and obesity among South Africans.

A number of studies have focused on single nutrients and foods in evaluating the association of diet with obesity (39,47,135–137). However, since people eat a combination of different foods, it is important that studies analyse nutrient pattern rather than individual food types. As proven in two studies conducted in the United States of America, nutrient pattern analysis allowed the effect of a complete diet on health as well as body composition parameters such as obesity status to be analysed (71). In view that nutrients are universal, the nutrient patterns can be compared across varied ethnicities. Only one study has evaluated the association of 68utriant patterns with obesity in a black South African population and this was performed in adolescents (138). However, findings from this study might not be transferrable to adults as these two groups are likely to have different eating patterns (86,139). Therefore, more studies assessing the nutrient patterns association with body composition are required in black South Africans, particularly women, who carry the largest burden of obesity. Therefore, our study aimed to evaluate the association of nutrient patterns with body composition parameters in middle-aged black South African women.

3.3 MATERIALS AND METHODS

3.3.1 Study Population and Setting

We adopted a cross sectional study design nested in the longitudinal Bt20+ study, now referred to as the MASC study, which is based at the Developmental Pathways for Health Research Unit, Chris Hani Baragwanath Hospital in Soweto, Johannesburg (112,115). For the

first aim of this PhD study, data collected from 498 black South African female caregivers from the MASC study and the SWEET sub-study was used (117). From the original cohort, 902 women were invited to participate and of this, 702 women were eligible for the SWEET study using the following exclusion criteria: <40 years and >60 years, pregnant, did not give consent, and ethnicity other than black African (117). Within this group of 702 women, 498 women had nutrition related data and therefore were included in this study.

In order to determine whether a sample size of 498 was sufficient to meet the aims of our study, we used the guidelines for factor analyses produced by Mundfrom et al. (140) and Kline (141). According to the method of Kline, a minimum sample of 100 participants is sufficient while Mundfrom et al. recommends a minimum sample size of 160. According to both these guidelines, the sample is adequate for this study.

3.3.2 Dietary Intake

Dietary intake data were collected using a standardised QFFQ, designed for the South African population (142). The QFFQ consists of 214 food items that are representative of foods consumed by this population. This tool has been piloted and utilised extensively for the same population as described elsewhere (119,120,143). Trained interviewers used high quality photographs of food items to assist with participant recall of food and beverage items consumed during the last seven days. The participants were asked to arrange the cards into three piles: foods eaten in the last seven days, occasionally, or never eaten and

this was recorded. In the case of food items consumed in the last seven days, additional data on the frequency and quantity of consumption was collected. The participants were asked to estimate their habitual intake by selecting the most accurate representation of their portion size from either two dimensional actual size drawings of foods, household utensils, or three dimensional validated food models as described and validated by Steyn et al. (121). Although this validation has been conducted in the South African adolescents population, the validation is applicable to the South African adult population. These household measures were then converted to grams for the computation of average intake over a seven-day period. The QFFQ took approximately 40–60 min to administer and was captured and managed using the Research Electronic Data Capture (RedCap) software package, an electronic data capture tool hosted at University of the Witwatersrand (122).

Nutrient intake was calculated from the conversion of single food items using the nutrient analysis software FoodFinder3 developed and hosted by the SAMRC (144). Over- and under-reporting of dietary EI was corrected by removing participants with total EI that was <3000 and >30,000 kJ (124). Plausibility of EI reporting was evaluated using the ratio of EI to EER (3). EER was calculated using the IOM gender specific equations using the participant's age, weight, height, and PA as shown below.

EER = 354 – ((6.91·Age (yrs)) + PA·(9.36·Weight (kgs)) + 726·Height (m)).

A PA factor of 1.12 arising from the ratio of total energy expenditure and basal energy expenditure was considered for this study in view of the moderate physical activity that has

been reported for this population. Dietary EI reporting was classified using categories derived from the EI:EER ratio. Participants with EI:EER ratio less than 0.7 were classified as under-reporters, 0.7 to 1.42 plausible reporters, and greater than 1.42 as over-reporters (3). In multivariable linear regression models, the plausible reporters were used as the reference group.

3.3.3 Body Composition

3.3.3.1 Simple Body Anthropometry

Height and weight measurements were assessed while participants were barefoot and wearing light clothing. Height was measured using a wall mounted stadiometer (Holtain Ltd., Crosswell, UK) and reported to the nearest 0.1 cm and weight was measured using a digital scale (Dismedinc., Anjou, QC, Canada) and reported to the nearest 0.1 kg. These measurements were used to calculate BMI. A soft measuring tape was used to measure hip (greatest circumference at the hips) and waist (smallest girth above the umbilicus) circumferences to the nearest 0.5 cm. Trained technicians performed all body measurements.

3.3.3.2 Dual-Energy X-ray Absorptiometry

Dual-Energy X-ray Absorptiometry scans were performed using a Hologic (Londonderry, NH, USA) by a single trained technician. These scans were conducted to measure VAT and SAT. The only specified criteria was that participants remove clothing and all metal objects, wore

surgical gowns for the procedure, and had whole body scans analysed using whole body less head as many participants wore wigs or hair weaves that could not be removed and these hairpieces are similar in density to soft tissue and may have caused measurement artefact. During data collection, the DXA phantom was scanned each morning to examine the CV of the DXA machine which were <2% for total fat mass and 1% for fat-free soft tissue mass. Furthermore, for this study, participants had FM and lean mass measured as described in a previous study (145). Fat mass was used to calculate whole body FMI (fat mass (kg)/height (m^2)) and lean mass was used to calculate whole body LMI (whole body lean mass (kg)/height (m^2)) (128).

3.3.4 Statistical Analysis

Data were analysed using SPSS Statistics software version 25 (IBM, Chicago, USA) and STATA version 15 (Statacorp, Texas, United States) (146,147). Normality tests were conducted using Q-Q plots for continuous variables. These variables are described using median and interquartile range (IQR) for non-parametric data or mean ± standard deviation (SD) for normally distributed data.

Principal component analysis was applied to the 25 nutrients derived from the QFFQ. Total available carbohydrates were divided into starch and total sugar. The nutrients were log transformed to remove the bias due to the different scales used to quantify the nutrients. The nutrient density approach of dividing the nutrient intake by the total EI was adopted to adjust for energy intake variation (148). This was used to capture variability of nutrient

intake independently from changes of energy intake. The PCA procedure was performed using the covariance matrix of the log transformed nutrients that were selected to capture the whole dietary intake as previously reported by Pisa et al. (138). Varimax rotation was performed to improve the interpretability of the factor loadings (149). Three PCs representing three independent nutrient patterns were selected according to the percentage of total variance explained, their eigenvalues, and visual presentation on the scree-plot of eigenvalues (Appendix 7A).

Multivariable linear regression models were performed to assess the association of the selected body composition parameters as outcome variables and nutrient patterns as predictors while adjusting for dietary intake reporting (under-, plausible-, and over-reporting), employment status, age, PA, and smoking status. Statistical significance was accepted at a level of $p < 0.05$.

3.4 RESULTS

3.4.1 Descriptive Characteristics

Of the 498 women, 11.2% were underweight or normal weight (Table 1). The body composition indicators VAT, SAT, FMI, LMI, gynoid fat, and hip and waist circumference were significantly higher in individuals classified as having overweight or obese status compared to the lean participants ($p < 0.001$ for all comparisons). The VAT/SAT ratio was not different between the groups ($p = 0.171$). Most of the women (65.4%) were classified as

physically active and only 2.8% were active smokers. The proportion of EI from dietary carbohydrate and fat was similar across the groups (54.4% and 53.5% for carbohydrate intake and 31.3% and 30.1% for fat intake for lean and overweight/obese participants, respectively).

Table 1 Descriptive characteristics for the study population disaggregated by BMI

	Overall	**Underweight and Normal Weight (*N* = 56)**	**Overweight and Obese (*N* = 441)**	***p* Value**
Demographic Characteristics				
Age (years)	49 (45–53)	47 (44–52)	49 (45–53)	0.162
Current smoker *n* (%)	14 (2.8%)	2 (3.6%)	12 (2.7%)	0.063
Physically active *n* (%)	325 (65.4%)	41 (73.2%)	284 (64.4%)	0.191
Employed *n* (%)	295 (59.5%)	29 (52.7%)	266 (60.3%)	0.280
Body Composition Indicators				
Visceral Adipose Tissue (VAT) (cm^2)	99.7 (74.1–127.6)	42.3 (29.0–58.5)	103.1 (83.9–131.5)	<0.001
Subcutaneous Adipose Tissue (SAT) (cm^2)	455.8 (356.2–561.8)	215.8 (168.9–266.0)	482.0 (387.2–576.5)	<0.001

VAT/SAT ratio	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.171
Whole body fat mass index (FMI) (kg)	32.0 (25.6–39.3)	16.8 (12.8–19.4)	33.1 (28.4–40.7)	<0.001
Whole body lean mass index (LMI) (kg)	40.4 (35.8–44.8)	31.1 (28.7–34.6)	41.5 (37.2–45.4)	<0.001
Gynoid fat	58.6 (47.2–70.8)	32.7 (27.9–39.3)	61.3 (51.7–72.5)	<0.001
Hip circumference (cm)	117.5 (109.0–128.0)	97.0 (91.5–101.3)	119.0 (112.5–129.0)	<0.001
Waist circumference (cm)	99.5 (90.0–108.0)	77.0 (74.3–81.3)	101.5 (93.0–110.0)	<0.001
Dietary Information				
Total energy (kJ)	9759 (7628–13271)	10737 (8717–12659)	9614 (7523–13354)	0.291
% Carbohydrates	53.5 (48.8–58.4)	54.4 (49.4–58.8)	53.5 (48.8–58.3)	0.598
% Protein	11.6	11.0	11.8	0.026

	(10.3–13.3)	(9.7–12.4)	(10.4–13.4)	
% Fat	30.2	31.3	30.1	0.756
	(25.9–34.3)	(25.5–35.1)	(26.0–34.2)	

Values are represented as Median (25P-75P) unless specified otherwise

3.4.2 Nutrient Patterns of Population

Table 2 shows that three nutrient patterns were extracted using PCA analysis, explaining 59% of the total variation of nutrients consumed by the study participants. The first nutrient pattern, the "*Plant driven nutrient pattern*" with higher factor loadings of plant protein, starch, and B vitamins, explained 25% of the variance; while the second (the "*Animal protein driven nutrient pattern*"), characterised by animal protein and saturated fat, and third (the "*Vitamin C, sugar and potassium driven nutrient pattern*") accounted 23% and 11%, respectively.

Table 2 Factor loadings and explained variances for the nutrient patterns.

	Plant Protein Driven Nutrient Pattern	**Animal Protein Driven Nutrient Pattern**	**Vitamin C, Sugar, and Potassium Driven Nutrient Pattern**
Plant protein	**0.921**	0.121	−0.017
Animal protein	0.080	**0.834**	0.175

Saturated fat	0.284	**0.729**	0.136
Monounsaturated fat	0.352	**0.695**	0.062
Polyunsaturated fat	0.443	0.549	−0.059
Cholesterol	0.009	**0.776**	−0.003
Starch	**0.712**	0.046	−0.304
Total sugar	0.050	0.095	**0.721**
Dietary fiber	**0.686**	0.044	0.455
Calcium	−0.110	0.507	0.431
Iron	**0.785**	0.413	0.170
Magnesium	0.574	0.169	0.273
Phosphorus	0.156	0.560	0.171
Potassium	0.028	0.089	**0.688**
Zinc	**0.758**	0.471	0.117
Retinol	0.073	0.345	0.110
Beta carotene	0.033	−0.038	0.229
Thiamine	**0.861**	0.341	0.158
Riboflavin	0.415	**0.630**	0.314

Vitamin B6	**0.906**	0.199	−0.019
Folate	0.618	0.080	0.111
Vitamin B12	0.113	**0.691**	0.101
Vitamin C	0.193	0.063	**0.897**
Vitamin D	0.265	**0.837**	−0.124
Vitamin E	0.370	0.482	0.088
Explained variance%	24.678	22.945	10.884
Cumulative explained variance%	24.678	47.623	58.507

Bold factor loadings used to indicate factor loadings > ± 0.60 used for naming the nutrient patterns.

3.4.3 Factors Associated with Varying Body Composition Measurements

Statistically significant associations were found for the animal protein driven nutrient pattern with all body composition indicators (Table 3). A one SD increase in the animal protein driven nutrient pattern resulted in a 1.19 $kg \cdot m^{-2}$ ($p = 0.002$) increase in BMI, 10.17 cm^2 ($p < 0.001$) increase in VAT, 24.43 cm^2 ($p = 0.009$) increase in SAT, 0.01 ($p = 0.009$) increase in VAT/SAT ratio, 0.69 $kg \cdot m^{-2}$ ($p = 0.005$) increase in FMI, and 0.48 $kg \cdot m^{-2}$ ($p = 0.002$) increase in LMI. There was a marginal association for plant driven nutrient pattern, with an increase in this pattern resulting in an increase in SAT of 20.45 cm^2 ($p = 0.045$). Underweight participants were associated with over-reporting energy intake, while

overweight individuals were noted to underreport. Notably, over-reporting EI, when compared to plausible EI, was associated with decreases of 3.51 kgm^{-2} in BMI, 17.61 cm^2 in VAT, 76.15 cm^2 in SAT, 1.95 $kg \cdot m^{-2}$ in FMI, and 1.47 $kg \cdot m^{-2}$ in LMI ($p < 0.001$ for all associations). Engagement in physical activity was associated with BMI, VAT, SAT, FMI, and LMI and inverse associations were reported at 1.51 $kg \cdot m^{-2}$, 9.21 cm^2, 37.76 cm^2, 0.91 $kg \cdot m^{-2}$, and 0.56 $kg \cdot m^{-2}$, respectively. Increasing age showed a direct statistically significant association with VAT ($p < 0.001$) and VAT/SAT ratio ($p = 0.002$). The plant protein and starch driven nutrient pattern was only associated with modest increases in SAT compared to the animal protein and fat driven pattern (Table 3).

Table 3 Multivariable linear regression models showing the association of body composition indicators (BMI, VAT, SAT, VAT/SAT, FMI, and LMI; outcome variables) with the three nutrient patterns (predictor variables) and possible confounding factors (energy intake reporting, physical activity, employment status, education, smoking, and alcohol intake).

	BMI	**VAT**	**SAT**	**VAT/SAT ratio**	**Whole body FMI**	**Whole body LMI**
	Adjusted β (95% CI)	Adjusted β (95% CI)	Adjusted β (95% CI)	Adjusted β (95% CI)	Adjusted β (95% CI)	Adjusted β (95% CI)
PCA1	0.65 (-0.17;1.47)	2.06 (-3.12;7.23)	**20.45**** (-0.47;40.43)	-0.01 (-0.01;0.01)	0.46 (-0.07;0.99)	0.20 (-0.12;0.53)
PCA2	**1.29***** (0.54; 2.04)	**9.88***** (5.13;14.63)	**26.30**** (7.97;44.63)	**0.01**** (0.00;0.02)	**0.74**** (0.25;1.22)	**0.53***** (0.23;0.83)
PCA3	0.30 (-0.31; 0.91)	-1.09 (-4.97;2.80)	-5.05 (-20.04;9.94)	0.00 (-0.01;0.01)	0.21 (-0.19;0.60)	0.08 (-0.16;0.33)
Under reporting	1.85 (-0.40; 4.10)	5.41 (-8.83;19.64)	54.44 (-0.53;106.41)	-0.00 (-0.04;0.02)	**1.49**** (0.03;2.95)	0.37 (-0.53;1.26)
Over reporting	**-3.51***** (-5.18; -1.85)	**-17.61***** (-28.13;-7.09)	**-76.15***** (-116.77;-35.53)	-0.00 (-0.02;0.02)	**-1.95***** (-3.03;-0.87)	**-1.47***** (-2.13;-0.80)
Physically Active	**-1.51****	**-9.21****	**-37.76****	-0.00	**-0.91****	**-0.56****

	(-2.76; -0.26)	(-17.14;-1.28)	(-68.37;-7.15)	(-0.02;0.01)	(-1.73;-0.10)	(-1.06;-0.06)
Employed	**1.41****	-2.10	26.07	**-0.02****	**0.81****	**0.55****
	(-0.21; 2.62)	(-9.79;5.58)	(-3.59;55.73)	(-0.03;-0.01)	(-0.03;1.60)	(-0.06;1.03)
Attended high school	-0.33	-2.77	12.35	-0.02	0.07	-0.39
	(-2.31; 1.64)	(-15.27;9.74)	(-35.92; 60.63)	(-0.05; 0.04)	(-1.21;1.35)	(-1.17;0.40)
Completed high school	-2.13	-8.28	-23.53	-0.02	-1.00	**-1.07*****
	(-4.28; 0.02)	(-21.87;5.31)	(-76.04; 28.95)	(-0.05; 0.001)	(-2.39;0.39)	(-1.93;-0.22)
Ex-smoker	-2.00	7.76	-31.28	0.00	**-1.56****	-0.40
	(-4.38; 0.36)	(-22.67; 7.16)	(-88.88;26.31)	(-0.03;0.03)	(-3.10;-0.31)	(-1.34;0.55)
Current smoker	-0.24	-12.76	-23.53	-0.04	-0.32	0.09
	(-4.00; 3.52)	(-15.27;9.74)	(-76.01;28.95)	(-0.09;0.01)	(-2.76;2.11)	(-1.41;1.59)
Alcohol intake	-0.02	0.54	-0.83	**0.00****	-0.01	-0.05
	(-0.15; 0.10)	(-0.27; 1.36)	(-3.96; 2.30)	(0.00; 0.00)	(-0.09; 0.74)	(-0.07;0.04)
Age	0.03	**1.68*****	2.61	**0.00****	0.17	-0.03
	(-0.08; 0.15)	(0.94;2.42)	(-0.27;5.49)	(0.00;0.00)	(-0.02;0.35)	(-0.07;0.02)

*** p<0.01; ** p<0.05 NOTE **bold** values denote statistical significance at p<0005

PCA1 = Plant protein and starch driven nutrient pattern; PCA2 = Animal protein driven nutrient pattern; PCA3 = Vitamin C, sugar, and potassium driven nutrient pattern.

3.5 DISCUSSION

This study aimed to determine the nutrient patterns of middle-aged black women in Soweto and their association with body composition measurements. Three nutrient patterns, namely, plant driven nutrient pattern, animal protein driven nutrient pattern and vitamin C, sugar and potassium driven nutrient pattern, were found to explain 59% of the variance in nutrient intake in the study population. The animal protein driven nutrient pattern was positively associated with BMI and DXA-derived body composition parameters.

Three studies have previously characterised nutrient patterns of black Africans. Pisa et al. (150) and Visser et al. (5) studies were conducted in children and adolescents, while the study by Chikowore et al.(4) was done in adults. Pisa et al. found that the animal protein driven nutrient pattern comprising of animal protein, saturated fat, cholesterol, riboflavin, vitamin B12, retinol, vitamin D, and zinc were the most commonly consumed nutrient pattern, while in our study, this was the second most consumed pattern. Visser et al. reported similar results to our study where the plant driven nutrient pattern comprising of protein, carbohydrate, iron, and vitamin B was the most consumed followed by the animal protein and saturated fat pattern. Additionally, Chikowore et al. also reported similar findings to our study where the plant driven pattern comprising of magnesium, phosphorus, and plant protein was the most consumed pattern followed by fat and animal protein and starch driven nutrient patterns. Regardless of the small differences in the naming of the patterns, Visser et al. and Chikowore et al. studies, conducted in both urban and rural areas, reported similar nutrient patterns to those that we found in our study (4,5). This thereby

suggests that these patterns are common in the black South African population. These nutrients are most likely derived from the consumption of staple foods such as maize meal, which are fortified with B vitamins (5,151). However, the differences in the variances of the animal protein driven nutrient pattern reported by Pisa et al. are expected, since the principals analysis approach used is data driven and thus, is expected to capture the unique patterns (138). However, this pattern was also noted in our study and the high loadings of animal protein and saturated fat are suggestive of the shift towards western diets, which has been reported in this population group.

Very few studies have been conducted to assess the association between nutrient patterns and obesity. One such study conducted in American adults evaluated nutrient patterns and their relationship with general and central obesity (71). Results from this study showed that the odds ratios of obesity increased across quartiles of the major nutrient pattern of saturated/mono-unsaturated fatty acids. Relative to the first quartile, the fourth quartile was associated with odds ratio of 1.5 (95%CI, 1.3–1.7) for central obesity and odds ratio of 1.3 (95%CI: 1.1–1.5) for general obesity. These findings are similar to those of the present study, which showed that the animal protein driven nutrient pattern is significantly associated with increases in BMI, VAT, SAT, VAT/SAT ratio, FMI, and LMI.

The plant driven nutrient pattern was correlated significantly with SAT, but not as strongly as the animal protein driven pattern. Although it has been demonstrated that a plant-based protein diet, through its anabolic properties, could be an effective strategy to enhance lean mass index, when eaten in excess, these foods may result in increasing SAT, hip, and waist

circumference (77). The increase in body parameters may be due to the fact that these plant-based foods may contain hidden sugars and refined carbohydrates, which contribute to weight gain.

The animal protein driven nutrient pattern, characterised with higher loadings for animal protein and fat driven nutrients, increased body composition parameters, potentially due to the storage of animal fat as triglycerides in adipose tissue (152,153). However, protein is known to lead to increased energy expenditure and improved satiety through the release of hormones, which favours weight loss (154). In fact, protein weight loss effects have been reported when it contributes approximately 20% or more of the energy intake (66). This weight loss effect was less likely in our study, which comprised of protein intake at 11% of total energy intake. Furthermore, it must be noted that when energy demand is low, excess protein can be converted to ketone bodies or glucose (via gluconeogenesis) and contribute to a positive energy balance, which can result in increased weight (155). Previous research done in both animals and humans demonstrated that a high fat diet promotes obesity (156). This explains the association of the animal protein driven nutrient pattern with increases in body fat as measured by BMI.

A strength of this study included using more accurate DXA body composition measurements when compared to simple measurements. Additionally, this study adjusted for energy intake misreporting in the linear regression models, which improved the accuracy of these models. Notably, having overweight/obese status was noted to under report dietary energy intake as has been indicated in other studies (157,158). The nutrient density method was

implemented in order to adjust for total energy, thereby allowing for the independent association of the nutrients with body composition to be determined.

The study has some limitations. The QFFQ is known to have recall bias, Iing the potential for food measurement errors, resulting in inaccurate reporting of food consumption (159). Recall bias was reduced by using actual food models to help with accurate recall of food quantities consumed. Similarly, physical activity was self-reported, however interviewers provided contextual examples of moderate and vigorous intensity activities during the interview to assist with recollection of recent activity. Accurate PA measurements using electronic activity monitors would ideally remove the potential for recall bias, however such tools are expensive and not feasible for large scale epidemiological studies (160).

3.6 CONCLUSION

This study showed that in this population, the animal protein driven nutrient pattern was significantly associated with increases in body fat and its distribution was indicated by the selected measures (BMI, VAT, SAT, VAT/SAT ratio, FMI, and LMI). These findings and recommendations are intended to be used to design nutrient pattern studies that are necessary for proving causality between nutrient patterns and body composition changes in black Sub-Saharan African women, thus contributing to the body of knowledge in this population group.

CHAPTER 4: PAPER 2

(Appendix 8)

4.1 ABSTRACT

This study aimed to evaluate the longitudinal association of nutrient patterns with adiposity in a cohort of 132 black South African middle-aged women over five years. Nutrient patterns were identified using PCA at baseline and 5-year follow-up. Associations between nutrient patterns and 5-year adiposity measures were evaluated using generalized estimating equations (GEE). The animal-driven nutrient pattern was associated with increases in VAT (β coefficient, 5.79cm2, [95%CI, 0.01-11.57]), FMI (0.47kg m-2 [0.01-0.93]) and LMI (0.50kg m-2 [0.18-1.17]) after adjusting for baseline education and repeated measures of age, SES, PA and employment ($p<0.05$). Vitamin C, sugar, and potassium-driven nutrient pattern was associated with increases in FMI and LMI (0.50kg m-2 [0.12-0.88] and 0.58kg m-2 [0.07-1.10], respectively) after adjustment ($p<0.05$). These findings suggest that dietary interventions to curb obesity in black middle-aged South African women should focus on attenuation of nutrient patterns centred on added sugar, animal fat and animal protein.

4.2 INTRODUCTION

Metabolic syndrome, high blood pressure, and T2D are all more common in black women, who have higher rates of obesity, than in white women, according to research conducted in South Africa (161,162). In South Africa, it has been estimated that NCDs account for ~51% of all fatalities with ~19% of all deaths attributable to CVD (82). Additionally, this increased risk of NCD onset may be due to black women showing a unique body composition phenotype

by storing more SAT than other ethnic groups (163). Furthermore, the same study demonstrated that ageing in black women is characterized by significant increases in VAT and a relative redistribution of fat from the peripheral to the central region.

Obesity has been shown to have strong links with dietary behaviours (152,154,156). As a result, designing health promotion programmes that focus on dietary behaviours is critical (43,71). To create appropriate and effective health promotion programs, information on long-term nutritional behaviours and associations with health outcomes is required.

The United Nations' FAO and the WHO have formed a joint consultation to develop national FBDGs, which provide context-specific advice and principles on healthy diets based on scientific evidence (23). This has resulted in the FAO/WHO 2004 Global approach on food, PA, and the amended 2012 South African FBDGs, which drew on research findings among other recommendations and reports. Unfortunately, these dietary guidelines have not been updated since 2012 therefore, the impact of adult South Africans continues to be not well documented leaving this population vulnerable (164).

The body composition of women has also been proven to be significantly influenced by their ethnicity, and numerous cross-sectional studies have documented these racial disparities in body composition and associations with nutrient patterns. Nutrient patterns of middle-aged black South African women have not been studied longitudinally to date. There have only been five cross-sectional studies examining the nutrient patterns of adult men and women aged 18 and older in South Africa (4,76,150,165,166). These cross-sectional studies unfortunately are unable to show sustainability of the dominant nutrient patterns in the

target populations. Longitudinal nutrient pattern studies are needed to show sustainability of the nutrient patterns and association of adiposity. Results of such studies may be used to updated the dietary guidelines. Of these studies, two documented specific nutrient patterns in black South African women, the same cohort for this study, and evaluated the association between these nutrient patterns and adiposity with the one study looking at sex differences in the associations of nutrient patterns with total and regional adiposity (76,165). This study showed that a plant driven nutrient pattern explained the greatest variance in nutrient intake in this population and was positively associated with SAT (165). This study aims to investigate changes in body composition over 5.5 years, and to determine if these changes are attributable to nutrient patterns extracted at baseline and follow-up. Furthermore, this study aims to examine if baseline and nutrient patterns extracted 5.5 years later remained the s

4.3 MATERIALS AND METHODS

4.3.1 Study population and setting

At baseline, a total of 902 women who were the biological mothers of the MASC cohort were invited to participate in the SWEET study (165). The following inclusion criteria were applied: between 40 and 60 years of age, not pregnant, black African women (Figure 1) (112,117). Between January 2017 and August 2018, 498 black South African female caregivers from the MASC study and the SWEET sub-study formed the baseline study population using the following exclusion criteria: <40 years and >60 years, pregnant, did not give consent, and ethnicity other than black African (117). Of these women, the cohort was

randomly sampled and the women were invited to participate in a new follow-up study which was subsequently referred to as the GSK study. After excluding participants that were not contactable, deceased, relocated, not interested, missing anthropometry measurements or not included in sampling, did not have baseline nutrition data, or were a mismatched participant, the final sample size of women with DXA body composition measurements and dietary data at baseline and follow-up was n=132 (Figure 13). The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (ethics numbers: M160604 and M160975).

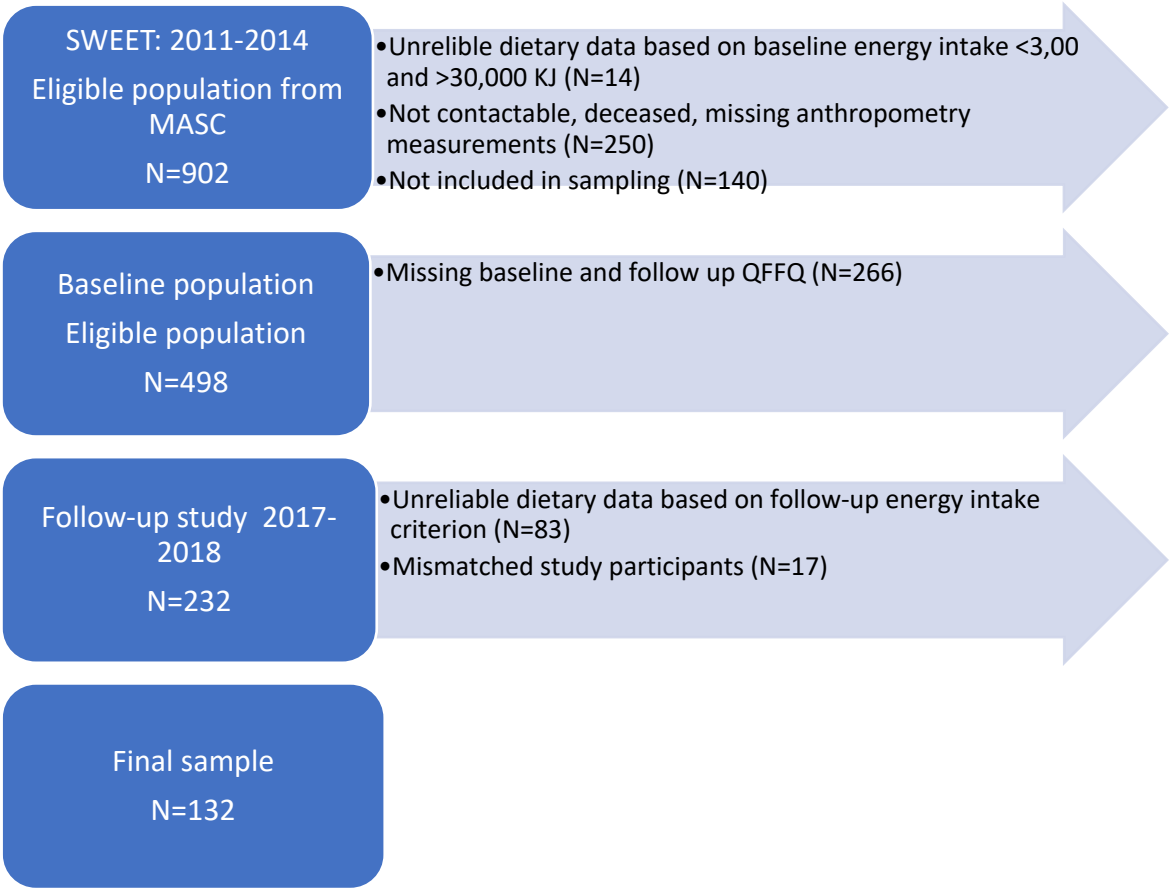

Figure 12 Flow chart of study participants. (SWEET = Study of Women in and Entering Endocrine Transition; MASC = Middle-aged Soweto Cohort).

4.3.2 Dietary intake

Dietary intake data was collected at baseline and follow-up using a standardised QFFQ, designed for the South African population as previously described (119–121,165). The QFFQ consists of 214 food items that are representative of foods consumed by most of the South African population currently (143). Although this tool was tested in adolescents, it has since been piloted and utilised extensively in the general population (121). Trained interviewers used high quality photographs of food items to assist with participant recall of food and beverage items consumed during the last seven days. In the case of food items consumed in the last seven days, additional data on the frequency and quantity of consumption were collected. The participants were asked to estimate their habitual intake by selecting the most accurate representation of their portion size from either two-dimensional actual size drawings of foods, household utensils, or three-dimensional validated food models as described by Steyn et al. (121). These household measures were then converted to grams for the computation of average intake over a seven-day period. Food items were then converted to nutrients using the nutrient analysis software FoodFinder3 developed and hosted by the SAMRC. Participants with unreliable dietary data based on energy intake <3000 and >30,000 kJ, as described by Vorster et al. (124), were excluded (*n* = 20 with either unreliable dietary data at baseline (*n* = 14) or follow-up (*n* = 6)).

Baseline and follow-up plausibility of EI reporting was adjusted in GEE and this variable was calculated using the ratio of the dietary EI and EER which is the average dietary EI that is predicted to maintain energy balance in healthy, normal weight individuals of a defined age, gender, weight, height, and level of PA consistent with good health (3,165). The EER was

calculated using the IOM gender-specific equations shown below for females as previously described with a PA coefficient of 1.12:

EER=354-((6.91*Age (years))+ PA coefficient (9.36*Weight (kgs))+726*Height (m))

EI:EER ratio was used to classify participants into 3 categories as previously described:

Less than 0.7 were classified as under-reporters,

0.7 to 1.42 as plausible reporters (this was the reference category,

Greater than 1.42 as over-reporters.

For adult women reporting a low active lifestyle, the PA coefficient is 1.12 as moderate intensity physical activity has been reported for this same population which is in line with findings from this study as well(127). Participants with EI:EER ratio less than 0.7 were classified as under-reporters, 0.7 to 1.42 as plausible reporters, and greater than 1.42 as over-reporters. Plausible energy intake was used as the reference category in statistical models for the EI: EER ratio (3). There are several recommendations about the optimal sample size and Mundfrom highlighted that it is not feasible to set a single, one-size-fits-all number for minimum sample size (140). Furthermore, following Kline's recommendation of a minimum sample size of 100, this sample is sufficient to generate accurate findings (140,141) .

4.3.3 Body composition measures

Participants were requested to remove their shoes and to wear lightweight clothing prior to weigh in. Weight was recorded to the nearest 0.01 kg. Height was measured and recorded to

the nearest 0.1 cm. Body mass index was calculated as weight (in kilograms) divided by the square of baseline height (in metres).

A single trained technician performed DXA scans at baseline and follow-up using a Hologic Discovery software version 12.1 (Hologic Inc., Bedford, MA, USA, APEX software). Whole-body scans were completed to determine FM and FFSTM. The CV for the DXA parameters were <2% for total fat mass and 1% for fat-free soft tissue mass. Fat mass was used to calculate FMI (fat mass (kg)/baseline height (m^2)), and lean mass was used to estimate LMI (whole-body lean mass (kg)/ baseline height (m^2))(128). Visceral adipose tissue, SAT and gynoid fat were also estimated using DXA(167).

4.3.4 Socio-demographics and physical activity

Details of socio-demographic and lifestyle characteristics were collected at baseline and at follow-up and questionnaires were administered by research assistants. Socio-demographic factors included age, self-reported employment status (Yes/No) and highest education level achieved (completed primary school, incomplete high school and completed high school). Household socio-economic status was estimated using a count of the following major household amenities: electricity, radio, motor vehicle, fridge, washing machine, mobile, microwave, MNET and digital satellite television, and reported as a score out of nine.

Self-reported PA was collected using the GPAQ and participants were categorised as active or inactive at baseline and follow-up according to the GPAQ criteria. Active were participants were classified as having a total reported >600 MET minutes per week and the

remaining (<600 MET minutes per week) as inactive at both baseline and follow-up. This PA was further divided into three categories: active participants at both time points, inactive participants at both time points, and PA bidirectional participants defined as individuals who changed their physical activity in any direction between the two study time points.

4.3.5 Statistical analysis: Analysis of longitudinal data

All statistical analyses were performed using STATA version 15 (StataCorp, College Station, TX, USA) and IBM SPSS version 27 software packages (IBM, Chicago, IL, USA) (146,147). Q-Q plots were used to perform normality tests on the continuous variables and normally distributed continuous variables are presented as mean ± SD, while non-parametric data are presented as median and IQR (25P,75P) for the descriptive tables. The Student paired *t*-test, Chi-square test or the Wilcoxon signed rank test were used to explore differences between baseline and follow-up measurements for normally distributed continuous data, categorical data, and not normally distributed continuous data, respectively.

Principal component analysis was applied at both baseline and follow-up to extract nutrient patterns from 25 nutrients derived from the QFFQ (140). Total available carbohydrates were divided into starch, total sugar and dietary fibre. The nutrients were log transformed to remove bias due to variance as a result of the different measures of scale used to quantify the nutrients (165). As previously described, the PCA approach was performed using the covariance matrix of the log transformed nutrients that were selected to capture the entire dietary intake (4,150,165). Varimax rotation was used to make the factor loadings more understandable (149,168). The top three PCs indicating three distinct nutrient patterns were

selected and plotted on a circular plot based on their eigenvalues and visual appearance on the scree-plot of eigenvalues and the proportion of total variance explained. Nutrient patterns at baseline and follow-up were used as predictor variables.

Generalized estimating equation analyses were used to analyse longitudinal associations of both baseline and follow-up measures of nutrient patterns with six repeated measures of the following body composition measures: BMI, VAT, SAT, FMI, LMI and gynoid fat. The GEE analysis extends the generalized linear model to allow for the analysis of repeated measurements. It combines within-subject relationships with between-subjects relationships into a single regression coefficient (169,170). Furthermore, GEE allows for the explicit modelling of a wide range of correlation structures and is more interpretable than repeated measures analysis of variance (ANOVA). The GEE models examined time-varying nutrient patterns while accounting for the correlation of baseline and follow-up (repeated measures) for the same participant. Two GEE models were constructed:

1. GEE model 1 was adjusted for repeated measures of all nutrient patterns and repeat measures of EI reporting and
2. GEE model 2 was adjusted for the same variables as model 1 plus baseline education and the following repeat measures: age, household SES, PA and employment. Furthermore, the GEE models for the regional adiposity measures (VAT, SAT, and gynoid fat mass) were further adjusted for FMI to explore the effects of regional adiposity as opposed to total adiposity.

The β coefficients and the 95% confidence interval (CI) from GEE models were reported as the measures of association.

4.4 RESULTS

4.4.1 Descriptive Characteristics

The characteristics of the participants are summarized in Table 4.

Table 4 Baseline and follow-up descriptive characteristics (N=132)

	Baseline	5.5- years follow-up	p value
Demographics			
Age, y	48 (44; 52)	53 (50; 58)	**<0.001**
Socio-economic status score (/9)	5 (4; 6)	5 (3; 5)	0.572
Physically active (%)	94 (71.2%)	93 (70.5%)	0.882
Body composition indices			
BMI (kg m^{-2})	33.7 (28.1; 39.0)	33.7 (28.1; 39.2)	0.767
Whole-body fat mass (kg)	60.5 (47.1; 73.3)	63.4 (49.1; 76.3)	**0.015**
Fat mass index (FMI) (kg m^{-2})	23.5 (20.5; 30.3)	26.6 (20.7; 31.8)	**<0.001**
Whole body lean mass (kg)	41.9 (36.9; 45.1)	41.0 (34.4; 52.4)	**0.029**
Lean mass index (LMI) (kg m^{-2})	16.6 (14.7; 18.2)	16.4 (14.5; 20.8)	0.055
Gynoid fat mass (kg)	32.1 (25.7; 41.5)	36.3 (29.8; 46.3)	**<0.001**

Subcutaneous Adipose Tissue (cm^2)	452.7 (360.2; 574.5)	457.6 (349.1; 576.4)	0.547
Visceral Adipose Tissue (cm^2)	102.6 (75.2; 128.9)	107.8 (70.4; 137.3)	**0.001**
Nutritional intake data			
Energy intake (mJ)	9.3 (7.9; 12.0)	6.4 (5.1; 8.4)	**<0.001**
Carbohydrates (%)	54.5 (49.7; 58.1)	52.6 (48.6; 56.5)	0.052
Protein (%)	11.4 (10.1; 12.8)	11.2 (9.7; 12.7)	0.871
Fat (%)	30.1 (26.0; 34.3)	31.7 (27.0; 35.5)	**0.035**
Energy intake (EI) estimated energy reporting			
Plausible	72 (57.1%)	67 (50.8%)	**0.021**
Under reporters	17 (13.5%)	58 (43.9%)	
Over reporters	37 (29.4%)	7 (5.3%)	

Values are reported as median (25P, 75P)

Despite no significant changes in BMI, LMI and SAT, DXA-derived measures of fat mass, FMI, VAT and gynoid fat mass increased over the 5-year follow-up while lean mass decreased. At baseline and follow-up, over 65% of the women were classified as having overweight or obesity.Total energy intake significantly decreased over the 5.5- yearss (from 9.3mJ to 6.4mJ respectively) although the proportion of carbohydrates and protein consumed remained stable across the two time points, while fat consumption significantly increased over the

5.5- yearss. Whole-body FM, FMI, gynoid fat mass and VAT all increased at follow-up (p value <0.05) while whole body lean mass decreased at follow-up (p value = 0.029).

4.4.2 Nutrient Patterns

Baseline and follow-up nutrient patterns were extracted using PCA and results of the nutrient patterns displayed as a radar plot and a table (Figure 14 and Appendix 8A respectively).

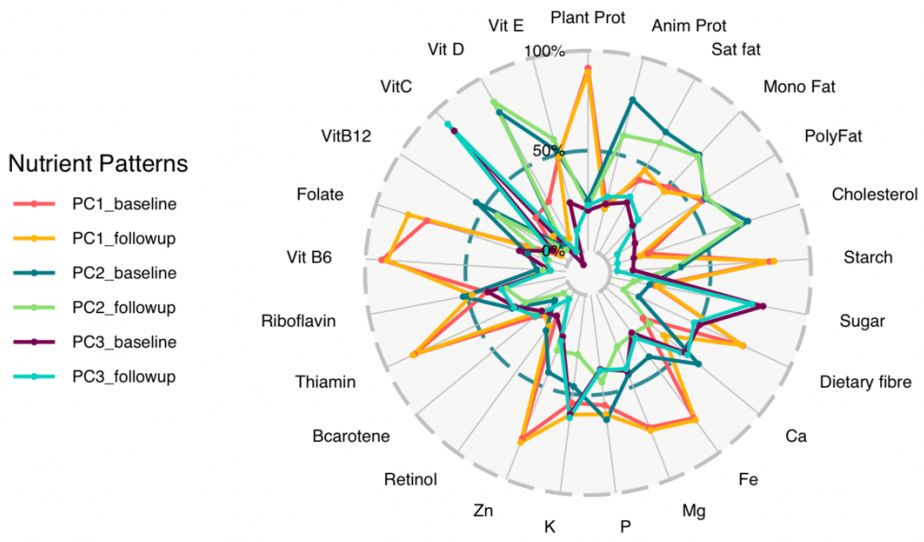

Figure 13 Baseline and follow-up PC derived nutrient patterns.

This is based on 25 nutrient items in middle-aged black South African women (N =132). Identified by principle component analysis, on the circular plot, the first 3 PCs that met the Scree test and eigenvalue criterion are shown by different coloured lines (referred to as PCA nutrient pattern 1-3) for both baseline and follow-up and each component represents an independent nutrient pattern. PC = principal component; Plant Prot = plant protein; Anim prot = animal protein; Sat fat = saturated fat; Mono Fat = monounsaturated fat; Poly Fat =

polyunsaturated fat; Ca = Calcium; Fe = Iron; Mg = magnesium; P = phosphorus; K = potassium; Zn = zinc; Vit B6 = vitamin B6; Vit C = vitamin C; Vit D = vitamin D; Vit E = vitamin E; PC1 = plant driven nutrient pattern; PC2 = animal protein driven nutrient pattern; PC3 = vitamin C, sugar and potassium driven nutrient pattern. The orange and yellow line (PC1) represents the factor loadings related to the plant driven nutrient pattern (baseline and follow-up respectively), the dark and light green lines (PC2) represents factor loadings related to the animal protein driven nutrient pattern (baseline and follow-up) and the purple and light blue lines (PC3) represents factor loadings related to the vitamin C, sugar and potassium driven nutrient pattern (baseline and follow-up).

These nutrient patterns were identified and driven by the same nutrients at baseline and follow-up (Figure 14). These nutrient patterns explained 69% and 64% of the total variation of nutrients consumed by the study participants at baseline and follow-up, respectively. Although the value of the factor loadings of the individual nutrients changed between baseline and follow-up, the nutrients with the highest loadings for each PC did not change therefore the nutrient patterns remained the same. The first nutrient pattern extracted was the “Plant driven nutrient pattern” with high factor loadings of plant protein, starch, and B vitamins, followed by the “Animal protein driven nutrient pattern”, PC2, characterized by high factor loadings of animal protein and saturated fat, and PC3 was the “Vitamin C, sugar and potassium driven nutrient pattern” characterized by high factor loadings of sugar, vitamin C and potassium.

4.4.3 Longitudinal association between nutrient patterns and body composition indices

Univariate analysis using GEE and individual body composition measures showed that the associations of the plant based nutrient pattern with FMI and LMI were significant, however these associations were no longer significant after adjusting for covariates in multivariate analysis (Table 5).

Table 5 Longitudinal GEE β coefficients for adiposity body measurements and nutrient patterns (N=132)

	BMI		VAT		SAT	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Plant driven nutrient pattern	0.14	0.29	0.95	-4.04	-1.43	-2.14
95% CI	[-0.38; 0.65]	[-0.56; 1.14]	[-5.03; 6.94]	[-10.83; 2.75]	[-18.21; 15.34]	[-18.47; 19.03]
Animal protein driven nutrient pattern	0.31	0.24	**10.12*** **	**5.79** **	1.45	-7.10
95% CI	[-0.11; 0.73;]	[-0.48; 0.96]	**[4.56; 17.02]**	**[0.02; 11.57]**	[-12.13; 15.03]	[-13.63 ;16.56]
Vitamin C, Sugar and Potassium Driven nutrient pattern	0.16	0.40	3.53	0.01	6.10	-2.79
95% CI	[-0.23; 0.55]	[0.19; 1.00]	[0.82; 7.87]	[-4.66; 4.78]	[-6.31; 18.50]	[-6.56; 1.96]

	FMI		LMI		GYNOID	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Plant driven nutrient pattern	**0.46 ****	0.36	**-0.89 ****	0.15	0.13	-0.79
95% CI	**[0.12; 0.81]**	[-0.36; 0.90]	**[-1.45; -0.15]**	[-0.65; 0.95]	[-1.27; 1.52]	[-2.37; 0.80]
Animal protein driven nutrient pattern	**0.45 *****	**0.47 ****	**0.47 ***	**0.50 ****	**0.72 ****	0.01
95% CI	**[0.17; 0.73]**	**[0.01; 0.93]**	[-0.05; 0.98]	**[0.18; 1.17]**	**[0.40; 1.85]**	[-1.35; 1.36]
Vitamin C, Sugar and Potassium driven nutrient pattern	**0.43 *****	**0.50 *****	**0.50 ****	**0.58 ****	0.31	-0.62
95% CI	**[0.16; 0.69]**	**[0.13; 0.88]**	**[0.06; 0.94]**	**[0.07; 1.10]**	[-0.74; 1.36]	[-1.74; 0.49]

**** p<0.01, ** p<0.05 Note: Bold values denote statistical significance at p<0.05.*

Model 1: Adjusted for all nutrient patterns and energy intake

Model 2: Adjusted for M1 plus employment, baseline education, energy intake reporting, physical activity, socioeconomic score. * FMI included only for VAT, SAT and gynoid GEE models.

The animal protein driven nutrient pattern was associated with significant increases in FMI, LMI and VAT for model 1 and this remained significant after adjustment (note, the GEE VAT model was further adjusted with FMI). Repeated measures of the vitamin C, sugar, and potassium driven nutrient pattern was significantly associated with an increase in FMI and LMI before and after controlling for household SES and related covariates. None of the nutrient patterns were associated with BMI, SAT and gynoid fat when adjusted for all the model covariates (the animal protein nutrient pattern was significantly associated with increases in gynoid fat in unadjusted model).

4.5 DISCUSSION

In this study, we explored the longitudinal relationship between nutrient patterns and various measures of body composition in black middle-aged South African women. Three nutrient patterns were extracted at baseline and follow-up which were plant driven, animal protein driven and vitamin C, sugar, and potassium driven nutrient pattern. The identified nutrient patterns described 69% and 64% of the total variation in nutrient consumption at baseline and follow-up respectively. Repeated measures of VAT, FMI and LMI were positively associated with repeated measures of the animal-driven nutrient pattern, while repeated measures of FMI and LMI was positively associated with repeated measures of the vitamin C, sugar, and potassium-driven nutrient pattern. The plant driven nutrient pattern was associated with repeated measures of FMI and LMI, however, after adjusting for co-variates, significance was lost.

The nutrient patterns of adult black South Africans have previously been studied in 4 cross-sectional studies (4,76,165,166). Chikowore et al examined associations between nutrient patterns and fasting glucose and glycated haemoglobin levels while Conradie et al examined the quality of pregnant women diet. Makura-Kankwende et al and Ratshikombo et al, both cross sectional studies, examined associations of nutrient patterns with body composition measurements with the later study aimed at examining the association between nutrient patterns and DXA-derived body fat and regional adiposity in middle-aged black South African men and women and determine if this differed by sex. The patterns extracted from these previous studies are comparable to those found in our study despite minor discrepancies in the nomenclature of the patterns and suggests that these nutrient patterns are common and consistent in the black South African population.

The amount of total energy consumed over the course of five years dramatically decreased (from 9.3 mJ to 6.4 mJ, respectively), despite the proportion of carbohydrates, fat and protein remaining unchanged. This may be due to measurement error in self-reported food intake data, which is a well know limitation of self-reported dietary food intake data collection. Validation studies have demonstrated that estimation of energy intake (EI) is particularly impacted by this measurement error. Our capacity to define nutrient consumption-disease relationships may be enhanced by identifying the most effective strategies to address EI misestimation, through the use of biomarker-based investigations. There were no significant changes in BMI, only significant changes in DXA-derived measures namely fat mass, FMI, VAT, lean mass and gynoid were observed over the 5-year follow-up period. Despite the paucity of research on the longitudinal effects of nutrient patterns on adiposity, our findings are consistent with other cross-sectional studies that show that the

animal protein driven nutrient pattern, was positively associated with repeated measures of FMI and VAT, measures of total and central body fat, respectively (152,171,172). Makura-Kankwende et al, Rosqvist et al and Fischer et al showed that saturated fats or animal protein driven diet were associated with an increase in VAT in adults which is in line with findings from this study. The storage of animal fat as triglycerides in adipose tissue resulting in increased adiposity is thought to explain the association of animal protein driven nutrient patterns with increased VAT and FMI [6,39](152,171). These changes to total and central body fat may be attributable to aging which in turn, may contribute to increased risk of development or progression of NCDs in LMIC. This may result in over-burdened health care facilities and limited resources thus impacting the management of NCDs.

Our investigation also found that consumption of the animal protein driven nutrient pattern was associated with an increase in LMI. These findings are similar to other longitudinal studies that also reported that nutrients from animal food sources resulted in increases in lean body mass (65,173,174). Lean mass and adiposity indices were positively associated with animal protein intake in the Osteoporosis Risk Factor and Fracture Prevention Study [42]. Furthermore, a meta-analysis study which included 18 studies found that a diet high in animal protein was associated with increased lean mass compared a diet high in plant protein which supports the findings from this study (65). It is thought that the consumption of animal protein may cause the activation of protein synthesis leading to an increase in lean mass (154,175). This suggests that a diet high in animal protein is advantageous for lean mass growth however, this nutrient pattern was also associated with higher fat mass therefore recommendations are needed on how to leverage this nutrient pattern to promote lean mass and not increase fat mass. The variance of the animal protein driven

nutrient pattern which relates to consumption, was far less than the plant driven nutrient pattern.

Our findings showed that a vitamin C, sugar, and potassium driven nutrient pattern was associated with higher FMI and LMI. Foods and beverages that are rich in these nutrients include starchy vegetables such as white potatoes and sweet potatoes and sugar-sweetened beverages for example which may contribute to increased adiposity, while foods such as green leafy vegetables such as spinach may lead to increased LMI (176). This nutrient pattern has a wide diverse range of food which includes both beneficial and harmful food choices, therefore further research is needed to help identify which foods are beneficial. Observational studies have revealed that high potassium intake may be associated with increased lean mass, along with other body composition changes, consistent with our findings where the vitamin C, sugar and potassium driven nutrient pattern had higher factor loadings for potassium (177,178). In addition, results from a longitudinal study conducted in adult men and women over a 3-year follow up period showed that a higher intake of foods rich in potassium, supported preservation of lean mass [48]. It is suggested that potassium preserves lean mass by neutralising an acidic environment which promotes protein-energy wasting and loss of lean mass (179). Other epidemiological research has found that high consumption of foods high in vitamin C, have been linked to increased fat mass, possibly due to higher sugar levels (42,180,181). Dietary sugar may be converted to fat and stored in the body which might explain the association of the sugar-based nutrient pattern with FMI in this and other studies.

Dietary assessment studies are often hampered by inconsistencies in the self-reporting of food intake, which we worked to minimise by correcting for energy intake reporting. The study population underreported their energy intake more noticeably during the second visit compared to the first visit and these could be due to consumption of more recent food items that were not consumed at baseline but are now consumed at follow-up that might not be recorded on the QFFQ. Despite these limitations, to our knowledge, this is the first longitudinal study of nutrient patterns and body composition conducted in black African women which provides evidence on consistent, stable nutrient patterns of the population compared to a cross sectional nutrient pattern study. Furthermore, our investigation used DXA-derived measurements of body composition which provide more accurate assessments of body composition where we were able to examine associations between nutrient pattern and both lean mass and fat mass. Previous studies used simple anthropometric body composition measurements. Finally, while the study was focused on black South African middle-aged women, gender and age specific studies should be conducted to investigate whether the same nutrient patterns are dominate in men or the younger population and if the nutritional recommendations can be applied across the various age and gender groups.

4.6 CONCLUSION

This study documented the nutrient patterns of black South African middle-aged women, and indicated that these patterns remained the same over 5.5- yearss. The consumption of animal based nutrient patterns and vitamin C, sugar, and potassium based nutrient patterns were associated with both increased adiposity and lean mass. More importantly, we found that animal protein was associated with visceral adiposity which is a major risk factor of

cardiometabolic diseases. This is in line with recent data from large cohorts that confirmed that total and animal proteins are associated with the risk of cardiovascular disease. Additional research including larger sample sizes and a longer follow-up time is needed to verify these results.

CHAPTER 5: PAPER 3

5.1 ABSTRACT

Objective: This study aimed to elucidate the longitudinal associations of blood pressure with nutrient patterns over 5.5 years follow-up period and to explore if BMI mediates the relationship between nutrient patterns and blood pressure using structural equation modelling (SEM).

Materials and methods: Nutrient patterns were identified using principal component analysis at baseline and 5.5 years follow-up. Generalized estimating equation analyses were used to analyse longitudinal associations of baseline and follow-up measures of nutrient patterns with baseline and follow-up measures of BMI. SEM was applied to determine whether BMI mediates the effect between BMI and blood pressure.

Results: During the 5.5 year follow-up, less than 40% of study participants developed HTN, and of these, approximately 81% were classified as having an obese status. Of those classified as normotensive people, 58% were classified as having obese status (p=0.008). Both baseline and follow-up measurement of the animal protein driven nutrient pattern were significantly associated with only systolic blood pressure. Significant relationships were observed between energy intake and SES; energy intake and age; BMI and consistent physical activity status; and BMI and mean SBP. Indirect relationships were observed between physical activity and mean SBP through BMI, as well as baseline education and mean SBP through BMI.

Conclusion: There is evidence on the direct association between BLOOD PRESSURE and an animal protein driven nutrient pattern. To prove causality, Mendelian randomization must be implemented at the study design phase.

5.2 INTRODUCTION

Hypertension impacts over 1 billion people globally (182). The 2016 South African Demographic Health Survey showed that HTN affects 50% of adult women and 47% of adult men (183), which is significantly higher than the overall global prevalence of HTN, which is reported to be 32% and 34% for women and men, respectively (184). Controlling HTN could result in a significant reduction in CVD morbidity and mortality . However, only 25-57% of South African adults living with HTN are controlled, usually with multiple blood pressure lowering medications (185,186). Furthermore, there is an urgent need for HTN care in South Africa as it has been reported that 91% of the hypertensive population was unscreened, undiagnosed, untreated, or uncontrolled (187). An observational study conducted in an adult South African population with HTN found that dietary interventions play a critical role in the prevention, and management of HTN (188). As shown in the DASH intervention study, the risk of HTN can be reduced by eating a diet low in sodium chloride or salt, high in both fruits and vegetables (189).

Dietary intake and blood pressure are known to have a complex relationship (190) however, relationships between blood pressure and individual nutrients such as sodium have been widely examined (191). This has led to recommendations for reduced sodium intake as it has been demonstrated that even 5 grams per day decrease, can aid with lowering blood pressure (189–191). However, nutrients are not consumed in isolation but are constituents of composite diets that include varied foods. This makes it difficult to associate the impact of a diet to a single nutrient (192). In nutritional epidemiology, nutrient pattern analysis has been regarded as a suitable method for investigating diet-disease associations as it considers the whole diet .

Obesity is on the rise, and a high BMI has been shown to be significantly and positively associated with both systolic and diastolic blood pressure in several prior studies (33,193–197). In South Africa, middle-aged women are particularly vulnerable due to the high obesity rates. Previous studies have shown that BMI mediates the relationship between HTN or blood pressure and either; dietary patterns, diet quality (DASH) including frequency of eating away from home or individual nutrients, however, no study has examined whether BMI mediates the relationship between nutrient patterns and blood pressure (198–203). Furthermore, it has not yet been established in the South African population whether nutrient patterns have a direct or indirect effect on blood pressure or whether its association with blood pressure is mediated through BMI. Therefore, this study aimed to elucidate the longitudinal association of nutrient patterns and blood pressure and to explore whether this is an indirect effect mediated by BMI.

5.3 MATERIALS AND METHODS

5.3.1 Study population and dietary data

The MASC study, which was initially open to biological mothers of the Bt20+ cohort and the following exclusion criteria were used at baseline: aged younger than 40 years, aged 60 years or older, pregnant, did not give consent, and ethnicity other than black African (Figure 15) (112,115). Of the 902 women initially enrolled, a random sample was selected and had

follow-up dietary data collected between January 2017 and August 2018 (n=498). The final sample size was 117 after participants were eliminated due to the following reasons:

1) Unreachable, dead, relocated, refused to participate, missing anthropometric measurements,
2) Lacking follow-up nutrition data,
3) Mismatched participants at baseline and follow-up,
4) Suspected of underreporting energy intake.

This is illustrated in Figure 15.

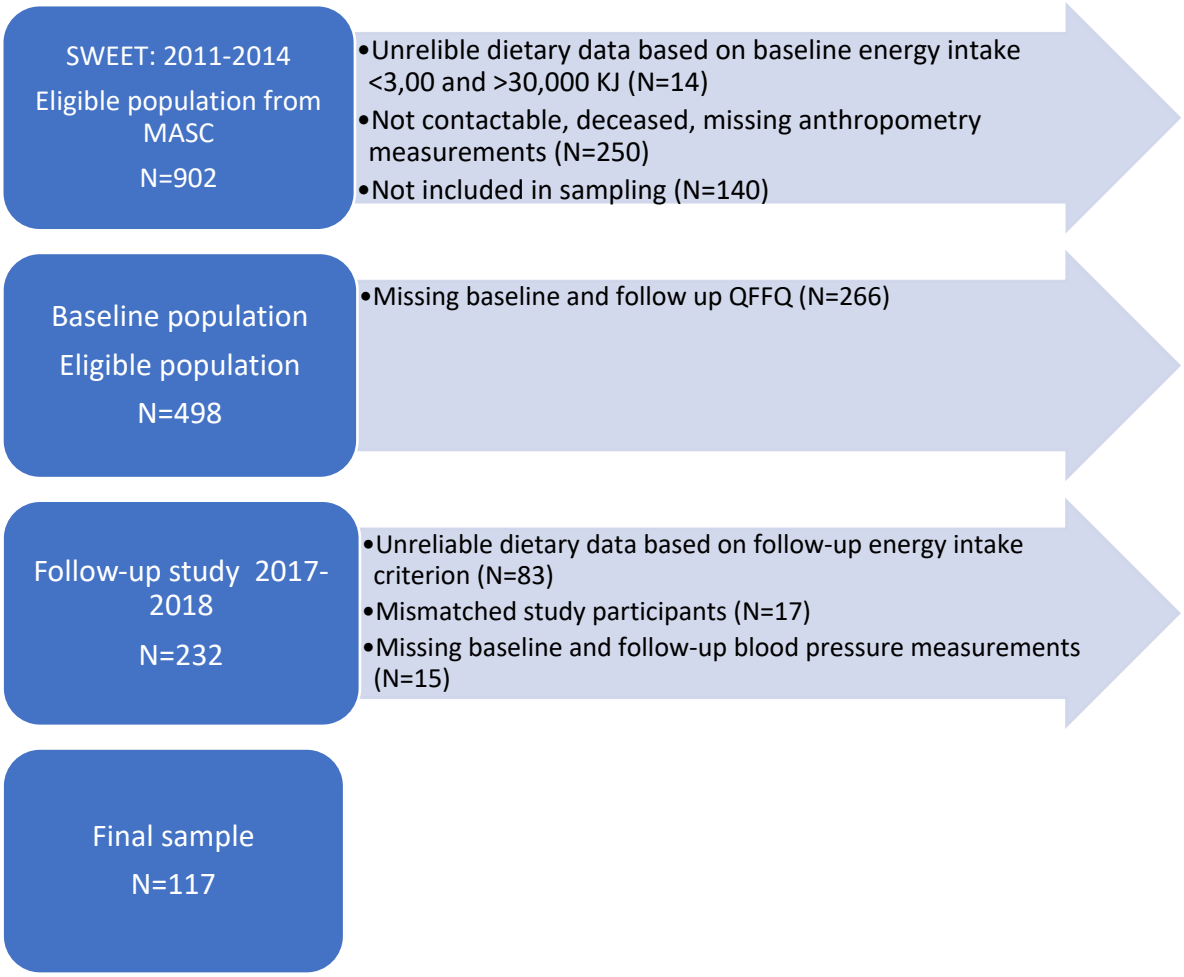

Figure 14 Flow chart of study participants (MASC= Middle-aged Soweto Cohort)

Dietary consumption data was gathered at baseline and follow-up using a standardised QFFQ, that has previously been highlighted and discussed (Chapter 2) (165,204). Three nutrient patterns were identified for this cohort: the first nutrient pattern was a "Plant driven nutrient pattern" with high factor loadings of plant protein, starch, and B vitamins, followed by an "Animal protein driven nutrient pattern", which was characterized by high factor loadings of animal protein and saturated fat. This nutrient pattern was associated with higher body mass index (BMI). The third nutrient pattern was a "Vitamin C, sugar and potassium driven nutrient pattern".

5.3.2 Assessment of lifestyle and clinical measures

At baseline and follow-up, data on socio-demographic and lifestyle variables were collected, and questionnaires were administered by research assistants. Age, employment status (yes/no), and highest educational level attained were all socio-demographic characteristics that were collected. Self-reported physical activity was collected using the GPAQ as previously described in Chapter 4.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in triplicate with a 2-minute interval using after the participant had been seated for at least 5 minutes. A digital blood pressure monitor (Omron M6, Kyoto, Japan) and appropriate cuffs were used to measure blood pressure. For each participant a blood pressure cuff was tightly wrapped around the upper arm and placed at the height of the heart, and only two measurements were used to compute an average SBP and DBP average based on their measurements (first

discarded due to white coat effect). Resting SBP of 140 mmHg or higher and/or DBP 90 mmHg or higher was defined as HTN.

5.3.3 Statistical analysis: Analysis of longitudinal data

STATA version 15 and IBM SPSS version 27 software packages were used for all statistical analyses (146,147). Differences between baseline and follow-up measurements were analysed using the Student paired t-test, Chi-square test, or Wilcoxon signed rank test where appropriate.

Generalized estimating equation (GEE) models were used to examine time-varying nutrient patterns while accounting for the correlation of repeated measures of SBP and DBP for the same participant with adjustment for baseline and follow up EI reporting, age and BMI. GEE models were constructed using repeat measures of the variables listed below and the models were designed as follows:

GEE model 1: nutrient patterns adjusted for energy intake reporting

GEE model 2: nutrient patterns adjusted for model 1 variables plus age

GEE model 3: nutrient patterns adjusted for model 2 variables plus BMI

The β coefficients from GEE models were reported as the measures of association.

Structural equation modelling (SEM) was used to determine whether there was an indirect effect of the nutrient patterns on blood pressure through BMI. The direct, indirect, and total effects were computed and recorded, and the best fitting model was evaluated using

goodness-of-fit indicators. Multivariable analyses performed using SEM were guided by an *a priori* conceptual model.

5.4 RESULTS

5.4.1 Descriptive Characteristics

The characteristics of the participants were disaggregated by HTN status at follow-up and summarized in Table 6. The baseline cohort comprised of normotensive population and over 5.5 years, 37% of the baseline population developed hypertension.

Table 6 Demographic and behavioural characteristics of study participants by hypertension status at follow-up.

		Hypertension status at follow-up		
Characteristics	Baseline N=114	Hypertension N=42 (37.2%)	Normotensive N=71 (62.8%)	P value
Age, y	48 (44-52)	53 (50-57)	54 (51-58)	0.181
Smoking status:				
Non-smoker (%)	106 (90.6%)	39 (92.9%)	69(97.2%)	0.359
Current smoker (%)	11 (9.4%)	3 (7.1%)	2 (2.8%)	
Alcohol consumption (%)	11 (11.1%)	8 (19.1%)	8 (11.3%)	0.275
Socio-economic status (score out of 9)	5 (4-6)	5 (3-5)	5 (3-5)	0.946

Employed (%)	64 (54.7%)	17 (40.5%)	24 (34.3%)	0.548
Baseline education:				
Incomplete high school/ lower (%)	77 (68.1%)	29 (70.7%)	45 (66.2%)	0.676
Body mass index, kg m^{-2}				
Normal and underweight (%)	17 (14.5%)	1 (2.4%)	15 (21.1%)	**0.008**
Overweight (%)	22 (18.8%)	7 (16.7%)	15 (21.1%)	
Obese (%)	78 (66.7%)	34 (81.0%)	41 (57.8%)	

All characteristics reported as n (%) except for age and socio-economic status and nutritional intake which is reported as median (25P-75P).

Over the 5-year follow-up, 37.2% of participants developed HTN and of those classified as hypertensive, 81.0% were living with obesity compared to 57.8% of normotensive individuals (p=0.008). Behavioral and socioeconomic factors were not significantly different between the 2 groups.

5.4.2 Longitudinal association between nutrient patterns and blood pressure

Principal component analysis was applied at baseline and follow-up to determine nutrient patterns as previously described (Chapter 2) and results of the nutrient patterns are displayed in Appendix 9. As previously identified in both chapters 3 and 4, PC1 was the "Plant driven nutrient pattern" followed by the "Animal protein driven nutrient pattern"

(PC2) and the third nutrient pattern (PC3) was the “Vitamin C, sugar and potassium driven nutrient pattern”. These nutrient patterns remained at baseline and 5.5 years follow-up.

Only repeated measures of the animal protein driven nutrient pattern were significantly associated with repeated measures of SBP in both unadjusted and adjusted GEE models (Table 7). Increasing consumption of the animal protein driven nutrient pattern was associated with a 1mmHg increase in unadjusted SBP (95%CI: 0-3mmHg, p=0.050) which increased to 4mmHg (1-8mmHg, p=0.012) when adjusted for repeated measures of BMI, age, and energy intake misreporting. No associations were found between repeated measures of any of the three nutrient patterns and repeated measures of DBP.

Table 7 Longitudinal GEE analyses of blood pressure and nutrient patterns (n = 117).

	Systolic blood pressure			Diastolic blood pressure		
	Unadjusted β (95% CI)	Model 1 β (95% CI)	Model 2 β (95% CI)	Unadjusted β (95% CI)	Model 1 β (95% CI)	Model 2 β (95% CI)
Plant Protein Driven NP	-0.244 (-2.456; 1.968)	-0.467 (-3.212; 2.789)	-4.385 (-8.192; 0.939)	0.083 (-1.415; 1.582)	0.797 (-0.892; 2.486)	0.538 (-1.162; 2.238)
Animal Protein Driven NP	0.935 ** (0.036; 2.664)	1.361 ** (0.084; 3.389)	4.144 ** (0.630; 7.539)	-0.224 (-1.468; 1.020)	0.242 (-1.142; 1.625)	-0.125 (-1.508; 1.257)
Vit C, Sugar, and Potassium Driven NP	-0.056 (-2.219; 1.705)	-0.140 (-2.368; 2.050)	-2.050 (-5.533; 1.076)	0.472 (-0.863; 1.807)	0.609 (-0.710; 1.929)	0.211 (-1.117; 1.539)

** p<0.05; Model 1: Nutrient patterns + energy intake misreporting; Model 2: Model 1 + age + BMI

5.4.3 Structural Equation Modelling results

The results of the analysis to determine the direct and indirect effect of the animal protein driven nutrient pattern on SBP through BMI are presented in Figure 16 and Appendix 9. Although there was a significant relationship between repeated measures of PC2 and SBP, there was no indirect effect of the animal nutrient pattern on SBP through BMI.

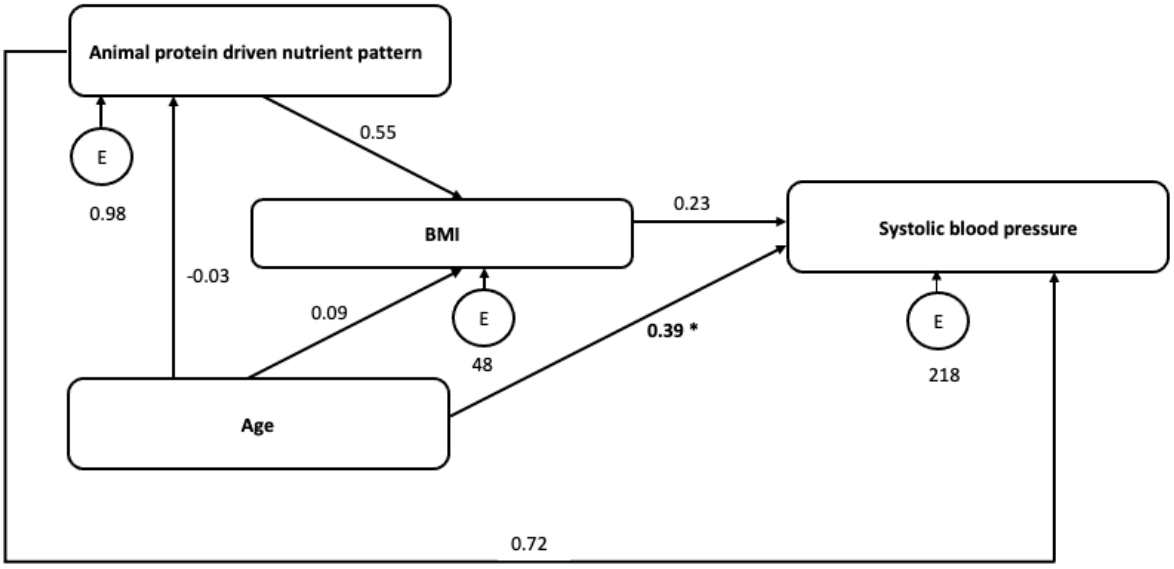

Figure 15 Structural equation models for the relationship between the animal protein driven nutrient patterns and SBP

5.5 DISCUSSION

In the current study, we examined the longitudinal association between nutrient patterns and SBP and DBP in a study population that was normotensive at baseline by applying GEE analysis to the repeated measures of both nutrient patterns and blood pressure. In addition,

we applied structural equation modelling to conduct mediation analysis to examine if nutrient patterns are associated with systolic or diastolic blood pressure through BMI. We found that repeated measures of the animal protein driven nutrient pattern was significantly associated with repeated measures of SBP in both unadjusted and adjusted models, however, no significant indirect pathways between this nutrient pattern and SBP through BMI was found. Therefore, for this population, BMI does not mediate the effect of the animal driven nutrient pattern on SBP.

Our previous work has shown that the animal protein driven nutrient pattern explained approximately 20% of total nutritional variance and was the second most consumed nutrient pattern in this sample of middle-aged women from Soweto (165,204). This pattern had higher loadings for animal protein, saturated fat, monounsaturated fat, cholesterol and vitamin D. Over the course of a 5.5-year follow-up period, our research showed that this nutrient pattern was significantly and positively associated with repeated measures of high SBP.

Previous nutritional studies that have examined the association between animal protein and HTN resulted in inconsistent results that showed that animal protein is both positively and inversely associated with HTN .(205–208) Increased consumption of animal protein has been shown to cause increased levels of trimethylamine N-oxide (TMAO) in the blood which has been shown to damage the lining of blood vessels, cause inflammation, and promote the growth of cholesterol plaques all of which lead to increased blood pressure (209). On the other hand, there is evidence that certain amino acids, classified as cardio-protective amino acids, present in animal protein have antihypertensive properties thus decreasing

oxidative stress and reducing arterial stiffness. This prior study identified these cardioprotective amino acids as: arginine, cysteine, glutamic acid, glycine, histidine, leucine, and tyrosine.

The animal protein driven nutrient pattern also reported high factor loadings of cholesterol and high cholesterol levels have been demonstrated to cause aortic stiffening as well as aortic narrowing, both changes which result in increased blood pressure, findings that are consistent with our study (50)[NO_PRINTED_FORM]. Some studies have shown that even moderately elevated cholesterol levels have been demonstrated to affect blood pressure (183,207). In addition, excess vitamin D, which also had reported high factor loadings in the nutrient pattern, has been demonstrated to increase blood pressure as this vitamin results in excessively high blood calcium levels, which has been associated with increased blood pressure (210).

When we explored the indirect relationships between the animal protein driven nutrient patterns with blood pressure through BMI, we found no mediating effect of BMI on SBP. As BMI has been shown to directly affect blood pressure, a larger study is required to further examine whether BMI truly has no mediating effect between the animal protein driven nutrient pattern and SBP. In our study population, we found a direct effect of age on mean SBP which may be due to the hardening of the arteries that has been observed with aging (211).

This study has some limitations and the first is inconsistencies in self-reporting of food intake impede dietary assessment research, which we tried to attenuate by adjusting for

energy intake reporting. Additionally, a limitation of this study is the small sample size which may not allow for generalizability or cause and effect principles to be applied, therefore a study with a larger sample size is recommended.

5.6 CONCLUSION

In conclusion, our findings provide evidence for the direct association between repeated measures of an animal protein driven nutrient pattern characterized as having high loadings of animal protein, saturated fat, monounsaturated fat, cholesterol and vitamin D, and repeated measures of systolic blood pressure. To prove causality, Mendelian randomization can be integrated in the study design. Mendelian randomization is an approach that helps eliminate biases in observational studies while providing greater confidence in data regarding the influence of dietary variables in illness and health. Four distinct relations must be assessed in a normal Mendelian randomization analysis: 1) an exposure of interest (e.g., nutrition, lifestyle, obesity, and biomarker) and an outcome (continuous phenotypic or disease risk); 2) a selected genotype and exposure; 3) a selected genotype and outcome; and 4) the predicted relationship between the genotype and outcome. This study did not find a significant indirect effect of the animal protein driven nutrient pattern on systolic blood pressure through BMI therefore, in our sample, BMI did not mediate the relationship between the animal protein driven nutrient pattern and SBP.

This is the first study to examine the longitudinal associations between nutrient patterns and blood pressure amongst adult black sub-Saharan African women. The results of our investigation could be used to design nutritional interventions that focus on reducing the

consumption of foods rich in animal protein, saturated fat, monounsaturated fat and cholesterol. Although previous nutritional studies focused on the relationship between individual macro or micronutrients, our research looked at the total diet by assessing nutrient patterns and investigating possible associations with blood pressure.

Further research with a larger sample size is needed to support the nutrient pattern approach, especially a pattern with high loadings for animal protein, saturated fat, monounsaturated fat, cholesterol and vitamin D and its impact on blood pressure for both clinical and public health interventions.

Good health is an investment in economic growth, and nutrition is one of the recommended preventive measures to manage these chronic diseases –

Kern and Mitmesser 2018

Previous chapters' findings are summarized here in relation to the PhD research objectives. The aim of the PhD research was to track the nutrient patterns of middle-aged black South African women over time and see if there was any correlation between these patterns and changes in body composition or HTN risk. Table 9 provides an overview of the PhD's goals and findings.

Table 8 Summary table of PhD objectives and subsequent findings.

Number	Objectives	Chapter	Key findings
1.	To examine changes in body composition indices by two groups (lean and obese status) at baseline	3	Compared to the lean group, individuals with overweight and obese status showed an increase in a variety of body composition parameters, including VAT, SAT, and FMI, as well as hip and waist circumferences and gynoid fat (p 0.001).
2.	To investigate the proportion of energy intake from macronutrients across the 2 groups	3	Proportion of energy intake from carbohydrate and fat was similar across the groups (54.4% and 53.5% for carbohydrate intake and 31.3% and 30.1% for fat intake for lean and overweight/obese participants, respectively).

			Participants who had obese or overweight status had greater protein consumption percentages than lean participants (11.8 percent vs. 11.0 percent; p = 0.02)
3.	To determine the nutrient patterns of the study population		The first nutrient pattern, the "*Plant driven nutrient pattern*" had higher factor loadings of plant protein, starch, and B vitamins, explained 25% of the variance; The second nutrient pattern (the "*Animal protein driven nutrient pattern*"), was characterised by animal protein and saturated fat explained 23% of variance The third pattern the "*Vitamin C, sugar and potassium driven nutrient pattern*" accounted for 11% of variance.
4.	To evaluate the association of nutrient patterns with body composition parameters in middle-aged black South African women.	3	Statistically significant associations were found for the animal protein driven nutrient pattern with all body composition indicators

			A one standard deviation increase in the animal protein driven nutrient pattern resulted in a 1.19 $kg \cdot m^{-2}$ (p = 0.002) increase in BMI, 10.17 cm^2 (p < 0.001) increase in VAT, 24.43 cm^2 (p = 0.009) increase in SAT, 0.01 (p = 0.009) increase in VAT/SAT ratio, 0.69 $kg \cdot m^{-2}$ (p = 0.005) increase in FMI, and 0.48 $kg \cdot m^{-2}$ (p = 0.002) increase in LMI. The plant driven nutrient pattern was associated with an increase in SAT (p = 0.045).
5	To confirm if the population's nutrient patterns remain the same at baseline and follow-up	4	Nutrient patterns as baseline and follow-up were examined and although the value of the factor loadings of the individual nutrients changed between baseline and follow-up, the nutrients with the highest loadings for each principal component (PC) did not change therefore based on the nutrient pattern naming convention, the nutrient patterns remained the same at follow-up.

6	To determine body composition indices changes over the 5-year follow up	4	Only DXA-derived measurements of whole body FM, FMI, VAT, and gynoid fat mass increased with time, with no significant changes in BMI. Significant decrease for whole body lean mass was observed.
7	An investigation of associations between body composition measures and nutrient patterns over a 5.5- yearss of follow-up amongst black women in South Africa.	4	Increases in FMI, LMI, and VAT were shown to be closely linked to the animal-driven nutrition pattern. The rise in FMI and LMI was shown to be substantially correlated with repeated measurements of the vitamin C, sugar, and potassium-driven dietary pattern,
8	To elucidate the longitudinal associations in HTN with nutrient patterns during 5.5- yearss follow-up	5	Repeated measures of animal protein driven nutrient pattern was significantly associated with repeated measures of high systolic blood pressure
9	Explore the direct and indirect effects or nutrient patterns and	5	Although results from the longitudinal GEE analysis showed a significant relationship between nutrient patterns

	HTN through BMI using structural equation modelling		and SBP, SEM results showed that there is a complex relationship between age, BMI and nutrient patterns as only a significant relationship between age and systolic blood pressure was found

6.1 EMERGING SCIENTIFIC AREAS FROM THE RESULTS:

6.1.1 The consistency of nutrient patterns of middle-aged black South African woman

At baseline, the PhD study population reported the following nutrient patterns:

1. “Plant driven nutrient pattern” which had higher factor loadings of plant protein, starch, and B vitamins (explaining the largest variance).
2. “Animal-driven nutrient pattern” which is known for its high factor loadings of animal protein and saturated fat.
3. Lastly, “the vitamin C, sugar and potassium driven nutrient pattern” .

At 5.5- years follow up, PCA was applied, as previously described, to the follow up nutritional data collected. The individual factor loadings for each of the selected 25 nutrients imputed into the PCA model did change between baseline and follow-up. The same naming convention was used at both timepoints and naming the nutrient patterns was

done by the including the nutrients with the reported highest factor loadings for each retain principle component. As the rank of the nutrients in the factor loading matrix did not change, the nutrient patterns remained the same at baseline and follow-up.

Over the course of 5.5 years, the quantity of total energy consumed reduced considerably, despite the percentage of macronutrients remaining constant. This might be related to measurement error in self-reported food intake data, which is a well-known problem of self-reported dietary food consumption data.

It has been proven that nutrition transition is impacted by urbanization, increased wealth, modernization and ageing. However, in this population there was no observed shift in the nutrient patterns as validated by the findings of this research therefore an assumption can be made that there was no nutrition transition in this specific community. While the population experienced ageing, socio-economic status remained the same during the study as reported which may contribute to the population consuming the same diet or food items. Furthermore, consumption of the same nutrient patterns may also be related to the population not experiencing modernization as the population may be culturally rooted in their nutritional habits.

The consistency of these nutrient patterns poses a public health concern as this population may resist changes to nutritional recommendations and new habits. Unfortunately as the

study baseline data was collected in 2011, there is no evidence that prior to the start date that demonstrated that there has been any attempt to introduce a diet change in this community. This may explain why the nutrient patterns remained consistent over the 5.5-year and therefore, with this in mind, investing in resources to develop interventions aimed at an integrated approach to behavioural changes is key to ensure that food or diet recommendations and guidelines may be successfully adopted.

6.1.2 The animal protein driven nutrient pattern and significant associations with adiposity

At baseline, the animal protein driven nutrient pattern was positively associated with BMI, SAT, VAT, VAT/SAT ratio, FMI and LMI. Therefore, by increasing consumption of this nutrient pattern may result to increasing these body composition measurements. Adiposity was linked to a high animal protein-driven nutrient pattern defined by high loadings of animal protein, saturated fat, cholesterol, riboflavin, vitamin B12, retinol, vitamin D, and zinc. In terms of BMI and the DXA-derived body composition parameters (VAT, SAT, VAT/SAT ratio, FMI and LMI), this pattern was strongly linked at baseline with most body composition parameters, and at follow-up, associations remained for only the DXA-derived body composition parameters (FMI, LMI, and VAT).

The significant relationship between repeated measures of the animal- driven nutrient pattern and repeated measures of VAT is a concern as it has been shown that abnormally high deposits of visceral adipose tissue is associated with medical disorders such as

cardiometabolic conditions, several malignancies such as breast cancer in women and cardiovascular disease to name a few. Thus, over time, the animal protein- driven nutrient pattern may result in an increased risk of developing these conditions.

Furthermore, as women age, it is known that adiposity changes as demonstrated by a magnitude of studies which reported that fat mass increases. This study found that FMI increased over time, coupled to known adiposity changes in ageing women, an increased risk of cardiometabolic comorbidities may occur. FMI, compared to BMI, is not influenced by muscle mass, making it a better indicator of fat or lean body types than the latter. The incidence of type 2 diabetes (T2D) is greater among black Africans, and it has been proven that a high fat mass index (FMI) increases the risk of cardiovascular disease, particularly in those with type 2 diabetes. This may led to an increased burden on healthcare facilities which may result on a strain on the healthcare budget and available resources. On an individual level, cardiometabolic comorbidities may result in decreased life expectancy and increased pill burden for medical management for the multiple conditions.

6.1.3 Methodological challenges on assessing nutrient patterns

Dietary data was collected using QFFQ that have been developed for the South African population and included indigenous foods that are commonly consumed by this population. Collecting dietary information using QFFQ is a preferable method of measuring intake for nutrients with very high day-to-day variability. The challenge with QFFQ is participant recall

bias when providing historical dietary information and this tool is less sensitive to measures of absolute intake for nutrients. The latter challenge was addressed through the nutrient pattern analysis methodology that was used. This is where 25 selected nutrients were imputed into the PCA for factor reduction and identification of nutrient patterns.

Dietary or nutrient pattern analysis is one of various methodologies for examining the correlations of dietary consumption and this PhD study, conducted nutrient pattern analysis over dietary pattern analysis. Although dietary patterns provide results that are more interpretable, the selection of food groups in dietary pattern analysis is based on arbitrary groupings, which is subjective. By conducting nutrient pattern analysis, the combined effect of nutrients is conducted. There may be additional information regarding the interactions, synergistic effects, and underlying processes of nutrients with the nutrient pattern analysis technique which this PhD study used.

Although each diet analysis approach has its advantages, it also has some limitations. A recommendation from this PhD study would be investigate and explore a diet method that combines both methodologies. This can be through applying the dimension reduction technique to the food items as well as the nutrients. Clustering of food items and nutrient co-occurrence patterns may be possible as a result. Only one such study has been done in Africa, and several have been undertaken in developed countries. It is possible that the development of new statistical tools for food composition data might lead to better dietary guidance and policy development.

Another recommendation to reduce EI bias, is to consider incorporating the use of biomarkers which are thought to offer near-unbiased estimations of energy and protein consumption. Biomarkers, like short-term nutritional assessment approaches, are impacted by within-person variance, which necessitates repeat measurements to estimate usual intake within a population by characterising and controlling for within-person random error. This will give a steady dose-response connection to consumption and has the potential to reduce the inaccuracy that comes with self-report data.

6.1.4 Recommendation of accurate body composition measurements to use in practice

BMI is a tool that was developed in the 1970s to quantify body mass composition and became commonly used in the 1990s. BMI is now used by clinicians to assess the risk of various diseases related to obesity. According to the Centers for Disease Control and Prevention, BMI is generally used as a screening tool to categorize people as underweight, healthy weight, overweight, or obese. However, it is now becoming widely demonstrated that BMI is not an accurate measure of body composition as it is not gender, race or age specific.

A significant finding from this study was that, while blood pressure increased, BMI remained constant, however, VAT increased over the same period. This highlights the importance of revising clinical guidelines to use more accurate body composition measurements such as

VAT or FMI, which may benefit population health through enabling clinicians to diagnose obese and overweight status much earlier, reducing the prevalence of obesity and, as a result, the prevalence of cardiometabolic diseases.

6.2 CONCEPTUAL RELEVANCE

With the majority of the population residing in metropolitan areas, South Africa is in the midst of an epidemiological transformation. The PhD findings, albeit population-specific, add to the limited information on nutrient patterns and their relationships with body composition indices and HTN risk in black African women.

According to the conceptual framework developed from Skelton & Dinan-Young, 2008, an ageing female cohort that is physically inactive is more likely to be obese, and to develop cardiometabolic complications such as HTN. Nutritional epidemiological studies have shown that nutrition plays a role in obesity risk which leads to HTN risk therefore, nutrition needs to be considered when developing interventions to lower these risks in ageing women.

This PhD study found that the prevalence of obesity and HTN in middle-aged black African women is significant, and the findings add to the limited information on the factors that contribute to HTN in African women. Hypertension and obesity may be linked, as seen in the

conceptual framework presented in Chapter 1 (Figure 17). This study also provided evidence that age is positively associated with systolic blood pressure. Findings that many other studies have shown.

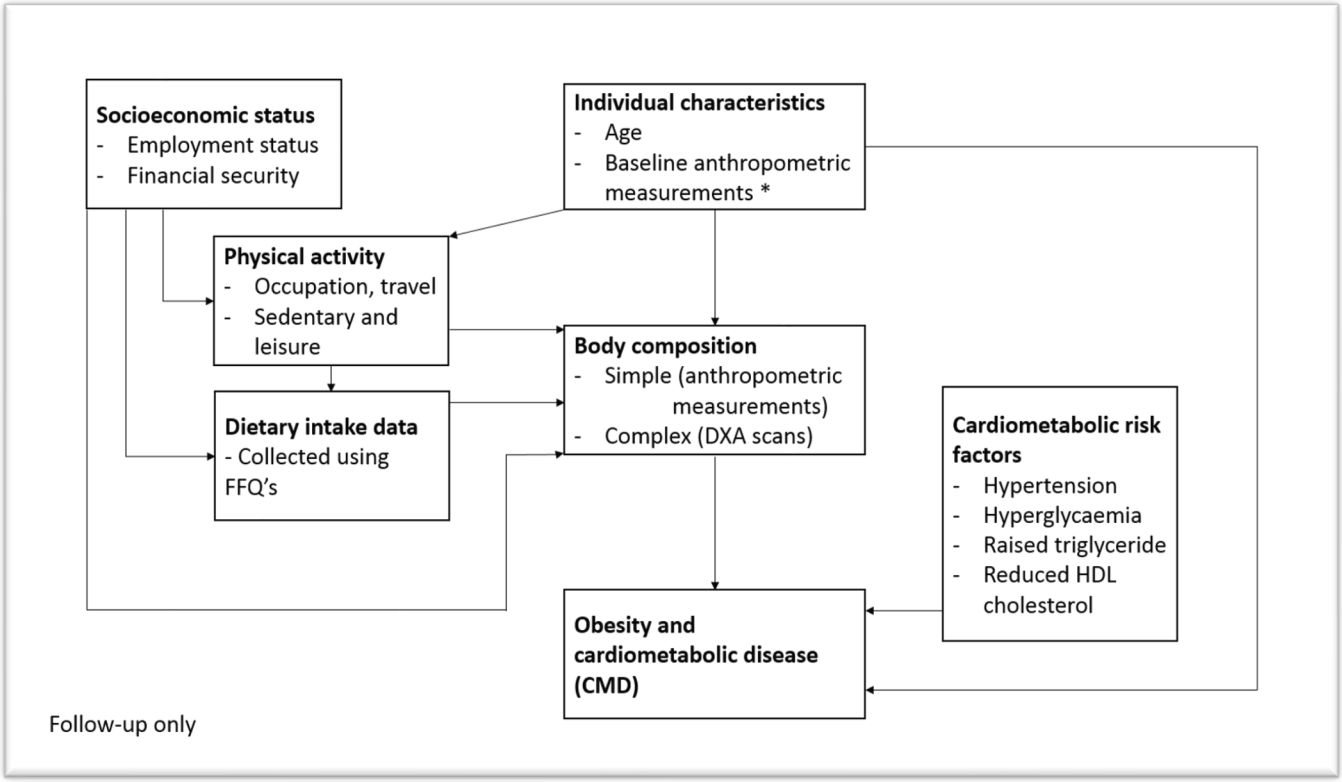

Figure 16 A conceptual framework of ageing and how lifestyle behaviours affect the development of cardiometabolic risk factors (adapted from Ageing and older people (Skelton & Dinan-Young, 2008)

The PhD results substantiated the conceptual framework through the evidence that proved the relationships and associations as shown in the conceptual framework as indicated. Furthermore, this PhD study further substantiated that the general conceptual framework was applicable to the population under study (black middle aged African women).

6.3 IMPLICATIONS

In South Africa, this is the first study of its kind to examine the longitudinal nutrient patterns of middle-aged black South African women and the correlations between their body composition indices and HTN. The implications from this study are listed below:

Implication on Research

The following recommendations are made for further research:

- Electronic activity monitors should be used to collect physical activity data to ensure that there is no potential recall bias as this study used self-reported physical activity data
- A larger sample size and longer follow-up time to confirm these findings
- Investigating alternative nutritional analytic methodologies such as combining both diet and nutrient pattern methodologies as previously mentioned to address both limitations of both methodologies and to leverage on their strengths. This can be through applying the dimension reduction technique to the food items as well as the nutrients
- Explore adaptation of the QFFQ for self-administration by participants during the study period to reduce participant recall bias

Implication on Practice

- This PhD contributes to literature on nutrient patterns, adiposity and BLOOD PRESSURE in middle-aged black African women
- The results from this PhD provided information on the most consumed nutrient patterns which can be used in practice to advise and raise awareness on the impact of these nutrient patterns on health and body composition
- The peer reviewed academic articles published under the banner of this PhD have highlighted associations between identified nutrient patterns and adiposity indictors

Implication on Policy

- Using the proposed recommendations, policymakers may create interventions that are tailored to the needs of specific populations
- It is critical to utilize these PhD results to build the body of knowledge of nutritional epidemiology and associations with body composition of black middle-aged women. Furthermore, this study
    - highlighted the gaps in nutritional epidemiology in this vulnerable population group highlighting a need for further research;
    - allowed for practical recommendations on study design for future studies where results from these studies may contribute to the body of research;
    - identified the nutrient patterns of this population group and this should be taken into consideration when designing diet interventions.

This showed that foods with high factor loadings of plant protein, starch, and B vitamins, as well as foods with high factor loadings of animal protein and saturated fat may be associated with both increased adiposity and lean mass over time therefore a policy guideline could include highlighting examples of these foods with guidance to limit them.

6.4 LIMITATIONS AND ADVANTAGES

Each of the three papers has its own set of limitations, but one of the most critical for this PhD research is the attrition rate of the sample that provided nutritional data. A recommendation to improve this rate, would be to consider revised study protocol to ensure improved attrition rate over time. At baseline, the sample size was 498, the same sample can be invited for a follow-up QFFQ resulting in a study period that is longer than 5.5 years which this study was set on.

Another limitation of this study is dietary recall. To address this limitation, I would recommend revising the QFFQ to ensure an accurate representation of foods that are consumed. Furthermore, to improve recall bias, other methods can be incorporated into the dietary data collection such as analysis of participants biomarkers to reduce EI bias. A final recommendation would be to review lessons learnt from the 2 diet data collection points from this study as well as other lessons learnt from other diet studies in a similar setting and to consider how these can be incorporated for improved diet data collection.

Lastly, the use of self-reported physical activity was a limitation of this study. With hindsight accurate physical activity assessments could have been recorded using electronic activity monitors thus eliminating recall bias. This information was gathered during follow-up however, a direct comparison between baseline and follow-up physical activity could not be done. Should a follow-up nutritional study be conducted and electronic activity monitors are used, this information can be used in post-doctoral studies.

This PhD thesis has a number of key advantages as follows:

- This is the first investigation of nutrient patterns, blood pressure and body composition in black African women over time.
- The study leverages on the Bt20+ trial which spans over 20 years.
- Women in Soweto, Johannesburg, a densely populated township, made up the majority of the study's participants.
- Body composition data was collected utilizing advanced equipment and standardised laboratory methods by an established network of researchers allowing a rigorous examination of a wide range of risk factors and covariates linked to obesity and BLOOD PRESSURE.
- The research team comprised a skilled team of multilingual research assistants making data collection successful.
- Further, regression models in this PhD were adjusted for energy intake misreporting, which enhanced accuracy of the models.

- Previous nutritional research focused on the association between individual macro and micronutrients; however, our study examined nutrient patterns across the entire diet.

6.5 RECOMMENDATIONS FOR FURTHER RESEARCH

From the research key findings, a key research question following this study would be to conducted a follow up study at 10 years with the same cohort thus addressing the recommendation for a longer follow-up time. At follow-up, approximately half of the baseline cohort that was recruited for the follow-up study had dietary data collected however, 35% of this sample were excluded due to unreliable follow-up EI criterion. Therefore a recommendation would be to repeat the study with the same follow-up sample and ensure to implement strategies to reduce EI misreporting such as reducing measurement error.

Secondly, I would explore the use of the alternative nutritional analytic methodologies which would provide results on both food based diet patterns and co-occurring nutrient patterns. These results would easier to interpret and will be ready for incorporation into policy and diet guidelines. By translating nutrient patterns into diet, recommendations from research findings will be more practical and easier to interpretate.

An additional recommendation is to explore the use of self-administered QFFQ to reduce recall bias or explore providing participants with a food scale or to amend the study protocol

and collect two QFFQs one month apart in addition to one 24 hour dietary recall questionnaire. The advantage of self-administered QFFQ is that these can be completed concurrently when consuming food thus reducing the recall bias which may address the EI variance found between baseline and follow-up nutrient patterns. Secondly, by transitioning to collecting the actual weight of the food rather than estimated food portions, a more accurate intake estimate may be made thus minimizing EI misreporting. Finally, by collecting two QFFQs one month apart for one time point as well as one 24 hour dietary recall questionnaire will allow for correlations between the nutrient patterns to be analysed in addition to increasing the statistical significance to be increased.

Lastly, based on the idea of Mendel's law of independent assortment, Mendelian randomization is a recently created methodology that combines genetic and traditional epidemiological analysis to infer causality for exposures. Mendelian randomization can be integrated into the study design and may eliminate biases in observational studies while providing greater confidence in data regarding the influence of dietary variables in illness and health. One example would be to include analyzing participants biomarkers which would subsequently result in reduce EI bias.

6.6 CONCLUSION

This PhD examined a group of black middle-aged African females with a high prevalence of obesity and focused on association of nutrient patterns with adiposity and cardiometabolic complications including blood pressure. A key finding that is of public concern is that this

study showed that the animal protein-driven nutrition pattern, which was the second most consumed pattern, was positively associated with FMI and VAT over a 5.5-year period thus contributing to visceral obesity which is a known risk factor of cardiometabolic conditions. This is of concern as the population already has reported high rates of obesity, which when coupled to this increased risk may led to cardiometabolic co-morbidities.

These PhD study results, albeit population specific, can be used to add to the body of knowledge about the nutrient patterns of black South African women. Additionally, recommendations from this study could be used to design more efficient nutritional studies with the hope that the results from those studies, may inform policies and nutritional guidelines for middle-aged black women not only in South Africa, but recommendations from this study can be adopted to other countries that are comparable to South Africa. Ahead of implementing these policies, the finding that the nutrient patterns remained unchanged shows that health promotion interventions on nutritional behavioral changes must be first conducted ahead of implementing diet changes. This may lead to an increased update of diet recommendations. Furthermore, if this is implemented successfully followed by updates to nutritional guidelines of middle-aged women, obesity risk may be lowered thus lowering the risk of cardiometabolic conditions including increasing blood pressure.

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Appendix 1: GSK informed consent

PARTICIPANT INFORMATION SHEET 1

Determinants of type 2 diabetes mellitus (T2D) risk in middle-aged black South African (SA) men and women: dissecting the role of sex hormones, inflammation and glucocorticoids

Invitation

Hello, my name is Lisa Micklesfield and I am an Associate Professor at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) from the University of the Witwatersrand.

I would like to invite you to participate in the research study entitled; Determinants of type 2 diabetes mellitus (T2D) risk in middle-aged black South African men and women: dissecting the role of sex hormones, inflammation and glucocorticoids. Before, agreeing to participate, it is important that you understand the purpose of the study, the study procedures, benefits and risks as well as your right to withdraw from the study at any time. If you have any questions, do not hesitate to ask me. You should not agree to take part unless you are satisfied with all the procedures involved. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will also be given a copy to keep.

Why is the study being done?

Studies have shown that body composition (the amount of muscle and fat in your body) and body fat distribution (where the fat is located in your body, for example, fat around your stomach) changes as one gets older, and that this is associated with an increased risk of certain diseases such as type 2 diabetes (sugar disease). However, most of these studies were undertaken in white populations from developed countries. It is important to study this in the black African population as many factors such as socio-economic status, physical activity and nutrition, are specific to this population, and may have an important impact on how your body works and how these changes with aging. Further, it is not known how body fat distribution and the risk of diabetes is affected by HIV infection, which is known to change body fat and its distribution. This study will examine different changes that happen in the body with aging and how these influence body fat distribution and type 2 diabetes risk in middle-aged black men and women. The factors that we are interested in examining include male and female sex hormones (for example, testosterone and oestrogen), inflammatory markers (involved in the immunity) and circulating cortisol (stress hormone).

Who can participate?

We are going to be testing women who participated in the Birth to Twenty Study of Women Entering and in Endocrine Transition (SWEET study) between 2011 and 2014, and men who participated in the H3Africa/AWIGEN study in 2014.

You will only be eligible for this follow-up study if you participated in these studies.

What will happen if you decide to take part in the study?

If you meet the criteria and decide to take part in the study, you will be required to complete two testing sessions described below, at DPHRU at Chris Hani Baragwanath Hospital in Soweto.

You are under no obligation to take part in the study and are not required to give a reason if you do not wish to participate. If you decide to take part in the study, you are free to withdraw at any time and without giving a reason and without prejudice. If you decide to withdraw from the study, we will discuss with you what will happen to any information or samples that you have provided. If the incomplete samples and information can usefully contribute to the study, we will ask your permission to store them and use them in our analysis. Alternatively, on your request all your information and samples will be destroyed.

Testing Session 1: Testing at any time of the day and will take 2 hours of your time.

You will be requested to complete a series of questionnaires by interview, which will include questions on measures of socioeconomic status (i.e. housing, employment and income), personal and family history of disease (e.g. diabetes, obesity, high blood pressure, and cardiovascular disease), reproductive history, access to food, and stress. In addition, questions on lifestyle factors, including smoking and alcohol intake, medication and supplement use will be included. Furthermore, your dietary food intake over the past 7 days will be measured using a questionnaire that lists all possible foods. You will also be asked to wear two small devices, one on your hip and one on your thigh, for 7 days to measure your

physical activity and sedentary time. Your weight and height will be measured, as well as your waist and hip circumferences. In addition, you be required to undergo a scan to measure your body fat, muscle mass, and bone density, using a special X-ray scan. If there is any risk that you may be pregnant, you will be requested to have a pregnancy test prior to the scan, as the scan will expose you to a small amount of radiation. Your blood pressure will also be measured and you will also be requested to have an HIV test. You will also be required to bring your ID document, clinic card as well as any medication that you are currently taking.

Further details of these procedures are provided below:

Demographic, socioeconomic status and lifestyle questionnaire

You will be asked questions about various measures of social and economic status (e.g. if you are employed or not, what do you do at work, your source (s) of income) and questions about whether there are people in your family with diseases such as high blood pressure or heart problems. You will also be asked questions about your personal health, stress and reproductive history. In addition questions on lifestyle factors including smoking and alcohol intake, medication and supplement use will be included. We will fill in the answers for you. You can skip any questions that make you uncomfortable.

Food frequency questionnaire (FFQ)

You will also be asked to fill in a food frequency questionnaire to measure your usual dietary intake (what you normally eat). This questionnaire will give us a sense of what and how much you have eaten over the last week. In addition, we will ask you a few questions about your food security, in other words, your access to food and if you or your family ever experience periods of hunger.

Body composition and DXA (dual-energy X-ray absorptiometry) measurements

Your weight, height, waist and hip circumference will be measured as part of your body composition assessment. In addition, you will undergo a special X-ray scan (DXA) that will tell us about your muscle mass, body fat and bone density. The scan will take approximately 20 minutes to perform during which you will lie quietly on the scanning table in a medical gown provided. You will be asked whether or not you are pregnant. If there is any possibility that you may be pregnant please tell the technician and we will perform a pregnancy test. If you are pregnant, you will not have the scan. The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray.

After a 5-minute relaxation period, blood pressure will be measured 3 times in a row, separated by 5 minutes of relaxation between readings. A standard blood pressure monitor will be used.

Physical activity

You will be asked to wear two motion sensors (accelerometer and activPAL) for 7 days, to measure your activity and sedentary patterns. They are the size of a small matchbox, and one is worn on the waist with a lightweight belt and the other is attached to the thigh using a waterproof plaster. You will be instructed on how to use the monitors. There are no side-effects associated with wearing the monitors.

Grip strength

Your grip strength will be measured using a hand dynamometer. In a standing position you will be required to squeeze the dynamometer with as much force as possible with your non-dominant hand. You will be required to do this 3 times with a 10-20 second rest in between.

Sleep

To help us understand your personal sleep habits, we will ask you to complete three questionnaires relating to your sleep quality, daytime sleepiness levels and your chronotype (i.e. your personal preference for mornings or evenings). You will also keep a sleep diary for one week. Together these will allow us to measure your sleep habits such as how long you sleep for, when you go to bed and wake up and how efficient your sleep is.

HIV test

We would like to test your HIV status. This is very important as it may have effects on your body composition, risk for diabetes and the factors that we will measure in your blood. The HIV test is voluntary, however If you have previously been tested as HIV positive this is not necessary. If you refuse the test, you will not be able to participate in the study. It is also important to note that if you decide to test, you will be given pre-test counselling. This test is always strictly confidential and can only happen if you agree. There is no way in which anyone can link your HIV status to your name as all results in this study are coded with a number. No one including your doctor, family, or work colleagues will be told about this test without your permission. The advantage of a rapid test is that you do not have to return to get your test result. Results will be available when your check out, after all the other tests, measurements and questionnaires are completed.

You will have your finger pricked with a sterile needle and the drop of blood will be tested on specific HIV testing kits to check for HIV antibodies. Test results will be given to you in private by a registered trained counsellor. If the report states negative it means that there are no antibodies to HIV. The window period will be explained. If the report states positive, it means that you are HIV positive and that there are antibodies to HIV. You will be given a letter to refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result. Sometimes we cannot clearly tell if the results are negative or positive. We will then refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result.

There is a chance that some of the questions in the questionnaire might trigger some

emotional distress. If this happens, we will refer you to our counselling nurse on site and she will make the necessary appointment for you to see a psychologist at the Psychology Unit at Chris Hani Baragwanath Hospital

Testing session 2: Testing in the early morning (between 7:30 and 8:00 am) and will take 2**½** hours of your time

You will be requested to visit the DPHRU offices at the Chris Hani Barangwanath Hospital between 7:30 and 8:00 am in the morning approximately 7 days after testing session 1, but after an overnight fast (nothing to eat or drink, except water, from 10pm the night before). You will be asked to donate a sample of blood and then undergo an oral glucose tolerance test (OGTT) to determine whether you are at risk for developing diabetes. You will also be required to return the two physical activity monitors that were given to you in the first testing session.

Blood sampling and oral glucose tolerance test (OGTT)

Blood sampling and the OGTT can only be performed in the morning after an overnight fast. Therefore it is important that you do not eat or drink anything (except water) from 10 PM the night before, or for 10-12 hours before your test begins. You are not allowed to take any medication, food or drink, chew gum or sweets, before your blood test.

Blood sampling will be performed by inserting a small plastic tube into a vein in your arm and a small plastic tap or valve will be attached onto it so that blood samples can be drawn before

the 2 hour OGTT test. The first blood sample (50 ml) will be drawn for the measurement of blood lipids (fats), glucose (sugar) and various hormones (e.g. insulin and cortisol) and inflammatory markers in the fasted state (i.e. before you eat anything). You will then be asked to drink a cup of water containing 75 g of glucose (sugar). Thereafter, blood samples (5 ml or 1 teaspoon each) will be drawn from your arm at 30, 60, 90 and 120 min after the glucose ingestion (total 20ml, 4 teaspoons) for the later measurement of changes in glucose and insulin levels. After your test you we will give you something to eat and drink.

What are the risks and discomforts of this study?

There are no risks or discomforts associated with the administration of the questionnaires. Strict confidentiality of results will be maintained. Your name will be removed from all data, and you will be assigned a number, which will be used to identify data relating to you. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

There are no risks associated with the use of the physical activity monitors. The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray (11.3 microSieverts).

There are no appreciable risks associated with the fasting blood sampling and OGTT, other than those associated with routine blood sampling. Sometimes when blood is taken you may feel a prick at the place where the needle enters your body. Afterwards there may be a little

bruise, and pain (which are associated with normal blood sampling), and in very unusual circumstances, local infection. Very occasionally participants may 'faint'. This is a stress response to a trigger (e.g. the sight of blood) and has no long-term effects. This test is used routinely for both research and medical purposes. The total amount of blood drawn will be 70 ml, which is substantially less ($1/7^{th}$) than that of a standard blood donation (500 ml). All procedures will be supervised and carried out by a nursing sister and appropriately trained medical personnel using sterile techniques to minimise any risks of infection.

If we discover that you have any health problems based on our tests, you will be contacted and referred to the appropriate clinic for treatment and/or management.

Are there any benefits to you for being in the study?

You will receive your own results from the study, including your body composition (e.g., muscle and fat mass), your blood pressure, lipids (fats in your blood) and glucose tolerance (your risk for diabetes). In addition, you will contribute to our understanding of the how body fat distribution and the risk for type 2 diabetes differs between black South African men and women (pre-menopausal and post-menopausal), with and without HIV. This information can be used to provide evidence for future strategies for the prevention, treatment and management of diabetes risk in middle-aged black South Africans.

What will happen when the study is over?

Detailed analysis of the samples will take some time, but once these analyses have been completed, the final results of the study will be shared with you. In addition, the results of the study will be published in scientific journals, as well as in the local media. Your name will not be used in any publication of the results. You may be contacted for a follow-up study.

Will you receive reimbursement for transport?

You will receive R150 to cover transport costs to DPHRU for each of the two testing sessions. The transport money will be paid to you at the end of each session.

Who will see the information that is collected about you during the study?

Strict confidentiality of results will be maintained. Your name will be removed from all data, and you will be assigned a number, which will be used to identify data relating to you. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

Who do I contact if I have any questions about the study?

If you have any questions or you experience any problems during or after the tests, please contact Associate Professor Lisa Micklesfield or Associate Professor Julia Goedecke, or a representative of the Human Research Ethics Committee.

Associate Professor Lisa Micklesfield (PhD)

Principal Investigator

MRC/Wits Developmental Pathways for Health Research Unit

Chris Hani Baragwanath Hospital

Soweto Johannesburg

Tel: 021-650 3153(w) 083 9433172 (cell)

Email : lisa.micklesfield@wits.ac.za

Associate Professor Julia Goedecke (PhD)

Principal Investigator

Honorary Associate Professor, School of Clinical Medicine, Wits University

Address: Room 230 RIND Building, South African Medical Research Council, Francie van Zijl Drive, Parow, 7725, Cape Town

Tel: 021-9380862(w) 0828255616 (cell)

Email : julia.goedecke@mrc.ac.za

Human Research Ethics Committee contact details:

Prof P Cleaton-Jones, Tel 011 717 2301, email peter.cleaton-jones1@wits.ac.za or Ms Zanele Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za

INFORMED CONSENT 1

Determinants of type 2 diabetes mellitus (T2D) risk in middle-aged black South African (SA) men and women: dissecting the role of sex hormones, inflammation and glucocorticoids

Consent to participate in the study:

"I, _____, hereby give consent to participate in this research trial to be conducted by DPHRU, within the Department of Paediatrics at the University of Witwatersrand.

I understand that the study will involve completion of questionnaires by interview, an HIV test, routine body measurements (i.e. height, weight, hip and waist circumference), an X-ray scan to measure my body fat and muscle mass, and bone density, blood pressure, grip strength, collection of blood samples after an overnight fast (10-12 hours) and during a 2-hour oral glucose tolerance test, as well as wearing an accelerometer and ActivPAL for 7 days to measure physical activity and sedentary time, respectively. The purpose and all the details of this study have been explained to me.

I have read and have had explained to me the procedures described. I have had an opportunity to ask questions and my questions have been answered in a satisfactory way.

I understand that all the information collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. All samples will be kept in a freezer in a secure facility with access limited to research personnel. All records will be kept in a locked room and in a secure computer database in the research unit. My name will not be used in any publication of the results. I understand that for data verification and quality control purposes regulatory authorities and/or members of the Wits Human Research Ethics Committee (Medical) may be allowed access to my personal data under conditions of strict confidentiality.

I understand that I may be contacted for a follow-up study.

I agree to participation in the study on the condition that:

1. I can withdraw voluntarily from the study at any time and that no adverse consequences will follow on withdrawal from the study.

2. I have the right not to answer any or all questions posed in the questionnaire.

3. The University of the Witwatersrand's Human Research Ethics committee has approved the study protocol and procedures.

4. All results will be treated with the strictest confidentiality.

5. Only group results, and not my individual results, will be published in scientific journals and in the media.

6. The study scientific team are committed to treating participants with respect and privacy through interviews conducted in private and follow-up counselling available on request.

7. I will receive a referral note to psychological services if I experience any psychological distress during the study.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

RESEARCH ASSISTANT:

Printed Name and Time	Signature/Mark or Thumbprint	Date

Copy provided to participant _____ (initialed by researcher)

WITNESS: (If applicable)

Printed Name Date and Time	Signature/Mark or Thumbprint

Appendix 2: HREC (Medical) Clearance Certificate M010556

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Richter

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M01-05-56

PROJECT Birth To Twenty: A Longitudinal Population-Based Study of Reproductive And Psychosocial Maturation In Urban South African Youth Aged 10 To 20 Years

INVESTIGATORS Prof LM Richter

DEPARTMENT School of Clinical Medicine, CH Baragwanath Hospital

DATE CONSIDERED 01-05-25

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 01-07-09 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor:
Dept of ,

Works2\Iain0015\HumEth97.wdb\M 01-05-56
==========
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3: HREC (Medical) Clearance Certificate M090620

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Richter

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M01-05-56

PROJECT Birth To Twenty: A Longitudinal Population-Based Study of Reproductive And Psychosocial Maturation In Urban South African Youth Aged 10 To 20 Years

INVESTIGATORS Prof LM Richter

DEPARTMENT School of Clinical Medicine, CH Baragwanath Hospital

DATE CONSIDERED 01-05-25

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 01-07-09 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor:
Dept of ,

Works2\Iain0015\HumEth97.wdb\M 01-05-56
==========
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4: HREC (Medical) Clearance Certificate M170718

R14/49 Ms CBT Makuru & Professor SA Norris

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170718

NAME: (Principal Investigator)	Ms CBT Makuru & Professor SA Norris
DEPARTMENT:	School of Clinical Medicine Division of Paediatrics Developmental Pathways to Health Research Unit CH Baragwanath Hospital
PROJECT TITLE:	Nutrition of ageing Black South African women and correlates with anthropometry and cardiometabolic outcomes
DATE CONSIDERED:	28/07/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr P Gradidge
APPROVED BY:	Professor CB Penny, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	26/09/2017

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature — Date: September 29, 2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 5: Food frequency questionnaire

Study ID **Date**……..

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
DAIRY-BLUE										
	1.Tea	Ordinary		4038						
		Herbal		4053						
		Rooibos		4054						
	1. Sugar in tea			3989						
	2. Milk in tea	Full cream		2718						
		Low fat 2%		2772						
		Skim fat free		2775						

		Other								
	1. Coffee			4037						
	2. Milk in coffee	Full cream		2718						
		Low fat 2%		2772						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		Skim fat free		2775						
		Other								
	2. Sugar in coffee			3989						
	2. Milk as a drink	Full cream		2718						
		Low fat 2%		2772						
		Skim fat free		2775						
	3. Buttermilk/maas	Buttermilk		2713						
		Maas		2787						
	4. Milk drinks, flavoured									

X	**5. Yoghurt**	Fruit, LF, sweetnd		2732						
		Plain LF		2734						
	3. Breakfast cereals									
	2. Milk added to cereal									
	1. Sugar added to cereal			3989						
	9. Ice cream	Full cream		3519						
		Low fat		4325						
	9. Ice lollies			3982						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	ASK ABOUT TYPE OF BREAD,THICKNESS OF SLICES AND THE SPREAD									
	1. Bread/rolls	White		3210						
		Brown		3211						
		Whole wheat		3212						
		Traditional		3210						
		Roti								
	Spread? Y / N	**Brand name**	*Do you use spread every day on your bread?*							
	1. Brick margarine	HM		3484						
	1. Tub margarine	PUM		3496						
	1. other tub e.g. floro			3521						

	1. Butter			3479						
	Cheese spread	WM		2730						
	Fish paste			3109						
	Honey/syrup			3988						
	Jam			3985						
circle	**Marmite/Bovril**			4030 / 4029						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	Sandwich spread			3522						
	Peanut butter			3485						
	Chocolate spread			9999						
	Cold meats	Ham		2969						
		Polony		2919						
		Salami		2948						
	6. Cottage cheese	Low fat		2729						
		Full fat		2759						
X	**7. Cheddar**			2722						

	7. Gouda			2723						
	7. Other cheese									
	8. Cheese wedges			2728						
	2. Fat cakes			3257						
	Any topping?									
(Red)	**QUARTER**	*Tick toppings:*								
	White bread			3210	¼ loaf = 225					

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	Fried Chips			3740	1 portion = 90					
	Cheese			2722	___ slices x 20 =					
	Polony / French			2919	___ slices x 15=					
	Vienna (Russian)			2936	___ unit x 40 =					
	Fried egg			2869	___ medium x 45 =					
	Atchar			3117	___ Tsp x 40 =					
	Tomato sauce			3169	___Tsp x 25 =					
Other										
	Mashed potato			3876	¼ cup = 62.5					
	Mince meat, regular			2987						

	4. Maize porridge stiff			3400						
	4. Maize porridge soft									
	2. Milk on soft porridge									
	1. Sugar on soft porridge			3989						
	1. Fat on soft porridge									
	4. Mabele/maltabella stiff			3241						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	4. Mabele soft									
	2. Milk on Mabele									
	1. Sugar on Mabele			3989						
	1. Fat on Mabele									
	4. Oats			3239						
	2. Milk on Oats									
	1. Sugar on Oats			3989						
	4. Maize & pumpkin porridge									
	5. Pasta/Pasta dishes									

	Remember to ask about added cheese! Grated?									
	6. Spaghetti Bolognaise	Regular mince		3260						
	6. Macaroni and cheese	WH, HM		3301						
	6. Lasagne	Regular		3261						
	7. Rice	White		3247						
		Brown		3315						
		With fat?								

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	7. Samp/mealie rice	With fat?								
	7. Samp and beans	With fat?		3402						
	7. Wheat rice			3249						
X	**8. Pizza**	Vegetable topping								
		Meat topping								
		Meat & vegetables								
	8. Savoury tart									
EGGS-YELLOW										

	Boiled/poached			2867						
	Fried	HM		2877						
		PUM		2878						
		Sun oil		2869						
tick	**Scrambled**	LFM, PUM		2888						
		LFM, Sun oil		2889						
		WM, HM		2890						
		WM, Sun oil		2873						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		WM		2872						
	Omelette (tick scrambled & add topping here)									
FRUITS-ORANGE										
X	**1. Apples**			4222						
X	**2. Bananas**	Fresh		3540						
		Fried		3611						
	3. Berries	Fresh								
		Candied								

X	**4. Figs/prickly pears &**	Fresh								
	Coconuts, dates	Candied								
	5. Fruit salad	Fresh								
		Sugar added?								
		Canned juice		3667						
		Canned syrup		3580						
X	**6. Grapes**			3550						
X	**7. Guavas**	Fresh		3551						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		Canned juice		3628						
		Canned syrup		3553						
X	**8. Mango**	Fresh		3556						
		Canned syrup		3633						
	8. Pawpaw	Fresh								
	8. Kiwi fruit			3660						
	8. Leechies			3632						
	9. Watermelons			3576						
X	**9. Sweet melon**									

X	**10. Naartjies**	Mineola fresh		4227						
		Naartjie fresh		3558						
		Naartjie canned in syrup		3635						
X	**11. Oranges**	Fresh		3560						
	11. Grapefruit	Fresh		3546						
X	**12. Peaches**	Fresh		3565						
		Canned juice		3640						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		Canned syrup		3567						
X	**12. Nectarines**	Fresh		4228						
X	**13. Pears**	Fresh		3582						
		Canned juice		3643						
		Canned syrup		3583						
	14. Pineapple	Fresh		3581						
		Canned juice		3647						
		Canned syrup		3648						
X	**15. Plums**	Fresh		3570						

	15. Apricots			3534						
	16. Dried fruit									
	16. Dry stewed fruit									
	16. Raisins			3552						
	17. Fruit juice	Fresh								
		Sweetened								
		Unsweetened								

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
SOUPS, LEGUMES, NUTS										
X	**1. Soups**	Powdered made with water		3165						
		Vegetable		3162						
		Meat, lentil veg		3153						
		Dried bean, meat, veg		3145						
		With bones								

	2. Beans and lentils	Anything added								
	3s. Nuts									
	3. Peanuts									
	Peanuts & raisins									
FISH & SEAFOOD- BEIGE										
	1. Fried fish									
	1. Fish cakes									
	1. Fish fingers									
	1. Calamari									

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
Circle one	**2. Grilled/smoked/dried fish**									
	2. Haddock									
	3. Pilchards & sardines	Canned water								
	Canned	Tomato sauce								
		Mayonnaise		3488						
	3. Tuna	Brine water								
	3. Tuna	In oil								
		Mayonnaise		3488						
	3. Pickled fish									

MEAT- RED *How do you eat meat? 1= with fat 2= fat trimmed*										
	1. Roast beef									
	1. Beef chops									
	1. Beef steak (bone)									
	1. Beef steak (no bone)									
	1. Beef stir-fry									
	1. Beef stew / veg			3120						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	1. Beef stew / cabbage			3006						
	2. Beef patties			2984						
	2. Mince	Regular		2987						
		With vegetables								
X	**2. Meatballs**	*Egg / no egg*								
	2. Cottage pie			3009						
	3. Burgers	Bun		3210						
		Patty: Beef		2984						
		Cheese slice		2722						

	please tick, quantify, *brand names (* *McDonalds bigmac)*	Tomato sauce		3169						
		Mayonnaise		3488						
Meal?	**Chips (fried)**	*Size?*		3740						
	Soft drink	*Size?*		3981						
	3. Hot dog	HD roll								
		Vienna								
		Tomato sauce		3169						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	3. Pita with…									
	3. Chicken burger	Bun		3210						
	please tick, quantify, *name of burger (e.g KFC twister)*	Crumbed, fried		3011						
		Cheese slice		2722						
		Tomato sauce		3169						
		Mayonnaise		3488						
Meal?	**Chips (fried)**	*Size?*		3740						
	Soft drink	*Size?*		3981						

	3. Chicken nuggets			3018 ?						
	4. Chicken stew									
	4. Fried chicken pieces	With skin								
		Without skin								
	4. Roast chicken	With skin								
		Without skin								
	4. Chicken stir-fry									
	5. Chicken other									

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	6. Fat cakes & mince									
	7. Meat pies									
	7. Samoosas	Meat		3355						
		Vegetable		3414						
	7. Sausage rolls			2939						
	7. Spring rolls			2918						
	8. Mutton stew no veg									
	8. Mutton stew with veg									
	8. Mutton leg chop									

	8. Mutton loin chop			2927						
	8. Roast mutton			2947						
	9. Spare ribs			3010						
	9. Pork chops			2930						
	9. Bacon			2906						
	9. Roast pork									
	10. Pork sausages			2932						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	10. Viennas			2936						
circle	**10. Frankfurters**	*Beef/pork/chicken*								
	10. Boerewors			2931						
	11. Traditional / organ meats									
	Chicken livers			2970						
	Chicken giblets			3014						
	Chicken head			2999						
	Chicken feet			2997						
	Liver & fat			2920						

	Sheep intestine / lungs			4342						
	Shank *pork/lamb/beef*									
	Mopani worms	*How cooked?*								
	12. Vegetarian products									
	13. Dry sausages			2949						
	13. Biltong			2912						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
VEGETABLES – GREEN										
	1. Asparagus	Fresh boiled		3695						
		Canned boiled		4094						
	2. Avocado	Fresh		3656						
	3. Baby marrows	Fresh boiled		4171						
		Fat added								
		Sauce								
	4. Beetroot	Boiled		3698						
	Grated / sliced	Salad		3699						
	Chutney, onion,	Salad								

	5. Butternut	Boiled		3759						
		Boiled Sugar added		3989						
		Sugar & fat added		4273						
	5.Pumpkin	Boiled		4164						
		Sugar & fat added		3893						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	6. Broccoli, *Fresh/frozen*	Boiled								
		Fat added		3808						
	6. Cauliflower	Boiled		3716						
		Boiled + cheddar		3715						
	Quantify white/cheese sauce Pg 23									
	7. Cabbage		Boiled	3756						

	7. ..with potato, onion	HM		3813						
	7. ..with potato, onion	Sun oil		3815						
	7. coleslaw (mayo)			3705						
	7. Cabbage, *fried*	HM		3810						
	7. Cabbage, *fried*	Sun oil		3812						
	8. Carrots	Raw		3709						
	8. Carrots	Boiled		3757						
		Sugar added		3818						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	9. Gem squash	Boiled		3760						
		Boiled + sugar		3754						
	10. Green beans	Boiled		3696						
	10.. with potato, onion	No fat		3933						
	10.. with potato, onion	HM		3792						
	10.. with potato, onion	Sun oil		3794						
	11. Mealies	Whole, boiled		4133						
		Whole, boiled canned		4134						
	11. Sweet corn									

	12. Mixed vegetables	Canned, boiled		4264						
	carrot, corn, peas, green beans	Frozen, boiled		3727						
	caulif', carrot, green beans	Frozen, boiled		4265						
		Fresh boiled, PUM		3836						
		Fresh boiled, HM		3835						
	13. Mushrooms	Fried HM		3893						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	13. Mushrooms	Fried in Sun oil		3841						
	14. Peas	Frozen boiled		4146						
		Boiled, sugar, HM		3859						
	15. Potatoes, Baked	(ate flesh & skin)		3736						
		(ate skin only)		3970						
		(ate skin, sour cream & chives		3973						

	Boiled	(ate skin & flesh)		4155						
		(ate no skin)		3737						
		HM		3867						
		PUM		3868						
	Croquette/cake			3915						
	Fried/sautéed	HM		3871						
		Sun oil		3873						
	Frozen, boiled			4156						
	Mashed	(SM, PUM)		3875						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		(WM, HM)		3876						
	Roasted in..	.. beef fat		3878						
		.. chicken fat		3923						
		.. lamb fat		3735						
		.. pork fat		3956						
		..Sun oil		3979						
	15. Potato Salad	(mayonnaise)		3928						
	Potatoes, whole canned			4159						
	16. Potato chips	Fried in Sun oil		3740						

		Oven baked		3945						
		Mayonnaise		3488						
		Tomato sauce		3169						
	If take away where from?									
	And what size?									
	17. Salad vegetables	French		3921						
		Greek		4271						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
		Mixed green		3927						
	Dressing? See pg 19!									
	17. Raw tomato			3750						
	17. Cucumber			3718						
	17. Peppers raw, green			3733						
	17. Onion, raw			3755						
	17. Onion, fried	HM		3844						
	17. Onion, fried	Sun oil								
	18. Spinach/morogo	boiled		3913						
		Boiled + HM								
		Boiled + PUM								

	18.. with potato, onion	HM		3901						
	18.. with potato, onion	Sun oil								
	19. Sweet potatoes	Baked, ate skin only		3748						
		Boiled, no skin		3903						
		Sugar + HM		3749						
	20. Tomatoes, cooked	Fried, onion		3925						
		Fried, onion, peppers								
		Fried, onion, peppers, carrots								

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
FATS-TAN 'prompt'										
	1. Tub margarine	Where used								
		Number of spoons								
		Number in family								
	1. Butter									
	1. Brick Margarine									
	2. White margarine (type of fat)									

	3. Cream and substitutes (brand, real dairy/plant fats)									
	4. Oils	Where used								
		Number of spoons								
		Number in family								
	5. Salad dressings	Homemade								
		Shop bought								
	5. Mayonnaise	Where used								
		Number of spoons								
		Number in family								

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
BISCUITS, CAKE & PUDDING - PURPLE										
	1. Biscuits/cookies	Commercial plain		3216						
brand		Commercial, filling		3217						
		Homemade, plain		3341						
	2. Biscuits/savoury	Provita		3235	___ x 6					
		Ry-vita		3236	___ x 5					
		Cream crackers		3230	___ x 8					

	High fat *(circle which one)*	Tuc/salticrax/kips		3331						
	Harvest wheat	Whole wheat		3391						
	Spread?									
	Topping?									
	3. Special buns *(hot cross, Danish, raisin)*			3409						
	3. Muffins	Bran		3407						
		plain		3408						
	3. Scones	plain		3237						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	4. Tart									
	4. Cake *(circle)*	Iced/Cream								
		Plain								
	5. Doughnuts	Plain		3232						
	5. Doughnuts	Jam		3423						
	5. Doughnuts	Icing		3422						
	5. éclairs			3268						
	5. Koeksisters			3231						
X	**6. Pancakes/crumpets**	WM/Sun oil		3238						

	Toppings?									
	6. Waffles	WM/Sun oil		3238						
	Toppings?									
	7. Baked pudding									
	7. Trifle									
	7. Custard									
	7. Instant pudding									

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	8. Rusks	Commercial		3329						
	9. Special breads	Banana loaf		3333						
		Date loaf		3256						
		Raisin bread		3214						
	Spread?									
SNACKS, SWEETS & COLD DRINKS-PINK										
	1. Carbonated cold drinks e.g. coke			3981						

	1. Diet cold drinks e.g. diet coke			3990						
	2. Mageu			4056						
	2. Cold drinks (powder)									
	2. Energy drinks			4007						
	2. Squashes			3982						
brands	**3. Crisps, potato** *(lays)*			3417						
Size ***packets***	**3. Crisps Savoury,** *(nik* *naks, fritos, ghost pops)*			3418						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	3. Popcorn, plain			3332						
	3. Popcorn, sugar coated			3359						
	4. Sweets									
	Lollipops									
	4. Chocolates									
	Brands describe size									
SAUCES & CONDIMENTS-GREY										
	1. Cheese sauce	LFM, HM, cheese		3126						

		LFM, PUM, cheese		3127						
		SM, PUM, cheese		3128						
		WM, HM, cheese		3125						
	1. White sauce	LFM, HM		3143						
		LFM, PUM		3164						
		SM, PUM		3141						
		WM, HM		3142						
	2. Chakalaka									

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	I. Grams (g) / day
	2. Atjar			3117						
	2. Tomato sauce & other									
	2. Chutney									
ALCOHOLIC DRINKS-GREY										
	1. Beer **(regular/low alcohol)**									
	1. Cider									
	Coolers									
	2. Wine									

	2. Champagne									
	3. Spirits (any carbonated drink e.g. coke added)									
	4. Liqueurs & Fortified wine									
	OTHER									

Appendix 6: Participant questionnaire

University of the Witwatersrand, Johannesburg

Developmental Pathways for Health Research Unit

SWEET STUDY: Study of Women in and Entering Endocrine Transition

PARTICIPANT QUESTIONNAIRE

DATE: **Day** ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER: ☐☐☐☐☐☐☐

Consent Table

	Yes	**No**
Caregiver Questionnaire		
Food Frequency Questionnaire		
Caregiver Anthropometric Measurements		
Caregiver DXA		
Caregiver Bloods		

Contact details of a relative or a friend who will **always** know where you live (different to information on contact sheet):

Name: _____ Relationship: _____

Landline number: _____ Cell number: _____

Work number: _____ Other: _____

Address: _____

SECTION A: GENERAL INFORMATION, EMPLOYMENT AND INCOME

The information you give us from this section will give us general information about you

	Y	N
1. Are you currently employed?		

1.1 If YES, what type of employment?

_____

	Y	N
1.2 If NO to question 1, do you get any income from other sources?		

If YES to question 1.2, what type of sources do these include?

_____

SECTION G: (Modified STEPs Core data set)			
The next questions are about the time you spend doing different types of physical activities. This includes activities you do at home, at work, travelling from place to place and during your spare time. You are requested to answer the questions even if you don't consider yourself to be an active person.			
• **Occupation-related Physical Activity (paid or unpaid work):** *When answering the following questions, think back over the **past 12 months** and consider (think of) **a usual week**:*			
1	Does your work involve <u>mostly</u> sitting or standing still, **OR** walking	YES ..... 1 NO 2	—4

	for very short periods (less than 10 minutes)?		
2a	Does your work involve vigorous activities, (like heavy lifting, digging, or heavy construction) for **at least 10 minutes** at a time?	YES .... 1 NO 2	→3a
2b	In **a usual week**, how many days do you do vigorous activities as part of your work?	DAYS _ [ ][ ]	
2c	On a usual day on which you do vigorous activities, how much time do you spend doing such work?	________HOURS ________ MINUTES	
3a	Does your work involve moderate-intensity activities (like brisk walking or carrying light loads) for **at least 10 minutes** at a time?	YES .... 1 NO...2	→4
3b	In **a usual week**, how many days do you do moderate-intensity activities as part of your work?	DAYS _ [ ][ ]	
3c	On a usual day on which you do moderate-intensity activities, how	___________ HOURS ________ MINUTES	

	much time do you spend doing such work?		
4	How long is your usual workday?	_____ HOURS _____ MINUTES	
Travel-related Physical Activity: *Other than activities that you've already mentioned; I would like to ask you about the way you travel to and from places (to work, to shopping, to market, to church, etc).*			
5a	Do you walk or use a bicycle (pedal cycle) for **at least 10 minutes** at a time to get to and from places?	YES..... 1 NO.....2	—6
5b	In **a usual week**, how many days do you walk or cycle for at least 10 minutes to get to and from places?	DAYS _	
5c	On a usual day, how much time do you spend walking and cycling for travel	_____HOURS _____ MINUTES	
Non-work related and leisure time Physical Activity: *The next questions ask about activities you do in your leisure or spare time, for recreation or fitness. Do not include the physical activities you do at work or for travel already mentioned*			
6	In your leisure or spare time do you do any vigorous or moderate-intensity physical activity lasting **more than 10 minutes** at a time?	YES..... 1 NO 2	—9
7a	In your leisure or spare time, do you do any <u>vigorous</u> activities (<u>like</u>	YES..... 1 NO 2	—8a

	running or strenuous sports, weightlifting) for **at least 10 minutes** at a time?		
7b	IF YES, in **a usual week**, how many days do you do <u>vigorous</u> activities as part of your leisure or spare time?	DAYS _	
7c	How much time do you spend doing this on a usual day?	_____HOURS _____ MINUTES	
8a	In your leisure or spare time, do you do any <u>moderate-intensity</u> activities (<u>like</u> brisk walking, cycling or swimming) for **at least 10 minutes** at a time?	YES..... 1 NO 2	——9
8b	IF YES, in **a usual week**, how many days do you do <u>moderate-intensity</u> activities as part of your leisure or spare time?	DAYS _	
8c	How much time do you spend doing this on a usual day?	_____HOURS _____ MINUTES	

Sitting / Resting Activity: Now I would like to ask you about the time spent *sitting or resting, not including sleeping, **in the <u>past 7 days</u>**. This may include time sitting at a desk, visiting friends, reading, or sitting down to watch television **during working hours and leisure or spare time**.*			
9.	Over the **past 7 days**, how much time did you spend sitting or reclining (lying) on a **usual day (exclude sleeping)**?	_____HOURS _____ MINUTES	

We are very interested in the amount of time people spend sitting. I am going to ask you a few questions about your sitting behavior. These questions all refer to a usual day, and please answer in hours and minutes per day.

	Question	Week day (hours:minutes)	Weekend day (hours: minutes)
1a.	How much time do you spend sitting while you are at work? (per day)	Hrs: Min:	Hrs: Min:
1b.	How many days per week do you work? _____ days		
1c.	Do you work on the week-end? (Please circle answer) Yes No		
2.	How much time do you spend sitting while watching TV per day?	Hrs: Min:	Hrs: Min:

3. How much time do you spend sitting while using the computer per day, excluding your normal working hours	Hrs: Min:	Hrs: Min:
4. How much time do you spend sitting while travelling from place to place (e.g. in car, bus, train)	Hrs: Min:	Hrs: Min:
5. How much time do you spend sitting while eating and socialising and relaxing on a week day (Monday – Friday)?	Hrs: Min:	Hrs: Min:
6. How much time do you spend sitting while reading and/or listening to music (Monday – Friday)?	Hrs: Min:	Hrs: Min:
7. How much time do you spend sitting while at church?	Hrs: Min:	Hrs: Min:
Sleep 8. a. What time do you go to bed? b. What time do you wake up?		

SECTION J: PARTCIPANT MEASUMENTS

- STANDING HEIGHT: (mm)
- WEIGHT: (kg)
- WAIST CIRCUMFERENCE: (mm)
- HIP CIRCUMFERENCE (mm)

BLOOD PRESSURE (1) (2) (3)

- SYSTOLIC BP
- DIASTOLIC BP
- PULSE
- TIME OF BP **H**

RESEARCH ASSISTANT: **DATE:**

COLLECTION OF SPECIMENS:

ROUTINE BLOOD SAMPLE **Y** **N**

RESEARCH NURSE: **DATE:**

DXA	**Y**	**N**	**OPERATOR**	**DATE:**	

FFQ	**Y**	**N**	**RESEARCH ASSISTANT**	

QUALITY CHECKED BY:		**DATE:**	

nutrients

MDPI

Article

Nutrient Patterns and Body Composition Parameters of Black South African Women

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Abstract: Obesity is more prevalent in black South African women than men. However, little is known about the nutrient patterns associated with body composition indices in black African women. Principle Component Analysis (PCA) was applied to 25 nutrients derived from quantified food frequency questionnaires (QFFQs) in 498 middle aged black South African women. Three nutrient patterns, the plant driven, animal driven and Vitamin C, sugar and potassium driven nutrient patterns, accounted for 59% of the variance of nutrient intake. Linear models of the body composition parameters as outcome variables indicated that a standard deviation increase in the animal driven nutrient pattern was significantly associated with increases in body mass index (BMI) (1.29 kg·m^{-2} (95% CI, 0.54–2.04; $p = 0.001$), subcutaneous adipose tissue (SAT) (26.30 cm^2 (7.97–44.63); $p = 0.005$), visceral adipose tissue (VAT) (9.88 cm^2 (5.13–14.63); $p < 0.001$), VAT/SAT ratio (0.01 (0.00–0.02); $p = 0.018$), whole body fat mass index (0.74 kg·m^{-2} (0.25–1.22); $p = 0.003$), and whole body lean mass index (0.53 kg·m^{-2} (0.23–0.83); $p = 0.001$). An increase in plant driven nutrient pattern was significantly associated with an increase in SAT of 20.45 cm^2 (0.47–40.43); $p = 0.045$. This study demonstrates that animal driven nutrient pattern, characterised by the consumption of more animal protein and fat nutrients, similar to the western diet is associated with increased body fat and lean mass.

Keywords: African women; nutrient patterns; obesity; South Africa

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1. Introduction

The obesity pandemic remains a growing global concern, having a direct impact on non-communicable diseases (NCDs) such as hypertension, type 2 diabetes, and cardiovascular diseases [1]. The prevalence of obesity is increasing in low and middle income countries (LMICs) such as South Africa, in part due to the ongoing nutritional transition and rapid urbanisation [2,3]. In a study conducted in black South African women residing in Soweto, Johannesburg, the prevalence of obesity was found to be 67.8% and the prevalence of morbid obesity (BMI $\geq$ 40) was 16.8% [4]. Similarly, results from the South African National Health and Examination Survey (SANHANES-1) conducted in 2012 reported that 40.1% of women were obese and 25.0% were overweight. These rates were higher than those in males, who reported obesity and overweight prevalence rates of 11.6% and 19.6%, respectively [5]. It is therefore pivotal to understand the determinants of obesity and body composition parameters that increase NCD risk in black African women as they carry a larger proportion of this burden compared to men.

Obesity is thought to develop from the interaction of genetic, environmental, and modifiable lifestyle factors such as diet and physical activity [6,7]. South Africa has experienced

a shift from a more active lifestyle to a sedentary lifestyle, associated with increased overweight and obesity rates [2]. The adoption of a more Western diet, which mainly consists of a diet high in total and saturated fats and sugars and low consumption of whole grains such as legumes and home grown vegetables, is becoming prevalent [2]. More nutrition studies are now being conducted to understand the association of diet and obesity among South Africans.

A number of studies have focused on single nutrients and foods in evaluating the association of diet with obesity [8–12]. However, since people eat a combination of different foods, it is important that studies analyse nutrient pattern rather than individual food types. Nutrient pattern analysis enables the effect of the whole diet on health as well as body composition to be evaluated [13]. In view that nutrients are universal, the nutrient patterns can be compared across varied ethnicities. Only one study has evaluated the association of nutrient patterns with obesity in a black South African population and this was performed in adolescents [14]. However, findings from this study might not be transferrable to adults as these two groups are likely to have different eating patterns [15,16]. Therefore, more studies assessing the nutrient patterns association with body composition are required in black South Africans, particularly women, who carry the largest burden of obesity. Therefore, our study aimed to evaluate the association of nutrient patterns with body composition parameters in middle aged black South African women.

2. Materials and Methods

2.1. Study Population and Setting

We adopted a cross sectional study design nested in the longitudinal Birth to Twenty plus (Bt20) study, which is based at the Developmental Pathways for Health Research Unit, Chris Hani Baragwanath Hospital in Soweto, Johannesburg. The Bt20 study commenced in 1990 and recruited neonates, their mothers, and caregivers, who are being followed up at set time points [17]. Data for the current study was collected among 498 black South African female caregivers from a Bt20 sub-study called Study of Women Entering and in Endocrine Transition (SWEET) study [14,15]. From the Bt20 original cohort, 902 women were invited to participate and 702 women were eligible for the SWEET study using the following exclusion criteria: <40 years and >60 years, pregnant, did not give consent, and ethnicity other than black African [18]. Within this group of 702 women, 498 women had nutrition related data and therefore were included in this study.

In order to determine whether a sample size of 498 was sufficient to meet the aims of our study, we used the guidelines for factor analyses produced by Mundfrom et al. [19] and Kline [20]. According to the method of Mundfrom et al., a sample size calculation table is used, which considers the number of variables and factors used in the factor analysis. The current study used 25 variables (nutrients) and 3 factors (nutrient patterns) in a principal component analysis (PCA) and this would require a minimum sample size of 160, which is well below that of the actual sample size. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Human Research Ethics Committee (Medical) of University of the Witwatersrand (ethics number: M170718 (2017-09-26) and M110627 (2011-06-24).

2.2. Dietary Intake

Dietary intake data were collected using a standardised quantitative food frequency questionnaire (QFFQ), designed for the South African population [21]. The QFFQ consists of 214 food items that are representative of foods consumed by at least 3% of the population. This tool has been piloted and utilised extensively for the same population as described elsewhere [22–24]. Trained interviewers used high quality photographs of food items to assist with participant recall of food and beverage items consumed during the last seven days. The participants were asked to arrange the cards into three piles: foods eaten in the last seven days, occasionally, or never eaten and this was recorded. In the case of food items consumed in the last seven days, additional data on the frequency and quantity of

consumption was collected. The participants were asked to estimate their habitual intake by selecting the most accurate representation of their portion size from either two dimensional actual size drawings of foods, household utensils, or three dimensional validated food models as described and validated by Steyn et al. [25]. These household measures were then converted to grams for the computation of average intake over a seven-day period. The QFFQ took approximately 40–60 min to administer and was captured and managed using the Research Electronic Data Capture (RedCap) software package, an electronic data capture tool hosted at University of the Witwatersrand [26].

Nutrient intake was calculated from the conversion of single food items using the nutrient analysis software FoodFinder3 developed and hosted by The South African Medical Research Council [27]. Over- and under-reporting of dietary energy intake was corrected by removing participants with total energy intake that was <3000 and >30,000 kJ as described by Vorster et al. [28]. Plausibility of energy intake reporting was evaluated using the ratio of energy intake (EI) to estimated energy requirement (EER) [29]. Estimated energy requirement (EER) was calculated using the Institute of Medicine (IOM) gender specific equations using the participant's age, weight, height, and physical activity level (PA) as shown below [29]. A physical activity factor (PA) of 1.12 arising from the ratio of total energy expenditure and basal energy expenditure was considered for this study in view of the moderate physical activity that has been reported for this population.

$$EER = 354 - ((6.91 \cdot Age\ (yrs)) + PA \cdot (9.36 \cdot Weight\ (kgs)) + 726 \cdot Height\ (m)).$$

Dietary energy intake reporting was classified using categories derived from the EI:EER ratio. Participants with EI:EER ratio less than 0.7 were classified as under-reporters, 0.7 to 1.42 plausible reporters, and greater than 1.42 as over-reporters [29]. In multivariable linear regression models, the plausible reporters were used as the reference group.

2.3. Body Composition

2.3.1. Simple Body Anthropometry

Height and weight measurements were assessed while participants were barefoot and wearing light clothing. Height was measured using a wall mounted stadiometer (Holtain Ltd., Crosswell, UK) and reported to the nearest 0.1 cm and weight was measured using a digital scale (Dismedinc., Anjou, QC, Canada) and reported to the nearest 0.1 kg. These measurements were used to calculate BMI. A soft measuring tape was used to measure hip (greatest circumference at the hips) and waist (smallest girth above the umbilicus) circumferences to the nearest 0.5 cm. Trained technicians performed all body measurements.

2.3.2. Dual-Energy X-ray Absorptiometry

Dual-energy X-ray Absorptiometry (DXA) scans were performed using a Hologic (Londonderry, NH, USA) DXA by a single trained technician. The scans were conducted to measure visceral adipose tissue and subcutaneous adipose tissue. Women removed clothing and all metal objects, wore surgical gowns for the procedure, and had whole body scans analyzed using whole body less head as many participants wore wigs or hair weaves that could not be removed and these hairpieces are similar in density to soft tissue and may have caused measurement artefact. During data collection, the DXA phantom was scanned each morning to examine the CV of the DXA machine and the CV was found to be less than 0.5% for all parameters. For this study, participants had subtotal (whole body less head) fat mass and lean mass measured as described in a previous study [30]. Fat mass was used to calculate whole body fat mass index (FMI) (fat mass (kg)/height (m^2)) and lean mass was used to calculate whole body lean mass index (LMI) (whole body lean mass (kg)/height (m^2)) [31]. (CVs for DXA parameters were <2% for total fat mass and 1% for fat-free soft tissue mass).

2.4. Statistical Analysis

Data were analysed using SPSS Statistics software version 25 (IBM, Chicago, IL, USA) and STATA version 15 (Statacorp., College Station, TX, USA) [32], [33]. Normality tests were conducted using Q-Q plots for continuous variables. These variables are described using median and interquartile ranges (IQR) for non-parametric data or mean ± standard deviation (SD) for normally distributed data.

Principal component analysis (PCA) was used to extract nutrient patterns from 25 nutrients derived from the QFFQ. Total available carbohydrates were divided into starch and total sugar. The nutrients were log transformed to remove the bias due to the different scales used to quantify the nutrients. The nutrient density approach of dividing the nutrient intake by the total energy intake was adopted to adjust for energy intake [34]. This was used to capture variability of nutrient intake independently from changes of energy intake. The PCA procedure was performed using the covariance matrix of the log transformed nutrients that were selected to capture the whole dietary intake as previously reported by Pisa et al. [14]. Varimax rotation was performed to improve the interpretability of the factor loadings [35]. Three principal components (PCs) representing three independent nutrient patterns were selected according to the percentage of total variance explained, their eigenvalues, and visual presentation on the scree-plot of eigenvalues (Supplementary Materials Figure S1).

Multivariable linear regression models were performed to assess the association of the selected body composition parameters as outcome variables and nutrient patterns as predictors while adjusting for dietary intake reporting (under-, plausable-, and over-reporting), employment status, age, physical activity, and smoking status. Statistical significance was accepted at a level of $p < 0.05$.

3. Results

3.1. Descriptive Characteristics

Of the 498 women, 11.2% were underweight or normal weight (Table 1). The body composition indicators visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), FMI, LMI, gynoid fat, and hip and waist circumference were significantly higher in the overweight and obese group compared to the lean participants ($p < 0.001$ for all comparisons). The VAT/SAT ratio was not different between the groups ($p = 0.171$). Most of the women (65.4%) were classified as physically active and only 2.8% were active smokers. The proportion of energy intake from dietary carbohydrate and fat was similar across the groups (54.4% and 53.5% for carbohydrate intake and 31.3% and 30.1% for fat intake for lean and overweight/obese participants, respectively). However, the proportion of energy intake from protein was significantly different, with those obese or overweight consuming higher levels of protein compared to the lean participants (11.8% and 11.0%, respectively; $p = 0.026$).

3.2. Nutrient Patterns of Population

Table 2 shows that three nutrient patterns were extracted using PCA analysis, explaining 59% of the total variation of nutrients consumed by the study participants. The first nutrient pattern, the "*Plant driven nutrient pattern*" with higher factor loadings of plant protein, starch, and B vitamins, explained 25% of the variance; while the second (the "*Animal driven nutrient pattern*"), characterised by animal protein and saturated fat, and third (the "*Vitamin C, sugar and potassium driven nutrient pattern*") accounted 23% and 11%, respectively.

Table 1. Descriptive characteristics for the study population according to BMI.

	Overall	Underweight and Normal Weight (*N* = 56)	Overweight and Obese (*N* = 441)	*p* Value
Demographic Characteristics				
Age (years)	49 (45–53)	47 (44–52)	49 (45–53)	0.162
Current smoker *n* (%)	14 (2.8%)	2 (3.6%)	12 (2.7%)	0.063
Physically active *n* (%)	325 (65.4%)	41 (73.2%)	284 (64.4%)	0.191
Employed *n* (%)	295 (59.5%)	29 (52.7%)	266 (60.3%)	0.280
Body Composition Indicators				
Visceral Adipose Tissue (cm^2)	99.7 (74.1–127.6)	42.3 (29.0–58.5)	103.1 (83.9–131.5)	<0.001
Subcutaneous Adipose Tissue (cm^2)	455.8 (356.2–561.8)	215.8 (168.9–266.0)	482.0 (387.2–576.5)	<0.001
VAT/SAT ratio	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.171
Whole body fat mass index (FMI) (kg)	32.0 (25.6–39.3)	16.8 (12.8–19.4)	33.1 (28.4–40.7)	<0.001
Whole body lean mass index (LMI) (kg)	40.4 (35.8–44.8)	31.1 (28.7–34.6)	41.5 (37.2–45.4)	<0.001
Gynoid fat	58.6 (47.2–70.8)	32.7 (27.9–39.3)	61.3 (51.7–72.5)	<0.001
Hip circumference (cm)	117.5 (109.0–128.0)	97.0 (91.5–101.3)	119.0 (112.5–129.0)	<0.001
Waist circumference (cm)	99.5 (90.0–108.0)	77.0 (74.3–81.3)	101.5 (93.0–110.0)	<0.001
Dietary Information				
Total energy (kJ)	9759 (7628–13271)	10737 (8717–12659)	9614 (7523–13354)	0.291
% Carbohydrates	53.5(48.8–58.4)	54.4 (49.4–58.8)	53.5 (48.8–58.3)	0.598
% Protein	11.6 (10.3–13.3)	11.0 (9.7–12.4)	11.8 (10.4–13.4)	0.026
% Fat	30.2 (25.9–34.3)	31.3 (25.5–35.1)	30.1 (26.0–34.2)	0.756

3.3. Factors Associated with Varying Body Composition Measurements

Statistically significant associations were found for the animal driven nutrient pattern with all body composition indicators (Table 3). A one standard deviation increase in the animal driven nutrient pattern resulted in a 1.19 $kg \cdot m^{-2}$ ($p = 0.002$) increase in BMI, 10.17 cm^2 ($p < 0.001$) increase in VAT, 24.43 cm^2 ($p = 0.009$) increase in SAT, 0.01 ($p = 0.009$) increase in VAT/SAT ratio, 0.69 $kg \cdot m^{-2}$ ($p = 0.005$) increase in FMI, and 0.48 $kg \cdot m^{-2}$ ($p = 0.002$) increase in LMI. There was a marginal association for plant driven nutrient pattern, with an increase in this pattern resulting in an increase in SAT of 20.45 cm^2 ($p = 0.045$). Underweight participants were associated with over-reporting energy intake, while overweight individuals were noted to underreport. Notably, over-reporting energy intake, when compared to plausible energy intake, was associated with decreases of 3.51 kgm^{-2} in BMI, 17.61 cm^2 in VAT, 76.15 cm^2 in SAT, 1.95 $kg \cdot m^{-2}$ in FMI, and 1.47 $kg \cdot m^{-2}$ in LMI ($p < 0.001$ for all associations). Engagement in physical activity was associated with BMI, VAT, SAT, FMI, and LMI and inverse associations were reported at 1.51 $kg \cdot m^{-2}$, 9.21 cm^2, 37.76 cm^2, 0.91 $kg \cdot m^{-2}$, and 0.56 $kg \cdot m^{-2}$, respectively. Increasing age showed a direct statistically significant association with VAT ($p < 0.001$) and VAT/SAT ratio ($p = 0.002$). The plant protein and starch driven nutrient pattern was only associated with modest increases in SAT compared to the animal protein and fat driven pattern (Table 3).

Table 2. Factor loadings and explained variances for the nutrient patterns.

	Plant Protein Driven Nutrient Pattern	Animal Protein Driven Nutrient Pattern	Vitamin C, Sugar, and Potassium Driven Nutrient Pattern
Plant protein	**0.921**	0.121	−0.017
Animal protein	0.080	**0.834**	0.175
Saturated fat	0.284	**0.729**	0.136
Monounsaturated fat	0.352	**0.695**	0.062
Polyunsaturated fat	0.443	0.549	−0.059
Cholesterol	0.009	**0.776**	−0.003
Starch	**0.712**	0.046	−0.304
Total sugar	0.050	0.095	**0.721**
Dietary fibre	**0.686**	0.044	0.455
Calcium	−0.110	0.507	0.431
Iron	**0.785**	0.413	0.170
Magnesium	0.574	0.169	0.273
Phosphorus	0.156	0.560	0.171
Potassium	0.028	0.089	**0.688**
Zinc	**0.758**	0.471	0.117
Retinol	0.073	0.345	0.110
Beta carotene	0.033	−0.038	0.229
Thiamine	**0.861**	0.341	0.158
Riboflavin	0.415	**0.630**	0.314
Vitamin B6	**0.906**	0.199	−0.019
Folate	0.618	0.080	0.111
Vitamin B12	0.113	0.691	0.101
Vitamin C	0.193	0.063	**0.897**
Vitamin D	0.265	**0.837**	−0.124
Vitamin E	0.370	0.482	0.088
Explained variance %	24.678	22.945	10.884
Cumulative explained variance %	24.678	47.623	58.507

Bold factor loadings used to indicate factor loadings > ±0.60 used for naming the nutrient patterns.

Table 3. Multivariable linear regression models showing the association of body composition indicators (BMI, VAT, SAT, VAT/SAT, FMI, and LMI; outcome variables) with the three nutrient patterns (predictor variables) and possible confounding factors (energy intake reporting, physical activity, employment status, education, smoking, and alcohol intake).

		BMI		**Visceral Adipose Tissue (VAT)**		**Subcutaneous Adipose Tissue (SAT)**		**VAT/SAT Ratio**		**Whole Body Fat Mass Index (FMI)**		**Whole Body Lean Mass Index (LMI)**	
		Adjusted β (95% CI)	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**
Nutrient patterns	PCA1	0.65 (−0.17; 1.47)	0.118	2.06 (−3.12; 7.23)	0.434	20.45 (0.47; 40.43)	0.045	−0.01 (−0.01; 0.01)	0.309	0.46 (−0.07; 0.99)	0.088	0.20 (−0.12; 0.53)	0.220
	PCA2	1.29 (0.54; 2.04)	0.001	9.88 (5.13;14.63)	<0.001	26.30 (7.97; 44.63)	0.005	0.01 (0.00; 0.02)	0.018	0.74 (0.25; 1.22)	0.003	0.53 (0.23; 0.83)	0.001
	PCA3	0.30 (−0.31; 0.91)	0.328	−1.09 (−4.97; 2.80)	0.581	−5.05 (−20.04; 9.94)	0.508	0.00 (−0.01; 0.01)	0.567	0.21 (−0.19; 0.60)	0.300	0.08 (−0.16; 0.33)	0.506
EI-EER	Plausible	1		1		1		1		1		1	
	Under reporting	1.85 (−0.40; 4.10)	0.107	5.41 (−8.83; 19.64)	0.456	54.44 (−0.53; 106.41)	0.064	−0.00 (−0.04; 0.02)	0.695	1.49 (0.03; 2.95)	0.045	0.37 (−0.53; 1.26)	0.421
	Over reporting	−3.51 (−5.18; −1.85)	<0.001	−17.61 (−28.13; −7.09)	0.001	−76.15 (−116.77; −35.53)	<0.001	−0.00 (−0.02; 0.02)	0.972	−195 (−3.03; −0.87)	<0.001	−1.47 (−2.13; −0.80)	<0.001
Physical activity	Inactive	1		1		1		1		1		1	
	Physically Active	−1.51 (−2.76; −0.26)	0.018	−9.21 (−17.14; −1.28)	0.023	−37.76 (−68.37; −7.15)	0.016	−0.00 (−0.02; 0.01)	0.671	−0.91 (−1.73; −0.10)	0.027	−0.56 (−1.06; −0.06)	0.028
Employment	Unemployed	1		1		1		1		1		1	
status	Employed	1.41 (0.21; 2.62)	0.023	−2.10 (−9.79; 5.58)	0.590	26.07 (−3.59; 55.73)	0.085	−0.02 (−0.03; −0.01)	0.023	0.81 (0.03; 1.60)	0.043	0.55 (0.06; 1.03)	0.027
Education	Completed primary	1		1		1		1		1		1	
	Attended high school	−0.33 (−2.31; 1.64)	0.739	−2.77 (−15.27; 9.74)	0.643	12.35 (−35.92; 60.63)	0.615	−0.02 (−0.05; 0.04)	0.096	0.07 (−1.21; 1.35)	0.914	−0.39 (−1.17; 0.40)	0.336

Table 3. *Cont.*

		BMI		Visceral Adipose Tissue (VAT)		Subcutaneous Adipose Tissue (SAT)		VAT/SAT Ratio		Whole Body Fat Mass Index (FMI)		Whole Body Lean Mass Index (LMI)	
		Adjusted β (95% CI)	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**	**Adjusted β (95% CI)**	***p* Value**
	Completed high school	−2.13 (−4.28; 0.02)	0.052	−8.28 (−21.87; 5.31)	0.232	−23.53 (−76.04; 28.95)	0.379	−0.02 (−0.05; 0.001)	0.132	−1.00 (−2.39; 0.39)	0.157	−1.07 (−1.93; −0.22)	0.014
Cigarette use	Never smoked	1		1		1		1		1		1	
	Ex-smoker	−2.00 (−4.38; 0.36)	0.097	7.76 (−22.67; 7.16)	0.307	−31.28 (−88.88; 26.31)	0.286	0.00 (−0.03; 0.03)	0.909	−1.56 (−3.10; −0.31)	0.046	−0.40 (−1.34; 0.55)	0.406
	Current smoker	−0.24 (−4.00; 3.52)	0.899	−12.76 (−15.27; 9.74)	0.290	−23.53 (−76.01; 28.95)	0.379	−0.04 (−0.09; 0.01)	0.145	−0.32 (−2.76; 2.11)	0.794	0.09 (−1.41; 1.59)	0.905
Alcohol	No	1		1		1		1		1		1	
	Yes	−0.02 (−0.15; 0.10)	0.714	0.54 (−0.27; 1.36)	0.187	−0.83 (−3.96; 2.30)	0.603	0.00 (0.00; 0.00)	0.026	−0.01 (−0.09; 0.7)	0.849	−0.05 (−0.07; 0.04)	0.566
Age		0.03 (−0.08; 0.15)	0.569	1.68 (0.94; 2.42)	<0.001	2.61 (−0.27; 5.49)	0.075	0.00 (0.00; 0.00)	0.002	0.17 (−0.02; 0.35)	0.073	−0.03 (−0.07; 0.02)	0.205

PCA1 = Plant protein and starch driven nutrient pattern; PCA2 = Animal protein and fat driven nutrient pattern; PCA3 = Vitamin C, sugar, and potassium driven nutrient pattern.

4. Discussion

This study aimed to determine the nutrient patterns of middle aged black women in Soweto and their association with body composition measurements. Three nutrient patterns, namely, plant driven nutrient pattern, animal driven nutrient pattern and vitamin C, sugar and potassium driven nutrient pattern, were found to explain 59% of the variance in nutrient intake in the study population. The animal driven nutrient pattern was positively associated with BMI and DXA-derived body composition parameters.

Three studies have previously characterised nutrient patterns of black Africans. Pisa et al. [14] and Visser et al. [36] studies were conducted in children and adolescents, while the study by Chikowore et al. [37] was done in adults. Pisa et al. found that the animal driven nutrient pattern comprising of animal protein, saturated fat, cholesterol, riboflavin, vitamin B12, retinol, vitamin D, and zinc were the most commonly consumed nutrient pattern, while in our study, this was the second most consumed pattern [14]; Visser et al. reported similar results to our study where the plant driven nutrient pattern comprising of protein, carbohydrate, iron, and vitamin B was the most consumed followed by the animal protein and saturated fat pattern [36]. Additionally, Chikowore et al. also reported similar findings to our study where the plant driven pattern comprising of magnesium, phosphorus, and plant protein was the most consumed pattern followed by fat and animal protein and starch driven nutrient patterns [37]. Regardless of the small differences in the naming of the patterns, Visser et al. and Chikowore et al. studies, conducted in both urban and rural areas, reported similar nutrient patterns to those that we found in our study [36,37]. This thereby suggests that these patterns are common in the black South African population. These nutrients are most likely derived from the consumption of staple foods such as maize meal, which are fortified with B vitamins [36,38] However, the differences in the variances of the animal driven nutrient pattern reported by Pisa et al. are expected, since the principals analysis approach used is data driven and thus, is expected to capture the unique patterns [14]. However, this pattern was also noted in our study and the high loadings of animal protein and saturated fat are suggestive of the shift towards western diets, which has been reported in this population group.

Very few studies have been conducted to assess the association between nutrient patterns and obesity. One such study conducted in American adults evaluated nutrient patterns and their relationship with general and central obesity [13]. Results from this study showed that the odds ratios of obesity increased across quartiles of the major nutrient pattern of saturated/mono-unsaturated fatty acids [13]. Relative to the first quartile, the fourth quartile was associated with odds ratio of 1.5 (95% CI, 1.3–1.7) for central obesity and odds ratio of 1.3 (95% CI: 1.1–1.5) for general obesity [13]. These findings are similar to those of the present study, which showed that the animal driven nutrient pattern is significantly associated with increases in BMI, VAT, SAT, VAT/SAT ratio, FMI, and LMI.

The plant driven nutrient pattern was correlated significantly with SAT, but not as strongly as the animal driven pattern. Although it has been demonstrated that a plant-based protein diet, through its anabolic properties, could be an effective strategy to enhance lean mass index, when eaten in excess, these foods may result in increasing SAT, hip, and waist circumference [39]. The increase in body parameters may be due to the fact that these plant-based foods may contain hidden sugars and refined carbohydrates, which contribute to weight gain [39].

The animal driven nutrient pattern, characterised with higher loadings for animal protein and fat driven nutrients, increased body composition parameters, potentially due to the storage of animal fat as triglycerides in adipose tissue [40,41]. However, protein is known to lead to increased energy expenditure and improved satiety through the release of hormones, which favours weight loss [42]. In fact, protein weight loss effects have been reported when it contributes approximately 20% or more of the energy intake [43]. This weight loss effect was less likely in our study, which comprised of protein intake at 11% of total energy intake. Furthermore, it must be noted that when energy demand is low, excess protein can be converted to ketone bodies or glucose (via gluconeogenesis)

and contribute to a positive energy balance, which can result in increased weight [44]. Previous research done in both animals and humans demonstrated that a high fat diet promotes obesity [45]. This explains the association of the animal driven nutrient pattern with increases in body fat as measured by BMI.

A strength of this study included using more accurate DXA body composition measurements when compared to simple measurements. Additionally, this study adjusted for energy intake misreporting in the linear regression models, which improved the accuracy of these models. Notably, overweight/obese subjects were noted to under report dietary energy intake as has been indicated in other studies [46,47]. The nutrient density method was implemented in order to adjust for total energy, thereby allowing for the independent association of the nutrients with body composition to be determined.

The study has some limitations. The QFFQ is known to have recall bias, including the potential for food measurement errors, resulting in inaccurate reporting of food consumption [48]. Recall bias was reduced by using actual food models to help with accurate recall of food quantities consumed. Similarly, physical activity was self-reported, however interviewers provided contextual examples of moderate and vigorous intensity activities during the interview to assist with recollection of recent activity. Accurate physical activity measurements using electronic activity monitors would ideally remove the potential for recall bias, however such tools are expensive and not feasible for large scale epidemiological studies [49].

5. Conclusions

This study showed that in this population, the animal driven nutrient pattern was significantly associated with increases in body fat and its distribution was indicated by the selected measures (BMI, VAT, SAT, VAT/SAT ratio, FMI, and LMI). This information is essential for interventions aimed at addressing obesity in black sub-Saharan African women. Decreased consumption of foods high in animal protein and fat will result in decreased body fat levels. However, larger studies are required to validate these findings.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/1/6/s1, Figure S1: Scree plot of the nutrients and the extracted principal components.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Please refer to suggested Data Availability Statements in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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Appendix 7A: SCREE-PLOT OF PRINCIPLE COMPONENT ANALYSIS

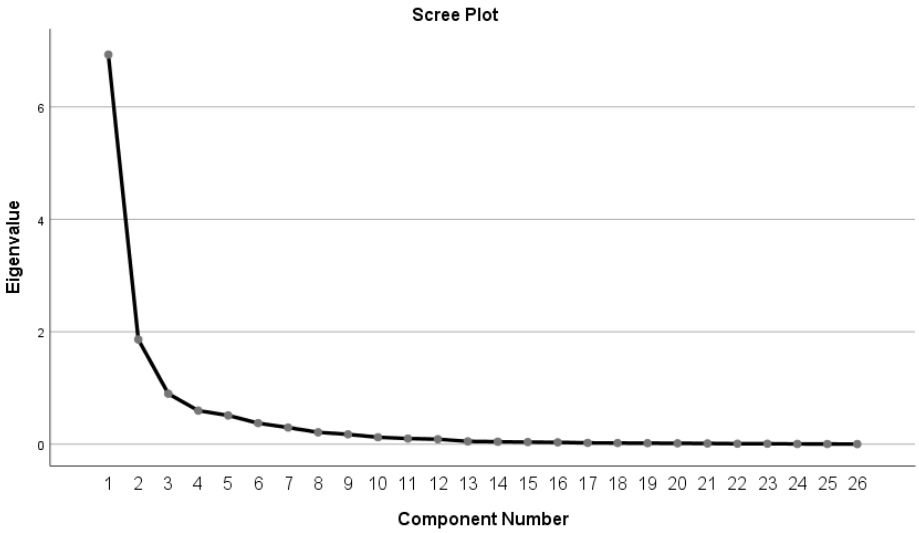

Figure S1. Scree plot of the nutrients and the extracted principal components.

Appendix 8: Manuscript 2 – Chapter 4

International Journal of
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Article

Association of Longitudinal Nutrient Patterns with Body Composition in Black Middle-Aged South African Women: A Five-Year Follow-Up Study

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Abstract: This study aimed to evaluate the association of longitudinal nutrient patterns with body composition in a cohort of 132 black South African middle-aged women over five years. Nutrient patterns were identified using principal component analysis at baseline and follow-up 5 years later. Associations between nutrient patterns and repeated body composition measures were evaluated using generalized estimating equations, before and after adjusting for baseline education and repeated measures of age, socio-economic status, physical activity and employment. The animal-driven nutrient pattern was associated with increases in repeated measures of visceral adipose tissue (VAT) (β coefficient, 5.79 [95% CI, 0.01–11.57] cm^2), fat mass index (FMI) (0.47 [0.01–0.93] $kg{\cdot}m^{-2}$) and lean mass index (LMI) (0.50 [0.18–1.17] $kg{\cdot}m^{-2}$) ($p < 0.05$) after adjustment. Vitamin C, sugar, and potassium-driven nutrient pattern was associated with higher FMI (0.50 [0.12–0.88] $kg{\cdot}m^{-2}$) and LMI (0.58 [0.07–1.10] $kg{\cdot}m^{-2}$) before and after adjustment ($p < 0.05$). These findings suggest that dietary interventions to curb obesity in black middle-aged South African women should focus on attenuation of nutrient patterns centred on added sugar, animal fat and animal protein.

Keywords: nutrient patterns; body composition; adiposity; African women

1. Introduction

Metabolic syndrome, high blood pressure, and type 2 diabetes are all more common in black women, who have higher rates of obesity, than in white women, according to research conducted in South Africa [1,2]. In South Africa, it has been estimated that NCDs account for ~51% of all fatalities with ~19% of all deaths attributable to cardiovascular diseases [3]. Additionally, this increased risk of NCD onset may be due to black women showing a unique body composition phenotype by storing more subcutaneous adipose tissue (SAT) than other ethnic groups [4]. Furthermore, it has been demonstrated that ageing in black women is characterized by significant increases in visceral adipose tissues (VAT) and a relative redistribution of fat from the peripheral to the central region [4].

Obesity has been shown to have strong links with dietary behaviours [5–7]. As a result, designing health promotion programmes that focus on dietary behaviours is critical

[8,9]. To create appropriate and effective health promotion programs, information on long-term nutritional behaviours and associations with health outcomes is required.

The United Nations' Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have formed a joint consultation to develop national food-based dietary guidelines (FBDGs), which provide context-specific advice and principles on healthy diets based on scientific evidence [10]. This has resulted in the FAO/WHO 2004 Global approach on food, physical activity, and the amended 2012 South African FBDGs, among other recommendations and reports [10]. These dietary guidelines are regularly updated with the most relevant information and therefore there is a need for frequent nutritional studies to provide both country and population specific results to inform such guidelines.

The body composition of women has also been proven to be significantly influenced by their race or ethnicity, and numerous cross-sectional studies have documented these racial disparities in body composition and associations with nutrient patterns. Nutrient patterns of middle-aged black South African women have not been studied longitudinally to date. There have only been five cross-sectional studies examining the nutrient patterns of adult men and women aged 18 and older in South Africa [11–15]. These cross-sectional studies unfortunately are unable to show sustainability of a populations nutrient patterns while longitudinal nutrient pattern studies results show sustainability of nutrient patterns in the population and longitudinal association of adiposity, therefore, appropriate long-term nutritional advice can be given. Of these studies, two documented specific nutrient patterns in black South African women, the same cohort for this study, and evaluated the association between these nutrient patterns and adiposity with the one study looking at sex differences in the associations of nutrient patterns with total and regional adiposity [11,15]. This study showed that a plant driven nutrient pattern explained the greatest variance in nutrient intake in this population and was positively associated with SAT [11]. This study aims to investigate changes in body composition over 5.5 years, and to determine if these changes are attributable to nutrient patterns extracted at baseline and follow-up.

2. Materials and Methods

2.1. Study Population and Setting

At baseline, a total of 902 women who were the biological mothers of the Birth to Twenty Plus (BT20) cohort were invited to participate in the Study of Women in and Entering Endocrine Transition (SWEET) study [11]. The following inclusion criteria were applied: between 40 and 60 years of age, not pregnant, black African women (Figure 1) [16,17]. Between January 2017 and August 2018, 527 women from the SWEET cohort were randomly sampled and invited to participate in a follow-up study which was subsequently referred to as the Middle-aged Soweto Cohort (MASC) [18]. After excluding participants that were not contactable, deceased, relocated, not interested, missing anthropometry measurements or not included in sampling, did not have baseline nutrition data, or were a mismatched participant, the final sample size of women with dual-energy X-ray absorptiometry (DXA) body composition measurements and dietary data at baseline and follow-up was $n = 132$ (Figure 1). The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (ethics numbers: M160604 and M160975).

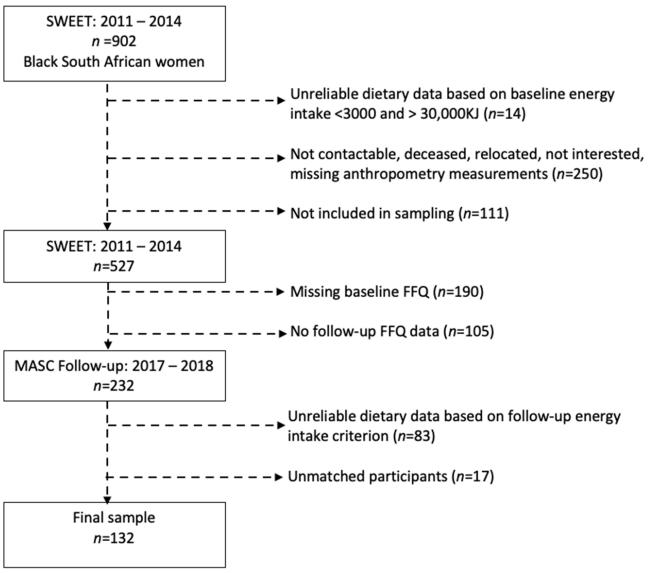

Figure 1. Flow chart of study participants. (SWEET = Study of Women in and Entering Endocrine Transition; MASC = Middle-aged Soweto Cohort).

2.2. Dietary Intake

Dietary intake data was collected at baseline and follow-up using a standardised quantitative food frequency questionnaire (QFFQ), designed for the South African population [11,19,20,21,22]. The QFFQ consists of 214 food items that are representative of foods consumed by most of the South African population currently [23]. Although this tool was tested in adolescents, it has since been piloted and utilised extensively in the general population [11,20,21,22]. Trained interviewers used high quality photographs of food items to assist with participant recall of food and beverage items consumed during the last seven days. In the case of food items consumed in the last seven days, additional data on the frequency and quantity of consumption were collected. The participants were asked to estimate their habitual intake by selecting the most accurate representation of their portion size from either two-dimensional actual size drawings of foods, household utensils, or three-dimensional validated food models as described by Steyn et al. [23]. These household measures were then converted to grams for the computation of average intake over a seven-day period. Food items were then converted to nutrients using the nutrient analysis software FoodFinder3 developed and hosted by The South African Medical Research Council [24]. Participants with unreliable dietary data based on energy intake <3000 and >30,000 kJ, as described by Vorster et al. [25], were excluded (n = 20 with either unreliable dietary data at baseline (n = 14) or follow-up (n = 6)).

Baseline and follow-up plausibility of energy intake reporting was adjusted in Generalized estimating equations and this variable was calculated using the ratio of the dietary energy intake (EI) and estimated energy requirements (EER) which is the average dietary energy intake that is predicted to maintain energy balance in healthy, normal weight individuals of a defined age, gender, weight, height, and level of physical activity consistent with good health [11,26]. The EER was calculated using the Institute of Medicine (IOM) gender-specific equations shown below for females:

EER = 354 − ((6.91 × Age (years)) + Physical Activity (PA) coefficient (9.36 × Weight (kgs)) + 726 × Height (m))

This equation considers the participant's age, weight, height, and standard pre-defined physical activity (PA) coefficient from the equation, which is gender and age specific and classified according to different ranges of physical activity levels. For adult women reporting a low active lifestyle, the PA coefficient is 1.12 as moderate intensity physical activity has been reported for this same population which is in line with findings from this study as well [27]. Participants with EI:EER ratio less than 0.7 were classified as under-reporters, 0.7 to 1.42 as plausible reporters, and greater than 1.42 as over-reporters. Plausible energy intake was used as the reference category in statistical models for the EI: EER ratio [26].

2.3. Body Composition Measurements

Participants were requested to remove their shoes and to wear lightweight clothing prior to weigh in. Weight was recorded to the nearest 0.01 kg. Height was measured and recorded to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of baseline height (in metres).

A single trained technician performed dual-energy X-ray Absorptiometry (DXA) scans at baseline and follow-up using a Hologic Discovery software version 12.1 (Hologic Inc., Bedford, MA, USA, APEX software). Whole-body scans were completed to determine whole body (minus head) fat mass (FM) and whole-body lean mass (fat-free soft-tissue mass). The coefficient of variation (CVs) for the DXA parameters were <2% for total fat mass and 1% for fat-free soft tissue mass. Fat mass was used to calculate fat mass index (FMI) (fat mass (kg)/baseline height (m^2)), and lean mass was used to estimate lean mass index (LMI) (whole-body lean mass (kg)/ baseline height (m^2)) [28]. Visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and gynoid fat were also estimated using DXA [29].

2.4. Socio-Demographics and Physical Activity

Details of socio-demographic and lifestyle characteristics were collected at baseline and at follow-up and questionnaires were administered by research assistants. Socio-demographic factors included age, self-reported employment status (Yes/No) and highest education level achieved (completed primary school, incomplete high school and completed high school). Household socio-economic status was estimated using a count of the following major household amenities: electricity, radio, motor vehicle, fridge, washing machine, mobile, microwave, electronic media network (MNET) and digital satellite television.

Self-reported physical activity was collected using the Global Physical Activity Questionnaire (GPAQ) [30]. Participants were categorised as active or inactive at baseline and follow-up according to the GPAQ criteria [30]. Participants were categorised as active if they reported >600 MET minutes per week [31]. Participants who did not meet these criteria were classified as inactive. Physical activity was further categorized into three groups: active participants at both time points, inactive at both time points, and those who changed their physical activity in any direction between the time points (spectrum of physical activity).

2.5. Statistical Analysis: Analysis of Longitudinal Data

All statistical analyses were performed using STATA version 15 (StataCorp, College Station, TX, USA) and IBM SPSS version 27 software packages (IBM, Chicago, IL, USA) [32,33]. Q-Q plots were used to perform normality tests on the continuous variables and normally distributed continuous variables are presented as mean ± SD, while non-parametric data are presented as median (interquartile range [IQR]) for the descriptive tables. The Student paired *t*-test, Chi-square test or the Wilcoxon signed rank test were used to explore differences between baseline and follow-up measurements for normally distributed continuous data, categorical data, and not normally distributed continuous data, respectively.

Principal component analysis (PCA) was applied at both baseline and follow-up to extract nutrient patterns from 25 nutrients derived from the QFFQ [34]. Total available carbohydrates were divided into starch, total sugar and dietary fibre. The nutrients were log transformed to remove bias due to variance as a result of the different measures of scale used to quantify the nutrients [35]. As previously described, the PCA approach was performed using the covariance matrix of the log transformed nutrients that were selected to capture the entire dietary intake [11,13,14]. Varimax rotation was used to make the factor loadings more understandable [36]. The top three principal components (PCs) indicating three distinct nutrient patterns were selected and plotted on a circular plot based on their eigenvalues and visual appearance on the scree-plot of eigenvalues and the proportion of total variance explained. Nutrient patterns at baseline and follow-up were used as predictor variables.

Generalized estimating equation (GEE) analyses were used to analyse longitudinal associations of both baseline and follow-up measures of nutrient patterns with six repeated measures of body composition: BMI, VAT, SAT, FMI, LMI and gynoid fat. The GEE analysis extends the generalized linear model to allow for the analysis of repeated measurements. It combines within-subject relationships with between-subjects relationships into a single regression coefficient [37,38]. Furthermore, GEE allows for the explicit modelling of a wide range of correlation structures and is more interpretable than repeated measures analysis of variance (ANOVA) [37,38]. The GEE models examined time-varying nutrient patterns while accounting for the correlation of repeated measures for the same participant. Two GEE models were constructed, GEE model 1 was adjusted for all nutrient patterns and energy intake reporting and GEE model 2 was adjusted for the same variables as model 1 plus the following: baseline education and repeated measures of age, household socio-economic status (SES), physical activity levels and employment. Furthermore, the GEE models for the regional adiposity measures (VAT, SAT, and gynoid fat mass) were further adjusted for FMI to explore the effects of regional adiposity as opposed to total adiposity. The β coefficients and the 95% confidence interval (CI) from GEE models were reported as the measures of association.

3. Results

3.1. Descriptive Characteristics

The characteristics of the participants are summarized in Table 1. Despite no significant changes in BMI, LMI and SAT, DXA-derived measures of fat mass, FMI, VAT and gynoid fat mass increased over the 5-year follow-up while lean mass decreased. At baseline and follow-up, over 65% of the women were classified as having overweight or obesity.

Table 1. Baseline and follow-up descriptive characteristics (n = 132).

	Baseline	**Follow-Up**	***p* Value**
Demographics			
Age, years	48 (44; 52)	53 (50; 58)	<0.001
Socio-economic status (a score out of 9)	5 (4; 6)	5 (3; 5)	0.572
Physically active (%)	94 (71.2%)	93 (70.5%)	0.882
Body composition			
BMI (kg m^{-2})	33.7 (28.1; 39.0)	33.7 (28.1; 39.2)	0.767
Whole-body fat mass (kg)	60.5 (47.1; 73.3)	63.4 (49.1; 76.3)	0.015
Fat mass index (FMI) (kg m^{-2})	23.5 (20.5; 30.3)	26.6 (20.7; 31.8)	<0.001
Whole body lean mass (kg)	41.9 (36.9; 45.1)	41.0 (34.4; 52.4)	0.029
Lean mass index (LMI) (kg m^{-2})	16.6 (14.7; 18.2)	16.4 (14.5; 20.8)	0.055
Gynoid fat mass (kg)	3.2. (2.6; 4.2)	3.6 (3.0; 4.6)	<0.001
Subcutaneous Adipose Tissue (cm^2)	452.7 (360.2; 574.5)	457.6 (349.1; 576.4)	0.547

Visceral Adipose Tissue (cm²)	102.6 (75.2; 128.9)	107.8 (70.4; 137.3)	0.001
Nutritional intake data			
Energy intake (mJ)	9.3 (7.9; 12.0)	6.4 (5.1; 8.4)	<0.001
% Carbohydrates (of total energy)	54.5 (49.7; 58.1)	52.6 (48.6; 56.5)	0.052
% Protein (of total energy)	11.4 (10.1; 12.8)	11.2 (9.7; 12.7)	0.871
% Fat (of total energy)	30.1 (26.0; 34.3)	31.7 (27.0; 35.5)	0.035

Total energy intake significantly decreased over the 5 years (from 9.3 mJ to 6.4 mJ, respectively) although the proportion of carbohydrates and protein consumed remained stable across the two time points, while fat consumption significantly increased over the 5 years. Whole-body fat mass, fat mass index, gynoid fat mass and visceral adipose tissue all increased at follow-up (p value < 0.05) while whole body lean mass decreased at follow-up (p value = 0.029).

3.2. Nutrient Patterns

Baseline and follow-up nutrient patterns were extracted using principal component analysis (PCA) and results of the nutrient patterns displayed as a radar plot and a table (Figure 2 and Table S1 respectively).

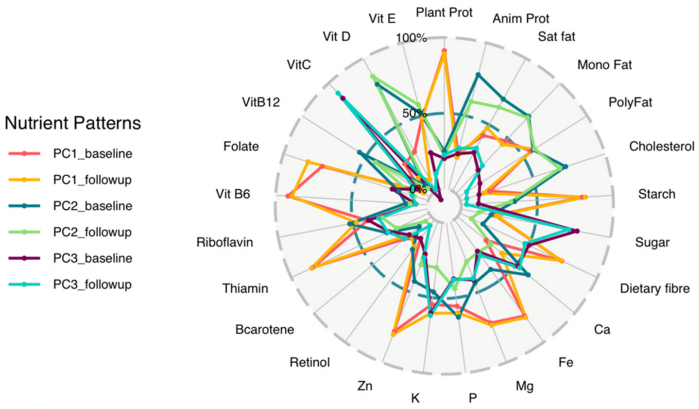

Figure 2. Baseline and follow-up PC derived nutrient patterns. This is based on 25 nutrient items in middle-aged black South African women (n = 132). Identified by principle component analysis, on the circular plot, the first 3 PCs that met the Scree test which was used to determine the number of PCs to retain and eigenvalues are shown by different coloured lines (referred to as PCA nutrient pattern 1–3) for both baseline and follow-up and each component represents an independent nutrient pattern. PC = principal component; Plant Prot = plant protein; Anim prot = animal protein; Sat fat = saturated fat; Mono Fat = monounsaturated fat; Poly Fat = polyunsaturated fat; Ca = Calcium; Fe = Iron; Mg = magnesium; P = phosphorus; K = potassium; Zn = zinc; Vit B6 = vitamin B6; Vit B12 = vitamin B12; Vit C = vitamin C; Vit D = vitamin D; Vit E = vitamin E; PC1 = plant driven nutrient pattern; PC2 = animal protein driven nutrient pattern; PC3 = vitamin C, sugar and potassium driven nutrient pattern. The orange and yellow line (PC1) represents the factor loadings related to the plant driven nutrient pattern (baseline and follow-up, respectively), the dark and light green lines (PC2) represents factor loadings related to the animal protein driven nutrient pattern (baseline and follow-up) and the purple and light blue lines (PC3) represents factor loadings related to the vitamin C, sugar and potassium driven nutrient pattern (baseline and follow-up).

These nutrient patterns were identified and driven by the same nutrients at baseline and follow-up (Figure 2). These nutrient patterns explained 69% and 64% of the total

variation of nutrients consumed by the study participants at baseline and follow-up, respectively. Although the value of the factor loadings of the individual nutrients changed between baseline and follow-up, the nutrients with the highest loadings for each principal component (PC) did not change therefore the nutrient patterns remained the same. The first nutrient pattern (PC1) was the "Plant driven nutrient pattern" with high factor loadings of plant protein, starch, and B vitamins, followed by the "Animal protein driven nutrient pattern" (PC2) characterised by high factor loadings of animal protein and saturated fat, and the third nutrient pattern (PC3) was the "Vitamin C, sugar and potassium driven nutrient pattern" characterised by high factor loadings of sugar, vitamin C and potassium.

3.3. Longitudinal Association between Nutrient Patterns and Body Composition Indices

Univariate analysis using GEE and individual body composition measures showed that the associations of the plant based nutrient pattern with FMI and LMI were significant, however these associations were no longer significant after adjusting for covariates in multivariate analysis (Table 2).

The animal protein driven nutrient pattern was associated with significant increases in FMI, LMI and VAT for model 1 and this remained significant after adjustment (note, the GEE VAT model was further adjusted with FMI). Repeated measures of the vitamin C, sugar, and potassium driven nutrient pattern was significantly associated with an increase in FMI and LMI before and after controlling for household SES and related covariates. None of the nutrient patterns were associated with BMI, SAT and gynoid fat when adjusted for all the model covariates (the animal protein nutrient pattern was significantly associated with increases in gynoid fat in unadjusted model).

Table 2. GEE β coefficients for repeated measures of nutrient patterns and repeated measures of body composition (n = 132).

	BMI		VAT		SAT		FMI		LMI		GYNOID	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
Plant Protein Driven NP	0.138	0.290	0.952	−4.040	−1.432	−2.135	**0.461** **	0.361	**−0.890** **	0.150	0.127	−0.787
Animal Protein Driven NP	0.307	0.243	**10.115** ***	**5.788** **	1.451	−7.103	**0.449** ***	**0.469** **	**0.466** *	**0.498** **	**0.724** **	0.002
Vit C, Sugar, and Potassium Driven NP	0.157	0.404	3.525	0.010	6.096	−2.785	**0.425** ***	**0.502** ***	**0.499** **	**0.584** **	0.308	−0.622

Note: *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$. Bold values denote statistical significance at $p < 0.05$. Model 1: Adjusted for all NPs and energy intake reporting. Model 2: Adjusted for M1 plus employment, baseline education, energy intake reporting, physical activity, socioeconomic score. * FMI included only for VAT, SAT and gynoid GEE models.

4. Discussion

In this study, we explored the longitudinal relationship between nutrient patterns and various measures of body composition in black middle-aged South African women. Three nutrient patterns were extracted at baseline and follow-up which were plant driven, animal protein driven and vitamin C, sugar, and potassium driven nutrient pattern. The identified nutrient patterns described 69% and 64% of the total variation in nutrient consumption at baseline and follow-up, respectively. Repeated measures of VAT, FMI and LMI were positively associated with repeated measures of the animal-driven nutrient pattern, while repeated measures of FMI and LMI was positively associated with repeated measures of the vitamin C, sugar, and potassium-driven nutrient pattern. The plant driven nutrient pattern was associated with repeated measures of FMI and LMI, however, after adjusting for co-variates, significance was lost.

The nutrient patterns of adult black South Africans have previously been studied in 4 cross-sectional studies [11–13,15]. Chikowore et al. examined associations between nutrient patterns and fasting glucose and glycated haemoglobin levels while Conradie et al. examined the quality of pregnant women diet. Makura-Kankwende et al. and Ratshikombo et al., both cross sectional studies, examined associations of nutrient patterns with body composition measurements with the later study aimed at examining the association between nutrient patterns and DXA-derived body fat and regional adiposity in middle-aged black South African men and women and determine if this differed by sex. The patterns extracted from these previous studies are comparable to those found in our study despite minor discrepancies in the nomenclature of the patterns and suggests that these nutrient patterns are common and consistent in the black South African population.

There were no significant changes in BMI, only significant changes in DXA-derived measures namely fat mass, FMI, VAT, lean mass and gynoid were observed over the 5-year follow-up period. Despite the paucity of research on the longitudinal effects of nutrient patterns on adiposity, our findings are consistent with other cross-sectional studies that show that the animal protein driven nutrient pattern, was positively associated with repeated measures of FMI and VAT, measures of total and central body fat, respectively [6,39,40]. Makura-Kankwende et al., Rosqvist et al. and Fischer et al. showed that saturated fats or animal protein driven diet were associated with an increase in VAT in adults which is in line with findings from this study [39,40]. The storage of animal fat as triglycerides in adipose tissue resulting in increased adiposity is thought to explain the association of animal protein driven nutrient patterns with increased VAT and FMI [6,39]. These changes to total and central body fat may be attributable to ageing, which, in turn, may contribute to increased risk of development or progression of NCDs in LMIC. This may result in over-burdened health care facilities and limited resources thus impacting the management of NCDs.

Our investigation also found that consumption of the animal protein driven nutrient pattern was associated with an increase in LMI. These findings are similar to other longitudinal studies that also reported that nutrients from animal food sources resulted in increases in lean body mass [41–43]. Lean mass and adiposity indices were positively associated with animal protein intake in the Osteoporosis Risk Factor and Fracture Prevention Study (OSTPRE-FPS) [42]. Furthermore, a meta-analysis study which included 18 studies found that a diet high in animal protein was associated with increased lean mass compared a diet high in plant protein which supports the findings from this study [43]. It is thought that the consumption of animal protein may cause the activation of protein synthesis leading to an increase in lean mass [7,44]. This suggests that a diet high in animal protein is advantageous for lean mass growth however, this nutrient pattern was also associated with higher fat mass therefore recommendations are needed on how to leverage this nutrient pattern to promote lean mass and not increase fat mass. The variance of the animal protein driven nutrient pattern which relates to consumption, was far less than the plant driven nutrient pattern.

Our findings showed that a vitamin C, sugar, and potassium driven nutrient pattern was associated with higher FMI and LMI. Foods and beverages that are rich in these nutrients include starchy vegetables such as white potatoes and sweet potatoes and sugar-sweetened beverages for example which may contribute to increased adiposity, while foods such as green leafy vegetables such as spinach may lead to increased LMI [45]. This nutrient pattern has a wide diverse range of food which includes both beneficial and harmful food choices, therefore further research is needed to help identify which foods are beneficial. Observational studies have revealed that high potassium intake may be associated with increased lean mass, along with other body composition changes, consistent with our findings where the vitamin C, sugar and potassium driven nutrient pattern had higher factor loadings for potassium [46,47]. In addition, results from a longitudinal study conducted in adult men and women over a 3-year follow up period showed that a higher intake of foods rich in potassium, supported preservation of lean mass [48]. It is suggested that potassium preserves lean mass by neutralising an acidic environment which promotes protein-energy wasting and loss of lean mass [48]. Other epidemiological research has found that high consumption of foods high in vitamin C, have been linked to increased fat mass, possibly due to higher sugar levels [49–51]. Dietary sugar may be converted to fat and stored in the body which might explain the association of the sugar-based nutrient pattern with FMI in this and other studies [49–51].

Dietary assessment studies are often hampered by inconsistencies in the self-reporting of food intake, which we worked to minimise by correcting for energy intake reporting. The study population underreported their energy intake more noticeably during the second visit compared to the first visit and these could be due to consumption of more recent food items that were not consumed at baseline but are now consumed at follow-up that might not be recorded on the QFFQ. Despite these limitations, to our knowledge, this is the first longitudinal study of nutrient patterns and body composition conducted in black African women which provides evidence on consistent, stable nutrient patterns of the population compared to a cross sectional nutrient pattern study. Furthermore, our investigation used DXA-derived measurements of body composition which provide more accurate assessments of body composition where we were able to examine associations between nutrient pattern and both lean mass and fat mass. Previous studies used simple anthropometric body composition measurements. Finally, while the study was focused on black South African middle-aged women, gender and age specific studies should be conducted to investigate whether the same nutrient patterns are dominate in men or the younger population and if the nutritional recommendations can be applied across the various age and gender groups.

5. Conclusions

This study documented the nutrient patterns of black South African middle-aged women and indicated that these patterns were sustained over 5 years of follow-up. The consumption of animal based nutrient patterns and vitamin C, sugar, and potassium based nutrient patterns were associated with both increased adiposity and lean mass. More importantly, we found that animal protein was associated with visceral adiposity which is a major risk factor of cardiometabolic diseases. This is in line with recent data from large cohorts that confirmed that total and animal proteins are associated with the risk of cardiovascular disease. Additional research including larger sample sizes and a longer follow-up time is needed to verify these results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph191912792/s1, Table S1: Baseline and follow-up extracted nutrient patterns and factor loadings for the Nutrient Patterns for the nutrient patterns.

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Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study after full explanation of the study's objectives and testing procedures.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix 8A: Baseline and follow-up extracted nutrient patterns and factor loadings for the Nutrient Patterns for the nutrient patterns

	Baseline			Follow-up		
	Plant Driven Nutrient Pattern	Animal Driven Nutrient Pattern	Vitamin C, Sugar and Potassium Driven Nutrient Pattern	Plant Driven Nutrient Pattern	Animal protein driven Nutrient Pattern	Vitamin C, Sugar and Potassium Driven Nutrient Pattern
Plant Protein	**0.913**	0.250	0.202	**0.892**	0.222	0.226
Animal Protein	0.248	**0.783**	0.244	0.219	**0.600**	0.282
Saturated fat	0.419	**0.693**	0.291	0.476	**0.632**	0.324
Monounsaturated Fat	0.478	**0.699**	0.213	0.446	**0.688**	0.254
Polyunsaturated Fat	0.562	0.580	0.166	0.586	0.591	0.062
Cholesterol	0.203	**0.727**	0.129	0.159	**0.686**	0.044
Starch	**0.797**	0.352	0.114	**0.818**	0.326	0.035
Sugar	0.229	0.202	**0.777**	0.239	0.120	**0.721**

Dietary fibre	**0.743**	0.169	0.502	**0.739**	0.086	0.474
Calcium	0.245	**0.604**	0.516	0.378	0.288	0.532
Iron	**0.786**	0.407	0.259	**0.799**	0.266	0.291
Magnesium	**0.720**	0.436	0.416	**0.736**	0.285	0.404
Phosphorus	0.556	**0.629**	0.375	**0.602**	0.440	0.381
Potassium	0.544	0.461	**0.601**	**0.603**	0.300	**0.619**
Zinc	**0.780**	0.424	0.231	**0.801**	0.307	0.256
Retinol	0.153	0.245	0.155	0.218	0.048	0.051
Beta carotene	0.233	0.103	0.188	0.186	0.048	0.228
Thiamin	**0.845**	0.306	0.289	**0.854**	0.235	0.297
Riboflavin	0.403	0.522	0.397	0.477	0.305	0.328
Vitamin B6	**0.919**	0.144	0.077	**0.892**	0.114	0.077
Folate	**0.733**	0.205	0.249	**0.831**	0.095	0.112
Vitamin B12	0.073	0.549	0.095	0.049	0.424	0.132
Vitamin Cal	0.269	0.074	**0.863**	0.141	0.048	**0.910**
Vitamin D	0.296	**0.805**	-0.065	0.086	**0.865**	0.009
Vitamin E	0.483	0.520	0.252	0.488	0.579	0.109
Explained variance %	32.157	23.748	13.577	33.583	17.133	13.212

Cumalative explained var. %	32.157	55.904	69.482	33.583	50.716	63.928

Bold factor loadings indicate factor loadings >±0.600 which were used for naming the nutrient patterns

Appendix 9: Structural equation models for the relationship between the animal protein driven nutrient pattern and SBP

Outcome	Pathway	Direct effects	Indirect effects (Through BMI)	Total effects
SBP	BMI->SBP	0.23 (-0.05; 0.51)		0.23 (-2.66; 1.23)
	PC2->SBP	**0.72*** (0.23; 2.66)	0.13 (-0.13; 0.39)	**0.84 *** (0.10; 2.79)
	Age->SBP	**0.39 *** (0.03; 0.75)	0.04 (-0.04; 013)	**0.43 *** (0.08; 0.79)

*P<0.005

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