The relationship between the inflammatory potential of the diet and metabolic syndrome in Black South Africans

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North-West University

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ABSTRACT

Introduction and aim:

The prevalence of the metabolic syndrome (MetS) is increasing globally. In South Africa it is mainly linked to the high prevalence of obesity, sedentary lifestyles and poor dietary habits, which are now also observed in rural areas. MetS and its components raise major public health concerns due to their strong connection to the pathophysiology of cardiovascular disease (CVD) features. Recent studies suggest that chronic low-grade inflammation, associated with obesity, plays a central role in the development of MetS, and contributes to the development of other metabolic dysfunctions. Several studies have closely linked diet and inflammation; thus dietary inflammatory indices have been developed with the aim to determine the inflammatory potential of whole diets. Since diet influences inflammation and CVDs, and with MetS rising exponentially in South Africa, we aim to investigate whether the dietary inflammatory potential of a Black South African population is associated with blood levels of inflammatory markers and ultimately the MetS.

Methods

The proposed mini-dissertation is affiliated to the South African arm of the international PURE study (PURE-SA-NW). This study was of cross-sectional design and included 2 010 individuals who participated in the PURE-SA-NW data collection of 2005. The study participants were Tswana-speaking, Black South Africans residing in rural and urban areas of the North-West Province, who were apparently healthy individuals, older than 30 years, and not using any acute or chronic medication in exclusion of blood pressure medication. The dietary intake of the participants was assessed through a validated quantitative food frequency questionnaire, from which the Energy-adjusted Dietary Inflammatory Index (E-DII) was calculated. MetS was diagnosed according to the guidelines of the International Diabetes Federation (IDF). The anthropometrical measurements included weight, height, BMI, hip and waist circumference. The biochemical tests used included inflammatory markers including C-reactive protein (CRP), albumin, interleuken-6 (IL-6), homocysteine (Hcy), fibrinogen, plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$) and others, including glucose and blood lipids.

Results

The main aim of this study was to compare the inflammatory index of the diet of the PURE-SA-NW study participants diagnosed with MetS with those without MetS. The literature-based E-DII, as outlined by Shivappa *et al.* (2014), was utilised to calculate the inflammatory index of the PURE study participants' diets.

The MetS group comprised of more women compared to the controls, lived mainly in urban areas, and were older than the control group. Individuals with MetS consumed less alcohol and used less tobacco than the control group.

There was no statistically significant difference between the mean E-DII of the MetS and control groups. The MetS participants consumed higher amounts of pro-inflammatory as well as anti-inflammatory food components than the controls.

In men, hypertensive status, fasting glucose, waist circumference and LDL-cholesterole were all significantly higher and HDL-cholesterol significantly lower in the MetS group than in the controls, while in women, WC reached borderline significance only with no significant difference reported for HDL-C. Anthropometric indices as well as inflammatory markers measured were all higher in the MetS group compared to the control group, as expected.

The mean E-DII did not differ between individuals with different numbers of MetS components and the number of participants with MetS was evenly distributed across the E-DII quartiles. Regarding the MetS, there were no significant differences observed between the E-DII quartiles for any of the MetS components or MetS itself, even after adjusting for covariates. There was also no significant difference in any of the inflammatory markers investigated across the quartiles before or after adjustment for covariates, except for a borderline significant decrease in albumin, after full adjustment.

Conclusion

The findings of this study suggest that MetS may be influenced by factors other than the inflammatory potential of the diet in Black South Africans, particularly in this context of limited dietary intakes. Further studies are thus needed to substantiate the inflammatory role of the diet in the development of MetS in Black South Africans. The adoption and maintenance of a healthy lifestyle, however, remains important to reduce chronic disease risk.

Keywords: Metabolic syndrome; Inflammatory potential; E-DII; African; Central obesity

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AxSYM	Abbott automated immunoassay analyser
BCOPS	Buffalo cardio-metabolic occupational police stress
bDBP	Brachial diastolic blood pressure
BMI	Body mass index
bSBP	Brachial systolic blood pressure
C-DII	Children dietary inflammatory index
CI	Confidence interval
CRP	C-reactive protein
CUADHS	Chinese urban adults' diet and health study
CVD	Cardiovascular disease
DII	Dietary inflammatory index
E-DII	Energy-adjusted dietary inflammatory index
EDIP	Empirical dietary inflammatory pattern
EDTA	Ethylenediamine tetra acetic acid
EGIR	European group for the study of insulin resistance
ELISA	Enzyme-linked immunosorbent assay
FBG	Fasting blood glucose
FCT	Food composition tables
Fe	Iron
FFA	Free fatty acids
g	Gram
g/d	Grams per day
GWAS	Genome-wide association studies
GRS	Genetic risk score
HbA1C	Glycated haemoglobin

Hcy	Homocysteine
HIV	Human immunodeficiency virus
HDL-C	High-density lipoprotein cholesterol
hsCRP	High sensitivity C-reactive protein
IDF	International Diabetes Federation
IL-1β	Interleukin -1beta
IL-4	Interleuken-4
IL-6	Interleuken-6
IL-10	Interleuken-10
IL-18	Interleuken-18
ISAK	International Society for the Advancement of Kinanthropometry
kcal	Kilocalorie
kg/m^2	Kilogram per square metre of body height
kJ	Kilojoule
KoGES-HEXA LDL-C	Korean Genome and Epidemiological Studies Health Examination low-density lipoprotein
LGSI	Low-grade systemic inflammation
MEDIS	Mediterranean Islands
MetS	Metabolic syndrome
Mg	Magnesium
mg	Milligram
mg/dL	Milligram per decilitre
mg/L	Milligram per litre
mmHg	Millimetre of mercury
mmol/L	Millimols per litre
MRC	Medical Research Council
MSc	Master of Science
MUFA	Mono-unsaturated fatty acids

NCEP-ATP III	National Cholesterol Education Programme-Adult Treatment Panel III
NCD	Non-communicable diseases
ng/ml	Nanogram per millilitre
NHANES	National Health and Nutrition Examination Study
NHS	Nurses' Health Study
NO	Nitric oxide
NWU	North-West University
OR	Odds ratio
ORISCAV-LUX	Observation of Cardiovascular Risk Factors in Luxembourg study
p	p-value
$PAI\text{-}1_{act}$	Plasminogen activator inhibitor-1 activity
pg/mL	Picogram per millilitre
PUFA	Poly-unsaturated fatty acids
PURE	Prospective Urban Rural Epidemiology Study, South Africa, North West
PURE-SA-NW	Prospective Urban Rural Epidemiology, South Africa, North West
RE	Retinol equivalents
ROS	Oxygen reactive species
RRR	Reduced rank regression
SAT	Subcutaneous adipose tissue
SD	Standard deviation
Se	Selenium
SFA	Saturated fatty acids
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package for Social Sciences
SU.VI.MAX	Supplementation on Vitamins and Minerals Antioxidant Study TAG triacylglycerol
TE	Total energy
T2D	Type 2 diabetes

TC	Total cholesterol
TG	Triglycerides
tHcy	Total homocysteine
TNF-R2	Tumour necrosis factor receptor 2
QFFQ	Quantitative food frequency questionnaire
VAT	Visceral adipose tissue
VLDL	Very low-density lipoprotein
WAT	White adipose tissue
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip-ratio
µg	Microgram
Ω3	Omega-3
Ω6	Omega-6
%	Percentage
Zn	Zinc

CHAPTER 1 INTRODUCTION

1.1 Background

Non-communicable diseases (NCDs), including cardiovascular diseases (CVDs), different types of cancer, diabetes and chronic respiratory disease, are increasingly becoming the leading cause of morbidity and mortality in every country, and account for 70% of all deaths worldwide (Geto *et al.*, 2021). Metabolic Syndrome (MetS) is a main public health concern because of its high prevalence and contribution to the onset of developing chronic diseases (Andersen *et al.*, 2016a; Kumaran & Paari, 2021; Kumari *et al.*, 2019; Monteiro & Azevedo, 2010). MetS is estimated to affect more than 50% of the elderly in different ethnic groups (Kumar *et al.*, 2013). This prevalence seems to be increasing due to a corresponding increase in obesity prevalence (Akhigbe & Ajayi, 2021; Grundy, 2008b; Kumar *et al.*, 2013). Whilst obesity and physical inactivity are the main drivers behind the MetS, metabolic susceptibility is the other factor necessary for this syndrome to become evident (Grundy, 2008a). Factors of metabolic susceptibility include adipose tissue disorders and typically present as abdominal obesity, aging, racial and genetic factors, and endocrine disorders (Grundy, 2008b; Kumar *et al.*, 2013; Kumaran & Paari, 2021). In addition, MetS, together with obesity, is associated with chronic inflammation, atherogenic dyslipidaemia, endothelial dysfunction and insulin resistance (Andersen *et al.*, 2016a), consequently increasing an individual's risk of developing cardiovascular diseases (CVD) by two-fold, diabetes mellitus type 2 by seven-fold (Aparajita *et al.*, 2020; Awoyemi *et al.*, 2018; Grundy, 2015) and all-cause mortality by 1.5-fold (Awoyemi *et al.*, 2018). Regarding the prevalence rate of MetS, the highest rates are usually in developed countries, while African nations exhibit the lowest prevalence rates (Izadi *et al.*, 2021). The prevalence of MetS in the general South African population varies from 5% to 62% (Kruger & Nell, 2017; Sekgala *et al.*, 2018; Sekokotla *et al.*, 2017), depending on the group being studied and the MetS definition used (Kruger & Nell, 2017). Most studies have associated MetS with the female sex, rather than male gender (Erasmus *et al.*, 2012; Motala *et al.*, 2011), Black ethnicity and ageing populations, mostly residing in urban areas (Erasmus *et al.*, 2012; Peer *et al.*, 2015).

Even though it has existed for decades, there has not been an exclusive definition given to the syndrome, and a number of expert groups have strived to come up with a unifying definition for MetS (Alberti *et al.*, 2005). Different bodies, such as the WHO, EGIR and NCEP-ATPIII, have compiled definitions with the main components of MetS being insulin resistance, obesity, dyslipidaemia, and hypertension, although they offer different clinical criteria to classify the pattern (Alberti *et al.*, 2005; Bentley-Lewis *et al.*, 2007; Kumar *et al.*, 2013). The numerous

definitions exist because of contradictory views on the thresholds that should be applied for certain criteria, such as fasting glucose levels or blood pressure, and disagreement on which components are crucial and most clinically appropriate for diagnosis. However, all the definitions include a measure of central obesity, dyslipidaemia, hypertension and glucose level (Bentley-Lewis *et al.*, 2007).

Inflammation is a key contributor to obesity-related chronic diseases, including MetS (Robinson *et al.*, 2007). Metabolic overload induces several stress reactions, such as inflammatory, organelle, oxidative, and cell hypertrophy stresses, bringing about vicious cycles that strengthen each other resulting in dysfunction (Monteiro & Azevedo, 2010). Metabolic disorders lead to immune activation in tissues, for example pancreas, liver, adipose tissue and the vasculature; individuals typically present with chronic low-grade inflammation (Andersen *et al.*, 2016a) demonstrated by elevated of C-reactive protein (CRP) levels and pro-inflammatory cytokines for example tumour necrosis factor alpha (TNF-α) and interleukin (IL-6) (Awoyemi *et al.*, 2018; Eckel *et al.*, 2005; Robinson *et al.*, 2007), and adipokines, which are an expanding collection of adipose tissue-derived proteins and hormones (Andersen *et al.*, 2016b; Robinson *et al.*, 2007). Adipokines influence metabolic processes that include adipose tissue mass, energy balance, carbohydrate and lipid metabolism, vascular endothelial function, and insulin sensitivity, making them recognised metabolic mediators that link obesity with MetS development (Monteiro & Azevedo, 2010; Robinson *et al.*, 2007). The oxidative stress that accompanies excess ingestion of fat and/or other macronutrients without associated consumption of antioxidant-rich foods/beverages, possibly contribute to the inflammatory markers ascribed to obesity (Monteiro & Azevedo, 2010).

Prudent dietary intake is a vital modifiable factor that contributes to attaining and sustaining a healthy metabolic profile (Kouvari *et al.*, 2020; Taheri *et al.*, 2022). Thus, overall dietary patterns are more informative regarding the habitual diet of an individual than single foods or nutrients (Cavicchia *et al.*, 2009; Kouvari *et al.*, 2020). However, knowing an individual's general dietary pattern provides only limited information on its overall inflammatory potential (Cavicchia *et al.*, 2009), thus, the Dietary Inflammatory Index (DII) was designed to overcome this limitation by considering all food items combined (both pro-inflammatory and anti-inflammatory). The development of the DII was aimed at better addressing the overall inflammatory potential of an individual's diet (Wang *et al.*, 2021). The DII score, developed by Shivappa et al., in 2009 and updated in 2014 to also include an energy-adjusted dietary inflammatory index (E-DII) (Shivappa *et al.*, 2014), intends to measure the inflammatory potential of an individual's diet based on the balance of pro-and anti-inflammatory properties of its components, including macronutrients, minerals, vitamins, flavonoids, and particular food

ingredients. DII scores are computed and standardised to an international reference developed from 11 population groups, which allows for their application across different ethnic populations and dietary patterns (Jia *et al.*, 2022).

The DII have been used to investigate the relationship between diets of varying inflammatory potential and blood levels of inflammatory markers such as IL-6 and CRP (Shivappa *et al.*, 2017a; Shivappa *et al.*, 2017b; Julia *et al.*, 2017; Mayr *et al.*, 2018; Shin *et al.*, 2019b;), cancer (Alkerwi *et al.*, 2015; Ge *et al.*, 2015; Li *et al.*, 2018; Maisonneuve *et al.*, 2016; Tabung *et al.*, 2015; Zamora-Ros *et al.*, 2015), CVD incidence (Garcia-Arellano *et al.*, 2015; Ramallal *et al.*, 2015), risk factors of CVD (O'Neil *et al.*, 2015; Pimenta *et al.*, 2015b; Ruiz-Canela *et al.*, 2015; Sokol *et al.*, 2016a; Wirth *et al.*, 2014), and mortality (Graffouillere *et al.*, 2016; Deng *et al.*, 2017; Hodge *et al.*, 2018a).

Although it is clear the DII is associated with several CVD risk factors, there is little investigation in the Sub-Saharan region in relation to MetS (Ojwang *et al.,* 2020). While some evidence exists for the relationship between the inflammatory index of the diet and the prevalence of MetS (Sokol et al., 2016), the dietary patterns of African populations and their association with MetS have not been explored (Carvalho *et al.,* 2014). Traditional African diets are different from Western and Mediterranean diets even though they are shifting due to urbanisation, therefore, its inflammatory characteristics should be explored. These studies are required to increase our understanding with regard to the possible pathways through which diet can influence an individual's risk to develop MetS and to inform intervention strategies. We, therefore, aim to investigate whether the inflammatory index of the diet, based on the E-DII, consumed by individuals who took part in the South African arm of the PURE study conducted in the North West Province (PURE-SA-NW), is associated with MetS in this Black South African population.

1.2 Aims and objectives

Aim

The aim of this study is to determine the relationship between the inflammatory potential of the diet and MetS in Black South Africans who participated in PURE-SA-NW study. These relationships will be investigated in a cross-sectional design.

Objectives

- To calculate and describe the inflammatory index of the PURE-SA-NW participants' diet, making use of the E-DII

- To determine the prevalence of MetS in the PURE-SA-NW participants in 2005
- To determine the relationship between the E-DII and MetS in the PURE-SA-NW study in 2005.

1.3 Research team

Team member	Qualification	Role in study
Ms. Kgomotso Tshiamiso	B.Sc. Food Science and Chemistry	M.Sc. student: Critical evaluation of the literature, protocol drafting and editing. Determining the prevalence of MetS in the participants of the PURE-SA-NW study who took part in 2005. Using the E-DII to calculate and describe the inflammatory index of the diet of the participants who took part in the 2005 PURE-SA-NWU study. Determining the relationship between the E-DII and MetS in the PURE-SA-NW study in 2005. Statistical analysis and writing of mini dissertation.
Prof. Marlien Pieters	Ph.D. Dietetics	Supervisor of M.Sc. student: study conceptualisation, guidance with regard to protocol writing, reviewing literature, statistical analysis, interpretation of results, writing of mini dissertation.
Dr. Tertia van Zyl	Ph.D. Dietetics	Co-supervisor of M.Sc. student: guidance with regard to protocol writing, reviewing literature, interpretation of results, writing of mini dissertation
Dr. Albe C Swanepoel	Ph.D. Physiology	Co-supervisor of M.Sc. student: guidance with regard to reviewing literature, statistical analysis, interpretation of results, writing of mini dissertation.

1.4 Structure of mini dissertation

This mini dissertation is written in chapter format and comprises of five chapters. Editing of all the chapters was performed by a competent language editor, technical requirements were as prescribed by the North-West University, and the referencing followed the Harvard style, as outlined by the North-West University.

Chapter 2 is a literature review, providing background information as required for the analysis of the study results. The chapter gives details on the definition of the MetS and the MetS criteria used and stipulates its pathogenesis. The chapter also describes the link between central obesity and chronic low-grade inflammation leading to the development of MetS and its components. The role of diet in the development of MetS is described as well as the development of the literature-based dietary inflammatory index (DII). In addition, there is a description of the association between the DII and MetS, as well as the difference between the DII and empirically-based dietary indices.

Chapter 3 provides an overview of the study design used and the PURE study population characteristics. There is an outline of the methods used for the anthropometrical and biochemical tests, as well as the description for the Quantitative Food Frequency Questionnaire (QFFQ). The chapter tabulates the food items included in the PURE E-DII and provides a detailed description of the calculation of the E-DII. The chapter describes the MetS classification used in this study, as well as a description of the statistical analyses employed.

Chapter 4 provides the results in table format. This includes comparisons of the E-DII food components consumed between the participants with and without MetS. The chapter also presents the study variables, which include demographics, MetS components and other factors linked to MetS. The mean E-DII across the number of MetS components are also reported together with the demographics, inflammatory markers and MetS components across the E-DII quartiles.

Chapter 5 is a discussion of all the findings of the study. Current literature provides validation for the findings of this study. The limitations and strengths of the study are included in this chapter and a conclusion drawn. This mini dissertation concludes with a list of references, enlisting the sources cited from chapter 1 to 5, along with the supporting documents provided in Annexure A to C.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Metabolic syndrome (MetS) is considered a main public health concern since its prevalence is increasing globally (Nikniaz *et al.*, 2018). It is, furthermore, an important risk factor for cardiovascular diseases (CVDs) and type 2 diabetes, both of which are considered the foremost causes of death globally (Dandona *et al.*, 2005a). Since MetS is a collection of diverse risk factors, identifying targets that influence the overall metabolic syndrome risk instead of its individual factors, is highly relevant in the clinical setting (Wirth *et al.*, 2014). There have been numerous diagnostic criteria offered by different organisations over the past 10-year period to define the MetS (Alberti *et al.*, 2006). Of late, these have originated from the National Cholesterol Education Programme Adult Treatment Panel III (NCEP: ATP III) as well as the International Diabetes Federation (IDF) who all focus specifically on abdominal circumference, as a proxy measure of central obesity, which is the central component of MetS (Bruce & Byrne, 2009; Kassi *et al.*, 2011). According to the NCEP: ATP III criteria, the diagnosis of MetS requires three of five clinical components, namely increased waist circumference, decreased high-density lipoprotein cholesterol (HDL-C) levels, elevated triglycerides (TGs), increased fasting blood glucose (FBG), and hypertension (Vassallo *et al.*, 2016).

MetS is also related to chronic low-grade inflammation, being an integral part of central obesity (Neufcourt *et al.*, 2015a). Although the exact pathogenesis is unclear; various factors are known to increase MetS prevalence such as ethnic and/or genetic predisposition, environmental factors and lifestyle choices which include dietary habits and inactivity, increasing age as well as elevated body mass index (Nikniaz *et al.*, 2018). The suggested mechanisms include elevated pro-inflammatory cytokine and adipokine levels and decreased anti-inflammatory cytokine and adipokine levels, consequently producing a pro-inflammatory environment (Neufcourt *et al.*, 2015a).

Many dietary nutrients and components are associated with markers of systemic inflammation (Guinter *et al.*, 2019). Diet is one of the regulators of subclinical inflammation, assessed in humans through inflammatory marker levels, for instance interleukin-6 (IL-6), C-reactive protein (CRP), tumour necrosis factor-α (TNF- α), or cell adhesion molecules (Mazidi *et al.*, 2018). Diet can influence the balance between pro- and anti-inflammatory adipokines and cytokines by providing nutrients that exhibit potential anti- or pro-inflammatory properties (Guinter *et al.*, 2019).

Conventionally, most studies have concentrated on the relationship between a specific food or nutrient and disease risk (Shivappa *et al.*, 2018). However, more recently, numerous dietary indices have been developed to quantity the effects of the diet as a whole, as dietary indices permit the capturing of nutrient interactions within the food matrix (Shivappa *et al.*, 2014). Furthermore, the dietary pattern approach is better to reveal nutritional behaviour, as individuals do not consume individual nutrients but rather meals consisting of diverse combinations of nutrients and foods (Neufcourt *et al.*, 2015a). Worldwide, numerous epidemiologic studies have reported a link between several dietary patterns and inflammatory markers (Neufcourt *et al.*, 2015a). Generally, a Westernised dietary pattern is positively associated with inflammation, whereas a Mediterranean dietary pattern, has a negative association with inflammation due to its anti-inflammatory properties (Neufcourt *et al.*, 2015a). An example of a dietary index specifically aimed at inflammation, namely the Dietary Inflammatory Index (DII), was developed in 2009 and updated in 2014 (Hébert *et al.*, 2019; Shivappa *et al.*, 2014). It is used to evaluate the inflammatory potential of the diet based on the pro-inflammatory properties as well as with the anti-inflammatory properties of several dietary components, comprising of macronutrients, minerals, vitamins, flavonoids and particular food items, which have been shown to affect markers of inflammation (Carvalho *et al.*, 2019).

Although some evidence exists for the relationship between the inflammatory index of the diet and the prevalence of the MetS (Sokol *et al.*, 2016b), little information is available on Africans, and specifically the African diet, and its association with inflammation. This chapter provides a background on the MetS and different dietary inflammatory indices, as well as the evidence linking the use of these indices to the context of MetS.

2.2 Metabolic syndrome

2.2.1 The definition

Metabolic and immune systems are some of the most central necessities for survival and are therefore highly interconnected. The proper operation of each is reliant on the other creating a central homeostatic mechanism, which when dysfunctional, may cause a cluster of chronic metabolic disorders, known as the MetS (Figure 2.1) (Grundy, 2006; Hotamisligil, 2006). The MetS is defined as a clustering of components that allude to over-nutrition, an inactive lifestyle and subsequent excess adiposity, characterised by abdominal obesity, hypertension, dyslipidaemia and insulin resistance (Cornier *et al.*, 2008). All these features are risk factors for atherosclerosis, thereby making MetS a major contributing factor towards coronary heart diseases, which are a great threat to global human health and all-cause mortality (Dandona

et al., 2005b; Hotamisligil, 2006). These conditions are interconnected and have common underlying mediators, pathways and processes (Kassi *et al*., 2011), and tend to cluster together in the same individual.

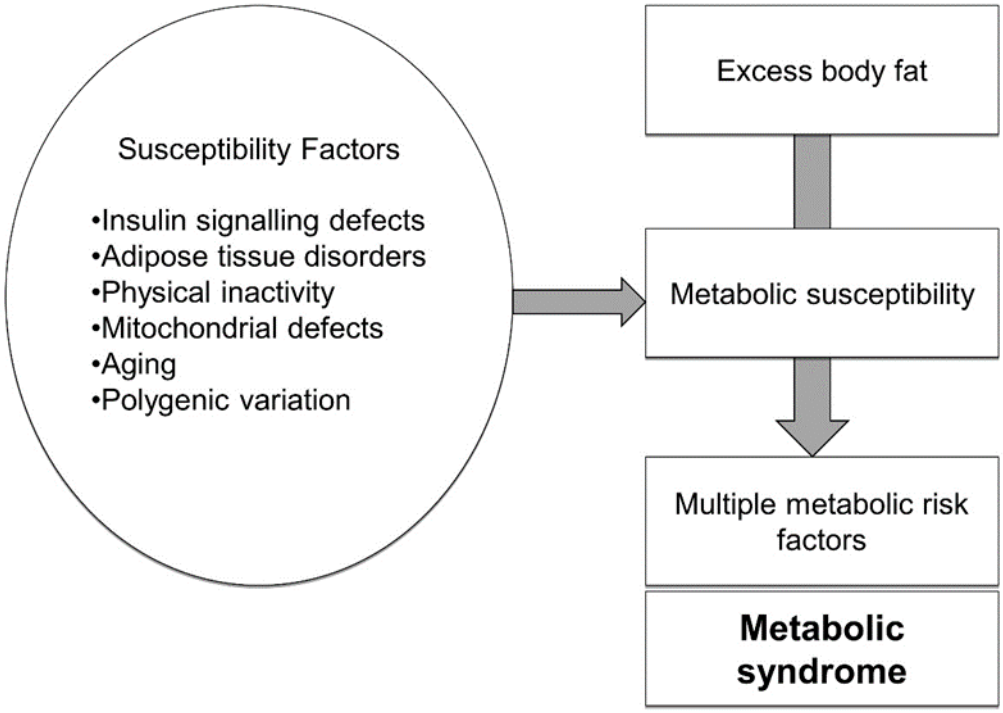

Figure 2-1 Proposed scheme for the pathogenesis of the metabolic syndrome

According to Lo (2016), MetS clusters around central obesity, which is indicative of increased visceral fat, and believed to have a greater flux of adipose tissue-derived free fatty acids into the liver through the splanchnic circulation. This leads to the increased production of low-density lipoproteins, along with hypertriglyceridaemia and increased glucose release from the liver into the systemic circulation and subsequently, insulin resistance and hyperinsulinemia (Lo, 2016). Originally, this syndrome was envisioned as insulin resistance, resulting in glucose intolerance, hyperinsulinaemia, type 2 diabetes, hypertriglyceridaemia and low HDL-C concentrations (Dandona *et al.*, 2005a). All these factors could be accounted for by resistance to the actions of lipid and carbohydrate metabolism (Dandona *et al.*, 2005a; Kassi *et al.*, 2011; Retondario *et al.*, 2019). Reaven (1988) provided the fundamental step in the development of the pathophysiological concepts surrounding MetS, and named it “Syndrome X” (Reaven, 1988). The original description of the MetS by Reaven (1988) comprised of obesity, hypertension, impaired glucose tolerance or diabetes, insulin resistance, hyperinsulinaemia

and dyslipidaemia characterised by elevated TGs and low HDL-C levels (Dandona *et al.*, 2005a)

There has however, been a long-standing controversy regarding the definition of the MetS and its utility (Huang, 2009), which has led to other investigators giving several different names and suggesting different diagnostic approaches (Bonora, 2006). Ultimately, the World Health Organization (WHO) appointed a group of experts tasked with the identification of the most appropriate criteria for the syndrome (Bonora, 2006). Their criteria were however, deemed unsuitable due to the inclusion of insulin resistance, which is difficult to assess because it is labour intensive (Bonora, 2006; Bruce & Byrne, 2009). Currently, central obesity is favoured above insulin resistance for inclusion in the definition of the MetS by organisations such as the WHO, the European group for the study of Insulin Resistance (EGIR), NCEP: ATP III and IDF, who all focus specifically on waist circumference as a proxy measure of central obesity (Bruce & Byrne, 2009; Kassi *et al.*, 2011). In 2001, the NCEP ATP III resolved that MetS was to be defined by three or more of five criteria, which included increased waist circumference of greater or equal to 94 cm for men and 80 cm for women, blood pressure over 130/85 mmHg, fasting TG level over 1.7 mmol/L, HDL-C levels below 1.03 mmol/L and 1.29 mmol/L for men and women, respectively, and fasting blood sugar above 6.1 mmol/L (Bruce & Byrne, 2009; Huang, 2009). This definition was widely used because of its simplicity and feasibility, since it is based on measurements and laboratory results that are readily available to physicians, thereby enabling its clinical and epidemiological application (Huang, 2009). In 2005, however, the IDF published a new set of criteria, with a stronger emphasis on the requirement of obesity as a prerequisite unlike the NCEP: ATP for which central obesity is not a requirement, as long as there are three of the five criteria met. The obesity cut points were also made population-specific to account for the differences in distribution norms for body weight, and waist circumference in different ethnic groups. Table 2.1 gives a summary of the ATP-III and IDF definitions of the MetS.

Table 2-1 Definition of metabolic syndrome according to the NCEP-ATP-III and IDF

	NCEP ATP-III (NCEP Expert panel on detection, 2001)	IDF (www.idf.org)
Central Obesity	Waist circumference ≥102cm (men) Waist circumference >88 cm (women)	Waist circumference >94cm (European men) Waist circumference >90cm (Asian men) Waist circumference >80cm (women)
Blood pressure (mmHg)	BP >130/85 mmHg or treated for hypertension	SBP >130 mmHg or DBP >85 mmHg DBP or treated for hypertension
Dyslipidaemia (mmol/l)	TAGs ≥1.7 mmol/l HDL-cholesterol: <1.0 mmol/l (men), <1.3 mmol/l (women)	TAGs ≥1.7 HDL-cholesterol: <1.04 mmol/l (men), <1.29 mmol/l (women)
Hyperglycaemia (mmol/l)	Fasting plasma glucose ≥6.1 mmol/L	Fasting glucose ≥5.6 mmol/l or previous diagnosis of impaired glucose tolerance or diabetes

BMI: Body Mass Index; DBP: Diastolic blood pressure; HDL: high-density lipoprotein; SBP: Systolic blood pressure; TAG: Triacylglycerol; WHR: Waist/hip ratio

2.2.2 Central obesity: A pro-inflammatory state that links metabolic syndrome components

Obesity is associated with inflammation and can serve as a precursor for metabolic disease (Sokol *et al.*, 2016b); it is thus favoured as a central component of the MetS because of its causal link (Grundy, 2015). Inflammation is the primary response invoked by the body to deal with injuries, which includes swelling, redness, pain and fever. Obesity is a low-grade inflammatory condition metabolically triggered by, amongst other things, nutrients and metabolic surplus (where the body has more to metabolise than it requires), which lay the foundation for adiposity and its associated problems (Dandona *et al.*, 2005b). The most primitive response systems assimilate pathogen-and-nutrient sensing pathways such that nutrients can provoke immune responses and pathogens can induce and control metabolic responses (Dandona *et al.*, 2005b); however, this has not been adapted to nor is it beneficial in the presence of continuous nutrient surplus, which is experienced in metabolic dysfunction (Dandona *et al.*, 2005b). Obesity, insulin resistance and type 2 diabetes (T2D) are closely related to metaflammation (metabolically triggered inflammation), characterized by increased acute-phase reactants, abnormal cytokine production, additional mediators and activation of a system of inflammatory signalling pathways (Hotamisligil, 2017). Undeniable epidemiological, clinical, and experimental evidence produced in recent years, causally linked

inflammation, molecules, and networks essential to inflammatory responses to the development of metabolic diseases (Dandona *et al.*, 2005b). Furthermore, Bruce and Byrne (2009) hypothesised, through a study conducted in extremely obese subjects, that visceral fat could possibly be at the heart of an underlying mechanism linking inflammation to the development of metabolic syndrome. Visceral fat has been demonstrated to release substances that are causal to metabolic abnormalities, showing that IL-6 concentrations were significantly raised and directly correlated with CRP, which suggests that visceral fat provides a contributory link to systemic inflammation (Bruce & Byrne, 2009). Furthermore, white adipose tissue (WAT) represents most of the adipose tissue and consist of many types of cells, the most abundant of which are adipocytes (Fantuzzi, 2005). WAT, no longer deemed an inert tissue primarily devoted to energy storage, may play a recognisable role in regulating physiologic and pathologic processes, which include immunity and inflammation (Fantuzzi, 2005). Macrophages are active components of adipose tissue. Adipocytes as well as macrophages found within adipose tissue secrete several hormones and cytokines that could promote the characteristic pathophysiologic alterations seen in the MetS, as well as local inflammation within adipose tissue (Balistreri *et al.*, 2010). The increases in pro-inflammatory cytokines, particularly CRP IL-6, and TNF-α, suggest overproduction by the expanded adipose tissue mass and have been persistently found to be elevated in the serum, WAT, or both, of obese individuals, thereby contributing to the development of obesity-linked complications (Eckel *et al.*, 2005; Fantuzzi, 2005; Ouchi *et al.*, 2011).

2.3 Inflammatory index of the diet

Dietary habits are of central importance in modifying inflammation associated with MetS and general recommendations comprise of reducing obesity, consuming an anti-atherogenic diet that focuses on low total fat intake, as well as increasing physical activity (Feldeisen & Tucker, 2007). Table 2.2 details dietary recommendations for the different components of the MetS.

Table 2-2 Dietary recommendations for the metabolic syndrome

Metabolic components	Dietary recommendations
Abdominal/central obesity	Healthy dietary pattern Reduced caloric intake if weight loss is needed Low saturated and trans fats, and cholesterol intake High fruit, vegetable and whole grain intake Low refined carbohydrate intake
Elevated blood pressure	Dietary approaches to stop hypertension (DASH diet) Reduced sodium intake Low to moderate alcohol intake
Elevated triglyceride levels and low High-density lipoprotein	Reduced caloric intake if weight loss is needed Mediterranean-style diet Low saturated fat and trans fat High fruit, vegetable, whole grain and dietary fibre Moderate fat consumption from unsaturated sources, especially from monounsaturated fats Low to moderate alcohol intake
Elevated fasting glucose concentration	Reduced caloric intake if weight loss is needed Low refined carbohydrate intake Moderate carbohydrate intake rich in whole grains
Pro-inflammatory/pro-thrombotic state	Reduced caloric intake Low saturated and trans fat and cholesterol intake

[Adapted from Feldeisen and Tucker, 2007]

In addition to the MetS, inflammation is also linked to other chronic conditions, such as cardiovascular disease and cancer, with more evidence also amassing on the role of chronic inflammation in diabetes, asthma and depression (Cavicchia *et al.*, 2009). Even though acute inflammation is essential as the body's natural response to tissue injury, in wound healing and to promote tissue regeneration, without proper regulation it may result in a chronic, low-grade condition that involves several inflammatory proteins created by distinct and diverse cell types in the immune system and adipocytes (Agostinis-Sobrinho *et al.*, 2016). Regulating inflammation is an important step in the inhibition or treatment of these disorders, and diet has been consistently presented as a tool to control inflammation (Cavicchia *et al.*, 2009; Shivappa *et al.*, 2015). There have been a number of dietary factors connected to inflammation, for instance the Westernised diet, which is high in refined carbohydrates, high-fat intakes and red meat, is linked to high CRP, IL-6 and fibrinogen levels, all inflammatory markers connected to these chronic illnesses (Shivappa *et al.*, 2015). Conversely, the Mediterranean diet, which

incorporates mainly whole grains, green vegetables, fruit and fish, and reasonable alcohol intake and olive oil ingestion, is linked to lower levels of inflammation (Shivappa *et al.*, 2015). Some specific nutrients have also been reliably shown to be related to lower levels of inflammation, such as the unrefined carbohydrates, n-3 PUFA, fibre, vitamin E, vitamin C, β-carotene and magnesium (Shivappa *et al.*, 2015).

To enable research into the inflammatory effects of diet on health in humans, researchers developed a number of inflammatory indices envisioned to assess the inflammatory potential of individuals' diets as a whole, rather than focusing on individual nutrients or foods (Cavicchia *et al.*, 2009). A number of indices have been developed, and they can be grouped into two groups. The first is empirical-based scores, where dietary intakes in large epidemiological studies are related to a number of inflammatory markers measured in the same study and then through the use of statistical analyses, the dietary components with the strongest association to the inflammatory markers, are included in the empirical score (Tabung *et al.*, 2016). As an alternative to an empirical score, a literature-based score has also been developed, based on the outcomes of an extensive literature review (Cavicchia *et al.*, 2009). This score is based on associations observed between dietary components and inflammatory markers, as reported in the available literature (Cavicchia *et al.*, 2009). An example of such a score is the Dietary inflammatory index (DII), which classifies an individual's diet on a range from maximally anti-inflammatory to maximally pro-inflammatory by generating a score for each food and constituent alleged to positively or negatively affect the degree of inflammation (Cavicchia *et al.*, 2009; Shivappa *et al.*, 2014). The DII was developed to offer a measure of diet-associated inflammation that is comparable across populations (Hébert *et al.*, 2019). As part of its development, a systematic literature review was conducted focusing on the relationship between diet and markers of inflammation (Cavicchia *et al.*, 2009). Dietary factors included in the scores (macronutrients, micronutrients, flavonoids and food items) had to have been associated with >1 of 6 inflammatory markers: Interleukin-1beta (IL-1β), interleukin-4 (IL-4), IL-6, interleukin-10 (IL-10), TNF-α and CRP (Khan *et al.*, 2018a). The original version of the DII was presented in 2009 and was based on scoring 929 peer reviewed articles published in the biomedical literature through 2007, connecting any aspect of diet to ≥1 of the 6 inflammatory biomarkers with no restrictions made on dietary factors (Cavicchia *et al.*, 2009). One of three likely values was allocated to each article based on the effect of the specific dietary factor on an inflammatory marker: pro- inflammatory scored +1, if no change in inflammatory marker was observed scored 0, and anti-inflammatory was scored -1 (Cavicchia *et al.*, 2009). Study design was used to weigh the articles, the highest weight of 10 assigned to intervention studies and the lowest weight of 3 assigned to cell culture studies (Cavicchia *et al.*, 2009) The weighted values were then utilised to compute a score for each food and

constituent by first dividing the weighted pro- and anti-inflammatory articles by the total number of weighted articles (Cavicchia *et al.*, 2009). The pro-inflammatory fraction was then deducted from the anti-inflammatory fraction. The scores were then adjusted by the total number of articles in which a subjective cut-off point of 100 was chosen. This number was used to adjust scores by weighting the number of articles. All foods and constituents with a number of articles greater than or equal to 100 were assigned full value of the score, those with articles less or equal to 100 were adjusted first by dividing the number of weighted articles by 100 then the fraction was multiplied by the score for that particular constituent, leading to a new adjusted score. The food and constituent specific scores were then multiplied by the intake of each participant. Each food and constituent score product was summed to create the overall Inflammatory Index score for each participant. The Inflammatory Index score was ultimately rescaled by dividing by 100, to aid in interpreting results (Cavicchia *et al.*, 2009). In the initial DII, literature review-based scores were multiplied by individuals' actual food parameter intakes; no attempt was made to relate to any external standard of intake such as energy intake (Cavicchia *et al.*, 2009).

While the original DII epitomised the fruitful development of a literature-derived index that could be comprehensively functional across a range of human studies, there were some methodologic difficulties causing complications to its application, hence the development of an upgraded, second version that exhibits a number of developments over the original (Hébert *et al.*, 2019). The updated DII was created as follows:

The journal review database was updated to include articles published until December 2010. The resultant 1943 articles linked to a total of 45 food parameters were considered. Calculating the null, anti-inflammatory and pro-inflammatory effects for each of the 45 food parameters and tallying the total weighted number of articles causal to every category, based on the study design and the number of articles using that design, determined the pro-inflammatory and anti-inflammatory fractions for each food parameter. The 'food parameter-specific overall inflammatory effect score' was then calculated by subtracting the anti-inflammatory fraction from the pro-inflammatory fraction. Literature robustness was accounted for by a cut-off point of 236 which was chosen as the median of the total weighted number of articles across all of the food parameters. The raw overall inflammatory effect score was then multiplied by the total weighted number of articles and then divided by 236, the resulting value, being the 'overall food parameter-specific inflammatory effect score.' Table 3 presents the effect scores for the 45 food parameters, which are then used in the subsequent calculations. Next, a world composite database for the 45 food parameters was developed, based on data from 11 countries and used to calculate a world mean and standard deviation

for each of the 45 food parameters (Shivappa *et al*., 2014). This dataset represents an assortment of dietary intakes used as references to provide comparative consumption data for the 45 food parameters at hand (Cavicchia *et al.*, 2009). Based on available dietary intake data, Z-score and centred percentiles were calculated for each of the food parameters for each individual in the study based on the world average and standard deviation, which was then multiplied by the respective 'overall food parameter-specific inflammatory effect score' to obtain the 'food parameter-specific DII score.' This helped to avoid dependence on reported raw amounts of food consumed (Hébert *et al.*, 2019). Expressing the values as collective proportions with values ranging from 0-1, to reduce the effect of right skewing, avoided the arbitrariness of using raw consumption amounts. Centering the data on zero was achieved by multiplying each of the cumulative proportions by 2 and then subtracting 1 (Hébert *et al.*, 2019). Table 2.3 illustrates the food parameter effect score, which is used to calculate the food parameter specific DII score.

Table 2-3 List of the DII Food parameters and their effect scores

Food parameter	Effect score
Alcohol (g)	-0.278
Vitamin B12 (µg)	0.106
Vitamin B6 (mg)	-0.365
B-carotene(µg)	-0.584
Caffeine (g)	-0.110
Carbohydrate (g)	0.097
Cholesterol (mg)	0.110
Energy (kcal)	0.180
Eugenol (mg)	-0.140
Total fat (g)	-0.663
Fibre (g)	-0.190
Folic acid (mg)	-0.412
Garlic (g)	-0.453
Fe (mg)	0.032
Mg (mg)	-0.484
MUFA (g)	-0.009
Niacin (mg)	-0.246
n-3 Fatty acids (g)	-0.436
n-6 Fatty acids (g)	-0.159
Onion (g)	-0.301
Protein (g)	0.021
PUFA (g)	-0.337
Riboflavin (mg)	-0.068
Saffron (g)	-0.140
Saturated fat (g)	0.373
Se (g)	-0.191
Thiamine (mg)	-0.098
Trans fat (µg)	0.229
Turmeric (mg)	-0.785
Vitamin A (RE)	-0.401
Vitamin C (mg)	-0.424
Vitamin D (µg)	-0.446
Vitamin E (mg)	-0.419
Zn (mg)	-0.313
Green/ black tea (g)	-0.536
Flavan-3-ol (mg)	-0.415
Flavones (mg)	-0.616
Flavonols (mg)	-0.467
Flavonones (mg)	-0.250
Anthocyanidins (mg)	-0.131
Isoflavones (mg)	-0.593
Pepper (g)	-0.131
Thyme/ Oregano (mg)	-0.102
Rosemary (mg)	-0.013
Score range	-7.65 to 6.67

[Adapted from Shivappa et al., 2014]

Next, the DII scoring algorithm was reversed so that more anti-inflammatory scores are in the negative range and more pro-inflammatory scores are in the positive range; i.e. a larger, positive DII score correlating to a more pro-inflammatory the diet, while greater negative

values signify diets that are more anti-inflammatory. It was necessary to create the DII because previously, most of the dietary indices used in epidemiologic research were based on dietary recommendations, such as the Healthy Eating Index, associated with adherence to a certain cuisine, such as the Mediterranean Dietary Index, or derived from a particular study using regression techniques, for example reduced rank regression (Tabung *et al.*, 2016). Hence, the DII was anticipated to reflect all evidence from a wide assortment of human populations using different studies and dietary assessment methods (Hébert *et al.*, 2019). There was an additional adaption to the DII to adjust for energy intake. To compute the Energy-adjusted DII (E-DII) score, all intakes were normalised to 1000kCal, to take into account varying amounts of energy consumption between people (Hébert *et al.*, 2019; Khan *et al.*, 2018b; Phillips *et al.*, 2018).

Due to children's unique nutritional needs, there was also a need to create an index that can be applied specifically to deal with enteric and other infections, which children are most susceptible to (Khan *et al.*, 2018a). The Children-Dietary Inflammatory Index (C-DII) was created with only 25 food parameters compared to the 45 for adults primarily due to the different diets consumed by adults and children (Khan *et al.*, 2018a). The C-DII was developed, like the original DII, based on the literature review through 2010, using dietary data obtained from children in 16 countries to create a reference database for consumption of macronutrients, vitamins, minerals and whole foods (Khan *et al*, 2018a). The dietary data were collected from three diverse sources, including i) datasets derived from the study principal investigators (n=11), ii) from published articles (n=3) and iii) National Health and Nutrition Examination (NHANES) survey reports (n=2). The C-DII is calculated in a manner similar to the approaches described above to calculate adult DII scores (Khan *et al.*, 2018a).

Contrary to the literature-based Dietary Inflammatory Index, a number of empirical Dietary Inflammatory Indices have been developed. For the first empirical index developed, the authors used reduced rank regression (RRR) to develop an empirical dietary inflammatory index (EDIP) using the dietary and inflammatory marker data from the Nurses' Health Study (NHS in the United States) (Tabung *et al.*, 2018). It is a hypothesis-driven index focused on finding a dietary pattern that could be predictive of biological markers of inflammation. The use of 39 predefined food groups in RRR models, followed by stepwise linear regression analyses to identify a dietary pattern that relates to three plasma inflammatory markers, IL-6, CRP and TNF-R2, derived a dietary pattern (Tabung *et al.*, 2016). The construct validity was then assessed in two independent samples from the NHS and the Health Professional follow-up study, using multivariate-adjusted linear regression models. The intake of the food groups identified in the stepwise linear regression analyses was weighted by the regression coefficients derived from the final stepwise linear regression model and then summed to

constitute the EDIP score. The EDIP was rescaled by dividing by 1000 to reduce the magnitude of the scores and aided in interpretation of statistical analyses. The EDIP evaluates the inflammatory potential of an individual's diet on a range from maximally pro-inflammatory to maximally anti-inflammatory (Tabung *et al.*, 2016). The EDIP was soon followed by the development of more empirical-based inflammatory indices in other population groups to account for differences in dietary intakes around the world. A multinational study, conducted by the MEDIS group, sought to assess the inflammatory potential of dietary habits of older adults living in the Mediterranean basin. The nutrition-related inflammatory score was calculated based on the validated MEDIS food Frequency Questionnaire, with a total of seven food components utilised (Tyrovolas *et al.*, 2018).

Another study conducted by Kaluza *et al.* (2018), used an empirically developed dietary questionnaire to predict low-grade systemic chronic inflammation in a Nordic population. From the 123-item food frequency questionnaire, they indicated statistically significant correlations with hs-CRP in 20 food groups (comprising 62 food items) among women with high CRP concentrations adjusted for age, smoking status, lean body mass, age of menopause and total energy intake. Fifteen of the food groups had an anti-inflammatory potential and five had a pro-inflammatory potential (Kaluza *et al.*, 2018).

Altough both types of dietary indices evaluate the inflammatory potential of an individual's diet they vary in origin and design. The DII is an a priori index developed from scientific evidence available in the literature. The empirical indices are hypothesis driven and based on statistical exploratory methods, and focused on identifying a dietary pattern that can be predictive of biological markers of inflammation (Tabung *et al.*, 2017) measured in the same study population. The DII is also nutrient–based, whilst its counterpart, EDIP, is based on food groups alone. Table 2.4 presents a comparison of the methodologic differences between the two types of dietary scores referred to above.

Table 2-4 Methodological differences between DII and EDIP scores

	EDIP	DII
Derivation approach	Empirical hypothesis-oriented	A priori
Statistical methods	Reduced rank regression, stepwise linear regression	Standardisation, normalisation
Predictors	Foods and food groups exclusively	Mainly nutrients
Outcomes	Three circulating inflammatory markers (CRP, TNFαR2, IL-6)	Six inflammatory markers reported in the literature (CRP, TNFαR2, IL-6, IL-1β, IL-4, IL-10)
Number of components	18 components (nine anti-inflammatory, nine pro-inflammatory) but population specific	45 components (36 anti-inflammatory, nine pro-inflammatory)
Weights	Derived from regression analyses	Derived from the literature
Final score	18 components, weighted and summed to derive the score for each individual (population specific)	45 components, weighted and summed to derive the score for each individual
Influenced by supplement use	No	Yes
Score interpretation	Higher scores indicate pro-inflammatory diets; lower scores indicate anti-inflammatory diets	Higher scores indicate pro-inflammatory diets; lower scores indicate anti-inflammatory diets

CRP, C-reactive protein; DII, Dietary inflammatory Index; EDIP, Empirical dietary inflammatory pattern; TNFαR2, TNF-α receptor 2. [Adapted from Tabung et al., 2017]

2.4 The association between the DII and MetS

With respect to the MetS, associations with the DII have been inconsistent (Neufcourt *et al.*, 2015b) and the research to date limited. While some studies found an association between the MetS and the DII, others showed no association, even though individual components of the MetS were associated with the DII (Shivappa *et al.*, 2018). For instance, the research of Ahluwalia *et al.* (2013), focussing on the simultaneous association of dietary patterns with inflammation in relation to the MetS, reports negative associations between the MetS and a priori indices such as the DII (Pimenta *et al.*, 2015a). Dietary patterns that are consistent with healthy eating also show a negative association, supporting the idea that healthy diets decrease inflammation and the MetS through their connotation to weight control (Ahluwalia *et al.*, 2013). In a study conducted by Mazidi *et al.* (2018), in US adults, they suggested a possible association between CVD risk factors, including the MetS and its individual

components, markers of subclinical inflammation and glucose homeostasis, and the DII (Mazidi *et al.*, 2018). They reported MetS prevalence as 28.3% and DII scores ranging from -5.66 to 4.24 in age, gender and race adjusted logistic regression models, with the odds of having the MetS increasing across DII quartiles (Mazidi *et al.*, 2018). Participants in the fourth quartile (Q4) of DII had 65% (95% CI: 44-89%) higher odds of having the MetS compared to those in the first quartile (Mazidi *et al.*, 2018). These results strengthen the hypothesis that inflammation related to diet is linked to chronic diseases such as the CVD and MetS. Conversely, Wirth and his associates (2014) conducted a study in a defined cohort of workers, applying the DII; the outcome was that elevated DII scores were related to increased CRP levels as well as the glucose intolerance component of the MetS, which contributed the least to MetS diagnosis compared to the other components (Wirth *et al.*, 2014). Although the study was well-characterised with information on comprehensive work history, diet, and various biological outcomes, there was only a statistically significant association detected between the DII and CRP as a dichotomous variable, reflecting a non-linear association between CRP and diet, which was also detected utilising other dietary indices, such as the Healthy Eating Index (Wirth *et al.*, 2014). However, the dietary patterns of African populations and their association with the MetS have not been explored (Carvalho *et al.*, 2019). Traditional African diets, which mainly consist of indigenous foods, (although in a transition into Western dietary patterns), are different from both Western diets and Mediterranean diets and need to be investigated, especially concerning its inflammatory characteristics hence, this investigation into the association of the inflammatory potential of the diet of an African study population with the MetS.

2.5 Conclusion

MetS is a multifactorial condition characterised by central obesity, high BP, hyperglycaemia, elevated TG, and decreased HDL-C (Alberti *et al.*, 2005). These factors are interconnected and share underlying mediators, processes and pathways, and they tend to cluster together in the same individual (Kassi *et al.*, 2011). MetS is associated with chronic low-grade inflammation, being an integral part of central obesity (Grundy, 2008b). Dietary factors can influence inflammatory status through nutrients that exhibit potential anti- or pro-inflammatory properties (Guinter *et al.*, 2019). The dietary inflammatory indices have consequently been developed to characterise the inflammatory potential of the diet as a whole (Shivappa *et al*., 2014). These indices can be divided into empirical or literature-based scores, such as the DII. The DII is a score obtained from an individual's food intake based on the inflammatory potential of foods, nutrients, or food constituents, with evidence showing an effect on inflammatory markers (Carvalho *et al.*, 2019). The DII was established to provide a possible

measurement of diet-associated inflammation that is comparable across populations (Hébert *et al.*, 2019). Regulating inflammation is an important step in the reduction or treatment of inflammation-based diseases, such as the MetS, and diet has been consistently presented as a tool to control inflammation (Cavicchia *et al.*, 2009; Shivappa *et al.*, 2015). While there is some evidence linking the inflammatory index of the diet to MetS, the dietary patterns of African populations and their association with MetS have not been explored (Carvalho *et al.*, 2019). This study therefore investigates the association of the inflammatory index of the diet with the MetS in Black South Africans. This study will make use of the literature-based DII, as the number of inflammatory markers measured in this study is too limited to allow the use of an empirical index.

CHAPTER 3 METHODOLOGY

3.1 Introduction

Metabolic syndrome (MetS) is increasingly becoming a prevalent and everyday concern worldwide (Dandona *et al.*, 2005a). The prevalence of MetS in South Africa is particularly associated with a high prevalence of obesity, sedentary lifestyle and poor dietary habits, which are now also reported in rural areas (Motala *et al.*, 2009). MetS and its components raise major public health concerns considering their strong involvement in pathophysiological cardiovascular disease (CVD) features (Kouvari *et al.*, 2020). Recent studies propose that chronic low-grade inflammation, associated with obesity, plays a central role in the development of MetS, further contributing to the development of a number of different metabolic dysfunctions (Grundy, 2008b). Several studies have shown that diet and inflammation are closely linked, and therefore dietary inflammatory indices have been developed with the aim to determine the inflammatory potential of whole diets (Guinter *et al.*, 2019; Shivappa *et al.*, 2014). Since diet influences inflammation and CVDs, such as MetS, which are increasing exponentially in South Africa, the study aimed to investigate whether the dietary inflammatory potential of 2 010 Black South Africans, who participated in the Prospective Urban and Rural Epidemiology (PURE) study in 2005, was associated with blood levels of inflammatory markers and ultimately the MetS.

The proposed study forms part of the North West Province, South African arm of the international PURE study (PURE-SA-NW). The PURE-SA-NW study consists of data collection at three time points, in 2005, 2010 and 2015. This proposed study, however, will be of a cross-sectional design on the 2 010 individuals who participated in PURE-SA-NW in 2005.

The objectives of the study were:

- To calculate and describe the inflammatory index of the PURE-SA-NW participants' diet, making use of the E-DII.
- To determine the prevalence of MetS in the PURE-SA-NW participants in 2005.
- To determine the relationship between the E-DII and MetS in the PURE-SA-NW study in 2005.

3.2 Ethical approval

Ethical approval (NWU-00034-17-A1-03) for this mini dissertation was obtained from the Health Research Ethics Committee of the North-West University (NWU), Potchefstroom campus (Annexure A). This study is affiliated with the PURE-SA study conducted in North West Province, South Africa (PURE-SA-NW) (NWU-00034-17-S1).

3.3 Study design

The international PURE study is a large-scale, prospective, epidemiological study, with the aim of investigating the relationship between lifestyle behaviours, risk factors and the incidence of non-communicable diseases in rural and urban communities across 27 low-, middle- and high-income countries, including South Africa. The North West Province and the Western Cape are currently the only two sites in South Africa. This MSc study only used the data collected in 2005 in North West province (PURE SA-NW).

3.3.1 Study population

Permission to access and recruit individuals within the community was granted on a jurisdictive (national) and goodwill (tribal/community and household) basis. The South African Department of Health, as a judicial body, and the Potchefstroom and Ganyesa city mayors, tribal chiefs, community leaders (acting as gatekeepers) and household heads representing the tribal and community leadership granted permission to access and recruit individuals.

There was equal selection of individuals from areas representing urban, urban-rural (transitional), and rural areas of North West Province. Thus, Setswana speaking individuals residing in Ikageng, Zonderwater, Extension 7 or Extension 11 (urban settlements), Ganyesa (urban-rural town), or Tlakgameng (rural area) were recruited for the PURE study. Although there was consent given for the study in its entirety, commencing in 2005 and completion in 2015, there was permission sought from the tribal and community leaders for each follow-up data collection time point out of respect for the community's leadership structure. The approaching of potential participants commenced once all the above-mentioned stakeholders had approved. Upon verbal consent, they received all the information regarding their possible participation. Individuals had one week to consider their participation with their family members, after which there was written voluntary informed consent collected by the fieldworkers from the individual's residence. It was still the participants' decision as to whether they would participate on the date of the data collection, irrespective of their initial decision to be involved. Consequently, the individuals who decided not to participate in the PURE study were not approached with any further requests of involvement.

3.3.2 Recruitment

The selection of individuals who wished to participate in the study ocurred randomly. Any address or homestead in the community, and consequently all possible participants, were chosen at random by trained field workers doing door-to-door visits. As a way to ensure effective communication, fieldworkers who already formed part of these communities were involved as translators with the participant groups. A sample size of 2 000 or more participants was necessary for the PURE study, as determined by a power calculation published by Vorster (2002). There were 6 000 households approached during recruitment. The communities and households included were considered to be stable regarding movement in and out of the area.

3.3.3 Inclusion and exclusion criteria

The inclusion criteria were as follows: participants had to be healthy, over 30 years of age, and not use medication for any chronic medical condition (apart from blood pressure medication). Individuals did not receive any incentives for participating in the study and only those willing to participate were included. Excluded from the study were individuals who were not capable of being fully informed, or inebriated at the time of giving informed consent. Availability and willingness to take part in follow up data collection for the subsequent 10 years were also taken into consideration. The head of the households was approached first and had to approve the participation of the household members. If the head of the household refused to participate, there was a non-complier questionnaire completed, and the entire household was excluded. If the head of the household agreed to participate, the participants were fully informed and thereafter signed the informed consent form. Participants had to complete a questionnaire regarding their physical and psychological health, lifestyle, socio-economic status and background. The individuals received the dates and travel arrangements relating to the applicable data collection, and all the necessary information regarding their participation was provided beforehand. Participation in the data collection process was voluntary throughout the process. Upon initial consent, 3 750 participants completed the questionnaires and 2 010 formed part of the baseline data collection. Participants requiring any additional information had the opportunity to ask the researchers questions any time during the data collection process.

3.4 Anthropometric measurements

Anthropometric measurements took place in closed, separate rooms as a way of giving privacy to participating individuals. To ensure accuracy of all measurements, participants were asked to take their shoes off and only wear minimal clothing. There were standard procedures used,

anthropometric assessments performed in duplicate and the mean reported, in accordance with guidelines of the International Society for the Advancement of Kinanthropometry (ISAK) (Lohman *et al.*, 1988).

3.4.1 Height

A calibrated Invicta Stadiometer (IP 1465, Invicta, London, UK, Stadiometer) was used to measure height to the nearest 0.1 cm. Participants had to stand upright with their arms hanging freely at their sides, shoulders relaxed, legs straight and heels together. The participant's shoulder blades, buttocks and heels had to be in contact with the stadiometer. The participant's head was held in the Frankfurt position, where the lowest point of the eye orbit is in line with the tragion of the ear. The participant was asked to inhale fully and while maintaining this posture, the measurement was taken.

3.4.2 Weight

An electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) was used to measure the weight to the nearest 0.01 kg. To ensure accurate assessment of weight, participants were instructed to keep their arms freely at their sides. The scale was placed on an even, stable, horizontal floor and calibrated daily with standard weights.

3.4.3 Body Mass Index (BMI)

BMI is a measure used to normalise weight in relation to height, in order to compare individuals with diverse heights and is calculated using the following formulae:

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$

Table 3.1 shows the categories commonly used in nutritional epidemiology studies (WHO, 2000).

Table 3-1 BMI categories as stipulated by the WHO

Underweight	BMI ≤ 18.5 (kg/m^2)
Normal weight	BMI >18.5 (kg/m^2) and < 25 (kg/m^2)
Overweight	BMI ≥ 25 (kg/m^2) and < 30 (kg/m^2)
Obese class I	BMI ≥ 30 (kg/m^2)
Obese class II	BMI ≥ 35 (kg/m^2)
Obese class III	BMI ≥ 40 (kg/m^2)

3.4.4 Waist and hip circumference

A Holtain non-stretchable metal tape was used to measure the circumference of both the waist and hip. The waist circumference measurement, as outlined by the WHO (2011), was taken at the approximate midpoint in the middle of the lower margin of the last palpable rib and the top of the iliac crest. The hip's circumference was measured using the widest portion of the buttocks. Waist hip ratio (WHR) was then calculated as a division of waist circumference by the hip circumference (waist circumference/hip circumference). The WHO recommends that a healthy WHR should be 0.9 or less for men and 0.85 or less for women.

3.5 Blood pressure

Blood pressure i.e., brachial systolic and diastolic blood pressure (bSBP, bDBP) was measured using the Omron HEM-757 (Omron Healhcare, Kyoto, Japan), and was taken in duplicate within 10-minute intervals and the second value was used. Measurements were taken after being seated for 5 minutes.

3.6 Biochemical analysis

A registered nurse collected blood samples from the antecubital vein from patients who had fasted from 22:00 the previous evening, using sterilised winged infusion sets and syringes, which had disposable needles. The collection of blood samples occurred between 7:00 am and 11:00 am. The samples were preserved at -80 ^{o}C until analysis after being centrifuged at 2,000 x g for 15 minutes within 30 minutes of collection.

3.6.1 Inflammatory markers

The inflammatory markers were quantified using blood collected in tubes without anti-coagulant. The Konelab20i auto-analyser, by Thermo Fischer Scientific (Vantaa, Finland), was used to quantify both high sensitivity C-reactive protein (CRP) and albumin. Serum interleukin-6 (IL-6) concentration was measured through enzyme immunoassays (ELISA), using the Elecsys 2010 from Roche, Basel, Switzerland. Blood for homocysteine (Hcy) analysis was collected in ethylenediamine tetra acetic acid (EDTA) tubes and the Hcy concentrations measured using the Abbott automated immunoassay analyser (AxSYM). The analysis of citrated plasma for quantification of total fibrinogen was conducted and obtained through the modified Clauss method on the Dade Behring BCS coagulation analyser (Multifibrin U-test Dade Behring, Deerfield, IL, USA). Citrated plasma was also used for the measurement of plasminogen activator inhibitor-1 activity (PAI-1_{act}), using an indirect enzymatic method (Spectrolyze PAI-1; Trinity Biotech, Bray, Ireland).

3.6.2 Glucose and glycated haemoglobin (HbA1c)

Blood used to quantify glucose was collected in fluoride tubes. Plasma glucose was quantified by a Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA). HbA1c was determined from EDTA tubes with the D-10 Haemoglobin testing system (Biorad, Hercules, California, USA).

3.6.3 Blood lipids

The Konelab20i™ auto-analyser (Thermo Fischer Scientific, Vantaa, Finland), was used to quantify triglyceride, high-density lipoprotein and cholesterol from serum whilst the Friedewald–Levy–Fredrickson formula was used to calculate low-density lipoprotein cholesterol (Roberts, 1988). However, this equation was not used in the existence of chylomicrons, or when plasma triglyceride concentrations were above 400 mg/dL (4.52 mmol/L), nor in patients with dysbetalipoproteinaemia (type III hyperlipoproteinaemia).

3.7 Quantitative food frequency questionnaire

A country-specific, validated quantitative food frequency questionnaire (QFFQ) (MacIntyre *et al*., 2002) (Annexure B), was used to capture data regarding food consumption and dietary habits. Fieldworkers received training on how to administer the QFFQ and to quantify the participants' food intake. All the necessary equipment, be it food replicas, household measures, utensils and/or a food picture atlas, was provided to the fieldworkers to accurately estimate the food portion sizes. The fieldworkers had to ensure the questionnaire was completed and make corrections if necessary, on site, in the presence of the participants. The researchers, together with trained research assistants, were responsible for data coding and capturing. Coding of foods was in accordance with the Medical Research Council's (MRC) Food Composition Tables (FCT) (Wolmarans, 2010), and the amounts were recorded in grams, according to the Medical Research Council Food Quantities Manual. Standard tables were utilised for converting household measures of food to grams, as recommended by Langenhoven et al. (1991). The South African MRC Biostatistics Unit performed the food intake and nutrient analyses based on the South African FCT (Wolmarans, 2010).

It should be noted that a limitation of using a FCT is that it depends on the nutrient data available and could therefore, it any given time point, include missing data in two ways: 1) nutrient data being available for some but not all foods, e.g. β-carotene and fatty acids, or 2) in the form of nutrients not yet analysed, for instance flavonoid content of foods. This was the case for the MRC's FCT used in this study. FCTs are continually being updated to expand the nutrient coverage as well as to direct any fortification legislation that will modify the nutrient

content of the fortified food items; the FCTs initially employed to compute the PURE dietary intakes of 2005 have since been updated. Nevertheless, it was elected to use the original nutrient analyses instead of re-analysing the intakes with the latest FCT, with the intention of preventing incorrect information caused by any mandatory food fortification that came in effect after 2005.

It is essential to firstly establish the completeness of the nutrient data in the FCT prior to investigating dietary intakes. This process requires comprehensive preparation to ensure accurate nutrient intakes and shows the significance of detailed planning and careful selection of the FCT to be utilised when analysing the dietary intake of a population. This process of addressing missing data for B-carotene and fatty acids, as well as the non-availability of flavonoid data, formed part of the mini dissertation of another student (M. Ferreira) and will not be addressed in detail here. This mini dissertation will make use of the E-DII developed in M. Ferreira's MSc project, and will focus on its association with MetS.

3.8 Dietary Inflammatory Index

The study used the E-DII published by Shivappa *et al*. (2014), adapted to the PURE-SA-NW participants' diets. There was exclusion of some nutrients and/or food components from the analyses if they were not included in the original PURE-SA-NWU-2005 data set, as is often common practice when using the E-DII (Garcia-Arellano *et al.*, 2015; Hodge *et al.*, 2018b; Phillips *et al.*, 2018; Yang *et al.*, 2019). To curb the confounding effect that may be imposed on the outcome of the score by different energy intakes, the E-DII additionally adjusts all intakes to 4180 kJ. The following steps were undertaken before creating the E-DII for each participant:

- Initially all the data of under and over reporters were removed from the dataset by excluding participants who reportedly consumed fewer than 3000 kJ or more than 30 000 kJ

- A separate data sheet was generated to include only the foods in the E-DII list

- There was a calculation of the total amounts for tea, caffeine and onions consumed. The caffeine consumed by each participant was obtained by adding the total coffee intake, dividing it by 100, and multiplying the answer by 68, which is the amount of caffeine contained in 100 mL of the type of coffee commonly consumed by the PURE participants. Also included was caffeine in brewed black tea. The total intake for each participant was determined, divided by 100 and then multiplied by 20, considering that 100 mL of the type of tea consumed mostly by the PURE participants contained 20 mg of caffeine. To obtain the total caffeine intake, there was a summing of the the final answers resulting from the coffee and tea calculations.

• The total tea intake for each participant was obtained by adding the intake of both rooibos and brewed black tea.

• To calculate the onion intake for each participant, there was a determination of the weighted onion intake of each recipe. The total amount of a specific onion-containing food or meal consumed was divided by 100 and multiplied by the amount of onion (g) per 100 g of food, as seen in Table 3.2. Next, there was the summing of the different onion intakes to determine the total onion intake for each participant,

Table 3.2 Weighted onion intakes

Recipe	Ingredients	Amount of boiled onion (g)/100g
Spinach	Swiss Chard, cooked with potato, onion, sunflower oil	10
Green Beans	Cooked with potato, onion and sunflower oil	10
Carrot	Cooked with potato, onion and sunflower oil	10
Cabbage	Cooked with potato, onion and sunflower oil	10
Chicken	With skin, stew, tomato and onion	14
Fish	Casserole low fat fish, tomato and onion sauce	25
Tomato and onion	Tomato, onion, stewed with no sugar	25
Tomato and onion	Tomato, onion and stewed with sugar	25
Tomato and onion	Canned tomato and onion	25
Beef	Mince lean, savoury tomato, onion	25
Beef	Corned, savoury, potato, onion	15

After cleaning the data set and determining the consumed amounts for the specific foods, the population-specific E-DII was created.

The steps detailed below were followed to calculate the E-DII of the diet for the PURE-SA-NW study participants:

Step 1: The total energy consumed by each participant was normalised to 1000 kcal (4180 kJ). To do this, multiply the nutrient intake of each participant by 1000 kcal (4180 kJ) and divide it by the energy intake of the participant.

Step 2: Determine the normalised mean and standard deviation (SD) of nutrients and food components consumed by the PURE-SA-NW population.

Step 3: Standardise intakes of participants by subtracting the mean intake of the respective food components/nutrients of the PURE population from the individual's intake calculated in Step 2 and divide the difference by the SD of the PURE-SA-NW population.

Step 4: Step 3 provides a z-score that has to be converted into a central percentile score. To reduce the effect of right skewing, this z-score is multiplied by 2 and then 1 subtracted.

Step 5: Multiply each individual's standardised nutrient or food component intake (calculated in Step 4 by the appropriate score (Table 2.3).

Step 6: The energy-adjusted standardised dietary intake (Steps 1 to 4) of each food component or nutrient will be summed for each participant to obtain that individual's overall E-DII score. E-DII scores are positively correlated to inflammatory potential. The higher an individual's score, the higher the dietary inflammatory potential. Negative scores, conversely, indicate an anti-inflammatory diet.

There were some adaptations made to the original index based on recommendations by other researchers who have used the E-DII (Hodge *et al.*, 2018b; Yang *et al.*, 2019). These include the exclusion of total energy since the energy intake was normalised to 4180 kJ for all participants. Total fat was also excluded, as individual fat components, such as poly- and mono-unsaturated and saturated fat combined, provide the value of total fat consumed by an individual. Lastly, alcohol provides an anti-inflammatory effect only when there is less than 40 g/d consumed (Calder *et al.*, 2011); thus, alcohol consumption of more than 40 g/d was excluded, since this is no longer considered anti-inflammatory. Table 3.3 presents the final list of nutrients and food components included in the E-DII, considering the adaptations mentioned above and whether the nutrients/food components were a) consumed by the PURE-SA-NW participants, and b) depending on the availability of the data in the South African FCT.

Table 3.3 E-DII Nutrients and food components included in PURE 2005 data

Food or nutrient	Present in PURE 2005 Yes/No Total: 37
Alcohol (g)	Yes
Vitamin B12 (µg)	Yes
Vitamin B6 (mg)	Yes
b-Carotene (µg)	Yes
Caffeine (g) [a]	Yes
Carbohydrate (g)	Yes
Cholesterol (mg)	Yes
Energy (kcal) [1]	Yes
Eugenol (mg)	No
Total fat (g)	Yes
Fibre (g)	Yes
Folic acid (µg)	Yes
Garlic (g)	Not consumed
Ginger (g)	Not consumed
Fe (mg)	Yes
Mg (mg)	Yes
MUFA (g)	Yes
Niacin (mg)	Yes
n-3 Fatty acids (g) [b]	Yes
n-6 Fatty acids (g) [c]	Yes
Onion (g) [d]	Yes
Protein (g)	Yes
PUFA (g)	Yes
Riboflavin (mg)	Yes
Saffron (g)	No
Saturated fat (g)	Yes
Se (µg)	Yes
Thiamin (mg)	Yes
Trans fat (g)	Yes
Turmeric (mg)	No
Vitamin A (RE)	Yes
Vitamin C (mg)	Yes
Vitamin D (µg)	Yes
Vitamin E (mg)	Yes
Zn (mg)	Yes
Black tea / Rooibos (g) [e]	Yes
Flavan-3-ol (mg)	Yes
Flavones (mg)	Yes
Flavonols (mg)	Yes

Flavonones (mg)	Yes
Anthocyanidins (mg)	Yes
Isoflavones (mg)	Yes
Pepper (g)	No
Thyme/ Oregano (mg)	No
Rosemary (mg)	No

Score range: -7.65 (anti-inflammatory) to 6.67 (pro-inflammatory)

[a] Caffeine 68mg/100ml coffee; caffeine 20mg/100ml tea, brewed

[b] n-3 fatty acids compiled by summing C18_3, C18_4, C20_5, C22_5 and C22_6

[c] n-6 fatty acids compiled by summing C18_2 and C20_4

[d] compiled by adding individual intake of onion (boiled), onion (raw), onion (sautéed in sunflower oil), spinach (Swiss Chard, cooked with potato, onion, sunflower oil), Green beans (cooked with potato, onion and sunflower oil), carrot (cooked with potato, onion and sunflower oil), cabbage (cooked with potato, onion and sunflower oil), chicken (with skin, stew, tomato and onion), fish (casserole low-fat fish, tomato and onion sauce), tomato and onion (stewed no sugar), tomato and onion (stewed with sugar), tomato and onion (canned), beef (mince lean, savoury tomato, onion), beef (corned, savoury, potato, onion) and weighted according to the onion concentration in the recipe

[e] compiled by adding intake of both rooibos and black tea

[1] Energy was not included in the E-DII calculation as intakes were standardised to 4180 kJ

3.9 Metabolic syndrome classification

The diagnosis of the MetS was according to the harmonised definition (Alberti *et al.,* 2006). Three or more of the following five components define MetS, namely 1) waist circumference, 2) hypertension, 3) fasting triglyceride level, 4) HDL-C level and 5) fasting blood sugar. The IDF stressed the need to use different cut-off values for waist measurement in different ethnic groups, thus the waist circumference cut-off for African women was set at 80 cm and at 94 cm for African men. The cut-off points for the other components were as follows: systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or the use of antihypertensive treatment; fasting triglycerides level above 1.7 mmol/L; HDL-C levels below 1.0 mmol/L and 1.3 mmol/L for men and women, respectively and/or use of lipid lowering medicaiton, and fasting blood glucose above 5.6 mmol/L and/or use of hypoglycaemic agents (Alberti *et al.,* 2009).

3.10 Statistical Analyses

Statistical analyses were performed with SPSS® version 27 (IBM Corp, 2015) and Statistica® software version 13 (Statsoft Inc., Tulsa, OK, USA). Type-I error was set at 5% ($\alpha = 0.05$). Continuous variables were described using mean ± standard deviation or median and interquartile range based on their distribution, visualised using histograms and Q-Q plots. Data not normally distributed was log transformed to improve normality and used in further analyses. Counts and percentages were used for categorical variables.

Participants with a CRP >10 mg/L were excluded, as well as individuals using arthritis medication, as these might artificially obscure the contribution of the inflammatory potential of the diet to serum inflammatory markers' concentration.

Firstly, independent t-tests were used to compare data (E-DII food components and other study variables) between individuals, with or without MetS, and analysis of variance (ANOVA) when comparing data between the number of MetS components (0-5), and also when dividing the study participants into E-DII quartiles. Secondly, the demographic and lifestyle variables (gender, rural/urban status, age, alcohol consumption and smoking) were compared across the E-DII quartiles. Next the MetS (as categorical exposure) and its individual components (WC, blood pressure, triglycerides, HDL-C and fasting glucose, as continuous variables, with the exception of blood pressure which is represented as a categorical variable; hypertension) were compared across the E-DII quartiles. Analysis of covariance (ANCOVA), adjusted for demographic and lifestyle variables that differed across the E-DII quartiles, was performed to determine whether any differences observed in MetS components related to differences in the demographic profile between the E-DII quartiles or to the E-DII itself. All MetS and components analyses were additionally adjusted for anti-inflammatory drug and aspirin use. Marginal least squared means were derived by groups and compared following the Tukey-Kramer method to take into account groups of unequal sizes (Kramer, 1956). Visual inspection of the scatter plot of regression residuals and Levene's test evaluated the model assumptions of linearity and variance homogeneity.

CHAPTER 4 RESULTS

The main objective of this study was to compare the inflammatory index of the diet of PURE-SA-NW study participants diagnosed with the MetS with those without the MetS. The literature-based E-DII, as outlined by Shivappa et al.(2014), was utilised to calculate the inflammatory index of the PURE study participants' diets. The E-DII range of the total PURE participants was also compared to the theoretical E-DII range, in order to interpret the overall anti- or pro-inflammatory status of the diet of the participants.

This mini dissertation reports on data of 1315 of the original 2010 participants. Data for 695 participants were not included since there was exclusion of over- and under-reporters in terms of energy intakes, as explained in the Methods chapter, as well as individuals with a CRP ≥ 10 mg/L, individuals using arthritis medication and those with missing data, for whom we could not determine MetS status. Of the 1 315 participants included in the analysis, there were 199 (15%) diagnosed with the MetS and 1 116 (85%) did not have the MetS. These numbers are in agreement with the MetS prevalence in the total study cohort, where 16% (n=325) had MetS.

The mean E-DII of the PURE cohort was +0.37 ± 1.25, which points to a general pro-inflammatory diet. The minimum and maximum scores were -3.8 and +5.0 respectively. Since our E-DII comprised of 37 food components, this should provide, a theoretical range of -7.67 to +6.67. This study's data, therefore, indicates the PURE dietary intakes only extended over 61% of the theoretical range; the most pro-inflammatory diet was 74% of the theoretical maximum pro-inflammatory potential while the most anti-inflammatory index was only 50% of the theoretical maximum anti-inflammatory potential. Ginger, garlic, pepper, rosemary, oregano, eugenol, saffron, and turmeric were excluded from the original 45-component E-DII as these components were either not consumed by the PURE-SA-NW study participants or were not captured by the FFQ used.

Firstly, there was a comparison of the E-DII, its food components and other study variables between individuals with MetS and the controls using independent t-tests. Next, there was a comparison of the mean E-DII across groups created based on the number of MetS components (0-5) using analysis of variance (ANOVA). Following this, there was a comparison of the MetS and its individual components across the E-DII quartiles. Analysis of covariance (ANCOVA) was used to adjust for demographic and lifestyle variables (age, rural/urban status, gender, alcohol and smoking as well as anti-inflammatory medication use, aspirin use) that differed across the E-DII quartiles to determine whether any differences

observed in MetS components were associated with differences in the demographic profile between the E-DII itself or its quartiles.

Table 4-1: Comparison of the E-DII food components in diets of individuals with and without MS

Food components	MetS (n=199)	Control (n=1116)	p-value
E-DII	0.38 ± 1.31	0.40 ± 1.25	0.77
Pro-inflammatory			
Energy (kJ)	7513 (5439 ; 9992)	7233 (5528 ; 9917)	0.87
Carbohydrates (g)	267 (201 ; 337)	261 (202 ; 344)	0.36
Carbohydrates % of TE	60.4 (45.5 ; 76.3)	61.3 (47.5 ; 80.9)	0.81
Protein (g)	49.3 (36.6 ; 72.7)	49.4 (36.1 ; 70.6)	0.57
Protein % of TE	11.2 (8.3 ; 16.5)	11.6 (8.48 ; 16.6)	0.87
SFA (g)	11.5 (7.26 ; 17.7)	9.49 (6.03 ; 15.5)	0.03
Trans fat (g)	0.28 (0.08 ; 0.71)	0.17 (0.07 ; 0.49)	0.01
Cholesterol (g)	159 (93.0 ; 285)	145 (81.4 ; 250)	0.12
Iron (mg)	13.0 ± 5.56	13.8 ± 6.27	0.12
Vitamin B12 (µg)	2.72 (1.55 ; 4.89)	2.68 (1.24 ; 4.99)	0.95
Anti-inflammatory			
Alcohol (g)	0.00 (0.00 ; 10.8)	0.00 (0.00 ; 17.1)	0.01
Alcohol consumers *(n=70 and =513)*	11.5 (0.00 ; 30.9)	14.9 (1.93 ; 35.0)	0.01
Fibre (g)	21.6 ± 10.0	21.6 ± 9.92	1.00
Magnesium (mg)	283 (210 ; 372)	291 (212 ; 419)	0.14
Zinc (mg)	10.0 ± 4.5	10.2 ± 4.72	0.63
Selenium (µg)	21.8 (12.9 ; 36.5)	22.2 (12.7 ; 35.0)	0.56
Vitamin A (RE)	629 (357 ; 1021)	588 (342 ; 1021)	0.90
Thiamine (mg)	1.61 ± 0.71	1.72 ± 0.79	0.07

Food components	MetS (n=199)	Control (n=1116)	p-value
Riboflavin (mg)	1.23 ± 0.79	1.27 ± 0.79	0.57
Niacin (mg)	13.0 (9.54 ; 18.53)	12.9 (1.19 ; 18.6)	0.97
Vitamin B6 (mg)	1.47 ± 0.65	1.50 ± 0.79	0.59
Folate (µg)	349 (258 ; 472)	371 (273 ; 493)	0.32
MUFA (g)	13.0 (7.35;19.7)	10.4 (6.27;17.3)	0.01
PUFA (g)	14.5 (8.94 ; 20.3)	13.2 (8.43 ; 19.3)	0.23
Vitamin C (mg)	20.5 (11.5 ; 42.4)	17.4 (10.9 ; 35.1)	0.02
Vitamin D (µg)	2.10 (1.11 ; 3.40)	2.07 (1.10 ; 3.64)	0.29
Vitamin E (mg)	10.5 (6.36 ; 14.0)	9.74 (6.23 ; 14.2)	0.70
ω-3 PUFA (g)	0.42 ± 0.24	0.41 ± 0.28	0.62
ω-6 PUFA (g)	13.3 (8.19 ; 19.7)	11.9 (7.48 ; 18.0)	0.14
β Carotene (mcg)	1006 (406 ; 2051)	931 (277 ; 1957)	0.30
Anthocyanidins (mg)	0.11 (0.00 ; 0.61)	0.11 (0.00 ; 0.40)	0.003
Flavan-3-ol (mg)	95.7 (30.7 ; 228)	81.5 (17.8 ; 191)	0.004
Flavonones (mg)	0.82 (0.15 ; 12.2)	0.76 (0.15 ; 7.09)	0.05
Flavones (mg)	2.74 (0.68 ; 6.95)	2.87 (0.77 ; 6.37)	0.84
Flavonols (mg)	24.8 (10.0 ; 40.8)	18.0 (5.30 ; 38.4)	0.005
Isoflavones (mg)	0.03 ± 0.06	0.03 ± 0.05	0.11
Onion (g)	6.20 (0.00 ; 78.2)	20.4 (0.00;79.0)	0.38
Onion (g) consumers (n= 104 ; =625)	76.6 (43.2 ; 112.1)	72 (42 ; 106.1)	0.86

Food components	MetS (n=199)	Control (n=1116)	p-value
Caffeine (mg) consumers (n= 87; =523)	450 (300 ; 1260)	528 (308 ; 1428)	0.54
Tea (g)	0.00 (0.00 ; 1500)	0.00 (0.00 ; 1500)	0.59
Tea (mg) consumers (n= 74; =441)	2100 (1245 ; 3080)	1540 (1100 ; 2127.5)	0.50

SFA: saturated fat, MUFA: mono-unsaturated fat, PUFA: poly-unsaturated fat, ω-3 PUFA: omega-3 poly-unsaturated fat, ω-6 PUFA: omega-6 poly-unsaturated fat, β Carotene: beta-carotene

Normally distributed variables are indicated as mean value (standard deviation)

Not normally distributed variables are indicated as median (25 ; 75th percentiles)

Table 4.1 reports the E-DII and its components of both the MetS participants and the participants in the control group. The mean E-DII of the two groups were 0.38 ± 1.31 for the MetS group and 0.40 ± 1.25 for the control group, indicating both groups consumed, on average, a pro-inflammatory diet since the mean was above 0. There was no statistically significant difference between the mean E-DII of the MetS and control groups, possibly because the MetS participants consumed higher amounts of both pro- and anti-inflammatory components. While most of the pro-inflammatory components did not differ significantly between the two groups, the MetS participants consumed higher amounts of saturated fatty acids (SFA) (11.5 g vs 9.49 g) and trans fatty acids (0.28 g vs 0.17 g) than the controls. There was also no significant difference for most of the anti-inflammatory components between the two groups except for alcohol consumption, which was lower in the MetS group than in the control group (11.5 g/L vs 14.9 g/L). The consumption of mono-unsaturated fatty acids (MUFA) (13.0 g vs 10.4 g), vitamin C (20.5 mg vs 17.4 mg), anthocyanidins (0.11 mg vs 0.11 mg), flavan-3-ol (95.7 mg vs 81.5 mg), flavonones (0.82 mg vs 0.76 mg) and flavonols (24.8 mg vs 18.0 mg) were, conversely, higher in the MetS compared to the control group.

Table 4-2: Comparison of the study variables between individuals with and without MS

Study Variables	MetS (n=199)	Controls (n=1116)	p-value
Demographics			
Women n (%)	149 (74.9)	640 (57.3)	<0.0001
Urban n (%)	125 (62.8)	526 (47.1)	<0.0001
Alcohol use n (%)	70 (35.4)	513 (46.3)	0.004
Smokers n (%)	89 (45.2)	661 (59.5)	<0.0001
Alcohol & Smoker n (%)	54 (27.4)	428 (38.7)	0.002
Age (y)	53.1 (45.4 ; 61.3)	47.1 (41.3 ; 54.9)	<0.0001
HIV infected n (%)	26 (13.1%)	183 (16.5%)	0.23
Metabolic syndrome components			
Hypertensive status n (%) **Men** **Women**	181 (91.0) 49 (98) 132 (88.6)	501 (45.1) 224 (47.4) 277 (43.5)	<0.0001 <0.0001 <0.0001
Waist circumference (cm) **Men** **Women**	88.2 (82.5 ; 96.6) 89.2 (81.7 ; 96.2) 87.4 (82.6 ; 96.8)	74.3 (68.8 ; 81.9) 74.00 (69.7 ; 79.3) 74.9 (68.0 ; 84.2)	<0.0001 <0.0001 0.06
Fasting glucose (mmol/L) **Men** **Women**	5.50 (4.90 ; 6.20) 5.70 (5.00 ; 6.50) 5.45 (4.80 ; 6.10)	4.70 (4.20 ; 5.10) 4.70 (4.20 ; 5.20) 4.70 (4.30 ; 5.10)	<0.0001 <0.0001 <0.0001
HDL-C (mmol/L) **Men** **Women**	1.02 (0.83 ; 1.42) 0.99 (0.80 ; 1.18) 1.05 (0.84 ; 1.52)	1.55 (1.20 ; 2.00) 1.61 (1.20 ; 2.07) 1.50 (1.20 ; 1.96)	<0.0001 <0.0001 0.14

Study Variables	MetS (n=199)	Controls (n=1116)	p-value
TG (mmol/L)	2.00 (1.58 ; 2.58)	0.97 (0.76 ; 1.32)	<0.0001
Men	2.28 (1.74 ; 3.35)	0.95 (0.76 ; 1.32)	<0.0001
Women	1.96 (1.54 ; 2.42)	0.99 (0.76 ; 1.33)	<0.0001
Other factors			
BMI (kg/m^2)	28.0 (24.6 ; 33.0)	21.6 (18.9 ; 25.3)	<0.0001
HbA1c (%)	5.80 (5.50 ; 6.20)	5.50 (5.20 ; 5.70)	<0.0001
hsCRP (mg/L)	3.29 (1.43 ; 5.97)	1.86 (0.64 ; 4.12)	<0.0001
IL-6 (pg/mL)	3.06 (1.95 ; 4.99)	2.12 (0.75 ; 4.08)	<0.0001
tHcy (µmol/L)	9.37 (7.43 ; 12.50)	9.18 (7.46 ; 11.9)	0.31
PAI-1$_{act}$ (U/ml)	7.57 (4.03 ; 12.94)	3.58 (0.89 ; 6.60)	<0.0001
Fibrinogen (g/L)	2.90 (2.30 ; 4.50)	2.60 (2.10 ; 3.60)	0.01
Albumin (g/L)	44.9 (41.0 ; 61.1)	43.4 (39.8 ; 57.2)	0.003
TC (mmol/L)	5.35 (4.46 ; 6.64)	4.78 (4.03 ; 5.82)	<0.0001
LDL-C (mmol/L)	3.26 (2.49 ; 4.29)	2.72 (2.05 ; 3.52)	<0.0001

E-DII: energy-adjusted dietary inflammatory index, BMI: body mass index, HbA1c: glycated haemoglobin, hs-CRP: high-sensitivity C-reactive protein, IL-6: interleukin 6, tHcy: total homocysteine, TC: total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: triglyceride, PAI-1$_{act}$: Plasminogen activator inhibitor-1 activity

Normally distributed variables are indicated as mean value ± standard deviation

Not normally distributed variables are indicated as median (25;75th percentiles)

Table 4.2 reports the results of the analysis of the demographic variables between the MetS group and controls. The MetS group comprised of more women, almost 75% of the group, compared to the controls, which consisted of around 57% women. The participants in the MetS group lived mostly in urban areas (62.8%), with only 37.2% being rural dwellers. Individuals with the MetS were also older than the control group (53.1 years vs 47.1 years). Less alcohol consumption (35.4% vs 46.3%) and tobacco use (45.2% vs 59.5%) were observed in the MetS group than the control group. There was no significant difference observed between the participants with MetS and those without regarding HIV status.

Regarding the MetS components, as expected, these components were significantly higher (lower in the case of HDL-C) in the MetS group than the controls when both genders were included in the analysis. However, when separated by gender, the difference in WC between women with MetS and those in the control group, reached borderline significance only ($p = 0.06$), with no significant difference reported for HDL-C. In men, HT status, fasting glucose, WC and LDL-C were all significantly higher and HDL-C significantly lower in the MetS group than in the controls.

Regarding the other factors, BMI was higher in the MetS group than in the controls, which is in agreement with the higher WC observed. Similarly, HbA1c, in agreement with the higher blood glucose, was also higher in the MetS group. All inflammatory markers measured, namely hs-CRP (3.29 mg/L vs 1.86 mg/L), IL-6 (3.06 pg/mL vs 2.12 pg/mL), PAI-1_{act} (7.57 U/ml vs 3.58 U/ml), fibrinogen (2.90 g/L vs 2.60 g/L) and albumin (44.86 g/L vs 43.36 g/L), except homocysteine, were significantly higher in the MetS compared to the control group. TC (5.35 mmol/L vs 4.78 mmol/L) and LDL-C (3.26 mmol/L vs 2.72 mmol/L) were also higher in the MetS group than the controls.

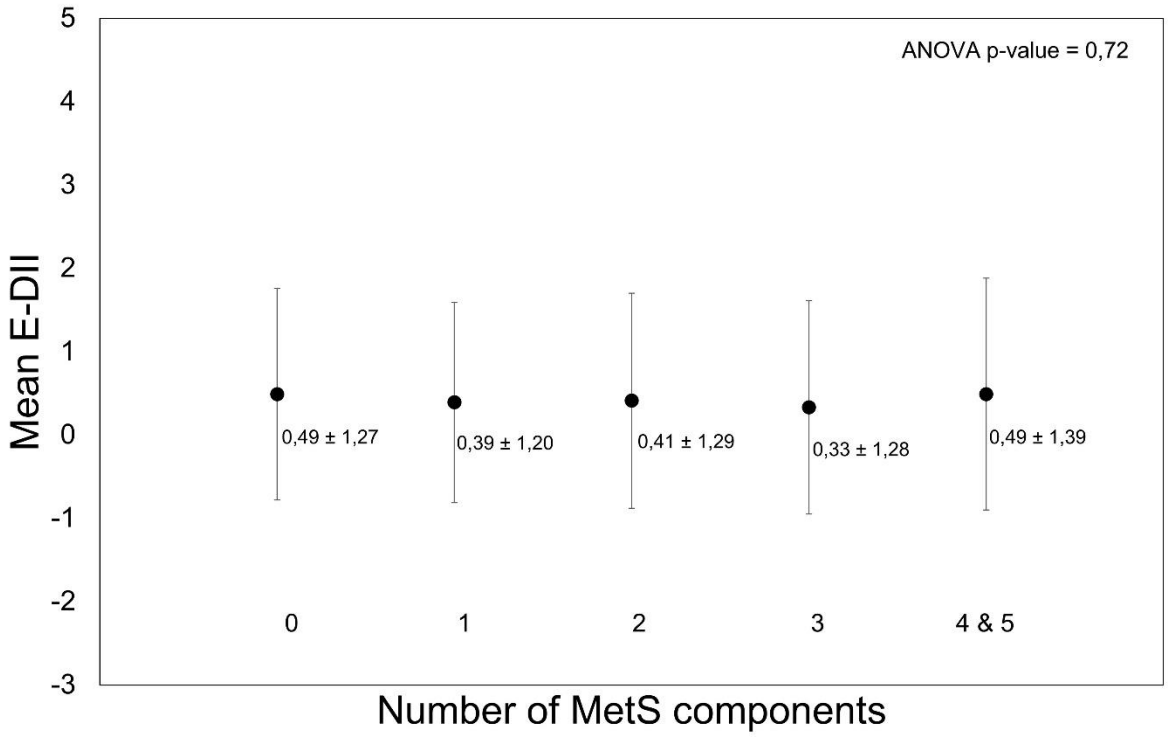

Figure 4-1: Mean E-DII across number of MetS components

Next, we investigated the mean E-DII between individuals stratified according to the number of MetS components, ranging from zero to those with four and five components combined due to the small number of individuals in the last two groups (4 components, n = 15 and 5 components, n = 3). The mean E-DII for individuals grouped according to the number of MetS components (0-5) were as follows: 0 = 0.49 ± 1.27; 1 = 0.39 ± 1.20; 2 = 0.41 ± 1.29; 3 = 0.33 ± 1.28; 4 and 5 = 0.49 ± 1.39. As illustrated in Figure 4.1, there was no significant difference in the mean E-DII between the individuals with differing number of MetS components.

Table 4-2 Demographics, MetS components and inflammatory markers across E-DII quartiles

E-DII quartiles	**1 (-3.70 to <-0.44)**	**2 (-0.44 to < 0.32)**	**3 (0.32 to < 1.17)**	**4 (1.17 to 5.08)**	**p-value ANOVA**	**p-value ANCOVA**
Demographics						
Age (y)	49.3 (43.2 ; 57.4)[a,b]	48.5 (42.4 ; 56.6)[c]	47.3 (41.1 ; 54.9)[a,c]	46.6 (41.3 ; 55.7)[b]	0.002	
Women n (%)	244 (67.8)	210 (58.3)	215 (59.7)	212 (58.9)	0.03	
Alcohol use n (%)	131 (36.6)	158 (44.3)	167 (46.6)	174 (48.7)	0.07	
Alcohol consumption (g/day)[#]	11.6 (5.04 ; 23.5)[a]	15.4 (7.40 ; 30.9)[b]	15.4 (5.71 ; 34.2)[a]	25.7 (11.4 ; 61.7)[a,b]	<0.0001	0.32*
Tobacco use n (%)	178 (49.9)	203 (56.9)	200 (55.9)	229 (64.0)	0.02	
Urban n (%)	203 (56.4)	214 (59.4)	174 (48.3)	144 (40.0)	<0.0001	
HIV infection n (%)	47 (13.2)	56 (15.8)	55 (15.4)	61 (16.9)	0.57	
MetS components						
Hypertensive status n (%)	192 (53.3)	211 (58.9)	179 (50.3)	183 (51.1)	0.09	
WC (cm)	77.3 (70.0 ; 85.3)	76.6 (69.7 ; 86.0)	76.5 (69.8 ; 84.8)	76.0 (69.8 ; 85.5)	0.99	0.83
Fasting glucose (mmol/L)	4.70 (4.30 ; 5.23)	4.80 (4.25 ; 5.30)	4.80 (4.30 ; 5.20)	4.80 (4.30 ; 5.30)	0.34	0.58
HDL-C (mmol/L)	1.50 (1.16 ; 1.98)	1.47 (1.10 ; 1.90)	1.47 (1.08 ; 1.92)	1.47 (1.12 ; 1.93)	0.45	0.38
TG (mmol/L)	1.09 (0.82 ; 1.57)	1.11 (0.82 ; 1.64)	1.01 (0.76 ; 1.40)	1.04 (0.80;1.49)	0.06	0.11
MetS n (%)	52 (15.8)	54 (16.7)	37 (11.3)	56 (16.6)	0.18	
Inflammatory markers						
hsCRP (mg/L)	2.09 (0.64 ; 5.24)	1.97 (0.62 ; 4.15)	2.08 (0.82 ; 4.49)	2.11 (0.74 ; 4.08)	0.76	0.21
IL-6 (pg/mL)	2.25 (0.75 ; 3.93)	2.05 (0.75 ; 4.36)	2.20 (0.75 ; 4.22)	2.54 (0.75 ; 4.56)	0.48	0.10
tHcy (µmol/L)	9.15 (7.51 ; 12.19)	9.26 (7.39 ; 12.13)	9.25 (7.37 ; 11.88)	9.21 (7.48 ; 12.26)	0.49	0.98
PAI-1_{act} (U/ml)	3.78 (1.28 ; 6.89)	4.44 (1.40 ; 7.59)	4.15 (1.32 ; 7.58)	3.95 (1.11 ; 8.01)	0.45	0.90
Fibrinogen (g/L)	2.60 (2.10 ; 3.90)	2.7 (2.20 ; 4.30)	2.60 (2.10 ; 3.70)	2.60 (2.00 ; 3.40)	0.16	0.22
Albumin (g/L)	44.58 (41.00 ; 59.55)	43.64 (39.63 ; 58.08)	43.31 (39.15 ; 57.07)	43.32 (40.00 ; 57.00)	0.11	0.05

E-DII: energy-adjusted dietary inflammatory index, WC: Waist circumference, HDL-C: High-density lipoprotein cholesterol, TG: triglyceride, MS: Metabolic syndrome

Normally distributed variables are indicated as mean value ±standard deviation

Not normally distributed variables are indicated as median (25; 75th percentiles)

[#] Mean intake of alcohol consumers only

Alcohol use * adjusted for: gender and age

MetS, components and Inflammatory markers ANCOVA – adjusted for: gender, rural/urban status, smoking, alcohol and age, anti-inflammatory medication use, aspirin use

[a,b,c,d] Means with the same symbol differ significantly between groups as shown by post-hoc tests

Table 4.3 provides the demographic characteristics, MetS components and inflammatory markers as compared across the E-DII quartiles. The first E-DII quartile represents a more anti-inflammatory profile at -3.70, whilst the fourth E-DII quartile is most pro-inflammatory. Individuals with the most anti-inflammatory profile (E-DII quartile 1) were mainly women (67.8%), older (49.3 years) and mostly residing in urban areas (56.4%), whilst individuals with the most pro-inflammatory profile (fourth E-DII quartile) were younger (46.6 years) and comprised of a larger proportion of rural dwellers (60%). The number of alcohol consumers was highest in the fourth quartile, although there was no significant difference across the quartiles. Alcohol consumption (g/d) was significantly higher in the fourth E-DII quartile than the other quartiles. After adjusting for gender and age, however, this difference was no longer significant, indicating the higher alcohol consumption in the fourth E-DII quartile is likely explained by this group consisting of proportionally younger-aged men. Sixty-four percent of individuals in the fourth E-DII quartile smoked, while only 49.9% in the first E-DII quartile smoked. There was no significant difference in HIV prevalence between the E-DII quartiles.

Regarding the MetS, no significant differences were observed between the E-DII quartiles for any of the MetS components or MetS itself, even after adjusting for gender, rural/urban status, smoking, alcohol use, age, anti-inflammatory medication and aspirin use. The number of participants with MetS was evenly distributed across the E-DII quartiles.

There was also no significant difference for any of the inflammatory markers investigated across the quartiles before or after adjustment for gender, rural/urban status, smoking, alcohol and age, anti-inflammatory medication and aspirin use; only for albumin was there a borderline significant decrease ($p = 0.05$) observed after full adjustment.

CHAPTER 5 DISCUSSION

5.1 Introduction

The prevalence of metabolic syndrome (MetS) and cardiovascular disease (CVD) has increased, with CVD continuing to be the leading cause of mortality worldwide (Aguilera-Méndez *et al.*, 2021). South Africa is not spared from this public health challenge (Peer *et al.*, 2015) owing to the country's nutritional transition (Gradidge & Crowther, 2017). The prevalence of MetS in South Africa is currently at 21.8%; (15.6% in men and 24.8% in women) (Seloka *et al.*, 2020). This has led to the advent of cardiometabolic diseases in Black South Africans, particularly women, which could be attributable to the adoption of sedentary lifestyle behaviours associated with this transition, whereby there is less physical activity and increased alcohol consumption and smoking (Peer *et al.*, 2015; Sekgala *et al.*, 2018). High consumption of unbalanced diets, mainly high in fat and sugar and low in fruit and vegetables (Gradidge & Crowther, 2017; Sekgala *et al.*, 2018; van Woudenbergh *et al.*, 2013) are major contributors to the development of insulin resistance, hyperinsulinaemia, central obesity, hypertension, elevated total cholesterol, low-density lipoprotien cholesterol (LDL-C), and triglycerides (TG) and a decrease in high-density lipoprotein cholesterol (HDL-C) (Sekgala *et al.*, 2018). Furthermore, the development and progression of several non-communicable diseases (NCDs), which include obesity, cancer, diabetes mellitus, CVD and MetS, have been credited to chronic systemic inflammation as reviewed by Namazi *et al.*, (2018). Diet is considered one of the modifiable factors involved in the inflammation development and related diseases (Ghorabi *et al.*, 2020). This has drawn significant attention to the pro- and anti-inflammatory properties of nutrients and foods. Recently it was confirmed that the composition of the total diet is more significant in the risk prediction of chronic diseases and mortality than the consumption of specific nutrients, considering that the final effect of a certain dietary component on inflammatory responses and health outcomes can be modified by interactions among nutrients (Wang *et al.*, 2021). The present study investigated whether the inflammatory potential of the diet (measured as E-DII) is associated with the prevalence of MetS in 1 315 Black South Africans who participated in the Prospective Urban and Rural Epidemiology (PURE) study. This study is, to our knowledge, the first of its kind in a Black South African population.

The objectives of the study were:

- To calculate and describe the inflammatory index of the PURE-SA-NW participants' diet, making use of the E-DII.
- To determine the prevalence of MetS in the PURE-SA-NW participants in 2005.

- To determine the relationship between the E-DII and MetS in the PURE-SA-NW study in 2005.

This chapter presents an interpretation of the main findings of this study and discusses how it contributes to the current literature.

5.2 E-DII and MetS in the PURE-SA-NW participants

The mean E-DII of the PURE population was +0.37 ± 1.25, indicating the overall pro-inflammatory characteristic of their diet. The minimum and maximum scores were -3.8 and +5.0 respectively. The E-DII used in this study comprised of 37 food components, thus providing a theoretical range of -7.67 to +6.67. The data shows that the PURE dietary intake only spanned 61% of the theoretical range; the most pro-inflammatory diet being 74% of the theoretical maximum pro-inflammatory potential while the most anti-inflammatory index was only 50% of the theoretical maximum anti-inflammatory potential. Some foods may not be easily available or even affordable for the participants to access sufficient safe and nutritious food that meets the dietary needs and requirements for a healthy life (Hardin-Fanning *et al.*, 2019), especially those residing in rural areas thus limiting their choice of food. Gradidge and Crowther (2017) deduced that African populations do not consume sufficient amounts of fruit and vegetables, and when comparing rural Black South African women to urban-dwelling women, they suggested that the latter consume more total fat, saturated fat, and sugar in their diet. This indicates a relatively limited diet that tends to be more pro- than anti-inflammatory.

Fifteen percent of the participants were diagnosed with the MetS in our study; this is slightly lower than the 21% obtained by Owolabi and associates in a study conducted in the Eastern Cape in 2017 (Owolabi *et al.*, 2017). Increased age, ethnic and/or genetic predisposition, environmental and lifestyle factors, such as diet and and an inactive lifestyles, and elevated body mass index (BMI) are all associated with increased prevalence of MetS (Nikniaz *et al.*, 2018). The prevalence of MetS in this population does not come as a surprise, since the components of MetS, especially obesity, hypertension and high fasting glucose, have become highly prevalent in Black South Africans (Peer *et al.*, 2015). The majority of those with MetS were women (74.9%) and individuals residing in urban areas (62.8%), as presented in Table 4.2. Additionally, alcohol was consumed by only 35.4% of those with MetS, while 46.3% of individuals without MetS consumed alcohol. Of those who consumed alcohol, the mean intake in the MetS individuals was less than the mean intake in the control group (11.5 g/day vs 14.9 g/day) suggesting lower alcohol consumption in individuals with MetS than without.

5.3 Relationship between E-DII and MetS and its components

5.3.1 MetS and E-DII

According to our results, both the MetS group and the control group presented with a generally pro-inflammatory diet, with no difference in mean E-DII between the two groups. This similarity could be attributable to the fact the two groups were of the same ethnicity, and their food patterns and/or diets are the same. The lack of association between the E-DII and MetS in this study is not an anomaly as some previous studies (employing either the E-DII or the DII) have yielded similar outcomes in different studies and settings. For example, the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) study is a cross-sectional study conducted among 464 police officers, with a mean age of 42.8 ± 8.5 years, doing shift work (Wirth *et al.*, 2014). There was no statistically significant association found between MetS and DII, and dysglycaemia was the only MetS component associated with the DII (Wirth *et al.*, 2014). Another study, which aimed to examine the association between the DII and MetS and its components, made use of data from the Observation of Cardiovascular Risk factors in a study in Luxembourg (ORISCAV-LUX) involving 1 352 healthy adults (Alkerwi *et al.*, 2014). They also did not find a noteworthy relationship between the DII and MetS and its components, but noted that participants with higher DII scores were significantly younger and had higher BMI, waist circumference and systolic blood pressure levels (Alkerwi *et al.*, 2014). They also observed that participants with pro-inflammatory DII scores had increased odds of having low HDL-C compared to those with anti-inflammatory scores (Alkerwi *et al.*, 2014). The Chinese Urban Adults Diet and Health study (CUADHS), a cross-sectional study wherein they explored the association between the DII, the inflammatory marker, C-reactive protein (CRP), and MetS in 1 712 Chinese urban adults (Ren *et al.*, 2018), also found no significant relationship between the MetS and DII, and of the MetS components, only blood pressure associated with the DII (Ren *et al.*, 2018).

Conversely, there are a number of studies that did find a positive association between the DII and Mets. A cross-sectional study conducted in urban Ecuadorian women, by Wang *et al.* (2021), found an association between the E-DII and MetS. Another cross-sectional study, by Abdollahzad *et al.* (2020), also found a significant association between DII and MetS in 6 538 apparently healthy Iranians. The longitudinal study, Supplementation on Vitamins and Minerals AntioXidant (SU.VI.MAX), also found a positive association between DII and MetS (Neufcourt *et al.*, 2015a). In a meta-analysis conducted recently by Yi *et al.* (2021), they found an overall positive association between higher DII score and MetS with an OR of 1.23 (95% CI 1.10,1.37). The different findings between studies that found an association and those that did not, may be due to the varying sample sizes, study designs, different food parameters

used for DII calculation, different criteria used for definition of MetS or different tools used for dietary intakes assessment such as the specific foods listed in the dietary assessment tools like the FFQ (Ghorabi *et al.*, 2020).

Although the diet of the participants in the two groups in this study had a similar mean E-DII, the MetS group consumed more anti-inflammatory food components, such as mono-unsaturated fatty acids (MUFA), vitamin C, anthocyanidins, flavon-3-ol, flavanones and flavonols. They also had a lower alcohol consumption, which has been related to lower levels of inflammation (Corley *et al.*, 2019). However, the MetS group also consumed higher amounts of pro-inflammatory components, saturated fatty acids (SFA) and trans fats, which are considered unhealthy fats and are associated with increased risk of high TGs, low HDL-C, larger waist circumference (WC) and high fasting blood glucose (Hardin-Fanning *et al.*, 2019; Zhang *et al.*, 2017). It seems that although the MetS group consumed more anti-inflammatory foods, the beneficial effects thereof were cancelled by the concomitant consumption of higher amounts of pro-inflammatory fat intakes. Since the E-DII is similar between the MetS group and controls, the inflammation leading to MetS development in this population is likely not diet-induced, as discussed below.

The mean E-DII did not differ across the number of MetS component categories, as shown in Figure 4.1. A number of studies have looked at the association between the number of MetS components and the DII, and have reported varied outcomes. One such study is the cross sectional study by Ghorabi *et al.* (2020), which studied the association of DII with MetS and its components among 404 Iranian adults, and they reported only an association between higher DII score with lower levels of HDL-C, contrary to the review by Yi *et al.* (2021), who reported no relationship between the DII and HDL-C. Imran *et al.* (2020) utilised data from 3 507 participants from the Korean Genome and Epidemiological Studies Health Examination cohort (KoGES_HEXA), to evaluate the relationship between DII and the risk of MetS and its components; all five of the MetS components were positively associated with higher DII scores in women, whilst only three, being low HDL-C, elevated WC and high blood pressure, were positively related to higher DII scores in men (Imran *et al.*, 2020). Another study, conducted among Mexican adults, observed positive associations between the DII and abdominal obesity, hypertriglyceridemia and hypertension (Canto-Osorio *et al.*, 2020). This is in agreement with the meta-analysis by Yi *et al.* (2021), which also reported associations with all the components of MetS except with decreased HDL-C.

5.3.2 Individual MetS components and E-DII

Although modification of diet and lifestyle is proposed as the first factor to prevent and treat MetS, the evidence remains inconclusive whether the improvement is in a specific component

or in overall MetS (Sajjanar & Nimbal, 2019). The individual MetS components, which include hypertension, central obesity, measured as WC, dyslipidaemia (elevated TG and reduced HDL-C) and increased fasting blood glucose (Namazi *et al.*, 2018; Sajjanar & Nimbal, 2019), and their association with the inflammatory potential of the diet will now be discussed.

There was no noteworthy variation in any of the MetS components across the E-DII quartiles of this study population. This lack of association may be attributable to the monotonous diet of the PURE population and lack of variety in terms of their dietary inflammatory potential. The mean E-DII of the PURE cohort spanned mainly over the middle of the theoretical E-DII range, therefore it was neither strongly pro- or anti-inflammatory. Additionally, the E-DII range of the PURE participants spanned only 61% of the theoretical range, indicating the study participants consumed a comparatively monotonous diet, most of the diets being only modestly pro- or anti-inflammatory. While a lack of association between the E-DII and the different MetS criteria has also been observed in a small number of other studies, it is generally in contrast with most of the available literature.

5.3.2.1 Central obesity

There was no significant difference in WC across the E-DII quartiles in this study population, even after adjusting for sex, rural/urban status, smoking, alcohol, age, anti-inflammatory medication, and aspirin use. This study's findings coincide with those of Naja *et al.* (2017), who also did not find any significant associations between the DII and any of the MetS components, including WC or body mass index (BMI). Even so, Ruiz-canella *et al.* (2016) suggests that a pro-inflammatory diet is associated with higher indices of central and abdominal obesity as they observed consumption of a higher pro-inflammatory diet in participants with higher BMI, waist circumference and WtHR (Ruiz- Canella *et al*., 2016). Similarly, a significant and positive correlation was observed between the DII and weight, BMI and % body fat by Alam *et al*. (2018). These findings suggest that adherence to high DII scores is independently associated with obesity (Varkaneh *et al.*, 2018). Conversely, Oliveira *et al.* (2020) found a clear relationship between E-DII and obesity, as participants in the most pro-inflammatory E-DII quartile had higher BMI and prevalence of overweight and obesity compared to those in the most anti-inflammatory E-DII quartile. Canto-Osorio and associates also linked more pro-inflammatory diets to a larger waist size in their cohort of apparently healthy Mexican adults (Canto-Osorio *et al.*, 2020; Tan *et al.*, 2021). A positive association between E-DII scores and WC was also found by Wang *et al.* (2021), wherein they examined the relationship between the E-DII and MetS and cardiometabolic risk in 276 Ecuadorian urban adult women. Central obesity is associated with a variety of metabolic disorders, such as insulin resistance, hypertension and dyslipidaemia, and is at the core of the risk of MetS

development (Tan *et al.*, 2021). It is suggested that while obesity may not be the primary cause of MetS, it may be a marker of the underlying metabolic dysfunction that potentially drives its development (Després & Lemieux, 2006; Weiss *et al.*, 2013). Furthermore, adipose tissue from obese individuals has been shown to be permeated by large numbers of macrophages, referred to as adipose tissue macrophages, which is a major source of pro-inflammatory cytokines (Shin *et al.*, 2019a), and which contributes to MetS as a pro-inflammatory state.

Cornier *et al.* (2008) further acknowledge that genetic factors may also be at play in determining the association between central obesity and MetS, and Nagrani *et al.* (2020) attest to this, saying that genetic inclination towards MetS is mainly attributed to genes of obesity and lipid metabolism, with numerous single nucleotide polymorphisms (SNPs) associated with individual metabolic components having been studied and reported in genome-wide association studies (GWAS). Alsulami *et al.* (2021) has since investigated the interaction of 10 metabolic disease-related SNPs, combined in a genetic risk score (GRS), with dietary intake on metabolic traits in Brazilian young adults. They, however, reported no significant associations between the GRS and metabolic disease-related traits except for an association with BMI (Alsulami *et al.*, 2021).

5.3.2.2 Fasting Blood Glucose

Fasting blood glucose did not differ significantly across the E-DII quartiles, suggesting the inflammatory potential of the diet did not relate to altered fasting blood glucose. This is consistent with the findings of the SU. VI. MAX cohort study, conducted in a French population aged 35 to 60 years over a period of 12 years, that did not find any association between the DII and fasting blood glucose (Neufcourt *et al.*, 2015a). Conversely, in a meta-analysis on E-DII and fasting blood glucose and hypertension, conducted by Farhangi *et al.* (2020), they reported that of the 21 manuscripts on hyperglycaemia, 14 reported a positive association, whilst six found no significant association, and only one reported an inverse association. Obesity and abdominal fat deposition trigger numerous metabolic abnormalities that bring about increased hepatic glucose production and reduced insulin sensitivity in adipose and liver tissue as well as skeletal muscle (Frankenberg *et al.*, 2017). Build-up of visceral fat decreases production of adiponectin, thus some studies have shown that decreased adiponectin levels were linked to increased levels of MetS components, including fasting glucose (Frankenberg *et al.*, 2017). Tissue inflammation upturns levels of C-reactive protein (CRP) and inflammatory cytokines (tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)) activating hepatic gluconeogenesis, further reducing insulin sensitivity in muscle and liver, resulting in increased circulating glucose levels (Farhangi *et al.*, 2020; Frankenberg *et al.*, 2017). Possibly, the lack

of difference in degree of obesity and inflammation across the E-DII quartiles might explain why there was also no difference in fasting glucose.

5.3.2.3 Dyslipidaemia

The two foremost types of dyslipidaemia related to MetS are hypertriglyceridemia and decreased HDL-C (Okafor, 2012). In this study, there was no significant association between E-DII and HDL-C or TG levels, as presented in Table 4.3. This is contrary to the findings of a Luxembourg study, where they investigated the relationship between DII and MetS, as well as its components in 1 352 participants of European descent using data from the Observation of Cardiovascular Risk Factors study (ORISCAV) (Alkerwi *et al.*, 2014). They reported a noteworthy inverse association between the DII and HDL-C levels (Alkerwi *et al.*, 2014), similar to the findings of an Iranian study by Ariya *et al.* (2020), which showed HDL-C had a significant inverse association with E-DII in both men and women, but that TG had a significant relationship with E-DII in men only. Wang *et al.* (2021) also found an association between the E-DII and TG but not with HDL-C in Ecuadorian women. An increase in the inflammatory potential of the diet may increase the risk of MetS by affecting serum concentrations of HDL (Ghorabi *et al.*, 2020), in which not only are HDL levels altered, but also its metabolism and distribution pattern, leading to dysfunction of HDL particles (Stadler & Marsche, 2020). The mechanism for decreased HDL concentration is multifactorial and includes both the heightened uptake of HDL_2 (a sub-population of HDL which is less dense, and cholesterol- and phospholipid-enriched) by adipocytes and an upsurge in the catabolism of apolipoprotein A-1 on HDL particles (Rashid & Genest, 2007), which is also linked to higher blood TGs. In an insulin resistant state, hepatic TG synthesis is driven by an increase in the inflow of free fatty acids from outside the liver, which in turn promotes the assembly and secretion of TG-containing very low density lipoprotein (VLDL) and elevates TG levels (Kumar *et al.*, 2013).

5.3.2.4 Hypertensive status

There was no statistically significant difference in the number of hypertensive participants across the E-DII quartiles. From this we deduce that being hypertensive is likely not related to the inflammatory potential of the diet in our study population. This is inconsistent with the findings of the SU.VI.MAX study (Neufcourt *et al.*, 2015a) and the study by Ariya *et al.* (2020), where they found a positive association between high E-DII scores and blood pressure (Neufcourt *et al.*, 2015a). Inflammation, an underlying component of MetS, is believed to negatively affect the blood vessels and the kidneys, thereby increasing blood pressure (Ariya *et al.*, 2020). Inflammatory adipocytokines, insulin and atherogenic dyslipidaemia directly act on the endothelial cells, monocytes and platelets to heighten their collaboration, thereby promoting atherosclerosis and ultimately plaque rupture and thrombosis (Kumar *et al.*, 2013).

Lv *et al.* (2018) posits that inflammation assist in renal fibrosis progression; the recruitment of fibrogenic cells and leukocytes to the glomerulus and renal interstitium, where combined with activation of resident kidney immune cells, they lead to augmented pro-inflammatory cytokine production. Furthermore, inflammatory cytokines are effective inducers of hypertension by way of disrupting the renin-angiotensin system, causing vascular inflammation and reducing nitric oxide (NO) production (Farhangi *et al.*, 2020). The lack of association between the E-DII and blood pressure in this study population could be due to the fact that Black South Africans are generally prone to having high blood pressure, regardless of dietary intake (Rayner, 2010). This could be due to genetic or environmental factors, such as lifestyle, as alluded to by Ariya *et al.* (2020). In a 5-year prospective study conducted in Black South Africans, Schutte *et al.* (2012) suggested that behavioural and conventional risk factors, such as increased *y*-glutamyltransferase, which is an indication of alcohol misuse and obesity, are the strongest contributors of hypertension development in Black South African communities. As obesity and inflammatory markers did not differ between the E-DII quartiles, the possilble effect of the inflammatory potential of the diet on HT in this study population becomes negligible compared to effects of other risk factors.

5.4 Relationship between inflammation, MetS and E-DII

Although unhealthy diets can be linked to MetS and other NCDs, at least in part, through diet-induced inflammation, we did not observe an association between the E-DII and serum inflammatory markers in the current study. Most of the inflammatory markers were, however, higher in individuals with MetS than those without. Also noteworthy, while the MetS group consumed more SFA and trans fats (pro-inflammatory), they also consumed more MUFA, vitamin C, anthocyanidins, flavan-3-ol, flavonones and flavonols (all anti-inflammatory), which likely balanced the effects of the pro-inflammatory components. Results of several studies show that high consumption of olive oil, whole grains, vegetables, fruit, and fish accompanied by low consumption of red meat and butter and low to moderate intake of wine (i.e. Mediterranean dietary pattern) are associated with reduced levels of inflammatory markers and are negatively associated with components of MetS (Aslani *et al.*, 2020; Giugliano *et al.*, 2006). Conversely, the Western dietary pattern, which is typically high in red meat, refined grains, and high fat products, correlates with elevated levels of inflammatory biomarkers, and thus inflammation (Aslani *et al.*, 2020). While inflammation is clearly implicated in MetS pathophysiology, and there is ample evidence that dietary intakes can mediate inflammation, the dietary inflammatory potential of the PURE-SA-NW participants was not associated with inflammatory markers in this study and thus does not seem to contribute to the increased inflammation observed in the individuals with MetS.

Chronic inflammation plays a pivotal role in the development and progression of cardiometabolic diseases owing to the exposures that fuel unbalanced production of reactive oxygen species (ROS), and generate pro-inflammatory cytokines, adipokines and acute phase proteins (Wang *et al.*, 2021). These accumulate over time, thus leading to metabolic disorders, MetS and cardiometabolic diseases. Circulating markers of inflammation, such as CRP, some interleukins (IL-6, IL-18) and TNF-α (Giugliano *et al.*, 2006) are significantly associated with metabolic abnormality (Syauqy *et al.*, 2018). CRP and IL-6 have previously been studied as pro-inflammatory markers associated with DII and cardiometabolic diseases because of their roles in the development of MetS (Cohen *et al.*, 2021; Wang *et al.*, 2021; Zuliani *et al.*, 2009). Increased levels of CRP are associated with the future incidence of MetS and its components (Espinola-Klein *et al.*, 2011), and is thought to play a role in MetS by impairing insulin signalling and promoting atherothrombosis via endothelial dysfunction (Reddy *et al.*, 2019). In addition, inflammatory markers have been associated with the individual components of the MetS (Syauqy *et al.,* 2018; Reddy *et al.,* 2019), as well as the number of MetS components (Espinola-Klein *et al.*, 2011).

Table 4.3 presents the only inflammatory marker associated with the E-DII in this study - albumin, with a borderline significant (p=0.05) negative association after full adjustment. According to Menon *et al.* (2005), serum albumin levels may partly reflect the role of chronic low-grade inflammation in inducing malnutrition through appetite suppression and enhanced protein catabolism by pro-inflammatory cytokines, thus lower serum albumin is regarded as an indicator of longer term malnutrition, inflammation and liver disease (Cho *et al.*, 2012). The inverse association between the E-DII and albumin in this study population could therefore suggest the E-DII is more likely associated with chronic, low-grade, rather than acute inflammation, since the other measured inflammatory markers (CRP, IL-6, PAI-1 and fibrinogen) are often used to depict an acute inflammatory state, which explains why these markers did not demonstrate a significant relationship with the E-DII.

We have previously demonstrated that the PURE-NW-SA cohort has elevated acute inflammatory marker levels (as shown in Table 3: median IL-6 of 2.84pg/mL and CRP of 3.31mg/L). Myburgh *et al.* (2018) recognised several factors, other than diet, that are specifically associated with CRP and include age, genetics, socio-economic status, and infectious diseases, that likely dilute the involvement of the inflammatory potential of the diet to inflammatory markers. Sex may be another contributing factor. The mechanism through which women may have higher CRP levels than men is possibly due to the higher secretion of oestrogen in women that may play a role in the cause of inflammation, thereby leading to a more pronounced effect on CRP levels (Cohen *et al.*, 2021). Sproston and Ashworth (2018) suggest that combining estrogen and oxidised low-density lipoproteins (oxLDLs) increases

CRP expression in postmenopausal women and older men. In this population, women had higher levels of total body and visceral fat than men. Visceral fat produces more pro-inflammatory cytokines than subcutaneous fat, hence increasing inflammatory markers (Cohen *et al.*, 2021; Nari *et al.*, 2020). Chronic smoking is further known to increase inflammation through inducing the release of pro-inflammatory markers, such as acute phase proteins and IL-6, as well as reducing anti-inflammatory cytokine production (Corley *et al.*, 2019). The observation was that cigarette smoking plays a role in the progression of CVD by increasing endothelial dysfunction, thrombosis, and inflammation, as well as reducing oxygen supply (Campbell *et al.*, 2015)

5.5 Strengths and Limitations

This study's strengths include the use of a variety of biomarkers, and the use of 37 out of 45 food parameters for calculating the E-DII, which is a fairly good representation of the DII score. A limitation to this study is that by virtue of it being a cross-sectional design study, causality could not be determined. Information on diet may have been exposed to recall bias considering the tool used for data collection in this study. In spite of all precautions during data collection, there always remained the scope for intentional fabrication by the respondents on particular information pertaining to the diet; this ultimately affects the accuracy of the E-DII. Potential residual confounding of variables not measured in this study can also not be excluded.

5.6 Conclusion

The findings of this study suggest MetS may be influenced by factors other than the inflammatory potential of the diet and it wass difficult to see a clear difference in such a monotonous diet, as there was no relationship found between the inflammatory index of the diet and MetS diagnosis or number of MetS criteria. It is likely that in the African setting, in the context or urbanisation and general limited dietary intakes, other lifestyle factors play a more prominent role in the development of MetS in Black South Africans. While further studies are needed to substantiate the inflammatory role of the diet in the development of MetS in Black South Africans, the adoption and maintenance of a healthy lifestyle is still important to reduce chronic disease risk.

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ANNEXURE A: ETHICS CERTIFICATE

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: http://www.nwu.ac.za/

North-West University Health Research Ethics Committee (NWU-HREC)

Tel: 018 299-1206
Email: Ethics-HRECApply@nwu.ac.za (for human studies)

12 April 2021

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Health Research Ethics Committee (NWU-HREC) on 12/04/2021, the NWU-HREC hereby approves your study as indicated below. This implies that the NWU-HREC grants its permission that, provided the general 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: The relationship between the inflammatory potential of the diet and metabolic syndrome in Black South Africans
Principal Investigator/Study Supervisor/Researcher: Prof M Pieters
Student: K Tshiamiso - 31460488

Ethics number:

N	W	U	-	0	0	1	6	2	-	2	1	-	A	1
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 12/04/2021
Expiry date: 30/04/2022

Risk: **Minimal**

Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of April annually until completion.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-HREC:*
  - *Annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and*
  - *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.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU-HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for active monitoring.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-HREC reserves 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 NWU-HREC or that information has been false or misrepresented;*
  - *submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
  - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-HREC can be contacted for further information via Ethics-HRECApply@nwu.ac.za or 018 299 1206*

Special conditions of the research approval due to the COVID-19 pandemic:

Please note: Due to the nature of the study i.e. (statistical analysis of previously collected data from the PURE-NW study), this study will be able to proceed during the current alert level, following receipt of the approval letter. No additional COVID-19 restrictions have been placed on the study except that the researcher must ensure that before proceeding with the study that all research team members have reviewed the North- West University COVID-19 Occupational Health and Safety Standard Operating Procedure.

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

Yours sincerely,

Digitally signed by
Prof Petra Bester
Date: 2021.04.14
13:43:03 +02'00'

Chairperson NWU-HREC

Current details:(23239522) G:\My Drive\9. Research and Postgraduate Education\9.1.5.4 Templates\9.1.5.4.2_NWU-HREC_EAL.docm
20 August 2019
File Reference: 9.1.5.4.2

ANNEXURE B: PARTICIPANT INFORMED CONSENT FORM

YUNIBESITI 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**

YUNIBESITI YA BOKONE-BOPHIRIMA
NORTH WEST UNIVERSITY
NOORDWES UNIVERSITEIT

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)

ANNEXURE C: QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

PURE Quantitative Food Frequency Questionnaire

Subject ID **Subject Initials**

☐☐ ☐☐☐ ☐☐☐ ☐☐ ☐☐☐

Centre # Community # Household # Subject # *F M L*

Today's date: ☐☐☐☐ ☐☐ ☐☐

year month day

1. Name: _____ _____

2. Not applicable in South Africa

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

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** **Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will go through a list of foods and drinks with you and I would like you to tell me:

- If you eat the food
- 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. Please say which picture is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

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?

FOOD FREQUENCY QUESTIONNAIRE

1 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 few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____							
Do you pour milk on your porridge or cereal? Ye **1** No **2** If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you put sugar on your porridge or cereal?		Yes 1		No 2				
If yes, how much sugar							3989	
							3989	
							3989	
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)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	
	Bought: Specify topping _____ _____						3353 (base+ch)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
You are being very helpful. Can I now ask you about meat? **<u>CHICKEN, MEAT, FISH</u>** **How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?** _____								
Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							
Do you eat chicken skin?	Always **1**		Sometimes **2**		Never **3**			
Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____ ___								
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit **<u>VEGETABLES AND FRUIT</u>**								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat ……...							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……...							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat ……							
	Boiled, little sugar and fat Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat ……							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat ……							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you like fruit?		Ye 1	No 2					
Apples							3592	
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	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit?		Ye 1	No 2					
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
<u>BREAD AND BREAD SPREADS</u>								
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you spread anything on the bread?		Always 1	Sometimes 2	Never 3				
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			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						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks **Coke, fanta, etc**	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							
<u>SNACKS AND SWEETS</u>								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	_____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____ _____							
Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____ _____							
Jelly							3983	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
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**								
Specify								

Thank you very much for your cooperation and patience.

Good-bye!

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