

Dietary and plasma fatty acids in association with obesity in black South Africans

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DECLARATION

I, **Alice Achieng Ojwang**, declare that this PhD thesis entitled “***Dietary and plasma fatty acids in association with obesity in black South Africans***” is my original work and has not been submitted elsewhere for any degree or examination. This work is in fulfilment of the requirements of Doctor of Philosophy in Dietetics under the guidance and supervision of Prof HS Kruger, Prof CM Smuts, and Dr M Zec at the North-West University, Potchefstroom, South Africa. The literature reviewed in the form of scientific publication, reports, books and websites, is acknowledged and referenced in the text.

A handwritten signature in black ink, appearing to read 'Ojwang', with a stylized flourish at the end.

Date: 23rd January 2019

ABSTRACT

Title: Dietary and plasma fatty acids in association with obesity in black South Africans

Background

South Africa is currently experiencing rapid nutritional, economic, demographic and epidemiological transitions with grave consequences for lifestyle and health. Obesity has escalated to epidemic levels and is a significant risk factor for metabolic syndrome (MetS) and cardiovascular disease. The increasing prevalence of obesity and related risk factors is compounded by the increased fat intake observed in many countries in Africa. Research reports and findings indicate that specific dietary and circulating fatty acids (FAs) as well as subsequent FA patterns may be beneficial or detrimental to human health. Despite the extensive use of circulating FAs in research in Asia, Europe and the USA, there is limited epidemiological research on the association of dietary FAs and circulating FA with obesity and MetS in black populations in Africa.

Aim

The PhD study aimed to investigate the association of dietary FAs and plasma phospholipid FAs and FA patterns with obesity and MetS, as well as comparison of dietary FAs and plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes in a selected group of black South Africans. This study forms part of the South African arm of the international Prospective Urban and Rural Epidemiological (PURE) study.

Subjects and methods

The study sample consisted of 711 (273 men and 438 women) adults; a sub-cohort of apparently healthy black men and women aged 30 to 93 years, from the rural and urban areas of the North West Province, South Africa. A random sample was selected from the original 2010 volunteers with both dietary and plasma phospholipid FAs after exclusion of 305 participants with human immunodeficiency virus and 305 matched controls due to previous use of plasma for analysis. The number of participants was randomly selected from stratified groups (rural male, rural female, urban male, and urban female). The sample included 125 rural men, 148 urban men, 218 rural women and 220 urban women. All the participants gave written and informed consent at each follow-up. The Ethics Committee of the North-West University approved the South African PURE study (Ethics number 04M10) and additional ethical clearance for this affiliated PhD study (NWU-00346-16-S1).

This PhD study include two cross-sectional studies and one longitudinal study of the 711 participants. The first cross-sectional study compared the levels of dietary and plasma phospholipid FAs of metabolically healthy and unhealthy phenotypes. The second study cross-sectionally investigated the association of dietary and plasma phospholipid FAs patterns with measures of adiposity including, body mass index (BMI), waist circumference (WC), and waist: height ratio (WHtR) and MetS. The third study prospectively investigated the association of baseline plasma phospholipid FA patterns with 10-year changes in anthropometric indices (weight, BMI, WC, and WHtR), as well as the reproducibility of plasma FA patterns at the 10-year time point. This study included 412 (173 men and 275 women) of 711 participants, followed up for 10 years and with complete anthropometric and plasma phospholipid FA data at baseline and follow-up.

Results

Study I: Adults from the South African arm of the Prospective Urban and Rural Epidemiology study (n=711) were categorised into four groups, namely normal weight without metabolic syndrome (MetS) (MHNW), normal weight with MetS (MUNW), metabolically healthy overweight/obese (MHO) and metabolically unhealthy overweight/obese (MUO). Dietary and plasma phospholipid FAs were measured by a quantitative food frequency questionnaire and gas chromatography-tandem mass spectrometry respectively. MetS was present in 35% of the participants. Compared to the MHNW reference group, dietary saturated FAs (C14:0 to C18:0) and alpha-linoleic acid intakes were higher in both overweight/obese groups (MHO and MUO), while most mono-unsaturated FAs (MUFAs), linoleic and arachidonic acid intakes were higher in the MUO group only. Plasma levels of most very long chain saturated FAs (C18:0 to C22:0) and PUFAs were higher, whereas selected MUFAs, palmitic acid and estimated desaturase activities were lower in the overweight/obese groups after adjustment for age and total energy intake.

Study II: Two patterns were derived from dietary FAs and six patterns from plasma phospholipid FAs, which explained the cumulative variance of 89% and 73%, respectively. The association between FA patterns with adiposity and MetS, respectively, was weaker for dietary FA patterns than for plasma phospholipid FA patterns. The plasma phospholipid FA pattern with high loadings of saturated FAs (SFAs) (high-Satfat) and another with high loadings of n-3 very long chain PUFAs (n-3 VLC-PUFA) were positively associated with measures of adiposity and MetS, while patterns with positive loadings of n-9 long chain monounsaturated fatty acids (n-9 LC-MUFA) and positive loading of n-3 essential FA (n-3 EFA) showed inverse associations with MetS and some measures of adiposity.

Study III: Six plasma phospholipid FAs patterns were identified at baseline and four similar FA patterns were identified at follow-up. A comparison of quartiles of factor scores at baseline and follow-up showed good reproducibility for four of the six FA patterns identified at baseline. The high-Satfat pattern presented with high positive loadings of saturated FAs (stearic, arachidic, behenic and lignoceric acids) both at baseline and 10-year follow-up, and was positively associated with 10-year changes in all anthropometric outcomes. This pattern that was maintained over 10 years may indicate unique FA metabolism associated with weight gain over time in this group of black South African adults. At 10-year follow-up plasma phospholipid levels of SFAs, stearic, arachidic and behenic acids were higher in overweight/obese participants of both sexes, confirming the association between plasma VLC-SFAs and adiposity. The baseline n-9 LC-MUFA pattern, with positive loadings of MUFAs (nervonic and gondoic acid), was positively associated with 10-year increases in all anthropometric outcomes, but this pattern was not maintained over 10 years. Most of the plasma MUFAs, notably nervonic and gondoic acid decreased significantly over ten years, while BMI, waist circumference and WHtR increased. Therefore, a sustained positive association between n-9 LC-MUFAs and adiposity could not be confirmed.

Conclusion

The results from this study show that overweight groups generally had higher fat intakes than normal weight (MHNW and MUNW) groups. The lower plasma levels of *de novo* lipogenesis pathway FAs and estimated desaturase activities may be biomarkers of abnormal metabolic health in overweight/obese study participants. In dimension reduction analysis, the n-9 LC-MUFA and n-3 EFA patterns seem to provide possible protective associations with adiposity and MetS, whereas the high-Satfat and n-3 VLC-PUFA patterns were associated with adiposity and MetS in our study participants in the cross-sectional study. In the longitudinal study, both the baseline n-9 LC-MUFA pattern and the high-Satfat pattern were associated with adiposity gain over 10 years. The high-Satfat pattern may be indicative of dietary intake and *de novo* lipogenesis involving elongation of FAs and was associated with adiposity gain over 10 years. This pattern derived at baseline was reproducible in the study participants after 10 years and may indicate a unique FA metabolism. On the other hand, the n-9 LC-MUFA pattern was not sustained over 10 years, thus a conclusion of an association between this FA pattern and adiposity gain cannot be made. This publication adds to the understanding of plasma phospholipid FA patterns and their prospective associations, as well as the reproducibility of some of these patterns. Further work is required to improve the interpretation and application of circulating FA patterns in health and disease globally, but especially in Africa, where data are lacking.

Keywords

Phospholipid fatty acid patterns, dietary fatty acid patterns, very long chain saturated fatty acids, black Africans, metabolically healthy and unhealthy, waist: height ratio

LIST OF ABBREVIATIONS

%E	-	Percentage of energy
AA	-	Arachidonic acid
ALA	-	α -linolenic acid
BMI	-	Body mass index
BP	-	Blood pressure
CHD	-	Coronary heart disease
CRP	-	C-reactive protein
CVD	-	Cardiovascular disease
D5D	-	Delta 5 desaturase
D6D	-	Delta 6 desaturase
DGLA	-	Dihomo- γ -linolenic acid
DHA	-	Docosahexaenoic acid
DHS	-	Demographic health survey
DNL	-	<i>De novo</i> lipogenesis
DPA _n -3	-	Docosapentaenoic acid
EPA	-	Eicosapentaenoic acid
FA	-	Fatty acids
FBDG		Food based dietary guidelines
FFA	-	Free fatty acids
GLA	-	γ -linolenic acid
HDL-C	-	High-density lipoprotein cholesterol

HREC	-	Health research ethics committee
IL-6	-	Interleukin-6
LA	-	Linoleic acid
LDL-C	-	Low-density lipoprotein cholesterol
LMICs	-	low- and middle-income countries
MA	-	Myristic acid
MetS	-	Metabolic syndrome
MHO	-	Metabolically healthy overweight/obese
MUFA	-	Monounsaturated fatty acid
NWU	-	North-West University
OA	-	Oleic acid
PA	-	Palmitic acid
POA	-	Palmitoleic acid
PUFA	-	Polyunsaturated fatty acid
PURE	-	Prospective Urban and Rural Epidemiological study
QOL	-	Quality of life
RBC	-	Red blood cells
SA	-	Stearic acid
SBP	-	Systolic blood pressure
SCD	-	Stearoyl-CoA desaturase
SFA	-	Saturated fatty acid
T2DM	-	Type 2 diabetes mellitus
TC	-	Total cholesterol

TNF- α	-	Tumour necrosis factor alpha
VLC	-	Very long chain
WHO	-	World Health Organisation
WHR	-	Waist: hip ratio
WHtR	-	Waist: height ratio

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PREFACE

This thesis is presented in article format and consists of six chapters.

Chapter 1 is an introduction and includes study aims and objectives of the PhD research topic.

Chapter 2 provides an overview of the relevant literature on adiposity and obesity-related outcomes in relation to FAs as biomarkers for cardiometabolic disease risk. Obesity-related outcomes include MetS, T2DM, insulin resistance and inflammation. The literature discusses the aetiology and risk factors of obesity and their association with dietary fats and FAs, plasma phospholipid FAs and identified dietary and plasma phospholipid FA patterns in relation to metabolic health. This chapter offers a comprehensive overview of the relevant literature needed for the interpretation of the data pertaining to the articles in this thesis.

Chapter 3 describes the research methodology of this PhD study.

Chapter 4 is an article titled: ***“Plasma phospholipid fatty acid patterns are associated with adiposity and metabolic syndrome in black South Africans: A cross-sectional study”***. This cross-sectional study included 711 (men 273, women 438) participants randomly selected from the South African arm of the PURE study participants.

Chapter 5 is an article titled: ***“Associations between plasma phospholipid fatty acid patterns and changes in anthropometric indices in black South African adults over ten years: a prospective cohort study”***. This study included a total of 412 (137 men and 275 women) participants with anthropometric, metabolic-related biochemical parameters and plasma phospholipid FAs at baseline and ten-year follow-up.

Chapter 6 is an article titled: ***“Comparison of dietary and plasma phospholipid fatty acids between normal weight and overweight black South Africans according to metabolic health: The PURE study”***. This cross-sectional study included 711 (men 273, women 438) participants randomly selected from the South African arm of the PURE study participants.

Chapter 7 is the final chapter that comprises a general discussion, recommendations and conclusion of this PhD study.

The relevant references used in Chapters 1, 2, 3 and 7 are provided at the end of each chapter according to the required style of the North-West University. The references of Chapters 4, 5 and 6 are provided at the end of each chapter according to the required style of the respective journals.

RESEARCH TEAM

The research team members and their contribution to this thesis are described in **Table 1-2**.

Table 1-1 List of members of the research team and their contributions to this study

Team member	Contribution	Affiliation
Alice Achieng Ojwang (25822829@nwu.ac.za)	PhD student: Conceptualised the protocol titled, "Dietary and plasma fatty acids in association with obesity in black South Africans". This protocol included the use of baseline PURE data collected in 2005 and 10-year follow-up data collected in 2015. She cleaned and prepared the 2005 data used in this sub-study. Also, she participated in the anthropometric data collection of the 10 th year follow-up PURE study in 2015, quantification of plasma phospholipid fatty acids, literature review, statistical analysis, interpretation of results and writing of all seven chapters in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom and Technical University of Kenya.
Prof Herculina Salome Kruger, Professor of Nutrition (salome.kruger@nwu.ac.za)	Promoter: Supervised this thesis, formulated research questions, supervised the data collection of the PURE study, quality control of data, statistical analysis support and interpretation of results. She also co-authored the three manuscripts (Chapters 4, 5 & 6) in this thesis.	Centre of Excellence for Nutrition, Medical Research Council Hypertension and Cardiovascular Disease Research Unit, North-West University, Potchefstroom, South Africa.
Prof Cornelius Matheus Smuts Director of Centre of Excellence for Nutrition. marius.smuts@nwu.ac.za	Co-promoter: Introduced the study, experience in fatty acids in health and disease and responsible for plasma phospholipid fatty acid analysis. Also, co-supervised this thesis and involved in the interpretation of results, co-authored the three manuscripts (Chapters 4, 5 & 6) in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.
Dr Manja Zec Postdoctoral fellow manjazec@gmail.com	Assistant Promoter: contributed to the initial development of research protocol, critical input and co-authored the three manuscripts (Chapters 4, 5 & 6) in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.

Team member	Contribution	Affiliation
Prof Edelweiss Wentzel-Viljoen edelweiss.wentzel-viljoen@nwu.ac.za	Conceptualised the dietary methodology, supervised data collection, coding, analysis and quality control of dietary intake data for the PURE study (2005-2015). Co-authored two manuscripts (Chapters 4 & 6) in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.
Dr Cristian Ricci Postdoctoral fellow Cristian.Ricci@nwu.ac.za	Statistical support and contributed to writing of the 1 st manuscript (Chapter 4) in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.
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Prof Marlien Pieters Professor of Nutrition Marlien.Pieters@nwu.ac.za	PURE collaborator. Contributed writing of the 1 st manuscript (Chapter 4) in this thesis.	Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.
Marike Cokeran Marike.cokeran@nwu.ac.za	Statistics support and consultation during the development of the research proposal.	Statistical Consultation Services, North-West University, Potchefstroom, South Africa.
Prof Faans Steyn Professor of Statistics	Statistical support for all the three manuscripts (Chapters 4, 5 & 6) in this thesis.	Statistical Consultation Services, North-West University, Potchefstroom, South Africa.

SIGNED DECLARATION BY THE RESEARCH TEAM

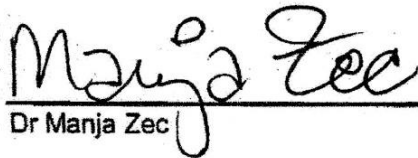
I declare with my signature that as a co-author I have approved the articles mentioned above, that my role in the study as indicated above is representative of my actual contribution and that I hereby give consent that it may be published as part of the PhD thesis of Ms OJWANG A.A.



Prof Herculina Salome Kruger



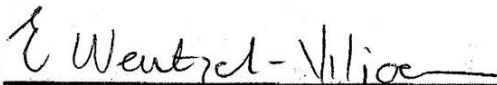
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CHAPTER 1 INTRODUCTION

1.1 Introduction and background information

1.1.1 Identification of obesity

Obesity is described by the World Health Organisation (WHO) as a condition of atypical or excessive body fat accumulation in adipose tissue (WHO, 2000). It is further defined as excessive storage of energy as fat and consequently is associated with adverse effects on health (O'rahilly & Farooqi, 2008). It is not only the degree of the excess fat that is detrimental, but its distribution within the body also significantly determines the health risks associated with the condition (WHO, 2000; Snijder *et al.*, 2006). Obesity is caused by a complex network of biological, environmental, societal and emotional factors (Faith & Kral, 2006). Fortunately, overweight and obesity, as well as their related comorbidities, are preventable; however, they have become a burden for society due to the increase in the burden of mortality and morbidity of obese people. Therefore, developing and sustaining healthy eating and physical activity habits, which would lead to the decrease in obesity prevalence, remains a challenge for society and policymakers (Butland *et al.*, 2007; Faith & Kral, 2006).

Body mass index (BMI) has been extensively used in epidemiological studies as a measure of obesity (WHO, 2000; WHO, 2007; Berrington de Gonzalez *et al.*, 2010; Di Cesare *et al.*, 2016; Abarca-Gómez *et al.*, 2017). BMI cut-points of 25 kg/m² and 30kg/m² have been used to diagnose the conditions of overweight and obesity, respectively (NHLBI Obesity Education Initiative Expert Panel, 1998). Even though various different measures of adiposity may categorise individuals as overweight or obese, studies that have compared BMI with other methods of measuring fat have concluded that BMIs ≥ 30 kg/m² contribute comparable results to other more advanced approaches, such as dual-energy x-ray absorptiometry (Okorodudu *et al.*, 2010).

In South Africa, health professionals have also recommended lower BMIs for the diagnosis of cardio-metabolic risk factors. To accurately diagnose populations with metabolic syndrome (MetS) and obesity-related risk factors, Kruger *et al.* (2015) have recommended BMI cut-points for use in black South Africans for the identification of cardiometabolic risk. They concluded that the WHO BMI obesity cut-off point of 30 kg/m² for black South African men and women might underestimate the risk of cardio-metabolic disease. They, therefore, recommended optimal BMI cut-points for black South African adults be 22 kg/m² for men and 28 kg/m² for women (Kruger *et*

al., 2015). Although BMI has been used widely, it has its limitations, which should be considered (Lam *et al.*, 2015).

Waist circumference (WC) is widely used to estimate abdominal obesity and is an excellent indicator to estimate cardio-metabolic risk in populations (Janssen *et al.*, 2004; Jacobs *et al.*, 2010; Cerhan *et al.*, 2014). The National Institute of Health cut-points for WC help to identify those at increased risk within the healthy weight, overweight and obesity categories. Due to differences across populations, some countries have made their own recommendations for use in their communities (WHO, 2004; Ohkubo *et al.*, 2006; Oka *et al.*, 2008). Crowther and colleagues (Crowther & Norris, 2012) aimed to determine appropriate WC cut-points for diagnosing the MetS of a high-risk group of urban African females with metabolic disorders. They endorsed a new cut-point for WC to be used for diagnosis of the MetS of 91.5 cm for women instead of the recommended 80 cm (Crowther & Norris, 2012).

The waist: hip ratio (WHR) has also been used as a useful indicator for abdominal fat accumulation and a strong predictor of cardiovascular risk (WHO, 2000; Dalton *et al.*, 2003; Mbanya *et al.*, 2015). However, increased WHR can reflect both increased visceral fat and reduced gluteo-femoral muscle mass. A high WHR of more than one unit in men and 0.85 in women predicts cardiovascular mortality, CVD risk factors including MetS in some populations, and to some extent, is independent of the degree of obesity as assessed by BMI (WHO, 2000; Jaeschke *et al.*, 2015; Gamit *et al.*, 2017). However, a higher WHR in the elderly may be a poor gauge of CVD risk, but rather an indication of visceral obesity combined with muscle loss (Snijder *et al.*, 2006).

A waist: height ratio (WHtR) is a threshold measurement that has been used successfully to determine cardiovascular risk in Asian and Caucasian populations (Li *et al.*, 2013; Savva *et al.*, 2013). A South African study, which evaluated WHtR to predict five-year cardiometabolic risk in sub-Saharan African adults, concluded that the recommended WHtR cut-point of > 0.5 was a significant predictor of all metabolic risk factors over five years for both men and women (Ware *et al.*, 2014). Mbanya and colleagues (2015) investigated the difference between BMI, WC, hip circumference, WHR and WHtR in screening for diabetes, and reported that WHtR is a good indicator of diabetes in a Cameroonian study (Mbanya *et al.*, 2015). Integrating height into an assessment of central obesity may supplement information about risk factors. Additionally, incorporating height into the primary obesity assessment may also reduce the variability observed between populations due to differences in height (Ware *et al.*, 2014).

Therefore, using a combination of measures, one that includes a measure of general adiposity and a measure of central obesity (BMI and WHtR) would be appropriate in the identification of cardiovascular disease (CVD) risk and MetS (Lam *et al.*, 2015). A comparison of these tools is shown in **Table 1-1**.

Table 1-1 Comparison of anthropometric methods used for measuring obesity

Methods	Description	Strengths and limitations	References
Body mass index	Defined as weight in (kg)/height (m ²) and is the most used index to measure adiposity. Used to predict disease incidences and mortality	Simple and easy to calculate. BMI has been used widely in epidemiological studies and in all age groups. Standardised cut-off-points have been determined for use globally. Less appropriate predictor of fat in the elderly than in middle-aged adults.	(WHO, 2000)
Waist circumference	Direct measure of abdominal obesity and has been validated against measures of abdominal fat by DXA and CT scan and has been shown to predict disease incidences and mortality.	Measurement is relatively easy but not standardised. WC is currently included as key criterion to define metabolic syndrome.	(Janssen <i>et al.</i> , 2004; WHO, 2004; Ohkubo <i>et al.</i> , 2006; Oka <i>et al.</i> , 2008)
Waist: hip ratio	An indirect measure of abdominal fat.	Interpretation of WHR is more complicated because the hip circumference does not have any biological meaning. However, studies recommend it as a good indicator of cardiovascular risk factors.	(WHO, 2000; Dalton <i>et al.</i> , 2003)
Waist: height ratio	Waist and height are easy to measure and can be obtained easily in any setting.	Superior over WC and BMI to detect cardiometabolic risk factors in both men and women.	(Li <i>et al.</i> , 2013; Savva <i>et al.</i> , 2013; Ware <i>et al.</i> , 2014)

1.1.2 Worldwide prevalence of overweight and obesity

The latest WHO estimates are that obesity has approximately tripled since 1975 (WHO, 2015). Global prevalence of overweight and obesity in adults is 39% and 13%, respectively (WHO, 2015). In 2016, more than 1.9 billion adults had BMI > 25kg/m², and 650 million were obese. A global survey of 200 countries from 1975 to 2014 (19.2 million people) reported a high mean BMI in men and women in northern and southern Africa (Di Cesare *et al.*, 2016). If recent trends continue, by 2030, up to 57.8% of the world's adult population will be classified either as overweight or obese (Kelly *et al.*, 2008), and not only will the world not meet the global obesity target, but severe obesity will also surpass underweight in women by 2025 (Di Cesare *et al.*, 2016). The recent increase in the prevalence of overweight and obesity in almost every country in the world (Finucane *et al.*, 2011; Ng *et al.*, 2014; Di Cesare *et al.*, 2016; Abarca-Gómez *et al.*, 2017) led to obesity becoming a global public health concern (Di Cesare *et al.*, 2016). Obesity is associated with adverse health consequences throughout the lifecycle and more deaths globally than underweight (WHO, 2015; Berrington de Gonzalez *et al.*, 2010).

1.1.3 Obesity in South Africa

Obesity and its co-morbidities negatively affect the lives of many South Africans and the consequent burden of disease contributes to the increasing cost of healthcare, both at a state level and in the private sector (Bradshaw *et al.*, 2003). According to the South African National Health and Nutrition Examination Survey in 2014, the prevalence of overweight and obesity in women and men increased from the Demographic and Health Survey (DHS) 2003. The most recent DHS (2016) reported a prevalence of overweight and obesity in women at 68%, while the prevalence remained the same for men at 31% (National Department of Health, 2017). Urgent leadership is needed in order to aid countries to plan and execute successful interventions to curb the obesity epidemic (Ng *et al.*, 2014).

1.1.4 Metabolic syndrome in South Africa

The prevalence of MetS in South Africa is increasing due to the high incidence of obesity and poor dietary habits, which are now prevalent in rural areas (Vorster *et al.*, 2014). Based on the available data of various South African studies, the overall crude prevalence of MetS in rural black South Africans in KwaZulu-Natal was 26.5%, with a higher prevalence in women (30%) than in men (12%) (Motala *et al.*, 2011). In the Western Cape Province, a study carried out in the rural wine farms showed a prevalence of 29.3% in men and 46.3% in women (Kruger & Nell, 2017),

whereas, in the Free State Province, the prevalence of MetS was 52.2% and 39.7% for rural and urban areas, respectively (Van Zyl *et al.*, 2012).

1.1.5 Fat intake in South Africa

Many African diets are low in fat but are currently changing because of the nutrition transition (Steyn & Mchiza, 2014; Vorster *et al.*, 2011). Due to these changes, people are consuming more fat in their diets, and therefore there is an emerging need for the adjustment of national recommendations. Due to changes in nutrition transition, many countries, with the exception of a few, have experienced an increase in fat supply and therefore the availability of cheap vegetable oils and other fats has influenced the increased consumption in many populations in Africa (Steyn & Mchiza, 2014).

The South African food-based dietary guidelines (FBDG) recommended fat intake of <30% of the total percentage of energy (%E) consumed (Smuts & Wolmarans, 2013). The quantitative dietary goals always recommended on the reduced intake of saturated fatty acids (SFA), in favour of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs). The focus of the current guidelines is more on the type of fatty acids (FA) rather than on the total amount of fat consumed (Smuts & Wolmarans, 2013). This is based on the new understanding of the impact of different individual FA on health and disease (Diekmann *et al.*, 2009; Smuts & Wolmarans, 2013). Therefore, blood levels of specific FAs are evolving as modifiable biomarkers for risk of major chronic diseases (Jackson & Harris, 2018). The conventional sources of SFA among South Africans include oils (coconut, palm kernel and palm oils) fats (animal and dairy fat, including butter, cheese), and processed products made with these fats and oils (Smuts & Wolmarans, 2013). Similarly, MUFA intake is derived mainly from meat, and vegetable oils including canola, olive, sunflower, soy and nuts, peanut butter and avocados (Smuts & Wolmarans, 2013).

A review of fat consumption between 1975 and 1996 up to 2005 in South Africa showed that the total fat consumption increased from 21% to 30% of energy in urban African women and from 15.5% to 21% of energy in rural African women (Vorster *et al.*, 2011). Increased intake of energy from total fat was observed in the urban areas, with figures approaching or already at the upper dietary goal of 30%. However, the percentage of energy from total fat was still low, at 21% of energy in the rural areas in 2005 (Vorster *et al.*, 2011). Recent publication findings show that dietary fat intake in South Africa is significantly higher in urban than in rural areas and women consume more energy from fat compared to men. Absolute intake of total fat, SFA, MUFA, PUFA, linoleic acid (LA, C18:2n-6) and α -linolenic acid (ALA, C18:3n-3) did not differ significantly between black men and women in the North West Province. However, when calculated as %E

intake, additional to the significant urbanisation effects, women had significantly higher intakes than men (Richter *et al.*, 2014). The South African white and black obese women, consumed more total fat, SFAs and n-6 PUFA as well as individual dietary FAs, LA, arachidonic acid (AA, C20:4n-6) eicosapentaenoic acid (EPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3) expressed as %E than the normal-weight groups (Joffe *et al.*, 2014).

1.2 The current state of the research in the field of dietary and circulating fatty acids

Recent research suggests that there is a need to consider the quality as well as the quantity of fat in the diets of a population (Smuts & Wolmarans, 2013; Micha *et al.*, 2014; Simopoulos, 2016b). The field of FA research has developed, and knowledge on the metabolism of FAs and their effects on health has been studied and documented in different parts of the world. Few studies have investigated the prospective association between dietary and plasma phospholipid FAs with obesity and MetS and such information is scarce in Africa. It is therefore imperative that we investigate the association of dietary FAs and plasma phospholipid FAs with obesity and MetS, to provide data and to shed light on its relationship with obesity and MetS.

Current knowledge on the association of dietary and circulating FA with obesity and MetS indicate the following:

1. For a long time, diets high in SFA were reported to be associated with obesity, insulin resistance and progression to MetS and even mortality in large prospective studies. However, well-designed observational studies do not support a robust association of saturated fats with all-cause mortality, ischemic stroke, or diabetes in healthy individuals; but the choice of comparison nutrient (n-6 and/or n-3 PUFA, MUFA, refined or high-quality carbohydrate) must be carefully considered (Bjermo *et al.*, 2012; de Souza *et al.*, 2015; Fretts *et al.*, 2016). On the other hand, plasma SFAs, myristic acid (MA, C14:0), palmitic acid (PA, C16:0) and stearic acid (SA, C18:0) have been associated with obesity and MetS (Mayneris-Perxachs *et al.*, 2013; Pickens *et al.*, 2015; Yammine *et al.*, 2018).
2. Evidence from a limited number of in vivo studies in animals supports the potential for long-chain (LC)-MUFA-rich diets in the prevention of MetS, type 2 diabetes mellitus (T2DM), and atherosclerosis (Qian, 2016; Yang *et al.*, 2016). Furthermore, human studies suggested a possible link between LC-MUFA-rich diet and CVD risk protection (Yang *et al.*, 2016) and lower prevalence of MetS (Qian, 2016). On the contrary, MUFAs in plasma, e.g. palmitoleic acid (POA, C16:1n-7), have been associated with obesity (Aglago, 2017; Paillard *et al.*, 2008;

Pickens *et al.*, 2015) and metabolic risk factors (Mayneris-Perxachs *et al.*, 2014; Mozaffarian D *et al.*, 2010).

3. Dietary n–3 PUFA intake has mostly been associated with less weight gain in some cohort studies (Albracht-Schulte *et al.*, 2018). Modest inverse association between n–3 LC-PUFA intake and risk of MetS has been observed in prospective cohort studies (Kim *et al.*, 2015). Similarly, plasma and erythrocyte n–3 PUFAs have been associated with a lower prevalence of obesity and MetS (Micallef, 2009; Kim *et al.*, 2013; Dai *et al.*, 2016).
4. The global increase in n–6 PUFA intake, and a decrease in n–3 PUFA intake increase the ratio of n–6/n–3 PUFA, and are associated with obesity and could enhance inflammatory processes and subsequently inflammatory diseases (Patterson, 2012; Simopoulos, 2016a; Torres-Castillo *et al.*, 2018). High dietary n–6/n–3 PUFA ratio is positively associated with measures of adiposity such as WC. In addition, subjects with higher dietary n–6/n–3 PUFA ratio had worse metabolic profiles, specifically regarding insulin and HOMA-IR values (Torres-Castillo *et al.*, 2018). In addition, specific n–6 PUFAs in plasma and serum have been positively associated with obesity and MetS (Huang *et al.*, 2010; Vanhala *et al.*, 2012; Howe, 2014; Ni *et al.*, 2015; Wang, 2016).

However, studies in different populations have reported mixed results of FA as biomarkers for specific health conditions, thereby creating reservations in their application. Furthermore, most of these outcomes are based on studies done outside of Africa and therefore cannot necessarily be used to draw conclusions from for recommendations in people of African descent.

Only recently, the first studies that investigated associations between dietary FA intake and blood lipid profiles in South Africans recorded the following findings; 1) dietary ALA intake was positively associated with total cholesterol (TC) and triglyceride in men, but not in women; 2) different dietary FAs and lifestyle characteristics were associated with adverse blood lipids profiles [(TC, triglycerides and low-density lipoproteins cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)], in both sexes (Richter *et al.*, 2014). Furthermore, a study that investigated the interactions between interleukin-6 (IL-6) polymorphisms and dietary fat intake on obesity and serum lipids in black and white South African women reported that an increased n–6/n–3 ratio was associated with increasing BMI in white women. On the contrary, elevated measures of adiposity in black women was associated with increasing dietary fat intake, rather than the quality of specific FAs, even though n–6/n–3 ratios was higher in the black women as compared to white women, (26.4:1 vs 16.1:1) respectively (Joffe *et al.*, 2014).

There is a great need to understand the association of dietary FA intake and plasma phospholipid FA with obesity and MetS in black South Africans. Consequently, investigating obesity and related risk factors and outcomes related to fat intake and endogenous metabolism will go a long way in supporting recommendations to develop policies that will have a significant impact in its prevention of obesity and MetS (Mokdad *et al.*, 2003).

Increasing obesity prevalence and associated obesity-related outcomes negatively affect the lives of many South Africans, together with the growing cost of healthcare. Current recommendations from international public health bodies, including the WHO and Centres for Disease Control on body weight/fat loss, are based on increasing physical activity and eating a healthy balanced diet. However, some meta-analysis showed that almost half of the initial weight loss achieved through diet or exercise is regained after one year and intervention programmes require rigorous strategies that are sustainable and not only applicable to small groups of people (Curioni, 2005; Greaves *et al.*, 2011). Therefore, as the prevalence of obesity continues to rise, more acceptable and sustainable strategies to reduce body fat must be found.

1.3 Problem statement

Investigating the relationship between dietary patterns and risk factors for disease can lead to a new understanding, which is essential for the development of dietary guidelines. Pattern analysis using principal component analysis (PCA) has become a popular method to study the total dietary intake or nutrients in a population and its association with health and disease. Although there are several dietary pattern studies, there is scarcity of data on dietary FAs and circulating FA pattern studies globally, and there are none that have been investigated in the black South African population. We attempted to address this key gap in the existing literature by investigating associations of dietary and plasma phospholipid FAs and subsequent patterns in obesity and related parameters (weight, BMI, WC and WHtR) and risk factors in black South African Adults.

The primary goal of the proposed study is to explore the associations of dietary and plasma phospholipid FAs and resulting patterns with obesity and MetS in black South African adults. The findings of this study may complement the data in the field and further supplement the knowledge and importance of FA status as potential novel biomarkers of obesity-related health outcomes. In addition to public health importance, the new knowledge may benefit policies that will direct nutrition intervention to recommend modulation of current FA intake guidelines within the black South African population. Furthermore, results from this PhD study may generate new hypotheses for future studies that may investigate various phases of FAs metabolism, specifically in the black South African population, as well as black Africans.

1.4 Research questions

The following research questions were formulated:

1. Are dietary FA intake and plasma phospholipid FAs associated with obesity-related parameters (adiposity) and risk factors in the black South African population?
2. What is the plasma phospholipid FAs profile of black South Africans in relation to obesity related parameters and MetS?
3. Are one or more distinctive dietary and plasma phospholipid FAs patterns protective of metabolically healthy and unhealthy phenotypes in the black South Africa population?

1.5 Aims and objectives

1.5.1 Aim

The PhD study aimed to investigate the association of dietary FAs and plasma phospholipid FAs and FA patterns with obesity and MetS, as well as comparison of dietary FAs and plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes in a selected group of black South Africans. This study forms part of the South African arm of the international Prospective Urban and Rural Epidemiological (PURE) study.

1.5.2 Objectives

1. To compare the composition of dietary FAs and plasma phospholipid FAs of metabolically healthy and unhealthy phenotypes classified according to Joint Interim Statement (JIS) of the International Diabetes Federation Task Force, (Alberti et al., 2009) MetS criteria and BMI categories in a group of black South African adults.
2. To investigate cross-sectional associations between dietary and plasma phospholipid FA patterns with obesity-related parameters and MetS, in black South Africans.
3. To investigate the associations between individual plasma phospholipid FAs and derived FA patterns with a 10-year change in obesity-related parameters, among a selected group of black South African adults.

1.5.3 Hypothesis

Hypotheses were not formulated because the objectives of this study were to identify distinctive dietary and plasma phospholipid FA patterns in this group of black South African adults and the composition of these FA patterns were unknown prior to the study. Associations between these dietary and plasma phospholipid FA patterns with measures of adiposity and MetS were investigated. However, based on studies investigating circulating FA patterns and individual FA profiles/proportions in plasma, we hypothesised that plasma phospholipid FAs patterns derived at baseline may be associated with 10-year changes in anthropometric indices. To test our hypothesis, we prospectively explored the associations of plasma phospholipid FA patterns derived at baseline with 10-year changes in anthropometric indices of adiposity among a sub-cohort of black South Africans participating in the Prospective Urban and Rural Epidemiology (PURE) study. This hypothesis was only formulated for the 2nd manuscript in **Chapter 5** of this PhD thesis.

1.6 Ethical approval

Ethical approval for the original PURE study was obtained from the Ethics Committee of the North-West University (NWU) for the period January 2005 to December 2009 (ethics number 04M10) (see Addendum 12.1.3), as well as for the period 2010 (ethical no NWU-0016-10-A1), January 2015 (ethical number NWU-0016-10-A1). An extension of the current ethical approval was obtained from the Human Research Ethics Committee (HREC) of the Faculty of Health Sciences from the North-West University until December 2020 (ethics number NWU-00016-10-A1). Additional ethical approval for this affiliated study of the PURE study was obtained from the HREC of the North-West University (NWU-00346-16-S1). HREC is registered at the National Health Research Ethics Council of South Africa.

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CHAPTER 2 LITERATURE REVIEW

2.1 Obesity and its associated risk factors for non-communicable diseases

2.1.1 Obesity and co-morbidities

Obesity is connected to several co-morbidities, including T2DM, CVD and other NCDs (Ezzati & Riboli, 2012), which are major health challenges of the 21st century (Mendis *et al.*, 2015). NCDs, including cancer, CVD, T2DM, and chronic respiratory diseases, are responsible for approximately two-thirds of deaths worldwide, with nearly 80% in low- and middle-income countries (LMICs) (Echouffo-Tcheugui *et al.*, 2018). A substantial amount of the worldwide NCD burden is attributable to behavioural, dietary, environmental and metabolic risk factors (Di Cesare *et al.*, 2013). In Africa, obesity-related diseases are projected to exceed communicable, maternal, perinatal, and nutritional diseases as the most common causes of death by 2030 (WHO, 2011). If these obesity trends continue, the possibility of meeting the global obesity target will not be possible (Di Cesare *et al.*, 2016).

The biggest risk factor for obesity and other chronic diseases is diet (Micha *et al.*, 2014). Many forms of dietary intake have been implicated in the prevalence of obesity, including total energy intake, excess starch, refined starch, fat and added sugar (Vorster *et al.*, 2014; Wentzel-Viljoen *et al.*, 2018). In recent years, innumerable studies have shown that obesity may cause CVD via multiple disease mechanisms such as subclinical inflammation, endothelial dysfunction, increased sympathetic tone, atherogenic lipid profiles, and enhanced thrombogenic factors and also through obstructive sleep apnoea (López-Jiménez & Cortés-Bergoderi, 2011) as outlined in **Figure 2-1**. Obese individuals have a poor quality of life (QOL) and work-related-QOL, including physical functioning, public distress, sexual functioning, self-esteem, as well as shorter life expectancy than their normal-weight counterparts (Baskurt *et al.*, 2018; Kolotkin *et al.*, 2006; Manaf, 2018). In extreme cases, obesity was found to be associated with a high risk of mortality (Kitahara *et al.*, 2014; Zheng *et al.*, 2011).

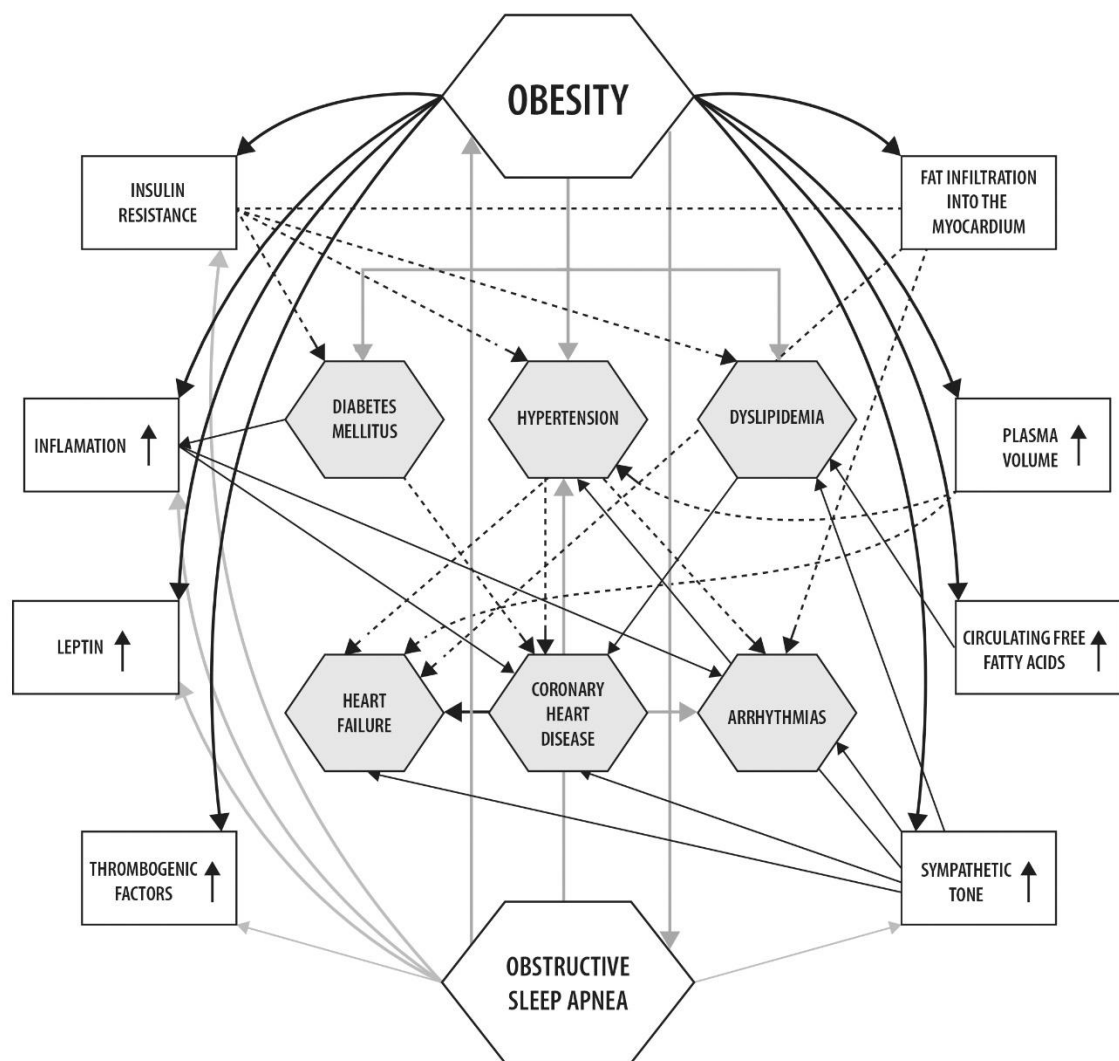


Figure 2-1 Pathophysiology of obesity, modified from López-Jiménez & Cortés-Bergoderi (2011)

South Africa is experiencing a quadruple burden of disease, a combination of poverty-related infectious disease, lifestyle-related NCDs, violence-related trauma and human immunodeficiency virus/acquired immune deficiency syndrome epidemic (Coovadia *et al.*, 2009; Mayosi *et al.*, 2012; National Department of Health, 2017). Recently, a condition (multi-morbidity), classified as the co-existence of two or more chronic diseases in an individual, has also been associated with obesity and is a growing problem with prevalence at 4% in South Africa (Alaba & Chola, 2013). Over 70% of adults with multi-morbidity were women (Alaba & Chola, 2013). Studies that evaluated the extent of this problem in primary healthcare settings in Europe and Australia found that half of the patients with NCD had multi-morbidity (Britt *et al.*, 2008; Fortin *et al.*, 2005). In

Europe, the prevalence of multi-morbidity was at 3.5% to 98.5% in primary care settings and 13% to 72% nationally (Fortin *et al.*, 2012), even though definitions of multi-morbidity may vary from country to country (Johnston *et al.*, 2018). As much as the prevalence was not as high as in high-income countries, rates of comorbidity ranged from 65.2% in diabetes, and these persons also were likely to have hypertension (Lalkhen & Mash, 2015). Multi-morbidity is, therefore, becoming an increasing challenge and a burden to primary healthcare in South Africa (Folb *et al.*, 2015). Evidently, those with multi-morbidity should essentially be targeted for health interventions (Waterhouse *et al.*, 2017). Furthermore, the consequent burden of disease contributes to the increasing cost of healthcare, both at a state level and in the private sector (Bradshaw *et al.*, 2003; Mayosi *et al.*, 2009). A body mass index (BMI) between 30 and 35 kg/m² and a BMI greater than 35 kg/m² are associated with an 11% and 23% increase in healthcare expenditures respectively in South Africa (Sturm *et al.*, 2013).

The epidemiological transition seen in South Africa is characterised by increases in overweight, obesity and several risk factors for NCDs (Vorster *et al.*, 2007), including tobacco use, physical inactivity and unhealthy diets (Mayosi *et al.*, 2009; Mchiza *et al.*, 2015). The freedom of movement of the black South African population and an increase in exposure to the global market economy have led to a shift from nutritious traditional meals low in fat and high in fibre, towards meat and dairy products containing high levels of SFA and more highly refined and energy dense foods (Bourne *et al.*, 2002; MacIntyre *et al.*, 2002; Vorster *et al.*, 2014; Wentzel-Viljoen *et al.*, 2018). Fat intake has increased from 16.4% to 26.2%, and carbohydrate intake has decreased from 69.3% to 61.7% over the past 50 years (Bourne *et al.*, 2002); in addition, sugar intake has also significantly increased even in rural areas (Vorster *et al.*, 2014).

2.1.2 Adiposity

Adipose tissue is a structural term for loose connective tissue composed of adipocytes or fat cells. Obesity is a condition in which the number and size of fat cells increase with an additional increase in weight or total fat mass (Jo *et al.*, 2009). Obesity is not dependent on the body weight, but on the amount of body fat, specifically within adipose tissue (Lee *et al.*, 2013). Fat distribution is determined by many factors, but especially sex and ageing (de Mutsert *et al.*, 2018; Geer & Shen, 2009). Women have higher fat mass as compared to men, but men tend to present with truncal obesity while women present with higher gluteo-femoral fat mass (fat in the lower body) (de Mutsert *et al.*, 2018; Geer & Shen, 2009). On the other hand, ageing increases the accumulation of both abdominal and visceral fat (Kuk *et al.*, 2009; Pérez *et al.*, 2016).

In a medical setting, obesity is frequently defined by a BMI above 30 kg/m² and is a useful indicator of general adiposity (Okorodudu *et al.*, 2010). However, WC is an easily obtainable and an imprecise measure of abdominal adiposity, because it is an outcome of both the subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) compartments (**Figure 2-2**) (Shuster *et al.*, 2012). The metabolic risks associated with obesity depend on overall adiposity as measured by BMI, but also vary according to different regional fat accumulation. It is well known that VAT is more closely associated with obesity-related metabolic diseases and CVD than SAT (Hwang *et al.*, 2015; Lopes *et al.*, 2016; Tu *et al.*, 2017; Wildman *et al.*, 2008). Both visceral and abdominal obesity is associated with a number of important metabolic and cardiovascular abnormalities, including ectopic fat accumulation, insulin resistance, low-grade inflammation and increased oxidative stress, impaired glucose homeostasis, hypertriglyceridemia, low HDL-C levels, and hypertension, all contributing to the increased risk of T2DM, CVD and MetS (Despres, 2006; Genske *et al.*, 2018; Grundy, 2015; Lopes *et al.*, 2016). In summary, adipose tissue is no longer viewed as a passive organ for triglyceride storage, but adipocytes are recognised as active endocrine-secreting cells that release adipokines, including IL-6, and tumour necrosis factor- α (TNF- α) acting as inflammation mediators (Ding *et al.*, 2015; Figueroa *et al.*, 2016).

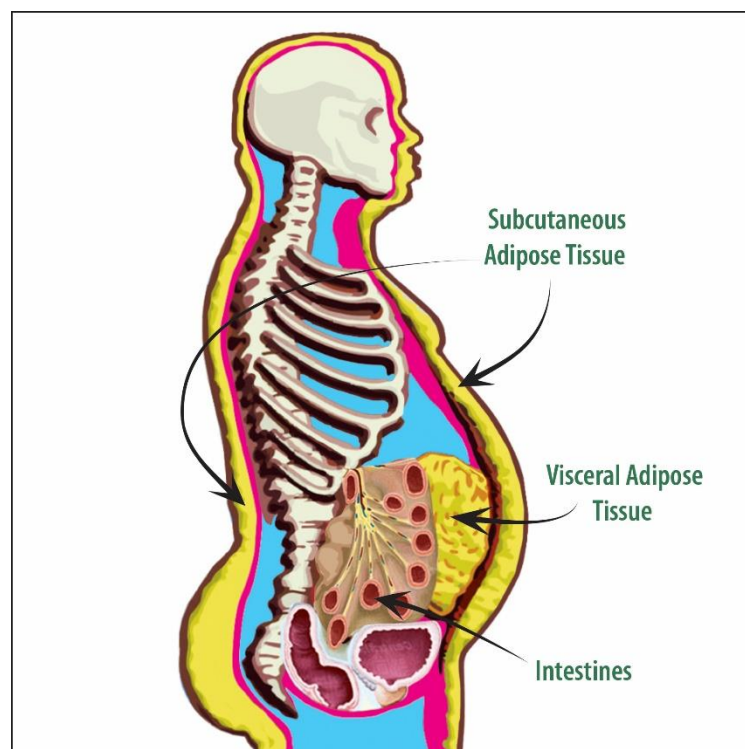


Figure 2-2 Adipose tissue compartments, modified from Walker *et al.*, (2014)

2.1.3 Metabolically healthy obesity

Despite obesity being a risk factor for several conditions, recent clinical evidence indicates that not all obese subjects are at increased risk of cardiometabolic risk factors (Goncalves *et al.*, 2016). Regardless of a high-fat mass, some obese individuals may present with normal metabolic profiles (Guo & Garvey, 2016). Approximately one-third of obese adults do not present with metabolic disorders, nor deranged lipid profiles, hypertension or insulin resistance (Wildman *et al.*, 2008). These obese persons without disorders are referred to as metabolically healthy obese/overweight (MHO) (Bell *et al.*, 2014). Therefore, MHO is defined as or characterised by the absence of at least some of the increased risks for degenerative diseases that accompany typical adult obesity, such as metabolic disorders and CVD risk factors (Goncalves *et al.*, 2016). In a study in Ireland, MHO and non-obese individuals presented with lower concentrations of complement component 3, C-reactive protein (CRP), TNF- α , IL-6, and plasminogen activator inhibitor-1; higher adiponectin levels; and reduced white blood cell count compared to their metabolically unhealthy counterparts (Phillips & Perry, 2013). Stefan and colleagues (2008) demonstrated that individuals with MHO exhibited 54% less fat accumulation in the liver than was measured in metabolically unhealthy obese (MUO) subjects. Furthermore, a recent study showed that the MHO phenotype in healthy obese postmenopausal women has a lower fatty liver index, and lower serum activities of hepatic enzymes, and this could be protective and may be associated with lower risk of development of T2DM, compared with MUO (Messier *et al.*, 2010). Based on these conclusions, Messier and colleagues (2010) proposed that hepatic enzymes could be considered as simple markers to categorise MHO individuals. On the other hand, MUO phenotypes present with MetS and T2DM, increased risk of total mortality from cardiovascular events, as well as elevated blood pressure, inflammatory markers and deranged lipid profile (Guo & Garvey, 2016; Wang *et al.*, 2017).

Several classifications have been recommended for definitions of MHO and MUO. Meigs and colleagues (2006) conducted a longitudinal study in the USA that included 2 902 participants without T2DM. Their study aimed to assess the risk of diabetes and CVD stratified by BMI and presence or absence of MetS or insulin resistance. They recommended the use of up to three BMI categories, risk factor clustering and insulin resistance to classify MHO and MUO accordingly. The uses of homeostatic model assessment (HOMA), of insulin resistance and serum high-sensitivity-CRP are some of the characteristics recommended for use in the classification of MHO and MUO (Meigs *et al.*, 2006; Phillips & Perry, 2013).

Cumulatively, this evidence strongly indicates that relative amounts of body fat, and the relative distribution of body fat between visceral and peripheral sites, and not BMI per se, are important in determining whether an individual's metabolic profile may be favourable or unfavourable (Goncalves *et al.*, 2016; Guo & Garvey, 2016). It remains uncertain whether adults who are metabolically healthy with normal weight (MHNW), but become obese later, are also exposed to an increased risk for T2DM over time given the increasing prevalence of obesity and associated metabolic risk factors (Guo & Garvey, 2016). However, sub-groups of overweight and obese individuals were documented to have normal metabolic profiles (Phillips & Perry, 2013), while a high risk of developing T2DM has been demonstrated in those who maintained MHO status over time, indicating the temporary nature of this condition (Appleton *et al.*, 2013). The MHO status was significantly associated with disadvantaged socio-economic status, physical inactivity, smoking history and other lifestyle conditions (Appleton *et al.*, 2013; Bradshaw *et al.*, 2012). Furthermore, lower central adiposity, young age, physical activity, moderate alcohol intake and compliance with food pyramid recommendations increased the likelihood of MHO (Appleton *et al.*, 2013; Phillips *et al.*, 2013; Wildman *et al.*, 2008). What is more, MHO was identified as associated with a lower risk of insulin resistance and atherosclerosis (Stefan *et al.*, 2008).

The identification of MHNW and MHO and those at risk of metabolic abnormalities in the South African population is of importance in further strengthening of the primary and secondary prevention strategies of obesity and related chronic diseases. Furthermore, generating evidence about which individuals are at higher risk for MetS, in the black South African population, could have implications for public health and clinical practice.

2.1.4 Metabolic syndrome

The MetS represents the clustering of risk factors, including dyslipidaemia, hypertension, and glucose metabolism disorders and is associated with accelerated arterial ageing (Alberti *et al.*, 2009; Scuteri *et al.*, 2015). Furthermore, MetS is recognised as an important risk factor for CVD, which now represents the first cause of mortality in the world, even in LMICs (Grundy, 2007; Grundy, 2015; Motala *et al.*, 2011). Non-diabetic MetS subjects have an up to five-fold higher risk of developing T2DM than those without MetS (Kaur, 2014). A report from the Framingham Heart Study indicated that a weight increase of ≥ 2.25 kg over a period of 16 years was associated with an up to 45% increased risk of progression to MetS in both men and women (Wilson *et al.*, 1999). It is documented that each 11cm increase in WC is associated with an adjusted 80% increased risk of developing the syndrome within five years in a culturally diverse population (Palaniappan *et al.*, 2004). Persons with MetS are also susceptible to other conditions including polycystic

ovarian syndrome (POS) (Kahal *et al.*, 2018; Mortada & Williams, 2015), non-alcoholic fatty liver (Al Rifai *et al.*, 2015; Asabamaka Onyekwere *et al.*, 2016) and obstructive sleep apnoea, among other disorders (Drager *et al.*, 2013; Pedrosa *et al.*, 2018).

There are various criteria to define MetS according to outputs from different international organisations. The National Cholesterol Education Program (NCEP) and Adult Treatment Panel III Report (ATP III) defined the condition of MetS as the presence of at least three of the following five factors: WC as a measure of abdominal obesity, elevated triglycerides, reduced high-density lipoprotein cholesterol (HDL-C), elevated blood pressure (BP), and elevated fasting plasma glucose, or T2DM. NCEP/ATP III identified MetS as a multiplex risk factor for CVD and recognised CVD as a major outcome of MetS (NCEP Panel, 2002). The WHO and European Group for the Study of Insulin Resistance agreed to include either glucose intolerance or insulin resistance as an essential component for defining MetS (Balkau & Charles, 1999). The diagnosis of the syndrome, according to the WHO criteria, includes markers of insulin resistance, impaired glucose tolerance, or T2DM plus 2 additional risk factors out of the following; obesity, hypertension, high triglyceride level, reduced HDL-C level, or microalbuminuria (Alshehri, 2010). In 2009, an additional guideline of MetS was proposed as a Joint Interim Statement (JIS) of the International Diabetes Federation Task Force, an attempt to harmonise the definition of MetS (Alberti *et al.*, 2009). The new harmonised guidelines, for the diagnosis of MetS, recommend that sub-Saharan African populations should use the European WC cut-points (Alberti *et al.*, 2009). The guidelines and cut-point values used to define each altered component are shown in **Table 2-1**.

In sub-Saharan Africa, the exact definitions of MetS criteria and its cut-points, together with the molecular basis underlying the development of risk factors, are of crucial importance and will further allow better grouping of risk factors and fewer therapeutic misjudgements (Alberti & Zimmet, 1998). The available information on the prevalence of MetS in sub-Saharan Africa is mainly limited to epidemiological reports on west Africans in Cameroon (Fezeu *et al.*, 2007), Benin (Ntandou *et al.*, 2009), and Nigeria (Oladapo *et al.*, 2010). In these West African countries, central obesity was reported as a key determinant in the prevalence of MetS in both urban and rural areas (Fezeu *et al.*, 2007; Ntandou *et al.*, 2009; Oladapo *et al.*, 2010). These studies utilised the JIS classification-recommended MetS criteria for African descendants (Alberti *et al.*, 2005). However, Fezeu and colleagues (2007) have stated that the MetS definitions might not be appropriate for populations of African descent.

There have been attempts in South Africa to classify MetS in regional studies of rural and urban populations. A study done in a rural community of Zulu descent (n=947) reported the prevalence

of MetS was 26.5%, with a higher prevalence in women (30.2%) than in men (11.6%) using the 2009 JIS criteria (Motala *et al.*, 2011). The MetS prevalence in this population was lower (23.3%) with the IDF definition and even lower (18.5%) with the NCEP/ATPIII criteria (Motala *et al.*, 2011). By using the 2009 JIS definition, a prevalence of 42.1% of MetS was reported in an urban population of middle-aged black women from Johannesburg (Crowther & Norris, 2012).

Table 2-1 Criteria for clinical diagnosis of metabolic syndrome according to the Joint Interim Statement of the International Diabetes Federation Task Force¹

Measure	Categorical points	
	Men	Women
Elevated waist circumference* Population and country-specific definitions	≥94 cm	≥80 cm
Raised triglycerides (drug treatment for elevated triglycerides is an alternate indicator*)	≥150 mg/dL (1.7 mmol/l)	≥150 mg/dL (1.7 mmol/l)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator*)	≤40 mg/dL (1.0 mmol/l)	≤50 mg/dL (1.3 mmol/l)
Raised blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic blood pressure of ≥130, and/or diastolic blood pressure of ≥85 mm Hg	Systolic ≥130 and/or diastolic ≥85 mm Hg
Elevated fasting glucose# (drug treatment of elevated glucose is an alternate indicator)	≥100 mg/dL (5.6 mmol/l)	≥100 mg/dL (5.6 mmol/l)
Notes: *The most commonly used drugs for elevated triglycerides and reduced high-density lipoprotein cholesterol are fibrates and nicotinic acid. A patient taking one of these drugs can be presumed to have high triglycerides and low high-density lipoprotein cholesterol. # Patients with type-2 diabetes mellitus will be on drug treatment for elevated fasting glucose.		

The high prevalence of MetS in this cohort was largely driven by the high prevalence of abdominal obesity and low fasting HDL-C levels (Crowther & Norris, 2012). Kruger and Nell (2017) used the

¹ Adopted from "Table 11, Criteria for Clinical Diagnosis of the Metabolic " <https://www.ncbi.nlm.nih.gov/books/NBK305897/table/lipid_risk-mgt-hbc.table11cri>. (viewed; 16 Oct. 2018)

IDF classification of MetS to investigate its prevalence in farm workers in the Winelands district of the Western Cape. The prevalence of MetS was higher in women (46.3%) than men (29.3%), and women were more at risk of developing MetS and had a higher prevalence of individual risk factors for MetS (Kruger & Nell, 2017). Crowther and colleagues recommended cut-off points for WC to be used for the diagnosis of MetS within the South African population and recommended further investigations for its use in sub-Saharan Africa. They recommended that a higher WC of 91.5 cm should be used for women instead of 80 cm recommended for diagnosis of MetS, which is too low and may exaggerate the prevalence of adiposity among women in South Africa (Crowther & Norris, 2012). Furthermore, an ideal WC cut-point for identifying men and women at increased cardiometabolic risk is recommended at ≥ 81.2 cm instead of the current guidelines ≥ 94.0 cm for use in sub-Saharan Africa (Ekoru *et al.*, 2018). A different study focused on BMI as a major cardio-metabolic risk factor (Kruger *et al.*, 2015). The study concluded that the WHO BMI obesity cut-point of 30 kg/m^2 for black South African men and women might underestimate the risk of cardio-metabolic disease. The authors recommended a lower cut-point of 22 kg/m^2 for men and BMI cut-point of 28 kg/m^2 for women for the diagnosis of MetS (Kruger *et al.*, 2015).

MetS is becoming a global epidemic as well as a health issue in metabolically distinctive African populations in both rural and urban areas (Fezeu *et al.*, 2007; Motala *et al.*, 2011; Ntandou *et al.*, 2009). Therefore, there is a necessity to accurately determine the prevalence of MetS within the South African population, according to more recent proposed criteria for central obesity cut-points, as measured by WC, WHR, WHtR and BMI (Crowther & Norris, 2012; Ekoru *et al.*, 2018; Kruger *et al.*, 2015; Ware *et al.*, 2014). The recommended new BMI, WC and WHtR cut-off points developed for the identification of persons at risk for MetS in black South Africans should be investigated for possible use in sub-Saharan Africa (Crowther & Norris, 2012; Ekoru *et al.*, 2018; Kruger *et al.*, 2015; Ware *et al.*, 2014).

2.1.5 Insulin resistance

Insulin resistance is linked to a decreased number of insulin receptors on the insulin-sensitive cell membranes due to a number of possible causes (Flie, 1983). A major cause of insulin resistance is associated with decreased beta cell function (Cerf, 2013). As beta cell dysfunction and insulin resistance intensify, hyperglycaemia increases, leading to the progression of T2DM (Cerf, 2013; Eguchi *et al.*, 2012). Although it is generally accepted that hyperinsulinaemia results from insulin resistance, studies also suggest that a high serum level of insulin may lead to insulin resistance, especially in the presence of free FAs (FFAs) in the plasma (Eguchi *et al.*, 2012; Ye, 2007). In humans, an increase in plasma insulin occurs within hours after the elevation of FFA in the

circulation due to fat ingestion (Boden, 1997). Furthermore, prolonged exposure to high levels of plasma FFA results in impaired peripheral glucose utilisation and promotion of hepatic glucose overproduction, leading to T2DM (Boden, 1997). Palmitic acid induces beta cell dysfunction in vivo by activating inflammatory processes within islets (Eguchi *et al.*, 2012). In addition, earlier studies have reported that PA, the most abundant SFA in blood, inhibits insulin signalling in liver, muscle, and fat cells in vitro (Nakamura *et al.*, 2009).

Insulin resistance increases with increasing BMI, WC and, in particular, WHR (Després, 1998; Aronne & Segal, 2002). However, abdominal obesity particularly associated is with resistance to the effects of insulin, peripheral glucose and FFA utilisation, often leading to T2DM and MetS (Boden, 2011). A relatively new study in sub-Saharan Africa reported that insulin resistance was related to higher BMI and WC, and a key determinant in impaired fasting blood glucose (Meeks *et al.*, 2017). The insulin resistance seen in obesity is believed to involve primarily muscle and liver, with increased adipocyte-derived FFA promoting triglyceride accumulation in these tissues (Perseghin *et al.*, 2003). Insulin resistance has repeatedly been associated with increased adipose tissue mass (de Mutsert *et al.*, 2018).

2.1.6 Inflammation

Inflammation is a physiological reaction of an organism to harmful, biological, chemical or physical stimuli (Kotas & Medzhitov, 2015). The response sometimes restores normal function of homeostasis, otherwise the inflammatory process persists, and a state of chronic inflammation is induced (Kotas & Medzhitov, 2015). Circulating mediators of inflammation are secreted directly from adipocytes and adipose tissue-derived macrophages (Berg & Scherer, 2005). Some environmental factors, such as smoking (McEvoy *et al.*, 2015) and obesity (Cottam *et al.*, 2004; Esser *et al.*, 2014) have long been recognised as promoters of inflammation and disease development. One common distinctive feature of obesity, T2DM and MetS, is excessive production of inflammatory mediators, including eicosanoids and cytokines (Bertoni *et al.*, 2010; de Luca & Olefsky, 2008). A number of studies (Hak *et al.*, 1999; Lasselin *et al.*, 2013; Visser *et al.*, 1999) have found that increased BMI and adipose tissue correlate with increases in systemic circulating levels of inflammatory proteins such as hs-CRP, IL-6 and TNF- α . Prolonged inflammatory states significantly influence the development T2DM, insulin resistance and MetS (Bertoni *et al.*, 2010; de Luca & Olefsky, 2008; Donath & Shoelson, 2011; Esser *et al.*, 2014). Raised hs-CRP has been associated with an increased risk of myocardial infarction (MI), cerebrovascular disease, peripheral arterial disease and coronary heart disease (CHD) death in apparently healthy men and women (Berg & Scherer, 2005; Wennberg *et al.*, 2012). Therefore,

inflammatory markers such as hs-CRP and IL-6 plasma levels may be of prognostic value to explain the effect of obesity on MetS, T2DM and CVD risk (Esser *et al.*, 2014; Guarner & Rubio-Ruiz, 2015).

2.2 Non-modifiable and behavioural risk factors for obesity

2.2.1 Early life programming and genetics

Early life programming is the developmental origins to disease and health, especially NCDs in later life (Barker & Fall, 1993; Cooper *et al.*, 1994; Crowther *et al.*, 1998; Levitt *et al.*, 2000). Early epidemiological studies, steered by Barker (1989), verified a relationship between birth-weight and risk of death due to ischemic heart disease in adulthood. This led to the “*Barker hypothesis*”. The hypothesis postulates that adverse environmental exposure during prenatal life induces developmental adaptations that result in the increased risk of glucose intolerance, hypertension, T2DM, insulin resistance, and obesity in middle and old age (Barker, 1990; Hales *et al.*, 1991; Phillips *et al.*, 1994; Ravelli *et al.*, 1999). The environment also predisposes epigenetic processes of gene expressions, which play a key role in the developmental programming of disease conditions in adulthood (Dolinoy, 2007).

Research on early life programming and genetics by Bouchard and colleagues (1991) reported that approximately 75% of the variation in percentage adipose tissue and total fat mass are determined by culture and lifestyle, whereas 25% can be attributed to genetic factors (Bouchard, 1991; Bouchard *et al.*, 1990). There are other estimates that indicate that up to 80% of the variation could be attributable to genetic influences (Faith *et al.*, 1999). Single or multiple gene effects may directly cause overweight and obesity in some individuals, but this does not seem to be the crucial determinant in the majority of people. Genes involved in weight gain probably increase the susceptibility of an individual to the development of obesity when exposed to an adverse environment (Grundy, 1998).

Levitt and colleagues (2000) investigated the association between low birth weight and metabolic conditions in young, non-obese, urban South African adults with a high prevalence of both NCD and low birth weight. Authors found that small size at birth in full-term pregnancies was linked to the successive programming of the major features of the MetS, i.e. impaired glucose tolerance, increased blood pressure, dyslipidaemia and increased mortality from CVD. The link between low birth weight and adult glucose intolerance and blood pressure elevation occurs in young adults in a high risk, disadvantaged population despite a lack of full catch-up growth (Levitt *et al.*, 2000). In a separate study, Levitt and colleagues (2005) followed up 20-year-olds among women of

mixed ancestry who had low birth weight and observed that “foetal origins” manifestation of the chronic diseases phenotype is not dependent on birth weight alone, but, also on its interaction with fat accumulation (Levitt *et al.*, 2005). Despite great advancement in science regarding maternal nutritional status and infant health and disease risk, data are still lacking in Africa, and more research on the mechanisms behind “*Barker hypothesis*” and its relationship to chronic diseases in later life needs to be made clear.

2.2.2 Dietary intake

Poor diet is believed to be an important modifiable cause of poor health in the world (Di Cesare *et al.*, 2013; Micha *et al.*, 2014). By 2020, diet will likely contribute to approximately 75% of all deaths from heart disease, T2DM, obesity, and cancer (Micha *et al.*, 2014). High intakes of energy-dense foods, carbohydrates and fat increase the risk of obesity (Crino *et al.*, 2015; Dehghan, 2017; Fogelholm *et al.*, 2012). Horton and colleagues (1995) studied the effect of fat and carbohydrate overfeeding in humans and different effects on energy storage. They concluded that excess dietary fat is stored with very high efficiency (Hill *et al.*, 2000; Horton *et al.*, 1995). Dehghan and colleagues (2017) recently investigated the association of fat and carbohydrate intake with CVD and mortality in 18 countries from five continents. In this study, a high carbohydrate intake (at least 60% of energy) especially from refined sources (such as white rice and white bread), was associated with an unfavourable effect on total mortality, especially in black Africans, whereas saturated and unsaturated FAs were related to lower risk of total mortality and stroke (Dehghan, 2017). Interestingly, they also reported that a high carbohydrate diet is typically accompanied with low fat intake (Dehghan, 2017). Black African diets, particularly in rural areas, are often very rich in unrefined carbohydrates and low in fat (Freese *et al.*, 2015).

A study of 1 950 black South African men and women in the North West Province reported that total dietary FA intakes were positively associated with higher BMI, TC, LDL-C, and triglyceride (Richter *et al.*, 2014). Similarly, dietary intakes of total fat, SFA, n-6 PUFAs, LA, AA, EPA, and DHA expressed as percentage energy were associated with obesity in black and white women in South Africa in the greater Cape Town area (Joffe *et al.*, 2014). The conventional sources of SFAs among South Africans include coconut, palm kernel, and palm oils, as well as animal and dairy fat, and processed products made with these fats and oils (Smuts & Wolmarans, 2013). Sources of PUFAs are mainly from sunflower and soybean oil, and products made from these oils (e.g. soft type margarines, mayonnaise and walnuts) MUFA intake is mainly derived from meat and vegetable oils (Smuts & Wolmarans, 2013). The increased consumption of sugar has been associated with an increased risk of NCDs in a group of black South Africans (Vorster *et al.*,

2014). A recent review and meta-analysis concluded that free sugars and sugar-sweetened beverages were positively associated with increased body weight (Te Morenga *et al.*, 2013). Unfortunately, sugar consumption has increased in South Africa, even reaching the inaccessible rural parts of the country (Vorster *et al.*, 2014), increasing the prevalence of obesity. Generally, the change in the direction to higher energy intake of animal-based dietary products and fat at the expense of lower plant-based proteins and starchy foods as well as lower intake of fibre, may well intensify the risk of NCD in black South Africans (Wentzel-Viljoen *et al.*, 2018).

2.2.3 Physical activity

Physical activity is documented and generally accepted as primary prevention of chronic diseases and lowered risk of all-cause mortality (Cavanagh *et al.*, 1998; Kruk, 2007). It is also widely used as treatment and rehabilitation of patients (Thompson *et al.*, 2003). Furthermore, physical activity has beneficial effects on a person's psychological health and well-being (Fox, 1999; Gunnell *et al.*, 2014).

Regardless of the benefits of regular physical activity, the proportion of sedentary adults in the world is approximately 31% and increasing (Hallal *et al.*, 2012). Physical inactivity has been documented as an epidemic (Kohl *et al.*, 2012). It is identified as the fourth leading cause of death responsible for more than three million preventable deaths globally (Lee *et al.*, 2012), as well as a risk factor for NCDs and reduced life expectancy (WHO, 2009). Physical inactivity is partly responsible for the global increase in obesity and NCDs (Church *et al.*, 2011; Lee *et al.*, 2012; Ng *et al.*, 2014).

In South Africa, there are indications of the protective role of physical activity in the development of obesity and associated co-morbidities (Kruger *et al.*, 2003), and these are reported as a modifiable risk factor for the development of hypertension (Kolbe *et al.*, 1999). On the other hand, physical inactivity in South Africa is reported as a growing problem in adults and even adolescents (Joubert *et al.*, 2007; Micklesfield *et al.*, 2014). The Transition and Health during Urbanization of South Africa study showed that physical inactivity was a principal determinant of obesity in adult black women of the North West Province (Bourne *et al.*, 2002; Kruger *et al.*, 2002). A study in a peri-urban community in the Western Cape also reported T2DM to be associated with physical inactivity (Levitt *et al.*, 1993). Physical inactivity was also associated with some CVD risk factors in overweight women, including high mean SBP, TC and LDL-C in rural and urban communities (Kruger *et al.*, 2003).

2.3 Circulating fatty acids

2.3.1 Introduction

Fatty acids are components of complex lipids and are found in the human diet, bloodstream, cells and tissues (FAO, 2008). They perform a number of key roles in metabolism and are essential structural components of all membranes, as well as gene expression regulators (Burdge & Calder, 2015). The FAs absorbed from the diet act to influence cell and tissue metabolism, function and responsiveness to hormonal and other signals, finally influencing health and disease risk (Calder, 2015).

The quality of fat is generally quantified by the relative content of SFA, MUFA, and PUFA, including the proportion or amount of essential PUFA of the n-6 and n-3 families; that is, LA and ALA, as well as the proportion or amount of their LC-metabolites, AA, EPA and DHA (Das, 2006; FAO, 2008). The array of FAs that are universally present in the human diet is also found in human tissues.

Some FAs such as high proportions of PA, POA, and dihomo- γ -linolenic acid (DGLA, C20:3n-6), and a low proportion of LA, AA, EPA and DHA, negatively impact metabolic diseases (Bhagat & Das, 2015) such as obesity, T2DM, hypertension and inflammatory diseases, insulin resistance, metabolic syndrome and CVDs (Chalut-Carpentier *et al.*, 2015; Galli & Calder, 2009; Hodge *et al.*, 2007; Lottenberg *et al.*, 2012; Rasmussen *et al.*, 2006). On the other hand, other FAs such as MUFAs are protective of MetS and CVD risk factors (Gillingham *et al.*, 2011). Also, n-3 PUFAs have been shown to improve body composition and counteract obesity-related metabolic changes through mechanisms such as; lipid metabolism modulation; alleviation of adipose tissue inflammation and promotion of adipogenesis amongst other mechanism (Albracht-Schulte *et al.*, 2018).

The longer-chain metabolites of ALA and LA are precursors for the formation of hormone-like substances known as lipid mediators or eicosanoids that influence and regulate key physiological functions including vasodilation and vasoconstriction, blood fluidity and platelet aggregation, inflammatory responses, cell proliferation and pain inhibition and transmission (Bagga *et al.*, 2003; Patterson, 2012). Very high intakes of n-6 PUFAs lead to increased synthesis of AA from LA and trigger the eicosanoid metabolic products from AA to contribute to the development of pro-inflammatory disorders, shifting the physiological state to that of pro-inflammatory (Fritsche, 2008; Patterson, 2012). However, studies show that individuals consuming high levels of LA had the lowest inflammatory status. Also, recent findings suggest that LA and AA are involved in both

pro- and anti-inflammatory signalling pathways. Thus, the evidence does not support reducing LA intake below current consumption levels (Fritsche, 2008).

The n-6 PUFAs may increase the risk of metabolic diseases because they may compete with n-3 PUFA for incorporation into target tissues and desaturation enzymes to produce inflammatory eicosanoids (Schmitz & Ecker, 2008; Simopoulos, 2002). The opposing effects of n-3 and n-6 PUFAs have been shown to be a major influence on health, and impact the pro-inflammatory, thrombogenic and anti-inflammatory and anti-thrombotic state, respectively (Simopoulos, 1999; Schmitz & Ecker, 2008; Simopoulos, 2008). Furthermore, the function and numerous cellular processes such as cell death and survival are significantly influenced by the n-6/n-3 PUFA ratio content of cell and membranes (Schmitz & Ecker, 2008).

Consumption of n-3 LC-PUFAs (DHA and EPA) and ALA is suggested to afford benefits for both primary and secondary prevention of cardiovascular disease (Adkins & Kelley, 2010). The n-3 PUFAs, from diet and circulation, both act directly, by replacing arachidonic acid as an eicosanoid substrate, inhibiting arachidonic acid metabolism and increasing anti-inflammatory resolvins. Consequently, n-3 LC-PUFAs are useful anti-inflammatory agents and have benefits in patients at risk of various acute and chronic inflammatory disorders (Calder, 2006).

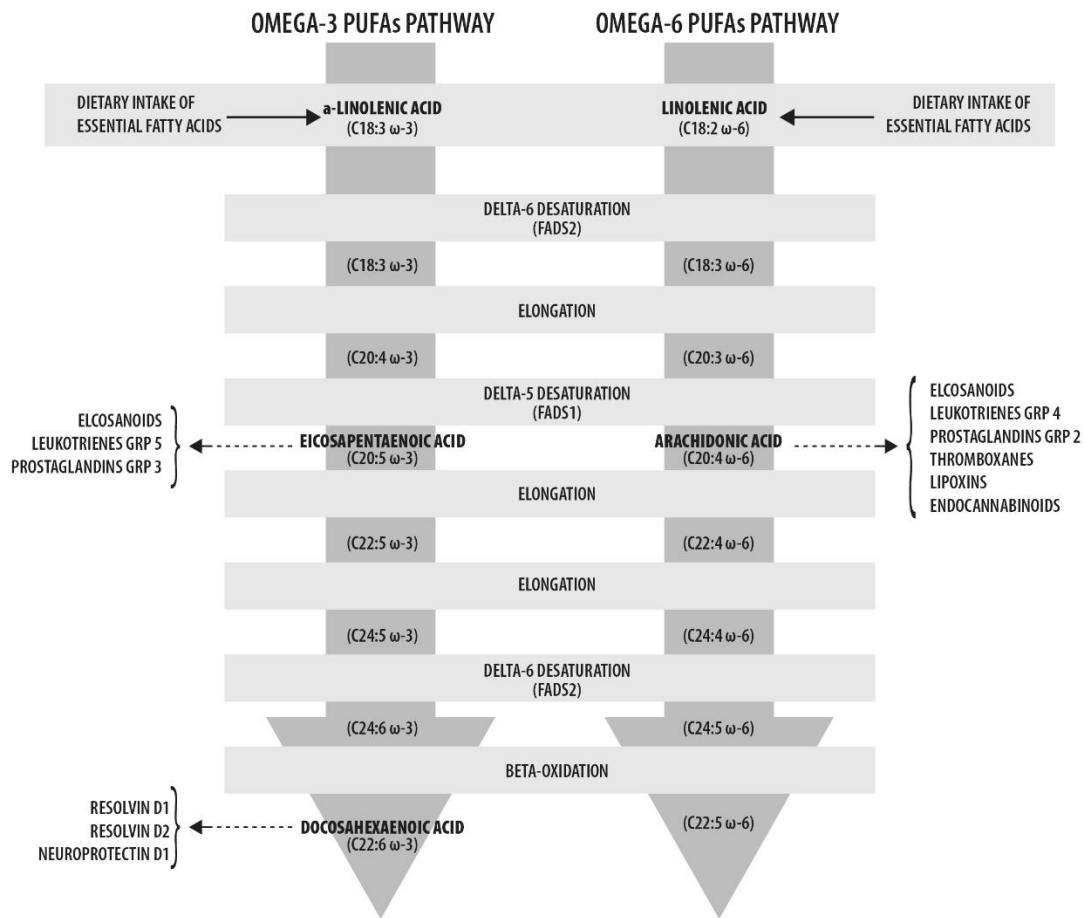


Figure 2-3 The n-3 and n-6 fatty acid metabolism pathways with desaturase and elongase (Tosi *et al.*, 2014) (PUFA = polyunsaturated fatty acids).

2.3.2 Biomarkers of fatty acids

FA levels have been tested in blood and lipid fractions as well as tissues and are reflective of dietary intake (Hodson *et al.*, 2008). Lipid pools most often described in the literature for the depiction of FAs include plasma phospholipids, plasma cholesteryl esters, plasma triacylglycerols, total plasma lipids, erythrocyte and platelet lipids, as well as subcutaneous adipose tissue from the abdomen or buttock (Hodson *et al.*, 2008). Different studies have used different lipid pools, including erythrocytes (Dai *et al.*, 2016; Wang, 2016; Knoll *et al.*, 2011; Shearer *et al.*, 2009), plasma phospholipids (Lauritzen & Hellgren, 2015; Lemaitre *et al.*, 2015), cholesteryl esters (CE) (Kurotani *et al.*, 2012; Van Woudenberg *et al.*, 2012), plasma (Kawabata *et al.*, 2011; Stark *et al.*, 2016), and adipose tissue (Cespedes *et al.*, 2015; Dahm, 2011; Jakobsen, 2017) to investigate the association with various health and disease conditions.

Circulating FA levels reflect dietary intake, endogenous metabolism, including *de novo* lipogenesis (DNL) and FA desaturation as well as elongation (Baylin & Campos, 2006). The phospholipid and cholesteryl ester fractions of lipoprotein particles represent the dietary fat quality of shorter periods, i.e. few days and weeks, whereas FA levels in adipose tissue reflect long-term intake (Hodson *et al.*, 2008). Fasting whole blood is recommended as a suitable biomarker of EFA status in epidemiological studies, since it is readily accessible, requires minimal sample processing time, and can predict intake more accurately compared to plasma (Baylin *et al.*, 2005). Triglycerides and phospholipids are some of the different types of lipids found in the body. Triglycerides are esters, containing a single glycerol molecule which is attached to three FA molecules. Phospholipids are a derivative of triglycerides and are the main structural constituent of the cell membrane (Martini, 2010).

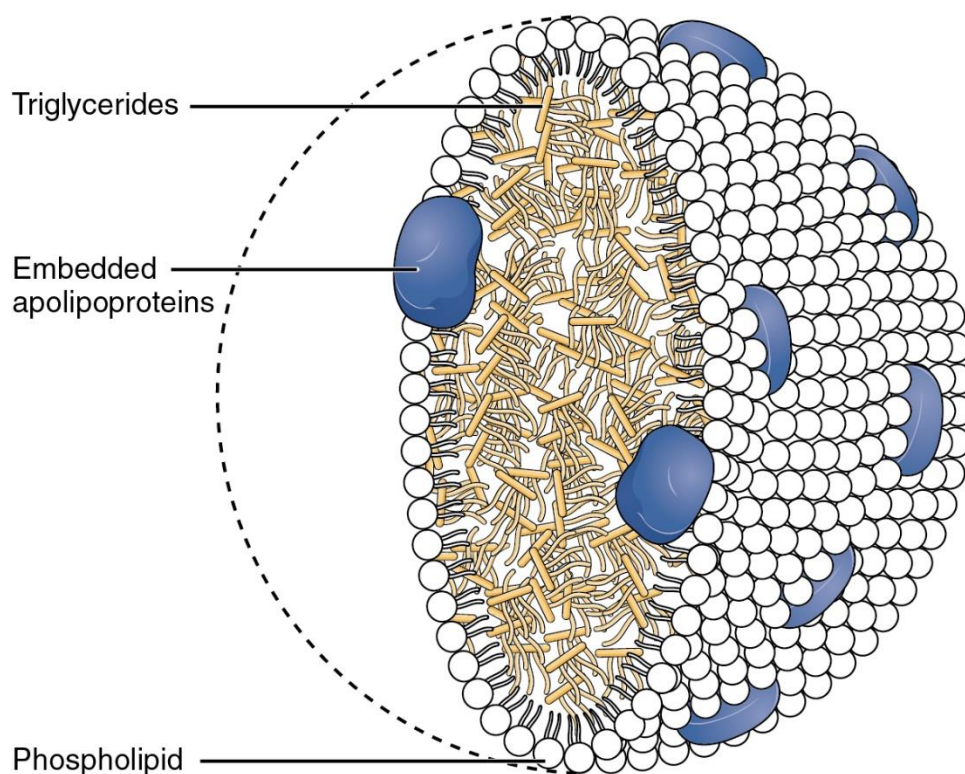


Figure 2-4 Phospholipids are a derivative of triglycerides and are the main structural constituent of the cell membrane adopted from (Martini, 2010).

Specific FAs have been used as dietary biomarkers. Its use as a biomarker is only possible when two conditions are satisfied. That is, when the food item is the primary source of the FA and if this specific FA is stable in the test food group or sampling medium. An example of such is pentadecanoic acid (15:0) and heptadecanoic acid (17:0), present in milk (Wolk *et al.*, 1998), and the n-3 PUFAs, EPA and DHA conventionally derived from marine sources (Hjartåker *et al.*, 1997;

Kobayashi *et al.*, 2001). Nevertheless, they may not always accurately reflect absolute intake because FA concentration is transformed by endogenous metabolism (Arab, 2003). Certain FAs such as LA, EPA and DHA provide a better reflection of dietary intake, while SFA and MUFA provide a less sensitive estimate of dietary intake because of endogenous metabolism (Arab, 2003; Hodson *et al.*, 2008). However, the use of biomarkers may provide a more valid estimation of dietary intakes (Arab, 2003; Baylin & Campos, 2006) since traditional methods of dietary assessment such as QFFQ and 24hr recall are prone to errors as well as under- or over-reporting of energy intake (Markussen *et al.*, 2016; Subar *et al.*, 2015).

2.3.3 Factors influencing fatty acids in circulation

The pattern of FA consumption is affected by several factors, including climate, dietary patterns, season, ageing, geographical locations and availability, and cultural and religious practices. The quantities and quality of fat in the diet are further affected by processing, storage and cooking methods (Calder, 2015; Smuts & Malan, 2016). Black African diets, especially in rural areas, are often very rich in unrefined carbohydrates, vegetables and fruit, with high consumption of legumes and fruit and low in fat (Freese *et al.*, 2015; Pretorius & Sliwa, 2011). In urban areas, the diets are replaced by highly refined carbohydrate sources, increased consumption of processed and convenience foods and the diet lacks the variety of essential nutrients (Govender *et al.*, 2016; Pretorius & Sliwa, 2011). Very low intakes of dietary fat might compromise the sufficient intake of EFAs (Freese *et al.*, 2015). On the other hand, increased consumption of high LA foods and oils, combined with reduced intakes of ALA, could lower the total n-3 LC-PUFA status. The latter FA dietary pattern is typical of rural black South Africans with generally low dietary fat intake (Richter *et al.*, 2014).

The proportions of n-3 and n-6 PUFA in tissues and cells are modified by the dietary intake, activities of the enzymes including desaturases and elongases that are needed for LC-PUFA synthesis, as well as the enzymes that incorporate FA into lipid particles (Hodson *et al.*, 2008; Tosi *et al.*, 2014). Blood and tissue levels of the EFAs (LA and ALA) in addition to their biologically active LC-PUFA derivatives, are influenced not only by diet, but to a large extent also by genetic variants common in European and Canadian populations. Consequently, relative to genetic variants, population sub-groups may differ in requirements of dietary PUFA or LC-PUFA intakes to achieve comparable biological effects. (Glaser *et al.*, 2010; Smith *et al.*, 2015).

Circulating FAs reflect dietary intake; however, the associations of particularly circulating SFAs and MUFAs with dietary SFAs and MUFAs are weak (Volk *et al.*, 2014). Nevertheless, most dietary FAs can be synthesized *de novo*; thus, their source of origin is inconclusive. Also, the

extent to which individual exogenous FAs reflect total dietary fat intake is unknown (King *et al.*, 2006). Carbohydrate diets have been shown to significantly influence plasma levels of SFAs, as opposed to dietary saturated fat intake (Forsythe *et al.*, 2008; Volk *et al.*, 2014). In a study that investigated an isocaloric carbohydrate restricted high saturated fat diet compared to a carbohydrate-restricted diet high in unsaturated fat on plasma SFAs, they reported that a low carbohydrate diet with a high SFA content failed to influence plasma SFA levels (Forsythe *et al.*, 2010). Also, Cassady *et al.* (2007), found that plasma PA and SA were not dependent on saturated fat content of a carbohydrate-reduced diet high in SFAs and PUFAs. Reduced plasma levels of SFA in response to diets that contained higher levels of SFAs and lower carbohydrate than standard intakes was observed in two controlled studies (King *et al.*, 2006; Raatz *et al.*, 2001). Proportionally, POA is the most responsive FA to carbohydrate overfeeding (Aarsland & Wolfe, 1998) and it drops swiftly when carbohydrate is limited to less than 50 g/day (Forsythe *et al.*, 2010; Forsythe *et al.*, 2008). There is reliable evidence that plasma SFA correlates poorly with dietary saturated fat, but SFA levels in plasma is enhanced with a high carbohydrate diet (Volk *et al.*, 2014).

A randomised, controlled, dietary intervention trial study compared FA changes in red blood cells (RBC), plasma phospholipids, and cholesterol esters in response to a low-fat diet and a moderate-fat diet and the baseline diets of women randomly assigned to both the controlled diets. The levels of individual and total FAs increased and decreased accordingly in relation to the FA pool. Saturated FAs, MUFA, n-6 LC-PUFAs, as well as exogenous FAs greatly differed in their response to the low-fat diet and a moderate-fat diet (King *et al.*, 2006). Plasma phospholipid levels of PA, SA and total SFAs were not affected by both low and moderate fat diets, whereas the levels of behenic and lignoceric acids decreased in low-fat diet and increased in moderate fat diet accordingly. For MUFAs, only POA showed a marked increase as compared to all other MUFAs in the low-fat diet and decreased levels in moderate fat diet, while total MUFAs showed minimal change in levels (King *et al.*, 2006). In addition, the consumption of a low-fat diet also promotes an increase in n-3 LC-PUFAs levels and a decrease in the total n-6 LC-PUFAs content of plasma phospholipid FAs (King *et al.*, 2006; Raatz *et al.*, 2001).

Sex and ageing influence FAs concurrently. Certainly, dietary habits can be quite different according to age, ethnic origin and lifestyle (Centritto *et al.*, 2009; Waijers *et al.*, 2006). Ageing may influence circulating EFAs (LA and ALA) due to poor nutritional status impacted by changes in dietary intake experienced in this stage of life (Leslie & Hankey, 2015). The diet of the elderly is impacted by reduced energy expenditure, physiological changes, anorexia due to ageing and pathological changes (Leslie & Hankey, 2015). Mean serum LA, ALA, total n-6 EFA, as well as

delta 6 desaturase (D6D) function were shown to decrease in both elderly women and men due to ageing (Lohner, 2013; Sfar *et al.*, 2010). Similarly, the sex differences in FA status can be explained by hormonal and physiological changes caused by ageing and dietary habits, which may influence the composition of plasma FAs (Lohner, 2013). Sex differences in the synthesis of n-3 LCPUFA from ALA may contribute to higher circulating levels of DHA seen in women than in men (Lohner, 2013). Other factors such as desaturase enzyme activity, health and disease conditions, and genetic features may alter the inter- and intra-individual biological variations of the actual FA measured in the plasma (Glaser *et al.*, 2010; Smith *et al.*, 2015; Tosi *et al.*, 2014).

Absolute amounts of FAs in circulation are also affected by other FAs in the same medium. This is because FAs are expressed as a percentage of total FAs; as levels of one FA increase, the levels of the other FAs are affected (Baylin & Campos, 2006). Other factors that affect the FAs in circulation are BMI, use of supplements such as fish oil capsules, micronutrient deficiencies, disease conditions such as malabsorption, liver cirrhosis, diabetes and laboratory procedures (Arab, 2003). Dietary intake and metabolism remain the principal contributors to plasma FAs status (Arab, 2003; Baylin & Campos, 2006).

Hepatic DNL is an endogenous pathway for the synthesis of fat from excess carbohydrates and protein into SFAs and MUFAs (Chong *et al.*, 2008). A high-carbohydrate feeding upregulates DNL and leads to unfavourable changes in metabolic traits in humans (Chong *et al.*, 2008; Hellerstein, 1999). The key saturated and monounsaturated FAs products of DNL synthesis include PA and SA, POA, oleic acid (OA, C18:1n-9), *cis*-vaccenic acid (C18:1n-7), MA and 7-hexadecenoic acid (C16:1n-9), as shown in **Figure 2-4** (Wu *et al.*, 2011). Even though these FAs are also consumed in the diet, endogenous synthesis appears to contribute considerably to their circulating levels (King *et al.*, 2006; Raatz *et al.*, 2001; Siler *et al.*, 1999). In addition, higher carbohydrate and protein intake and alcohol use were independently associated with higher levels of PA, POA and *cis*-vaccenic acid in free-living adults (Wu *et al.*, 2011). Circulating levels of FAs in the DNL pathway have been linked to the risk of several major CVD cardiometabolic conditions, including T2DM (Hodge *et al.*, 2007; Wang *et al.*, 2003), CHD (Simon *et al.*, 1995; Warensjö *et al.*, 2008; Wu *et al.*, 2011) and hypertensive heart failure (Yamagishi *et al.*, 2008).

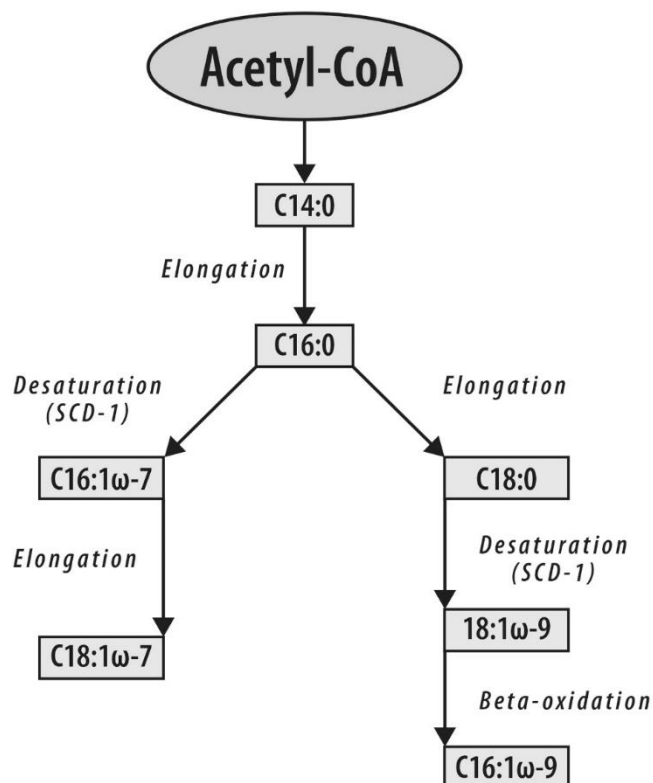


Figure 2-5 De novo fatty acid synthesis, modified from Wu *et al.*, (2011).

2.3.4 Fatty acids in association with obesity

Obesity is a major risk factor for several conditions, including liver, cardiac, pulmonary, endocrine and reproductive system, and the development of obesity-related disorders is associated with lipid abnormalities (Mika & Sledzinski, 2017). Recent trials suggest that certain types of fats are associated with abdominal obesity (Alsharari *et al.*, 2017). SFAs are largely reflected as harmful to health. However, the literature on SFAs being a risk factor for various CVD is mixed, with many studies showing no clear effects, emphasising a need for further investigation of these outcomes (Micha & Mozaffarian, 2010). Perreault *et al.* (2014) documented that serum SFAs and TFAs are associated with obesity, and the presence of MA and SA predominantly predisposes to concomitant inflammation; however, odd-chain FAs (OCFAs) may exert beneficial biological effects on health (Perreault *et al.*, 2014). A study that investigated the association of serum phospholipids with adiposity reported positive correlations of total SFAs with BMI in men (Yammine *et al.*, 2018). Also, plasma levels of total SFAs, MA and PA were higher in obese as compared to normal weight counterparts in a selected group of Turkish participants (Yerlikaya *et al.*, 2011). A recent study examined the relationship between BMI and plasma phospholipid FA composition in men aged between 48 and 65 years and reported higher levels of PA and SA in

obese Caucasians in the USA (Pickens *et al.*, 2015). Other studies have also reported the positive association of SA with obesity (Patel *et al.*, 2010; Zong *et al.*, 2013), and PA in both cholesterol esters and phospholipids was positively associated with specific adipose tissue content (Rosqvist *et al.*, 2017). Furthermore, plasma concentrations of SA were positively associated with risk of total mortality in men and women in a prospective study in the USA (Fretts *et al.*, 2016).

Several studies have investigated the association of short and medium chain FAs with obesity. However, recently, VLC-SFAs are gaining interest in association with obesity and related risk factors. Lemaitre and colleagues (Lemaitre *et al.*, 2018) described plasma levels of circulating sphingolipids with VLC-SFA in association with type 2 diabetes in American Indians with normal BMI followed for up to 5.4 years. Higher levels of stearic, arachidic, behenic and lignoceric acids were prospectively associated with lower fasting insulin in participants with normal BMI; however, at very high BMI status, higher concentrations of these FAs were associated with higher insulin levels (Lemaitre *et al.*, 2018). The association of stearic, arachidic, behenic and lignoceric acids with insulin in this population was highly dependent on BMI categories (Lemaitre *et al.*, 2018). In an earlier study, Lemaitre and colleagues (Lemaitre *et al.*, 2015) reported the association of arachidic, behenic and lignoceric acids in plasma phospholipids with the risk of diabetes in older adults. In analyses classified by three classes of BMI, the association of these FAs indicated a markedly lower risk of diabetes among participants with normal BMI (Lemaitre *et al.*, 2015). Fretts and colleagues (Fretts *et al.*, 2016) described a lower risk of the cause of mortality with stearic, behenic and lignoceric acids in plasma phospholipids in older adults. However, they lacked evidence of association of behenic, lignoceric as well as PA and SA with BMI (Fretts *et al.*, 2016). Mika *et al.* (2016) conducted a comprehensive study of SFAs in serum. Lower levels of stearic, arachidic, behenic and lignoceric acids were recorded in participants with excess weight, whereas levels of MA and PA were lower compared to the controls (Mika *et al.*, 2016). In a study in the USA, VLC-SFAs in plasma were correlated with favourable profiles of CVD risk markers and significantly associated with a lower risk of CHD incidence in men and women in the USA after adjustment for age, smoking, alcohol use, BMI, and dietary intake (Malik *et al.*, 2015). Further research is recommended to explain the mechanisms fundamental to the observed results (Malik *et al.*, 2015).

Warensjo *et al.* (2006) reported positive correlations of proportions of POA in serum cholesteryl esters with markers of obesity in men and women. Higher plasma levels of POA were associated with higher WC in healthy men in France and were therefore considered a marker of abdominal obesity (Paillard *et al.*, 2008). A positive correlation of POA with BMI was reported in Lebanese adults (Yammine *et al.*, 2018) and higher plasma levels of POA were observed in obese

participants (Yerlikaya *et al.*, 2011). Furthermore, POA in plasma phospholipids was positively associated with BMI in men and women in different geographical locations (Aglago, 2017; Pickens *et al.*, 2015). A study that investigated POA in phospholipids with the risk of metabolic abnormalities reported independent associations of higher levels of POA with higher BMI and greater WC in men and women followed up between 1992 and 2006 (Mozaffarian D *et al.*, 2010). Gong *et al.* (2011) also observed a positive association between the adipose tissue concentration of POA and BMI in 1 926 adults from Costa-Rica. POA is nearly absent in typical dietary intake but is the main product of desaturation from PA through the stearoyl-CoA desaturase-1 (SCD-1) activity in the liver and adipose tissue (Paillard *et al.*, 2008). Furthermore, OA can also be synthesised *de novo* in the liver by SCD-1 activity and has been shown to influence utilisation and storage of fat in mouse models (Burhans *et al.*, 2015). *Cis*-vaccenic acid in serum phospholipid has been inversely associated with obesity (Aglago, 2017).

Omega-3 and n-6 FAs may prompt opposing effects (Schmitz & Ecker, 2008). Specifically, linoleic acid (LA, C18:2n6) competes for the same metabolic enzymes as ALA, and increased dietary intake of main n-3 PUFAs, including ALA may be a worthy approach to decrease elongation of n-6 FAs leading to reduced plasma AA (Barceló-Coblijn & Murphy, 2009). DHA must be acquired in the diet, but can also be synthesised within the body from ALA (Domenichiello *et al.*, 2015). Another observational study that considered total plasma n-3 PUFAs, in free-living men and women in Australia, reported that BMI, WC and hip circumference were inversely correlated with plasma levels of total n-3 PUFA, as well as EPA and DHA in the obese group. In addition, obese individuals had significantly lower plasma concentrations of total n-3 PUFA compared to normal-weight individuals (Micallef, 2009). Furthermore, both DHA and n-3 index (EPA + DHA) in erythrocytes correlated inversely with BMI, WC and body fat in women, and docosapentaenoic acid (DPA n-3, C22:5n-3) correlated inversely with body fat in men in a cross-sectional study (Howe, 2014). A study investigating the association of abdominal obesity in older women reported inverse associations of ALA and LA in serum cholesteryl esters with abdominal obesity in a recent cross-sectional study of 60-year-old men and women (Alsharari *et al.*, 2017). However, EPA was not associated with abdominal obesity as expected in this same study (Alsharari *et al.*, 2017). A recent study showed that LA in plasma was inversely associated with WC and body fat (Li *et al.*, 2018) from the Irish National Adult Nutrition Survey, while total n-6 and n-3 PUFAs, also in plasma, were inversely associated with body fat in elderly men and women in Oslo, Norway (Vinknes, 2013). A 10-year longitudinal study in the USA involving older adult men and women reported inverse associations between plasma phospholipid levels of total n-3 PUFAs and baseline BMI (Hastert, 2018). In the Women's Health Study, baseline erythrocyte

n-3 FAs were inversely associated with weight gain over 10.4 years in healthy normal-weight women (Wang, 2016).

Obesity studies also propose that an increased intake of n-3 LC-PUFAs, ALA, EPA, and DHA, could reduce the risk of CVD, and could have potential anti-obesity properties (Abete *et al.*, 2011; Mozaffarian & Wu, 2011). Thorsdottir and colleagues (2007) reported more than 1kg of weight loss after supplementation of n-3 PUFAs from seafood and fish oils in 20- to 40-year-old adults from Spain and Ireland. Within four weeks, there was more weight loss in the group that had the fish oil supplement compared to a similar diet without seafood or supplement of marine origin (Thorsdottir, 2007). Similarly, Parra and colleagues (2008) also reported successful weight loss with a diet high in n-3 LC-PUFAs. The n-3 LC-PUFAs intake modulated postprandial satiety in overweight and obese volunteers, thus improving weight loss. Similarly, weight loss was achieved in obese females who received a three-week controlled inpatient treatment with n-3 LC-PUFAs and very low calorie diet as compared to the control group that had only very low calorie diet (Kunesova, 2006). However, the number of participants was very low (n=27). Krebs *et al.* (2006) reported successful weight loss of up to 10%; however, very high doses of n-3 LC-PUFAs were used, and this is not achievable through typical dietary intake. All these studies concluded that n-3 FA supplementation (EPA+DHA) reduced body weight in lean, overweight and obese individuals (Krebs, 2006; Kunesova, 2006; Parra, 2008; Thorsdottir, 2007). However, all these studies reported restricted energy intake, which may also significantly influence weight loss (de Souza *et al.*, 2012; Harvie *et al.*, 2013). Nevertheless, there is very little information regarding the effects of n-3 LC-PUFA on body composition (Buckley, 2010). Further research on the association between n-3 LC-PUFA and body composition is recommended.

In contrast, Warensjo *et al.* (2006) reported a positive association of EPA in serum lipids with obesity in the Swedish Longitudinal Study of Adult Men. A very recent study that investigated the association of serum cholesteryl esters and phospholipids in association with visceral and subcutaneous adipose tissue content in elderly persons also reported positive associations of EPA and DHA with an accumulation of body fat (Rosqvist *et al.*, 2017). These discrepancies in results may be due to the convergent properties of n-3 and n-6 FAs (Simopoulos, 2016b). Nevertheless, a high intake of LA has been shown to increase the endogenous synthesis of AA, which, in turn, reduces the endogenous synthesis of LC-PUFAs from ALA (Patterson, 2012; Simopoulos, 2016b). High intakes of LA were reported in this same study (Warensjo, 2006).

Pickens *et al.* (2015) reported higher plasma phospholipid levels of DGLA in overweight and obese individuals. Furthermore, positive associations of serum phospholipid DGLA with BMI, as

well as total n-6 PUFAs with waist-hip ratio were also documented in a study of Mexican women (Aglago, 2017). The examination of serum cholesteryl ester FAs with obesity in Swedish men and women reported positive associations of DGLA, AA, and γ -linolenic (GLA, C18:3n-6), and inverse associations of LA with markers of obesity, including WC, BMI and sagittal abdominal diameter (Warensjo, 2006). However, when they adjusted for BMI in the sex-specific analysis, a positive association between LA and sagittal abdominal diameter was found in men (Warensjo, 2006). Furthermore, total n-6 PUFAs in plasma were inversely associated with body fat in elderly men and women (Vinknes, 2013). LA in both cholesterol esters and phospholipids was inversely associated with all body fats, whereas PA was associated with specific adipose tissue content (Rosqvist *et al.*, 2017). In a prospective study, significant positive associations of total n-6 PUFAs as well as DGLA, LA, and GLA with weight gain were recorded at 10.4 years follow-up (Wang, 2016). Similarly, a longitudinal study reported positive associations of total n-6 PUFAs in plasma phospholipids with baseline BMI, as well as of total n-6 PUFAs with an increased change in BMI over time (Hastert, 2018).

Furthermore, animal studies report that a high intake of n-6 and n-6/n-3 PUFAs ratio promotes fat accumulation in white adipose tissue, abdominal obesity as well as chronic inflammation, which are distinctive features of obesity whereas, n-3 PUFAs are protective of cardio-metabolic risk factors (Alarcon *et al.*, 2016; Nuernberg *et al.*, 2011).

In summary, there is varied evidence for the perception that obesity is associated with dietary and circulating FAs in various lipid pools as well as the difference in geographical regions, different dietary patterns, creating some inconsistency in the literature. Plasma FA levels are a complex reflection of metabolism, influenced by dietary intake, climate and seasons affecting food supply, ageing and geographical locations, processing, storage and cooking methods and further influenced by cultural and religious dietary practice. Several factors play a role in aetiology of obesity. However, FA biomarkers is an emerging field with great potential in understanding biological characteristics of obesity. Obesity is associated with alterations of many specific lipid compounds, and these may serve as markers of obesity-related metabolic disorders. Further studies addressing all these issues are reasonable and may provide more information to support existing policies and public health strategies on fats and FA recommendations.

2.3.5 Fatty acids in association with metabolic conditions

The adverse metabolic effects of obesity may in part be facilitated by disorders of lipid metabolism (Kazdova, 2015).

The results from the Prevención con Dieta Mediterránea study reported higher plasma levels of the saturated FAs MA and PA in subjects presenting with MetS as compared to non-MetS participants (Mayneris-Perxachs *et al.*, 2013). Women with MetS had higher plasma levels of MA than men with MetS (Mayneris-Perxachs *et al.*, 2013). Another study that investigated the relationships between plasma phospholipids and MetS in Chinese subjects reported inverse associations of MA and PA with MetS in both men and women (Huang *et al.*, 2010). In a different study, MA, PA and SA in plasma were positively associated with MetS, while longer-chain SFAs, arachidic acid, behenic acid and lignoceric acids were inversely associated in men and women from Taiwan (Yang *et al.*, 2018). Another study also reported lower levels of plasma behenic and lignoceric in MetS participants (Yamazaki *et al.*, 2014).

Studies also showed associations between MUFAs and metabolic risk. Higher plasma levels of POA were associated with multiple metabolic risk factors in men and women (Mayneris-Perxachs *et al.*, 2013; Mozaffarian D *et al.*, 2010). In a study that investigated plasma FA composition in men with abdominal obesity or MetS showed that the MetS group had higher levels of POA and OA than the control (Kawashima *et al.*, 2009). In a two-year intervention study, POA in serum FFAs was reported as predictor of MetS in a group of obese subjects (Ni *et al.*, 2015). Furthermore, significantly reduced proportions of nervonic acid (C24:1n-9) in serum lipids were reported in MetS as compared to non-MetS subjects (Yamazaki *et al.*, 2014). Huang *et al.* (2010) reported inverse associations of total n-3 PUFAs, n-6/n-3 ratios in plasma phospholipids, as well as DHA and EPA with MetS (Huang *et al.*, 2010). Furthermore, the n-3 PUFAs in RBCs showed inverse associations with MetS in Chinese men and women (Dai *et al.*, 2016). Higher concentrations of erythrocyte DHA were associated with lower odds of MetS, whereas higher concentrations of ALA were associated with increased odds among middle-aged and older Chinese adults (Zhang *et al.*, 2012). Higher plasma levels of ALA were also observed in men with MetS (Kawashima *et al.*, 2009).

Kabagambe *et al.* (2008), on the other hand, reported no associations of n-3 PUFAs with MetS. Unfortunately, reports in the literature on the association between circulating n-3 PUFAs and the presence of MetS are not consistent. Some (Dai *et al.*, 2016; Huang *et al.*, 2010) report inverse associations, and Zhang *et al.* (2012) reported both inverse and positive associations of specific n-3 PUFAs, but Kabagambe *et al.* (2008) reported no association with n-3 PUFAs. The disparities in associations in these studies mentioned above may partly be explained by differences in sample sizes and low dietary intake possibly affecting circulating levels (Kabagambe *et al.*, 2008) as well as the difference in FA medium used. Other confounding dynamics that may mask the associations of n-3 PUFAs in human studies include, the quantity

and source of n–3 PUFA, proportions of other fatty acids in the n–3 PUFA source, subject selection, genetic make-up, living conditions, quality and quantity of daily food intake and others, as outlined in this recent review (Huang, 2016).

Omega–6 PUFAs may increase the risk of metabolic diseases because it may compete with n–3 PUFAs in desaturation enzymes for incorporation into target tissues and desaturation enzymes to produce inflammatory eicosanoids (Simopoulos, 2002). A study in Spain reported higher quartiles of LA in plasma to be inversely associated with MetS, and decreased proportions of LA were associated with adverse profiles of several metabolic risk factors (Mayneris-Perxachs *et al.*, 2013). Kabagambe *et al.* (2008) reported inverse associations of total n–6 PUFAs in erythrocytes with MetS. In a prospective study, lower serum levels of n–6 PUFAs was associated with the incidence of MetS over 6.4 years in a cohort of Finnish descent (Vanhala *et al.*, 2012). DGLA was reported as predictive of MetS in a group of obese subjects (Ni *et al.*, 2015). Another study that investigated the association of FFA with the metabolic status of obesity reported higher levels of n–6 PUFAs in MUO as compared to the MHO and MHNW. Furthermore, FFAs have also been implicated in the pathogenesis of obesity-related insulin resistance, T2DM and CVD (Boden, 2011).

In contrast, higher plasma phospholipid levels of total circulating n–6 PUFAs were shown to be protective of risk factors for MetS, including both systolic and diastolic BP and plasma triglycerides in a multi-ethnic study (de Oliveira Otto *et al.*, 2013). Furthermore, higher serum total n–6 PUFAs, LA and AA concentrations were associated with a lower risk of developing MetS after 11 years of follow-up in Finnish men (Yary *et al.*, 2016). Kawashima *et al.* (2011) reported lower plasma levels of LA in the MetS group than the control, and Warensjo *et al.* (2005) reported lower serum LA levels at baseline, whereas and DGLA increased in persons who developed MetS after 20-year follow-up.

Further research is recommended to understand the prospective role of n–6 and n–3 PUFAs in the development of obesity and MetS (Fekete, 2015), as well as the reason for the differences in results from different populations.

In conclusion, fats and oils influence metabolic health. However, their effect may vary according to their precise FA compositions and molecular structures. Some FAs such as LA, PA, and oleic acids are consumed in comparatively large quantities; however, others, such as ALA and n-3 LC-PUFAs (EPA and DHA) are consumed in small quantities yet have potent biologic effects. Also, obesity, in particular, abdominal obesity, alters the composition of plasma and tissue FAs, which contributes to inflammation and insulin resistance, leading to MetS. Several studies investigating

associations of FAs with MetS used different classifications of MetS including, JIS criteria, insulin levels, HOMA index and others used circulating levels of adiponectin or C-peptide concentrations which currently have no clinically defined biological cut-offs. These differences in biochemical parameters used in investigating the association of FAs with MetS provide results that cannot be compared across all populations. Standardisation is therefore necessary to enable comparison of findings from different studies.

2.3.6 Estimated desaturases, elongases and stearoyl-CoA-desaturase activity

Desaturases are important enzymes for human FA metabolism and have been associated with dietary intake, metabolic health and obesity (Alsharari *et al.*, 2017; Murakami *et al.*, 2008; Vinknes, 2013). The commonly estimated desaturase activities include delta 9 desaturase, also known as SCD-1, D6D and delta 5 desaturase (D5D). Elongases are the products of the elongation-of-VLC-FAs (Elovl1-6) genes and work concurrently together with desaturases enzymes to form LC-FAs in the endoplasmic reticulum (Hodson & Fielding, 2013; Jakobsson *et al.*, 2006). Stearoyl-CoA desaturase synthesises MUFAs, POA and OA from PA and SA, respectively. The estimated desaturase activities can be assessed as the ratio of FA product and precursor of circulating or adipose tissue FAs, according to the following formulas: SCD-16 (POA/PA), SCD-18 (OA/SA), D6D (GLA/LA), and D5D (AA/DGLA) (Nakamura, 2004). A high estimated SCD-16 may both reflect a high intake of SFA, low PUFA and (or) metabolic disorders (Saadatian-Elahi *et al.*, 2009). Additionally, D5D and D6D catalyse the synthesis of long-chain n-6 and n-3 PUFAs. Decreased D5D and increased D6D activities have been associated with the development of abdominal adiposity and increased the risk of diabetes and MetS (Kawashima *et al.*, 2009; Warensjo, 2006).

In obesity studies, D5D activity was negatively associated with abdominal obesity in Japanese men (Kawashima *et al.*, 2009). In the Swedish study mentioned at the outset, the increased SCD-16 and SCD-18 in men was also explained by higher abdominal obesity (Warensjo *et al.*, 2005). Similarly, Alsharari and colleagues (2017) also reported the positive association of SCD-1 and D6D with abdominal obesity, and D5D was inversely associated with abdominal obesity. Kawashima *et al.* (2009) observed low D5D and high SCD-16 activity to be predictive of the development of abdominal obesity compared to controls. SCD-16 and SCD-18 have been documented as a marker of abdominal obesity in healthy men (Paillard *et al.*, 2008) and have been associated with increased body fat in elderly men and women (Vinknes *et al.*, 2013). Furthermore, another study by Warensjo and colleagues (2006) investigating the association of

circulating FAs with BMI, WC and sagittal abdominal diameter in obese men and women reported positive associations of SCD-16 and D5D with markers of obesity.

A cross-sectional study that investigated the association of phospholipid FAs with MetS in 50- to 79-year-old Korean men reported increased SCD-16 and SCD-18 and decreased D5D activities in participants with MetS (Kim *et al.*, 2013). Mayneris-Perxachs *et al.* (2013) reported higher estimated activities of SCD-1, and D6D; and decreased activities of D5D were associated with adverse profiles of several metabolic risk factors. Strong differences were observed in the proportions of several FAs and estimated desaturase activities in adverse profiles of MetS risk factors. Women with MetS had higher levels of SCD-16 and D6D, and lower levels of SCD-18 and D5D than men with MetS (Mayneris-Perxachs *et al.*, 2013). In other prior studies, estimated SCD-18 activity did not differ between subjects with and without MetS (Kawashima *et al.*, 2009). However, the estimated SCD-16 activity was a predictive factor to develop metabolic syndrome from abdominal obesity in Japanese men (Kawashima *et al.*, 2009). Also, in a Swedish cohort, the prognostic value of SCD-18 was not significant for MetS in Swedish people (Warensjö *et al.*, 2005). A Swedish study that followed up men for 20 years found that both SCD-16 and D6D were directly associated with the incidence of MetS, and increased D5D activity predicted lower incidence even after adjustment for BMI and other lifestyle factors (Warensjö *et al.*, 2005). In addition, a longitudinal study that investigated the association of serum FFA ratios with metabolic abnormalities in healthy young obese individuals reported that baseline levels of SCD-18 were positive predictors, whereas SA/PA ratios and D5D were negative predictors of metabolic abnormalities in MHO subjects even after adjusting for age, sex and BMI (Zhao *et al.*, 2016).

D5D and D6D are well recognised as the main predictors of PUFA variability influencing several biological mechanisms (Martinelli *et al.*, 2009). However, the complex and diverse effects of desaturase activities and their influence from PUFA intake make it challenging to utilise these enzymes fully in translational research (Tosi *et al.*, 2014). Data are completely lacking in populations of Africa. Therefore, there is great need to investigate these enzyme activities in these populations.

All these results prompt to hypothesize that desaturase activity plays a role in the pathogenesis of MetS and may influence the individual susceptibility to diabetes. Taking into account the pleiotropic effects of PUFA, it appears biologically that different desaturase activities can have an impact on several pathological conditions. Therefore, although the mechanisms by which desaturase activity and PUFA concentration influence health remain largely unknown, studies

analysing PUFAs play a crucial role in understanding these desaturase activities and their influence in health.

2.4 Dietary fatty acids

The World Health Organization recommends that total fat should not exceed 30% of energy intake (WHO, 2015). Most important dietary sources of FAs are vegetable oils, dairy products, meat products, and fatty fish or fish oils (Saini & Keum, 2018; Smuts & Wolmarans, 2013) as well as seeds (Smuts & Malan, 2016). PA is the most common SFA in animals, plants and microorganisms (Rustan & Drewnowski, 2005). SA is a major FA in animals and some fungi, and a minor component in most plants. MA is present in many foods, while shorter-chain SFA with 8 to 10 carbon atoms is found in milk and coconut triglycerides. The sources of circulating VLC-SFAs may be both dietary and metabolic (Lemaitre *et al.*, 2015). In a study investigating association of plasma phospholipid FAs with T2DM of communities in the USA, higher levels of behenic and lignoceric acids were associated with a greater peanut intake (Lemaitre *et al.*, 2015). In addition, these VLC-SFAs are found in some foods, including peanuts, macadamia nuts and canola oil (USDA, 2013). SFAs are derived not only from the diet, but also from hepatic DNL catalysed by a high intake of refined carbohydrates (Mozaffarian, 2014). Nevertheless, it is generally unspecified whether these circulating VLC-SFAs are more heavily influenced by diet or endogenous production (Fretts *et al.*, 2016).

MUFAs include OA and POA. OA is the most common monoenoic FA in plants and animals. Benefits relating to dietary OA, the principal dietary fat of MUFA, are attributable to components of a diet rich in olive oil, which comprises phenolic composites, which have antioxidant and anti-inflammatory properties (Covas *et al.*, 2009). In the South African population, MUFAs are derived largely from meat (Smuts & Wolmarans, 2013). A study that investigated the level of OA in human plasma and its association with olive oil consumption showed minor differences in plasma OA between the high and low olive oil consumers (Buckland *et al.*, 2017). POA also occurs widely in animals, plants and microorganisms, and is a major component in some seed oils; however, it is virtually absent from standard dietary intake in humans, but is a major product of desaturation from PA through the SCD-1 activity in liver and adipose tissue (Liu *et al.*, 2013; Paillard *et al.*, 2008). Other MUFAs include *cis*-vaccenic and gondoic acids. *Cis*-vaccenic acid is derived from chain elongation of POA and has been used as a marker of elongase activity (Lankinen *et al.*, 2015; Yee *et al.*, 2012). Gondoic acid is found in wild fruits (Ouoba *et al.*, 2018), mustard and rapeseed in small quantities (Chowdhury *et al.*, 2007); although it is found in large amounts in

some marine fish, it can also be formed by *de novo* synthesis through the elongation of OA and converted to other FAs (Yang *et al.*, 2016b).

The principal longer-chain n-3 PUFAs are derived from ALA, an EFA. ALA is found in higher plants, green leaves, soybean oil, rapeseed oil, seeds and nuts (Burdge & Calder, 2005). EPA and DHA, derivatives of ALA, are the major FAs of marine algae, fatty fish and fish oils (Abedi & Sahari, 2014).

The major n-6 PUFAs are derived from LA, which is also an EFA as it cannot be synthesised by mammals, and is found in a variety of nuts, seeds and vegetable oils (Abedi & Sahari, 2014). Arachidonic acid is obtained from food such as poultry, animal organs and meat, fish, seafood, and eggs as well as by means of desaturation and chain elongation of the plant-rich essential FA LA (Taber *et al.*, 1998; Tallima & El Ridi, 2018). Higher plasma proportions of AA have been documented among people with a high intake of red and white meat (Sfar *et al.*, 2010). Eicosadienoic acid (C20:2n-6) is a rare, naturally occurring n-6 PUFA, and is widely distributed in animal tissues. It is elongated from LA and can also be metabolised to DGLA and AA (Huang *et al.*, 2011).

Global changes in dietary intake of n-3 PUFAs have reduced while n-6 PUFAs have increased showing n-6/n-3 ratios of ~15:1, and this has created a public health concern (Patterson, 2012). In the USA, this ratio is almost 12:1, while in the Swedish diet it is 5:1 (Råd, 2005; Wijendran & Hayes, 2004). This change in an imbalance of dietary n-6 and n-3 PUFAs may be parallel to the startling increase in the prevalence of overweight and obesity (Ailhaud, 2006). These imbalances of n-6 and n-3 PUFAs have also been associated with inflammatory processes (Simopoulos, 2008) and MetS. A recent cross-sectional study concluded that high dietary n-6/n-3 PUFA ratio was positively associated with measures of adiposity such as WC. In addition, subjects with higher dietary n-6/n-3 PUFA ratio had worse metabolic profiles, especially insulin and HOMA-IR values (Torres-Castillo *et al.*, 2018).

2.4.1 Diet and obesity

General dietary intake is a major risk factor associated with the rising prevalence of obesity and MetS (Echouffo-Tcheugui *et al.*, 2018; Lim *et al.*, 2013; Micha *et al.*, 2014). Although the type of fat and FA composition in diet has a specific, substantial impact on health, and consequently plays an important role in the development of overweight, obesity, MetS and NCDs, specific dietary and lifestyle factors are individually associated with long-term weight gain (Mozaffarian *et al.*, 2011). For obesity prevention, the WHO recommends less than 30% of energy intake from

the total fat, while limiting SFAs to less than 10% of energy intake by replacing them with unsaturated fatty acids reduces the risk of NCDs (WHO, 2015). However, recently, several meta-analyses of prospective cohort studies mainly investigated in European and North American countries presented either a lack of relationship or a lower risk between SFAs intake with total mortality and CVD events (de Souza *et al.*, 2015; Hooper *et al.*, 2015; Siri-Tarino *et al.*, 2010). More data are needed to elucidate whether CVD risks are likely to be influenced by the specific nutrients used to replace saturated fat. Also, further consideration of results of clinical trials as well as information regarding the effects of saturated fat on underlying disease mechanisms, are necessary to confirm the trends observed (Siri-Tarino *et al.*, 2010). A recent prospective cohort study investigating the association of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries reported that higher intake of total fat and individual types of fat such as SFAs, among others, was each associated with lower total mortality risk (Dehghan, 2017). However, evidence on the association of SFAs with weight gain and increased WC in several studies compiled in a systematic review were inconsistent (Fogelholm *et al.*, 2012). In a South African study, total dietary fat intakes expressed as percentage energy (%E) were associated with obesity in black and white women (Joffe *et al.*, 2014). Furthermore, the availability of cheap fats due to the nutrition transition experienced in sub-Saharan Africa is associated with an increase in obesity (Steyn & Mchiza, 2014). A strong and positive association between SFA intake and sagittal abdominal diameter, WC and BMI was documented in Swedish men and women (Warensjo, 2006). Today, industrialised societies are characterised by an increase in SFAs, n-6 PUFA, and trans fatty acid intake, and a decrease in n-3 PUFA intake (Simopoulos, 2011), and these changes are associated with the prevalence of obesity (Simopoulos, 2016a) and other serious health conditions (Patterson, 2012). On the other hand, dietary n-3 PUFAs intake has mostly been associated with less weight gain in cohort studies, but results are not consistent as outlined in this current review (Albracht-Schulte *et al.*, 2018). Development of reliable and valid methods of assessing FA intakes in sizeable longitudinal cohort studies with sufficient follow-up to observe clinical events and deaths must remain a priority to improve the quality of the evidence on which dietary recommendations are based (de Souza *et al.*, 2015).

On the contrary, higher refined carbohydrate intake was associated with a higher risk of total mortality (Dehghan, 2017), positively associated with atherosclerotic progression in postmenopausal women (Mozaffarian *et al.*, 2004a), but also starches or refined carbohydrates have been positively associated with weight gain (Mozaffarian *et al.*, 2011). In addition, a high intake of refined grains as well as and sweets and desserts was associated with predicting more weight gain, and for refined (white) bread and high energy density in predicting larger increases

in WC (Fogelholm *et al.*, 2012). It is documented that high carbohydrate feeding increases the DNL production of circulating FAs such as MA, PA, SA and POA, which have been associated with weight gain (Paillard *et al.*, 2008; Pickens *et al.*, 2015; Yerlikaya *et al.*, 2011). Therefore, as much as higher dietary fat intake is associated with an increase in obesity and MetS, the high consumption of carbohydrates should be a concern and dietary guidelines should recommend a reduction of refined carbohydrate intake.

Obesity/overweight-associated inflammation is mainly facilitated by the increased fat mass; but, it may be modulated by prolonged or acute exposure to dietary fat (Telle-Hansen *et al.*, 2017). Prior studies have shown that “healthy dietary patterns”, characterised by replacement of SFAs with PUFAs, are associated with reduced chronic low-grade inflammation, in particular, the low level of TNF α and IL-6 (Calder *et al.*, 2011). A study that compared a butter meal (14 %E from SFA) and a margarine meal (8.7 %E from PUFA of which 8.0 %E was LA) reported that the PUFA meal decreased several inflammatory markers including IL-6, TNF α , sTNFR1 and sTNFR2, and sVCAM as compared to the SFA meal (Masson & Mensink, 2011). In a cross-over study, men consumed three high-fat shakes containing SFA, MUFA or n-3 PUFAs. The MUFA and n-3 PUFA challenge, compared to the SFA challenge, induced higher plasma concentrations of IL-1 β . Also, the TNF α level was lower after the intervention in lean compared with obese and obese diabetic subjects (van Dijk *et al.*, 2012). Moreover, Bjermo *et al.* (2012) investigated the effects of a high-PUFA diet and a high-SFA diet (mostly from butter) on systemic inflammation, and metabolic disorders in a randomized study. The IL1Ra, and sTNFR2 were lower in the PUFA group as compared to the SFA group at the end of a 10-week the study. Interestingly, no group difference was found for plasma levels of CRP, IL-1 β , IL-6, and IL-10 (Bjermo *et al.*, 2012). In a different randomized study of overweight and obese women with dyslipidemia, the CRP levels decreased after 3 weeks with a high-PUFA diet compared to a high-SFA diet (Lesna *et al.*, 2013). Lesna and colleagues (2013) concluded that the substantial change of dietary FA composition may influence the pro-inflammatory effect in addition to traditional cardiovascular risk factors. Thus, it is acceptable to conclude that a healthy FA composition (high-PUFA and low-SFA) as part of a healthy dietary pattern may be of importance in reducing systemic low-grade inflammation when overweight or obese (Telle-Hansen *et al.*, 2017).

Some studies examined the relationship between consuming a healthy dietary pattern and markers of low-grade inflammation. In the Nurses’ Health Study, a “prudent pattern” was categorised by higher intakes of fruit, vegetables, legumes, fish, poultry and whole grains, and a “Western-type pattern” was characterised by higher intakes of red and processed meats, sweets, desserts, French fries and refined grains (Lopez-Garcia *et al.*, 2004). The prudent pattern was

inversely associated with plasma concentrations of CRP, whereas the Western type pattern showed a positive relationship with CRP, IL-6, sE-selectin, sICAM-1 and sVCAM-1 (Lopez-Garcia *et al.*, 2004). Using data from the Nurses' Health Study, Schulze *et al.* (2005) identified a dietary pattern that was significantly associated with increased concentrations of CRP, IL-6, sTNFR2, sE-selectin, sICAM-1 and sVCAM-1. This pattern was high in sugar-sweetened soft drinks, refined grains, diet soft drinks and processed meat but low in wine, coffee, cruciferous vegetables and yellow vegetables, and was also associated with an increased risk of diabetes (Schulze *et al.*, 2005). Hoebeeck and colleagues (2011) evaluated the relationship between adherence to food-based dietary guidelines and inflammatory markers among healthy Belgian men and women aged 35–55 years. The dietary index consisted of dietary quality, diversity and equilibrium, according to adherence to the Flemish food-based dietary guidelines. Higher dietary scores were inversely associated with IL-6 concentration in the bloodstream. Calder and colleagues compiled a comprehensive review on dietary factors and low-grade inflammation and overweight and obesity. They concluded that key protective features in the diet consist of whole grains and fibre, fruits and vegetables, fish and PUFA, and especially n-3 PUFA, vitamin C, vitamin E, and carotenoids (Calder *et al.*, 2011). They further established that the Mediterranean diet, classified as a healthy dietary pattern, is associated with decreased low-grade inflammation in both healthy and obese individuals (Calder *et al.*, 2011).

2.4.2 Diet and metabolic conditions

Diet and lifestyle play an important role in the development of MetS, and nutritional modulation may modify the development of MetS (Ilanne-Parikka *et al.*, 2008; Bo *et al.*, 2007; Oh *et al.*, 2010; Orchard *et al.*, 2005). Total fat and type of dietary fats consumed have been associated with MetS (Risérus *et al.*, 2009; Rodriguez-Monforte *et al.*, 2017). The dietary FA composition of patients with MetS is typically characterised by high intakes of SFA and low intakes of PUFA (Warensjo *et al.*, 2006b), with adverse health effects (Ebbesson *et al.*, 2007). High SFA diets, with physical inactivity, adversely affect insulin sensitivity (Stettler *et al.*, 2005). Diets high in total fat and SFAs have been shown to exacerbate the chronic inflammatory state of MetS (Mozaffarian *et al.*, 2004b). Saturated FA intake was positively associated with MetS through their effect on adiposity, serum lipids, insulin, and BP (Kabagambe *et al.*, 2008).

Although the mechanisms accountable for the development of MetS have not been fully documented, nutritional modulation, such as increasing the intake of n-3 PUFAs, may be beneficial in decreasing the storage of triglyceride and body fat, lowering BP, and improving insulin resistance (Abete *et al.*, 2011; Poudyal *et al.*, 2011). Several reviews (Carpentier *et al.*,

2006; Lottenberg *et al.*, 2012; Poudyal *et al.*, 2011) have summarised the effects and probable underlying mechanisms of action of marine-derived n-3 FA intake on the multiple risk factors for MetS. This evidence suggests that individual n-3 PUFAs may exert differential effects on some risk factors for MetS, including improving the lipid profile, BP, insulin sensitivity, and inflammation status (Carpentier *et al.*, 2006; Lee *et al.*, 2008b; Poudyal *et al.*, 2011). Increasing the proportion of n-3 LC-PUFAs intake is protective of obesity-related insulin resistance and inflammation, particularly when combined with a reduction in n-6 FAs in experimental animals (González-Pérez *et al.*, 2009; White *et al.*, 2010). A recent meta-analysis concluded that a modest inverse association between fish consumption or n-3 LC-PUFA intake and risk of MetS has been observed when combining available data from prospective cohort studies, but not from cross-sectional studies (Kim *et al.*, 2015). Favourable associations between fish or marine-derived n-3 PUFAs and MetS were also found in several cross-sectional studies (Huang *et al.*, 2010; Noel *et al.*, 2010; Zhang *et al.*, 2012). Similar results were observed in 1 689 Korean men during a four-year follow-up (Choi *et al.*, 2013).

2.5 Pattern analysis in dietary exposure

Dimension reduction techniques such as principal component analysis (PCA) and clusters have been used extensively in nutrition epidemiology to define dietary exposure (Newby & Tucker, 2004). Principal component analysis, also referred to as factor analysis, decreases data into patterns based upon inter-correlations between identified dietary items/food groups referred to as patterns or factors, whereas cluster analysis condenses data into patterns based upon individual differences in defined mean intakes (Newby & Tucker, 2004). The factor loadings, also called component loadings in principal component analysis, are the correlation coefficients between the variables and factors. Many patterns are named according to the input variable with the highest factor loading (i.e., quantitatively) (Newby & Tucker, 2004). If a pattern is classified as SFAs pattern, it means that there are more loadings of individual SFAs within the pattern as compared to other single FAs.

Treelet transform is another dimension reduction technique developed and recommended by Lee and colleagues (2008a). It integrates the quantitative pattern extraction abilities of PCA with the interpretability advantages of cluster analysis (Gorst-Rasmussen *et al.*, 2011). Appreciation of the complex interaction of single nutrients and other food components has led to the frequent use of methods assessing complete dietary intake of nutrients in relation to disease and health (Tucker, 2010).

2.5.1 Dietary patterns

Dietary patterns mostly describe dietary behaviour and also explain the relationship of diet to health outcomes (Moeller *et al.*, 2007). Dietary pattern analysis describes the overall diet; individual foods, food groups, and diverse nutrients; their combination and variety; and the regularity and quantities with which they are customarily consumed (Hu, 2002). Nutritional epidemiologists give the following reasons for investigating dietary patterns: (1) people eat meals with composite of nutrients that are likely to be interactive or synergistic, (2) several nutrients are highly interrelated, making it difficult to examine their isolated effects, (3) the effect of an individual nutrient may be insufficient to detect, but the collective effects of various nutrients incorporated in a dietary pattern may be adequate to detect, and (4) analyses of individual nutrients may be confounded by the effect of dietary patterns (Kant, 2004; Michels & Schulze, 2005; Newby & Tucker, 2004).

The most popular dietary patterns based on population studies and randomised control trials include the Mediterranean diet and the Dietary Strategies to Stop Hypertension (DASH), respectively (Appel *et al.*, 1997; Bach-Faig *et al.*, 2011). The DASH diet is a dietary pattern rich in fruits and vegetables and low-fat dairy products (Appel *et al.*, 1997). The Mediterranean dietary pattern is high in fruits, vegetables, nuts, seeds, legumes and cereals as well as fish, but is limited in meat and dairy products (Bach-Faig *et al.*, 2011). Both these dietary patterns have been shown to reduce the risk of chronic disease and lower hypertension (Appel *et al.*, 1997; Bach-Faig *et al.*, 2011). Furthermore, the Mediterranean dietary pattern has been shown to lower the risk of chronic diseases in both observational studies and randomised control trials (Appel *et al.*, 1997; Estruch *et al.*, 2013; Sofi *et al.*, 2014).

Other dietary patterns are exploratory and are specific to the population being investigated. Dietary intakes are assessed by means of the use of validated FFQ (Maghsoudi *et al.*, 2016), 24-hour dietary recall (Wagner *et al.*, 2012), three-day records (Leite & Nicolosi, 2009) and sum of 24-hour and three-day dietary recall (Kimokoti *et al.*, 2012). Dietary data are then extracted and analysed into dietary patterns using the statistical models mentioned at the outset. Several studies have investigated associations of dietary patterns with obesity (Machado Arruda *et al.*, 2016; Naja *et al.*, 2011) and MetS (Mazidi *et al.*, 2017; Xia *et al.*, 2016) as well as components of MetS (Ndanuko *et al.*, 2017; Weber *et al.*, 2016), to mention a few. The outcomes of these studies suggest that healthy dietary patterns are generally high in fruits, vegetables, fish and seafood, legumes and whole grains, nuts and seeds, poultry, as well as fat-free and low-fat dairy products. On the other hand, unhealthy dietary patterns (Western) are characterised by high meat intake,

processed meats, refined grains, sweets, desserts, fast food, snack foods, sodas, sweetened beverages and high salt foods (Naja *et al.*, 2011; Rodriguez-Monforte *et al.*, 2017; Xu *et al.*, 2016). The healthy dietary patterns are associated with favourable effects on metabolic abnormalities (Duffey *et al.*, 2012; Panagiotakos *et al.*, 2007; Xia *et al.*, 2016), and lowered T2DM risk by up to 14% (Maghsoudi *et al.*, 2016). On the contrary, unhealthy dietary patterns, characterised by high-fat foods, were associated with obesity (Kimokoti *et al.*, 2012; Naja *et al.*, 2011) and metabolic abnormalities (Duffey *et al.*, 2012; Panagiotakos *et al.*, 2007; Xia *et al.*, 2016; Xu *et al.*, 2016).

As research in dietary patterns are advancing and the indication for public health importance is reinforced, the benefits that dietary patterns offer as an approach for informing public health recommendations are increasingly being recognized (Cespedes & Hu, 2015).

2.5.2 Circulating fatty acid patterns

A few studies have applied dimension reduction statistical techniques mentioned in **section 2.5** to study FA biomarkers in obesity (Dahm, 2011), metabolic health (Li *et al.*, 2016; Warensjo *et al.*, 2006), blood pressure (Yang *et al.*, 2016a) and cardiovascular disease (Imamura *et al.*, 2012; Skidmore *et al.*, 2010), and identified various patterns characterised as SFA, MUFA, and n-3 and n-6 LC-PUFA pattern.

To the best of our knowledge, only two studies have investigated the association between FA patterns and metabolic health in adults (Li *et al.*, 2016; Warensjo *et al.*, 2006). Patterns derived by Warensjo and colleagues (2006) included a low-LA pattern (with positive loadings of POA and OA, SCD-16 and D6D), dietary SFA patterns (positively loaded with PA, SA, SCD-18) and factor 3 pattern (positively loaded with SA, DGLA, EPA and DHA, SCD-18, and D5D). Higher scores of low-LA and lower scores of factor 3 significantly predicted the development of MetS over 20 years of follow-up, independent of lifestyle factors (Warensjo *et al.*, 2006). Clusters 3 (consisted of arachidic, behenic, lignoceric acid and others) and 2 (consisted of EPA, DPAn-3 and DHA), identified by Li and colleagues (2016), were cross-sectionally associated with the healthy metabolic phenotypes. On the other hand, cluster 4 (consisting of GLA and DGLA and low in LA and other FAs) was positively associated with a higher risk of MetS in Irish adults (Li *et al.*, 2016). There were differences between these two studies. Firstly, these two studies identified different FA patterns. Secondly, Warensjo and colleagues (2006) derived their patterns from serum FA, while Li and colleagues (2016) used plasma FAs. Thirdly, Warensjo and colleagues (2006) used 11 FAs and also included estimated desaturase activities to identify patterns, while Li and his colleagues (2016) used 26 FAs and included odd-chain FAs. Lastly, they both identified a different number of FA patterns and used different statistical methods to define their patterns.

Both studies, however, defined the MetS according to the NCEP/ATPIII criteria for MetS 2001 (NCEP Panel, 2002).

Dahm *et al.* (2011), on the other hand, investigated the association of adipose tissue FA patterns with five-year changes in anthropometric indices. FA patterns with high levels of trans-FAs were inclined to be positively associated with 5-year changes in weight and WC for men and women. On the other hand, patterns with high levels of n-6 LC-PUFA tended to be protective against increases in weight and WC in men, and deleterious in women. Patterns with high loadings of MUFAs were negatively associated with linear change in WC_{BMI} in women, defined as residual values from equations of BMI and WC (Dahm, 2011). Patterns with high levels of trans-FAs could be linked to weight gain, but patterns with high n-3 LC-PUFA were not associated with changes in weight gain. These adipose tissue patterns were obtained for men and women separately and used a total of 34 FAs including trans-FAs (Dahm, 2011).

An analysis of circulating FA patterns (indicating recent diet) may explain the combined effects of multiple FAs acting simultaneously in the body (Baylin & Campos, 2006), which is not possible with studies of single FAs.

2.6 Summary of the literature review

This review of cross-sectional and longitudinal studies provided information about dietary and circulating FAs, in association with obesity and MetS. Evidence from FA research is, however, very advanced in Europe, Asia and the USA. Available data on research on dietary and circulating FAs in Africa are limited to levels of individual FAs in various conditions, but very limited pertaining to obesity and MetS.

Specific FAs, both in diet and circulation, have been associated either positively or negatively with obesity and MetS. MA, PA and SA were positively associated with obesity, MetS and CVDs, in most of the studies, while studies on VLC-SFAs have reported inverse associations with CVD risk factors. This shows that different SFAs work in opposing directions, which provides clear evidence that SFAs are not a solitary homogeneous group. MA, PA and SA have been studied extensively in both obesity and MetS, but VLC-SFAs have mostly been investigated in T2DM and CVDs. Further research is needed to understand their association with obesity, but especially in populations of Africa. It is apparent that dietary n-3 PUFAs are generally protective of obesity and MetS, while some studies indicate that particular dietary n-6 PUFAs are deleterious. This is consistent with circulating n-3 and n-6 PUFAs, but with some conflicting results in the literature. Results from various studies show that not all n-6 PUFAs are equal. For example, LA was

inversely associated with obesity and MetS in most of the studies, but a longitudinal study showed otherwise. On the other hand, DGLA and GLA were mostly positively associated with both obesity and MetS. The differences in outcomes in n-3 and n-6 PUFAs may be attributed to several factors, including geographical regions influencing dietary patterns, sample sizes, and FAs medium and also errors associated with dietary intake assessment. Consequently, better designed longitudinal studies are recommended to ascertain these differences.

Another area of research where there is a gap in the literature is FA pattern analyses both in diet and plasma. This methodology is especially useful because foods contain a combination of FAs. Research on the cumulative effect of various foods may be more useful in setting priorities to change dietary patterns in populations through public health initiatives. Dietary pattern study outcomes may be especially useful in providing evidence towards the development of national food-based dietary guidelines.

Despite advanced research in FA and health, a lack of data in black Africans presents a challenge in making recommendations to support policies that are targeted at the prevention of obesity and related risk factors in our population. Further high-quality research inclusive of pattern analysis of dietary and circulating FAs, as well as estimated desaturases activity, is required to better understand the potential role of SFAs, MUFAs, n-3 and n-6 PUFAs in the development and consequences of obesity and MetS specific to black Africans. The evidence from these studies may support policies that exist on fat recommendations or provide a new direction to prevent obesity and related risk factors.

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CHAPTER 3 RESEARCH METHODOLOGY

3.1 Introduction

This PhD study is an affiliated study under a larger study. This is because data from the South African leg of the PURE study were used in this affiliated study. This chapter on research methodology describes (1) the PURE research methodology, and (2) the PhD study research methodology.

3.2 SECTION 1: RESEARCH METHODOLOGY OF THE PURE STUDY

3.2.1 Study design

The PURE study is a large-scale multi-national cohort study, coordinated by the Population Health Research Institute (PHRI) in Ontario, Canada. It is aimed at addressing the general health implications of nutritional transitions in 17 developing and developed countries worldwide (Teo *et al.*, 2009; Dehghan, 2017). In the current analysis, the data collected at baseline (2005), as well as the ten-year follow-up collections (2015), were used to examine the dietary intake, dietary FA and plasma phospholipid FA status in association with obesity-related outcomes in black South Africans in the North West Province. The information discussed in the methodology section of this protocol was limited to the parameters that were used for the proposed PhD study titled “**Dietary and plasma fatty acids in association with obesity in black South Africans**”. The data has been collected and analyses performed.

3.2.2 Study setting

The South African leg of the PURE study is set in rural and urban African communities in the North West Province of South Africa. The participants were recruited accordingly: urban households were selected from two communities in Ikageng, Potchefstroom, and rural households were selected from two communities in the Ganyesa District. Maps of all communities were used to divide the communities into wards and a local fieldworker was allocated to each ward to select a number of households in proportion to the number of houses in the community. All measurements of the urban participants during 2005, 2010 and 2015 were performed at the metabolic unit of the North-West University, Potchefstroom with excellent infrastructure to draw blood, perform interviews and measure body composition. Measurements of the rural participants were done in a field laboratory in Ganyesa in rural North West Province.

3.2.3 PURE study population and sampling

3.2.3.1 Participant recruitment: 2005

Recruitment was performed by stratified random selection of 6000 houses from maps of the four communities by trained fieldworkers. Specific eligibility criteria were used to make a paper selection from the data obtained from the 6000 households. One thousand participants from each community were selected, resulting in 2000 participants from the rural community and 2 000 from the urban community, totalling to 4000 participants. In 2005, from the 4000 identified individuals, a total of 2010 (1006 rural and 1004 urban) apparently healthy participants turned up for measurements, provided informed consent and were enrolled for participation in the study.

3.2.3.1.1 Eligibility criteria

From the data obtained from the census, a paper-based selection of possible subjects was made based on the following inclusion and exclusion criteria: For inclusion in the study, participants had to reside in the approached households, be apparently healthy volunteers of either sex and be older than 30 years.

Inclusion criteria:

- ≥ 30 years of age
- ≤ 70 years of age
- Males and females
- No reported diseases of lifestyle, acute TB or HIV
- Not using any chronic medication
- Eligible participants have to reside in the households
- Consented

Exclusion criteria:

- < 30 years of age
- > 70 years of age

- Using chronic medication (except hypertension and T2DM)
- Existing known chronic conditions (except hypertension and T2DM)
- Did not sign the informed consent
- Pregnant or lactating
- Known tuberculosis and confirmed HIV infection. The non-consenters were included and their status was indicated as unknown, but this was a small number of participants (n=11).

The South African PURE study was planned to run over a period of 10 years, and all participants who planned to move away from the study area in the near future were also excluded.

3.2.3.2 Role of the mediators

The fieldworkers acted as the mediators between the research team and the participants and the life within the communities involved in the study. They fulfilled an important role; not only did they track the participants enrolled in the study, but they also acted as advisors and offered support to the participants regarding basic health issues and health education.

3.2.3.3 Goodwill permission

The principal investigator (PI) of the PURE study made appointments with the mayors of both Potchefstroom and Ganyesa. During the meetings, the PI fully informed them about the aims of the study and the possible outcomes and benefits of the study. The research procedures were also explained, and permission was granted to proceed with the planned study.

After permission was obtained from the mayors, the Inkosis (tribal chief) of the rural communities in Ganyesa and the community leaders in the urban areas (acting as the gatekeepers for each community) were approached. Verbal permission was obtained from the gatekeepers to perform the study within their communities. This was done again prior to the 2015 follow-up study as an act of respect.

3.2.4 PURE study population

In 2005, a total of 2792 were recruited to participate in the study. Participants were excluded due to unavailability during blood collections despite previously indicating that they would be available, and acute illness on the day of assessments, such as a cold. The final number of 2010, apparently

healthy participants were enrolled and participated in the study. They were 1260 women and 750 men from both rural (n=1006) and urban (n=1004) communities. The first follow-up measurements of the PURE study were done in 2010. There were 1284 (440 men and 844 women). The decrease in the number of participants from 2005 to 2010 was due to various reasons: 1) participants' death, 2) participants moved away outside the study area, 3) participants refused to participate or have a blood sample taken, 4) participants failed to show up on the day of blood collection, 5) participants withdrew from the study, and 6) participants were lost to follow-up. In 2015, there were 924 participants.

3.2.5 Study procedures

3.2.5.1 The process of obtaining informed consent for PURE participants

Fieldworkers were recruited from the communities being researched. They were systematically trained to perform well-outlined tasks such as: completing questionnaires and consent forms and assisting with the recruitment of the participants. They were supervised by the research team. The fieldworkers spoke the languages of the participants, namely Afrikaans, Setswana and English. All questionnaires were translated into these languages. The fieldworkers performed house visits to every participant, at least three months before the commencement of the study. Participants gave pre-consent; then, they signed the main consent form. Consent was confirmed upon arrival at the study site for the data collection. Additional informed consent was obtained prior to testing for HIV. The fieldworkers ensured that the participant knew and understood that he/she was not to consume food or drink (except water) from 22.00pm on the evening prior to his/her appointment for the data collection and therefore arrive at the study site in a fasting state.

3.2.5.1.1 HIV consent and testing

HIV testing was performed if the participants consented. Pre-test counselling was offered in groups of 10, and during the process of obtaining written informed consent for the HIV test from the participant, the participant was asked whether or not they would like their status to be disclosed to them. Individual participants who chose to know their HIV status received individual post-test counselling upon receiving their results. If a subject tested positive, the test result was confirmed with follow-up tests. Feedback on results including HIV status was given by trained counsellors during individual sessions. Participants with a positive HIV test were given a referral letter to their nearest clinic for a confirmation CD4 test and treatment. The non-consenters were included in the study, and their status was indicated as unknown, but this was a small number of participants. Participants were not excluded if HIV positive because they could be stigmatised.

Their status was recorded in the database despite initial exclusion criteria. The total exclusion of apparently healthy HIV positive people would result in a non-representative sample because around 12% of the SA adult population was HIV positive in 2005.

The HIV status was also checked at follow-up and recorded. They were included in the data base in order to be more representative of the SA adult population.

3.2.5.2 The process of data collection from PURE participants

All of the measurements were performed, and questionnaires were administered by experienced members of the research team.

During each data collection, consent was reconfirmed and the fact that the participant was participating entirely voluntary and could withdraw from the study at any time, without being penalised in any way, was emphasised. As soon as written informed consent was confirmed, the tympanic temperature (ear temperature) was measured and each participant gave a blood sample (33ml). The same measurements were repeated in 2015.

Thereafter, a light breakfast was served followed by data collection at the following stations (in no specific order):

- Cardiovascular measurements
- Blood pressure – a non-invasive technique
- Physical measurements
- Anthropometry – Height, weight, waist circumference

Validated questionnaires and time necessary to complete:

- Household questionnaire: 30 min
- Adult participant questionnaire: 60 min
- Physical Activity Index (PAI) questionnaire: 10 min
- International Physical Activity Questionnaire (IPAQ): 10 min
- Quantitative Food Frequency Questionnaire (QFFQ): 45 min
- Patient Health Questionnaire (PHQ-9) 5 min
- Information regarding medication usage: 5 min

3.2.5.3 Precautions to ensure the participants were comfortable:

- (i) Participants were provided with breakfast and lunch. Clean and safe drinking water and juice were also available in dispensers in the data collection area where participants accessed it freely at any time they needed, but after blood collection.
- (ii) Research assistants were available to show the participants around and guide them through the data collection points.
- (iii) Clean toilets and bathrooms were clearly marked and running water, soap and tissue paper were provided for their use.
- (iv) At the point where they had their blood taken, all precautions were taken to ensure the safety of both the researcher and the participants. The participants were informed about the procedure before they gave their permission for the blood to be drawn.

3.2.6 Research tools

Trained fieldworkers administered all questionnaires. To ensure that the administration of all questionnaires was standardised, the fieldworkers were trained extensively by a group of experts. The adult questionnaire was completed by the trained fieldworkers who were also responsible for tracking the participants, conducting the house visits and obtaining informed consent. The QFFQ was completed by fieldworkers from the participating communities in Potchefstroom and Ganyesa, who were specifically trained to administer dietary questionnaires.

3.2.6.1 Adult questionnaire

An extensive standardised interviewer-administered adult questionnaire was completed for each participant in his/her preferred language (Afrikaans, Setswana or English). Fieldworkers received instructions on how to administer and complete the questionnaires. The first part of the questionnaire was set to obtain information about gender, ethnicity, age, marital status, the level of education, occupation and employment status. The next set of questions investigated the socio-economic status and background, lifestyle practices, physiological health and support of the participants. In 2015, data collection questions regarding the current habits (such as the use of tobacco, alcohol and medication) and current physical activity and health status (particularly occurrence of new illnesses since 2005) were included.

3.2.6.2 Dietary questionnaire

Dietary data were collected using a culture-sensitive QFFQ (135 food items with additional elaboration of preparation details and different varieties where appropriate for each; e.g, for carrots, there were 13 additional sub-questions related to different ways carrots were prepared in these different communities), which was developed and validated for the population in the Transition, Health and Urbanization in South Africa (THUSA) project (MacIntyre *et al.*, 2001b) and for which reproducibility was proven (MacIntyre *et al.*, 2001a). The QFFQ recorded foods eaten over the previous 30 days (Wentzel-Viljoen *et al.*, 2011). A photograph book specifically developed for use in the African population in the North West Province was used to estimate portion sizes (Venter *et al.*, 2000) and reported using household measures. Thereafter, standard tables were used to convert dietary information to weights (Langenhoven, 1991). Nutrient intakes were calculated using the South African Food Composition Database System of the South African Medical Research Council (SA-MRC) (Wolmarans *et al.*, 2010). It was analysed to obtain the nutritional intake of the participants in 2005. Quality control of the QFFQs was done by a registered dietician during the data collection at the research facility to ensure that all QFFQs were administered correctly, thereby ensuring the quality of the data gathered.

3.2.6.3 Physical activity questionnaire

A validated physical activity questionnaire developed specifically for use in this South African population was used to determine physical activity levels of the Setswana-speaking people of the North West Province (Kruger *et al.*, 2000; Kruger *et al.*, 2002). The questionnaire reported physical activity in the form of commuting, stair climbing, sports participation, occupational activity and leisure time activity and the total physical activity index was calculated by combining the indices mentioned above. A physical activity index score of 1 to 3.33 was classified as low, 3.34 to 6.67 as moderate and >6.67 as high (Kruger *et al.*, 2002). Trained Biokinetics students collected the physical activity data in the participant's local language.

3.2.7 Anthropometric, blood pressure and biochemical measurements

3.2.7.1 Anthropometric measurements

All measurements were done by trained postgraduate Biokinetics or Nutrition students with the participants in minimum clothing in private rooms. Measurements were done according to standard as prescribed by the International Society for the Advancement of Kinanthropometry methods (Marfell-Jones *et al.*, 2012). For this affiliated PhD study, the following anthropometric

measurements were used: weight, height, hip circumference, WC and body fat percentage. The BMI, WHR and WHtR were calculated.

BMI was calculated using $(Wt \text{ (kg)}/ht \text{ (m}^2\text{)})$

Waist: hip ratio was calculated by $(WC \text{ (cm)}/Hip \text{ circumference (cm)})$

Waist: height ratio was calculated by $(Waist \text{ (cm) } /height \text{ (cm)})$

3.2.7.1.1 Weight

A portable electronic scale (Precision health scale, A&D Company, Tokyo, Japan) was used to measure the weight of the participants in kilogrammes. Before taking each measurement, the scale was secured in a stable horizontal position on the floor and reading “0” (zero). Standard 10kg weights were used to calibrate the scales on a daily basis. Measurements were taken barefoot, with arms hanging freely at the sides and with minimal clothing.

3.2.7.1.2 Height

Height was measured using a calibrated stadiometer (Seca, Humburg, Germany) with the participants standing in an upright position, arms hanging freely at the sides, relaxed shoulders, legs straight and knees together. Participants were barefoot, standing with their feet flat and heels together. The following body parts were in contact with the stadiometer: shoulder blades, buttocks and heels. The participant faced forward with their head held in the horizontal Frankfort plane. Height was recorded to the nearest 0.1 cm after the participant inhaled fully and maintained the erect position without altering the load on the heels. Weight and height were used to calculate the BMI of participants ($BMI = \text{weight (in kg) divided by height}^2 \text{ (in m)}$).

3.2.7.1.3 Waist circumference

Waist and hip circumferences were measured using steel tapes (Lufkin, Apex, NC, USA) attached to a spring balance exerting a force of 750g. Participants were required to stand upright with both arms at the sides, and the abdomen relaxed. The measurement was taken over an unclothed abdomen at the smallest diameter between the iliac crest (the hip) and the costal margin, with the tape kept in a horizontal line. Measurements were recorded to the closest 0.1 cm at the end of normal expiration.

3.2.7.2 Blood pressure

Blood pressure measurements were taken by members of the Hypertension in Africa research team of the Potchefstroom Campus of the North-West University, using the Omron automatic digital pressure monitor (Omron HEM-757, Kyoto, Japan). All participants had to sit for at least five minutes before the commencement of BP measurements. Additionally, they should not have smoked or eaten at least 30 minutes and should not have climbed stairs at least 15 to 30 minutes prior to the measurements being taken. All participants were seated upright and relaxed with his/her arm supported at heart level using the brachial artery. Both systolic and diastolic BP readings were recorded twice. This same procedure was repeated during the 2015 data collection.

3.2.7.3 Biochemical tests and analysis

The biochemical analysis discussed in this section is limited to be parameters that were used specifically for this affiliated study. All blood samples for 2005 and 2015 have been analysed. The same biochemical analysis procedures were used in 2005 and 2015. All safety precautions were taken as described in the PURE protocol and the Centre of Excellence for Nutrition laboratory manual.

3.2.7.3.1 Plasma sampling and storage

Fasting blood samples, with minimal stasis, were collected by a registered nurse with a sterile winged infusion set from the antecubital vein and finally stored at -80°C. Blood samples were immediately stored in ice-filled insulated containers and centrifuged, aliquoted and stored at -20°C within two hours of collection. Samples were then transferred to centralised long-term storage at -80 °C. In the rural areas, samples were rapidly frozen and stored at -18 °C (no longer than 5 days) until they could be transported to the laboratory facility and then stored at -80 °C. Each collection tube was filled to maximum capacity to ensure optimal blood: anticoagulant ratio and gently inverted five times to ensure thorough mixing of contents. Blood intended for investigating the serum lipid profile was collected in tubes without anticoagulants and was allowed to clot at room temperature. Fluoride tubes were used to collect blood intended to determine plasma glucose, samples for glycated haemoglobin test, commonly referred to as HbA_{1C}, were collected in ethylene diamine tetra-acetic acid (EDTA) tubes, and for insulin, CRP and IL-6 were collected in serum tubes. Between blood collection and processing, the tubes were stored on ice, while serum tubes without anticoagulant were left to clot for 30 minutes at room temperature.

Blood was centrifuged at 2 000g for 15 minutes at 10 °C. These same methodologies were used for the 2005 and 2015 samples.

3.2.7.3.2 Measurement of serum cholesterol fractions

The analysis of TC, HDL-C and triglycerides was performed at the North-West University, Potchefstroom. Quantitative determination of HDL-C, triglycerides and TC was performed using Sequential Multiple Analyser Computer (SMAC), using the KonelabTM auto analyser (Thermo Fisher Scientific Oy, Vantaa, Finland) (Pieters *et al.*, 2011), a clinical chemistry analyser for colorimetric, immuno turbid metric and ion-selective electrode methods. Low-density lipoprotein cholesterol was calculated using the Friedewald equation (Friedewald *et al.*, 1972). Calculation using the formula is a good estimation if the participant is fasted and their triglycerides are below (400 mg.dL⁻¹):

Equation: $HDL = C - L - kT$ where:

H	=	HDL-C
L	=	LDL
C	=	TC
k	=	0.20 (if H, C, L and T are measured in mg/dL ⁻¹) 0.45 (if H, C, L and T are measured in mmol/L ⁻¹)
T	=	triglycerides

3.2.7.3.3 Fasting plasma glucose

Plasma glucose concentration was determined by the hexokinase method using the Synchron® System (Beckman Coulter Co., Fullerton, CA, USA) and reagents. Samples and reagents were divided into cuvettes with a ratio of one-part sample to 100 parts reagent. Change in absorbance was relative to the glucose concentration of the reagent in the sample, monitored at 340 nm. Plasma glucose concentration was calculated and expressed using the change in absorbance.

3.2.7.3.4 Serum liver function tests

All serum liver function test concentrations were measured on a sequential multiple analyser computer (SMAC), using particle-enhanced immunoturbidimetric assay tests and were analysed using two sequential multiple analysers (Konelab 20i, Thermo Scientific, Vantaa, Finland in 2005; and Cobas Integra 400 Plus, Roche, Switzerland in 2010). Methods for specific liver enzymes are outlined below.

3.2.7.3.5 Alanine aminotransferase and aspartate aminotransferase

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the International Federation of Clinical Chemistry, without pyridoxal-5'-phosphate (Bergmeyer HU *et al.*, 1986; ECCLS, 1989).

3.2.7.3.6 γ -Glutamyltransferase

γ -Glutamyltransferase (GGT) was measured using enzymatic colorimetric assay (Bergmeyer HU *et al.*, 1986).

3.2.7.3.7 Kidney function

The creatinine clearance was analysed by means of the measurement of serum creatinine. This enzymatic colorimetric assay of serum creatinine is performed using the COBAS INTEGRA 400 plus analyser. Creatinine clearance is calculated using the Cockcroft-Gault formula: $= [(140 - \text{age}) * (\text{weight in kg}) * (0.85 \text{ if female})] / (72 * \text{creatinine in } \mu\text{mol/l})$.

3.2.7.3.8 HIV test

HIV status was determined according to the protocol of the South African Department of Health by using the First Response rapid HIV card test (PMC Limited, Daman, India).

After pre-test counselling, registered nurses performed the rapid HIV test, using the First Response HIV card test (PMC Medical, India). If tested positive, the test result was confirmed with the SD Bioline HIV 1/2 3.0 card test (Standard Diagnostics, INC, Korea). Counsellors provided individual counselling on the HIV results, and HIV-positive men and women were referred for a confirmation CD4 test and treatment.

3.2.8 Data entry and management

Data entry was done by qualified data entry clerks/captors in 2005. Data were coded before entry to ensure uniformity. The data clerk was trained on how to enter data. The research team checked entered data to ensure uniformity between the data entry clerks. The different sections of the data, e.g. anthropometric measurements and dietary intake data, were coded and entered by one person. Quality control of the QFFQs was done by a registered dietician during the data collection at the research facility as mentioned earlier.

All data entry was done in Microsoft Excel 2010, cleaned and exported to SPSS (version 23.0).

3.2.8.1 Access and management of data

Hard copies of all the data collected are locked in the PURE study office, and electronic data is archived in a password protected computer and backed up on an external hard drive.

3.2.9 Ethical considerations

3.2.9.1 Ethical approval

Ethical approval for the original PURE study was obtained from the Ethics Committee of the North-West University for the period January 2005 to December 2009 (ethics number 04M10) as well as for the period 2010 (ethical no NWU-0016-10-A1), January 2015 (ethics number NWU-0016-10-A1). These three ethical approval certificates are included in the annexures. An extension of the current ethics approval was obtained from the Health Research Ethics Committee (HREC) of the Faculty of Health Sciences from the North-West University until December 2020 (ethics number NWU-00016-10-A1). This HREC is registered at the National Health Research Ethics Council (NHREC) of South Africa.

3.2.9.2 Goodwill permission

The principal investigator (PI) of PURE made appointments with the mayors of both Potchefstroom and Ganyesa. During the meetings, the PI fully informed them about the aims of the study and the possible outcomes and benefits of the study. The research procedures were also explained, and permission was obtained to proceed with the planned study. After permission was obtained from the mayors, the Inkosis (tribal chief) of the rural communities in Ganyesa and the community leaders in the urban areas (acting as the gatekeepers for each community) were approached. Verbal permission was obtained from the gatekeepers to perform the study within their communities. This was done again prior to the 2015 follow-up study as an act of respect.

3.2.9.3 Participants' written informed consent

It is a legal obligation to attain written informed consent from participants before participating in any research. Since this is a longitudinal study and research is on-going, the fieldworkers obtained re-consent from all current active participants prior to the follow-up study visit. The fieldworkers (mediators) performed house visits to each individual, at least three (3) months prior to the start of the study. During these visits, they informed the participants about the next follow-up study. All of the procedures/measurements that were performed on the study day were discussed and explained in full to each individual. The participant was also given an opportunity to ask questions.

This process was done in the participant's preferred language (Setswana, English or Afrikaans). A repeat visit was done one month prior to the start of the study. During this visit, the fieldworkers fully informed the participant about the study and gave them another opportunity to ask questions. The participants were given one week to consider further participation in the study. After a week, the fieldworker went back to the participant, and if the participant agreed to participate in the study, the re-consent was confirmed and finally signed at the study site. In the case where a participant was illiterate, the right thumbprint was taken as a substitute for a signature.

The subjects were reminded that participation is completely voluntary and that they could withdraw from the study at any point before data analyses without any consequences or being penalised in any way. The fieldworkers scheduled an appointment, which suited the participant, and logistical arrangements were made. The day before the participant was scheduled to arrive at the research facility, the fieldworker performed one last house visit, to make final arrangements with the participants regarding pick-up time and to remind and explain to them to bring along all necessary documentation. The fieldworkers were once more reminded that the participants were to be in a fasting state and have to ensure that they understood that this entailed that they should not eat or drink anything from 22:00 pm onwards, except water.

On the day of the study, upon arrival at the study site, a senior researcher collected the signed written informed consent forms and again reminded the participants that participation is completely voluntary and that they could withdraw at any time (up to data analyses) without any consequences. The fieldworker, as well as the researcher, made sure that the participant understood everything before they confirmed and signed the written informed consent.

In the case where a participant refused in the past to further participate or in the event where a participant moved out of the community and recently returned, these participants were also visited again to re-invite them back to the study. If they were willing to participate/return to the study, they were fully briefed about the upcoming study and re-consent was obtained by the fieldworker.

3.2.9.4 Expertise, skills and legal competencies

All of the researchers and assistants (nurses, anthropometrists, counsellors, students) who are part of this study are experienced in their fields. Data collection and completion of questionnaires of all staff members were standardised prior to the study. Physical and anthropometric measures were gathered by staff using standardised protocols. Key staff (principal investigator, coordinator, and nutritionists) attended initial training. They, in turn, trained local staff. These staff were trained and tested on 'mock' participants in order to ensure that measured values between interviewer

and a local certified 'expert' (supervisor) were within an acceptable range. If differences between interviewer and supervisor variation were unacceptable, the staff member was re-trained. The fieldworkers were re-trained intensively every time prior to any form of data collection, among other things, all the procedures of the study, in order to give the participants a comprehensive overview of what was expected of them on the day of the study. The fieldworkers were also responsible for completing the following questionnaires associated specifically with this affiliated study on the data collection days: 1) Household questionnaire, 2) Adult questionnaire, and 3) Physical activity questionnaire. They were intensively trained beforehand on how to complete these questionnaires.

The anthropometric measurements were performed by Nutrition and Biokinetics students. They were trained on how to take different measurements including, but not limited to weight, height, waist circumference and skin folds. They also learned how to use the scales, height metre, skinfold callipers and how to standardise them to ensure reporting of correct results. Blood samples were collected by professional registered nurses, with experience in collecting blood samples for research purposes, minimising risk/harm to the participants and themselves.

3.2.9.5 Privacy of participants

Privacy of the participants was ensured by using private rooms and dedicated areas that were closed off from the general research area. In the case where a measurement required the participant to partially undress, it was ensured that there were two people present (researcher and an assistant) in the room or dedicated area. The researcher and assistant made sure that the participant was comfortable with the gender of the person taking the measurements. During the completion of questionnaires, only the fieldworker and/or assistant were present. Any other members of the research team or participants were not allowed within these dedicated areas. Privacy was guaranteed by ensuring that the conversation took place only between the HIV participant and trained counsellor.

3.2.9.6 Confidentiality

Confidentiality pertains to how information is treated after it has been disclosed to the researcher, whether it is verbal information or physical information. Each participant was assigned a unique participant number during the initial baseline data collection in 2005. This unique number was used in all stages of data gathering up to 2015. Upon arrival on the day of the study, the participant was to produce his/her South African identity document, in order to verify the identification number against his/her unique participant number. This was done only by the project coordinators. No

other researcher and/or assistant had access to this information or were involved in this process. Participants were not required to provide their personal information to the project coordinators, except in some cases where the date of birth was required for input into research apparatus. All of the data captured used the unique participant number. The data that were electronically captured were handed to the PI of the study, and he/she matched the new data with existing data in the master dataset.

3.2.10 Research facilities

3.2.10.1 Urban facilities

Participants were collected on a pre-arranged date at a central point within the community by a taxi and brought to the Metabolic Unit at the North-West University. The Metabolic Unit has several private rooms where all the stations were set up for measurements. Some of the measurements were performed at the Campus' Healthcare Centre as well as at the Hypertension Clinic, all with available private rooms.

3.2.10.2 Rural facilities

Participants were collected on a pre-arranged date at a central point within the community by a taxi and brought to the research facility situated at Setlhare Lodge, which is located within the rural community. Tents and gazebos were erected on the premises of Setlhare Lodge and served as the research facility. Each researcher received a dedicated area, which was closed off and privately set up to perform measurements.

Sensitive measurements that required participants to remove the top layers of clothing, such as anthropometry, body composition, ECG, and pulse wave velocity were performed within dedicated closed-off areas in the lodge's seminar room, which was closed off to ensure privacy.

The HIV counsellors also had a private room that was secluded from the rest of the research area to ensure complete privacy for pre- and post-counselling.

3.2.11 Benefits

3.2.11.1 Direct benefits

Participants taking part in the study directly benefited from feedback, which was given to them, regarding results that were immediately available. This included:

- Blood pressure
- Blood glucose levels
- HIV status (performed by professional counsellors)
- Anthropometry

Any abnormalities that were observed during the measurements were communicated to the participants through the lead researcher. These participants were provided with a referral letter that they presented to the nearest hospital or local clinic for medical attention. The PI visited the local hospital and clinics prior to the follow-up study and informed them to make pre-arrangements for possible referrals from the study participants.

3.2.11.2 Indirect benefits

Through participating in the PURE study, the participants help to provide the government of South Africa with a direct estimate of the health/disease burden attributable to established and emerging risk factors for obesity, diabetes and CVD.

Recent research suggests that there is a need to consider the quality as well as the quantity of fat in the diets of a population (Smuts & Wolmarans, 2013). Understanding obesity and related risk factors and outcomes related to fat intake will be useful in making recommendations to develop policies that significantly contribute to outcomes in its prevention, especially in South Africa.

3.2.12 Dangers/risks/discomforts and precautions

3.2.12.1 The risks associated with the PURE study

As with any research involving human participants, there is always some form of risk involved. Although most of the techniques and procedures that were used during the PURE study are non-invasive, there are some discomforts that might have been experienced during some of the measurements:

- (i) Participants were asked to give a blood sample (which is an invasive procedure), which might cause some discomfort or anxiety. As a precaution to the latter mentioned and to minimise risks of possible injuries to the participants as well as staff members, we made use of professional and registered nurses to collect blood samples. The nurse explained every step of the blood collection procedure to the participant as they went along, in order to put them at ease.

- (ii) During some of the measurements (anthropometry, body composition, ECG, and pulse wave velocity), the participants were requested to remove the top layer of their clothes (remaining only in their underwear or light clothing) in order for the researcher to conduct the necessary measurements. This made the participant feel uncomfortable. In order to minimise any discomfort, the areas dedicated to these measurements were closed off and private, and only the researchers and/or assistant were present.
- (iii) Participants arrived at the research facility in a fasting state for blood samples to be collected. Being in a fasting state might make the participant feel uncomfortable or perhaps light-headed. A light breakfast was provided to them as soon as the blood sample was collected to relieve their fasting state.
- (iv) All of the measurements were to take approximately a whole day, which might have been very exhaustive for some participants. In order to minimise their discomfort, participants were provided with lunch as well as tea/coffee/juice/water throughout the duration of the day at the seating/waiting area.
- (v) After finishing at a measurement station, research assistants helped the participants along to the next station to avoid them getting lost or not knowing where to go and consequently wasting their time and energy.

HIV testing was performed on the day of the study (with the participant's consent). Being newly diagnosed with a chronic disease such as HIV might cause some anxiety within some participants. Participants might also refuse to have their status disclosed to them due to them being in denial. As precautionary steps, a registered HIV counsellor performed pre-counselling on the participants as a group. Professional counsellors were to perform the testing. After each testing, regardless of the outcome, post-counselling was also performed on an individual level. During the post-counselling session, the counsellor provided necessary information regarding HIV/AIDS and support to each participant. In the event where participants agree to have their status disclosed to them, they also received a referral letter to their nearest clinic or hospital for further treatment. The non-consenters were included in the study and those who tested HIV positive were not excluded from the study because they could be stigmatised. The HIV status was also checked at follow-up and the same procedures of pre and post-test counselling and referrals was repeated.

3.2.12.2 Incentive and reimbursement for PURE participants

Participants were convened by transport provided by the research study (North-West University), and therefore no travel expenses were incurred by them.

Participants were required to arrive in a fasting state (at least 10 hours) at the research facility in order to provide a fasting blood sample. After the blood sample was collected, a breakfast was provided to the participants. Furthermore, in order to complete all of the measurements and questionnaires, participants spent approximately the whole day at the research facility. Lunch was provided, and tea/coffee/juice and water were available for their consumption throughout the day.

Participants were also financially compensated to the amount of R100.00 for any expenses or loss of income incurred due to their participation in the study. They were also provided with funds if they were referred to the local hospital/clinic.

If a participant was in full-time employment, their employer was contacted, and employment was confirmed. Daily remuneration was also confirmed with the employer, and the participant was compensated for the loss of income for their attendance at the study. Only one day was compensated.

3.2.13 Access to privileged data/information

Participants were asked to bring any medication that they were using for any chronic or non-chronic conditions as well as their clinic books and/or any other medical records within their possession to confirm any reported medical/health events (such as heart attack, stroke, asthma attack, injury etc.) with them when they came to the study site. Participants provided written informed consent in order for researchers to access this privileged information. Furthermore, in the case of HIV-infected participants, access to hospital records/patient files was also requested to obtain information regarding the history of the type of antiretroviral therapy (ART) used, year of diagnosis, years since diagnosis, history of CD4 counts, viral load and history of renal function. This because, participants who tested HIV positive post- recruitment, were included in the data base and hence the need for confidentiality.

Prior to access to this privileged information, written informed consent was obtained from the participant, and additional permission from the hospitals and/or clinics' supervisors was obtained.

There is a possibility of risk associated with loss of confidentiality if someone is able to access the database. The precautions that are in place to overcome this are that all data in the database are linked to a participant number only, with no identifying information, such as name or address.

3.2.14 Announcements of results

Measurements that are immediately available on the day of the study were reported back, verbally and individually. This took place privately, and they were given a copy of their results, which included the following:

- Blood pressure
- Pulse wave velocity
- ECG
- Intima-media thickness
- HIV status
- Anthropometry

The information was discussed and explained to them by the lead researcher of the day. If any abnormalities were present, it was discussed with the participant, and they were referred to the nearest hospital or local clinic. Feedback was also given to the North West Department of Health in the form of an official report regarding the disease burden within these communities.

Results of the proposed study are published in a PhD thesis; thereafter, the results are published in peer-reviewed academic journals, conference proceedings and health reports.

3.2.15 Storage and archiving of data

3.2.15.1 Hard copy data

All hard copies are stored in a locked office within the Africa Unit for Transdisciplinary Health Research at the North-West University (Potchefstroom Campus). Since the hard copies contained identifiable data (personal information) of each participant, strict control over the hard copies was applied. Therefore, only the PI and the co-research team had access to the hard copies. The hard copies are securely stored for up to five years after the study is completed. All hard copies of the questionnaires were destroyed according to the North-West University's rules and regulations for data /record management. The PhD student for this proposed affiliated study did not handle the hard copies of files.

3.2.15.2 Electronic data

Electronically captured data are stored on a central computer and password protected. The dataset is only accessible by the PI. For research and dissemination purposes, data are made available to other team members (researchers) via the PI upon request after proof of ethics approval is submitted. Data provided to the PhD student contained no personal information regarding the participants (de-identified dataset), and therefore researchers are not able to identify any participants based on the data received.

All electronic data are backed up on an external hard drive, which is locked up in a cupboard within a locked office at AUTHeR at the North-West University.

It is important to note that this is an international study, which includes 17 countries. PURE is supervised by the PHRI, Hamilton, Canada, under the administration and supervision of Prof Salim Yusuf. The PURE South Africa study PI/team is under contractual agreement to send data to PHRI on an annual basis. As a research institute, PHRI complies and accords to all research ethics to ensure data are handled privately, securely and confidentially.

Furthermore, no data were accessed by any person unless permission was granted by the PI/project leader, and even then only the necessary data are provided.

3.2.15.3 Sample storage and analyses

Blood and urine samples collected up to 31 December 2015 were stored for further analyses. These analyses only took place as financial funding became available. Samples were sent to laboratories outside of South Africa for analyses if expertise and/or equipment were not locally available. This is, however, done with HREC approval as well as the relevant permits from the Department of Health and only if the participants had indicated on their written informed consent form that their samples may be sent overseas. Samples are stored for an unspecified period of time. To ensure the integrity of the samples while in storage, blood and urine samples are stored at -80°C in a secure lockable bio-freezer until the determination of the variables examined for the PURE study. Access to these bio-freezers was limited to key personnel. These bio-freezers are monitored via a cellular-based monitoring system. Sample integrity is furthermore maintained via the connection of the freezers to uninterrupted power supplies and centralised power generators.

3.2.15.4 Declaration

It was declared that no participant was to be re-approached after 31 December 2015 to collect new additional data under the newly extended ethical approval. Only data collected up to 31 December 2015 were used for primary and secondary analyses. Primary and secondary analyses for this application were in line with the original scope and aims of the PURE study as stipulated within the affiliated study application.

3.2.16 Financial resources

The budget for major data collection of the PURE 2015 was sponsored by South Africa Sugar Association. The training of the fieldworkers and QFFQ data collection and coding, accommodation for the file research assistants collecting QFFQ data and plasma phospholipid FA profile tests were sponsored by Unilever. Contracts were signed between CEN and the two entities mentioned above.

3.3 SECTION 2: RESEARCH METHODOLOGY OF THE AFFILIATED STUDY

3.3.1 Title of the study

Dietary and plasma fatty acids in association with obesity in black South Africans

3.3.2 Study design

This study had three publications with different designs:

First manuscript: Cross-sectional study (2005)

Second manuscript: Prospective study (2005-2015)

Third manuscript: Cross-sectional study (2005)

3.3.3 Study population and sampling

A random sample of 711 participants was selected from the original 2010 volunteers with both dietary and plasma phospholipid FAs after exclusion of 305 participants with human immunodeficiency virus controls due to previous use of plasma for analysis. The number of participants was randomly selected from stratified groups (rural male, rural female, urban male, and urban female). The sample included 125 rural men, 148 urban men, 218 rural women and 220 urban women. Random sampling was necessary to select a smaller sample, because plasma phospholipid FA analysis is expensive, and we did not have sufficient funding to analyse all samples.

The required sample size for these research questions was not based on power calculations, but a review of similar studies showed that although some national studies included 3 000 to 6 000 participants, smaller regional studies included 343 to 775 participants (Rey-López *et al.*, 2014), and this was sufficient to answer the research question. The sample size limited us from carrying out specific sex-specific analysis to determine the association of the studied FAs and patterns in association with obesity-related parameters and MetS.

3.3.4 Data procedure

Two different datasets from the PURE study were used in the proposed PhD study. Secondary data analysis was performed on the baseline data collected in 2005 and at ten-year follow-up (2015) data.

3.3.5 Research tools

Research tools used in this study are described in the section 1 of the PURE research methodology under section 6.2.4.

3.3.6 Ethical considerations

3.3.6.1 Ethical approval

Ethics approval for this affiliated study of the PURE study was obtained from the Human Research Ethics Committee of the North-West University (NWU 00346-16-A1). A copy of the ethics form is included in the annexures.

3.3.6.2 Participants written informed consent form

No additional participants' consent was required for this affiliated study.

3.3.6.3 Permission to use PURE data

Permission to use both the secondary data collected in 2005 and data collected in 2015 (where the PhD student participated) was obtained from the PI. A formal request was sent to the PI after the proposal for this affiliated study was approved by the HREC of North-West University. Formal permission was granted, and this document is included in the annexures.

This study was considered of 'minimal risk'. Safety precautions in the laboratory, as outlined by CEN laboratory regulations, were strictly adhered to.

3.3.7 Benefits of the affiliated study

3.3.7.1 Direct benefit

Participants partaking in the data collection in 2015 directly benefited from the day's measurements. They received feedback regarding results that were immediately available. These included:

- Blood pressure
- Blood glucose levels
- Anthropometry

In cases of any abnormalities that were picked up on any of the measurements, the lead researcher for the day provided the participant with a referral letter that they presented at the nearest hospital or local clinic. The principal investigator visited the local hospital and clinics to inform them about the upcoming follow-up study and to make pre-arrangements for possible referrals.

3.3.7.2 Indirect benefits

The association of dietary and plasma FA with obesity-related outcomes including the MetS and associated risk is not well established in the black South African population. The major goal of the proposed study is to explore the associations of dietary and plasma FA biomarkers and resulting patterns with obesity and metabolic features, and its development over time. The findings of this study may complement the data in the field and further supplement the knowledge and importance of FA status as potential novel biomarkers of obesity-related health outcomes. In addition, the new knowledge will be used to support policies that direct nutrition intervention to recommend modulation of current FA intake guidelines in South African population, as well as general public health importance. Furthermore, the student will obtain a doctoral degree.

3.3.7.3 Incentive and reimbursement for this proposed study

Participants were financially compensated to the amount of R100.00 per day in 2005 and 2015 for any expenses or loss of income incurred due to their participation in the study. They were also provided with funds if they were referred to the local hospital/clinic and compensated for a day's income if they missed work due to data collection. No additional compensation was required in relation to this affiliated study.

3.3.8 The risk associated with the research to be conducted within the proposed study

The proposed research included comprehensive statistical analysis of existing PURE data and data collected in 2015, in order to fulfil the proposed aims and objectives of the affiliated study. In addition, further laboratory analysis was performed from the already sampled human plasma samples available from 2015. No additional analysis involving re-consenting and further sampling of biological human species was included in the research proposed herein. Furthermore, the data and results obtained from the proposed study are not of immediate clinical relevance, but of broad epidemiological significance. Therefore, the research conducted within this PhD project is of minimal risk.

3.3.9 Data access and management

The PhD student for this proposed study received coded data in an electronic format after an official request from the PURE study PI. Together with her supervisors and statistician, the data were stored in a password protected computer and backed up on an external hard drive. All the data in possession of the PhD student will be re-possessed.

3.3.10 Research methodologies specific to PhD study

3.3.10.1 Summary of laboratory measurements

Fasting blood samples were collected from the antecubital vein with a sterile winged infusion set with minimal stasis. The samples were collected by a registered nurse and stored at -80°C in the urban areas. In rural areas, the samples were stored at -18°C up to five days where after it was transported to the laboratory facility and stored at -80°C until used. Fasting plasma glucose concentration was determined by the hexokinase method using the Synchron® system (Beckman Coulter Co., Fullerton, CA, USA). The Sequential Multiple Analyser Computer (SMAC), using the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland), performed quantitative determination of HDL-C, triglycerides and TC. LDL was calculated using the Friedewald equation (Friedewald *et al.*, 1972).

EDTA plasma samples were thawed and extracted with chloroform:methanol (2:1 v/v) according to the modified Folch method (Folch *et al.*, 1957). The plasma phospholipid FA fraction was isolated by thin layer chromatography from the extracted lipids [33]. Subsequently, the phospholipid FA fraction was transmethylated to FA methyl esters and analysed by quadrupole gas chromatography electron ionisation mass spectrometry by Agilent Technologies 7890 A GC system, as described by Baumgartner *et al.* (2012). Thirty-two FAs were measured in fasted plasma samples from 711 participants. Six FAs, i.e. pentadecanoic acid (C15:0), margaric acid (C17:0), trans-vaccenic acid (C18:1n-7t), rumenic acid (C18:2n-7tt), stearidonic acid (C18:4n-3), and eicosatrienoic (C20:3n-3) were below the limit of quantification and therefore were not included. The remaining 26 plasma phospholipid FAs were quantified and expressed as a percentage of total FAs. Quality of data was assured with a separate calibration for each FA, monitoring of internal standard (1,2 – diheptadecanoyl – sn – glycerol – 3 phosphorylcholine, Matreya, Pennsylvania, USA) and Levey Jennings graphs for a pooled plasma control analysed with each batch. The coefficient of variation for the internal standards ranged between 4.76 and 18.5%, with a coefficient of variation smaller than 10% for 14 of the 26 FAs. The plasma FA samples for 2005, were analysed in 2012, 2010 samples were analysed in 2013 and 2015

samples were analysed in 2016. The samples were stored for a period of 8, 4, and 1 year, respectively. Plasma phospholipid FAs are very reliable when stored at -80 °C for up to 10 years and are suitable in establishing dietary fat-disease associations (Matthan *et al.*, 2010).

3.3.10.2 Estimation of desaturase activities

The estimated desaturase activities were assessed as the ratio of FA product to the precursor of individual plasma phospholipid FAs (Warensjo, 2006) according to the following formulas: SCD-16 activity was calculated as a ratio of POA to palmitic acid, SCD-18 activity was calculated as the ratio of oleic acid to stearic acid; D6D activity was calculated as the ratio of GLA to LA, and D5D activity was calculated as a ratio of AA to DGLA.

Table 3-1 List of plasma phospholipid fatty acids studied in the selected group of black South African adults

Plasma phospholipid fatty acids studied in black South African adults		
Fatty acid name	Chemical notation	Abbreviations
Saturated		
Myristic acid	C14:0	MA
Palmitic acid	C16:0	PA
Stearic acid	C18:0	SA
Arachidic acid	C20:0	
Behenic acid	C22:0	
Lignoceric acid	C24:0	
Monounsaturated		
Palmitoleic acid	C16:1n-7	POA
<i>Cis</i> -vaccenic acid	18:1n-7	
Oleic acid	C18:1n-9	OA
Gondoic acid	C20:1n-9	
Erucic acid	C22:1n-9	
Nervonic acid	C24:1n-9	
n-3 polyunsaturated		
α -linolenic acid	C18:3n-3	ALA
Eicosapentaenoic acid	C20:5n-3	EPA
Docosapentaenoic acid	C22:5n-3	DPA
Docosahexaenoic acid	C22:6n-3	DHA
n-6 polyunsaturated		
Linoleic acid	C18:2n-6	LA
γ -linolenic acid	C18:3n-6	GLA
Eicosadienoic	C20:2n-6	
Dihomo- γ -linolenic acid	C20:3n-6	DGLA
Arachidonic acid	C20:4n-6	AA
Docosadienoic acid	C22:2-n6	
Adrenic acid	C22:4n-6	
Osbond acid	C22:5n-6	
n-9 polyunsaturated		
Mead acid	C20:3n-9	
Trans fatty acid		
Elaidic acid	C18:1n9t	

3.3.10.3 Definition of overweight/obesity and metabolic status

For this specific study, underweight and healthy weight were defined as BMI < 25kg/m² and overweight and obesity as BMI ≥ 25kg/m², because a BMI above 25kg/m² has been associated with increased cardiometabolic risk in this population (Kruger *et al.*, 2015). MetS was defined according to the JIS recommendations (Alberti *et al.*, 2009). The existence of three or more of the following: (i) fasting plasma glucose levels ≥ 5.6 mmol/l or the use of oral hypoglycaemic medication; (ii) serum triglycerides ≥ 1.7 mmol/l; (iii) serum HDL-C ≤ 1.0 mmol/l for men and ≤ 1.3 mmol/l for women; (iv) BP ≥ 130/85 mmHg or the use of BP medication; and (v) WC of ≥ 94 cm for men and ≥ 80 cm for women (Alberti *et al.*, 2009) was considered to meet the requirement for diagnosis of MetS.

Table 3-2 Classification of metabolic syndrome (Alberti *et al.*, 2009)

Measure	Categorical points	
	Men	Women
Elevated waist circumference* Population- and country-specific definitions	≥94 cm	≥80 cm
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator*)	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator*)	≤40 mg/dL (1.0 mmol/L)	≤50 mg/dL (1.3 mmol/L)
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic ≥130 and/or diastolic ≥85 mm Hg	Systolic ≥130 and/or diastolic ≥85 mm Hg
Elevated fasting glucose# (drug treatment of elevated glucose is an alternate indicator)	≥100 mg/dL (5.6 mmol/L)	≥100 mg/dL (5.6 mmol/L)
Notes: *The most commonly used drugs for elevated triglycerides and reduced HDL-C are fibrates and atorvastatin. A patient taking one of these drugs can be presumed to have high triglycerides and low HDL-C. # Patients with type 2 diabetes mellitus on drug treatment for elevated fasting glucose.		

3.3.10.4 Additional variables used in the study

The variables used in all the three manuscripts are summarised in the table below.

Table 3-3 Variables used in the PhD study

Variables used in the study	First manuscript	Second manuscript	Third manuscript
Number of participants	(n=711)	(n=711)	(n=412)
Study design	Cross-sectional (2005)	Cross-sectional (2005)	Prospective (2005-2015)
Outcomes	Metabolic status classified according to newly harmonized JIS criteria. Normal weight: Metabolically healthy normal weight and metabolically unhealthy with normal weight Overweight/obese: Metabolically healthy and overweight/obese and metabolically unhealthy and overweight/obese	Baseline Body mass index, waist circumference, waist: height ratio, and metabolic syndrome	10-year changes in weight, Body mass index, waist circumference, and waist: height ratio
		Covariates used in the sequential regression models	
Age and sex		Age, men and women	Age, men and women
Lifestyle variables		Self-reported smoking (never smoked and current smokers), education (no school and formal education), and physical activity.	Self-reported smoking (never smoked and current smokers), education (no school and formal education), and physical activity.
Dietary variables		Alcohol intake, total fats, carbohydrates, dietary fibre and energy from added sugar.	Alcohol intake, total fats, carbohydrates, dietary fibre and energy from added sugar.
Exposures			
Diet-related	Dietary intake and individual dietary fatty acids	Dietary patterns	None
Plasma fatty acids	Individual plasma phospholipid fatty acids	Plasma phospholipid fatty acid patterns (n=711)	Baseline plasma phospholipid fatty acid patterns(n=412)
Number of fatty acids used	26	26	25

3.3.11 Statistical analysis

3.3.11.1 Planned statistical analysis

The planned statistical analysis was based on the manuscripts published from the proposed PhD project. All statistical analyses were performed on the baseline randomly selected cohort (n=711). The exploratory output, including data cleaning, outlier testing and normality testing was applied to the dataset before it was used for analysis for each publication.

A summary of statistical analysis, according to the expected publications is outlined below. All analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA) (SAS, 2016) and $p < 0.05$ was considered significant.

3.3.11.2 First manuscript

Title: “Dietary and plasma phospholipid fatty acids are associated with metabolic health in black South Africans: PURE study”.

Objective: To compare the levels of dietary and plasma phospholipid FAs of metabolically healthy and unhealthy phenotypes classified according to JIS criteria and BMI categories in a group of black South African adults.

Statistical analysis: Medians and interquartile ranges were used to provide overall descriptions of variables in the groups, since our data had a skewed distribution. A Kruskal-Wallis test with Dunnett's post hoc analysis was conducted to compare demographic, anthropometric, biochemical, dietary intake, dietary FA, plasma phospholipid FA and estimated desaturase activity data between the identified metabolic status groups, with the MHNW group as the reference. The effect size of the differences between MHNW vs MUNW and MHO vs MUO groups was calculated using the Man-Whitney U-value and sample size of the groups (Pautz et al., 2018). We also performed an ANCOVA test and adjusted for age and total energy intake to compare dietary FAs, plasma phospholipid FAs and estimated desaturase activity data between the identified metabolic status groups.

3.3.11.3 Second manuscript

Title: “Plasma phospholipid fatty acid patterns are associated with adiposity and metabolic syndrome in black South Africans: A cross-sectional study.”

Objective: To investigate the associations between dietary FAs and plasma phospholipid FA patterns, respectively, with adiposity measures BMI, WC and WHtR, and MetS in a selected group of black South African adults.

Statistical analysis: Non-normally distributed data were log transformed before inclusion in regression models. Participants' characteristics were compared by sex and BMI categories using the Mann-Whitney U-test or chi-squared test for continuous and categorical variables, respectively. Differences between individual FAs and ratios by BMI and sex groups were tested by the Mann-Whitney test. The effect size of the differences between groups was calculated using the Man-Whitney U-value and sample size of the groups (Pautz *et al.*, 2018). Principal-component-based varimax factor analysis of the correlation matrix was used to define dietary FA (based on the QFFQ) and plasma phospholipid FA patterns. The number of factors to retain was established by the Kaiser criterion (eigenvalues > 1) and scree-plot visual inspection. Loadings with absolute values higher than 0.5 were considered as relevant for the contribution to each FA pattern. The associations between FA patterns and outcomes were evaluated by sequential regression models, logistic regression for the dichotomous outcome (MetS), and generalised linear models for continuous outcomes (WC, BMI and WHtR). The first step of the sequential modelling analyses was based on models that contained only dietary FAs or plasma phospholipid FA patterns and was referred to as a crude model. The crude model was then adjusted for sex and age (adjusted model₁). This model was further adjusted for lifestyle confounders, including the level of education, physical activity, alcohol and total energy intake, and self-reported smoking status creating a fully adjusted model.

3.3.11.4 Third manuscript

Title: "Associations between plasma phospholipid fatty acid patterns and changes in anthropometric indices in black South African adults over ten years: a prospective cohort study."

Objective: To prospectively explore the associations of plasma phospholipid FA patterns derived at baseline with 10-year changes in anthropometric indices of adiposity among a sub-cohort of black South Africans participating in the PURE study.

Statistical analysis: The characteristics of the participants at two time points (baseline and 10-year follow-up) were assessed by Wilcoxon matched pairs test, and data are presented by sex. Differences between individual FAs and ratios by BMI and sex groups at 10-year follow-up were tested by means of the Mann-Whitney test. A BMI < 25 kg/m² was considered as underweight and/or normal-weight or lean, whereas BMI ≥ 25 kg/m² was considered overweight and/or obese.

The effect size of the differences between groups was calculated using the Man-Whitney U-value, and the sample size of the groups and effect size for differences at two time points were calculated by means of the Wilcoxon Signed Ranks Test Z-value and sample size (Pautz et al., 2018).

Categorical variables were classified as follows: sex, self-reported smoking and education . Continuous variables included age, weighted physical activity index and total energy and alcohol intake, as well as 10-year changes in weight, BMI, WC and WHtR. Exposure variables included six plasma phospholipid FA patterns identified at baseline.

To define the plasma phospholipid FA patterns at baseline, principal component analysis (PCA) based on the varimax rotation was conducted. We considered FA with an absolute factor loading of > 0.5 to make a significant contribution to the pattern. The same PCA procedure was repeated for the patterns derived at 10-year follow-up. Additionally, we divided the participants into quartiles of factor scores for each pattern and tested how many participants stayed in the same quartile or moved to the adjacent quartiles for the similar FA pattern after follow-up. The purpose of the analysis was to examine the reproducibility of baseline patterns with similar patterns at 10-year follow-up.

We determined the correlation between scores of the different FA patterns, as well as plasma FAs, which were correlated with percentage energy (%E) intake from total fat, %E from total SFA, total fat and alcohol intake.

The differences over ten years for all anthropometric indices (weight, BMI, WC, and WHtR) were included in the sequential adjusted linear regression models as continuous outcome variables. The association with FA pattern scores, with adjustment for covariates, was assessed in sequential models, starting with the crude model, which consisted of only plasma phospholipid FA patterns (exposure variables) and outcomes. In the first adjusted model, age and sex (men and women) were added as covariates, and finally, in the fully adjusted model, all lifestyle covariates, including self-reported smoking, education education, physical activity, alcohol and total energy intake, as well as the baseline anthropometric variable were added. Model goodness-of-fit was evaluated using R-square statistics.

3.3.12 Dissemination of research results

The manuscript will be submitted for publication in peer-reviewed journals, and part of the research has been presented at conferences. The planned submissions are outlined below.

First manuscript: Prostaglandins, Leukotrienes & Essential Fatty Acids

Second manuscript: Cardiovascular Journal of Africa

Third manuscript: Nutrition Research

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CHAPTER 4 MANUSCRIPT 1

4.1 Comparison of dietary and plasma phospholipid fatty acids between normal weight and overweight black South Africans according to metabolic health: The PURE study.

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4.2 Abstract

Background: Information regarding circulating fatty acids (FA) in association with metabolic health in black Africans is scarce, while the usefulness of circulating FAs as biomarkers of dietary fat intake and predictors for medical conditions is advancing globally.

Objective: We compared the levels of eleven dietary and 26 plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes in black South African adults.

Methods: Adults from the South African arm of the Prospective Urban and Rural Epidemiology study baseline (n=711) were categorised into four groups, namely normal weight without metabolic syndrome (MetS) (MHNW), normal weight with MetS (MUNW), metabolically healthy overweight/obese (MHO) and metabolically unhealthy overweight/obese (MUO). Dietary and plasma phospholipid FAs were measured by a quantitative food frequency questionnaire and gas chromatography-tandem mass spectrometry, respectively. We compared dietary FAs, plasma phospholipid FAs and estimated desaturase activity between the metabolic status groups using ANCOVA adjusted for age and energy intake.

Results: MetS was present in 35% of the participants. After adjustment for age and total energy intake, in comparison to the MHNW reference group, dietary saturated FAs (C14:0 to C18:0) and alpha-linolenic acid intakes were higher in both overweight/obese groups (MHO and MUO), while

linoleic acid intakes were higher in the MUO group only. Plasma levels of most saturated FAs (C18:0 to C22:0) and PUFAs were higher, whereas selected MUFAs, palmitic acid and estimated desaturase activities were lower in the overweight/obese groups.

Conclusions: The overweight groups generally had higher fat intakes than normal weight groups, but lower plasma levels of *de novo* lipogenesis pathway FAs and estimated desaturase activities. Therefore, lower plasma levels of *de novo* lipogenesis pathway FAs and decreased estimated desaturase activities may be biomarkers of abnormal metabolic health in overweight/obese study participants.

Keywords

Black Africans, dietary fatty acids, plasma phospholipid fatty acids, metabolic health, factor analysis, metabolically healthy and unhealthy phenotypes

Abbreviations

AA, arachidonic acid; ALA, α -linolenic acid; BP, blood pressure; BMI, body mass index; CVD, cardiovascular disease; D5D, delta 5 desaturase; D6D, delta 6 desaturase; DNL, *de novo* lipogenesis; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FPG, fasting plasma glucose; GLA, γ -linolenic acid HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MHNW, metabolically healthy with normal weight; MHO, metabolically healthy and overweight/obese; MUFA, monounsaturated fatty acid; MUNW, metabolically unhealthy with normal weight; MUO, metabolically unhealthy and overweight/obese; POA, palmitoleic acid; PUFA, polyunsaturated fatty acid; SCD, stearoyl-Co-A desaturase; SFA, saturated fatty acid; T2DM, type 2 diabetes mellitus; TG, Triglycerides; VLC-PUFA, very long-chain polyunsaturated fatty acid; VLC-SFA, very long-chain saturated fatty acid; WC, waist circumference; WHtR, waist: height ratio.

4.3 Introduction

The metabolic syndrome (MetS), characterised by a group of cardiovascular disease (CVD) risk factors including truncal obesity, hypertension, deranged lipid profiles, and abnormal glucose metabolism [1], is a significant risk factor for CVD and type 2 diabetes mellitus (T2DM) [2]. Obesity, a global epidemic, is a substantial risk factor for MetS [2]. The South African prevalence of overweight and obesity is exceptionally high, at 31% in men and 67% in women [3], and higher than that of the USA where 31.7% of men and 33.9% of women are overweight and obese [4].

The prevalence of MetS in South Africa is increasing due to the high prevalence of obesity and poor dietary habits, which are now also reported in rural areas [5, 6]. Based on the available data of various South African studies, the overall crude prevalence of MetS in rural black South Africans in KwaZulu-Natal was 26.5% [7]. In the Western Cape Province, a study carried out in the rural wine farms showed a prevalence of 29.3% in men and 46.3% in women [8], whereas, in the rural and urban areas of Free State, the prevalences were 52.2% and 39.7%, respectively [9].

Earlier different criteria for defining MetS have been recommended by the National Cholesterol Education Program, the Adult Treatment Panel III Report [10] and the World Health Organization (WHO)[11]. More recently, the harmonised Joint Interim Statement (JIS) of six international associations includes waist circumference (WC) cut-points for the different ethnic groups and recommends that sub-Saharan African populations use the European WC cut-points [12]. The available information on the prevalence of MetS in sub-Saharan Africa, based on the classification of JIS [1, 12], is limited to epidemiological reports on West Africans in Benin [13], Cameroon [14] and Nigeria [15]. In these West African countries, central obesity was reported as a critical determinant in the prevalence of MetS in both urban and rural areas [13-15]. Fezeu and colleagues [14] recommended that the MetS definitions might not be appropriate for populations of African descent. However, different recommendations for WC [16] have been suggested for possible use in black Southern African adults for identification of cardio-metabolic risk, but these cut-points have not been accepted internationally.

Certain dietary fatty acids (FA) have been associated with the risk of MetS, while others are considered to be protective. Dietary essential FAs, linoleic acid and α -linolenic acid (ALA) are converted to the longer chain FAs through endogenous metabolism [17]. Dietary n-3 FAs have been shown to be protective of MetS [18], while dietary intake of linoleic acid has been related to a lower risk of CVD in prospective observational studies [19]. On the other hand, an increased dietary ratio of n-6 polyunsaturated FAs (PUFA) to n-3 PUFAs has been positively associated with measures of adiposity and subjects with higher dietary n-6/n-3 PUFA ratio had worse metabolic profiles including insulin and HOMA-IR values [20], showing some inconsistencies in the literature.

The associations between the proportion and ratios of individual FAs in plasma phospholipids with MetS and related risk factors have been investigated in several cross-sectional studies. Overall, these studies propose that high proportions of palmitic and stearic acid in plasma phospholipids are positively associated with MetS [21] and adverse metabolic risk [22]. On the other hand, very long chain (VLC)-SFAs with chain lengths ranging from C20:0 to C24:0 in plasma

were found to be inversely associated with MetS [21]; however, another study observed mixed findings with VLC-SFAs in participants with MetS [22]. Selected studies have also reported positive associations of POA with the risk of MetS [23, 24].

Evidence from studies that have evaluated the associations between n-3 PUFAs in plasma phospholipids and plasma FA proportions with MetS is limited and inconsistent. Huang *et al.* [25] reported an inverse association of total n-3 PUFAs in plasma phospholipids with MetS. A study in Tunisia documented higher plasma levels of ALA and lower levels of docosahexaenoic acid (DHA) and total n-3 PUFAs in patients with MetS [23], whereas another study reported lower plasma levels of eicosapentaenoic acid (EPA) in coronary patients with MetS [26]. A study in a Mediterranean population at high risk of CVD reported no association between plasma n-3 PUFAs and MetS [24]. Total PUFAs in plasma phospholipids were significantly lower in MetS participants as compared to healthy subjects in Chinese adults [25]. Sethom *et al.* [23] reported decreased total n-6 and total n-3 PUFAs in patients with MetS. Lower plasma levels of LA and higher levels of DGLA were reported in participants with MetS in Spain and Tunisia, respectively [23, 24].

The stearoyl-Co-A desaturase (SCD)-1 is a rate-limiting enzyme in the synthesis of monounsaturated FAs (MUFAs). SCD-1 transforms the SFAs, palmitic and stearic acid into MUFAs, palmitoleic acid (POA) and oleic acid, respectively [27]. Delta 6 desaturase (D6D) and delta 5 desaturase (D5D) are the rate-limiting enzymes for PUFA conversion and are recognised as a main determining factor of levels of longer-chain PUFAs [28]. Alterations of D5D, D6D and SCD-1 activities have been associated with MetS [23, 27, 29, 30]. Higher estimated desaturase activities, SCD-16 (POA/palmitic acid) and SCD-18 (oleic acid/stearic acid) estimated in phospholipid fractions were recorded in participants with MetS [30]. Increased SCD-1 activity and decreased D5D activity were reported in adults with MetS [23]. In a longitudinal study, D5D activity was inversely associated with the risk of MetS, whereas D6D activity was directly associated with risk in men [29].

Very little is known about the association of specific FAs and estimated desaturase activities with obesity and MetS in black Africans. We, therefore, aimed to compare the levels of dietary FAs and plasma phospholipid FA of metabolically healthy and unhealthy phenotypes classified according to JIS criteria and BMI categories in a group of black South African adults.

4.4 Research design and methods

4.4.1 Study design

This cross-sectional study utilised baseline (year 2005) data from black South African adults. They were participants of the Prospective Urban and Rural Epidemiology (PURE) multi-centre cohort study that tracks the impact of diet and lifestyle on the development of non-communicable diseases (NCDs) in urban and rural areas of low- and middle-income countries in transition [31].

4.4.2 Study settings

The South African leg of the PURE study is set in rural and urban African communities in the North West Province of South Africa. Urban participants were recruited from Tlokwe, and rural participants from the Ganyesa District. All measurements of the rural participants were performed in a field laboratory in Ganyesa. Evaluation of the urban participants was conducted in the metabolic unit based at the Centre of Excellence for Nutrition of the North-West University, Potchefstroom. Infrastructure and resources to draw blood, administer questionnaires and measure body composition were available at both sites [32, 33].

4.4.3 Study population

Recruitment was performed from 6000 randomly selected houses by trained fieldworkers, and a paper selection was completed according to specific eligibility criteria from the data obtained from these households. One thousand participants from both rural and urban communities were selected, resulting in 4000 participants, forming the South African arm of the international PURE study. In 2005, a total of 2010 (1006 rural and 1004 urban) apparently healthy recruited participants turned up for measurements and were enrolled for participation in the study. A sub-cohort of 711 participants (273 men, 438 women) were selected randomly for this present study from the original 2010 volunteers with both dietary and plasma phospholipid FAs, after exclusion of 305 participants with human immunodeficiency virus infection. The number of participants was randomly selected from stratified groups (rural male, rural female, urban male, and urban female) and included 125 rural men, 148 urban men, 218 rural women and 220 urban women. All participants (n=711) had complete data of anthropometric and metabolic parameters, and plasma phospholipid FAs. Also, complete dietary data set excluding under- and over-reporters of total energy intake. All the participants provided written informed consent at each follow-up. Ethical clearance for the South African PURE study was given by the Ethics Committee of the North-West University (ethics number 04M10).

4.4.4 Anthropometric and blood pressure assessments

Anthropometric measurements were performed by trained research assistants according to standards prescribed by the International Society for the Advancement of Kinanthropometry [34]. All equipment was standardised. A portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) was used to measure the weight of the participants. Standing height was measured using a calibrated stadiometer (Seca, Hamburg, Germany). WC was recorded using steel tapes (Lufkin, Apex, NC, USA). The BMI and waist: height ratio (WHtR) were calculated using weight (in kg) divided by height (m²) and WC (cm) divided by height (cm), respectively. Two blood pressure (BP) readings were assessed at a seated position, and the average used for analysis for this study. Further details of anthropometric measurements and BP have been published elsewhere [32, 35].

4.4.5 Fatty acids and biochemical measurements

Fasting blood samples were collected from the antecubital vein with a sterile winged infusion set with minimal stasis. The samples were collected by a registered nurse and stored at -80°C in the urban areas. In rural areas, the samples were stored at -18°C up to five days, where after they were transported to the laboratory facility and stored at -80°C until analysed. Fasting plasma glucose concentration was determined by the hexokinase method using the Synchron® system (Beckman Coulter Co., Fullerton, CA, USA). The Sequential Multiple Analyser Computer (SMAC), using the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland), performed the quantitative determination of HDL, TG and total cholesterol (TC). Low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald equation [36].

EDTA plasma samples were thawed and lipids were extracted with chloroform:methanol (2:1 v/v) according to the modified Folch method [37]. The plasma phospholipid fatty acid fraction was isolated by means of thin layer chromatography from the extracted lipids [38]. Subsequently, the phospholipid fatty acid fraction was transmethylated to fatty acid methyl esters and analysed by gas chromatography electron ionisation mass spectrometry by means of an Agilent Technologies 7890 A GC system, as described by Baumgartner *et al.* [38]. Thirty-two FAs were measured in fasted plasma samples from 711 participants. Six FAs, pentadecanoic acid (C15:0), margaric acid (C17:0), trans-vaccenic acid (C18:1n-7t), rumenic acid (C18:2n-7tt), stearidonic acid (C18:4n-3), and eicosatrienoic (C20:3n-3) were below the limit of quantification and therefore were not included. The remaining 26 plasma phospholipid FAs were quantified and expressed as a percentage of total FAs. Quality of data was assured with a separate calibration for each fatty acid, monitoring of internal standard (1,2 – diheptadecanoyl – sn – glycerol – 3

phosphorylcholine, Matreya, Pennsylvania, USA) and Levey Jennings graphs for a pooled plasma control analysed with each batch.

4.4.6 Estimation of desaturase activities

The estimated desaturase activities were assessed as the ratio of FA product to the precursor of individual plasma phospholipid FAs [27] according to the following formulas: SCD-16 activity was calculated as a ratio of POA to palmitic acid, SCD-18 activity was calculated as the ratio of oleic acid to stearic acid, and both SCD-16 and SCD-18 represent SCD-1 activity. The D6D activity was calculated as the ratio of DGLA to LA, and delta-5 desaturase (D5D) activity was calculated as a ratio of AA to DGLA.

4.4.7 Definition of overweight/obesity and metabolic status

For this specific study, underweight and healthy weight were defined as BMI < 25kg/m² and overweight and obesity as BMI ≥ 25kg/m², because a BMI above 25kg/m² has been associated with increased cardiometabolic risk in this population [39]. MetS was defined according to the JIS recommendations [12]. The existence of three or more of the following: (i) FPG levels ≥ 5.6 mmol/l or the use of oral hypoglycaemic medication; (ii) serum TG ≥ 1.7 mmol/l; (iii) serum HDL ≤ 1.0 mmol/l for men and ≤ 1.3 mmol/l for women; (iv) BP ≥ 130/85 mmHg or the use of BP medication; and (v) WC of ≥ 94 cm for men and ≥ 80 cm for women [12] was considered to meet the requirement for diagnosis of MetS.

We categorised the study participants into four metabolic status groups, namely metabolically healthy with normal weight (MHNW), metabolically unhealthy with normal weight (MUNW), metabolically healthy and overweight/obese (MHO), metabolically unhealthy and overweight/obese (MUO).

4.4.8 Estimation of dietary nutrients

Dietary data were collected using a culturally sensitive quantified food frequency questionnaire (QFFQ) listing 135 food items with additional elaboration of different varieties and cooking methods [40], as described elsewhere [32]. Dietary macronutrients and FAs were calculated using the South African Medical Research Council food composition tables [41]. Twenty-eight dietary FAs were included initially, but FAs that had a daily median intake of less than 0.10 mg were excluded, leaving a total of 11 dietary FAs to be investigated in this study. Participants with reported total energy intakes ≥30,000 or ≤3000 KJ were excluded from the baseline as over-reporters and under-reporters, respectively.

4.4.9 Statistical analysis

Medians and interquartile ranges were used to provide overall descriptions of variables in the groups, since our data had a skewed distribution. A Kruskal-Wallis test with Dunnett's *post hoc* analysis was conducted to compare demographic, anthropometric, biochemical, dietary intake, dietary FA, plasma phospholipid FA and estimated desaturase activity data between the identified metabolic status groups, with the MHNW group as the reference. The effect size of the differences between MHNW vs MUNW and MHO vs MUO groups was calculated using the Man-Whitney U-value and sample size of the groups [42]. We also performed an ANCOVA test and adjusted for age and total energy intake to compare dietary FAs, plasma phospholipid FAs and estimated desaturase activity data between the identified metabolic status groups. Statistical analysis was performed using SAS version 9.4 [43]. A p-value of ≤ 0.05 was considered statistically significant.

4.5 Results

The percentage of participants with MetS was 35% (n=246) and 40% (n=287) were overweight/obese. Differences in demographic data and metabolic parameters between the four metabolic status groups are reported in **Table 4-1**. The MUO group was significantly older compared to all the other groups. All the anthropometric measurements, including weight, BMI, WC and WHtR were higher in the MHO, MUNW and MUO compared to the MHNW. As expected, the systolic and diastolic BP, as well as TC, TGs and FPG, were higher in the MetS groups (MUNW and MUO). On the other hand, LDL was higher for MHO, MUNW and MUO, while HDL was lower for both overweight/obese (MHO and MUO) groups than in the MHNW group.

We also compared the differences between the two normal weight groups (MHNW and MUNW). Even though the two groups had normal BMI, the MUNW group had higher anthropometric measurements compared to the MHNW group. As expected, all the metabolic parameters were significantly higher in the MUNW group compared to the MHNW group. We furthermore compared the differences between both the overweight/obese groups (MHO and MUO). WC, WHtR, systolic and diastolic blood pressure, TC, LDL, TC and FPG were higher in the MUO group compared to the MHO group. Surprisingly, HDL was also higher in this group. The MHO group were more physically active as compared to the MUO group.

Dietary intake and dietary FAs are indicated in **Table 4-2**. The MUO group had the lowest %E intake from carbohydrates, but highest %E intake from total fat compared to the MHNW group. Higher %E intake from PUFAs was observed in the MetS groups (MUNW and MUO) than in the MHNW group, while the MHO group had the highest %E intake from added sugar. The quantities

of most dietary FAs were highest in the MUO group, except POA, and ALA, which were higher in both the MUNW and MUO groups compared to the MHNW group.

Furthermore, we compared dietary intake and dietary FAs in the normal weight groups (MHNW and MUNW) as well as overweight/obese groups (MHO and MUO). The MUNW group had significantly higher levels of energy intake from total protein and total fat compared to the MHNW group. The MUO group had higher total energy intake, as well as higher intakes of most dietary SFAs and MUFAs. The MUNW group had higher intakes of dietary ALA compared to the MHNW group. Interestingly, dietary POA and AA were higher in both MetS groups (MUNW and MUO) as compared to their healthier counterparts, MHNW and MHO, respectively.

The composition of plasma phospholipid FAs and estimated desaturase activities according to metabolic status are outlined in **Table 4-3**. Proportions of most of the individual plasma phospholipid FAs from different FA classes (SFAs, MUFAs, n-3 and n-6 PUFAs) were significantly different in the overweight/obese (MHO and MUO) groups compared to the MHNW group. Plasma SFAs (C18:0 to C22:0), as well as n-3 and n-6 PUFAs (DHA, DGLA, AA) and total n-6 PUFAs were higher, whereas MUFAs, (POA and oleic acid) were lower for both the overweight/obese groups compared to the MHNW group. Total n-3 PUFA was higher in the MUO group compared to the MHNW group. Palmitic acid is the most abundant FA and was an exception among SFAs, because the proportion of palmitic acid was lower in the overweight/obese groups compared to the MHNW group. There were additional significant differences among groups for several FAs that were identified in very small proportions in the plasma phospholipids, for example EPA was lower in the MHO group compared to the MHNW group. The estimated desaturase activities, SCD-1, were lower in both the overweight/obese groups. Inconsistent differences were found for desaturase activities D6D and D5D. D6D was higher in the MUO group, while D5D was lower in the MUO group, compared to the MHNW group.

Comparison of the differences between the two normal weight groups (MHNW and MUNW)- showed that the MHNW had higher plasma levels of VLC--SFAs, VLC-MUFAs and linoleic acid compared to the MUNW group (**Table 4-3**). Higher plasma levels of EPA (effect size 0.50), higher SCD-18 activity and lower D5D activity were observed in the MUNW group compared to the MHNW group. There were other significant differences recorded between FAs that occur in low proportions in these two groups that are not highlighted in the results. Furthermore, the effect sizes for the difference between the groups were generally low.

We further present plasma phospholipid FA differences between the healthy and unhealthy overweight/obese groups (MHO and MUO) (**Table 4-3**). Plasma levels of palmitic acid, MUFAs

(POA, oleic acid), EPA, and SCD-1 activity, were higher in the MUO group compared to the MHO group, whereas plasma long-chain SFAs (C18:0 to C24:0), total n-6 PUFAs, and D5D activity were consistently higher in the MHO group compared to the MUO group. The effect sizes for the differences between the groups were low, ranging between 0.33 and 0.43.

ANCOVA tests, controlled for age and total energy intake, show differences between the four metabolic status groups as outlined in **Table 4-4**. Whereas most FAs were higher in the MUO group only, after adjustment for age and total energy intake most dietary saturated FA and ALA intakes were higher in both overweight/obese groups (MHO and MUO). Linoleic acid intakes were higher in the MUO group only compared to MHNW. Most of the plasma phospholipid FAs, remained significantly different in overweight/obese groups as compared to the MHNW group, whereas all estimated desaturase activities were lower in the MUO group as compared to the MHNW after adjustments for age and total energy intake.

4.6 Discussion

This cross-sectional study of the South African arm of the international PURE study adds new information about dietary FAs and plasma phospholipid FAs in relation to metabolically healthy and unhealthy phenotypes in black Africans. To our knowledge, this is the first study to compare the levels of dietary and plasma phospholipid FAs, as well as anthropometric and metabolic parameters among metabolically healthy and unhealthy phenotypes in black Africans. After adjustments for age and total energy intake, and comparisons of dietary and plasma phospholipid FAs between the four metabolic status groups, dietary saturated FAs and ALA intake were higher in both the overweight/obese groups, while linoleic acid intakes were higher in the MUO group only. Lower plasma levels of most MUFAs, ALA and GLA, and lower estimated desaturase activity in the overweight/obese groups (MHO and MUO) persisted. The overweight/obese groups had higher VLC-SFAs, DHA, DGLA, total n-6 PUFAs compared to the MHNW group.

Studies report that overweight or obese persons, characterised by an excessive visceral and ectopic fat, are at higher risk of developing metabolic complications categorised by hyperinsulinemia/glucose intolerance, hypertension, atherosclerosis, and adverse cardiac effects [44]. In line with our findings, older obese men and women with MetS have been reported to present with significantly higher abdominal visceral fat compared to non-MetS participants [45]. The MUO group had higher abdominal obesity as measured by WC and WHtR compared to the MHO group (**Table 6-1**). Over time, sub-groups of MHO individuals developed T2DM and other related CVD risk factors in an Australian longitudinal study, indicating the temporary nature of this condition [46]. Therefore, higher abdominal obesity measured by WC and WHtR observed in the

MUO group, compared to the MHO observed in our study participants, is consistent with what is reported in the literature [44, 45]. Indeed, an increase in WC predicts a shift to the MUO phenotype [46]. On the contrary, lower central adiposity, young age and physical activity increased the likelihood of MHO in adults [46]. These results are in support of our findings. The MHO group in our study participants were more physically active, had lower central adiposity and were younger as compared to the MUO group.

Higher %E intake from total fat observed in the MUO group in this present study may explain the abdominal obesity and higher intakes of most dietary FAs seen in this group. A robust association between excessive caloric intake and ectopic fat accumulation has been documented [2]. Generally, unhealthy dietary patterns, characterised by higher energy intakes, animal source food-based diet, high-fat foods, and lower intake of dietary fibre, as well as increased consumption of added sugar (as also observed in the MHO group) have been associated with obesity and high risk of NCDs in the black South African population from the North West Province [5, 6]. In addition, higher levels of dietary SFAs (palmitic and stearic) were recorded in the overweight and obese groups (**Table 4-4**).

Our study participants were high carbohydrate consumers (**Table 4-2**) and are therefore expected to have high plasma levels of the *de novo* lipogenesis (DNL) pathway FAs, including palmitic and stearic acids, which are caused by by high doses of ingested carbohydrates and fructose [47]. A high-carbohydrate diet upregulates DNL from excess carbohydrates and protein into SFAs and MUFAs associated with unfavourable changes in metabolic traits in humans [48], while a restricted carbohydrate diet generally improves the features of MetS [49]. In our participants, the levels of plasma stearic acid were higher in the overweight/obese groups (MHO and MUO), whereas palmitic acid was lower compared to the MHNW group. One possible explanation could be that the MUO group had significantly lower carbohydrate intakes as a percentage of energy than the MHNW group. Another study that documented the plasma FAs profile of patients with MetS in Tunisia observed the opposite, with higher plasma levels of palmitic acid and lower levels of stearic acid in their participants [23]. Furthermore, results from the Prevención con Dieta Mediterránea (PreDiMed) study indicate that higher plasma levels of palmitic acid were observed in subjects presenting with MetS [24]. Levels of stearic and palmitic acids, observed in our study participants, are comparable to levels in serum and plasma phospholipid fractions reported in Korean men [30], but higher than levels in plasma reported in the PreDiMed study in Spain [24]. These differences in plasma levels are attributed to different dietary patterns associated with diverse geographical regions [50, 51].

In the present study, higher plasma levels of VLC-SFAs, (C18:0 to C22:0) were found in the overweight/obese groups (MHO and MUO) compared to the MHNW group, and persisted after adjustments for age and total energy intake. These VLC-SFAs have been associated with a lower risk of MetS [21] and a lower risk of diabetes in older adults [52]. Furthermore, Fretts and colleagues [53] described a lower risk of total mortality associated with higher VLC-SFAs in plasma phospholipids in older adults. The VLC-SFAs are a minor FA component in human tissues and blood [50] and represented less than 3% of the plasma phospholipid FAs in the adults in this study. Circulating concentrations of FAs have been shown only to function as biomarkers of dietary intake for the non-endogenously synthesised FAs [54], but circulating VLC-SFAs are not considered to be suitable markers of dietary intakes of these FAs. Therefore, the higher plasma levels of VLC-SFAs observed in the overweight/obese groups in our participants could be due to the underlying metabolic impairment compounded by an overall unhealthy lifestyle, rather than from intake of VLC-SFAs [52]. Although higher plasma levels of VLC-SFAs (C18:0 to C22:0) were found in both overweight/obese groups compared to the MHNW group, it was interesting to note that higher plasma levels of VLC-SFAs were found in the MHO group compared to the MUO group after adjustment for age and total energy intake. This finding confirms that behenic and lignoceric acids may be associated with a lower risk of MetS among the overweight and obese participants in our study, as reported in a study in China [22]. Further studies are needed in populations of black Africans to determine the sources of plasma VLC-SFAs and long-term association with metabolic health.

Palmitoleic acid in plasma has also been associated with poor metabolic health and increased risk of MetS in participants from different geographical locations [23, 24]. On the other hand, dietary oleic acid has been reported to prevent palmitate-induced metabolic impairment [55]. In this present study, lower plasma levels of *cis*-vaccenic and gondoic acids were observed in the MUO participants, and lower POA and oleic acid acids were observed in both overweight/obese groups (MHO and MUO) (**Table 64-4**). A study in the USA observed plasma and erythrocyte levels of *cis*-vaccenic and gondoic acids to be inversely associated with the odds of diabetes in men and women [56]; furthermore, *cis*-vaccenic acid in plasma phospholipids was also associated with lower diabetes risk [57]. Our study provides data to support current evidence from cross-sectional studies that low proportions of DNL FAs such as *cis*-vaccenic and gondoic acid observed in the MUO group as compared to the MHNW group are associated with an elevated risk of metabolic disorders, and might play a significant role in metabolic regulation and influence the pathogenesis of T2DM [56, 57]. MUFAs (POA, *cis*-vaccenic, and oleic acid) can all be derived

from palmitic acid through the DNL pathway, and play a role in metabolic abnormalities [58], but more prospective studies are required, especially among populations of African descent.

Higher SCD-1 and lower D5D activities in participants with MetS have been reported in diverse populations [23, 30, 59]. In our study, lower SCD-1 was observed in the overweight/obese groups (MHO and MUO) (**Table 4-4**). Higher D6D and lower D5D activity were recorded in the MUO group. The lower SCD-1 activity is supported by the lower plasma POA and OA observed in overweight/obese participants as compared to the MHNW after adjustments for age and total energy intake. Reduced D5D activity in overweight subjects with a high visceral fat distribution may result in a disturbed FA metabolism [60]. Moreover, variations in FA composition and desaturase activities may appear long before the onset of MetS [27]. These changes may be influenced by age, sex (hormonal), and even genetic factors [27, 61]. In our study, the MUO group was the oldest and had the lowest D5D activity, but the difference persisted after adjustment for age and total energy intake. Lower D5D activity was also reported in 71 year old patients with hepatic steatosis, a feature of MetS [62]. Sethom *et al.* [23] also reported lower D5D activity in MetS participants who were generally older as compared to their healthier counterparts.

Results of plasma levels of specific n-3 PUFAs in association with MetS have been inconsistent in the literature. In the present study, we observed higher plasma levels of total n-3 PUFAs in the MUO group, which persisted after adjustment for age and total energy intake (**Table 4-4**). We also observed higher plasma levels of EPA in the MUNW group (effect size 0.50) after comparison with the MHNW group. Contrary to our findings, lower plasma levels of total n-3 PUFAs were reported in patients with MetS in Tunisia [23] and in China [25]. In a different study in Canada, lower levels of EPA in plasma FA profiles were observed in participants with MetS [26], while some cross-sectional and prospective studies in diverse populations (Europe and USA) did not observe any associations of MetS with plasma n-3 PUFAs [24, 63]. These disparities in associations between n-3 PUFAs and metabolic health could conceivably be due to differences in sample size, dietary intake associated with geographical regions [50, 51], FA related metabolic processes and different tissues/lipid medium used in the extraction of these FAs. In randomised controlled trials, supplementation with n-3 LC-PUFAs could possibly improve some obesity-related MetS features, such as insulin resistance and others as outlined in a review [64]. However, considering that EPA and DHA are important nutrients and the intake of total n-3 PUFAs in our participants was lower than WHO and Food and Agricultural Organization of the United Nations recommendations [65], plasma n-3 PUFAs may not be sensitive metabolic health indicators for our participants as the status may be more reflective of endogenous metabolic pathways. This may be substantiated by the fact that dietary intake of ALA was higher in both overweight/obese

groups (MHO and MUO), but plasma phospholipids of ALA were lower, while levels of DHA were higher in both groups. This may indicate some form of up-regulation for converting dietary ALA to n-3 LC-PUFAs in our selected group of black South Africans. This endogenous response could be a result of metabolic adaptation to this health condition. Consequently, further research specifically in the black African population is required in this regard.

Total n-6 PUFAs in plasma phospholipid FAs have been associated with MetS in Asia [25] while decreased n-6 PUFAs were observed in the patients with MetS in Africa [23]. In our participants, total n-6 PUFAs were higher in both the overweight/obese (MHO and MUO) groups than in normal weight groups (MHNW and MUNW) when adjusted for age and total energy intake (**Table 4-4**). In addition, higher plasma levels of DGLA and AA were observed in both the overweight/obese groups when all the groups were compared (**Table 4-3**). This finding confirms results of a previous study that investigated African American, Chinese, Hispanic, and white adults found that obese individuals had significantly higher plasma levels of DGLA and AA compared to their healthy weight counterparts [66]. Furthermore, lower plasma levels of AA [23] and higher levels DGLA have been associated with MetS [24]. DGLA and AA form series 1 and 2 of prostaglandins which are generally viewed as anti-inflammatory and pro-inflammatory respectively [67]. In obesity and MetS, DGLA is a critical player in the synthetic pro-inflammatory pathway, and elevated levels of both DGLA and AA may contribute to inflammatory response [68]. Furthermore, from the literature the main difference between the MHO and MUO groups was linked to the degree of chronic inflammation [69] and this is the link to MetS through obesity-related insulin resistance [70]. The higher levels of plasma DGLA in the overweight/obese participants in this present study may be indicative of impaired FA metabolism due to ageing, which may also cause the accumulation of DGLA, probably as a consequence of long-term abnormal metabolic risk factors, including obesity and insulin resistance, as has also been observed elsewhere [30]. Besides, plasma DGLA is converted from dietary LA, and our participants are high consumers of LA from sunflower oil [71]. Also, higher plasma DGLA and lower D5D activities have been associated with risk of MetS in Spain and Japan [24, 59]. Levels of plasma DGLA in our MUO participants were comparable to those of patients with hepatic steatosis [62].

In summary, our study adds knowledge of levels of dietary and circulating FAs in metabolically healthy and unhealthy phenotypes in a selected group of black South Africans. High proportions of palmitic and stearic acid in plasma phospholipids were previously positively associated with MetS and metabolic markers of lipid, hepatic, inflammation and glycaemic pathways in adults [21, 22, 24]. In line with these findings we also found higher plasma stearic acids in the overweight/obese groups, but the lower levels of plasma palmitic acid in the overweight/obese

groups (MHO and MUO) compared to the MHNW group in our study participants is difficult to explain. The lower DNL pathway FAs (palmitic acids, POA and *cis*-vaccenic acid), observed in our overweight/obese (MHO and MUO) participants are different from results documented in epidemiological studies [23, 24, 30, 56]. Lower estimated SCD-1 activity observed in our overweight/obese participants compared to normal weight groups is different from what has been reported in cross-sectional studies [23, 30] and longitudinal study [27], whereas lower estimated D5D activity is consistent with scientific literature [23, 30, 59, 62, 72]. Furthermore, higher plasma VLC-SFAs observed among the MHO participants compare to the MUO group in the present study may be related to a lower risk of MetS, as has been observed elsewhere [22]. Likewise, higher serum levels of DGLA observed in this study may be related to the decreased estimated D5D activity, which is downregulated in individuals with obesity and risk of abnormal glucose metabolism [72]. In addition, visceral obesity may facilitate the conversion of persons who are MHO to progress to MUO status [73]. The high adiposity status may explain why the levels of most of the plasma phospholipid FAs observed in the MHO and MUO in our participants are comparable. Furthermore, the MUO group had higher abdominal obesity, and this may be attributed to higher dietary fat intake observed in this group. To our knowledge, no prior studies have compared the levels of both dietary and plasma phospholipid FAs with metabolic health in black South Africans.

4.7 Strengths and limitations

The major strength of this study is that information on plasma phospholipid FAs, dietary intake including dietary FAs, was explored in all the metabolically healthy and unhealthy phenotypes. Our study participants are not only high carbohydrate consumers, but are also indeed relatively low-fat consumers providing a unique prospect for exploring probable associations between the DNL pathway FAs in metabolically healthy (MHNW and MHO) and unhealthy phenotypes (MUNW and MUO). There are limitations associated with our study. The size of the cohort, randomly selected from the PURE sample, is relatively small, and therefore did not allow us to carry out sex-specific analysis, and therefore, the sex-specific associations of lipid profile and WC on our results cannot be established. Our sample size was small, because plasma phospholipid FA analysis is expensive, and we did not have sufficient funding to analyse all samples. The cross-sectional design of the study limits a conclusion about causality. We did not evaluate other biological markers such as inflammation or insulin levels associated with metabolic health. Middle aged and elderly populations are at a higher risk of degenerative diseases, and for this reason we are not able of determining whether the detected associations are causal. The estimation of desaturase activities from FAs ratios is not sufficient to conclude actual metabolic/physiological

processes within the body. However, this provided us with an indication of levels of these estimated enzyme activities and metabolic status in our black South African participants. Despite these limitations, some of our study results are in agreement with both cross-sectional and longitudinal studies from diverse populations. Unfortunately, we could only compare our results with few populations of African descent [23] due to a lack of data. Therefore, the results of this study provide some evidence needed to embark on prospective studies to establish more broadly the association of dietary and circulating FAs with metabolic health in black Africans.

4.8 Conclusion

Lower levels of DNL pathway FAs, palmitic acid, POA, oleic, *cis*-vaccenic and gondoic acids, as well as estimated desaturase activities, D5D, and SCD-1, and higher D6D activity are suggestive of biomarkers of abnormal metabolic health in our study participants. Higher plasma levels of behenic and lignoceric acids and VLC-SFAs observed in the MHNW compared to MUNW, as well as MHO compared to MUO groups may be associated with a lower risk of MetS. Prospective studies should investigate the roles of both DNL FAs and VLC-SFAs in metabolic health in an African population with diverse dietary fat, alcohol and carbohydrate intake.

Author contributions

OAA designed the research, performed the statistical analysis and drafted the manuscript with supervision from **HSK**, and **CMS**. **EWV** was responsible for the design and dietary data collection and quality control for the PURE study. **CMS** was responsible for the measurement of, processing and quality control of all fatty acids. **HSK**, **CMS**, **EVW**, **ILK** and **MZ** reviewed and edited the manuscript. All authors provided valued recommendations and approved the final version.

Competing interests

Authors declare no competing interests.

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Table 4-1: Differences in demographics and metabolic parameters of metabolically healthy and unhealthy black South African adults (n=711)

Variables ^a	Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups
	MHNW (n=345)	MUNW (n=79)	p-value ^a	Effect size ^a	MHO (n=120)	MUO (n=167)	p-value ^b	Effect size ^b	(n=711)
	Median (Q1, Q3)	Median (Q1, Q3)			Median (Q1, Q2) ^c	Median (Q1, Q2)			p-value ^c
Demographics									
Age (years)	51 (44, 58)a	51.0 (46, 57)a	0.58		49 (43, 56)a	55 (48, 65)b	<.0001	0.34	<.0001
Physical activity	2.85 (2.55, 3.27)a	2.70 (2.45, 3.13)a	0.63		2.97 (2.55, 3.30)a	2.70 (2.51, 3.03)a	0.006	0.40	0.25
Weight (kg)	52.6 (46.8, 58.3)a	55.9 (49.9, 63)b	0.001	0.38	75.8 (68.6, 86.9)b	77.3 (68.4, 86.3)b	0.50		<.0001
Body mass index (kg/m²)	19.8 (17.8, 22.0)a	21.4 (19.1, 23.3)b	0.002	0.36	30.4 (26.9, 34.1)b	31 (27.6, 34.5)b	0.50		<.0001
Waist circumference (cm)	71.7 (66.5, 76.0)a	78.4 (69.5, 83.5)b	0.001	0.31	89.5 (82.3, 94.9)b	92.6 (86, 99.5)b	0.01	0.39	<.0001
Waist to height ratio	0.44 (0.41, 0.45)a	0.47 (0.44, 0.51)b	<.0001	0.30	0.56 (0.52, 0.61)b	0.59 (0.55, 0.63)b	0.003	0.37	<.0001
Metabolic parameters									
Systolic blood pressure (mm/Hg)	129 (116, 144)a	148 (132, 164)b	<.0001	0.29	122 (112, 138)a	143 (130, 161)b	<.0001	0.25	<.0001
Diastolic blood pressure (mm/Hg)	84.0 (76.0, 93.0)a	96.0 (87.0, 108)b	<.0001	0.28	83.0 (76.0, 94.0)a	94.0 (86.0, 100)b	<.0001	0.30	<.0001
Total cholesterol (mmol/L)	4.87 (4.19, 5.77)a	6.04 (4.76, 6.98)b	0.0003	0.30	4.65 (4.06, 5.53)a	5.54 (4.71, 6.51)b	0.0002	0.32	<.0001
Low-density lipoprotein cholesterol (mmol/L)	2.99 (2.32, 3.73)a	3.64 (2.57, 4.9)b	0.001	0.34	3.21 (2.65, 3.98)b	3.77 (2.99, 4.75)b	0.005	0.38	<.0001
High-density lipoprotein cholesterol (mmol/L)	1.60 (1.22, 2.18)a	1.63 (1.39, 2.16)a	0.52		1.19 (1.05, 1.42)b	1.45 (1.07, 1.73)b	<.0001	0.36	<.0001
Triglycerides (mmol/L)	0.97 (0.75, 1.28)a	1.83 (1.14, 2.49)b	<.0001	0.21	1.04 (0.87, 1.36)a	1.72 (1.14, 2.22)b	<.0001	0.26	<.0001
Fasting plasma glucose (mmol/L)	4.70 (4.20, 5.10)a	5.60 (4.90, 5.90)b	<.0001	0.18	4.70 (4.30, 5.10)a	5.30 (4.60, 6.30)b	<.0001	0.30	<.0001
^a P-value and effect size for MHNW vs MUNW (Mann-Whitney U-test)									

Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups (n=711)
MHNW (n=345)	MUNW (n=79)			MHO (n=120)	MUO (n=167)			
Median (Q1, Q3)	Median (Q1, Q3)	p-value ^a	Effect size ^a	Median (Q1, Q2) ^c	Median (Q1, Q2)	p-value ^b	Effect size ^b	p-value ^c

Variables^a

^b P-value and effect size for MHO vs MUO (Mann-Whitney U-test)

^c P-value is indicating group differences (Kruskal-Wallis test). The letter a is considered to be similar to the reference group (MHNW), and b is considered to be different from the reference group.

Abbreviations: MHNW, metabolically healthy with normal weight; MHO, metabolically healthy and overweight/obese; MUNW, metabolically unhealthy with normal weight; MUO, metabolically unhealthy and overweight/obese. Data presented as median (interquartile ranges).

Table 4-2: Differences in quantities of dietary intake and dietary fatty acids of metabolically healthy and unhealthy black South African adults (n=711)

	Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups
	MHNW (n=345)	MUNW (n=79)	p-value ^a	Effect size ^a	MHO (n=120)	MUO (n=167)		(n=711)	
	Median (Q1, Q3)	Median (Q1, Q3)			Median (Q1, Q2) ^c	Median (Q1, Q2)	p-value ^b		Effect Size ^b
Dietary intake (per day)									
Added sugar, %E	14.1 (8.8, 20.3)a	12.7 (8.05, 17.5)a	0.18		20.7 (13.6, 28.5)b	16.8 (11.4, 23.4)a	0.03	0.41	<.0001
Alcohol, % E	1.63 (0, 9.24)a	0.78 (0, 9.43)a	0.71		0(0, 0)b	0 (0, 2.84)b	0.03	0.43	<.0001
Total carbohydrates, % E	65.2 (59.2, 72)a	62.1 (55.9, 70.5)a	0.06		64.8 (56.5, 71)a	60.4 (55.0, 66)b	0.004	0.40	<.0001
Total fibre (mg/day)	19.0 (13.0, 27.0)a	20.8 (14.7, 30.0)a	0.06		17.0 (13.7, 24.2)a	20.7 (12.0, 29.0)a	0.10		0.09
Total protein, % E	11.4 (10.3, 12.9)a	12 (10.7, 13.2)a	0.03	0.44	11.2 (10.22, 12.8)a	12.3 (10.7, 13.4)b	0.0002	0.39	0.0004
Total energy, KJ	7130 (5190, 9945)a	8096 (5816, 11237)a	0.06		6612 (5038, 9052)a	7660 (5292, 10171)a	0.01	0.44	0.02
SFA, % E	4.91 (3.36, 6.71)a	5.33 (3.41, 6.77)a	0.26		5.46 (3.62, 6.84)a	6.30 (4.58, 7.73)b	0.002	0.40	<.0001
MUFAs, % E	5.22 (3.51, 7.11)a	6.51 (4.03, 8.16)a	0.17		5.56 (3.805, 7.97)a	7.03 (5.03, 8.86)b	0.046	0.47	0.0002
PUFAs, % E	6.39 (5.02, 8.32)a	7.58 (5.1, 9.19)b	0.06		7.06 (5.04, 8.72)a	7.25 (5.41, 9.20)b	0.67		0.009
Total fat, % E	21.7 (17.05, 26.3)a	23.7 (16.8, 28.5)a	0.03	0.43	22.3 (18.2, 28.3)a	25.4 (20.4, 29.9)b	0.04	0.43	0.0001
Dietary fatty acids (mg)									
Myristic acid (C14:0)	620 (301, 1001)a	671 (304, 1104)a	0.71		678 (326, 1213)a	717 (450, 1306)b	0.67		0.15
Palmitic acid (C16:0)	4556 (2705, 7477)a	5517 (3283, 10332)a	0.17		4601 (2825, 7902)a	6324 (3921, 10045)b	0.005	0.40	<.0001
Stearic acid (C18:0)	2659 (1544, 4133)a	3049 (1712, 5314)a	0.26		2631 (1506, 4102)a	3445 (2060, 5627)b	0.005	0.39	0.0001
Behenic acid (C22:0)	0.12 (0.06, 0.21)a	0.15 (0.07, 0.25)a	0.08		0.12 (0.07, 0.2)a	0.17 (0.08, 0.25)a	0.02	0.43	0.01
Palmitoleic acid (C16:1n-7)	0.43 (0.25, 0.81)a	0.64 (0.3, 1.16)b	0.02	0.40	0.46 (0.22, 0.795)a	0.68 (0.38, 1.06)b	0.0004	0.38	<.0001
Oleic acid (C18:1n-9)	8920 (5222, 14384)a	11701 (6179, 21613)a	0.11		9807 (5167, 15204)a	12666 (7824, 18254)b	0.005	0.40	0.0004
α-linolenic acid (C18:3n-3)	164 (100, 266)a	205 (127, 380)b	0.02	0.40	186 (101, 305)a	229 (149, 330)b	0.06		0.0005

	Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups
	MHNW (n=345)		MUNW (n=79)		MHO (n=120)		MUO (n=167)		(n=711)
	Median (Q1, Q3)		Median (Q1, Q3)	p-value ^a Effect Size ^a	Median (Q1, Q2) ^c		Median (Q1, Q2)	p-value ^b Effect Size ^b	p-value ^c
Eicosapentaenoic acid (C20:5n-3)	40.0 (17.6, 71.9)a		45.5 (24.4, 103.1)a	0.26	31.3 (11.8, 62.6)a		39.6 (19.9, 77.3)a	0.50	0.22
Docosahexaenoic acid (C22:6n-3)	67.7 (26.4, 125.5)a		93.6 (42.6, 173.8)b	0.06	52.2 (18.3, 121.2)a		65.7(25.7, 123.0)a	0.25	0.13
Linoleic acid (C18:2n-6)	11697 (6744, 17160)a		13462 (7047, 23112)b	0.11	11510 (6917, 16717)a		13892 (8092, 20877)b	0.10	0.14
Arachidonic acid (C20:4n-6)	53 (27, 101)a		76 (37, 121)a	0.01 0.41	44 (22, 85)a		74 (39, 119)b	0.01 0.38	0.0006

^a P-value and effect size for MHNW vs MUNW (Mann-Whitney U-test)

^b P-value and effect size for MHO vs MUO (Mann-Whitney U-test)

^c P-value is indicating group differences (Kruskal-Wallis test). The letter a is considered to be similar to the reference group (MHNW), and b is considered to be different from the reference group.

Abbreviations: MHNW, metabolically healthy with normal weight; MHO, metabolically healthy and overweight/obese; MUNW, metabolically unhealthy with normal weight; MUO, metabolically unhealthy and overweight/obese. Data presented as median (interquartile ranges).

Table 4-3 Differences in proportions of plasma phospholipid fatty acids and estimated desaturase activities of metabolically healthy and unhealthy black South African adults (n=711)

	Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups (n=711)
	MHNW (n=345)	MUNW (n=79)			MHO (n=120)	MUO (n=167)			
	Median (Q1, Q3)	Median (Q1, Q3)	p-value ^a	Effect size ^a	Median (Q1, Q2) ^c	Median (Q1, Q2)	p-value ^b	Effect Size ^b	p-value ^c
Plasma phospholipid fatty acids (% total FAs)									
Saturated									
Myristic acid (C14:0)	0.25 (0.20, 0.31)a	0.26 (0.21, 0.32)a	0.17		0.26 (0.21, 0.31)a	0.28 (0.22, 0.32)a	0.62		0.11
Palmitic acid (C16:0)	27.2 (24.9, 29.2)a	27.4 (25.1, 29.7)a	0.53		25.2 (23.7, 27.3)b	26.3 (24.4, 28.1)b	0.005	0.43	0.0004
Stearic acid (C18:0)	14.4 (12.8, 15.8)a	14.1 (12.5, 15.7)a	0.26		15.7 (14.5, 17.2)b	15.2 (14.1, 16.4)b	0.0007	0.41	<.0001
Arachidic acid (C20:0)	0.29 (0.23, 0.36)a	0.27 (0.21, 0.32)a	0.17		0.39 (0.34, 0.45)b	0.33 (0.28, 0.40)b	<.0001	0.33	<.0001
Behenic acid (C22:0)	1.01 (0.74, 1.27)a	0.82 (0.64, 1.15)b	0.03	0.40	1.36 (1.17, 1.64)b	1.18 (0.94, 1.49)b	0.0001	0.35	<.0001
Lignoceric acid (C24:0)	1.02 (0.83, 1.28)a	0.94 (0.73, 1.08)b	0.03	0.38	1.14 (1.02, 1.35)b	1.00 (0.81, 1.24)a	<.0001	0.35	<.0001
Monounsaturated									
Palmitoleic acid (C16:1n-7)	0.78 (0.47, 1.30)a	0.86 (0.48, 1.70)a	0.26		0.55 (0.40, 0.68)b	0.66 (0.45, 0.94)b	0.001	0.38	<.0001
Cis-Vaccenic acid (C18:1n-7)	1.42 (1.24, 1.65)a	1.49 (1.16, 1.71)a	0.53		1.33 (1.12, 1.51)a	1.25 (1.10, 1.49)b	0.09		0.0001
Oleic acid (C18:1n-9)	8.99 (7.15, 11.9)a	10.2 (7.41, 13.4)a	0.06		6.92 (6.06, 7.91)b	7.45 (6.35, 9.21)b	0.02	0.40	<.0001
Gondoic acid (C20:1n-9)	0.11 (0.10, 0.13)a	0.10 (0.08, 0.12)a	0.03	0.40	0.11 (0.09, 0.12)a	0.10 (0.08, 0.11)b	0.004	0.36	<.0001
Erucic acid (C22:1n-9)	0.06 (0.05, 0.09)a	0.06 (0.04, 0.08)a	0.03	0.43	0.07 (0.06, 0.10)b	0.06 (0.04, 0.08)a	0.004	0.36	0.001
Nervonic acid (C24:1n-9)	1.70 (1.48, 2.01)a	1.56 (1.36, 1.87)b	0.02	0.40	1.95 (1.66, 2.29)b	1.76 (1.42, 2.10)a	0.002	0.38	<.0001
n-3 polyunsaturated fatty acids									
α-linolenic acid (C18:3n-3)	0.08 (0.06, 0.12)a	0.09 (0.06, 0.12)a	0.17		0.08 (0.05, 0.11)b	0.08 (0.06, 0.10)b	0.85		0.25
Eicosapentaenoic acid (C20:5n-3)	0.59 (0.43, 0.86)a	0.69 (0.46, 0.88)a	0.03	0.50	0.52 (0.38, 0.75)b	0.63 (0.45, 0.87)a	0.02	0.39	0.01
Docosapentaenoic acid (C22:5n-3)	1.41 (1.16, 1.64)a	1.40 (1.26, 1.57)a	0.71		1.38 (1.14, 1.59)a	1.38 (1.13, 1.57)a	0.85		0.90
Docosahexaenoic acid (C22:6n-3)	4.05 (3.16, 5.12)a	4.09 (3.52, 5.35)a	0.52		4.92 (3.71, 5.67)b	4.95 (4.05, 5.95)b	0.85		<.0001
Total-n-3	6.31 (5.14, 7.4)a	6.57 (5.73, 7.82)a	0.25		6.76 (5.60, 8.0)a	6.97 (6.11, 8.39)b	0.37		<.0001

	Normal weight groups (n=424)				Overweight/obese groups (n=287)				All groups (n=711)
	MHNW (n=345)	MUNW (n=79)	p-value ^a	Effect size ^a	MHO (n=120)	MUO (n=167)	p-value ^b	Effect Size ^b	
	Median (Q1, Q3)	Median (Q1, Q3)			Median (Q1, Q2) ^c	Median (Q1, Q2)			
n-6 polyunsaturated fatty acids									
Linoleic acid (C18:2n-6)	16.2 (13.4, 18.5)a	14.6 (12.9, 18.3)a	0.02	0.44	16.8 (14.7, 19.1)a	15.9 (13.5, 18.3)a	0.14		0.06
γ-linolenic acid (C18:3n-6)	0.11 (0.08, 0.18)a	0.12 (0.08, 0.17)a	0.38		0.09 (0.06, 0.11)b	0.10 (0.08, 0.15)b	0.02	0.38	<.0001
Eicosadienoic acid (C20:2n-6)	0.36 (0.30, 0.41)a	0.33 (0.29, 0.39)a	0.02	0.41	0.39 (0.33, 0.44)b	0.37 (0.31, 0.43)a	0.01	0.43	0.0005
Dihomo-γ-linolenic acid (C20:3n-6)	2.71 (2.39, 3.20)a	2.82 (2.45, 3.32)a	0.26		3.02 (2.60, 3.48)b	3.22 (2.75, 3.82)b	0.10		<.0001
Mead acid (C20:3n-9)	0.23 (0.16, 0.38)a	0.28 (0.15, 0.42)a	0.26		0.17 (0.12, 0.25)b	0.18 (0.13, 0.28)b	0.50		<.0001
Arachidonic acid (C20:4n-6)	13.2 (11.7, 14.8)a	13.4 (11.7, 14.7)a	0.53		14.4 (12.7, 15.5)b	14.1 (12.6, 15.8)b	0.60		0.01
Docosadienoic (C22:2n-6)	0.02 (0.01, 0.03)a	0.02 (0.01, 0.02)b	0.002	0.46	0.03 (0.02, 0.03)b	0.02 (0.02, 0.03)a	0.007	0.36	<.0001
Adrenic acid (C22:4n-6)	0.59 (0.49, 0.71)a	0.59 (0.50, 0.70)a	0.90		0.58 (0.46, 0.69)a	0.57 (0.46, 0.67)b	0.44		0.40
Osbond acid (C22:5n-6)	0.56 (0.43, 0.75)a	0.59 (0.46, 0.74)a	0.26		0.54 (0.39, 0.79)a	0.57 (0.41, 0.75)a	0.37		0.56
Total n-6	34.3 (31.2, 37.5)a	33.9 (30.6, 37.1)a	0.53		36.7 (33.75, 38.8)b	35.8 (32.5, 37.6)b	0.01	0.43	<.0001
n-9 polyunsaturated fatty acid									
Mead acid (C20:3n-9)	0.23 (0.16, 0.38)a	0.28 (0.15, 0.42)a	0.26		0.17 (0.12, 0.25)b	0.18 (0.13, 0.28)b	0.50		<.0001
Trans-fatty acid									
Elaidic acid (C18:1n-9T)	0.33 (0.21, 0.64)a	0.26 (0.19, 0.58)a	0.03	0.43	0.38 (0.26, 0.71)a	0.26 (0.19, 0.50)a	0.0001	0.38	0.0002
Estimated desaturase activities									
SCD-16 (POA/PA)	0.03 (0.02, 0.05)a	0.033 (0.02, 0.06)a	0.71		0.02 (0.016, 0.03)b	0.03 (0.02, 0.04)b	0.001	0.38	<.0001
SCD-18 (OA/SA)	0.60 (0.46, 0.91)a	0.74 (0.48, 1.01)a	0.03	0.43	0.45 (0.37, 0.52)b	0.49 (0.42, 0.61)b	0.002	0.39	<.0001
D6D (DGAL/LA)	0.18 (0.14, 0.22)a	0.20 (0.16, 0.22)a	0.03	0.42	0.18 (0.16, 0.22)a	0.22 (0.17, 0.25)b	0.05	0.38	<0.0001
D5D (AA/DGLA)	4.77 (4.12, 5.45)a	4.61 (3.99, 5.30)a	0.03	0.45	4.56 (3.91, 5.44)a	4.30 (3.68, 5.18)b	0.005	0.37	0.045

^a P-value and effect size for MHNW vs MUNW (Mann-Whitney U-test)

^b P-value and effect size for MHO vs MUO (Mann-Whitney U-test)

^c P-value is indicating group differences (Kruskal-Wallis test). The letter **a** is considered to be similar to the reference group (MHNW), and **b** is considered to be different from the reference group.

Abbreviations: MHNW, metabolically healthy with normal weight; MHO, metabolically healthy and overweight/obese; MUNW, metabolically unhealthy with normal weight; MUO, metabolically unhealthy and overweight/obese. Data presented as median (interquartile ranges).

Table 4-4 Differences in dietary and plasma phospholipid fatty acids of metabolically healthy and unhealthy black South African adults controlled for age and total energy intake

	Normal weight status (n=424)		Overweight/obese groups (n=287)		P-value
	MHNW (n=345)	MUNW (n=79)	MHO (n=120)	MUO (n=167)	
Least squares means (95% CI)					
Dietary fatty acids (mg)					
Myristic acid (C14:0)	774.5 (714.94, 834.1)a	646.8 (522.1, 771.4)a	941.0 (839.6, 1042.4)b	936.9 (850.0, 1023.8)b	<.0001
Palmitic acid (C16:0)	5681 (5362, 6001)a	5980.7 (5313, 6649)a	6455.5 (5911, 7000)b	7135.2 (6669, 7601)b	<.0001
Stearic acid (C18:0)	3223.8 (3035, 3412)a	3169.8 (2775, 3564)a	3562.6 (3242, 3883)b	4021.8 (3747, 4297)b	<.0001
Behenic acid (C22:0)	0.16 (0.15, 0.18)a	0.16 (0.13, 0.20)a	0.17 (0.15, 0.20)a	0.19 (0.16, 0.21)a	<.0001
Palmitoleic acid (C16:1n-7)	0.60 (0.56, 0.64)a	0.66 (0.58, 0.75)a	0.65 (0.58, 0.72)a	0.74 (0.68, 0.80)b	<.0001
Oleic acid (C18:1n-9)	11211 (10552, 11869)a	11896 (10518, 13274)a	12602 (11481, 13723)a	13874 (12914, 14835)b	<.0001
α-linolenic acid (C18:3n-3)	201 (191, 212)a	219 (197, 241)a	236 (218, 254)b	257 (242, 272)b	<.0001
Eicosapentaenoic acid (C20:5n-3)	52.0 (47.3, 56.7)a	58.6 (48.7, 68.4)a	51.3 (43.3, 59.3)a	50.9 (44.1, 57.9)a	<.0001
Docosahexaenoic acid (C22:6n-3)	88.4 (80.0, 96.8)a	103.3 (85.7, 121.0)a	90.6 (76.3, 105.0)a	87.8 (75.5, 100.0)a	<.0001
Linoleic acid (C18:2n-6)	13047 (12374, 13720)a	14444 (13035, 15852)a	14260 (13115, 15406)a	14925 (13944, 15907)b	<.0001
Arachidonic acid (C20:4n-6)	69.9 (64.6, 75.1)a	74.9 (63.9, 85.8)a	70.0 (61.1, 79.0)a	84.6 (77.0, 92.3)b	<.0001
Plasma phospholipid fatty acids (% of total FAs)					
Saturated					
Myristic acid (C14:0)	0.25 (0.25, 0.28)a	0.26 (0.24, 0.28)a	0.26 (0.25, 0.28)a	0.27 (0.26, 0.28)a	0.0005
Palmitic acid (C16:0)	27.3 (27.0, 27.7)a	27.2 (26.4, 27.9)a	26.1 (25.5, 26.7)b	26.3 (25.8, 26.8)b	<.0001
Stearic acid (C18:0)	14.6 (14.4, 14.9)a	14.3 (13.7, 14.8)a	16.2 (15.8, 16.7)b	15.7 (15.3, 16.1)b	<.0001
Arachidic acid (C20:0)	0.31 (0.29, 0.32)a	0.28 (0.26, 0.30)a	0.40 (0.38, 0.41)b	0.34 (0.33, 0.36)b	<.0001
Behenic acid (C22:0)	1.06 (1.01, 1.10)a	0.94 (0.86, 1.04)a	1.39 (1.33, 1.47)b	1.22 (1.15, 1.28)b	<.0001
Lignoceric acid (C24:0)	1.08 (1.04, 1.11)a	0.95 (0.88, 1.02)b	1.19 (1.13, 1.24)b	1.04 (0.99, 1.09)a	<.0001

	Normal weight status (n=424)		Overweight/obese groups (n=287)		P-value
	MHNW (n=345)	MUNW (n=79)	MHO (n=120)	MUO (n=167)	
Least squares means (95% CI)					
Monounsaturated					
Palmitoleic acid (C16:1n-7)	0.99 (0.93, 1.06)a	1.10 (0.97, 1.23)a	0.62 (0.51, 0.73)b	0.78 (0.69, 0.87)b	<.0001
Cis-vaccenic acid (C18:1n-7)	1.47 (1.41, 1.52)a	1.60 (1.48, 1.72)a	1.35 (1.25, 1.44)a	1.31 (1.23, 1.40)b	0.0002
Oleic acid (C18:1n-9)	9.71 (9.40, 10.0)a	10.5 (9.83, 11.1)a	7.24 (6.71, 7.78)b	8.06 (7.65, 8.51)b	<.0001
Gondoic acid (C20:1n-9)	0.111 (0.108, 0.114)a	0.104 (0.10, 0.11)a	0.109 (0.105, 0.114)a	0.997 (0.096, 0.104)b	<.0001
Erucic acid (C22:1n-9)	0.07 (0.07, 0.072)a	0.06 (0.06, 0.07)a	0.08 (0.07, 0.09)b	0.07 (0.06, 0.07)a	<.0001
Nervonic acid (C24:1n-9)	1.76 (1.72, 1.81)a	1.64 (1.55, 1.74)a	1.97 (1.89, 2.04)b	1.76 (1.70, 1.83)a	<.0001
n-3 polyunsaturated fatty acids					
α-linolenic acid (C18:3n-3)	0.10 (0.09, 0.10)a	0.09 (0.08, 0.10)a	0.08 (0.08, 0.09)b	0.08 (0.08, 0.09)b	0.047
Eicosapentaenoic acid (C20:5n-3)	0.70 (0.66, 0.75)a	0.73 (0.64, 0.82)a	0.59 (0.52, 0.67)b	0.73 (0.67, 0.79)a	0.0007
Docosapentaenoic acid (C22:5n-3)	1.43 (1.39, 1.47)a	1.41 (1.33, 1.49)a	1.37 (1.30, 1.43)a	1.37 (1.31, 1.42)a	0.06
Docosahexaenoic acid (C22:6n-3)	4.11 (3.97, 4.26)a	4.41 (4.12, 4.72)a	4.71 (4.47, 4.96)b	5.07 (4.86, 5.28)b	<.0001
Total n-3	6.34 (6.16, 6.52)a	6.66 (6.27, 7.04)a	6.75 (6.45, 7.08)a	7.27 (6.98, 7.52)b	<.0001
n-6 polyunsaturated fatty acids					
Linoleic acid (C18:2n-6)	15.9 (15.6, 16.3)a	15.4 (14.7, 16.2)a	16.5 (15.9, 17.1)a	15.9 (15.4, 16.5)a	0.04
γ-linolenic acid (C18:3n-6)	0.13 (0.12, 0.14)a	0.13 (0.12, 0.14)a	0.09 (0.08, 0.11)b	0.11 (0.10, 0.12)b	<.0001
Eicosadienoic acid (C20:2n-6)	0.36 (0.36, 0.37)a	0.34 (0.33, 0.36)a	0.39 (0.37, 0.41)b	0.38 (0.36, 0.39)a	<.0001
Dihomo-γ-linolenic acid (C20:3n-6)	2.79 (2.72, 2.88)a	2.92 (2.77, 3.07)a	3.07 (2.95, 3.20)b	3.26 (3.15, 3.37)b	<.0001
Arachidonic acid (C20:4n-6)	13.3 (13.0, 13.5)a	13.4 (12.8, 13.9)a	14.0 (13.6, 14.4)b	14.1 (13.7, 14.4)b	<.0001
Docosadienoic acid (C22:2n-6)	0.21 (0.20, 0.02)a	0.02 (0.015, 0.02)b	0.03 (0.025, 0.029)b	0.23 (0.021, 0.024)a	<.0001
Adrenic acid (C22:4n-6)	0.62 (0.60, 0.63)a	0.61 (0.58, 0.65)a	0.59 (0.56, 0.62)a	0.58 (0.55, 0.60)b	<.0001
Osbond acid (C22:5n-6)	0.62 (0.59, 0.65)a	0.65 (0.59, 0.71)a	0.61 (0.56, 0.66)a	0.62 (0.58, 0.67)a	0.07
Total n-6	33.7 (33.2, 34.2)a	33.5 (32.4, 34.5)a	35.3 (34.4, 36.2)b	35.0 (34.2, 35.7)b	<.0001
n-9 polyunsaturated fatty acid					

	Normal weight status (n=424)		Overweight/obese groups (n=287)		P-value
	MHNW (n=345)	MUNW (n=79)	MHO (n=120)	MUO (n=167)	
	Least squares means (95% CI)				
Mead acid (C20:3n-9)	0.30 (0.28, 0.32)a	0.34 (0.30, 0.38)a	0.21 (0.17, 0.24)b	0.22 (0.19, 0.25)b	<.0001
Trans-fatty acid					
Elaidic acid (C18:1n-9T)	0.76 (0.64, 0.88)a	0.67 (0.42, 0.92)a	0.80 (0.60, 1.00)a	0.62 (0.45, 0.80)a	0.56
Estimated desaturase activities					
SCD-16 (POA/PA)	0.04 (0.03, 0.04)a	0.04 (0.036, 0.044)a	0.02 (0.02, 0.03)b	0.03 (0.026, 0.032)b	<.0001
SCD-18 (OA/SA)	0.71 (0.68, 0.74)a	0.78 (0.71, 0.84)a	0.46 (0.41, 0.52)b	0.54 (0.49, 0.58)b	<.0001
D6D (GLA/LA)	0.008 (0.008, 0.009)a	0.009 (0.008, 0.010)a	0.006 (0.005, 0.007)b	0.007 (0.006, 0.008)b	<.0001
D5D (AA/DGLA)	4.93 (4.79, 5.10)a	4.75 (4.43, 5.07)a	4.75 (4.50, 5.01)a	4.61 (4.39, 4.83)b	0.008
The letter a is considered to be similar to the reference group (MHNW), and b is considered to be different from the reference group. P-value is indicating group differences (ANCOVA test). Abbreviations: MHNW, metabolically healthy with normal weight; MHO, metabolically healthy and overweight/obese; MUNW, metabolically unhealthy with normal weight; MUO, metabolically unhealthy and overweight/obese. Data presented as least squares means, 95% CI.					

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CHAPTER 5 MANUSCRIPT 2

5.1 Plasma phospholipid fatty acid patterns are associated with adiposity and metabolic syndrome in black South Africans: A cross-sectional study

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Table 5-1: Details of authors

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5.2 Abstract

Background: Diets rich in n–6 polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFA) have been associated with increased risk of obesity and metabolic syndrome (MetS), whereas diets high in n–3 long-chain (LC)-PUFAs are associated with lower risk. There is limited information about the association between plasma phospholipid fatty acids (FAs) with obesity and MetS among black South Africans.

Objective: To investigate the association of dietary FAs and plasma phospholipid FA patterns, respectively, with measures of adiposity (body mass index, waist circumference, waist-to-height ratio) and MetS in black South Africans.

Design: Factor analysis was used to identify FA patterns from 11 dietary FAs and 26 individual plasma phospholipid FAs, respectively. Cross-sectional association of the identified patterns with measures of adiposity and MetS was investigated.

Participants and setting: A random sample of 711 black South African adults aged 30 to 70 years (273 men, 438 women) from the North West Province was selected from the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study.

Statistical analysis: Sequential regression models adjusted for confounders were applied to investigate the association between dietary FAs and plasma phospholipid FA patterns with measures of adiposity and MetS.

Results: Two patterns were derived from dietary FAs and six patterns from plasma phospholipid FAs that explained the cumulative variance of 89% and 73%, respectively. The association between FA patterns with adiposity and MetS, respectively, was weaker for dietary FA patterns than for plasma phospholipid FA patterns. The plasma phospholipid FA pattern with high loadings of saturated FAs (high-Satfat) and another with high loadings of n–3 very-long-chain PUFAs (n–3 VLC-PUFA) were positively associated with measures of adiposity and MetS, while patterns with positive loadings of LC monounsaturated fatty acids (n–9 LC-MUFA) and a positive loading of n–3 essential FA (n–3 EFA) showed inverse associations with MetS and some measures of adiposity.

Conclusions: The n–9 LC-MUFA and n–3 EFA patterns seem to provide possible protective associations with adiposity and MetS, whereas the high-Satfat and n–3 VLC-PUFA patterns were associated with adiposity and MetS in our study participants.

Five keywords: Phospholipid fatty acid patterns, dietary fatty acid patterns, adiposity, metabolic syndrome, waist: height ratio

5.3 Introduction and background

South Africa is currently experiencing rapid nutritional, economic, demographic and epidemiological transitions with likely consequences for lifestyle and health (1). The prevalence of overweight and obesity in South Africa was 31% in men and 64% for women in 2012 (2). This increased to 68% in women in 2016, but remained the same for men (3). Abdominal obesity among black South African women is particularly associated with elevated blood pressure (BP), lower high-density lipoprotein cholesterol (HDL), higher serum triglycerides, and elevated fasting plasma glucose, indicative of insulin resistance (4). Unhealthy diet is a major risk factor associated with the rising prevalence of obesity and metabolic syndrome (MetS) (5, 6).

Fat intake among the black urban population of South Africa increased from 16.4% to 26.2% of total energy over the past 50 years (7). The transition from more traditional to Western diets, characterised by an increase in n–6 polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), industrial *trans*-fatty acids (FAs) (8), as well as a decrease in n–3 PUFAs intake, is also prevalent in this population (9). Diets high in percentage energy from animal protein and total fat intake may increase the risk of NCDs in rural and urban black South Africans (10), and this may be related to meat intake, which is a major source of both MUFAs and SFA in South Africans (11). In contrast with this, however, a study that investigated dietary intake of carbohydrate and SFAs in 18 countries undergoing rapid change in nutrition transition documented that SFAs intake was associated with lower risk of mortality (12).

Studies investigating circulating FAs have also reported some conflicting results. A recent study examined the relationship between body mass index (BMI) and plasma phospholipid FAs composition in men aged between 48 and 65 years and reported higher plasma phospholipid levels of palmitic acid (C16:0) and stearic acid (C18:0) in obese individuals (13). Furthermore, plasma concentrations of C16:0 were positively associated with risk of total mortality in men and women in a prospective study in the USA (14). SFAs, myristic acid (C14:0), C16:0 and C18:0 in plasma were positively associated with MetS, while longer chain SFAs, arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0) were inversely associated in men and women from Taiwan (15). Another study also reported lower levels of plasma C22:0 and C24:0 in MetS participants (16). Palmitoleic acid (C16:1n-7) in plasma phospholipids was positively associated with BMI in men and women (13, 17) and higher levels of plasma C16:1n-7 were associated with multiple metabolic risk factors in men and

women (18, 19). In different populations, total n–3 FAs in plasma were associated with lower BMI, waist circumference (WC) and hip circumference (20) and inversely associated with MetS (21), while omega-6 polyunsaturated fatty acids (PUFA) have been associated with obesity and MetS. Pickens and colleagues (13) reported higher plasma phospholipid levels of dihomo- γ -linolenic acid (C20:3n-6) in overweight and obese individuals. Positive associations of serum phospholipid C20:3n-6 with BMI, as well as total n–6 PUFAs with waist-hip ratio were also documented in a study of Mexican women (17). Some studies also report positive associations of specific plasma phospholipids n–6 PUFAs with metabolic risk (18, 22), while other studies report inverse associations of total n–6 PUFAs in erythrocytes and serum, respectively, with MetS (23, 24). Due to inconsistent results in different studies relating to circulating n–6 PUFAs, further research to understand their role in association with obesity and MetS is highly recommended (25).

Since people consume food rather than individual nutrients, it is difficult to isolate the individual nutrients in the diet and link them to disease and health (26). Therefore, the analysis of food intakes into patterns derived from various combinations of nutrients or foods has developed as a preferred alternative to investigating associations between nutrients and diseases (27). Several studies have applied factor and cluster analysis to derive patterns from food and tissues in investigating the association of these patterns with health and diseases (28). FA patterns from adipose tissue and plasma have been employed to describe associations of FAs with obesity (29) and MetS (22, 30). Despite the extensive use of plasma FAs in research, there is limited epidemiological research on the use of both dietary and circulating FA patterns in association with obesity and MetS in black populations in Africa. To address the key gaps in the current knowledge, the aim of this study was to investigate the associations of dietary and plasma phospholipid FA patterns, respectively, with adiposity measures [BMI, WC and waist-to-height ratio (WtHR)], and MetS in a selected group of black South African adults. The present study is based on a random sub-sample of 711 participants selected from the South African site (North West Province) of the multi-country Prospective Urban and Rural Epidemiological (PURE) study. This study will make use of cross-sectional data collected at baseline during the months of August to November 2005.

5.4 Materials and methods

5.4.1 Participants and setting

A sub-sample of 711 black South African participants were randomly selected from 2 010 adults recruited at baseline (the year 2005), from urban (1 004) and rural (1 006) households in the North West Province, to assess dietary FA intake and plasma phospholipid FA status.

Those included were apparently healthy subjects older than 30 years at baseline, with no reported diseases of lifestyle, TB or HIV, and used chronic medication for diabetes and hypertension only. Ethical approval for the South African PURE study was obtained from the Ethics Committee of North-West University (Ethics number 04M10). Participants provided written informed consent and participation was voluntarily.

5.4.2 Data collection and measurements

Transportation was provided for the study subjects to reach the data collection centres in both rural and urban areas. During face-to-face interviews by trained fieldworkers, each participant completed questionnaires in his or her preferred language (Afrikaans, Setswana or English). The questionnaires included demographic (31), physical activity (32) and quantitative food-frequency questions (QFFQ (33, 34), and made use of, among others, validated food photo-books to estimate portion sizes (35). Reproducibility (33) and details of dietary assessments have been published elsewhere (10).

5.4.3 Estimation of dietary nutrients

Dietary macronutrients and FAs were calculated using the South African Medical Research Council food composition tables (36).

Twenty-eight dietary FAs were included initially, but FAs that had a daily median intake of less than 0.10 mg were excluded. A total of 11 dietary FAs were used to derive FA patterns for investigation in this study.

5.4.4 Anthropometry

Anthropometric measurements were performed by trained research assistants according to standards prescribed by the International Society for the Advancement of Kinanthropometry (37). A portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) was used to measure weight. Height was measured using a calibrated stadiometer (Seca, Hamburg, Germany). Waist and hip circumferences were recorded using steel tapes (Lufkin, Apex, NC, USA). BMI and WHtR were calculated using weight (kg)/height (m²) and waist (cm)/height (cm) formulas, respectively. Blood pressure (mmHg) was measured in duplicate (5 minutes apart) on the right upper arm. Appropriately sized cuffs were used for obese subjects.

5.4.5 Laboratory measurements

Fasting blood samples were collected from the antecubital vein with a sterile winged infusion set with minimal stasis. The samples were collected, plasma and serum prepared and aliquoted, by a registered nurse and then stored at -80°C in the urban areas. In rural areas, the samples were stored at -18°C up to five days, where after it was transported to the laboratory facility and stored at -80°C until analysed. Fasting plasma glucose concentration was determined by the hexokinase method using the Synchron® system (Beckman Coulter Co., Fullerton, CA, USA). The Sequential Multiple Analyser Computer (SMAC), using the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland), performed quantitative determination of HDL, triglycerides and total cholesterol (TC). Low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald equation (38).

EDTA plasma samples were thawed and extracted with chloroform:methanol (2:1 v/v) according to the modified Folch method (39). The plasma phospholipid fatty acid fraction was isolated by thin layer chromatography from the extracted lipids (40). Subsequently, the phospholipid fatty acid fraction was transmethylated to fatty acid methyl esters and analysed by quadrupole gas chromatography electron ionization mass spectrometry by means of an Agilent Technologies 7890 A GC system, as described by Baumgartner *et al.* (40). Thirty-two FAs were measured in fasted plasma samples from 711 participants. Six FAs, i.e. pentadecanoic acid (C15:0), margaric acid (C17:0), trans-vaccenic acid (C18:1n-7t), rumenic acid (C18:2n-7tt), stearidonic acid (C18:4n-3), and eicosatrienoic (C20:3n-3) were below the limit of quantification and therefore not included. The remaining 26 plasma phospholipid FAs were quantified and expressed as a percentage of total FAs. Quality of data was assured with a separate calibration for each fatty acid, monitoring of internal standard (1,2 – diheptadecanoyl – sn – glycerol – 3 phosphorylcholine, Matreya, Pennsylvania, USA) and Levey Jennings graphs for a pooled plasma control analysed with each batch.

5.4.6 Definition of metabolic syndrome

MetS was defined according to recommendations by the Joint Interim Statement of six international associations as the presence of three or more of the following: (i) fasting plasma glucose levels ≥ 5.6 mmol/l or the use of oral hypoglycaemic medication; (ii) serum triglycerides ≥ 1.7 mmol/l; (iii) serum HDL ≤ 1.0 mmol/l for men and ≤ 1.3 mmol/l for women; (iv) BP $\geq 130/85$ mmHg or the use of BP medication; and (v) WC of ≥ 94 cm for men and ≥ 80 cm for women (41).

5.4.7 Statistical analysis

Continuous variables were described as medians and interquartile ranges if data deviated from the normal distribution according to the Kolmogorov-Smirnov test, whereas categorical variables were presented as percentages. Non-normally distributed data were log transformed before inclusion in regression models. Participants' characteristics were compared by sex and BMI categories using the Mann-Whitney U-test or chi-squared test for continuous and categorical variables, respectively. Differences between individual FAs and ratios by BMI and sex groups were tested by the Mann-Whitney test. A BMI < 25 kg/m² was considered as underweight and/or normal-weight or lean, whereas BMI ≥ 25 kg/m² was considered overweight and/or obese. The effect size of the differences between groups was calculated using the Man-Whitney U-value and sample size of the groups (42).

Principal-component-based varimax factor analysis of the correlation matrix was used to define dietary FA (based on the QFFQ) and plasma phospholipid FA patterns. The identification and naming of 11 dietary FAs and 26 plasma phospholipid FAs used in this study are based on relevant literature and the levels of specific FAs observed in our population (43). The number of factors to retain was established by the Kaiser criterion (eigenvalues > 1) and scree-plot visual inspection. Loadings with absolute values higher than 0.5 were considered as relevant for the contribution to each FA pattern. The associations between FA patterns and outcomes were evaluated by sequential regression models, logistic regression for the dichotomous outcome (MetS), and generalised linear models for continuous outcomes (WC, BMI and WHtR). The first step of the sequential modelling analyses was based on models that contained only dietary FAs or plasma phospholipid FA patterns and was referred to as a crude model. The crude model was then adjusted for sex and age (adjusted model₁). This model was further adjusted for lifestyle confounders, including the level of education, physical activity, alcohol and total energy intake, and self-reported smoking status creating a fully adjusted model. We further adjusted this model for contraceptives (adjusted for in plasma phospholipid FA patterns models only) and dietary factors, including total fats, carbohydrates, dietary fibre and energy from added sugar as individual confounders and as combined covariates. Model fitting was evaluated using the adjusted R-square for linear regression and maximum re-scaled R-square statistic for logistic regression. Linear regression results are presented as standardised β and 95% confidence intervals (CI) with their significance levels, and odds ratio and 95% CI with significance levels for logistic regression. All analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA) (44) and p < 0.05 was considered significant.

5.5 Results

5.5.1 Demographic characteristics, health and dietary intake data

The baseline characteristics of the 711 participants are shown in **Table 4-2**. The majority were women (61.6%), and the median age was comparable between men and women. Men had higher HDL levels and were more likely to smoke. In contrast, the women had higher serum triglycerides, as well as higher levels of measures of adiposity ($p < 0.0001$). The dietary intake data revealed that men had higher total energy, total carbohydrate and total protein intakes, whereas the women, on the other hand, had higher total dietary fibre intake.

5.5.2 Differences between total energy, dietary fatty acids and plasma phospholipid fatty acids within BMI categories structured by sex

Table 4-3 shows that total intakes of fat, SFAs, and MUFAs were significantly higher in overweight/obese women compared to lean women. Plasma phospholipid FAs within SFAs, MUFAs and PUFAs classes differed across BMI categories and sex. In men, the plasma levels of SFAs, C18:0, C20:0, C22:0, C24:0 and n-6 PUFA C20:3n-6 were significantly higher in overweight men than in lean men, whereas C16:0, C18:3n-6 and MUFAs, C16:1n-7, *cis*-vaccenic acid (C18:1n-7), oleic acid (C18:1n-9) and gondoic acid (C20:1n-9) were higher in lean than in overweight men. Although significant differences were observed for these FAs between overweight and lean groups in men, most had a small effect size of ~ 0.30 to 0.40.

Plasma levels of similar SFAs, i.e. C18:0, C20:0, C22:0, C24:0, as well as nervonic acid (C24:1n-9), docosahexaenoic acid (C22:6n-3), C20:3n-6, arachidonic acid (C20:4n-6) and docosadienoic acid (C22:2-n6) were higher in overweight women than in their lean counterparts. Similar MUFAs, i.e. C16:1n-7, C18:1n-7, C18:1n-9, C20:1n-9, but also mead acid, were higher in lean than in overweight women. In women, small effect sizes (~ 0.35-0.44) were found for most FAs, except for C24:0, which had a medium effect size of 0.45.

5.5.3 Identification of fatty acid patterns

The factor analysis identified two dietary FAs and six plasma phospholipid FA patterns according to the Kaiser criterion and scree-plot visual inspection. Results are shown in **Table 4-4**. Eleven dietary FAs and 26 phospholipid FAs were entered into the analysis. The factors generated explained 89% of the cumulative variance in dietary FA patterns, and 73% in plasma phospholipid FA patterns. The Kaiser's measure of sampling adequacy was 0.84 and 0.78 for the dietary FAs and plasma phospholipid FA patterns, respectively. Loadings with absolute values higher than 0.5 were considered relevant for the contribution to each FA

pattern. The patterns are characterised and named according to the highest loadings of the specific FAs present in a given pattern.

Among dietary FAs, the first extracted pattern presented with high positive loadings of saturated FAs, MUFAs, α -linolenic acid (C18:3n-3) and n-6 FAs, and therefore was named the “*non-marine*” FA pattern. The second pattern was named the “*marine*” FA pattern because it was characterised by high positive loadings of eicosapentaenoic acid (C20:5n-3) and C22:6n-3.

The six plasma phospholipid FA patterns are discussed in the order in which they were derived. The first pattern presented with positive loadings of LC-SFAs, C18:0, C20:0, C22:0 and C24:0 and very high negative loadings of C16:1n-7, C18:1n-7, C18:1n-9; we named it “*high-Satfat*” pattern. The second pattern was named “*n-3 VLC-PUFA*” and presented with high positive loadings of docosapentaenoic acid (C22:5n-3), C22:6n-3, C20:5n-3 as well as C20:4n-6. The third pattern presented the highest positive loadings of C18:2n-6 and eicosadienoic acid (C20:2n-6) and was named accordingly as the “*high-LA*” pattern. The fourth pattern was named as “*n-6 VLC-PUFA*” since it was characterised with high positive loadings of adrenic acid (C22:4n-6), C22:2n-6 and C20:3n-6. The fifth pattern extracted was named as “*n-9 LC-MUFA*” pattern and presented with positive loadings of C24:1n-9 and gondoic acid (C20:1n-9). The sixth and last pattern had a positive loading of one FA, i.e. C18:3n-3, and we named it “*n-3 EFA*” pattern.

5.5.4 Association of dietary fatty acids and plasma phospholipid FA patterns with adiposity and metabolic syndrome

Dietary FA patterns were weakly associated with measured outcomes (**Table 4-5**). The non-marine FA pattern showed marginal positive associations with WC in the crude model and the association remained marginal after adjusting for age and sex ($\beta = 0.06$, 95% CI = -0.01, 0.13, $p = 0.09$). The association was lost after adjustment for lifestyle variables and energy intake. On the other hand, we did not find any associations with the marine FA pattern (**Table 4-5**). Neither pattern revealed any association with BMI, WHtR or MetS. Further adjustment to the regressions for total fat, fibre, carbohydrates and added sugar did not result in any significant associations. The variables in the adjusted models explained 0.02 to 27% of the variation in measures of adiposity and 0.4 to 20% of the variation in MetS.

Plasma phospholipid FA patterns resulted in stronger associations with measures of adiposity and MetS (**Table 4-6**). The high-Satfat and n-3 VLC-PUFA patterns were positively associated with all measures of adiposity and MetS. The associations remained significant in the fully

adjusted model. The omega-6 VLC-PUFA pattern showed marginal and positive associations with WC and WHtR in the crude model, but associations were lost after further adjustments. This pattern also showed higher odds for having the MetS and remained significantly associated in the fully adjusted model (odds ratio, OR = 1.25, 95% CI = 1.02, 1.54, $p = 0.03$). The n-9 LC-MUFA pattern was inversely associated with WC and WHtR in the crude model as well as after adjustment for age and sex. The associations were, however, lost after adjustments for lifestyle variables and energy intake. This pattern also showed lower odds for having the MetS and remained significantly associated in the fully adjusted model (OR = 0.61, 95% CI = 0.50, 0.75, $p < .0001$). The omega-3 EFA pattern showed an inverse association with BMI, WC and WHtR, but in the fully adjusted model marginal significance remained for BMI only. This pattern also showed lower odds for having MetS and remained significantly associated in the fully adjusted model (OR = 0.81, 95% CI = 0.66, 0.99, $p = 0.04$). The variables in all the adjusted models explained 14 to 34% of the variation in measures of adiposity, and 18 to 31% of the variation in MetS.

We further adjusted all regression models for use of contraceptives and intakes of total fat, fibre, carbohydrates, and energy from added sugar in association with plasma phospholipid FAs. Additional adjustment for these variables did not result in different associations with anthropometric indices. The association between high-LA pattern and MetS remained marginally significant after adjusting for additional variables, whereas the associations with the n-6 VLC-PUFA and n-3 EFA patterns were lost.

5.6 Discussion

The results of this study add new information about identified FA patterns both in diet and plasma phospholipids among a selected group of black South Africans from the North West Province. We identified for the first time two dietary FA patterns and six plasma phospholipid FA patterns (**Table 4-4**) by means of factor analysis in this group of black adults. The dietary non-marine FA pattern showed a weak positive association with WC, whereas the marine pattern did not show any associations with outcomes measured. On the other hand, two plasma phospholipid FA patterns (high-Satfat and n-3 VLC-PUFA) were positively associated with all measures of adiposity and MetS. The omega-6 VLC-PUFA pattern showed a positive association with MetS, but not with measures of adiposity. The n-9 LC-MUFA and the n-3 EFA patterns showed an inverse association with MetS in fully adjusted models and tended to be negatively associated with some measures of adiposity. The high-LA pattern was neither associated with measures of adiposity nor MetS. Our findings indicate that dietary FA patterns were weakly associated, whereas plasma phospholipid FA patterns were more strongly associated with measures of adiposity and MetS.

Previous studies have reported FA patterns, derived from different components of blood and tissue in association with obesity (29) and MetS (22, 30), but not with dietary patterns. These patterns were generated by varying numbers of FAs ranging from 9 to 34 FAs (22, 29, 30), and some included estimated desaturase activities (30), by means of use of factor (29, 30) and cluster (22) analysis. Consequently, these derived patterns differed from that obtained in our study.

A dietary pattern, consisting of SFAs, PUFAs, MUFAs and other nutrients, was not associated with obesity among Iranian adults (45). On the contrary, a multiracial study in the USA reported a positive association of intakes of total fat, total saturated fat, long-chain (LC)-SFAs, myristic acid (C14:0), C16:0 and C18:0, and MUFAs with BMI (46). Furthermore, a study investigating the association of dietary patterns with MetS concluded that a pattern high in meat products was associated with a higher prevalence of MetS (47). In our study, the dietary non-marine FA pattern showed marginal and positive associations with WC, but not with other measures of adiposity or MetS. The non-marine FA pattern had positive loadings of FAs from SFAs, MUFAs and PUFAs, specifically from two SFAs (C16:0 and C18:0), two MUFAs (C16:1n-7, C18:1n-9) and two PUFAs (C18:2n-6 and C18:3n-3). The dietary marine FA pattern showed no association with outcomes measured. Our results are in agreement with a study in the USA that also found no associations of n-3 LC-PUFAs with BMI due to low intakes of these FAs in their participants (46). In our study and the study in the USA, lower intakes of n-3 PUFA compared to the FAO/WHO recommendation of 0.25–2 g/day were found (48). Under-reporting of dietary intake may significantly influence nutrient pattern investigation and association with disease (49), however, in the PURE study, over- and under-reporters of dietary intake (subjects with reported energy intakes $\geq 30,000$ or ≤ 3000 KJ) were excluded prior to analyses (50). Apart from the marine FA pattern, we did not derive other clear dietary FAs patterns, likely due to the homogenous nature of food intake in this group of adults. Therefore, factor analysis may not be the most appropriate method to investigate dietary FAs in this population and the associations observed should be interpreted with caution.

The first plasma phospholipid FA pattern, high-Satfat, was positively associated with all measures of adiposity and MetS. This pattern had high positive loadings of SFAs C18:0, C20:0, C22:0 and C24:0, as well as negative loadings of MUFAs. In our study, the plasma phospholipid levels of these saturated FAs were also higher in the overweight men and women compared to their leaner counterparts, although effect sizes tended to be small. Plasma phospholipid VLC-SFAs, such as C20:0, C22:0 and C24:0 have previously been reported to be inversely associated with MetS among adults in Taiwan (15). In a study in Japan, serum VLC-SFAs were also inversely associated with MetS and positively associated with HDL-

cholesterol (16). The authors concluded that these VLC-SFAs may be indicative of healthier metabolic health (15, 16). Li and colleagues (22) derived a cluster that consisted of the same VLC-SFAs mentioned above. This cluster was also associated with the healthier metabolic profile (22), but was not identical to the high-Satfat pattern identified in this current study, as it did not have negative loadings of MUFAs. High intakes of MUFAs are generally considered the driving force behind the protective effect of the Mediterranean diet on cardiovascular diseases (51). The combined presence of high loadings of some SFAs, particularly C18:0 and low loadings on MUFAs may, therefore, explain the association with obesity and MetS found in our study. Plasma C18:0 levels were higher and plasma C18:1n-9 levels were lower in the overweight/obese groups than among their leaner counterparts in the current study, and the same FAs had positive and negative loadings, respectively, in the high-Satfat pattern. These two FAs make up a considerable proportion of the FAs in the plasma phospholipid profile and may be the driving force behind the positive association of the high-Satfat pattern with all measures of adiposity and MetS in the current study.

The second pattern, n-3 VLC-PUFA, had high positive loadings of C20:5n-3, C22:5n-3 and C22:6n-3 as well as the n-6 PUFA arachidonic acid (C20:4n-6). This pattern was positively associated with all measures of adiposity and MetS. In line with our findings, an n-3 FA pattern (with positive loading of C20:5n-3, and estimated delta 5 desaturase activity and negative loading of C20:3n-6) in the study by Warensjo *et al.* (30) predicted the development of MetS in Swedish men, independent of lifestyle factors. The main difference between our study and that of Warensjo and colleagues (30) is that they included estimated desaturase activity in their patterns and measured FAs in serum. Omega-3 PUFAs, especially C22:6n-3 and C20:5n-3, have multiple beneficial effects and are generally inversely associated with obesity and related risk factors as detailed in a recent review (52). Other studies have also reported the inverse association of circulating n-3 PUFAs with measures of adiposity and MetS (20, 21). It should be kept in mind that the PURE-SA study population reported very low intakes of n-3 FAs; however, despite these low intakes, their plasma levels were considered sufficient (43). Continuous low intake of n-3 LC-PUFAs, as reported in the present study, can result in up-regulation of the endogenous synthesis of n-3 LC-PUFAs from C18:3n-3. The possibility, therefore, exists that this upregulated conversion is a response to the CVD-risk milieu, reflecting reverse causality, rather than being the other way around. Further research is needed to elucidate the endogenous conversion of dietary n-3 PUFAs in black African populations. It is also possible that the positive association of this pattern with adiposity and MetS could have been driven by the C20:4n-6, which formed part of this pattern. Omega-3 and n-6 FAs compete for incorporation into target tissues and metabolism by common enzymes, which may lead to opposing health effects. (53). The eicosanoid metabolic products

from C20:4n-6 promote inflammatory responses. There is some evidence that a higher ratio of n-6 PUFAs to n-3 PUFAs is associated with a higher prevalence of obesity and MetS (54).

The fourth plasma phospholipid pattern, n-6 VLC-PUFA, had positive loadings of n-6 VLC-PUFAs, C20:3n-6, C22:4n-6 and osbond acid (C22:5n-6) and was positively associated with MetS. Mayneris-Perxachs *et al.*, (18) also reported a positive association between plasma phospholipid C20:3n-6 PUFAs and MetS among older adults in Spain. Higher concentrations of plasma phospholipid C20:3n-6 were observed in both overweight men and women compared to their leaner counterparts in our study, but the n-6 VLC-PUFA pattern was not associated with BMI in the fully adjusted model. Plasma phospholipid levels of C20:3n-6 were also positively associated with BMI in participants from the USA and Mexico (13, 17). There was, however, also a longitudinal study that found higher total circulating n-6 PUFAs, in particular linoleic acid and arachidonic acid, to be protective of risk factors for MetS, including both systolic and diastolic BP and plasma triglycerides in men (24), indicating that different n-6 FAs showed opposite associations with MetS. The association of C20:3n-6 with MetS requires further investigation (18).

The fifth pattern, n-9 LC-MUFA, loaded positively with C20:1n-9 and C24:1n-9, and negatively with myristic acid (C14:0). This pattern showed an inverse association with WC and WHtR, but lost association when adjusted for lifestyle variables and energy intake. However, lower odds for having the MetS remained after adjustment for covariates. In our study, levels of C20:1n-9 were significantly higher in lean men and women compared to their overweight counterparts, whereas C24:1n-9 was higher in overweight compared to lean women only. Nervonic acid (C24:1n-9) and C20:1n-9 are both products of endogenous metabolism by elongation from oleic acid (55), but plasma C24:1n-9 may also be related to fish intake (56). Since fish consumption was very low in our study population, this pattern could therefore reflect an upregulated metabolism of oleic acid in our study participants.

The sixth pattern, n-3 EFA, was positively loaded with C18:3n-3 and tended to be inversely associated with all measures of adiposity and showed lower odds for MetS. This is in agreement with a study that found C18:3n-3 in serum cholesteryl esters to be inversely associated with abdominal obesity in a recent cross-sectional study of 60-year-old men and women (57). Alpha-linolenic acid (C18:3n-3) is an essential FA and a precursor from which n-3 LC-PUFAs are synthesised. Increased consumption of C18:3n-3-rich foods elevates its tissue levels as well as levels of C22:6n-3 and C20:5n-3 in the liver lipids (58). Alpha-linolenic acid can be beneficial to health; firstly, C18:3n-3 intake was associated with a moderately lower risk of CVD in randomised controlled studies as outlined in reviews (59, 60). Secondly, as explained above, C18:3n-3 competes for the same metabolic enzymes, as does C18:2n-6,

and increased dietary intake may be a worthy approach to decrease elongation of n-6 FAs leading to reduced plasma C20:4n-6 levels and increased plasma levels of C22:6n-3 and C20:5n-3 (58). As C18:3n-3 is an essential FA, this pattern, identified in our study participants, is probably related to food intake and therefore indicative of a higher intake of vegetable oils, legumes, nuts and seeds (61).

5.7 Strengths and limitations

A rigorous methodological approach of sequential regression modelling enabled us to investigate the associations between dietary FAs and plasma phospholipid FA patterns, respectively, and measures of adiposity and MetS. Another strength of our study is the use of both dietary FA and plasma phospholipid FA patterns (27), which is a preferred method to investigate the association between diet and diseases (27). Our work is not free of limitations. Firstly, inaccuracies associated with collecting dietary intake data may have influenced the dietary FA results; however, in our population, fieldworkers collecting dietary data were intensively trained and supervised, and both under- and over-reporters of dietary intake were excluded (50). In addition, repeatability of the QFFQ was also demonstrated (10). Secondly, the cross-sectional design does not account for possible reverse causation between measures of adiposity and dietary FAs intake or plasma phospholipid FA concentration, nor can causality be inferred. Thirdly, a possible limitation of the present study is incomplete information on FA composition in the food composition databases. This third limitation was compensated for by our study design that also considered plasma phospholipid FAs. Fourthly, we assessed the associations with indirect measurements of adiposity, including BMI, WHtR and WC, as secondary markers of total and central adiposity, whereas imaging methods would better differentiate between lean and fat mass.

5.8 Conclusion

To our knowledge, this is the first study to investigate and document novel data on dietary FA and plasma phospholipid FA patterns and their association with measures of adiposity and MetS in a selected group of black South African adults. This study presents evidence that although marginal association was found with dietary FA patterns, some circulating plasma phospholipid FA patterns were more strongly and significantly associated with BMI, WC and WHtR and MetS. The high-Satfat and n-3 VLC-PUFA patterns were positively associated with adiposity and MetS, whereas the n-9 LC-MUFA and n-3 EFA patterns were inversely associated with adiposity. These patterns may suggest possible differences in FA metabolism between lean and overweight/obese individuals. It should also be considered that, in a study population with low-fat intakes, such as the PURE participants, plasma FA levels may reflect

endogenous FA generation rather than dietary intakes, which could result in different findings than those reported in other studies from affluent communities.

Our results are not sufficiently conclusive to make recommendations on dietary FA intakes in this population. Further prospective cohort studies that explain possible differences in characteristics of FA metabolism among black South African men and women are needed. More studies that apply the use of dietary FA and plasma or tissue FA patterns are required to determine whether the results from the current study can be generalised to the black population of African descent.

Table 5-2: Demographics, health and dietary intake data of apparently healthy cohort of 711 black South African adults participating in the PURE study

Variables	Men (n=273)	Women (n=438)	p-value ^c
	Median (Q ₁ , Q ₃) ^b	Median (Q ₁ , Q ₃) ^b	
Demographics			
Age (years)	52 (46, 60)	52 (45, 59)	0.80
Education (educated)	155 (57.6%)	263 (62.2%)	0.22
Tobacco use (current smoker)	163 (59.7%)	205 (46.8%)	0.0008
Alcohol (g week-1)	6.4 (0, 24.9)	0 (0, 3.9)	<0.0001
Physical activity index	2.8 (2.5, 3.1)	2.8 (2.5, 3.3)	0.71
Waist circumference (cm)	75.4 (69.7, 82.4)	82.0 (71.7, 92.6)	<0.0001
Waist to height ratio	0.45 (0.4, 0.5)	0.52 (0.5, 0.6)	<0.0001
Body mass index (kg/m2)	20.0 (18.1, 23.2)	26.0 (21.8, 31.9)	<0.0001
Systolic blood pressure (mmHg)	135 (121, 152)	132 (118, 150)	0.06
Diastolic blood pressure (mmHg)	88 (78, 98)	88 (70, 97)	0.84
Fasting glucose (mmol/L)	4.8 (4.3, 5.4)	4.9 (4.3, 5.4)	0.53
Total cholesterol (mmol/L)	5.0 (4.1, 6.0)	5.1 (4.4, 6.2)	0.35
High-density lipoproteins cholesterol (mmol/L)	1.54 (1.2, 2.1)	1.5 (1.2, 1.8)	0.04
Low-density lipoprotein cholesterol (mmol/L)	3.1 (2.3, 4.0)	3.4 (2.7, 4.2)	0.06
Triglycerides (mmol/L)	1.0 (0.8, 1.5)	1.2 (0.9, 1.8)	0.002
Dietary intake ^a			
Total energy (kcal/day)	1874 (1377, 2612)	1628 (1189, 2212)	0.001
Total carbohydrate (g/day)	285.4 (199, 378)	248.8 (180.6, 325.1)	0.01
Total fibre intake (g/day)	14.8 (25, 30)	17.9 (12.7, 25.2)	0.004
Total protein (g/day)	55.0 (38, 75.7)	46.2 (33.1, 65.0)	<0.0001
Total fat (g/day)	45.3 (28.5, 63.7)	40.5 (26.3, 64.4)	0.10
Total saturated fatty acids (g/day)	10.5 (6.5, 15.7)	9.5 (5.6, 16.6)	0.13
Total monounsaturated fatty acids (g/day)	11.4 (6.8, 17.8)	10.4 (6.0, 18.3)	0.14
Total polyunsaturated fatty acids (g/day)	13.6 (8.8, 19.6)	13.1 (7.5, 20.0)	0.47
Total n–3 polyunsaturated fatty acids (g/day)	0.4 (0.2, 0.6)	0.33 (0.20, 0.5)	0.15
Total n–6 polyunsaturated fatty acids (g/day)	13.3 (8.8, 19.2)	12.9 (7.3, 19.6)	0.55

^a Baseline demographic details of participants.

^b Data are presented as median (interquartile range): Q1, lower interquartile range; Q3, upper interquartile range.

^c Significance levels of differences in parameters between men and women, based on Mann-Whitney and chi-square tests for continuous and categorical variables, respectively.

Table 5-3 Dietary intake of fats, individual fatty acids and plasma phospholipid fatty acid profile by BMI categories and sex in 711 black South African adults

	Men (n=273)				Women (n=438)			
	BMI ^a <25 (n=233)	BMI≥25 (n=40) Median (Q ₁ , Q ₃)	p-value	Effect size	BMI <25 (n=191) Median (Q ₁ , Q ₃)	BMI≥25 (n=247) Median (Q ₁ , Q ₃)	p-value	Effect size
Total energy, dietary fats intake								
Total energy (kcal/day)	1856 (1374, 2599)	2052 (1370, 2681)	0.48		1579 (1139, 2188)	1651 (123, 2224.)	0.50	
Total fat intake (g/day)	44.8 (28.5, 63.0)	46.8 (31.5, 66.9)	0.71		35.5 (22.8, 59.2)	44.9 (27.9, 68.0)	0.002	
Total saturated fatty acids (g/day)	10.4 (6.5, 15.4)	12.4 (8.1, 16.3)	0.71		8.4 (4.7, 15.5)	10.8 (6.2, 17.1)	0.003	
Total polyunsaturated fatty acids (g/day)	13.8 (8.8, 19.6)	12.8 (8.7, 18.7)	0.32		11.8 (7.0, 19.5)	13.5 (8.2, 20.9)	0.29	
Total n–3 PUFAs (g/day)	0.4 (0.2, 0.6)	0.34 (0.2, 0.6)	0.58		0.3 (0.2, 0.5)	0.4 (0.2, 0.5)	0.09	
Total n–6 PUFAs (g/day)	13.5 (8.7, 19.1)	12.4 (9.2, 19.8)	0.39		12.1 (6.9, 18.9)	13.4 (8.1, 20.2)	0.27	
n–6/n–3 ratio	33.1 (24.4, 152.4)	34.2 (22.5, 53.1)	0.84		37.3 (27.57,49.73)	36.8 (26.1, 51.9)	0.76	
Total monounsaturated fatty acids (g/day)	11.2 (6.8, 16.4)	13.4 (7.2, 19.7)	0.48		8.5 (5.0, 16.4)	12.0 (6.4, 19.4)	<.0001	
Plasma phospholipid fatty acids (% total FAs) ^c								
Saturated fatty acids								
Myristic acid (C14:0)	0.25 (0.2, 0.3)	0.25 (0.2, 0.3)	0.75		0.26 (0.2, 0.3)	0.27 (0.2, 0.3)	0.21	
Palmitic acid (C16:0)	27.6 (25.2, 29.5)	26.3 (24.6, 28.0)	0.002	0.37	26.7 (24.8, 29.2)	25.7 (24.0, 27.6)	0.21	
Stearic acid (C18:0)	14.0 (12.4, 15.5)	15.1 (14.4, 16.2)	<.0001	0.31	14.8 (13.5, 16.3)	15.5 (14.3, 17.0)	0.0004	0.38
Arachidic acid (C20:0)	0.27 (0.21, 0.34)	0.34 (0.27, 0.39)	0.02	0.33	0.30 (0.25, 0.37)	0.36 (0.30, 0.43)	<.0001	0.35
Behenic acid (C22:0)	0.94 (0.66, 1.17)	1.22 (0.97, 1.43)	0.0006	0.32	1.03 (0.78, 1.35)	1.27 (1.07, 1.54)	<.0001	0.35
Lignoceric acid (C24:0)	1.01 (0.81, 1.23)	1.13 (0.91, 1.36)	0.02	0.40	1.00 (0.81, 1.24)	1.07 (0.9, 1.27)	0.03	0.45
Monounsaturated fatty acids								
Palmitoleic acid (C16:1n-7)	0.88 (0.5, 1.6)	0.6 (0.4, 0.8)	0.0007	0.34	0.8 (0.5, 1.2)	0.6 (0.4, 0.8)	0.003	0.40
<i>Cis</i> -vaccenic acid (C18:1n-7)	1.46 (1.26, 1.69)	1.19 (1.01, 1.44)	0.002	0.30	1.41 (1.20, 1.65)	1.30 (1.12, 1.5)	0.03	0.41
Oleic acid (C18:1n-9)	10.0 (7.6, 13.0)	7.5 (6.2, 9.3)	0.0002	0.30	8.2 (7.0, 11.3)	7.16 (6.3, 8.3)	<.0001	0.35
Elaidic acid (C18:1n9t)	0.31 (0.20, 0.56)	0.27 (0.20, 0.5)	0.32		0.34 (0.21, 0.81)	0.32 (0.21, 0.61)	0.50	

	Men (n=273)				Women (n=438)			
	BMI ^a <25 (n=233)	BMI≥25 (n=40) Median (Q ₁ , Q ₃)	p-value	Effect size	BMI <25 (n=191) Median (Q ₁ , Q ₃)	BMI≥25 (n=247) Median (Q ₁ , Q ₃)	p-value	Effect size
Gondoic acid (C20:1n-9)	0.11 (0.09, 0.12)	0.10 (0.08, 0.11)	0.04	0.36	0.11 (0.09, 0.12)	0.10 (0.09, 0.12)	0.02	0.42
Erucic acid (C22:1n-9)	0.06 (0.04, 0.08)	0.07 (0.04, 0.09)	0.29		0.06 (0.05, 0.09)	0.07 (0.05, 0.09)	0.50	
Nervonic acid (C24:1n-9)	1.66 (1.44, 1.95)	1.61 (1.35, 1.95)	0.75		1.69 (1.42, 2.02)	1.88 (1.55, 2.21)	0.005	0.41
n-3 polyunsaturated fatty acids								
α-linolenic acid (c18:3n-3)	0.09 (0.07, 0.12)	0.08 (0.05, 0.10)	0.18		0.08 (0.06, 0.1)	0.1 (0.05, 0.1)	0.77	
Eicosapentaenoic acid (C20:5n-3)	0.6 (0.5, 0.9)	0.6 (0.5, 0.9)	0.32		0.6 (0.4, 0.9)	0.6 (0.4, 0.8)	0.92	
Docosapentaenoic acid (C22:5n-3)	0.5 (0.4, 0.7)	0.6 (0.4, 0.8)	0.51		0.6 (0.5, 1.0)	0.5 (0.4, 0.8)	0.92	
Docosahexaenoic acid (C22:6n-3)	3.8 (3.0, 4.8)	4.29 (3.2, 5.6)	0.09		4.4 (3.5, 5.5)	5.0 (4.05, 5.8)	0.0008	0.39
n-6 & n-9 polyunsaturated fatty acids								
Linoleic acid (C18:2n-6)	16.1 (13.6, 18.5)	16.4 (14.1, 18.6)	0.71		15.9 (12.8, 18.4)	16.0 (13.8, 18.7)	0.50	
γ-linolenic acid (C18:3n-6)	0.13 (0.1, 0.2)	0.10 (0.07, 0.14)	0.04	0.39	0.1 (0.1, 0.2)	0.10 (0.07, 0.13)	0.07	
Eicosadienoic acid (C20:2n-6)	0.34 (0.29, 0.39)	0.35 (0.29, 0.4)	0.71		0.36 (0.31, 0.42)	0.38 (0.33, 0.44)	0.07	
Dihomo-γ-linolenic acid (C20:3n-6)	2.7 (2.4, 3.3)	3.1 (2.7, 3.9)	0.006	0.32	2.8 (2.4, 3.2)	3.1 (2.72, 3.61)	<.0001	0.35
Arachidonic (C20:4n-6)	12.9 (11.5, 14.3)	14.1 (12.6, 15.7)	0.08		13.5 (12.0, 15.0)	14.2 (12.7, 15.6)	0.007	0.44
Docosadienoic acid (C22:2-n6)	0.018 (0.013, 0.023)	0.018 (0.014, 0.022)	0.75		0.02 (0.014, 0.027)	0.025 (0.018, 0.032)	<.0001	0.37
Adrenic acid (C22:4n-6)	0.6 (0.5, 0.7)	0.6 (0.5, 0.7)	0.51		0.6 (0.5, 0.7)	0.6 (0.5, 0.7)	0.29	
Osbond acid (C22:5n-6)	1.4 (1.2, 1.6)	1.4 (1.1, 1.7)	0.71		1.4 (1.2, 1.6)	1.4 (1.1, 1.6)	0.15	
Mead acid (C20:3n-9)	0.3 (0.2, 0.4)	0.2 (0.1, 0.3)	0.09		0.2 (0.1, 0.3)	0.17 (0.1, 0.3)	0.0003	0.41

^a BMI = Body mass index; calculated as kg/m².

^b Median (Q₁, Q₃) = Differences between FAs across BMI and sex were tested by independent t-test according to BMI <25 and BMI ≥ 25. Data are presented as median; Q₁: lower interquartile range, Q₃: upper interquartile range.

^c Plasma phospholipid fatty acids (% total FAs) = Fatty acids are expressed as a percentage (%) of total FA. Significance levels of differences in parameters between men and women based on Mann-Whitney tests.

Table 5-4 Factor loadings for dietary and plasma phospholipid fatty acids

Dietary fatty acids	Dietary fatty acid patterns ^a					
	Non-marine	Marine				
	($\lambda_1=60\%$) ^b	($\lambda_2=29\%$)-				
Saturated fatty acids						
Myristic acid (C14:0)	0.78	0.10				
Palmitic acid (C16:0)	0.97	0.22				
Stearic acid (C18:0)	0.94	0.21				
Behenic acid (C22:0)	0.67	0.29				
Monounsaturated fatty acids						
Palmitoleic acid (C16:1n-7)	0.89	0.28				
Oleic acid (C18:1n-9)	0.95	0.23				
n-3 fatty acids						
α -Linolenic acid (C18:3n-3)	0.89	0.22				
Eicosapentaenoic acid (C20:5n-3)	0.21	0.96				
Docosahexaenoic acid (C22:6n-3)	0.23	0.96				
n-6 fatty acids						
Linoleic acid (C18:2n-6)	0.80	0.31				
Arachidonic acid (C20:4n-6)	0.70	0.48				
The Kaiser's measure of sampling adequacy =0.84						
Plasma phospholipid fatty acid patterns ^c						
	High-Satfat	n-3 VLC-PUFA	High-LA	n-6 VLC-PUFA	n-9 LC-MUFA	n-3 EFA
Plasma phospholipid fatty acids	($\lambda_1=24\%$)	($\lambda_2=11\%$)	($\lambda_3=11\%$)	($\lambda_4=10\%$)	($\lambda_5=10\%$)	($\lambda_6=7\%$)
Saturated fatty acids						
Myristic acid (C14:0)	-0.13	-0.28	-0.06	-0.06	-0.56	0.01
Palmitic acid (C16:0)	-0.10	-0.54	-0.47	-0.40	-0.34	-0.05
Stearic acid (C18:0)	0.80	-0.21	-0.04	0.08	-0.04	-0.16
Arachidic acid (C20:0)	0.86	-0.08	0.01	-0.04	0.36	-0.08
Behenic acid (C22:0)	0.92	-0.03	0.06	-0.07	0.24	-0.07
Lignoceric acid (C24:0)	0.81	-0.19	-0.01	-0.06	0.34	0.10
Monounsaturated fatty acids						
Palmitoleic acid (C16:1n-7)	-0.84	-0.13	-0.25	0.08	-0.12	0.23
Cis-vaccenic acid (C18:1n-7)	-0.73	0.10	0.16	0.08	0.40	-0.20
Oleic acid (C18:1n-9)	-0.82	-0.15	-0.26	0.11	-0.01	0.35
Elaidic acid (C18:1n9t)	0.44	-0.58	-0.25	-0.04	0.09	-0.02
Gondoic acid (C20:1n-9)	-0.17	-0.07	0.52	-0.05	0.54	-0.13
Erucic acid (C22:1n-9)	0.35	-0.01	0.05	0.22	0.42	-0.05
Nervonic acid (C24:1n-9)	0.16	0.09	0.07	-0.01	0.85	-0.04
n-3 fatty acids						
α -linolenic acid (C18:3n-3)	-0.23	0.02	0.28	-0.15	-0.09	0.71
Eicosapentaenoic acid (C20:5n-3)	-0.37	0.57	-0.25	-0.32	-0.01	0.42
Docosapentaenoic acid (C22:5n-3)	-0.08	0.71	-0.01	0.12	0.15	0.27
Docosahexaenoic acid (C22:6n-3)	0.05	0.79	0.25	-0.08	0.08	-0.24
n-6 & n-9 fatty acids						
Linoleic acid (C18:2n-6)	0.08	0.31	0.80	-0.10	0.05	0.16
γ -linolenic acid (C18:3n-6)	-0.41	-0.08	-0.17	0.27	-0.37	0.49
Eicosadienoic acid (C20:2n-6)	0.09	0.06	0.82	0.30	0.15	0.13
Dihomo- γ -linolenic acid (C20:3n-6)	0.00	0.29	0.14	0.65	-0.06	0.30
Arachidonic acid (C20:4n-6)	0.04	0.66	0.12	0.42	0.24	-0.31
Docosadienoic acid (C22:2-n6)	0.44	0.08	0.47	-0.06	0.45	-0.24
Adrenic acid (C22:4n-6)	-0.13	0.02	0.03	0.83	0.08	0.01
Osbond acid (C22:5n-6)	-0.12	-0.04	-0.09	0.82	0.01	-0.32
Mead acid (C20:3n-9)	-0.58	-0.15	-0.53	0.32	0.05	0.28
The Kaiser's measure of sampling adequacy =0.78						

^aFatty acid patterns derived from dietary fatty acids.

^bVariance explained by the single factor.

^cFatty acid patterns derived from plasma phospholipids. Abbreviations: High-Satfat, saturated fatty acid pattern; n-3 VLC-PUFA, n-3 very long chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; n-6 VLC-PUFA, n-6 very long chain polyunsaturated fatty acid pattern; n-9 LC-MUFA, n-9 long chain monounsaturated fatty acid pattern; n-3 EFA, n-3 essential fatty acid pattern

Table 5-5 Associations of dietary fatty acid patterns with adiposity and metabolic syndrome in 711 black South African adults in regression models

	Linear regression models						Logistic regression models	
	Body mass index		Waist circumference		Waist: height ratio		Metabolic syndrome	
	β^a & (95% CI) ^a	p-value	β & (95% CI)	p-value	β & (95% CI)	p-value	OR & (95%CI) ^b	p-value
Crude model^c								
Non-marine	0.05 (-0.02, 0.13)	0.15	0.07 (-0.004, 0.14)	0.07	0.06 (-0.02, 0.13)	0.14	1.13 (0.96, 1.32)	0.14
Marine	-0.015 (-0.09, 0.06)	0.69	0.007 (-0.07, 0.08)	0.85	0.004 (-0.07, 0.08)	0.92	0.99 (0.84, 1.16)	0.88
R ² (%)		0.03		0.02		0.03		0.43
Adjusted model₁^d								
Non-marine	0.04 (-0.02, 0.11)	0.20	0.06 (-0.01, 0.13)	0.09	0.04 (-0.02, 0.11)	0.19	1.12 (0.95, 1.33)	0.19
Marine	-0.02 (-0.09, 0.05)	0.55	0.004 (-0.07, 0.08)	0.91	-0.0015 (-0.07, 0.07)	0.97	0.98 (0.82, 1.16)	0.78
R ² (%)		20.48		6.35		17.25		17.69
Fully adjusted model^e								
Non-marine	0.04 (-0.02, 0.11)	0.21	0.06 (-0.01, 0.13)	0.09	0.04 (-0.03, 0.11)	0.25	1.15 (0.96, 1.38)	0.12
Marine	-0.02 (-0.10, 0.04)	0.47	0.002 (-0.07, 0.07)	0.96	-0.005 (-0.07, 0.06)	0.88	0.94 (0.78, 1.14)	0.53
R ² (%)		26.68		13.34		22.72		20.39

^a Standardised betas and standardised 95% confidence intervals (CI).

^b OR, odds ratio and 95% CI.

^c Crude model; consisted of plasma phospholipid fatty acid patterns only.

^d Adjusted model₁; crude model and additionally adjusted for age and sex.

^e Fully adjusted model; adjusted model₁, additionally adjusted for lifestyle confounders (physical activity, self-reported smoking, total dietary energy and alcohol intake (Kcal) and level of education).

Table 5-6 Associations of plasma phospholipid fatty acid patterns with adiposity and metabolic syndrome in 711 black South African adults in regression models.

	Linear regression models						Logistic regression models	
	Body mass index		Waist circumference		Waist: height ratio		Metabolic syndrome	
	β ^a & (95% CI) ^a	p-value	β & (95% CI)	p-value	β & (95% CI)	p-value	The exponent of β & (95%CI)	p-value
Crude model^b								
High-Satfat	0.37 (0.31, 0.44)	<.0001	0.28 (0.21, 0.35)	<.0001	0.31 (0.24, 0.38)	<.0001	1.62 (1.34, 1.96)	<.0001
n-3 VLC-PUFA	0.21 (0.15, 0.28)	<.0001	0.22 (0.15, 0.28)	<.0001	0.22 (0.15, 0.28.)	<.0001	1.76 (1.44, 2.15)	<.0001
High-LA	0.04 (-0.02, 0.11)	0.20.	-0.05 (-0.12, 0.02)	0.16	-0.04 (-0.10, 0.03)	0.29	1.09 (0.92, 1.30)	0.33
n-6 VLC-PUFA	0.05 (-0.0, 0.12)	0.13	0.06 (-0.005, 0.13)	0.07	0.07 (0.001, 0.14)	0.045	1.28 (1.08, 1.53)	0.006
n-9 LC-MUFA	-0.05 (-0.11, 0.02)	0.17	-0.10 (-0.16, -0.03)	0.007	-0.09 (-0.16, -0.02)	0.009	0.63 (0.52, 0.75)	<.0001
n-3 EFA	-0.12 (-0.18, -0.05)	0.0007	-0.06 (-0.13, 0.006)	0.07	-0.09 (-0.16, -0.02)	0.009	0.81 (0.68, 0.96)	0.02
R ² (%)	19.72		13.87		15.62		18.30	
Adjusted model₁^d								
High-Satfat	0.29 (0.22, 0.35)	<.0001	0.25 (0.18, 0.32)	<.0001	0.23 (0.16, 0.30)	<.0001	1.44 (1.17, 1.76)	0.0004
n-3 VLC-PUFA	0.18 (0.12, 0.24)	<.0001	0.20 (0.13, 0.27)	<.0001	0.18 (0.12, 0.24)	<.0001	1.70 (1.37, 2.10)	<.0001
High-LA	0.03 (-0.04, 0.09)	0.43	-0.04 (-0.11, 0.03)	0.26	-0.04 (-0.11, 0.02)	0.21	1.10 (0.89, 1.30)	0.43
n-6 VLC-PUFA	0.029 (-0.03, 0.09)	0.36	0.05 (-0.016, 0.12)	0.13	0.05 (-0.02, 0.11)	0.16	1.26 (1.04, 1.51)	0.02
n-9 LC-MUFA	-0.04 (-0.10, 0.02)	0.20	-0.10 (-0.16, -0.03)	0.005	-0.09 (-0.15, -0.02)	0.007	0.61 (0.5, 0.73)	<.0001
n-3 EFA	-0.06 (-0.13, 0.001)	0.05.	-0.060 (-0.13, 0.010)	0.09	-0.05 (-0.12, 0.011)	0.10	0.84 (0.69, 1.01)	0.07
R ² (%)	30.78		16.73		25.76		29.74	
Fully adjusted model^e								
High-Satfat	0.27 (0.20, 0.34)	<.0001	0.22 (0.15, 0.30)	<.0001	0.20 (0.13, 0.27)	<.0001	1.54 (1.21, 1.95)	0.0004
n-3 VLC-PUFA	0.14 (0.08, 0.20)	<.0001	0.16 (0.087, 0.23)	<.0001	0.15 (0.08, 0.21)	<.0001	1.72 (1.38, 2.16)	<.0001
High-LA	-0.004 (-0.070, 0.06)	0.90	-0.06 (-0.13, 0.01)	0.11	-0.06 (-0.13, 0.01)	0.07	1.14 (0.93, 1.4)	0.22
n-6 VLC-PUFA	0.029 (-0.04, 0.09)	0.37	0.05 (-0.02, 0.12)	0.14	0.05 (-0.02, 0.11)	0.15	1.25 (1.02, 1.54)	0.03
n-9 LC-MUFA	0.002 (-0.06, 0.07)	0.957	-0.06 (-0.13, 0.02)	0.13	-0.05 (-0.11, 0.02)	0.17	0.61 (0.50, 0.75)	<.0001
n-3 EFA	-0.06 (-0.13, 0.005)	0.07	-0.06 (-0.13, 0.014)	0.12	-0.05 (-0.12, 0.02)	0.17	0.81 (0.66, 0.99)	0.04
R ² (%)	33.69		20.38		28.38		31.09	

Linear regression models						Logistic regression models Metabolic syndrome	
Body mass index		Waist circumference		Waist: height ratio		The exponent of β & (95%CI)	p-value
β^a & (95% CI) ^a	p-value	β & (95% CI)	p-value	β & (95% CI)	p-value		

^a Standardised betas and standardised 95% confidence intervals (CI)

^b Crude model; consisted of plasma phospholipid fatty acid patterns only.

^c Adjusted model₁; crude model and additionally adjusted for age and sex.

^d Adjusted model₂; adjusted model₁, additionally adjusted for lifestyle confounders (physical activity, self-reported smoking, total dietary energy and alcohol intake (KJ) and level of education).

^e Fully adjusted model; adjusted model₁, additionally adjusted for contraceptive use.

Abbreviations: High-Satfat, saturated fatty acid pattern; n-3 VLC-PUFA, n-3 very long chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; n-6 VLC-PUFA, n-6 very long chain polyunsaturated fatty acid pattern; n-9 LC-MUFA, long chain monounsaturated fatty acid pattern; n-3 EFA, n-3 essential fatty acid pattern.

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CHAPTER 6 MANUSCRIPT 3

6.1 Associations between plasma phospholipid fatty acid patterns and changes in anthropometric indices in black South African adults over ten years: a prospective cohort study

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List of abbreviations

AA, arachidonic acid; ALA, α -linolenic acid; BMI, body mass index; CI, confidence interval; DGLA, Dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid, EFA, essential fatty acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ -linolenic acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; NCD, non-communicable disease; OA, oleic acid; OR, odds ratios; POA, palmitoleic acid; PUFA, polyunsaturated fatty acid; PURE, Prospective Urban and Rural Epidemiology; SFA, saturated fatty acid; VLC, very long chain; WC, waist circumference; WHO, The World Health Organization; WHtR, waist: height ratio.

6.2 Abstract

Individual fatty acids (FA) and FA patterns, both in the diet and tissues, have been associated with obesity in various populations in Europe and the USA, but the associations have been inconsistent. However, data from Africa are lacking. We used principal component analysis to derive factor analysis patterns with the use of 25 plasma phospholipid FAs from randomly selected participants of the cohort of the South African arm of the Prospective Urban and Rural Epidemiology (PURE) study. We examined prospective associations between six plasma phospholipid FA patterns derived at baseline with 10-year changes in anthropometric indices, including weight, body mass index (BMI), waist circumference (WC) and waist: height ratio

(WHtR) in 412 black South African adults. We applied sequential adjusted linear regression models to investigate the associations. Six plasma phospholipid FAs patterns were identified at baseline and at follow-up. A comparison of quartiles of factor scores at baseline and follow-up showed good reproducibility of four of the FA patterns identified at baseline. The high-satfat pattern presented with high positive loadings of saturated FAs (stearic, arachidic, behenic and lignoceric acids) and negative loadings of monounsaturated FA (MUFAs) and the n-9 long-chain (LC)-MUFA pattern presented with positive loadings of MUFAs (nervonic and gondoic). These patterns were positively associated with 10-year changes in all anthropometric outcomes. The high-satfat pattern derived at baseline and maintained over 10 years may indicate unique FA metabolism associated with weight gain over time in this group of black South African adults.

Keywords: Very-long-chain saturated fatty acids, anthropometric indices, black Africans, factor analysis, plasma phospholipid fatty acids, PURE study

6.3 Introduction

The World Health Organization (WHO) estimated that, globally, obesity has approximately tripled since 1975 (1). By 2030, up to 58% of the world's adult population may be classified either as overweight or obese. Mean body mass index (BMI) has been reported to be high in women from north and southern Africa, and severe obesity is predicted to exceed underweight in women by 2025 (2). South Africa has the highest prevalence of obesity reported in sub-Saharan Africa. One out of five women has a BMI $\geq 35\text{kg/m}^2$ (3), and its associated incidence of non-communicable diseases (NCDs) negatively affects the lives of many inhabitants. The consequent burden of disease contributes to the rising cost of healthcare, both at the state level and in the private sector (4). Furthermore, BMI between 30 and 35 kg/m² and greater than 35 kg/m² is associated with an 11% and 23% increase in healthcare expenditures, respectively (5).

A considerable amount of the global NCD burden is attributable to behavioural, dietary, environmental, and metabolic risk factors (6). Diet is one of the most amenable risk factors influencing obesity and its co-morbidities (7). The WHO recommends less than 30% of energy from the total fat intake to avoid unhealthy weight gain in adult populations (8). However, the recommendation is largely based on one meta-analysis from populations in Europe, North America and New Zealand (9). These recommendations have not considered studies done in Africa and may not, therefore, be generalised to its population. Increased fat consumption experienced in Africa is attributed to the nutrition transition characterised by an increase in the availability of cheap vegetable oils and fats in several countries (10). The conventional sources of saturated fatty acids (SFA) among South Africans include oils (coconut, palm kernel, and palm

oils), fats (animal and dairy fat), and processed products made with these fats and oils (11). Similarly, MUFA intake is mainly derived from meat, as well as vegetable oils, including canola, olive, sunflower, soy and nuts, peanut butter and avocados (11). Recent findings show that dietary fat intake in South Africa is significantly higher in urban than in rural areas, and women consume more energy from fat than men in rural and urban areas (12). Dietary intakes of total fat, SFA, n–6 FAs, LA, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) expressed as percentage energy were associated with obesity in black and white South African women and were modulated by polymorphism in the interleukin-6 gene (13). The South African food-based dietary guidelines emphasise the importance of the intake of specific polyunsaturated fatty acids (PUFAs), including essential fatty acids (EFAs), n–6 and n–3 PUFAs and reduction in SFA (11), but this recommendation is not specific to obesity. Moreover, current evidence is inconclusive regarding the role of EFAs, n–3 and n–6 PUFAs and their ratio in the gain of body fat and obesity (14). Understanding dietary fat consumption, total plasma phospholipid FA status and endogenous metabolism of black South Africans would provide data to support and further improve future recommendations on fat consumption in the country. Recommendations specific to the prevention of obesity in South Africa will be of utmost importance.

Dimension reduction techniques, such as principal component analysis (PCA), have been used extensively in nutrition epidemiology to identify particular distinctive dietary patterns relevant and applicable to obesity (15, 16). A few studies have applied this technique to FA biomarkers in obesity (17), metabolic syndrome (18) and cardiovascular disease (19–21). These studies identified PCA patterns and named them according to the FAs with the lowest or highest factor scores within the pattern.

To date, many studies have investigated the association between individual plasma phospholipid FAs and obesity. A study that investigated the association of serum phospholipids with adiposity reported positive correlations of total SFAs with BMI in men and women and a positive correlation of POA in Lebanese women (22). Furthermore, higher plasma levels of POA were associated with abdominal obesity in French men (23). Plasma total SFAs, myristic acid, palmitic acid and POA were higher in obese participants compared to normal weight ones in Turkey (24). A 10-year longitudinal study in the USA involving older adult men and women reported inverse associations between levels of total n–3 PUFAs and baseline BMI, positive associations of total n–6 PUFAs with baseline BMI, as well as of total n–6 PUFAs with increase in BMI over time (25). A recent study showed that LA in plasma was inversely associated with WC and body fat in Irish adults (26), while total n–6 and n–3 PUFAs also in plasma were inversely associated with body fat in elderly men and women in Norway (27).

Based on studies investigating circulating FA patterns (17-21) and individual FAs in plasma (23-27), we hypothesised that plasma phospholipid FAs patterns derived at baseline may be associated with 10-year changes in anthropometric indices. To test our hypothesis, we prospectively explored the associations of plasma phospholipid FA patterns derived at baseline with 10-year changes in anthropometric indices of adiposity among a sub-cohort of black South Africans participating in the Prospective Urban and Rural Epidemiology (PURE) study. We also derived new FA patterns at 10-year follow-up to study reproducibility of patterns identified at baseline. To our knowledge, no study has investigated prospective associations of plasma phospholipid FA patterns with adiposity in black South African adults.

6.4 Methods and materials

6.4.1 Study procedures and subjects

This prospective research forms part of the South African leg of the international PURE study, which is a multi-center longitudinal cohort investigation that tracks the impact of diet on cardiovascular diseases in diverse backgrounds (28). In South Africa, baseline data collection started in 2005 in the North West Province with follow-up at five-year intervals (2010 and 2015). Details of the study design, study population, inclusion and exclusion criteria, and data collection procedures have previously been reported (12, 29, 30). To facilitate data collection, participants were transported to the centers in the rural and urban areas. Participants completed administered questionnaires in their preferred language (Afrikaans, Setswana or English) in interviews with trained fieldworkers. Standardised questionnaires Structured demographic, socioeconomic, lifestyle, and physical activity questionnaires, which were standardised for the international PURE study and adapted for South Africa, were used (28, 31, 32). Anthropometric measurements, including weight, height, WC and their indices [(BMI and waist: height ratio (WHtR)], were assessed by trained research assistants using standardised methods at baseline (2005), and the same procedures were followed at five-year interval follow-up (2010 and 2015). As previously reported, 2 010 individuals aged 30 to 93 years were recruited in 2005 from rural and urban areas of the North West Province, South Africa. From this cohort, 711 participants were randomly selected, and plasma phospholipid FAs were measured. A random sample of 711 was selected from the original 2010 volunteers with both dietary and plasma phospholipid FAs, after exclusion of 305 participants with human immunodeficiency virus infection. The number of participants was randomly selected from stratified groups (rural male, rural female, urban male, and urban female) and included 125 rural men, 148 urban men, 218 rural women and 220 urban women. Those participants with available 10-year follow-up data for plasma phospholipid FAs and other

covariates were included in the prospective analyses, leaving a total of 412 participants (137 men and 275 women) aged between 45 and 93 years at baseline.

All the participants gave consent for data collection at baseline and follow-up. The researchers obtained ethical approval for the South African PURE study from the Ethics Committee of the North-West University (ethics number 04M10).

6.4.2 Measurements of fatty acid composition

EDTA plasma samples were thawed and extracted with chloroform:methanol (2:1 v/v) according to the modified Folch method (Folch *et al.*, 1957). The plasma phospholipid fatty acid fraction was isolated by thin layer chromatography from the extracted lipids (Baumgartner *et al.*, 2012). Subsequently, the phospholipid fatty acid fraction was transmethylated to fatty acid methyl esters and analysed by gas chromatography electron ionisation mass spectrometry by means of an Agilent Technologies 7890 A GC system, as described by Baumgartner *et al.* (Baumgartner *et al.*, 2012). Thirty-two FAs were measured in fasted plasma samples on the same instrument with the same methodology. Seven FAs, i.e. pentadecanoic acid (C15:0), margaric acid (C17:0), trans-vaccenic acid (C18:1n-7t), rumenic acid (C18:2n-7tt), stearidonic acid (C18:4n-3), eicosatrienoic (C20:3n-3) and elaidic acid (C18:1n9t) were below the limit of quantification and therefore were not included. The remaining 25 plasma phospholipid FAs were quantified and expressed as a percentage of total FAs. Quality of data was assured with a separate calibration for each fatty acid, monitoring of internal standard (1,2 – diheptadecanoyl – sn – glycerol – 3 phosphorylcholine, Matreya, Pennsylvania, USA), and Levey Jennings graphs for a pooled plasma control analysed with each batch. The 25 FAs used for baseline and prospective analysis included SFAs, MUFAs, n–3, n–6, and n–9 PUFAs (**Table 1**). At 10-year follow-up, the same FAs were measured and included in the analysis. Each FA is expressed as a percentage of total FAs (% weight/weight). The plasma phospholipid FAs included in this study are based on the distribution of FAs quantified in a selected group of black South Africans.

6.4.3 Measurements of 10-year outcomes

Changes in anthropometric indices, including weight, BMI, WC, and WHtR over ten years, were expressed as the difference between these measurements at 10-year follow-up and baseline.

6.4.4 Statistical analysis

The characteristics of the participants at two time points (baseline and 10-year follow-up) were assessed by Wilcoxon matched pairs test, and data are presented by sex. Differences between

individual FAs and ratios by BMI and sex groups at 10-year follow-up were tested by means of the Mann-Whitney test. A BMI < 25 kg/m² was considered as underweight and/or normal-weight or lean, whereas BMI ≥ 25 kg/m² was considered overweight and/or obese. We presented results as medians and interquartile ranges due to the skewed distribution of the variables. The effect size of the differences between groups was calculated using the Man-Whitney U-value, and the sample size of the groups and effect size for differences at two time points were calculated by means of the Wilcoxon Signed Ranks Test Z-value and sample size (33).

Categorical variables were classified as follows: sex (men and women), self-reported smoking (never or formerly smoked and current smokers) and education (no school and formal education). Continuous variables included age, weighted physical activity index and total energy and alcohol intake (all baseline values) as well as 10-year changes in weight, BMI, WC and WHtR. Exposure variables included six plasma phospholipid FA patterns identified at baseline.

To define the plasma phospholipid FA patterns at baseline, principal component analysis (PCA) based on the varimax rotation was conducted. Twenty-five plasma FA variables expressed as percentages of total FAs were used to derive six FA patterns. We considered FA with an absolute factor loading of > 0.5 to make a significant contribution to the pattern. The same PCA procedure was repeated for the patterns derived at 10-year follow-up. All FA patterns are listed in **Table 5-1**. Additionally, we divided the participants into quartiles of factor scores for each pattern and tested how many participants stayed in the same quartile or moved to the adjacent quartiles for the similar FA pattern after follow-up. The purpose of the analysis was to examine the reproducibility of baseline patterns with similar patterns at 10-year follow-up.

We determined the correlation between scores of the different FA patterns, as well as plasma FAs, which were correlated with percentage energy (%E) intake from total fat, %E from total SFA, total fat and alcohol intake. The purpose of this exploratory analysis was to determine the relationship between the identified plasma FA patterns with macro-nutrients commonly consumed with our study participants.

The differences over ten years for all anthropometric indices (weight, BMI, WC, and WHtR) were included in the sequential adjusted linear regression models as continuous outcome variables. The association with FA pattern scores, with adjustment for covariates, was assessed in sequential models, starting with the crude model, which consisted of only plasma phospholipid FA patterns (exposure variables) and outcomes. In the first adjusted model, age and sex (men and women) were added as covariates, and finally, in the fully adjusted model, all lifestyle covariates, including self-reported smoking, education, physical activity, alcohol and total energy

intake (baseline variables), as well as the baseline anthropometric variables were added. Model goodness-of-fit was evaluated using R-square statistics. Statistical analysis was performed using SAS Version 9.4 (SAS Institute, Inc., 2016) (34). A probability value of $p \leq 0.05$ was considered statistically significant.

6.5 Results

6.5.1 Identification of baseline fatty acid patterns using factor analysis

The factors identified at baseline explained 72% of the total variance ($n = 412$). The patterns were characterised and named according to the highest loadings of the specific FAs present in a given pattern, as is the norm in other studies (18-21). The results are presented in the order in which the FA patterns were derived: high-Satfat (positively loaded with stearic acid, VLC-SFAs and negative loadings of POA, *cis*-vaccenic acid, and oleic acid (OA)), n-9 LC-MUFA (positive loadings of nervonic acid and gondoic acids), n-6 VLC-PUFA (positive loadings on n-6 VLC-PUFAs), high-LA (positive loadings of n-6 LA, eicosadienoic acid and negative loading of palmitic acid), low-DHA [highest positive loading on α -linolenic acid (ALA) and γ -linolenic acid (GLA) and negative loadings of DHA], and n-3 very-long-chain (VLC)-PUFA [highest loading on EPA and docosapentaenoic acid (DPA)]. These patterns are listed in **Table 6-1**.

6.5.2 Identification and reproducibility of plasma phospholipid fatty acid patterns derived at baseline and 10-year follow-up

Table 6-1 also shows the factor loading of plasma phospholipid FA patterns obtained at 10-year follow-up ($n = 412$). The factor analysis identified six plasma phospholipid FA patterns from 25 individual phospholipid FAs. These factors explained 74% of the total variance. We named the six 10-year patterns *Factors 1 to 6*.

Similarities and reproducibility of 10-year follow-up patterns

The similarities between the patterns identified at baseline and at 10 years were determined by means of visual inspection of the FAs loaded within the patterns. The n-6 VLC-PUFA, high-Satfat, n-3-VLC-PUFA and low-DHA patterns identified at baseline were similar to factor 1, factor 2, factor 4 and factor 6, derived at 10-year follow up, respectively. The remaining two baseline FA patterns (n-9 LC-MUFA and the high-LA pattern) were not similar to any of the FA patterns derived at after 10 years.

In the n–6 VLC-PUFA patterns, n=101 of the participants stayed in the same quartile, and 286 moved to the adjacent quartiles. Therefore, after 10 years of follow-up, 387 out of a total of 412 participants (93%) either remained in the same quartile or moved to an adjacent quartile of scores for the n–6 VLC-PUFA pattern compared to baseline. This is an indication of stability of a plasma phospholipid pattern with high loadings on n–6 VLC-PUFAs at both baseline and end. Similar results were found for reproducibility of the high-Satfat pattern (n=92 of the participants stayed in the same quartile, but n=273 moved to the adjacent quartiles). Therefore, 88.6% of participants either remained in the same quartile or moved to an adjacent quartile of score for the high-Satfat pattern compared to baseline. In the n–3 VLC-PUFA pattern, n=152 of participants stayed in the same quartile and 260 moved to the adjacent quartiles. Therefore, 100% either remained in the same quartile or moved to an adjacent quartile of scores for the n–3 VLC-PUFA pattern compared to baseline. Likewise, for the low-DHA pattern, n=100 stayed in the same quartile and 277 moved to the adjacent quartiles. Therefore, 91.5% of participants either remained in the same quartile or moved to an adjacent quartile of scores for the low-DHA pattern compared to baseline.

6.5.3 Baseline and 10-year follow-up characteristics of the participants

In **Table 6-2**, we present baseline and 10-year follow-up characteristics of the participants by gender at two time points. Physical activity decreased significantly in both men and women. Increase in weight was only significant in women, not men. The WC increased significantly in both men and women, but the most substantial increase was recorded in women (effect size = 0.75).

All of the plasma phospholipid SFAs decreased significantly for both men and women at 10-year follow-up, except for myristic acid. The effect sizes for 10-year difference in SFAs for men and women ranged between 0.46 and 0.78, except for stearic acid, which had low effect sizes of 0.31 and 0.40 for men and women, respectively.

All the plasma phospholipid MUFAs decreased significantly for both men and women except for *cis*-vaccenic acid, which increased in men and POA increased in women at 10-year follow-up. The effect size for 10-year change in MUFAs ranged between 0.48 and 0.86 for erucic and nervonic acids in both men and women, and gondoic acid (effect size =0.64) in men. Changes in other MUFAs had low effect sizes.

In the n–3 PUFA group, ALA and EPA increased significantly for both men and women. However, DPA increased, whereas DHA decreased for women accordingly at the 10-year time point. The 10-year differences between the n–3 FAs had low effect sizes <0.38.

Most of the n–6 PUFAs increased for both men and women, except eicosadienoic and docosadienoic acids, which decreased at 10-year follow-up. Furthermore, mead acid increased significantly in women, but decreased in men at 10-year follow-up. Significant differences observed between the n–6 PUFAs at baseline and 10-year follow-up are supported by medium to large effect sizes ranging between 0.48 and 0.79, except for mead acid, GLA in men and adrenic acid in women.

6.5.4 Differences between plasma phospholipid fatty acids according to body mass index categories and sex of black South African adults at 10-year follow-up

All results are shown in **Table 6-3**. Plasma phospholipid levels of SFAs, stearic, arachidic and behenic acids were higher in overweight/obese participants of both sexes. Furthermore, lignoceric acid was higher in overweight/obese women, and PA was higher in leaner men. In the MUFAs group, POA, *cis*-vaccenic and OA were higher in leaner participants of both sexes, but gondoic acid was higher in leaner men only. Interestingly, nervonic acid was higher in overweight and obese women, but not men. Among the n–3 PUFAs, only DHA was higher in overweight and obese participants of both sexes; however, ALA was higher in lean women but not men. Among the n–6 PUFAs, dihomo- γ -linolenic acid (DGLA) was higher in overweight/obese participants of both sexes. Unexpectedly, eicosadienoic acid was higher in leaner men, and osbond acid was higher in leaner women. Despite the fact that we observed significant differences of FAs between overweight/obese and lean participants in both men and women, most had a small effect size of <0.45.

6.5.5 Associations of plasma phospholipid fatty acid patterns with dietary intake and changes in anthropometric indices over ten years

Scores of the baseline high-Satfat pattern, were correlated with %E from total fat, %E from total SFAs, total fat and alcohol intake. The high-Satfat pattern was negatively correlated to total fat intake ($r=-0.14$, $p=0.0016$) and alcohol intake ($r=-0.35$, $p<.0001$). Stearic acid was positively correlated to %E from total fat ($r=0.10$, $p=0.03$), %E from total SFAs ($r=0.12$, $p=0.018$), and negatively correlated to alcohol intake ($r=-0.25$, $p<0.0001$). Arachidic acid was positively correlated to %E from total fat ($r=0.12$, $p=0.013$) and %E from total SFAs ($r=0.12$, $p=0.02$), and negatively correlated to alcohol intake ($r=-0.15$, $p=0.002$). Behenic acid was positively correlated to %E from total fat ($p=0.12$, $p=0.016$) and %E from total SFAs ($r=0.09$, $p=0.07$), and negatively correlated to alcohol intake (-0.30 , $p<.0001$). Lignoceric acid was positively correlated to %E from total fat ($r=0.13$, $p=0.009$), and negatively correlated to alcohol intake ($r=-0.16$) and $p=0.001$.

Results of the sequential linear regression are shown in **Table 6-4**. Out of the six patterns identified at baseline, two patterns, the n-3 and n-6 VLC-PUFA patterns, were not associated with any of the outcomes at 10-year follow-up. The high-Satfat and n-9 LC-MUFA patterns were positively associated with 10-year changes in all anthropometric outcomes measured and remained positively associated with all the outcomes in the fully adjusted models. The high-LA pattern was positively associated with all outcomes measured in the crude model and in the model adjusted for age and sex; however, no association was found in the fully adjusted model. The low-DHA pattern was negatively associated with 10-year changes in WC and WHtR only in the crude model.

Among the covariates included in the linear regression models, age was negatively associated with a 10-year increase in all anthropometric indices, whereas male sex was associated with 10-year increase in WHtR, in the two adjusted models. Furthermore, baseline variables (weight, BMI, WC and WHtR) were all negatively associated with 10-year changes in all related outcomes.

6.6 Discussion

In this prospective cohort of the PURE study, we followed up a selected group of black South African men and women for ten years. We investigated the prospective associations between six distinct plasma phospholipid FA patterns derived at baseline and 10-year changes in anthropometric indices, including weight, BMI, WC and WHtR. Two patterns, i.e. high-Satfat and n-9 LC-MUFA, were positively associated with a 10-year increase in all anthropometric indices after adjustment for age, sex, lifestyle, and dietary intake variables. The high-LA pattern was associated with 10-year changes in the crude model, and change in weight and BMI in the model adjusted for age and sex. The low-DHA pattern was negatively associated with WC and WHtR only in the crude model. Our study is the first to prospectively examine the associations of plasma phospholipid FA patterns with changes in anthropometric indices as well as reproducibility of these patterns over time in black Africans.

Circulating FA levels reflect exogenous intake and endogenous metabolism, including *de novo* lipogenesis and FA desaturation and elongation (35). Notably, the bulk of studies examining cross-sectional associations between circulating FA concentrations and obesity have focused on individual FAs and used blood FA fractions from plasma phospholipids (25), plasma (23, 24, 27), erythrocyte membrane (36), and adipose tissue (37). To the best of our knowledge, few studies have investigated the prospective associations of plasma phospholipid FA patterns and obesity outcomes (17). None of these studies included black populations of African descent.

It should be considered that the plasma FA composition is expressed as a percentage of total FAs in this study, and as one FA increases, others decrease depending on which specific FAs are being modulated (35). We should, therefore, interpret FA patterns for the present study with caution. Although the trend is to interpret patterns according to the variables with high factor loadings, this may be deceiving and limits the experimental nature of PCA studies (38); however, interpretation using high factor loading has been frequently applied in dietary pattern studies (39). In our study, both high and low factor loadings have been considered in the interpretation of results, where applicable.

The high-Satfat pattern remains of interest, because, firstly, it has high loadings (>0.80) of individual LC and VLC-SFAs (stearic, behenic, arachidic, and lignoceric acid), but also negative loadings of MUFAs (POA, OA and *cis*-vaccenic acid). Secondly, this pattern was significantly associated with increases in all anthropometric variables over ten years. Thirdly, the leaner participants of both sexes had significantly higher plasma levels of the same individual MUFAs (POA, OA, and *cis*-vaccenic acid) at 10-year follow-up than their overweight/obese counterparts. Finally, the overweight/obese participants of both sexes had significantly higher plasma levels of most SFAs (stearic, arachidic, behenic and lignoceric) compared to their leaner counterparts at 10-year follow-up. This is in line with a study that reported higher serum levels of stearic, arachidic, behenic and lignoceric acids in women with excess weight in Poland (40), although their study had a small sample size (23 women with excess weight and 21 healthy women in the control group).

Higher plasma levels of VLC-SFAs, i.e. arachidic and behenic acids were associated with a higher intake of saturated fat and lower alcohol consumption in communities in the USA. Additionally, higher plasma levels of behenic and lignoceric acid were associated with a greater peanut intake (41). In our study, scores of the high-Satfat pattern and plasma levels of arachidic, behenic and lignoceric acids showed a positive correlation with %E intake from total fat, but a negative correlation with alcohol intake. Both arachidic and behenic showed positive correlations to %E intake from total SFAs. The weak association confirms that diet does not play an important role. Also, literature shows that high intake of SFAs does not contribute to accumulation of plasma SFAs (42). Nevertheless, it is not known if these circulating VLC-SFAs are influenced by diet or endogenous production, and further research is needed to better understand the relations of these VLC-SFAs with dietary intake and weight gain (43). VLC-SFAs are highly specialised and functional, and are exclusively found in sphingolipids. When extracted from phospholipid fractions, they represent the concentration of sphingomyelin (43, 44). Therefore, the relationship between these VLC-SFAs and obesity observed in our study may be explained by an association

with a possible higher relative quantity of sphingomyelin in the total plasma phospholipid pool (44, 45). However, most studies on the association between VLC-SFAs with NCD risk as well as diabetes have reported inverse associations with these FAs (41, 43, 46). Consequently, this finding is difficult to explain and supports the need for further investigation of the associations of these VLC-SFAs with weight gain or to investigate the associations between FAs from different phospholipid sub-fractions, and other lipid fractions, with measures of weight gain.

The high-Satfat pattern also had high negative loadings of specific MUFAs (POA, *cis*-vaccenic and OA). It has been shown that POA is virtually absent in standard dietary intake, but is a major product of desaturation from palmitic acid through the stearoyl-CoA desaturase-1 activity in liver and adipose tissue (23). On the other hand, *cis*-vaccenic acid is derived from chain elongation of POA and has been used as a marker of elongase activity (47). Furthermore, OA can also be synthesised *de novo* in the liver by SCD-1 activity and has been shown to influence utilisation and storage of fat in mouse models (48). The leaner participants of both sexes had significantly higher plasma levels of POA, OA, and *cis*-vaccenic acid at 10-year follow-up than their overweight/obese counterparts. In some studies, POA has been positively related to abdominal obesity and BMI (22, 23), while *cis*-vaccenic has been inversely associated with obesity (49). Although we did not assess estimated desaturase activity for the current study, higher desaturase and elongase activity could be reflected as higher plasma phospholipid levels of POA, OA and *cis*-vaccenic acid, as observed in leaner participants of both sexes in the current study.

The high-LA pattern had the highest positive loadings of LA and eicosadienoic acid, as well as a negative loading of palmitic acid. This pattern was positively associated with 10-year changes in all anthropometric outcomes in the crude model and changes in weight and BMI after adjustment for age and sex. At 10-year follow-up, the plasma levels of both eicosadienoic and palmitic acids were higher in leaner than overweight men, but not women, and the effect sizes for the differences were small. No significant differences were observed between plasma LA of lean and overweight study participants, or between baseline and 10-year follow-up levels. LA is a principal n-6 PUFA consumed from the diet and is a major FA in plant lipids (50), like sunflower oil that is commonly used in the PURE population. Eicosadienoic acid is found mainly in small amounts in animal tissues and is formed by a delta-9 elongase enzyme from LA. However, eicosadienoic acid has not been investigated extensively in association with obesity. Therefore, it is not clear how to interpret the association between this FA pattern and weight gain in this study.

The n-9 LC-MUFA pattern with the highest positive loadings on nervonic acid and gondoic acid was positively associated with 10-year changes in all outcomes measured. It is interesting to note

that different MUFAs had high positive and negative loadings, respectively in the high-Satfat (C16:1 and C18:1, negative loadings) and the n-9 LC-MUFA (C20:1 to C24:1, positive loadings) patterns at baseline and both patterns were positively associated with increases in adiposity-related outcomes. We also established that women with a BMI of ≥ 25 kg/m² had significantly higher plasma phospholipid levels of nervonic acid than lean women. Leaner men (BMI of ≤ 25 kg/m²) had higher levels of gondoic acid at 10-year follow-up than their overweight counterparts. However, most of the MUFAs decreased at 10-year follow-up, while the anthropometric indices increased (**Table 6-2**). Furthermore, the baseline n-9 LC-MUFA pattern was not similar to any of the plasma FA patterns identified at follow-up. Instead, the n-9 LC-MUFAs (C20:1 to C24:1) were included in the high-Satfat pattern (factor 2) at 10-year follow-up. Nervonic acid is found in seed oil plants (51), and gondoic acid is found in wild fruits (52), mustard seed and rapeseed (53) in small quantities, and in some marine fish in larger amounts. Nervonic and gondoic acids can also be formed by *de novo* synthesis through the elongation of OA and converted to other FAs (54). Therefore, this pattern may be related to *de novo* lipogenesis rather than dietary intake, because the study participants had low intakes of wild fruits, mustard seed and rapeseed and marine fish (12). To the best of our knowledge, no other studies have investigated the association of these n-9 LC-MUFAs in plasma phospholipids with obesity in black Africans. Consequently, it is not possible to compare our results with other studies. These findings suggest that increased hepatic desaturation of SFAs to MUFAs may indicate an underlying mechanism behind the positive association between plasma n-9 LC-MUFAs and weight gain, observed in our study. These findings need to be investigated further.

The low-DHA pattern with the highest positive loading of ALA and GLA and negative loading of DHA was negatively associated with 10-year changes in WC and WHtR only in the crude model. ALA is a major n-3 PUFA in green leafy vegetables, nuts and seeds (55). In our study, plasma ALA was higher in women with BMI < 25 kg/m² than in overweight women, but not in men, and decreased significantly among both men and women at 10-year follow-up, while BMI increased significantly. The higher plasma DHA in overweight/obese men and women than in the lean groups in the current study was unexpected and possibly related to higher DHA intakes (56). The lack of association between the n-3 VLC-PUFA pattern and 10-year changes in outcomes and the weak association between the pattern with positive loading of ALA and gains in adiposity are in contrast to earlier reports of an inverse association between plasma n-3 PUFAs and measures of adiposity in free-living adults in Australia (57). Our results are supported by a recent review on n-3 PUFAs for the primary and secondary prevention of cardiovascular disease, which reported a lack of association of n-3 LC-PUFAs with body weight, BMI and other measures of adiposity in

intervention studies (58). Nevertheless, further research involving the association between n-3 PUFAs and obesity in black Africans is required.

Relatively similar patterns as derived from our study were identified from a Danish longitudinal study that investigated the association of adipose tissue FA patterns and changes in anthropometry over five years. However, irrespective of the different lipid pools studied, there were typical differences between our patterns and those identified by Dahm *et al.* (17). Firstly, their patterns were obtained for men and women separately, whereas our pattern was obtained from a combined group. They used 34 FAs as compared to our 25 FAs, and their patterns included high loadings on trans-FAs not included in our patterns. Furthermore, the Danish participants consumed more fish, olives and olive oil (59, 60), as compared to our participants. Last but not least, the number of their study participants was higher (n=1100), compared to ours (n=412). We, therefore, cannot compare our patterns and those of Dahm and colleagues (17) directly. However, they reported a pattern (PC1) with positive loadings of SFAs and negative loadings of MUFAs and other LC-PUFAs, but this pattern was not associated with changes in WC_{BMI}, defined as residual values from equations of BMI and WC (17). The PC6 pattern had the highest level of arachidic acid and was positively associated with all outcomes measured. The PC7 pattern had positive loadings of LA and eicosadienoic and negative loading of trans-FAs. This pattern was negatively associated with a 5-year change in WC and marginally negatively associated with changes in WC_{BMI} in men (17).

Several studies investigating FA patterns have studied pattern stability (60-62). Dahm and colleagues (17) reproduced adipose tissue FA patterns through a random selection of men and women from the study population into a new group. They derived new patterns in adipose tissue FAs for this randomly selected group and assessed the stability of the new patterns by assessing the correlation between pattern scores derived for the total group and the new group. They were able to reproduce two patterns and had high correlation coefficients (> 0.80) between the two new pattern scores, indicating good stability (17). In our study, we reproduced four patterns at 10-year follow-up with inclusion of all participants. Consequently, after 10 years of follow-up, more than 88% of the participants either remained in the same quartile or moved to adjacent quartiles of factor scores for the individual patterns compared to baseline. This is an indication of stability of four of the six plasma phospholipid FA patterns (high-Satfat, n-6 VLC-PUFA, low-DHA and n-3 VLC-PUFA) identified in this selected group of black South Africans. Dietary intakes generally influence endogenous metabolism and circulating FA status (35). Therefore, the reproducibility of our patterns over time may be explained by sustained dietary intake over the 10-year period with minimal changes and no major impact in plasma phospholipid FAs status of our study participants.

In general, the major differences between our FA results and those of other studies include that this study has been done in a group of black South African adults, while most other studies included populations from different geographical locations and ethnicities and with different diets. The levels of phospholipid FA changed significantly over the 10-year period for most FA for both males and females. This may be explained by possible changes in dietary intakes over a period of 10-years, directly affecting FA status of our study participants. Plasma FAs are a complex reflection of metabolism, influenced by dietary intake, climate and seasons, affecting food supply, aging and geographical locations, processing, storage and cooking methods (35, 63), and further influenced by cultural and religious dietary practices (64). FAs derived from plasma and adipose tissue may not inform the potentially different roles that liver and adipose tissue metabolism play in the development of obesity (65). The challenge of interpretation of patterns derived using PCA persists, even though the use of pattern analysis is a more preferred method to investigate associations between nutrients and diseases (39).

These results are of particular importance because of concerns about the health and economic burden of increasing obesity being included among the global NCD targets (66). Obesity is a risk factor for metabolic syndrome and NCDs, and is a public health concern in both high-income and low- and middle-income countries (6, 7). In our study of 10-year follow-up, the weight and BMI of women increased more than in men. The increase of obesity indicators in women is a disturbing trend, considering that South Africa has the highest prevalence of obesity in sub-Saharan Africa and women are more obese compared to men with an alarming prevalence of 68% (3).

6.7 Strengths and limitations

The advantages of the study include accurate and standardised measurements of the anthropometric parameters of interest and a robust endpoint (change in anthropometric indices) with a relatively extended follow-up period (ten years) in a sub-cohort of the international PURE study. The measurements of plasma phospholipid FAs were performed at two different time points, and the reproducibility of four plasma phospholipid FA patterns may represent longer-term stability status of plasma FAs in this group of black South Africans. This study had some limitations, including that anthropometric measurements, are prone to error when repeated measurements are performed after extended follow-up, even though the research assistants were trained intensively according to International Society for the Advancement of Kinanthropometry guidelines (67) at all-time points. We did not assess biomarkers of inflammation. Clinical covariates and co-morbidities that influence inflammatory biomarkers, such as ageing, obesity, metabolic abnormalities may also affect the status of plasma phospholipid FAs in our participants.

Future studies should integrate biomarkers of inflammation. Furthermore, the numbers of participants were not large enough to derive patterns separately for men and women. We also lacked sufficient resources to evaluate plasma phospholipid FAs in a larger number of the participants.

6.8 Conclusion

This study pioneered the prospective investigation of plasma phospholipid FA patterns with 10-year changes in anthropometric indices in black Africans. Two patterns, i.e. high-Satfat (positively loaded with stearic acid, VLC-SFAs and negative loadings of POA, *cis*-vaccenic acid and OA) and n-9 LC-MUFA (positive loadings of nervonic acid and gondoic acids), consistently predicted adiposity gain, as measured by weight, BMI, WC and WHtR over 10 years. However, the n-9 LC-MUFA pattern was not maintained for 10-years when we studied its reproducibility and stability, by comparing the two patterns identified at baseline and at 10-year follow-up. The high-Satfat pattern derived at baseline was reproducible in the study participants after 10 years and may indicate a unique FA metabolism associated with weight gain over time in this group of black South African adults. This publication adds to the understanding of plasma phospholipid FA patterns and their prospective associations with measures of adiposity, as well as the reproducibility of these patterns. Further work is required to improve interpretation and application of circulating FA patterns in health and disease globally, but especially in Africa, where data are lacking.

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Table 6-1: Factor loadings of plasma phospholipid fatty acid patterns derived at two time points, i.e. baseline and 10-year follow-up

Factor loadings of patterns derived at baseline							Factor loadings of patterns derived at 10-year follow-u					
	High_Satfat	n-9 LC-MUFA	n-6 VLC	High-LA	Low-DHA	n-3 LC-PUFA	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Explained variance	(λ1=22%)	(λ2=12%)	(λ3=11%)	(λ4=11%)	(λ5=8%)	(λ6=8%)	(λ1=20%)	(λ2=18%)	(λ2=12%)	λ4=9%)	(λ5=9%)	(λ6=6%)
Fatty acid name and formula	Kaiser's Measure of Sampling Adequacy= 0.58						Kaiser's Measure of Sampling Adequacy= 0.70					
Saturated												
Myristic acid (C14:0)	-0.09	-0.48	-0.11	-0.14	0.18	-0.25	0.67	-0.01	0.14	0.21	-0.04	-0.01
Palmitic acid (C16:0)	0.05	-0.37	-0.48	-0.54	0.18	-0.40	-0.10	-0.18	0.86	-0.13	-0.09	-0.07
Stearic acid (C18:0)	0.82	-0.06	0.02	-0.14	-0.07	-0.26	-0.06	0.03	-0.82	-0.19	-0.01	0.00
Arachidic acid (C20:0)	0.87	0.32	-0.09	-0.12	-0.02	-0.10	0.23	0.70	-0.21	-0.13	-0.07	0.25
Behenic acid (C22:0)	0.91	0.18	-0.14	-0.02	-0.05	-0.11	-0.26	0.59	-0.40	-0.39	-0.21	0.24
Lignoceric acid (C24:0)	0.85	0.24	-0.12	-0.07	0.22	-0.05	0.07	0.69	-0.24	-0.23	-0.29	0.21
Monounsaturated												
Palmitoleic acid (C16:1n-7)	-0.76	-0.13	-0.04	-0.34	0.41	-0.02	0.56	-0.22	0.50	0.48	0.12	-0.12
Cis-vaccenic acid (18:1n-7)	-0.66	0.56	0.09	0.00	-0.13	0.00	0.04	0.20	0.28	0.76	0.29	-0.20
Oleic acid (C18:1n-9)	-0.76	-0.08	0.02	-0.27	0.48	0.05	0.42	-0.41	0.55	0.43	-0.04	-0.13
Gondoic acid (C20:1n-9)	-0.08	0.64	-0.02	0.49	-0.07	-0.12	0.45	0.71	-0.09	0.23	0.10	-0.12
Erucic acid (C22:1n-9)	0.26	0.37	0.21	0.02	-0.06	-0.10	0.09	0.76	0.17	-0.06	0.18	0.12
Nervonic acid (C24:1n-9)	0.18	0.84	0.01	-0.03	-0.01	0.15	-0.10	0.74	-0.01	0.40	0.00	0.12
n-3 polyunsaturated fatty acids												
α-linolenic acid (C18:3n-3)	-0.19	-0.17	-0.11	0.41	0.58	0.24	0.67	0.23	0.06	0.08	-0.23	0.17
Eicosapentaenoic acid (C20:5n-3)	-0.28	-0.08	-0.28	-0.13	0.00	0.74	0.08	-0.20	-0.07	0.64	-0.14	0.17
Docosapentaenoic acid (C22:5n-3)	-0.10	0.12	0.19	0.11	-0.04	0.80	0.24	0.24	0.06	-0.02	0.03	0.76

Factor loadings of patterns derived at baseline							Factor loadings of patterns derived at 10-year follow-u					
	High_Satfat	n-9 LC-MUFA	n-6 VLC	High-LA	Low-DHA	n-3 LC-PUFA	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Explained variance	(λ1=22%)	(λ2=12%)	(λ3=11%)	(λ4=11%)	(λ5=8%)	(λ6=8%)	(λ1=20%)	(λ2=18%)	(λ2=12%)	λ4=9%)	(λ5=9%)	(λ6=6%)
Fatty acid name and formula	Kaiser's Measure of Sampling Adequacy= 0.58						Kaiser's Measure of Sampling Adequacy= 0.70					
Docosahexaenoic acid (C22:6n-3)	-0.09	0.14	-0.10	0.28	-0.66	0.44	-0.31	0.22	-0.36	0.06	0.09	0.62
n-6 polyunsaturated fatty acids												
Linoleic acid (C18:2n-6)	-0.02	0.09	-0.08	0.88	-0.10	0.12	-0.24	0.18	-0.39	-0.28	-0.67	-0.29
γ-linolenic acid (C18:3n-6)	-0.38	-0.44	0.22	-0.12	0.51	0.07	0.87	0.03	0.08	-0.04	0.00	-0.04
Eicosadienoic acid (C20:2n-6)	0.04	0.26	0.32	0.76	0.07	-0.11	0.62	0.55	-0.30	0.09	-0.05	-0.11
Dihomo-γ-linolenic acid (C20:3n-6)	-0.10	-0.12	0.69	0.19	0.02	0.15	0.73	0.10	-0.19	-0.13	0.14	0.13
Arachidonic acid (C20:4n-6)	-0.52	0.03	0.19	-0.48	0.37	-0.04	-0.40	0.04	-0.21	-0.16	0.73	0.11
Docosadienoic acid (C22:2n-6)	-0.03	0.27	0.58	0.08	-0.51	0.31	0.05	0.85	-0.17	-0.20	0.07	0.03
Adrenic acid (C22:4n-6)	0.36	0.60	-0.06	0.36	-0.20	-0.10	0.69	0.29	0.00	0.01	0.48	-0.05
Osbond acid (C22:5n-6)	-0.08	0.09	0.88	-0.01	0.19	-0.01	0.51	0.05	-0.08	0.09	0.70	-0.15
n-9 polyunsaturated fatty acid												
Mead acid (C20:3n-9)	-0.11	0.06	0.83	-0.15	-0.10	-0.22	0.67	-0.03	0.40	0.30	0.25	0.04

^a Factor1-Factor 6= abbreviated names for the plasma phospholipid fatty acid patterns derived at 10-year follow-up.

Abbreviations: Satfat, saturated fatty acid pattern; n-3 VLC-PUFA, omega-3 very long chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; n-6 VLC-PUFA, omega-6 very long chain polyunsaturated fatty acid pattern; LC-MUFA, long chain monounsaturated fatty acid pattern; n-3 EFA, omega-3 essential fatty acid pattern.

Table 6-2: Sex-specific demographic and anthropometric data and plasma phospholipid fatty acids of black South African adults at two time points, i.e. baseline and 10-year follow-up

	Men (n=137)				Women (n=275)			
	Baseline	10-year follow-up	p-value ^c	Effect size	Baseline	10-year follow-up	p-value	Effect size
	Median (Q ₁ , Q ₂) ^b	Median (Q ₁ , Q ₂)			Median (Q ₁ , Q ₂)	Median (Q ₁ , Q ₂)		
Variables ^a								
Age (years)	53 (47, 60)	63.9 (57.2, 71.0)	<.0001		51 (44, 58)	61.5 (55.2, 68.6)	<.0001	
Physical activity index	2.83 (2.52, 3.27)	2.41 (1.90, 2.84)	<.0001		2.82 (2.53, 3.23)	2.15 (1.88, 2.52)	<.0001	
Anthropometric								
Weight (kg)	56.6 (50.7, 66.5)	58.1 (52.1, 66.3)	0.12		64.2 (54.4, 78.2)	67.8 (55.2, 80.5)	<.0001	0.19
Body mass index (kg/m ²)	20.1 (18.5, 23.9)	20.7 (18.6, 24.3)	0.03	0.18	25.8 (22.0, 31.6)	27.8 (22.9, 33.0)	<.0001	0.30
Waist circumference (cm)	75.4 (70.4, 83.5)	79.5 (74.2, 91.0)	<.0001	0.56	81.9 (72.3, 92.7)	93.0 (81.2, 101.6)	<.0001	0.75
Waist to height ratio	0.45 (0.42, 0.50)	0.48 (0.44, 0.54)	<.0001	0.58	0.52 (0.46, 0.58)	0.59 (0.52, 0.65)	<.0001	0.77
Plasma phospholipid fatty acids (% total FAs)								
Saturated								
Myristic acid (C14:0)	0.25 (0.19, 0.30)	0.32 (0.27, 0.38)	<.0001	0.55	0.26 (0.22, 0.32)	0.33 (0.28, 0.38)	<.0001	0.55
Palmitic acid (C16:0)	27.0 (25.1, 28.8)	25.5 (24.0, 27.0)	<.0001	0.49	26.1 (24.3, 28.6)	24.4 (23.4, 25.6)	<.0001	0.49
Stearic acid (C18:0)	14.4 (13.0, 15.5)	13.4 (12.5, 14.5)	<.0001	0.41	15.3 (14.1, 17.0)	14.5 (13.5, 15.4)	<.0001	0.46
Arachidic acid (C20:0)	0.27 (0.23, 0.36)	0.24 (0.2, 0.28)	<.0001	0.54	0.35 (0.29, 0.42)	0.28 (0.23, 0.33)	<.0001	0.65
Behenic acid (C22:0)	1.02 (0.72, 1.26)	0.92 (0.68, 1.08)	<.0001	0.31	1.23 (0.98, 1.54)	1.10 (0.86, 1.35)	<.0001	0.40
Lignoceric acid (C24:0)	1.04 (0.87, 1.28)	0.74 (0.64, 0.87)	<.0001	0.78	1.06 (0.90, 1.30)	0.74 (0.62, 0.91)	<.0001	0.78
Monounsaturated								
Palmitoleic acid (C16:1n-7)	0.70 (0.41, 1.34)	0.77 (0.53, 1.16)	0.31		0.62 (0.44, 0.89)	0.66 (0.49, 0.98)	0.001	0.19
<i>Cis</i> -vaccenic acid (C18:1n-7)	1.37 (1.18, 1.61)	1.4 (1.18, 1.71)	<.0001	0.09	1.33 (1.13, 1.56)	1.30 (1.12, 1.53)	<.0001	0.02
Oleic acid (C18:1n-9)	8.80 (7.08, 12.1)	7.97 (6.93, 10.3)	0.0008	0.14	7.37 (6.33, 9.09)	7.16 (6.16, 8.51)	0.002	0.19

	Men (n=137)				Women (n=275)			
	Baseline	10-year follow-up	p-value ^c	Effect size	Baseline	10-year follow-up	p-value	Effect size
	Median (Q ₁ , Q ₂) ^b	Median (Q ₁ , Q ₂)			Median (Q ₁ , Q ₂)	Median (Q ₁ , Q ₂)		
Variables ^a								
Gondoic acid (C20:1n-9)	0.11 (0.09, 0.12)	0.09 (0.07, 0.11)	<.0001	0.28	0.10 (0.09, 0.12)	0.08 (0.07, 0.10)	<.0001	0.64
Erucic acid (C22:1n-9)	0.06 (0.05, 0.08)	0.02 (0.01, 0.03)	<.0001	0.48	0.07 (0.05, 0.09)	0.02 (0.01, 0.03)	<.0001	0.86
Nervonic acid (C24:1n-9)	1.67 (1.45, 1.92)	1.45 (1.22, 1.66)	<.0001	0.86	1.81 (1.50, 2.13)	1.43 (1.24, 1.72)	<.0001	0.62
n-3 polyunsaturated								
α-linolenic acid (C18:3n-3)	0.09 (0.06, 0.12)	0.06 (0.04, 0.09)	<.0001	0.34	0.08 (0.06, 0.11)	0.06 (0.04, 0.08)	<.0001	0.34
Eicosapentaenoic acid (C20:5n-3)	0.64 (0.45, 0.91)	0.47 (0.34, 0.65)	<.0001	0.38	0.54 (0.40, 0.82)	0.43 (0.32, 0.64)	<.0001	0.28
Docosapentaenoic acid (C22:5n-3)	1.45 (1.22, 1.65)	1.50 (1.19, 1.84)	0.10		1.38 (1.15, 1.54)	1.44 (1.20, 1.74)	<.0001	0.26
Docosahexaenoic acid (C22:6n-3)	3.89 (3.16, 5.03)	3.94 (3.21, 4.69)	0.58		4.72 (3.71, 5.73)	4.48 (3.79, 5.27)	0.02	0.14
n-6 polyunsaturated								
Linoleic acid (C18:2n-6)	16.5 (14.2, 19.0)	16.0 (14.2, 17.5)	0.31		16.0 (13.6, 18.7)	16.0 (14.2, 18.4)	0.51	
γ-linolenic acid (C18:3n-6)	0.11 (0.07, 0.16)	0.10 (0.07, 0.16)	0.02	0.20	0.10 (0.06, 0.14)	0.09 (0.06, 0.13)	0.14	
Eicosadienoic acid (C20:2n-6)	0.35 (0.29, 0.41)	0.28 (0.25, 0.32)	<.0001	0.61	0.37 (0.32, 0.43)	0.29 (0.25, 0.35)	<.0001	0.65
Dihomo-γ-linolenic acid (C20:3n-6)	2.70 (2.33, 3.19)	3.31 (2.86, 3.81)	<.0001	0.57	2.95 (2.50, 3.40)	3.40 (2.88, 4.07)	<.0001	0.48
Arachidonic acid (C20:4n-6)	13.3 (11.6, 14.8)	17.8 (15.9, 19.5)	<.0001	0.85	13.7 (12.4, 15.2)	18.2 (16.5, 19.9)	<.0001	0.85
Docosadienoic (C22:2n-6)	0.02 (0.013, 0.023)	0.011 (0.01, 0.16)	<.0001	0.67	0.02 (0.02, 0.03)	0.01 (0.01, 0.02)	<.0001	0.74
Adrenic acid (C22:4n-6)	0.59 (0.49, 0.71)	0.69 (0.59, 0.81)	<.0001	0.44	0.57 (0.47, 0.69)	0.61 (0.52, 0.75)	0.0004	0.21
Osbond acid (C22:5n-6)	0.52 (0.40, 0.69)	0.95 (0.75, 1.28)	<.0001	0.79	0.57 (0.43, 0.79)	0.93 (0.69, 1.23)	<.0001	0.72
n-9 polyunsaturated								
Mead acid (C20:3n-9)	0.21 (0.15, 0.38)	0.23 (0.15, 0.37)	<.0001	0.05	0.18 (0.13, 0.28)	0.16 (0.11, 0.26)	<.0001	0.15

^a Data are presented as median and interquartile range.

^b Significance levels of differences measured between two-time points (baseline 2005 and 10-year follow-up in 2015), Wilcoxon matched pairs test.

Table 6-3: Differences between plasma phospholipid fatty acids according to body mass index categories and sex of black South African adults at 10-year follow-up

	Men (n = 137)				Women (n = 275)			
	Lean/normal weight (BMI < 25 kg/m ²) ^b	Overweight/obese (BMI ≥ 25 kg/m ²)	p-value ^d	Effect size	Lean/normal weight (BMI < 25 kg/m ²)	Overweight/obese (BMI ≥ 25 kg/m ²)	p-value	Effect size
	(n = 105)	(n = 32)			(n = 99)	(n = 176)		
	Median (Q ₁ , Q ₂) ^c	Median (Q ₁ , Q ₂)			Median (Q ₁ , Q ₂)	Median (Q ₁ , Q ₂)		
Plasma phospholipid fatty acids ^a								
Saturated								
Myristic acid (C14:0)	0.31 (0.26, 0.38)	0.32 (0.28, 0.35)	0.65		0.33 (0.27, 0.39)	0.33 (0.28, 0.38)	0.86	
Palmitic acid (C16:0)	25.8 (24.1, 27.1)	24.7 (23.5, 26.2)	0.02	0.37	24.5 (23.4, 25.7)	24.4 (23.3, 25.6)	0.36	
Stearic acid (C18:0)	13.2 (12.5, 14.3)	14.4 (13.1, 15.0)	0.004	0.33	14.3 (13.2, 15.0)	14.7 (13.8, 15.6)	0.01	0.41
Arachidic (20:0)	0.23 (0.20, 0.27)	0.25 (0.22, 0.31)	0.04	0.40	0.24 (0.18, 0.30)	0.30 (0.25, 0.35)	<.0001	0.29
Behenic (C22:0)	0.90 (0.65, 1.06)	0.99 (0.85, 1.25)	0.04	0.36	0.95 (0.68, 1.18)	1.20 (0.98, 1.40)	<.0001	0.30
Lignoceric (C24:0)	0.73 (0.64, 0.85)	0.80 (0.67, 0.92)	0.10		0.68 (0.56, 0.84)	0.77 (0.65, 0.94)	0.005	0.38
Monounsaturated								
Palmitoleic acid (C16:1n-7)	0.81 (0.59, 1.34)	0.64 (0.42, 0.87)	0.049	0.35	0.74 (0.56, 1.39)	0.64 (0.48, 0.90)	0.054	
Cis-vaccenic (C18:1n-7)	1.45 (1.23, 1.77)	1.30 (1.09, 1.42)	0.006	0.32	1.40 (1.18, 1.62)	1.25 (1.09, 1.47)	0.002	0.39
Oleic acid (C18:1n-9)	8.37 (7.24, 11.0)	7.53 (5.95, 8.17)	0.006	0.31	7.82 (6.69, 10.1)	6.70 (6.05, 8.02)	<.0001	0.33
Gondoic (C20:1n-9)	0.10 (0.08, 0.11)	0.08 (0.07, 0.09)	0.006	0.31	0.09 (0.08, 0.10)	0.08 (0.072, 0.10)	0.67	
Erucic (C22:1n-9)	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.45		0.02 (0.01, 0.03)	0.02 (0.02, 0.03)	0.41	
Nervonic (C24:1n-9)	1.46 (1.24, 1.68)	1.40 (1.13, 1.53)	0.72		1.32 (1.18, 1.59)	1.47 (1.30, 1.76)	0.005	0.39
n-3 polyunsaturated								

	Men (n = 137)				Women (n = 275)			
	Lean/normal weight (BMI < 25 kg/m ²) ^b	Overweight/obese (BMI ≥ 25 kg/m ²)	p-value ^d	Effect size	Lean/normal weight (BMI < 25 kg/m ²)	Overweight/obese (BMI ≥ 25 kg/m ²)	p-value	Effect size
	(n = 105)	(n = 32)			(n = 99)	(n = 176)		
	Median (Q ₁ , Q ₂) ^c	Median (Q ₁ , Q ₂)			Median (Q ₁ , Q ₂)	Median (Q ₁ , Q ₂)		
Plasma phospholipid fatty acids ^a								
α-linolenic acid (C18:3n-3)	0.06 (0.05, 0.09)	0.06 (0.04, 0.11)	0.96		0.07 (0.04, 0.10)	0.06 (0.04, 0.08)	0.007	0.42
Eicosapentaenoic acid (C20:5n-3)	0.47 (0.36, 0.64)	0.50 (0.30, 0.76)	0.65		0.44 (0.30, 0.68)	0.43 (0.32, 0.61)	0.86	
Docosapentaenoic acid (C22:5n-3)	1.48 (1.19, 1.84)	1.60 (1.19, 1.85)	0.65		1.47 (1.24, 1.73)	1.44 (1.17, 1.75)	0.50	
Docosahexaenoic acid (C22:6n-3)	3.78 (3.13, 4.51)	4.40 (3.71, 4.98)	0.04	0.36	4.13 (3.69, 5.17)	4.60 (3.91, 5.30)	0.04	0.44
n-6 polyunsaturated								
Linoleic acid (C18:2n-6)	15.9 (14.1, 17.5)	16.4 (14.2, 17.4)	0.39		15.7 (13.5, 17.7)	16.3 (14.6, 18.6)	0.11	
γ-linolenic acid (C18:3n-6)	0.10 (0.06, 0.17)	0.10 (0.07, 0.12)	0.65		0.09 (0.06, 0.15)	0.09 (0.06, 0.12)	0.86	
Dihomo-γ-linolenic acid (C20:3n-6)	3.25 (2.79, 3.68)	3.80 (3.07, 4.44)	0.01	0.36	3.31 (2.93, 3.78)	3.56 (2.88, 4.28)	0.04	0.41
Eicosadienoic (C20:2n-6)	0.29 (0.25, 0.33)	0.26 (0.23, 0.29)	0.05	0.32	0.29 (0.24, 0.34)	0.30 (0.25, 0.35)	0.56	
Arachidonic acid (C20:4n-6)	17.6 (15.6, 19.5)	18.5 (17.0, 19.5)	0.21		18.3 (16.3, 19.9)	18.1 (16.7, 19.9)	0.50	
Docosadienoic (C22:2n-6)	0.01 (0.001, 0.02)	0.01 (0.01, 0.02)	0.25		0.01 (0.01, 0.02)	0.02 (0.01, 0.02)	0.02	0.36
Adrenic acid (C22:4n-6)	0.69 (0.59, 0.79)	0.69 (0.56, 0.88)	0.96		0.64 (0.55, 0.79)	0.58 (0.5, 0.75)	0.09	
Osbond acid (C22:5n-6)	0.93 (0.75, 1.20)	1.02 (0.76, 1.49)	0.21		1.04 (0.81, 1.34)	0.89 (0.65, 1.16)	0.03	0.39
n-9 polyunsaturated								
Mead acid (C20:3n-9)	0.23 (0.16, 0.41)	0.18 (0.11, 0.27)	0.24		0.18 (0.12, 0.31)	0.15 (0.10, 0.24)	0.15	

^a Plasma phospholipid fatty acids at 10-year follow-up.

^b BMI=Body mass index categorised as lean/normal (BMI < 25 kg/m²), and Overweight/obese (BMI ≥ 25 kg/m²) at 10-year follow-up.

^c Data are presented as median and interquartile range.

^d Significance levels of differences in plasma phospholipid fatty acids according to BMI in men and women at 10-year follow-up, Mann-Whitney test.

Table 6-4 Prospective associations of baseline plasma phospholipid fatty acid patterns with changes in anthropometric indices of black South African adults followed up for 10 years (n = 412)

	Change in weight			Change in body mass index			Change in waist circumference			Change in waist: height ratio		
	β & (95% CI)	p-value ^a	SP Corr ^b	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr
<i>Crude model^c</i>												
High-Satfat	0.09 (-0.02, 1.58)	0.06	0.009	0.11 (0.05, 0.69)	0.02	0.012	0.19 (0.09, 0.28)	0.0001	0.033	0.19 (0.01, 0.02)	<.0001	0.035
LC-MUFA	0.07 (-0.19, 1.40)	0.13	0.006	0.12 (0.07, 0.70)	0.02	0.014	0.18 (0.08, 0.27)	0.0002	0.031	0.20 (0.006, 0.02)	<.0001	0.040
n-6 VLC-PUFA	0.004 (-0.77, 0.83)	0.94	0.00001	0.03 (-0.22, 0.41)	0.57	0.001	0.02 (-0.68, 1.02)	0.70	0.0003	0.03 (-0.004, 0.01)	0.53	0.001
High-LA	0.13 (0.03, 0.22)	0.01	0.016	0.13 (0.10, 0.73)	0.01	0.016	0.11 (0.106, 1.79)	0.03	0.011	0.10 (0.001, 0.011)	0.03	0.011
Low-DHA	-0.03 (-1.07, 0.53)	0.50	0.001	-0.03 (-0.42, 0.22)	0.53	0.001	-0.10 (-1.71, -0.01)	0.046	0.009	-0.10 (-0.19, -0.005)	0.04	0.010
n-3 LC-PUFA	-0.02 (-0.96, 0.63)	0.68	0.0004	-0.02 (-0.40, 0.24)	0.62	0.001	-0.06 (-1.39, 0.30)	0.20	0.004	-0.06 (-0.16, 0.03)	0.18	0.004
R-squared (%)			0.03			0.04			0.09			0.10
<i>Adjusted model^{1e}</i>												
High-Satfat	0.12 (0.02, 0.21)	0.02	0.009	0.12 (0.09, 0.72)	0.01	0.012	0.17 (0.07, 0.26)	0.0005	0.033	0.16 (0.004, 0.01)	0.0006	0.035
LC-MUFA	0.06 (-0.27, 1.25)	0.21	0.006	0.10 (0.03, 0.65)	0.03	0.014	0.16 (0.07, 0.25)	0.0006	0.031	0.18 (0.005, 0.02)	<.0001	0.040
n-6VLC-PUFA	0.01 (-0.68, 0.86)	0.81	0.0000	0.03 (-0.21, 0.41)	0.51	0.001	0.01 (-0.71, 0.91)	0.82	0.0003	0.02 (-0.004, 0.01)	0.69	0.001
High-LA	0.09 (-0.02, 1.52)	0.05	0.016	0.10 (0.004, 0.19)	0.04	0.016	0.08 (-0.09, 1.53)	0.08	0.011	0.08 (-0.0004, 0.01)	0.07	0.011
Low-DHA	-0.03 (-1.01, 0.56)	0.58	0.0011	-0.02 (-0.37, 0.26)	0.72	0.001	-0.05 (-1.26, 0.40)	0.31	0.009	-0.04 (-0.008, 0.003)	0.37	0.010

	Change in weight			Change in body mass index			Change in waist circumference			Change in waist: height ratio		
	β & (95% CI)	p-value ^a	SP Corr ^b	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr
n-3 LC-PUFA	0.002 (-0.75, 0.78)	0.97	0.0004	-0.002 (-0.32, 0.30)	0.97	0.001	-0.02 (-1.00, 0.61)	0.63	0.004	-0.02 (-0.006, 0.004)	0.61	0.004
Age	-0.28 (-0.32, -0.16)	<.0001	0.078	-0.24 (-0.33, -0.14)	<.0001	0.056	-0.22 (-0.29, -0.12)	<.0001	0.056	-0.19 (-0.28, -0.10)	<.0001	0.044
Sex							0.20 (0.11, 0.30)	<.0001	0.036	0.24 (0.02, 0.040)	<.0001	0.052
R-square (%)			0.11			0.10			0.18			0.20
Fully adjusted model^f												
High-Satfat	0.20 (0.09, 0.30)	0.0003	0.015	0.22 (0.10, 0.31)	0.0001	0.020	0.23 (0.13, 0.34)	<.0001	0.044	0.24 (0.007, 0.02)	<.0001	0.046
LC-MUFA	0.13 (0.03, 0.22)	0.008	0.015	0.18 (0.08, 0.27)	0.0002	0.029	0.19 (0.10, 0.28)	<.0001	0.041	0.22 (0.007, 0.02)	<.0001	0.052
n-6 VLC-PUFA	0.06 (-0.28, 1.32)	0.20	0.001	0.08 (-0.04, 0.60)	0.08	0.003	0.04 (-0.52, 1.24)	0.42	0.001	0.05 (-0.003, 0.01)	0.32	0.002
High-LA	0.09 (-0.10, 1.45)	0.09	0.013	0.09 (-0.03, 0.59)	0.07	0.012	0.07 (-0.20, 1.50)	0.13	0.008	0.07 (-0.001, 0.01)	0.13	0.008
Low-DHA	-0.08 (-1.43, 0.18)	0.13	0.002	-0.08 (-0.56, 0.08)	0.13	0.002	-0.08 (-1.61, 0.16)	0.11	0.010	-0.08 (-0.17, 0.02)	0.12	0.011
n-3 LC	-0.01 (-0.83, 0.69)	0.86	0.0003	-0.01 (-0.35, 0.26)	0.78	0.001	-0.02 (-1.00, 0.67)	0.70	0.002	-0.02 (-0.11, 0.07)	0.68	0.002
Age	-0.23 (-0.27, -0.11)	<.0001	0.069	-0.17 (-0.09, -0.02)	0.001	0.048	-0.17 (-0.24, -0.07)	0.0007	0.043	-0.13 (-0.22, -0.03)	0.01	0.032
Sex							0.19 (0.08, 0.28)	0.0003	0.026	0.25 (0.02, 0.04)	<.0001	0.040
Baseline variables	-0.22 (-0.32, -0.11)	<.0001	0.04	-0.28 (-0.18, -0.08)	<.0001	0.05	-0.13 (-0.16, -0.02)	0.01	0.01	-0.15 (-0.17, -0.03)	0.01	0.02
R-square (%)			0.15			0.17			0.19			0.21

Change in weight			Change in body mass index			Change in waist circumference			Change in waist: height ratio		
β & (95% CI)	p-value ^a	SP Corr ^b	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr	β & (95% CI)	p-value	SP Corr

^a Significance levels for crude and upon sequential adjusted linear regression.

^b Squared semi-partial correlation Type 1.

^c Crude model (plasma phospholipid fatty acid patterns only).

^d Model fitting was evaluated by means of R-square statistic.

^e Adjusted model1 (adjusted for age and sex)

^f Fully adjusted model - further adjusted for lifestyle covariates, self-reported smoking, education, weighted physical activity index, total energy, and alcohol intake and baseline anthropometric variable (only data for FA patterns and significant covariates shown).

Abbreviations: CI, confidence intervals; SP Corr, semi-partial correlation; high-Satfat, saturated fatty acid pattern n-9 LC-MUFA, omega-9 long chain monounsaturated fatty acid pattern; n-6 VLC-PUFA, omega-6 very long chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; low-DHA, docosahexaenoic acid pattern; n-3 VLC-PUFA; n-3 EFA, omega-3 essential fatty acid pattern.

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***Nutrition Research*: An International Publication for Nutrition to Advance Food and Life Science Research**

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CHAPTER 7 CONCLUSION AND RECOMMENDATIONS

7.1 Introduction

This PhD study expands current understanding of dietary and plasma phospholipid FAs and related FA patterns with measures of obesity and MetS in an African setting and helps to clarify the relations between these nutrients and the development of cardiometabolic disease risk in adults.

Nutrition research conventionally paid attention to single nutrients with health and disease conditions. Nevertheless, a clear understanding of the composite interactions of nutrients in foods has led to the appreciation of total dietary patterns or nutrient patterns (Tucker, 2010). Dimension reduction techniques have been used extensively in nutrition epidemiology to identify particular distinctive dietary patterns relevant and applicable to obesity (Machado Arruda *et al.*, 2016; Naja *et al.*, 2011) and FA biomarkers in obesity and MetS (Dahm, 2011; Warensjo *et al.*, 2006). In our study, we used principal component analysis to identify dietary and plasma phospholipid FA patterns and investigated their association with cross-sectional and prospective outcomes in the first two manuscripts in this PhD thesis. In the 3rd manuscript, we compare the proportions of individual dietary and plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes in black South African adults.

This concluding chapter provides a summary of the three manuscripts, included as part of this thesis in Chapters 4, 5 and 6 and makes recommendations from the main findings.

7.2 Aims and objectives

The aim and objectives are repeated in this section for ease of reference.

The PhD study aimed to investigate the association of dietary FAs and plasma phospholipid FAs and FA patterns with obesity and MetS, as well as comparison of dietary FAs and plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes in a selected group of black South Africans. This study forms part of the South African arm of the international Prospective Urban and Rural Epidemiological (PURE) study.

The objectives of this study were to:

1. To compare the composition of dietary FAs and plasma phospholipid FAs of metabolically healthy and unhealthy phenotypes classified according to JIS of the International Diabetes Federation Task Force, (Alberti *et al.*, 2009) MetS criteria and BMI categories in a group of black South African adults.
2. To investigate cross-sectional associations between dietary and plasma phospholipid FA patterns with obesity-related parameters and MetS, in black South Africans.
3. To investigate the associations between individual plasma phospholipid FAs and derived FA patterns with a 10-year change in obesity-related parameters, among a selected group of black South African adults.

These objectives are discussed below in the form of manuscripts.

7.3 Comparison of dietary and plasma phospholipid fatty acids and metabolic health in black Africans

In this manuscript, we classified the participants into metabolic status groups according to the new harmonised JIS criteria and further classification of normal BMI as $< 25\text{kg/m}^2$ and overweight and obesity ($\text{BMI} \geq 25\text{kg/m}^2$). We further compared the proportions of the dietary and plasma phospholipid FAs in these metabolically healthy and unhealthy phenotypes.

We observed higher dietary SFAs (MA, PA, and SA) and ALA in both the overweight/obese groups, while most MUFAs, LA and AA intakes were higher in the MUO group only. Plasma levels of VLC-SFAs and PUFAs were higher, whereas PA, OA, and estimated desaturase activities were lower in the overweight/obese (MHO and MUO) groups after adjustment for age and total energy intake.

PA is an abundant SFA and accounts for up to 20 to 30% of total FAs in membrane phospholipids. PA can be provided in the diet or synthesised endogenously via the DNL pathway, from high doses of ingested carbohydrates and fructose (Carta *et al.*, 2017; Chong *et al.*, 2008). Generally, our study participants were high carbohydrate consumers ($>60\%$ energy from carbohydrates), and therefore, high levels of plasma PA and SA were expected. Plasma PA levels were lower in the overweight/obese (MHO and MUO) groups, but the MUO group had the lowest carbohydrate intake of all groups, expressed as percentage energy from carbohydrates. To maintain the membrane phospholipid PA levels, a balanced dietary intake of rich sources of PA, n-3 and n-6 PUFAs is required (Carta *et al.*, 2017), but our participants are low consumers of n-3 PUFAs. Dietary intake of ALA was higher in both overweight/obese groups, but plasma phospholipids ALA

levels were similar, while plasma levels of DHA were higher in both overweight/obese groups, compared to the healthy normal weight group. This may indicate some form of up-regulation of the endogenous synthesis of primary dietary n-3 PUFAs in our selected group of black South Africans. An up-regulation of the endogenous synthesis of n-3 LC-PUFA from ALA as a precursor has been described in a group with continuous low intake of n-3 LC-PUFAs (Burdge & Calder, 2005).

It is also no surprise that we also recorded higher intake of primary n-6 PUFA, LA in the MUO group. As global intake of n-6 PUFAs increases, n-3 PUFA intakes decrease (Patterson, 2012). This increase in consumption of n-6 PUFAs is associated with higher ratios of n-6/n-3, which are associated with the prevalence of obesity and related risk factors (Patterson, 2012; Simopoulos, 2016). Severe obesity (National Department of Health, 2017) and MetS (Kruger & Nell, 2017; Motala *et al.*, 2011; Van Zyl *et al.*, 2012) are currently being experienced in South Africa. Moreover, current evidence is inconclusive regarding the role of EFAs, n-3, n-6 and their ratio in the gain of body fat and obesity (Lu Wang, 2015). Therefore, developing and sustaining healthy eating and physical activity habits, which would lead to the decrease in obesity prevalence, remains a challenge for society and policymakers (Butland *et al.*, 2007; Faith & Kral, 2006).

We found that lower plasma levels of *DNL* pathway FAs, including PA, *cis*-vaccenic and gondoic acids, as well as estimated desaturase activities (SCD-1) among the overweight/obese groups compared to the normal weight groups. We also found higher levels of D6D activity and lower levels of D5D in the MUO group. These FAs and estimated desaturase activities may be biomarkers of abnormal metabolic health in this selected group of black South African adults. Previous intervention studies have shown that *DNL* is upregulated in humans by relatively extreme diets such as increased intake of dietary carbohydrates, low-fat and high-fat diets, influencing circulating concentrations of several specific FAs (Hellerstein, 1999; King *et al.*, 2006; Raatz *et al.*, 2001). These estimated desaturase activities and *DNL* FAs have been associated with obesity and MetS in epidemiological studies (Kawashima *et al.*, 2009; Kim *et al.*, 2013; Mayneris-Perxachs *et al.*, 2014; Sethom *et al.*, 2011). Lower estimated SCD-1 activity observed in our participants is, however, different from what has been reported in other studies (Kim *et al.*, 2013; Sethom *et al.*, 2011; Warensjö, 2006), though, lower estimated D5D activity is consistent with scientific literature (Kawashima *et al.*, 2009; Kim *et al.*, 2013; Matsuda *et al.*, 2017; Sethom *et al.*, 2011; Warensjö *et al.*, 2009).

Furthermore, we observed higher plasma levels of VLC-SFAs in the MHO group compared to MUO group in our study participants, although both the overweight/obese groups had higher plasma levels of VLC-SFAs than the normal weight groups. Higher plasma levels of VLC-SFAs may have been protective against MetS among the overweight/obese participants. These VLC-SFAs are found in very low concentrations in plasma, but have been associated with lower risks of MetS, diabetes, total mortality, and with favourable profiles of blood lipids and CVD risk markers in diverse populations (Fretts *et al.*, 2016; Lemaitre *et al.*, 2015; Malik *et al.*, 2015; Yang *et al.*, 2018). Further research in black Africans is highly recommended.

Results of the plasma n–3 PUFAs in our groups of participants are mixed. We observed higher plasma levels of total n–3 PUFAs in the MUO group, which persisted after adjustment for age and total energy intake. We also observed higher plasma levels of EPA in the MUNW group after comparison with the MHNW group, as well as higher DHA levels in the overweight/obese groups compared to the MHNW group. Findings of plasma levels of specific n–3 PUFAs in association with MetS have been inconsistent in the literature. Some studies reported lower plasma levels of total n–3 PUFAs and EPA in MetS, contrary to our findings (Huang *et al.*, 2010; Nigam *et al.*, 2009; Sethom *et al.*, 2011), while other studies, did not report any associations of MetS with plasma n–3 PUFAs (Kabagambe *et al.*, 2008; Mayneris-Perxachs *et al.*, 2014). These inconsistencies in associations between n–3 PUFAs and metabolic health could possibly be related to low dietary intake as has been reported in our participants (Richter *et al.* 2014) as well as in a select group of participants from the USA (Kabagambe *et al.*, 2008).

Lower plasma levels of total n–6 PUFAs were observed in the patients with MetS in Tunisia (Sethom *et al.* 2011), this is different from what is observed in our participants. In addition, higher plasma levels of both DGLA and AA observed in both the overweight/obese groups compared to MHNW has also been reported in a multi-racial study in the USA (Steffen *et al.*, 2012). From DGLA and AA form series 1 and 2 prostaglandins which are generally viewed as anti-inflammatory and pro-inflammatory, respectively (Levin *et al.*, 2002). The higher levels of plasma DGLA in the overweight/obese (MHO and MUO) participants in this present study may be indicative of impaired FA metabolism due to ageing, which may also cause the accumulation of DGLA, probably as a consequence of long-term abnormal metabolic risk factors, including obesity and insulin resistance, as has also been observed elsewhere (Kim *et al.*, 2013). Besides, plasma DGLA is converted from dietary LA, and our participants are high consumers of LA from sunflower oil (Smuts & Wolmarans, 2013). The role of individual n–6 PUFAs on human health needs to be better evaluated in order to clearly identify which specific n–6 FAs exerts beneficial or harmful effects.

7.4 Cross-sectional association of dietary and plasma phospholipid fatty acid patterns with obesity and metabolic syndrome

In this cross-sectional study, we identified two dietary FA patterns and six plasma phospholipid FA patterns in a selected group of 711 black South Africans. The patterns were identified from 11 dietary FAs and 26 plasma phospholipid FAs.

Among dietary FAs, the first extracted pattern presented with high positive loadings of saturated FAs, MUFAs, ALA and n-6 FAs, and was therefore named the *non-marine* FA pattern. The second pattern was named the *marine* FA pattern because it was characterised by high positive loadings of EPA and DHA. Dietary FA patterns were weakly associated with measured outcomes. The non-marine FA pattern showed marginal positive associations with WC only, but the association was lost after adjustment for lifestyle variables and energy intake. On the other hand, we did not find any associations with the marine FA pattern. The weak association of the non-marine pattern in our study with the outcomes measured can be explained by the relative weakness of self-reported dietary data to quantify FA intakes compared to dietary FAs measured in the plasma or tissues (Hodson *et al.*, 2008). A multi-racial study in the USA that investigated the association between BMI and dietary FA intake did not find any significant associations between dietary n-3 FAs with BMI (Raatz *et al.*, 2017). Furthermore, Lai *et al.* (2013) failed to show an association of fish intake as well as dietary ALA with MetS and concluded that their results did not support an association between dietary n-3 FAs and MetS in a large US cohort. Our study participants had a low consumption of n-3 PUFAs as previously reported in this selected group of black South Africans (Richter *et al.*, 2014), and children and women from three different geographical areas in South Africa (Ford *et al.*, 2016) and adolescents in Mozambique (Freese *et al.*, 2015). Surprisingly, given the low consumption of n-3 PUFAs in our study participants the plasma DHA levels were high and probably more comparable to levels found in populations that have high n-3 PUFAs intake from fish and seafood (Stark *et al.*, 2016). This observation of higher plasma levels despite low intake of n-3 PUFAs in our participants has been reported in South Africa (Ford *et al.*, 2016) and Kenya (Knoll *et al.*, 2011). The mean plasma DHA levels expressed as % of total FAs in participants of this present study was 4.5%. In two other African studies, DHA levels were measured in red blood cells, and the levels were comparable to those reported by Ford *et al.* (2016) (4.6%) but higher than those reported by Knoll *et al.* (2011) (2.6%). EPA and DHA increase the formation of anti-inflammatory eicosanoids. Therefore, a higher intake of n-3 PUFAs is protective against inflammatory diseases, CVD, and other chronic diseases as outlined in Saini and Keum (2018). Although increased n-3 PUFAs intake have not yet shown reliable benefits in weight loss in humans, an improved metabolic profile of obese

individuals has been demonstrated and is promising (Albracht-Schulte *et al.*, 2018). In summary, n-3 PUFAs appear to regulate lipid metabolism, favouring fatty acid oxidation and suppression of lipogenesis, resulting in a favourable lipid profile and adipocyte metabolism. This process consequently limits fat storage in both adipose tissue and skeletal muscles (Albracht-Schulte *et al.*, 2018). We could not find any published research that investigated dietary FA patterns in association with obesity and MetS in Africa. We, therefore, could not make a comparison of our findings in that regard.

Studies examining cross-sectional associations between circulating FA concentrations and obesity have focused mainly on individual FAs as outlined in the literature review and three manuscripts. However, few studies have examined circulating FAs patterns in association with obesity and MetS. The six plasma phospholipid FA patterns included; the high-Satfat pattern with positive loadings of VLC-SFAs, the n-3 VLC-PUFA pattern with high positive loadings of DPA n-3, DHA, EPA as well as AA, high LA pattern with positive loadings of LA and eicosadienoic acid, the n-6 VLC-PUFA pattern, with high positive loadings of adrenic and docosadienoic acids, and DGLA, n-9 LC-MUFA pattern with positive loadings of nervonic and gondoic acids and the n-3 EFA pattern which had a positive loading of ALA.

The high-Satfat and the n-3 VLC-PUFA patterns were positively associated with measures of adiposity and MetS, while the n-9 LC-MUFA and the n-3 EFA patterns showed inverse associations with MetS and some measures of adiposity. The patterns we identified in this black population are unique and therefore not identical to any patterns that have been studied in association with obesity and metabolic health in Swedish, Irish and Danish cohorts (Dahm, 2011; Li *et al.*, 2016; Warensjo *et al.*, 2006). Plasma phospholipid FA patterns were strongly associated with measures of adiposity and MetS in this cross-sectional study. The dietary non-marine FA pattern showed marginal positive associations with WC whereas the marine pattern did not show any association with outcomes measured. On the other hand, the high-Satfat and n-3 VLC-PUFA plasma phospholipid FA patterns were positively associated with measures of adiposity and MetS, while the n-9 LC-MUFA and n-3 EFA patterns showed inverse associations with MetS and some measures of adiposity.

These associations between plasma FA patterns, adiposity and MetS may be suggestive of possible differences in FA metabolism between lean and overweight/obese individuals. In this study population with low-fat intakes, plasma FA levels may reflect endogenous FA metabolism rather than dietary intakes, which could result in different findings than those reported in other studies from affluent communities.

7.5 Prospective association of plasma phospholipid fatty acids with changes in anthropometric indices over 10 years

We identified six plasma phospholipid FA patterns at baseline in 412 participants with available 10-year follow-up data for plasma phospholipid FAs and other covariates included in the prospective analyses. The FA patterns were derived in the following order: high-Satfat (positively loaded with VLC-SFAs), n-9 LC-MUFA (positive loadings of nervonic acid and gondoic acids), n-6 VLC-PUFA (positive loadings on n-6 VLC-PUFAs), high-LA (positive loadings of n-6 LA and eicosadienoic acid), low-DHA (highest positive loading on ALA and GLA and negative loading of DHA), and n-3 VLC-PUFA (highest loading on EPA and DPA n-3). The baseline plasma phospholipid FA patterns were applied prospectively to investigate the association of these patterns with 10-year changes in anthropometric indices, namely weight, BMI, WC, and WHtR. We also derived new FA patterns at 10-year follow-up and studied their reproducibility. By evaluating the reproducibility of the plasma patterns over 10 years, we measured the stability of plasma FA patterns in our population. Four plasma FA patterns, i.e. n-6 VLC-PUFA, high-Satfat, n-3 VLC-PUFA and low-DHA were reproducible at 10-year follow-up, while the n-9 LC-MUFA and the high-LA patterns were not reproducible.

The high-Satfat and n-9 LC-MUFA patterns were positively associated with a 10-year increase in all measures of adiposity. The high-Satfat pattern was reproducible at 10-year follow-up, whereas the n-9 LC-MUFA pattern was not. Instead, the n-9 LC-MUFAs (nervonic and gondoic acid) were also included in the high-Satfat pattern (factor 2) at 10-year follow-up. The high-LA was weakly but positively associated with 10-year changes in anthropometric indices in the crude model, whereas the low-DHA pattern was inversely and weakly associated with WC and WHtR. The n-3 and n-6 VLC-PUFA patterns were not associated with outcomes measured. To the best of our knowledge, only one study has investigated prospective associations of adipose tissue FA patterns with five-year changes in anthropometry in Danish adults (Dahm, 2011); no study has examined the association of circulating FAs with obesity in black Africans. In this 5-year prospective study, adipose tissue FA patterns with high levels of n-6 VLC-PUFAs was negatively associated with changes in weight and WC in men but positively associated with women. The n-3 VLC-PUFA pattern was not associated with weight loss (Dahm, 2011). Due to several differences in their patterns, we could not make comparisons with our plasma phospholipid FA patterns. Plasma SA on its own has been associated with obesity (Pickens *et al.*, 2015). However, VLC-SFAs including arachidic, behenic and lignoceric, have not been associated with obesity. Most studies investigating these VLC-SFAs in plasma have reported inverse associations with these FAs in obesity, MetS, total mortality and coronary heart disease (Fretts *et al.*, 2016;

Lemaitre *et al.*, 2015; Malik *et al.*, 2015; Yang *et al.*, 2018). We, therefore, cannot explain the positive prospective association of the plasma high-Satfat pattern with 10-year changes all anthropometric indices, considering that the sources of plasma VLC-SFAs are not clear. Further research is recommended. On the other hand, the n-9 LC-MUFA pattern positively loaded with nervonic and gondoic acids was also positively associated with 10-year changes in all outcomes measured and these n-9 LC-MUFAs (nervonic and gondoic acid) were also included in the high-Satfat pattern (factor 2) at 10-year follow-up. Notably, nervonic acid was higher in overweight and obese women but not men; however, gondoic acid was higher in leaner men only. Nervonic acid is found in seed oil plants (Guo *et al.*, 2009), and gondoic acid is found in wild fruits (Ouoba *et al.*, 2018), mustard seed and rapeseed (Chowdhury *et al.*, 2007) in small quantities. Nervonic and gondoic acids can also be formed by *de novo* synthesis through the elongation of OA and converted to other FAs (Ratnayake & Galli, 2009). Therefore, this pattern may be related to *DNL* rather than dietary intake, because the study participants had low intakes of wild fruits, mustard seed, rapeseed and marine fish (Richter *et al.*, 2014). Unfortunately, global data in this field of research (FA patterns) is still lacking and presents a challenge of comparing our study against others.

7.6 Discussion

This study pioneered the investigation of (1) baseline associations of dietary and plasma phospholipid FA patterns with measures of adiposity and MetS in black Africans; (2) baseline comparison of dietary and plasma phospholipid FAs in metabolically healthy and unhealthy phenotypes; and (3) 10-year prospective association of plasma phospholipid FA patterns with changes in anthropometric indices in obesity in a selected group of black South Africans.

In the 1st manuscript, we investigated dietary FA patterns in association with obesity and MetS and in the 3rd manuscript, we report levels of dietary FAs in metabolically healthy and unhealthy phenotypes. In both manuscripts, we document a low intake of n-3 PUFAs in this population in comparison with the FAO/WHO recommendations. However, the higher intakes of particular dietary FAs observed in the MUO group in the 3rd manuscript may be due to generally higher dietary intakes of all nutrients. We found higher than expected levels of DHA in plasma phospholipids in this selected group of black South Africans and confirm the results documented earlier in the same population (Richter *et al.* 2014). Most countries in sub-Saharan Africa score very low for both seafood and plant n-3 PUFAs intakes (Micha *et al.*, 2014). Furthermore, the highest global mean intake of seafood n-3 PUFAs intakes were identified in island nations whereas and highest mean plant n-3 PUFAs consumption has been reported in some countries

in Africa; Tunisia, Angola, Senegal and Algeria. Blood levels of EPA and DHA vary across the globe, with most of the countries and regions reporting levels that are reflected as low to very low (Stark *et al.*, 2016), although global mapping of blood levels of EPA and DHA tend to agree with earlier reports of dietary intake of n-3 PUFAs from seafood (Micha *et al.*, 2014). Moreover, blood levels are less liable to errors, and thus blood level targets are better associated with specific chronic disease outcomes and events (Stark *et al.*, 2016). Our participants are not high consumers of seafood or plant n-3 PUFAs. Therefore, the presumed up-regulation of the endogenous synthesis of n-3 LC-PUFAs, and relationship to long-term risk of obesity should be investigated in prospective studies.

In the 1st and 2nd manuscripts, we identified some similar plasma FA patterns in 711 and 412 participants, respectively. The high-Satfat pattern was positively associated with measures of adiposity at baseline and changes in anthropometric indices at 10-year follow-up. The high-Satfat pattern had positive loadings on VLC-SFAs (SA, arachidic, behenic and lignoceric acid). Concurrently, the levels of these VLC-SFAs in the 1st manuscript were higher in overweight/obese participants than in normal-weight counterparts and higher in MHO participants when compared the MUO group in the 3rd manuscript. We speculate that higher plasma levels of VLC-SFAs observed in the MHO groups in our study participants may be protective of MetS among adults with a BMI higher than 25 kg/m² but, longitudinal studies are recommended to confirm this cross-sectional finding. This conclusion is based on evidence from other studies, that have demonstrated inverse associations of VLC-SFAs with various cardiometabolic risk factors (Fretts *et al.*, 2016; Lemaitre *et al.*, 2015; Malik *et al.*, 2015). The mechanisms underlying the relations of VLC-SFAs with health are not well established, even though evidence from animal experiments suggests that these VLC-SFAs may actively regulate lipid metabolism and insulin sensitivity through multiple pathways (Kawamura *et al.*, 1981). We, however, cannot explain why these VLC-SFAs, were positively associated with measures of adiposity, while as a single nutrient group, they tend to be protective against MetS. Also, literature is not clear as to whether these VLC-SFAs are associated with dietary intake or endogenous metabolism since they are also not common in the diet (Fretts *et al.*, 2016). Therefore, metabolic studies, which include OMICS (collective technologies used to explore the roles, relationships, and actions of the various types of molecules that make up the cells of an organism) in black South Africans are necessary.

The n-9 LC-MUFA pattern was inversely associated with MetS and some measures of adiposity at baseline, whereas at 10-year follow-up, it was positively associated with 10-year increases in all anthropometric indices. This pattern had positive loadings of both nervonic and gondoic acids at both time points. However, we observed that the n-9 LC MUFA pattern was not maintained for

10-years when we studied its reproducibility and stability, by comparing the two patterns identified at baseline and at 10-year follow-up. This may indicate that this pattern was protective of obesity and MetS in our study participants at baseline, but because this FA pattern was not sustained, the protection against weight gain was lost during the 10 years of follow-up. Notably, nervonic acid was higher in overweight and obese women but not men; however, gondoic acid was higher in leaner men only. Nervonic acid is found in seed oil plants (Guo *et al.*, 2009), and gondoic acid is found in wild fruits (Ouoba *et al.*, 2018), mustard seed and rapeseed (Chowdhury *et al.*, 2007) in small quantities. Nervonic and gondoic acids can also be formed by *de novo* synthesis through the elongation of OA and converted to other FAs (Ratnayake & Galli, 2009). Therefore, this pattern may be related to *DNL* rather than dietary intake. On the contrary, in the 3rd manuscript, lower plasma levels of gondoic acid were associated with unhealthy metabolic profiles as reported elsewhere (Ma *et al.*, 2015; Patel *et al.*, 2010). Gondoic acid observed in the MUO group maybe associated with an elevated risk of metabolic disorders, and might play a significant role in metabolic regulation and influence the pathogenesis of T2DM (Ma *et al.*, 2015; Patel *et al.*, 2010). Further research is required to establish whether these MUFAs are protective or deleterious for obesity and MetS in this selected group of black Africans.

In the three manuscripts, we observed higher plasma levels of POA and OA in leaner participants, even though the goals of the 1st and 2nd manuscripts were to investigate the association of FAs patterns rather than individual FAs. In the 1st manuscript, POA, *cis*-vaccenic acid, OA and gondoic acids were higher in leaner participants in both sexes, than in the overweight counterparts. In the 2nd manuscript, we demonstrated that the leaner participants of both sexes had significantly higher plasma levels of POA and OA than their overweight counterparts. In the 3rd manuscript, POA and OA were lower for both the overweight/obese (MHO and MUO) groups compared to the MHNW group. These higher levels in leaner participants may be indicative of the protective nature of POA and OA in obesity in this selected group of black South Africans. Other studies have demonstrated different associations between POA and OA with obesity (Aglago, 2017; Paillard *et al.*, 2008; Pickens *et al.*, 2015). In a French population of healthy men, higher plasma levels of POA were associated with higher WC and was therefore considered a marker of abdominal obesity (Paillard *et al.*, 2008). Also, POA in plasma phospholipids was positively associated with BMI in men and women in diverse populations (Aglago, 2017; Pickens *et al.*, 2015). In line with our findings, Cao *et al.* (2008) have shown that increased lipogenesis of POA in adipose tissue can efficiently suppress adipose-specific activation of *DNL* and may play a role in decreased total body weight with improved metabolic profiles in a genetic mouse model (Cao *et al.*, 2008). However, human studies are yet to prove this hypothesis. It has been shown that POA can benefit weight loss,

through increased energy expenditure of white adipose tissue stores, FA oxidation and oxygen consumption and, thus, raising cell energy metabolism (Cruz *et al.*, 2018). Furthermore, POA is virtually absent in standard dietary intake but is a key product of desaturation from PA through the SCD-1 activity in liver and adipose tissue (Paillard *et al.*, 2008). POA is principally produced by the liver, catalysed by intake of low-fat, high-carbohydrate diets, heavy alcohol use; or excess energy consumption (Chong *et al.*, 2008; Siler *et al.*, 1999; Warnet *et al.*, 1985). The difference between the results of this present study and the results of other studies include the following; first of all, higher levels of plasma POA have been observed in participants of other studies, namely 3.8% (Paillard *et al.*, 2008), 2.1% (Vinknes, 2013) 3.3% in men and 3.2% in women (Warensjo, 2006), in comparison with 0.77% in men and 0.66% women in our group of black South African adults. Aglago (2017) and Pickens (2015) also reported lower plasma levels of POA as compared to levels in this present study. Secondly, plasma FAs are a complex reflection of metabolism, influenced by dietary intake, climate and seasons affecting food supply, aging and geographical locations, processing, storage and cooking methods (Baylin & Campos, 2006; Calder, 2015) and further influenced by cultural and religious dietary practices (Smuts & Malan, 2016). Nevertheless, the significant difference in plasma POA and its association observed in this group of black South Africans, and in other studies from Europe (Paillard *et al.*, 2008; Vinknes, 2013; Warensjo, 2006), South America (Aglago, 2017) and USA (Pickens *et al.*, 2015) warrants further investigation to establish the benefit of higher POA levels in lean black Africans. Besides, dietary OA has also been reported to prevent palmitate-induced metabolic impairment (Salvadó *et al.*, 2013) as well as protects against insulin resistance, improves endothelial dysfunction, inflammation and reduces proliferation that may contribute to atherosclerotic process (Perdomo *et al.*, 2015).

As mentioned earlier, POA is a key product of desaturation through the SCD-1 activity in liver and adipose tissue and catalysed by low-fat, high-carbohydrate diets (Paillard *et al.*, 2008; Chong *et al.*, 2008). Therefore, these findings propose that low SCD-1 activity may have been an essential influence in the low proportion of plasma POA content in our overweight and obese participants. Furthermore, lower estimated SCD-1 activity observed in our overweight/obese (MHO and MUO) participants is different from what has been reported in cross-sectional studies (Kim *et al.*, 2013; Sethom *et al.*, 2011) as well as in a longitudinal study (Warensjo, 2006). This finding warrants further research to elucidate the influence of SCD-1 activity on plasma POA levels and its influence on obesity and metabolic health in black South Africans.

7.7 Conclusion

In summary, our results indicate that the dominating patterns of plasma phospholipid FAs, explaining most of the variance in our participants, may be associated with the long-term risk of obesity and MetS. In a study population with low-fat and high carbohydrate intakes, plasma FA levels may reflect endogenous FA production rather than dietary intakes which could result in different findings than those reported in the literature. This may further explain the weaker association of dietary patterns but stronger associations of plasma phospholipid FA patterns with obesity and MetS in the 1st manuscript. Plasma levels of VLC-SFAs, POA and DHA, reported in our study participants are possibly associated with endogenous FA metabolism. These results further add understanding of plasma phospholipid FA patterns and their associations, as well as the reproducibility of FA patterns we derived in our participants.

Prospective cohort studies generally provide the best epidemiological evidence (in addition to experimental studies) about causation and the most direct measurement of the risk of developing the disease after exclusion of bias and confounders. In our study, we add evidence of associations of plasma phospholipid FA patterns with obesity in both cross-sectional and the prospective study. In the cross-sectional study, we report lower levels of DNL pathway FAs, PA, POA, OA, *cis*-vaccenic and gondoic acids, lower estimated desaturase activities (D5D and SCD-1), as well as higher D6D activity in the MUO group. These differences from the normal weight healthy group may be suggestive of biomarkers of abnormal metabolic health in our study participants. However, being a cross-sectional study, no conclusions can be made of causal associations. Higher plasma levels of behenic and lignoceric acids (VLC-SFAs) observed in the healthy participants (MHNW and MHO groups) in our study participants may be associated with a lower risk of MetS. Prospective studies should investigate the roles of both DNL FAs and VLC-SFAs in metabolic health in African populations with diverse dietary fat, alcohol and carbohydrate intakes. In addition, the high-Satfat pattern was positively associated with adiposity, whereas the n-9 LC-MUFA pattern was inversely associated with adiposity. In the longitudinal study, the high-Satfat pattern was maintained over 10 years in an important proportion of the study participants and predicted adiposity gain, as measured by weight, BMI, WC and WHtR. The plasma phospholipid FAs patterns may suggest possible differences in FA metabolism between lean and overweight/obese individuals. In a study population with low-fat intakes, such as the PURE participants, plasma FA levels may reflect endogenous FA generation rather than dietary intakes, which could result in different findings than those reported in other studies from affluent communities with different diets.

We have also provided evidence from the literature that factors such as low-fat and high-carbohydrate intake may significantly impact FAs in circulation in this selected group of black Africans. Also, lifestyle factors may influence obesity and related risk factors in different directions. Therefore, to evaluate causation in an epidemiological study several dynamics have to be considered, such as consistency with previous studies, strengths of the associations, type of study design and lines of evidence that leads to the same conclusion. In the case of FA research, population studied, geographical location and habitual dietary intake are important considerations. In this PhD study, we have provided results that highlight new objectives and research recommendations that should be explored in future FA studies in black populations in Africa.

One of the strengths of this PhD study is the use of a rigorous methodological approach of sequential regression modelling enabled us to investigate the associations between dietary FAs and plasma phospholipid FA patterns, respectively, and measures of adiposity and MetS which is a preferred method to investigate the association between diet and diseases. Another strength of our study is the use of both dietary and plasma phospholipid FA patterns, which complemented the dietary FAs, that may be prone to recall bias or systematic errors from the calculation of nutrient composition of foods. The measurements of plasma phospholipid FAs were performed at two different time points, and the reproducibility of four plasma phospholipid FA patterns may represent the longer-term status of plasma FAs in this group of black South Africans. In addition, (1) these biomarkers have not been widely reported in Africa. (2) The cross-sectional design in the 1st and 3rd manuscripts allowed us to examine the association of FA patterns with obesity and MetS and levels of these individual FAs from diet and plasma phospholipids in metabolically healthy and unhealthy phenotypes in adults. (3) In the 2nd manuscript, the 10-year prospective design limited the possibility of reverse causation and allowed us to examine associations between plasma phospholipid FAs patterns and measures of adiposity as well as the stability of these patterns. Finally, this work as a whole adds new information on dietary and plasma phospholipid FAs and its relation to cardiometabolic risk in a population where these associations have not been investigated.

Our study is the first to examine the associations of dietary and plasma phospholipid FA patterns with obesity and MetS, as well as reports 10-year prospective changes in anthropometric indices in black Africans. Our study addresses a gap in the field and indicates that more research is necessary to investigate the association of FA patterns with obesity and MetS.

7.8 Recommendations

1. Research suggests that there is a need to consider the quality as well as the quantity of fat in the diets of different populations. The field of FA research has developed, and knowledge on metabolism and influence of FAs on obesity and MetS have been studied and documented in different parts of the world. However, data in populations of African descent are limited. It is therefore imperative that we investigate the association of dietary FAs, and plasma FAs in association with health and diseases in Africa, to provide data and to shed light on its influence on health. Some of the research recommendations include;
 - (i) The scope to which dietary and lifestyle exposures impact DNL or concentrations of SA, PA, POA, OA, *cis*-vaccenic and gondoic acids in our study participants is not established. Factors known to stimulate DNL include carbohydrate and alcohol intake. Further research is needed to determine how dietary intake and lifestyle habits may influence DNL synthesis in populations of African descent.
 - (ii) More research is necessary to investigate the influence of SCD-1 activity on plasma POA levels and its influence on obesity and metabolic health in black South Africans. Also, research is required to establish the association between selected MUFAs, such as nervonic and gondoic acids, obesity and MetS in black Africans.
 - (iii) The plasma DHA levels in this selected group of black Africans were higher than expected, given the low dietary intake of n–3 PUFAs reported in study participants of this present study. For this reason, it would be interesting to pursue further research that may explain and comprehend the endogenous conversion of n–3 PUFAs in black African populations with low dietary intake of n–3 PUFAs.
 - (iv) To improve the understanding of plasma VLC-SFAs, metabolic studies (which include collective technologies used to explore the roles, relationships, and actions of the various types of molecules that make up the cells of an organism) in black South Africans are necessary.
 - (v) The reproducibility of some of the plasma FA patterns identified demonstrates that these FA patterns of our study participants are likely to be consistent over a period of 10 years, thus, supporting the need to further explore plasma FAs patterns and cardiometabolic risk, longitudinally.

2. Dimension reduction studies, including dietary and nutrient pattern research, have great potential for use in nutrition research, particularly as it demonstrates the importance of total diet in health promotion. These dietary and nutrient patterns can be used for future research and development of dietary interventions in this black African population. The use of both FA biomarkers and dietary patterns could be appreciated in future studies to capture a complete picture of dietary practices in populations of African descent.
3. The findings of this study may complement the data in the field and further supplement the knowledge and importance of FA status as potential novel biomarkers of obesity-related health outcomes.

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ANNEXURES

ANNEX 1: ETHICAL APPROVAL 2005



Dr A Kruger
Bussie 594
Noordwes-Universiteit
(Potchefstroomkampus)

Etiëkkomitee
Tel (018) 299 2558
Faks (018) 297 5308
E-Pos dnvearl@puk.ac.za

2 September 2004

Geagte dr Kruger

GOEDKEURING VIR EKSPERIMENTERING MET MENSE

Hiermee wens ek u in kennis te stel dat u projek getiteld "*PURE study (Prospective Urban and Rural Epidemiology study)*" deur die Etiëkkomitee goedgekeur is met nommer 04M10.

Gebruik asseblief die nommer genoem in paragraaf 1 in alle korrespondensie rakende bogenoemde projek en let daarop dat daar van projekteleiers verwag word om jaarliks in Junie aan die Etiëkkomitee verslag te doen insake etiese aspekte van hulle projekte asook van publikasies wat daaruit voortgespruit het. U sal in Mei 2005 die dokumentasie hieroor ontvang.

Goedkeuring van die Etiëkkomitee is vir 'n termyn van hoogstens 5 jaar geldig (volgens Senaatsbesluit van 4 November 1992, art 9.13.2). Vir die voortsetting van projekte na verstryking van hierdie tydperk moet opnuut goedkeuring verkry word.

Die Etiëkkomitee wens u alle voorspoed met u werk toe.

Vriendelike groete

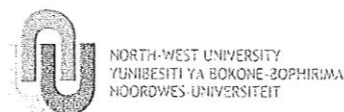
PROF. NT MALAN
VOORSITTER: ETIEKKOMITEE



POTCHEFSTROOMKAMPUS
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ANNEX 2: ETHICAL APPROVAL 2010



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ETHICS APPROVAL OF PROJECT

Ethics Committee
Tel: +27 18 299 4850
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Email: Ethics@nwu.ac.za

2010-02-23

This is to certify that the next project was approved by the NWU Ethics Committee:

Project title : <i>PURE study (Prospective Urban and Rural Epidemiology study)</i>	
Project leader / Student : Prof Annamari Kruger	
Ethics number:	NWU-00016-10-A1
Group 1 = Students 2 = Postgraduate 3 = Academic staff 4 = Non-academic staff 5 = Researchers	
Expiry date: 20/01/2015	

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

The formal Ethics approval certificate will be sent to you as soon as possible.

Yours sincerely

Me. Marietjie Halgryn
NWU Ethics Secretariate

ANNEX 3: ETHICAL APPROVAL 2015



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Email Ethics@nwu.ac.za

ETHICS APPROVAL OF PROJECT

The North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby approves your project as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: PROSPECTIVE URBAN AND RURAL EPIDEMIOLOGY STUDY (PURE STUDY)																														
Project Leader: Prof A Kruger																														
Ethics number:	<table border="1"><tr><td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>0</td><td>1</td><td>6</td><td>-</td><td>1</td><td>0</td><td>-</td><td>A</td><td>1</td></tr><tr><td colspan="3">Institution</td><td colspan="6">Project Number</td><td colspan="2">Year</td><td colspan="3">Status</td></tr></table>	N	W	U	-	0	0	0	1	6	-	1	0	-	A	1	Institution			Project Number						Year		Status		
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Institution			Project Number						Year		Status																			
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation																														
Approval date: 2015-01-20							Expiry date: 2020-01-20																							

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-RERC:
 - annually (or as otherwise requested) on the progress of the project;
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviation from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-RERC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-RERC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the NWU-RERC or that information has been false or misrepresented;
 - the required annual report and reporting of adverse events was not done timely and accurately;
 - new institutional rules, national legislation or international conventions deem it necessary.

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

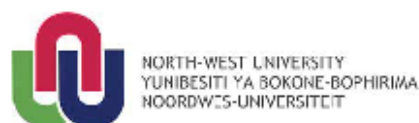
Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
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ou=Vaal Triangle Campus, ou=Vice-
Rector: Academic,
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Prof Linda du Plessis
Chair NWU Research Ethics Regulatory Committee (RERC)

ANNEX 4: ETHICAL APPROVAL FOR PHD STUDY



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Institutional Research Ethics Regulatory Committee

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ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by Health Research Ethics Committee (HREC) on 20/10/2016 after being reviewed at the meeting held on 16/11/2016, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Dietary and plasma fatty acids in association with obesity in black South Africans.																												
Study Leader/Supervisor:	Prof HS Kruger																											
Student:	AA Ojwang																											
Ethics number:	<table border="1"> <tr> <td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>3</td><td>4</td><td>6</td><td>-</td><td>1</td><td>6</td><td>-</td><td>A</td><td>1</td> </tr> <tr> <td colspan="3">Institution</td> <td colspan="3">Study Number</td> <td colspan="3">Year</td> <td colspan="3">Status</td> </tr> </table> Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation	N	W	U	-	0	0	3	4	6	-	1	6	-	A	1	Institution			Study Number			Year			Status		
N	W	U	-	0	0	3	4	6	-	1	6	-	A	1														
Institution			Study Number			Year			Status																			
Application Type:	Single study																											
Commencement date:	2016-10-20																											
Risk:	Minimal																											
Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.																												

Special conditions of the approval (if applicable):

- Translation of the Informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
 - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviation from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
 - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via Ethics-HREC@nwu.ac.za or 018 299 1200.

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

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
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Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

ANNEX 5: ANNUAL MONITORING REPORT FOR THE PHD STUDY



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Faculty of Health Sciences Ethics Office for
Research, Training and Support
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Email: minnie.greeff@nwu.ac.za

13 September 2017

Dear Prof Kruger

FEEDBACK ON HREC ANNUAL MONITORING REPORT: NWU-00346-16-A1

We would like to thank you for submitting the annual monitoring report for your project entitled, *"Dietary and plasma fatty acids in association with obesity in black South Africans"* to the Health Research Ethics Committee (HREC) in a timely manner. Please find below the decision of the HREC regarding the continuation of your project.

Classification	Mark with X	Comment	
Clarification			
Completion (Final report)			
Suspended			
Continuation	X	Date of next monitoring report:	30 September 2018
Termination			

Should you have any further queries, please feel free to contact Ms Leanie van Ronge at your earliest convenience (E-mail: Ethics-HRECMonitoring@nwu.ac.za; Tel: 018 299 2197). We wish you well in your future endeavours.

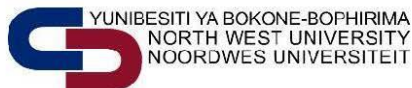
Yours sincerely

Prof Minnie Greeff
Head of Health Sciences Ethics
Office for Research, Training and Support

Dr Wayne Towers
Chairperson: HREC

Original details: (23239522) C:\Users\NMWUSER\Google Drive\HREC Monitoring\2017\NWU-00346-16-A1 (HS Kruger-AA Ojwang)\Continuation Letter 13_09
13 September 2017

ANNEX 6: INFORMED CONSENT FORM 2005



YUNIBESITHI YA BOKONE-BOPHIRIMA
NORTH WEST UNIVERSITY
NOORDWES UNIVERSITEIT

POTCHEFSTROOM CAMPUS

PURE-SA Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT FORM (including the PRIMER-study)

I, the undersigned(full names)

read / listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

I agree to be tested for HIV	Yes	No
I want to know my HIV-status	Yes	No
I agree to give a blood sample	Yes	No

I hereby also declare that I am aware that:

1. this blood sample will be used for the purpose of
 - a. Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
 - b. Testing for liver function by determining liver enzymes such as AST, GGT,
 - c. Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_{1c}, Blood glucose and Insulin
 - d. Analyses of clotting factors and hypertension markers
 - e. Analyses of bone health, iron and nutrition status
 - f. And may be stored until such time as the above measurements/analyses will be done.
2. A two hour glucose tolerance test will be done
3. Body measurements such as height, weight, skinfold thicknesses, arm and leg circumferences will be taken
4. Electrocardiograph be taken
5. Blood pressure to be taken
6. Pulse wave velocity measurements will be made
7. A urine sample to be collected to analyse for the presence of heavy metals such as lead and mercury,
8. A Spirometer test to be performed to determine lung function
9. A handgrip test to be performed to test muscle strength
10. A hair sample to be taken to test for fumonisin mycotoxins.

.....
(Signature of the subject)

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2005

Witnesses

1. 2.

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2005



PART 1

1. **School/Institute:**
Faculty of Health Sciences, North-West University
2. **Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study
3. **Full names, surname and qualifications of project leader:**
Dr. Annamarie Kruger, Ph.D. (Nutrition)
4. **Rank/position of project leader:**
Research Manager
- 5.. **Aim of this project**

PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.
6. **Explanation of the nature of all procedures, including identification of new procedures:**

Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire) with the help of field workers. A blood and urine sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and an ECG will be performed.
7. **Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**

It will take each participant quite a while (about two hours) to complete all the tests and discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.
8. **Precautions taken to protect the subjects:**

The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.
9. **Description of the benefits which may be expected from this project:**

When measures with immediate results are taken, such as blood glucose levels or blood pressure, the information will be communicated to the individual to seek professional help. Since this study is a longitudinal study, subjects that are high at risk will be identified from the dataset and personal feedback will be given.
10. **Alternative procedures which may be beneficial to the subjects:**

There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.



PART 2

To the subject signing the consent:

You are invited to participate in a research project. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:

1. **Participation in this project is voluntary.**
2. **It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.**
3. **You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.**
4. **The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.**
5. **We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.**
6. **The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.**
7. **All information will be kept CONFIDENTIAL and no personal information will be published without my consent.**

Dr ANNAMARIE KRUGER

Contact details: 082 771 5778 / 018 299 4037(Office)



ANNEX 7: INFORMED CONSENT FORM 2010

1497



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

PURE-SA Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT FORM (including the PRIMER-study)

I, the undersigned(full names and surname)

read / listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project. I understand that I have the right to withdraw at any time from the study.

I agree to be tested for HIV	Yes	No
I want to know my HIV-status	Yes	No
I agree to give a blood sample	Yes	No

I hereby also declare that I am aware that:

- This blood sample will be used for the purpose of
 - Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
 - DNA damage due to environmental factors such as cooking methods and smoke will be tested for.
 - Testing for liver function by determining liver enzymes such as AST, GGT,
 - Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_{1c}, Blood glucose and Insulin
 - Analyses of the clotting profile and hypertension markers
 - Analyses of bone health, iron and nutrition status

And may be stored until such time as the above measurements/analyses will be done.
- Body measurements such as height, weight, skin fold thicknesses, arm and leg circumferences will be taken
- Vascular sonar will be done
- Blood pressure to be taken
- Pulse wave velocity measurements will be made
- A Spirometer test to be performed to determine lung function
- Bone density will be determined with an osteometer to detect possible osteoporosis

.....

(Signature of the subject)

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2010

Witnesses

1. 2.

Signed at ... Potchefstroom / Ganyesa ... (delete not applicable option) on/...../ 2010

PART 1

1. **Research Unit and Faculty:**
Africa Unit for transdisciplinary Health research (AUTHeR); Faculty of Health Sciences, North-West University
2. **Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study
3. **Full names, surname and qualifications of project leader:**
Prof. Annamarie Kruger, Ph.D. (Nutrition)
4. **Rank/position of project leader:**
Research Director and project manager
5. **Aim of this project**
PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.
6. **Explanation of the nature of all procedures, including identification of new procedures:**
Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire, psychology questionnaire) with the help of field workers. A blood sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and bone density will be performed.
6. **Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**
It will take each participant quite a while (about 6 [six] hours) to complete all the tests and questionnaires. Discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.
8. **Precautions taken to protect the subjects:**
The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.
9. **Description of the benefits which may be expected from this project:**
When measures with immediate results are taken, such as blood glucose, haemoglobin, HIV status and blood pressure, the information will be communicated to the individual to seek professional help during an individual feedback session. Since this study is a longitudinal study, subjects that are high at risk will be identified over time and personal feedback and counselling will be given.
On the research site each participant will receive a cooked meal, a fruit and fruit juice. Also an amount of R50 will be paid to each participant to cover transport costs and enable each person to go for follow-up at a clinic of choice.
10. **Alternative procedures which may be beneficial to the subjects:**
There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.

PART 2

To the subject signing the consent:

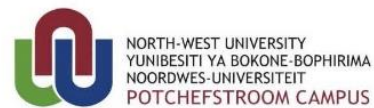
You are invited to participate in a research project. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:

1. Participation in this project is voluntary.
2. It is possible that you personally will not derive any benefit from participation in this project, although the health knowledge obtained from the results may be beneficial to you and other people.
3. You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.
4. The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.
5. We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.
6. The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.
7. All information will be kept CONFIDENTIAL and no personal information will be published without my consent.

Prof ANNAMARIE KRUGER

Contact details: 082 771 5778 / 018 299 2095(Office)

ANNEX 8: INFORMED CONSENT FORM 2015



INFORMATION LEAFLET AND WRITTEN INFORMED CONSENT FORM FOR PARTICIPANTS OF THE PURE STUDY

TITLE OF THE RESEARCH PROJECT: Prospective Urban and Rural Epidemiology
(PURE) study

REFERENCE NUMBERS: NWU-00016-10-A1

PRINCIPAL INVESTIGATORS: Prof A Kruger and Dr IM Kruger

ADDRESS: North-West University (Potchefstroom Campus), Africa Unit for
Transdisciplinary Health Research (AUTHeR), Building E8, Office G03.

Dear participant

You have been part of the PURE research since it started in 2005. Whether you took part in the research every year or even only some of the years, we would like to invite you once more to be part of this research.

This information document will provide you with all the information that you need to know about the research. A fieldworker who lives within your community will discuss all of the information in this document with you in order for you to understand why this research is done and why you are invited to be part of this research.

What is this research about?

This research has already started in 2005 and will finish in December 2015. This research study wants to look at different risks to your health and how these risks can develop over time.

What are some of the risks to your health?

- Being too fat (overweight or obese) or too thin (underweight),
- Having high blood pressure (hypertension) or too low blood pressure (hypotension),
- Having too high blood sugar levels (hyperglycaemia) or too low blood sugar levels (hypoglycaemia),

What might cause you to develop some of these risks?

- The type of foods you eat, for example too much salt or too much fat;
- If you don't do any exercise;
- Smoking or using tobacco products;
- Drinking alcohol;
- Some factors (genetic characteristics) that you inherit from your parents or even your grandparents,

Why have you been invited to take part in the study?

You have been invited to be part of the study because:

- In 2005:
 - you were between the ages of 35 and 70 years
 - you were a healthy man or woman

- you did not have any chronic disease or illness
- you permanently lived in a household within the community

Even though you might have developed some illness since 2005, you can still be part of the study. If you are part of the study now we want to see how you are doing.

Do you have to take part in the study?

You are not forced to take part in this research. It is important that you understand that you don't have to be part of this research study. You are invited, and therefore you can choose if you want to be part or not. You can agree to be part of the research now and change your mind later. If you do not want to be part of the research anymore, you will not be treated differently and you will not be punished or judged for not wanting to be part of the research.

This research was approved by a group of people (known as a committee) from the North-West University. These people looked at all the procedures of the research to make sure that if you decide to be a part of the research, you will not be hurt or harmed in any way. These people are known as the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University on the Potchefstroom campus. It might happen that during the research one or more of these committee members may come to check that the researchers are keeping to the procedures that was explained to you and no unnecessary procedures are performed on you.

What will you have to do if you decide to be part of the study?

If you decide to be part of the study the following will be done:

- A fieldworker that lives within your community will visit you at your house and he / she will explain everything to you and let you ask any questions if you don't understand anything. You will then be given 7 days to think about whether you want to continue to be part of the research.
- If you, after 7 days, agree to continue to be part of the research and you understand everything, you will sign a form, known as a written informed consent form, with the fieldworker.

- Once you have signed the written informed consent form an appointment will be made with you for you to come to the research facility. The date that will be scheduled with you, will be one that suits you the best. All of the arrangements will be made with you to bring you to the place where the research will take place.
- On the day of the appointment one of the taxis involved in the research will pick you up from a place that was arranged with you.
- When you arrive at the place where the research will take place the following will be done with your permission / consent:
 - A small amount of blood (33 ml) will be drawn from the inside of your arm.
 - Your blood pressure will be measured.
 - Your heart function will be checked (electrocardiogram). For us to be able to do this test, you will be asked to take off your shirt or blouse to reveal your chest. If you are a women, you are allowed to keep on your underwear. Little round stickers will be placed on your skin all around the area of your heart.
 - The speed that the blood travels through your blood vessels will be measured (pulse wave velocity). This will be done by placing a thing that looks like a pen on the skin of your neck while placing another one on the skin near your pubic area. For us to be able to do this test, the researcher will gently pull down your pants or skirt to reveal just a small area between the inside of your leg and your private area (pubic area) where the pen-like device will be placed.
 - Your blood vessels in your neck will be checked for any blockages or deposits (intima-media thickness) by placing some liquid gel on the skin of your neck and then by using a thing that looks like a torch to take a picture (sonar) of your blood vessels.
 - You will be weighed and your body length will be measured. You will be asked to remove your shoes and all of your extra clothing. You will be allowed to keep on your pants / skirt and your shirt / blouse.
 - The amount of body fat and amount of muscles you have will be measured. You will be asked to remove just your shirt / blouse. If you are a women, you may keep your underwear on. The thickness of your skin on

your stomach, arms and back will be measured using a tong-like instrument. The researcher will pinch the skin between their fingers and place the tong over the skin to measure how thick the skin fold is. A measuring tape will also be used to measure the circumference of your hip, waist and upper arm.

- The amount of fat and muscle will also be measured using a special machine called the BodPod. You will be asked to climb into the machine and sit on a little bench. A see-through lid will close around you. You will be asked to sit very still and make no movements at all. The machine will blow some air around you and this will then measure the amount of fat and muscle in your body. You will need to wear a tight pants and tight shirt. We will also give you a swimming cap to put on your head while you are in the machine.
- A photo (X-ray) will be taken of your forearm to see if the bones are still hard and strong (bone mineral density).
- Your lung function will be measured. A little plastic pipe will be placed between your lips. Then you have to take a deep breath and blow out the air as hard and as fast as you can through the little pipe.
- You will perform a test to see how strong you're muscles in your hands / forearm (hand grip strength) and legs are (chair stand / rise and walk test). To test how strong your muscles in your hand is you will be asked to squeeze your hand very tight around an instrument with a handle. To test the strength of your muscles in your legs you will have to sit down on a chair and then stand up without using your hands and arms. You will also be asked to walk on a straight line for 6 metres without any help.
- You will complete several questionnaires.
- You will be asked information regarding all the medicines that you currently use.
- You will be asked to give a little bit of urine in a small bottle.
- If you agree and give permission, a HIV test will be performed (If you do not want to be tested for HIV, you can still be part of the study). A needle will be used to prick one of your fingers to get a small drop of blood. This drop of blood will be used to do the HIV test.
- If you are newly diagnosed with HIV, or if you are currently HIV positive we will test your CD4 count. This test will show us how many CD4 cells

there are in your body. These cells keep your body healthy and prevent you from getting sick. If the number of cells are too low then you will need medicine (anti-retroviral therapy (ART)). This test is done by a small prick on one of your fingers to get a drop of blood. The drop of blood will be used to count the number of CD4 cells in your body.

- If you are HIV positive, and you agree and give permission, personal information will be collected from your personal files that are kept at the hospital or clinic that you attend. This will provide us with information to better understand how HIV and the treatment you receive for HIV influence your health.

What do you have to do before coming to the study?

- We ask that you do not eat or drink anything (except for water) from the previous night 22:00 (ten o'clock). This will help to see if your blood sugar levels are normal.
- Please bring along all the medication that you are currently drinking
- Please bring along your South African ID document
- Please bring along your clinic book or medicine sheet that contains all your medication that is prescribed to you

Will you benefit from taking part in this research?

When you are finished for the day, some of the results will be given back to you. This will include:

- Blood pressure
- Blood sugar levels
- Heart function (electrocardiogram)
- Lung function (spirometry)
- How hard the bone in your forearm is (bone mineral density)
- How strong your muscles in your hand / forearm (hand grip strength) and legs (chair stand / rise and walk test) are
- Your height and weight
- Your HIV status and most recent CD4 count – if you gave permission for the test to be done and you want to know your status

If you participate you will also help us to understand what the risks are to your health. If we know what the risks are we can help the government to set up policies that will address this health risks in order to help other South Africans, like you, to have better health.

Are there any dangers involved in you taking part in this research?

Most of the measurements that will be performed won't hurt or harm you in any way, but you might experience the following:

1. If you give permission to a blood sample, you might feel uncomfortable or scared. We want to make sure that you are not hurt in any way and therefore we will use a qualified nurse that has a lot of experience. She will talk to you and explain to you everything that she is going to do in order for you to feel at ease. Some of this blood will be used (tested) to help us to understand what the reason(s) are that some people get non-infectious (non-communicable) diseases (such as heart attack and stroke) as well as high blood sugar (Type 2 Diabetes) and high body weight (obesity) and others may not. All the blood tests and experiments will only look at the reasons that change your chance of getting non-communicable diseases of lifestyle.
2. Some of this blood will also be used to get genetic material (DNA and RNA) to look at genetic factors. Genetic factors (inherited from your parents) are like a book that tells your body how to work. Sometimes there are mistakes (genetic alterations) that cause an increase in your chance of getting non-communicable diseases (these are diseases of the heart and blood vessels (such as heart attack and stroke) as well as high blood sugar (Type 2 Diabetes) and high body weight (obesity). We want to look at these genetic mistakes to better understand how these diseases develop. We promise that all genetic tests and experiments will only focus on genetic factors that contribute to your chance of getting non-communicable diseases of lifestyle.
3. During some of the measurements (weight and height, body composition, ECG, and pulse wave velocity) you will be asked to remove some of your clothes keeping only your underwear or light clothing on. This might make you feel uncomfortable or shy. To help you feel less shy and uncomfortable the area where these measurements will be done will be private and closed off. This

means that no one will be able to see you and that only the person that will take the measurements and someone to help him / her will be with you.

4. You will be asked to do a test (strength test) in order to see how strong the muscles in your hand / forearm and legs are. Although this is not a very hard test to do, and it does not take very long (only a few minutes) you might get tired. The researcher will ask you to rise / stand up from a chair as well as to walk 6 meters. If you feel that you cannot do the test, you will not be forced to do it, and you can move on to the next station.
5. You will be asked not to eat or drink anything from 22:00 (ten o'clock) the night before you come to the study. You will only be allowed to drink water. You should also not eat any breakfast on the morning of the study and not drink coffee, tea, juice or cold drink. Not eating or drinking anything might make you feel uncomfortable or light headed (dizzy or faint). As soon as you arrive at the research study, we will take a blood sample (if you give us permission) and then we will give you a light breakfast to make you feel better.
6. Doing all of the measurements on the day of the study, will take the whole day. This might make you feel very tired. We will give you a lunch as well as tea / coffee / juice / water throughout the duration of the day at the seating / waiting area.
7. Being part of such a big study can be frightening and overwhelming. To prevent us from wasting your time and to make sure that you know where to go and what to do, there will be people available at all times to help you and show you where you have to go every time.
8. HIV testing will also be performed on the day of the study (with your permission). Being newly diagnosed with a long-lasting disease such as HIV might cause you to worry. To help reduce the worry, there will be a professional person (counsellor) that will come and talk to the whole group about HIV and the test they will do. They will explain everything to you. The counsellor will then take you alone to a private room where nobody can hear you when you talk to the counsellor. When they do the test, a small needle is used to prick your finger and

a little drop of blood is used to test for HIV. You can decide if you want to know your status. Whether the test is positive or negative, the counsellor will talk to you alone and give you all of the information that you need to know. If you are positive and you want to know your status, you will receive a letter that you can take to your clinic or hospital and they will help you with treatment.

There are more benefits than dangers / risks when you partake in the study.

Who will see my personal information (data)

When you arrive at the study, you will be given a unique number, and only this number will be used when you provide us with any personal information or when we do the measurements. No one, except the supervisor of this research will be able to identify you. All your information will be kept safe and secret and will be locked away. No one will have access to your information except the supervisors of this study. All of the information will be stored for 5 years after the study has finished.

The PURE study also takes place in other countries. The main country that is supervising this study is Canada. Because we have a contract with them, we have to share the data with them. Only the information you provide us within the questionnaires and the body measurements will be shared with them. We won't share your information from your HIV status or DNA with them. They will also keep your information safe and secret. They will not share your information with anyone else.

Will you be paid to take part in this study and are there any costs involved?

You will be provided with a breakfast when you arrive at the study. Furthermore, the measurements will take about a whole day, and therefore we will also provide you with lunch. Water / juice / coffee / or tea will also be provided.

There will not be any travel costs for you, since we will arrange for transport for you to the research facility and back to the point where you were picked-up. You will also receive R50.00 (fifty rand) for any expenses due to your attendance at the study. If you are referred to the hospital or clinic you can use this money for any expenses.

If you are fulltime employed we will make special arrangements with your employer for you to be able to take part in the study. If you lose your work income for the day you will be compensated for the day (one day of work).

Is there anything else that you should know or do?

- If you have any questions that the fieldworkers cannot answer you can ask that the researcher explains it to you. You can contact the researcher, Dr Lanthé Kruger at 018 299 2093 or lanthe.kruger@nwu.ac.za. You can also contact the researcher if you have any problems with the research.
- You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2089 or carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been fully addressed by the researcher.
- You will receive a copy of this information and consent form for you to keep with you.

How will you know about the findings?

Results that are immediately available will be given to you in private and in person on the day of the study. Other results of the research will be shared with you as a group (everyone that was a part of this study) and with your community leaders verbally during a personal visit from the lead researchers. When we share the results with the community leaders and with the group we will never share personal information. This means that people will not be able to identify you, and you will be safe from any harm such as stigmatization.

Storage of blood and urine samples

The blood and urine samples that we collect from you will be stored in a special freezer that will protect it. Only we will have access to your blood and urine samples, and no one else. The samples will be kept in these freezers for an unlimited period of time until we have money available to test (analyse) them. All of the tests (analyses) will be related to this study (PURE) and what you give permission for. Some of your blood samples may be sent to laboratories outside of South Africa for analysis if it cannot be done here. If we are finished with the tests of the blood, we will destroy it so that no one else can use it.

Declaration by participant

By signing below, I agree to take part in a research study entitled:

Prospective Urban and Rural Epidemiology study (PURE)

I declare / confirm that:

- I have read this information and consent form and it is written in a language that I understand and is comfortable with.
- I have had a chance to ask questions and all my questions have been answered to my satisfaction.
- I understand that taking part in this study is completely my choice and I have not been forced to take part.
- I may choose to leave the study at any time and will not be penalised or judged in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

If you want to get tested for HIV you have to give permission here below:

Yes, I want to get tested for HIV

☐

No, I don't want to get tested for HIV

☐

If you want to know your HIV status you have to indicate it to us here below:

Yes, I want to know my HIV status

☐

No, I don't want to know my HIV status

☐

Do you give permission that we may have access to your medical records at the hospital or clinic?

Yes, I give permission

☐

No, I don't give permission

☐

Do you give permission that we may collect your genetic material from your blood sample?

Yes, I give permission

☐

No, I don't give permission

☐

Do you give permission that your genetic samples may be analysed outside of South Africa if it cannot be done here?

Yes, I give permission

☐

No, I don't give permission

☐

Do you give permission that some of your blood samples may be analysed outside of South Africa if it cannot be done here?

Yes, I give permission

☐

No, I don't give permission

☐

Do you want to be contacted when there are any NEW research studies that will take place within your area?

Yes, I want to be contacted

☐

No, I don't want to be contacted

☐

PURE written informed consent 2015

Signed at (*place*) on (*date*) 20....

.....
Signature of participant

.....
Signature of witness

Declaration by person obtaining consent

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of person obtaining consent

.....
Signature of witness

Declaration by researcher

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of researcher

.....
Signature of witness

ADDENDUM A

SUMMARY FOR DATA COLLECTION (2005 – 2015) AND INTENDEND FUTURE USE FOR THE PROSPECTIVE URBAN AND RURAL EPIDEMIOLOGIC (PURE) STUDY

WHAT WAS COLLECTED?	WHAT INFORMATION DOES THIS GIVE US?
1. Blood sample	1.1 DNA damage due to environmental factors 1.2 Genetic factors related to non-communicable diseases 1.3 Indication of liver function 1.4 Clotting factors related to non-communicable 1.5 Risk markers for non-communicable diseases 1.6 Indication of bone health 1.7 Indication of nutritional status
2. Urine sample	2.1 Indication of kidney function 2.2 Indication of salt intake 2.3 Hypertension markers
3. Hair sample	3.1 Presence of toxins (fumonisin mycotoxins)
4. Body composition (Anthropometrics)	4.1 Height 4.2 Weight 4.3 Amount of fat in the body 4.4 Amount of muscles in the body 4.5 Amount of water in the body 4.6 Waist circumference 4.7 Hip circumference 4.8 Head circumference 4.9 Arm circumference 4.10 Leg circumference 4.11 Skinfolds
5. Blood pressure	5.1 To see if the blood pressure is too high or too low.
6. Pulse wave velocity	6.1 Indication of the flexibility of the arteries.
7. Electrocardiograph	7.1 Indication of heart function.
8. Vascular sonar (intima-media thickness)	8.1 Indication of blockages in the blood vessels.
9. Bone mineral density	9.1 Indication of the strength of the bones in the arm.
10. Lung function test (spirometry)	10.1 Indication of lung function.
11. Hand grip strength	11.1 Indication of the strength of the muscles in the hand.
12. Chair rise/stand test	12.1 Indication of the strength of the muscles in the legs.
13. 6 metre walk test	13.1 Indication of the strength of the muscles in the legs.
14. HIV test and CD4 count	14.1 Test for HIV and to test for the levels of CD4 cells in the body.
15. PURE Adult questionnaire	15.1 Provides demographic information. 15.2 Provides information regarding tobacco and alcohol use.

PURE written informed consent 2015

	15.3 Provides information regarding the use of medication. 15.4 Provides information regarding your health. 15.5 Provides information regarding violence and injuries. 15.6 Provides information on major personal events. 15.7 Provides information on female health. 15.8 Provides information on support systems used. 15.9 Provides information on consumer habits. 15.10 Provides information on work-, financial-, and home related stress. 15.11 Provides information regarding your neighbourhood. 15.12 Provides non-personal information regarding people living close to you who have HIV/AIDS.
16. PURE Family census questionnaire	16.1 Provides demographic- and health information on family members currently living within the household.
17. PURE Physical activity questionnaire	17.1 Provides information on the type, duration and frequency of physical activity.
18. International Physical Activity Questionnaire (IPAQ)	18.1 Provides additional information on the type, duration and frequency of physical activity.
19. PURE Health Services questionnaire	19.1 Provides information on health services, health related expenditures, medication use and health knowledge.
20. PURE Annual contact form	20.1 Provides mortality and morbidity information regarding participant and members of household.
21. PURE Event reports	21.1 Provides information regarding new diagnoses of non-communicable disease(s).
22. PURE Verbal Autopsy questionnaire	22.1 Provides information regarding death of participants.
23. PURE: EPOCH 1: Environmental Profile of a community's health	23.1 Provides information regarding community.
24. PURE: EPOCH 2: Environmental Profile of a community's health	24.1 Provides information regarding community.
25. PURE Community profile questionnaire	25.1 Provides information regarding community.
26. Assessing neighbourhood walkability	26.1 Provides information regarding community.
27. Mental health questionnaire: MHC-SF	27.1 Provides information on emotional-, social-, and psychological wellbeing.
28. Mental health questionnaire: SWLS	28.1 Provides information regarding satisfaction with life.
29. Mental health questionnaire: AFM	29.1 Affectometer provides information regarding positive mental health.
30. Mental health questionnaire: MLQ	30.1 Measures to what extent respondents feel their lives are of meaning. Measures how engaged and motivated respondents are in efforts to find meaning or deepen their understanding of meaning in their lives.
31. Mental health questionnaire: PHQ-9	31.1 Provides information on possible mental health disorders.

PURE written informed consent 2015

32. Mental health questionnaire: K6	32.1 Provides information psychological distress (depressive and anxiety symptoms).
33. Mental health questionnaire: MRG	33.1 Provides information on the most meaningful things, most important relationships and most important goals.
34. Mental health questionnaire: MMSE	34.1 Provides information on positive impairment.
35. Bone health questionnaire	35.1 Provides information regarding risk factors for bone health. 35.2 Provides information regarding bone fractures.
36. HIV and stigma questionnaire	36.1 Provides information regarding attitudes and stigma on HIV/AIDS.
37. Medicine use questionnaire	37.1 Provides information regarding use of chronic medication.
38. Quantitative Food Frequency Questionnaire (QFFQ)	38.1 Provides quantitative information regarding frequency of dietary intake. 38.2 Provides quantitative nutrient information. 38.3 Provides quantitative information on nutritional status.
39. 24-hour recall dietary intake	39.1 Provides information of dietary intake over the past 24 hours.

Declaration by participant

By signing below, I, ID number: give permission that all of my data (information obtained from questionnaires, personal communication and interviews, physical measurements, and biological samples) that was voluntarily collected from me during the entire duration of the **Prospective Urban and Rural Epidemiology study (PURE) study** (between January 2005 - December 2015) may be used for any further analyses (primary and secondary), however, with the understanding that any analyses and its related results will only be relevant to the scope of the **Prospective Urban and Rural Epidemiology study (PURE) study**, and for which I gave permission (written informed consent).

I further agree to the secure storage and archiving of all of my data on the premises of the North-West University, Potchefstroom Campus. I understand that all of my data that was collected between January 2005 and December 2015, will be stored for an unspecified period until such time is suitable for appropriate further analyses (primary and secondary), and that it will be kept anonymously to everyone except the principle investigator.

PURE written informed consent 2015

Signed at (*place*) on (*date*) 20....

.....
Signature of participant

.....
Signature of witness

.....
Signature of researcher

.....
Signature of witness

ADDENDUM B

AMENDED PROCEDURE ON PULSE WAVE VELOCITY MEASUREMENT WITHIN THE PROSPECTIVE URBAN AND RURAL EPIDEMIOLOGIC (PURE) STUDY

Dear participant,

I would like you to read the following letter. If you don't understand, please ask us to explain this to you. In your letter where you give permission to be part of the research, all of the tests (measurements) that you will do, are explained to you. On page number 2 of the permission letter, it is explained that we will measure how fast the blood travels through your blood vessels.

The words in the block below was used in your permission letter:

The speed that the blood travels through your blood vessels will be measured (pulse wave velocity). This will be done by placing a thing that looks like a pen on the skin of your neck while placing another one on the skin near your pubic area. For us to be able to do this test, the researcher will gently pull down your pants or skirt to reveal just a small area between the inside of your leg and your private area (pubic area) where the pen-like device will be placed.

But, the way we will do this test, will be different now:

You will be asked to lie down on a bed. You don't have to take off all your clothes. A strap (cuff) will be placed around your upper arm. This strap (cuff) is the same as the one your blood pressure is measured with. Another strap (cuff) will be placed around your upper leg. If you are wearing pants, the strap (cuff) on your leg will be placed over your pants. If you are wearing a skirt or dress, the researcher will gently move your skirt or dress upwards to place the strap (cuff) on the skin of your leg. The researcher will place a thing that looks like a pen and put it on the skin of your neck. You then need to lie very still while the test is done.

Declaration by participant

By signing below, I, ID
number: I
admit that I have been told that the test to measure the speed that the blood travels
through the blood vessels has changed. The new way of using a cuff and a pen like
object was explained to me and I understand how the new test will be done.

Signed at (*place*) on (*date*) 20....

.....
Signature of participant

.....
Signature of witness

.....
Signature of independent person

.....
Signature of witness

.....
Signature of researcher

.....
Signature of witness

ANNEX 9: PERMISSION LETTER TO USE PURE DATA



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOMKAMPUS

Permission for the use of PURE-SA data

Dear Chair and members of the HREC

I, Dr IM (Lanthé) Kruger, project leader of the South African arm of the Prospective Urban and Rural Epidemiological (PURE-SA) study, hereby grant the following student / researcher permission to use the following data from the PURE study in order to fulfill the requirements of their intended research.

Date signed

19/05/2016

Signature

Please complete ALL sections below

Student name and surname

ALICE ACHIENG OJWANG

Student number

25822829

Proposed title of research project

Dietary and plasma fatty acids in association with obesity in black South Africans.

Qualification

- ☐ Honours
☐ Masters
☒ PhD
☐ Not for qualification purposes

Supervisor / Primary researcher

Prof. H.S Kruger

Co-supervisor / Secondary researcher

Prof. M.C. Smuts and Assistant Supervisor-Dr. Manja Zec

Executive summary

Obesity is of public health concern in South African (SA). The role of dietary fat intake and plasma fatty acid (FA) status in relation to obesity and related morbidities are not well researched and understood. The aim of the proposed study is to investigate associations between dietary FA intake and plasma phospholipids (PLs) FA composition with obesity-related outcomes in black SA subjects followed up from 2005-2015. The study is part of the SA Prospective Urban and Rural Epidemiological study of apparently healthy black men and women aged 30 years and older from North West Province. The association of dietary FAs and PLs with inflammatory markers, glucose metabolism parameters and risk of obesity, type 2 diabetes and metabolic syndrome will be documented. New understanding on the impact of different individual FA on health and disease of black SA will provide scientific evidence to support SA policy and public health recommendations on fats and FAs.

Data from which time period:

- ☒ 2005
☒ 2010
☐ 2012
☒ 2015

Data from which community:

- ☒ Urban
☒ Rural

Data from which gender

- ☒ Males
☒ Females

Data requested from questionnaires:

- ☒ Demographic
- ☒ Alcohol and tobacco use
- ☐ Current disability
- ☒ Medical history
- ☐ Violence and injuries
- ☐ Self-help groups
- ☐ Major life events
- ☐ Stress levels
- ☐ Depression
- ☐ EPOCH
- ☐ HIV and stigma
- ☒ Medicine use
- ☐ Health services (2015 only)
- ☒ Nutrition (QFFQ)
- ☐ Positive Psychology
- ☐ General bone health
- ☒ Physical activity
- ☐ Event reports (verbal autopsies)

Physical measurements:

- ☒ Anthropometry
- ☐ Sarcopenia
- ☒ Cardiovascular
- ☐ Spirometry
- ☐ Bone mineral density

Biochemical analyses / indices:

- ☒ Cardiovascular
- ☒ Lipids
- ☒ Inflammatory
- ☐ Haemostasis
- ☒ Glycemic
- ☐ Bone turnover markers
- ☒ Nutrition
- ☒ HIV
- ☒ SMAC
- ☐ Hematology
- ☒ Urine
- ☐ Genetics

Shipping and transport of biological samples abroad

Will any biological samples be shipped / transported to another country for analyses?

- ☐ Yes
- ☒ No

If yes, please provide brief description regarding:

1. Company name and country to perform analyses
2. Sample type (serum, plasma, whole blood, urine, hair etc.) as well as the number of samples exported
3. Variables requested for analyses
4. Type of analyses to be performed
5. Procedure for sample disposal after completion of analyses

THIS SECTION IS FOR THE CASES OF MANIPULATION WITH SAMPLES ABROAD, SO YOU MAY LEAVE IT EMPTY

1. The blood tests will all be performed in the Centre of Excellence for Nutrition Laboratories in North West University.

2. No samples will be transported to outside of the country.

3. The biochemical analysis discussed in this section is limited to be parameters that will be used specifically for this affiliated study. All blood samples for 2005, 2010 and some samples for 2015 have been analyzed. However, some samples from 2015 are currently being analyzed. The same biochemical analysis procedures was used in 2005, 2010 and 2015.

4. Variables that will be used for this affiliated study include:

Confounders: Age, HIV status, Kidney function parameters (creatinine clearance and urinary albumin/creatinine ratio) Level of education, Medication use, Physical activity, Self-reported alcohol intake, Self-reported smoking status, Serum lipid profile, Liver function parameters (gamma-glutamyltranspeptidase, AST/ALT ratio) Sex, Total energy intake, Urbanization factor.

Exposures: Composite dietary FA intake, individual dietary FAs, plasma phospholipid FA profile, dietary patterns and plasma phospholipid FA groups according to the PCA.

Outcomes:

Primary outcomes: HOMA-Insulin resistance, hsCRP, IL-6 (Low grade Inflammation), MetS

Secondary Outcomes: Waist-to-height ratio, body mass index, waist circumference, obesity and overweight status

5. Samples are stored for an unspecified period of time. To ensure the integrity of the samples while in storage, blood and urine samples are stored at -80 degrees Centigrade in secure lockable bio-freezers until the determination of the variables examined the PURE study. Access to these bio-freezers is limited to key personnel. These bio-freezers are constantly monitored via a cellular-based monitoring system. Sample integrity is furthermore maintained via the connection of the freezers to uninterrupted power supplies and centralised

Additional documentation requested:

- ☐ PURE proposal
- ☒ Written informed consent form: 2005
- ☒ Written informed consent form: 2010
- ☒ Written informed consent form: 2015
- ☒ Ethical approval certificate: 2005
- ☒ Ethical approval certificate: 2010
- ☒ Ethical approval certificate: 2015
- ☐ Ethical approval certificate: Bone density
- ☒ Permission letter from North-West Department of Health
- ☒ Other (specify)

Please specify

Written informed consent for HIV testing, Letters given to study participants(2005,2010,2015), and referral letters.

Authorship in terms of PURE-SA publications

Please take note that the following researcher(s) has to be invited for co-authorship on any publication(s) that results from the use of PURE-SA data collected during any of the following time periods:

2005: Dr IM (Lanthé) Kruger
2010: Prof M (Minrie) Greeff
2012: Dr IM (Lanthé) Kruger
2015: Dr IM (Lanthé) Kruger

Signature of supervisor / primary researcher

Ajwang

Date signed

Ajwang 14/08/2016.

ANNEX 10: ADULT QUESTIONNAIRE

The PURE-SA project
ADULT QUESTIONNAIRE

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking
an X in ONE BOX on each line:
(unless otherwise instructed)

X

OR

By writing number(s) in the spaces provided :

1 8

OR

By specifying the answer on the line(s) provided

Adult Questionnaire

Subject Initials- F= 1st letter of 1st name
M= 1st letter of middle name
L= 1st letter of last name

3. National I.D.#

If not applicable please mark the N/A box

Ethnicity Codes

- 01 - South Asian (India, Sri Lanka, Pakistan, Bangladesh)
- 02 - Chinese (China, Hong Kong, Taiwan)
- 03 - Japanese
- 04 - Malays
- 05 - Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal, Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)
- 06 - Persian
- 07 - Arab
- 08 - Black African
- 09 - Coloured African (Subsaharan African only)
- 10 - European
- 11 - Native North/South American or Australian Aborigine
- 12 - Latin American (Latino)
- 13 - Bantu/Semi Bantu
- 14 - Hemitic/Semi Hemitic
- 15 - Nilotic/Hausa
- 16 - Pygmie
- 17 - Swahili
- 18 - Other (any other ethnoracial group not listed above)

Subject ID

Centre # Community# Household # Subject #

Today's date

--	--	--	--

--	--

--	--

year month day

1. Name: _____
 Given name *Surname*

2. Not applicable in South Africa

3. National identity # or equivalent: _____ N/A ☐

4. DOB:

 OR Age

 yrs

5. Sex: ☐ Female ☐ Male

6. Marital status: (check one only)

- ☐ Never married ☐ Currently married ☐ Common law/Living with partner
- ☐ Widowed ☐ Separated ☐ Divorced

7. Ethnicity:

--	--

 (Please refer to facing page for codes)

8. Caste/Tribe: _____

9. What level of formal education have you completed? (check highest level only):

- ☐ None
- ☐ Primary
- ☐ Secondary/highschool/higher secondary
- ☐ Trade School
- ☐ College/University
- ☐ Unknown

Subject Initials

--	--	--

F M L

Adult Questionnaire

1 1. Occupation

Group 1 : Legislators, senior officials and managers

Legislators and senior officials
Corporate managers
General managers
Businessman

Group 2: Professionals

Physical, mathematical and engineering science professionals
Life science and health professionals
Teaching professionals
Other professionals

Group 3: Technicians and associate professionals

Physical, mathematical and engineering-
science associate professionals/technicians
Life science and health associate professionals/technicians
Teaching associate professionals/technicians
Other associate professionals/technicians

Group 4: Clerks

Clerks
Customer service clerks

Group 5: Service workers and shop and market sales workers

Personal and protective services workers
Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers

Market-oriented skilled agricultural and fishery workers
Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers

Extraction and building trade workers
Metal, machinery and related trades workers
Precision, handicraft, printing and
related trades workers
Other craft and related trades workers

Group 8: Plant and machine operators and assemblers

Stationary plant and related operators
Machine operators and assemblers
Drivers and mobile plant operators

Group 9: Elementary occupations

Sales and services elementary occupations
Agricultural, fishery and related labourers
Labourers in mining, construction,
manufacturing and transport

Group 10: Armed forces

Armed forces

Group 1 1 : Homemaker

Housewife/Househusband

Subject ID

Centre #	Community#		Household #		Subject #		

10. Not applicable in South Africa

Subject Initials		
F	M	L

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.
(Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

- ☐ Group 1 ☐ Group 2 ☐ Group 3 ☐ Group 4 ☐ Group 5
☐ Group 6 ☐ Group 7 ☐ Group 8 ☐ Group 9 ☐ Group 10 ☐ Group 11

c) Not applicable in South Africa

d) What is your main source of income? _____

If occupation is group 1 1 (homemaker) go to question 13

12. Are you currently employed?

☐ No → (answer 12a - 12b) ☐ Yes → Go to #13
a) Are you retired/stopped work from your primary occupation due to old age? ☐ No ☐ Yesb) Have you stopped working due to illness? ☐ No ☐ Yes

Subject ID

Centre #	Community#		Household #		Subject #		

Subject Initials		
F	M	L

13. CURRENT DISABILITY:

	No	Yes
a) Do you have any problems using your 1.) fingers to grasp or handle?	☐	☐
b) Do you have any trouble walking about?	☐	☐
c) Do you have any trouble bending down and picking up an object from the floor?	☐	☐
d) Do you require a walking stick cane/walker to move about?	☐	☐
e) Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn)	☐	☐
f) Do you have trouble seeing a person from across the room? (1.2 feet/3.5 meters) (with glasses worn)	☐	☐
g) Do you have trouble speaking and being understood?	☐	☐
h) Do you have any trouble hearing what is said in a normal conversation?	☐	☐

Subject Medical History

14. Have you experienced any of the following in the last six months?

	No	Yes		No	Yes
a) Chest pain or tightness with usual activity	☐	☐	i) Vomiting	☐	☐
If Yes, → does the pain spread to the back, neck or inner border of arm	☐	☐	j) Loss of appetite	☐	☐
b) Breathlessness with usual activity	☐	☐	k) Painful or bleeding teeth/gums	☐	☐
c) Cough for at least 2 weeks	☐	☐	l) Jaundice	☐	☐
d) Any sputum while coughing	☐	☐	m) Burning while passing urine	☐	☐
e) Blood in sputum	☐	☐	n) Swelling of feet	☐	☐
f) Wheezing or whistling in the chest	☐	☐	o) Swelling of face	☐	☐
g) Early morning cough with chest tightness	☐	☐	p) Blood in urine	☐	☐
h) Loose stools/diarrhea for at least 3 days	☐	☐	q) Involuntary weight loss of > 3kg	☐	☐

15. Not applicable in South Africa

- a) 16 Do you use glasses/spectacles/contact lenses at present? No ☐ Yes ☐
 b) Do you use a hearing aid? No ☐ Yes ☐

Subject ID

Centre #	Community#	Household #	Subject #

Subject
Initials
F M L

17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis		No	Yes	#of yrs since diagnosis
a) Diabetes	☐	☐		i) COPD	☐	☐	
b) Hypertension/ high blood pressure	☐	☐		j) Asthma	☐	☐	
c) Stroke	☐	☐		k) Tuberculosis	☐	☐	
d) Angina/heart attack/ Coronary artery disease	☐	☐		l) Malaria	☐	☐	
e) Heart failure	☐	☐		m) Chagas	☐	☐	
f) Other heart disease	☐	☐		n) HIV/AIDS	☐	☐	
g) Cancer	☐	☐					
4= Small intestine	☐	☐					
9= Breast	☐	☐					
10= Cervical/Uterine cancer	☐	☐					
11= Prostate	☐	☐					
12= Head and neck cancer sites	☐	☐					
13= Other, specify	☐	☐					
7= Liver	☐	☐					
6= Pancreas	☐	☐					

1= Mouth
3= Stomach
2= Esophagus
5= Large intestine including rectum
h) Hepatitis/Jaundice

Cancer Sites

4= Small intestine
9= Breast
10= Cervical/Uterine cancer
11= Prostate
12= Head and neck cancer sites
13= Other, specify
7= Liver
6= Pancreas

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? ☐ No ☐ Yes

a) If yes, for what conditions:

	No	Yes
Blood pressure	☐	☐
Cholesterol lowering drugs	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Asthma	☐	☐
Chinese medicine	☐	☐
Others	☐	☐
Unknown	☐	☐

→ If Yes, specify _____

18b) If name of medication is unknown, please list as unknown.

Subject ID

Centre #	Community#	Household #	Subject #

Subject
Initials
F M L18b) List all the medications you are currently consuming at least once a week for the last month?

i) _____	ii) _____
iii) _____	iv) _____
v) _____	vi) _____
vii) _____	viii) _____

Men go to question #23For Women Only (Questions 19 - 22)19. Are you currently pregnant? ☐ No ☐ Yes → Go to #2120. Do you still have periods? ☐ No → (answer) ☐ Yes → Go to #21a) How many years since you stopped menstruating? years21. Have you ever used an oral/ injectable contraceptive? ☐ No ☐ Yes22a) How many live children have you given birth to? Boys Girlsb) Did you breast feed any of your children? ☐ No ☐ Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

- 1 = Factory/industrial place
- 2 = Office
- 3 = Agriculture field/farm
- 4 = Home
- 5 = Road
- 6 = Sport/game e.g. track, court, field, etc.
- 7 = Public building
- 8 = Mine/quarry
- 9 = Construction site e.g. building, road-works, etc.
- 10 = Other

Type of Injury

- 1 = Burns
- 2 = Scalds
- 3 = Fractures
- 4 = Muscle and ligament sprains/tears
- 5 = Cuts and lacerations
- 6 = Bruises and abrasions
- 7 = Suffocation
- 8 = Head injury (where person did not lose consciousness)
- 9 = Head injury (where person lost consciousness for some time)

PURE

Adult Questionnaire

Page 6

Subject ID

Centre # Community # Household # Subject #

Subject Initials F M L

23. During the past 12 months, have you had any injuries that were serious enough to limit your normal activities? (check all that apply)

☐ No → Go to #24 ☐ Yes → (answer 23a - 23s)

If yes, please provide details:

Please refer to facing page for Location and Type Codes
Absence from work or usual activities (Days)

Cause of injury

	☐ No	☐ Yes	Location	Type	Absence from work or usual activities (Days)
a) Motor vehicle accident (as a passenger)	☐	☐			
b) Motor vehicle accident (as a pedestrian)	☐	☐			
c) Struck by an object	☐	☐			
d) Explosion	☐	☐			
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	☐	☐			
f) Suffocation	☐	☐			
g) Poisoning	☐	☐			
h) Snake/scorpion bite	☐	☐			
i) Fall	☐	☐			
j) Fire/flames, resultant fumes	☐	☐			
k) Physical assault (gun, kidnapping, etc.)/violent crime	☐	☐			
l) Domestic violence (beaten by a family member)	☐	☐			
m) Drowning/submersion	☐	☐			
n) Hot or corrosive liquids/floods/substances	☐	☐			
o) Crush injuries (boulders, building materials, etc.)	☐	☐			
p) Accident caused by machinery	☐	☐			
q) Attempted suicide	☐	☐			
r) Armed conflict	☐	☐			
s) Other(specify) _____	☐	☐			

Adult Questionnaire

Location of Fractures

- 1= Hip/pelvis
- 2= Thigh
- 3= Leg
- 4= Forearm
- 5= Wrist
- 6= Hand/finger
- 7= Vertebrae (back)
- 8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter "0"

PURE

Adult Questionnaire

Page 7

Subject ID

Centre #	Community#	Household #	Subject #
----------	------------	-------------	-----------

Subject Initials

F	M	L
---	---	---

24. Have you ever fractured a bone? ☐ No (go to #25) ☐ Yes (if yes, answer a),b) and c)

a) Number of fractures

--	--

b) Years since last fracture

--	--

(yrs)

c) Bone (s) broken in the most recent fracture (if more than 3, list most severe sites)

(location) If other, specify

--

Please refer to facing page for fracture locations

--

--

Tobacco

25. Which best describes your history of tobacco use?

a) ☐ Formerly used tobacco products ☐ Currently use tobacco products ☐ Never used tobacco products → Go to #26

b) At what age did you start?

--	--

 yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

Past users only

	Average amount/day	Duration (years)	When Stopped (years ago)	If less than 1 yr (months ago)									
(i) Cigarettes (all kinds)	<table><tr><td></td><td></td><td></td></tr></table> number				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(ii) Beedies	<table><tr><td></td><td></td><td></td></tr></table> number				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(iii) Cigars	<table><tr><td></td><td></td><td></td></tr></table> number				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(iv) Pipes	<table><tr><td></td><td></td><td></td></tr></table> number				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(v) Sheesha/water pipe Hookah	<table><tr><td></td><td></td><td></td></tr></table> # of times				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(vi) Chewing tobacco	<table><tr><td></td><td></td><td></td></tr></table> # of times				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		
(vii) Snuff	<table><tr><td></td><td></td><td></td></tr></table> # of times				<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>			<table><tr><td></td><td></td></tr></table>		

(x) Other

Specify

--	--	--

--	--

--	--

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Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?

(Exposed is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

☐ No — Go to #27 ☐ Yes — Please answer questions 26a

a) Over the past 12 months, what has been your typical exposure to other people's smoke?

(Exposed is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke)

Select ONE only

☐ 1-2 times/week ☐ 3-6 times/week ☐ at least once a day ☐ 2-3 times/day ☐ 4 or more times/day

27. Not applicable in South Africa

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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28. Which best describes your history of alcohol use?

28c) Alcoholic Beverage: Regular use is defined as at least once a month.

a) ☐ Formerly used alcohol products ☐ Currently use alcohol products ☐ Never used alcohol products — Go to #29

b) At what age did you start?

--	--

 yrs

c) What forms of alcohol have you regularly used? (check all that apply)

Form of Alcohol	Approx. size of one "drink"	Frequency			Average # of drinks	Duration (years)	Past users only						
		Daily	Weekly	Monthly			When Stopped (years ago)						
(i) Spirits(rum,whisky, gin,vodka etc)	30ml	☐	☐	☐	<table><tr><td> </td><td> </td></tr></table>			<table><tr><td> </td><td> </td></tr></table>			<table><tr><td> </td><td> </td></tr></table>		
(ii) Wine	125ml	☐	☐	☐	<table><tr><td> </td><td> </td></tr></table>			<table><tr><td> </td><td> </td></tr></table>					

(vi) Beer	375ml	☐	☐	☐	☐	☐	☐
(vi) Country liquor/arrack/ sugar cane spirit	30ml	☐	☐	☐	☐	☐	☐

d) At least once a month, do you consume >5 alcoholic drinks/day? ☐ No — Go to #29 ☐ Yes

i) How many times per month do you consume >5 alcoholic drinks in a day?

--	--

 If yes, (i, ii)

ii) What is the average number of drinks that you consume each time?

--	--

29 a) During your longest or nocturnal sleep period, what time do you normally go to bed?

--	--

 (00:00-23:59)

b) During your longest or nocturnal sleep period, what time do you normally wake up?

--	--

 (00:00-23:59)

33. Civic organization: are defined as non-profit, voluntary organization societies, self help groups and (00:00-23:59)

c) Do you usually take naps/siestas? ☐ No ☐ Yes Total nap duration

--	--	--

 mins

Religious organization: are defined as different types of formal and informal groups set up on a religious basis.

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials	F	M	L
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30. Are you a member of any of the following:

How often do you participate in the activities of this group?

Per Month OR Per Year

- (i) Self help group, Co-operative, Social club, Sports club, ☐ No ☐ Yes
- (ii) Religious Group (e.g. church group, etc.) ☐ No ☐ Yes
- (iii) Other Specify ☐ No ☐ Yes

31. Please answer the following: (choose only one option for each)

Strongly Disagree Somewhat Disagree Somewhat Agree Strongly Agree

- (i) People are generally honest and want to help others. ☐ ☐ ☐ ☐
- (ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them. ☐ ☐ ☐ ☐

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each)

Not Applicable

- (i) Cooking oil ☐ ☐ ☐ ☐ ☐
- (ii) Flour ☐ ☐ ☐ ☐ ☐
- (iii) Rice/ Maize meal ☐ ☐ ☐ ☐ ☐

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

- (i) Soft drinks ☐ ☐ ☐ ☐ ☐
- (ii) Snacks ☐ ☐ ☐ ☐ ☐
- (iii) Cigarettes ☐ ☐ ☐ ☐ ☐
- (iv) Alcohol ☐ ☐ ☐ ☐ ☐

33. In a difficult situation, whose help can you count on from?(Please see facing page for definitions)

(i) Civic organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

(ii) Religious organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials	F	M	L
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34. Have you experienced any of the following events during the last 12 months?

No response No Yes

- (i) Loss of job ☐ ☐ ☐
- (ii) Retirement ☐ ☐ ☐
- (iii) Loss of crop/business failure ☐ ☐ ☐
- (iv) Household break in ☐ ☐ ☐
- (v) Marital separation/divorce ☐ ☐ ☐
- (vi) Other major intra-family conflict ☐ ☐ ☐ _____Please specify _____
- (vii) Major personal injury or illness ☐ ☐ ☐
- (viii) Violence ☐ ☐ ☐
- (ix) Armed conflict/war ☐ ☐ ☐
- (x) Death of a spouse ☐ ☐ ☐
- (xi) Death/major illness of another close family member ☐ ☐ ☐
- (xi i) Other major stress ☐ ☐ ☐ _____Please specify _____
- (xiii) Wedding of family member ☐ ☐ ☐
- (xiv) New job ☐ ☐ ☐
- (xv) Birth in the family ☐ ☐ ☐
- (xvi) Separation from family ☐ ☐ ☐
- (xvii) Unavailability of food/ food insecurity ☐ ☐ ☐

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

	No response	Never Experienced Stress	Some Period of Stress	Several Periods of Stress	Permanent Stress
a) How often have you felt stress at work in the last 12 months? (Mark here if not applicable: i.e. no longer working □)	☐	☐	☐	☐	☐
b) How often have you felt stress at home in the last 12 months?	☐	☐	☐	☐	☐

36. What level of financial stress have you felt in the last 12 months?

☐ No response ☐ Little/none ☐ Moderate ☐ High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

☐ No ☐ Yes —If yes, during those times, did you:

	No response	No	Yes
a) Lose interest in most things like hobbies, work or activities that usually give you pleasure?	☐	☐	☐
b) Feel tired or low on energy?	☐	☐	☐
c) Gain or lose weight?	☐	☐	☐
d) Have more trouble falling asleep than you usually do?	☐	☐	☐
e) Have more trouble concentrating than usual?	☐	☐	☐
f) Think a lot about death (either your own, someone else's, or death in general)	☐	☐	☐
g) Feel down on yourself, no good or worthless?	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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38. Please answer the following: (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	☐	☐	☐	☐
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	☐	☐	☐	☐
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	☐	☐	☐	☐
d) My neighbourhood streets are well lit at night.	☐	☐	☐	☐
e) I can see other people when I am walking in my neighbourhood.	☐	☐	☐	☐
f) I can speak to other people when I am walking in my neighbourhood.	☐	☐	☐	☐
g) There is a high crime rate in my neighbourhood.	☐	☐	☐	☐
h) There is a problem with unattended dogs in my neighbourhood.	☐	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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38a) Please answer the following: (Please check all that apply)

i) **Has your household been a victim of the following crime(s) in the last 12 months?**

	No	Yes
1. Armed robbery	☐	☐
2. Violent attacks	☐	☐
3. Murder	☐	☐
4. Vehicle hijacking	☐	☐
5. House breaking	☐	☐
6. Theft	☐	☐
7. Rape	☐	☐
8. Women abuse eg. (beat, swear-words, sexual) please specify _____	☐	☐
9. Child abuse eg. (burn, swear-words, rejection) please specify _____	☐	☐
10. Child sexual abuse	☐	☐
11. Other, please specify _____	☐	☐

ii) **Do you think that crime in your area has increased in the past 5 years?** ☐ No ☐ Yes
if yes, which of the following crime(s)?

- ☐ Armed robbery
☐ Violent attacks
☐ Murder
☐ Vehicle hijacking
☐ House breaking
☐ Theft
☐ Rape
☐ Women abuse
☐ Child abuse
☐ Child sexual abuse
☐ Other, please specify _____

Subject ID

Centre #	Community#	Household #	Subject #
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Subject Initials

F	M	L
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38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? ☐ No ☐ Yes

if yes, which of these people: (please mark all that apply)

- ☐ Your children
☐ Your grandchildren
☐ Your spouse
☐ Your family members
☐ Your friends
☐ People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

- ☐ Younger than 10 years ☐ Between 11-20 years ☐ Between 21-30 years
☐ Between 31-40 years ☐ Between 41-50 years ☐ Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- ☐ Spouse
☐ Parents
☐ Family member
☐ Child/children
☐ Friends
☐ Volunteer

38c) Do you care for any orphans in your family? ☐ No ☐ Yes

Subject ID

Centre #	Community #	Household #	Subject #
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Subject Initials

F	M	L
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39. How long would it take you to get from your house to the nearest facility if you walked?

	Minutes	Don't now		Minutes	Don't now						
i) grocery/convenience store	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐	iv) video store	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐
ii) bank	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐	v) non-fast food restaurant	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐
40b) Health History:			vi) fast food restaurant	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐			
iii) post office	<table border="1"><tr><td></td><td></td><td></td></tr></table>				☐						

Cancer Sites

1 = Mouth
2 = Esophagus
3 = Stomach
4 = Small intestine
5 = Large intestine including rectum
6 = Pancreas
7 = Liver
8 = Lung
9 = Breast
10 = Cervical/uterine/ovarian
11 = Prostate
12 = Head and neck
13 = Other, specify

40a) Total number of siblings

--	--

Health History: Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition																				
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes																					
Diabetes	☐	☐	☐	☐	☐	☐	☐	☐	☐	<table border="1"><tr><td></td><td></td></tr></table>																				
Coronary Heart Disease	☐	☐	☐	☐	☐	☐	☐	☐	☐	<table border="1"><tr><td></td><td></td></tr></table>																				
High Blood Pressure	☐	☐	☐	☐	☐	☐	☐	☐	☐	<table border="1"><tr><td></td><td></td></tr></table>																				
Stroke	☐	☐	☐	☐	☐	☐	☐	☐	☐	<table border="1"><tr><td></td><td></td></tr></table>																				
Cancer facing page for cancer sites	☐	☐	☐	☐	☐	☐	☐	☐	☐	<table border="1"><tr><td></td><td></td></tr></table>																				
4 if Yes, indicate site	<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>			<table border="1"><tr><td></td><td></td></tr></table>		

Other, Specify

Other, Specify

Other, Specify

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

Subject ID

Centre #	Community#	Household #	Subject #

 Subject
Initials
F M L
41. Physical Measurements
 Sitting
a) Right arm
blood
pressure #1 mmHg
Systolic Diastolic
#2 mmHg
Systolic Diastolic

 b) Heart
Rate #1 beats/min
#2 beats/min

 c) Wais? #1 cm ☐ minimal/no
clothing
#2 cm ☐ full clothing

 d) Weight kg ☐ minimal/no
clothing
☐ full clothing

 e) Hip #1 cm ☐ minimal/no
clothing
#2 cm ☐ full clothing

 f) Height cm (without shoes)

 42a) Circumference of
mid upper right arm: cm

 b) Circumference of right
calf: cm

 c) Head Circumference: cm

 d) Upper flexed arm
circumference cm

 43a) Right arm triceps
skinfold: #1 mm
#2 mm
#3 mm

 b) Right calf
skinfold: #1 mm
#2 mm
#3 mm

Subject ID

Centre #	Community#	Household #	Subject #

 Subject
Initials
F M L

 c) Biceps
skinfold #1 mm
#2 mm
#3 mm
d) Subscapular
skinfold #1 mm
#2 mm
#3 mm

 e) Supra spinal
skinfolds #1 mm
#2 mm
#3 mm

 44 a) Humerous breadth cm

 b) Femur breadth cm

45. Grip Strength (Maximal contraction):

 a) Non-dominant hand: #1 kg.
#2 kg.
#3 kg.
b) Dominant hand: #1 kg.
#2 kg.
#3 kg.

Adult Questionnaire

+ If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

American Thoracic Society criteria for acceptable spirometers:
Spirometers are acceptable if they are free from:

1. Cough during exhalation
2. Early termination or cut-off
3. Variable effort
4. Leaks
5. Obstructed mouth piece

PURE

Adult Questionnaire

Page 19

Subject ID

Centre #	Community#	Household #	Subject #

Subject
Initials
F M L

46. Spirometry:

a) FEV1 (Litre): #1 #2 #3

b) Does FEV1 obtained meet ATS criteria?

☐ No → (answer (i) to (iii)) ☐ Yes → Go to c)

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough ☐
- ii) Values not within 0.2L of each other ☐
- iii) Less than 3 values ☐

c) FVC (Litre): #1 #2 #3

d) Does FVC obtained meet ATS criteria?

☐ No → (answer (i) to (iii)) ☐ Yes → Go to e)

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough ☐
- ii) Values not within 0.2L of each other ☐
- iii) Less than 3 values ☐

e) PEFR (Litre/min): #1 #2 #3

f) Does PEFR obtained meet ATS criteria?

☐ No → (answer (i) to (ii)) ☐ Yes → Go to Q#47

Reasons for not meeting the ATS criteria: (check all that apply)

- i) Cough ☐
- ii) Less than 3 values ☐

Subject ID

Centre #

Community#

Household #

Subject #

Subject
Initials

F M L

47. Not applicable in South Africa

48. ECG obtained? No ☐ → Go to #49 Yes ☐a)
year month dayPlace
ECG File
Label Hereb) Please print ECG label # 49 a) Blood sample obtained? No ☐ → Go to #50 Yes ☐b) ☐ Fasting sample ☐ Non-fasting samplec) Time
year month day (00:00-23:59) Hours since any food/beverage consumed (excluding water) d) Please print Blood label # Place
Blood label
here50 a) Urine sample obtained? No ☐ → Go to #51 Yes ☐b) ☐ Fasting sample ☐ Non-fasting samplec) Please print Urine label # Place
Urine label
here51. Name of Interviewer: _____
(please print) First Initial Last NameInterviewer Code:

ANNEX 11: QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

PURE/South Africa 2010
Quantitative Food Frequency Questionnaire

	Name of fieldworker: _____
Subject Code	
Subject ID 	Subject Initials
Centre # Community # Household # Subject #	
Today's date: 2 0 1 0	Day of the week: _____
	year month day

Please think carefully about the food and drink you have consumed during the **PAST MONTH** (four weeks). We have divided the foods into different groups for example all the porridges and cereals together. I will go through a list of food groups and drinks with you and I would like you to tell me:

- Which foods you eat in each of the different food groups
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food as well as other food models, containers, etc.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

Before we start I would like to find out what type of margarine, oil and milk you **USUALLY** use in your home.

1. What type of **MARGARINE** do you USUALLY use in your home? Give brand name if possible
- ☐ Tub/Soft margarine (brand name) _____
- ☐ Brick/Hard margarine (brand name) _____
- ☐ Do not use margarine in home
2. What type of **OIL** do you USUALLY use in the preparation of food in your home?
- ☐ Sunflower oil (give brand name) _____
- ☐ Canola oil (give brand name) _____
- ☐ Olive oil (give brand name) _____
- ☐ Other (give brand name) _____
- ☐ Oil previously used _____
- ☐ Do not use OIL ever in the home
3. What type of **MILK** do you USUALLY use in your home? Mark only ONE
- ☐ Full cream milk / Fresh cow's milk/ Box milk full cream
- ☐ Low fat milk / 2% milk / Box low fat or 2% milk
- ☐ Fat free milk / Skim milk / Box fat free or skim milk
- ☐ Powder milk (eg Elite; give brand name) _____
- ☐ Do not use milk
4. What type of **CREAMER** do you USUALLY use in your home?
- ☐ Cremora, Ellis Brown, Coffee Mate, Tea Mate etc
- ☐ Cremora Lite
- ☐ Do not use creamer

QUANTIFIED FOOD FREQUENCY QUESTIONNAIRE 2010

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the **LAST MONTH**. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

PORRIDGE AND BREAKFAST CEREALS								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
Maize-meal porridge	Stiff (pap)						4401	
Maize-meal porridge	Soft (slappap)						4400	
Maize-meal porridge	Crumbly (phutu)						4402	
Ting	Maize meal						4429	
	Mabella						4427	
	Other							
Mabella	Stiff						3437	
	Soft							
Morvite	Soft						4404	
Oats							3239	
Tastee wheat	Soft						3240	
Other cooked porridge	Type							
Breakfast cereals	All bran flakes						3242	
	Corn flakes plain						3243	
	Weetbix						3244	
	Rice crispies plain						3252	
	Other							

Do you pour milk on your porridge or cereal?								
☐ Yes 1 ☐ No 2								
If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
If yes, how much milk	Whole milk/full cream milk/ fresh cow's milk						2718	
	Maas/sour milk						2787	
	Low fat / 2% milk						2772	
	Fat free / skim milk						2775	
	Other							

Do you put sugar on your porridge or cereal?								
☐ Yes 1 ☐ No 2								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
If yes, how much sugar WHITE or BROWN	Cooked porridge						3989	
	Cereal						3989	
	Other porridge / cereal						3989	
	Other							

OTHER STARCH								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp) 3458	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
	Any fat added?							
Pasta	Macaroni, plain						3262	
	Spaghetti, plain						3262	
	Spaghetti, canned in tomato sauce						3258	
	Macaroni & cheese Milk: Fat:							
	Other specify							
Pizza	Home made: Specify topping						3353 (base+ch)	
	Bought: Specify topping						3353 (base+ch)	

5

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week? _____

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Chicken	Meat & skin, boiled						2926	
	Meat & skin, roasted/grilled/fried in chicken fat						2925	
	Meat ONLY, boiled (no skin)						1559	
	Meat ONLY, roasted (no skin)						1545	
	White meat only, cooked (NO Skin)						2964	
	Kentucky / Chicken Licken (Fried in batter/crumbs)						3018	
	Nando's						2925	
	Other							
Do you eat chicken skin?			Always	1	Sometimes	2	Never	3
Chicken stew	With potato and onion WITH skin						4413	
	With tomato and onion WITH skin						2985	
	With vegetables WITH skin						3005	
	With tomato and onion NO skin						4379	
	With vegetables NO skin						4378	
	Other							
Chicken BONE stew	With potato and onion and tomato						4414	
	Other							
Chicken feet	Nothing added						2997	
	Stew with potato, onion and tomato						4415	
Chicken head							2999	
Chicken offal	Stew with tomato and onion and sunflower oil						4416	
	Liver, cooked						2970	
	Other							

6

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
RED MEAT	How do you like your meat? With fat OR Fat trimmed							
Red meat BEEF	BRISKET, boiled/fried without added fat						4363	
	CHUCK, boiled/fried without added fat						2945	
	BRISKET, fried in added fat						4363	
	Type of fat:							
	CHUCK, fried in added fat						2945	
	Type of fat:							
	Beef, stewed with cabbage						3006	
	Beef, stewed with potato and onion and tomato						4417	
	Beef, stewed with vegetables						3020	
	Mince, nothing added						2921	
	Mince, tomato & onion added						2987	
	Beef BONE stew with potato and onion and oil						4419	
	Other							
MUTTON	Meat, with fat, cooked						2947	
	Mutton, no fat, cooked						3036	
	Mutton, chop, grilled						2927	
	Mutton, stewed with vegetables						2916	
	Other							

BRISKET: more fat and cheaper
CHUCK: less fat than the brisket, but more expensive

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
Beef/mutton Offal	Offal, cooked						3003	
	Stewed with vegetables							
	Liver, beef, fried/cooked						2920	
	Liver, sheep, fried/cooked						2955	
	Kidney, beef, cooked						2923	
	Kidney, sheep, cooked						2956	
	Brain, sheep, cooked						2952	
	Lung, beef, cooked						3019	
	Lung, sheep, cooked						4337	
	"Gemaldes" (lung & fat)						4409	
	Heart, beef, cooked						2968	
	Heart, sheep, cooked						2969	
	Other							
Goat meat	Grilled/roasted/cooked						4281	
	Stewed with vegetables							
	Other							
Venison/ Wild buck							2913	
Horse/Donkey							4407	
Rabbit							4327	
Other type of meat	Specify							
What type of vegetables is usually put into meat stews?								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
Wors / Sausage							2931	
Bacon							2906	
Patties	Beef, fried						2984	
	Chicken, fried						3011	
Cold meats AND Processed meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Frankfurter, beef & pork						2937	
	Frankfurter/Sausage, chicken						3012	
	Russian/Salami						2948	
	Other							
Canned meat	Bully beef, plain						2940	
	Bully beef with potato & onion & oil						2994	
	Other							
Meat pie BOUGHT Or HOMEMADE	Beef						2939	
	Steak and kidney						2957	
	Sausage roll						2939	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought						4418	
	Other							
Biltong	Beef (with fat OR without fat)						3021	
Dried wors Dried sausage	Beef						2949	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
Dried beans	Baked beans in tomato sauce						3176	
	Bean salad / Sousbone						3174	
	Soup with dried beans, beef and vegetables						3145	
	Sugar beans, cooked						3205	
	Other							
Lentils	Whole, cooked						3203	
	Lentil soup with beef and vegetables						3153	
Soya products eg. Imana, Knorr, Jileleke, Toppers	Cooked						3196	
	Soup/Gravy						4431	
	Stewed with extra potato, onion and tomato						4430	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
Pilchards in tomato sauce or chilli or brine	Whole						3102	
	Mashed with fried onion						3102 (70%) 3730 (30%)	
	With tomato and onion						4420	
	Other							
Fish	Hake, fried with batter/crumbs						3072	
	Hake, fried in oil						3060	
	Hake, steamed						4373	
	Moddervis / Yellow fish fried in oil						3084	
	Moddervis / Yellow fish baked with onion (NO oil added)						3089	
	Other							
Other canned fish	Tuna in oil						3056	
	Other							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled (full cream milk & oil)						2890	
	Scrambled (NO milk, ONLY oil added)						2869	
	Scrambled (NO oil, ONLY full cream milk)						2872	
	Fried in oil						2869	
	Fried in brick margarine						2877	
	Other							

VEGETABLES								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and oil						3815	
	Boiled with potato and onion and brick margarine						3813	
	Fried in oil						3812	
	Fried in brick margarine						3810	
	Boiled with potato, onion and tomato and oil						4421	
	Raw with nothing added						3704	
	Other							
Spinach or morogo or beetroot leaves or other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with oil added							
	Boiled with brick margarine added						3898	
	Boiled with tub margarine added						3899	
	Boiled with potato, onion and tomato and oil						4422	
	Other							
Tomato and onion gravy	With oil						4423	
	Without fat						3925	
	Canned						4192	
	Thickened with packet soup powder						4432	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Pumpkin (yellow) Butternut Hubbard squash Table Queen Etc	Boiled, nothing added						4164	
	Boiled with sugar only (NO fat)						3728	
	Boiled with brick margarine & sugar						3893	
	Boiled with tub margarine and sugar						4433	
	Boiled with oil and sugar						4428	
	Other							
Carrots	Boiled, nothing added						3757	
	Boiled with oil added							
	Boiled with brick margarine added						3816	
	Boiled with tub margarine added						3817	
	Boiled with sugar only						3818	
	Boiled with oil and sugar							
	Boiled with brick margarine and sugar						3819	
	Boiled with tub margarine and sugar						3820	
	Boiled with potato, onion and oil						3824	
	Boiled with potato, onion and brick margarine						3822	
	Boiled with potato, onion and tub margarine							
	Chakalaka						4412	
	Raw, nothing added						3709	
	Other							
Mealies/ Sweet corn	On cob – fat added						3725	
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
	Whole kernel, frozen, boiled						4132	
	Other							

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FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
How do you cook potatoes?								
Potatoes	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Boiled with oil added						3873	
	Boiled with brick margarine added						3867	
	Boiled with tub margarine added						3868	
	Mashed with whole milk and brick margarine						3876	
	Mashed with whole milk and oil							
	Roasted in beef fat						3878	
	Roasted in oil						3979	
	French fries (chips) / Fried potatoes						3740	
	Other							
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Boiled with sugar and oil added						4434	
	Boiled with sugar and brick margarine added						3749	
	Other							

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FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
Broccoli	Boiled						3701	
	Raw						3702	
Cauliflower	Boiled						3716	
Green beans	Boiled, nothing added						3696	
	Cooked with potato, onion and oil						3794	
	Cooked with potato, onion and brick margarine						3792	
	Other							
Mixed vegetables	Canned						4264	
	Frozen, boiled						3727	
	Other							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Cucumber, raw						4119	
	Coleslaw (cabbage) (mayonnaise)						3705	
	Coleslaw (cabbage) (commercial)						3707	
	Potato salad (mayonnaise)						3928	
	Baked bean salad						4424	
	Other salad vegetables							
Mayonnaise / salad dressing	Mayonnaise						3488	
	Vinegar, oil						3487	
	Other: Specify							
Other vegetables, specify + preparation								

Now we come to fruit

FRUIT

Do you like fruit? ☐ Yes 1 ☐ No 2

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom /Never		
Apples							3592	
Banana							3540	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Watermelon	Fresh						3576	
Fruit salad	Fresh						3588	
	Canned						3580	
Fig (Vye)							3544	
Avocado							3656	
Wild fruit/berries	Specify type							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Dried fruit	Apple, dried, raw						3600	
	Peach, dried, raw						3568	
	Mixed fruit, dried, raw						3593	
	Mixed fruit, dried and cooked with sugar						3590	
	Fruit roll, dried (all types)						3655	
	Other							
Other fruit								

If subject eats canned fruit: Do you have custard with the canned fruit or fresh fruit salad?

Yes 1 No 2

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Custard	Homemade, full cream milk or fresh cow's milk						2716	
	Homemade, lowfat milk						2779	
	Homemade, skim milk						2717	
	Commercial eg Ultramel						2716	
	Other							

BREAD AND BREAD SPREADS								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	
Do you spread anything on the bread? Always 1 Sometimes 2 Never 3								
Margarine	What brand do you have at home now?							
	Tub, regular						3406	
	Tub, medium fat						4406	
	Tub, light/low fat						3524	
	Brick, regular						3484	
	Brick, medium fat						4405	
	Brick, lite/low fat						3528	
	Other							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fraybentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Cheddar						2722	
	Gouda						2723	
	Other							
Sandwich spread							3522	
Achaar							3117	
Other spreads	Specify							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom /Never		
Dumpling	White flour						3210	
	Whole wheat flour						3212	
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc	Provita						3235	
	Cream crackers						3230	
	Other savoury biscuits like Bacon kips, wheat crackers, etc						3331	

DRINKS								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom / Never		
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
White sugar	Tea						3989	
	Coffee						3989	
Brown sugar	Tea						4005	
	Coffee						4005	
Milk per cup of TEA	Do you use milk in your TEA? ☐ Yes ☐ No If YES, What type of milk do you use in <u>TEA</u> ?							
	Fresh / long life whole/full cream						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free / skim milk						2775	
	Creamer/whitener like Ellis Brown / Cremora						2751	
	Cremora Lite							
	Condensed milk						2714	
	Evaporated milk						2715	
	Other							
	None							
Milk per cup of COFFEE	Do you use milk in your COFFEE? ☐ Yes ☐ No If YES, What type of milk do you use in <u>COFFEE</u> ?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Creamer/whitener like Ellis Brown						2751	
	Cremora Lite							
	Condensed milk						2714	
	Evaporated milk						2715	
	Other							
	None							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom / Never		
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole / full cream milk						2718	
	Fresh/long life: 2% milk / low fat milk						2772	
	Fresh/long life: fat free / skim milk						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other							
Milk drinks	Flavoured milk						2774	
	Milo made with full cream milk						2735	
	Milo made with skim milk						2747	
	Drinking chocolate made with water						4287	
	Other							
Yoghurt	Drinking yoghurt low fat						2756	
	Plain low fat						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar or other						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other							
Fizzy drinks Coke, fanta, etc	Sweetened						3981	
	Diet							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom / Never		
Fruit juice	Fresh/Liquifruit/Ceres						2886	
	Tropica (Dairy –fruit juice mix)						2791	
	Other							
Mageu/Motogo							4056	
Home brew							4039	
Tlokwe							4039	
Beer							4031	
Cider	Sweet						4057	
Spirits Eg Brandy, gin, vodka, whisky, cane, etc							4035	
Wine red							4033	
Wine White							4033	
Other specify								
WATER	Tap, borehole, dam, river, etc						4042	
	Bottled						4042	

SNACKS AND SWEETS								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom / Never		
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Milk chocolate, plain						3987	
	Kit Kat etc						4024	
	Chocolate coated bars like Bar One, TV bar, etc						3997	
	Other							
Candies/Sweets	Sugus, gums, hard sweets, etc						4000	
Toffees / Fudge / caramels							3991	
Biscuits/cookies	Potch cookies						4425	
	Homemade, plain						3233	
	Commercial, plain						3216	
	Commercial, with filling						3217	
	Other							
Cakes	Butter cake, homemade with whole milk and brick margarine NO icing						3288	
	Chocolate cake, homemade with whole milk and brick margarine NO icing						3289	
	Icing for cake made with brick margarine						4014	
	Other							

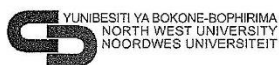
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN 2 columns to be completed				CODE	AMOUNT / WEEK
			Per day	Per week	Per month	Seldom / Never		
Tarts	Apple tart with a batter made with whole milk and brick margarine						3327	
	Other							
Scones	Plain made with whole milk and brick margarine						3237	
	Other							
Muffin	Bran						3407	
	Plain						3408	
	Other							
Rusks	Buttermilk, commercial						3329	
	Homemade, white						3222	
	Other							
Savouries	Sausage rolls						2939	
	Samosas: Meat filling						3355	
	Samosas: Vegetable filling						3414	
	Biscuits eg bacon kips						3331	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
Jelly							3983	
	Custard added made with whole milk						2716	
	Other							
Baked pudding	Baked in a syrup						3312	
	Baked without a syrup						3429	
	Custard added made with whole milk						2716	
	Other							
Instant pudding	Made with whole milk						3266	
	Made with low fat milk						3395	
	Other							
Ice cream	Regular						3483	
	Soft serve						3518	
	Other							
Sorbet							3491	
Other specify								

SAUCES, GRAVIES AND CONDIMENTS								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
Tomato sauce							3139	
Worcester sauce							4309	
Chutney							3168	
Pickles							3866	
White sauce	Made with whole milk and brick margarine						3142	
Packet soups	Dry powder (all types)						3158	
	Made with water (all types)						3165	
Gravy	Made from meat and thickened						3120	
Other								

WILD FRUITS, WILD BIRDS, ANIMALS OR INSECTS (hunted in rural areas or on farms)								
FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / WEEK
			2 columns to be completed					
			Per day	Per week	Per month	Seldom / Never		
MISCELLANEOUS: Please mention <u>ANY OTHER FOODS</u> used more than once/two times a week which we have NOT talked about								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS								
Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat PLEASE GIVE DETAILS								

ANNEX 12: PHYSICAL ACTIVITY QUESTIONNAIRE



PURE-SA Project (Prospective Urban and Rural Epidemiology)

Physical activity questionnaire

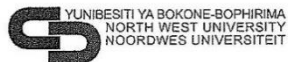
Date: _____ Place: _____ Interviewer: _____
The information on this questionnaire is confidential

1. Subject number _____
2. Gender ☐ Male ☐ Female
3. What is your main occupation? _____

Low level: office work, housework, scholar	
Middle level: factory work, carpentry, farming, hospital nurse, plumber	
High level ("sweat work"): construction work, digging, manual labour	
4. At work I sit ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
5. At work I stand ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
6. At work I walk ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
7. At work I lift heavy loads ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
8. At work I am tired ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
9. At work I sweat ☐ 1. never ☐ 2. seldom ☐ 3. sometimes ☐ 4. often
10. If you work away from home, how do you get to work/school?
☐ walk ☐ cycle ☐ car/taxi
11. How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station)
☐ 0-15 min ☐ 16-30 min ☐ 31-60 min ☐ 1-2 hours
12. If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station)
☐ casual stroll ☐ fairly brisk ☐ brisk/fast
13. Do you climb stairs often? ☐ yes ☐ no
14. If yes, how many flights of stairs do you climb each day? (1 flight = 10 steps)
15. How many days per week do you climb steps?
16. Do you play sport? ☐ yes ☐ no
17. Which sport do you play most frequently?

low level: bowling, golf, billiards	
middle level: tennis, athletics, cycling	
high level: soccer, rugby, netball	
18. How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4
 (Write appropriate code in space) 0.5, 1.5, 2.5, 3.5, 4.5^{a2}
19. How many months per year? <1/ 1-3/ 4-6/ 7-9/ >9
 (Write appropriate code in space) 0.04, 0.17, 0.42, 0.67, 0.92^{a3}

^{a1} intensity code of sport, ^{a2} time code for sport, ^{a3} proportion of year



20. If you play a second sport, which is it?

low level: bowling, golf, billiards	1	0.76 ^{a1}
middle level: tennis, athletics, cycling	2	1.26
high level: soccer, rugby, netball, boxing	3	1.76(27)
21. How many hours per week do you practice?
 <1/ 1-2/ 2-3/ 3-4/ >4
 0.5, 1.5, 2.5, 3.5, 4.5^{a2}
22. How many months per year?
 <1/ 1-3/ 4-6/ 7-9/ >9
 0.04, 0.17, 0.42, 0.67, 0.92^{a3}
23. During leisure time I watch TV/ do sitting activities (read, needle-work, play cards)

1. never	2. seldom	3. some-times	4. often	5. always	(34)
----------	-----------	---------------	----------	-----------	------
24. During leisure time I walk/ do standing activities (gardening, housework)

1. never	2. seldom	3. some-times	4. often	5. always	(35)
----------	-----------	---------------	----------	-----------	------
25. Other leisure-time activities: _____
 (leisure-time = time off from work/ school)

1. never	2. seldom	3. some-times	4. often	5. always	(36)
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ANNEX 13: LANGUAGE EDITING CERTIFICATE

To whom it may concern

Cecile van Zyl
Language editing and translation
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10 January 2019

Dear Mr / Ms

Re: Language editing of thesis (Dietary and plasma fatty acids in association with obesity in black South Africans)

I hereby declare that I language edited the above-mentioned thesis by Ms Alice Ojwang (student number: 25822829).

Please feel free to contact me should you have any enquiries.

Kind regards



Cecile van Zyl
Language practitioner
BA (PU for CHE); BA honours (NWU); MA (NWU)
SATI number: 1002391

The End