

Association of the inflammatory index of the diet with fibrinogen concentration and fibrin clot properties

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ABSTRACT

Introduction and aim:

Cardiovascular disease (CVD), morbidity and mortality are increasing significantly in South Africa. Inflammation is known to play an integral part in CVD. Fibrinogen, an acute-phase protein, is a well-recognised risk marker for CVD and inflammation significantly up-regulates its expression. Fibrinogen concentration is also a main determinant of blood clot structure, which plays an important role in CVD event outcome. Increased fibrinogen concentration results in a denser clot with increased stability that is difficult to lyse. Several studies have shown that diet and inflammation are closely linked, and therefore, dietary inflammatory indices (DII) have been developed with the aim of determining the inflammatory potential of an individual's diet. These dietary inflammatory indices have been used successfully to determine the relationship between the diet and several health outcomes, including CVD. Less is known regarding the dietary inflammatory potential of African population groups whose dietary patterns and intakes differ from populations included in the development of these dietary inflammatory indices. African diets consist mainly of indigenous food, with rural populations having more traditional intakes and urban populations transitioning to more Westernised dietary intakes. Since diet influences inflammation and CVD is increasing exponentially in South Africa, we aim to describe the inflammatory potential of the diet of Black South Africans from both rural and urban settings and determine the relationship between the inflammatory potential of the diet (calculated using DII) and inflammatory markers, fibrinogen concentration and fibrin clot properties.

Methods:

This dissertation investigated 2010 apparently healthy Black South Africans who were recruited into the South African, North-West Province arm of the international Prospective Urban and Rural Epidemiology (PURE) study. The PURE study is a prospective study but only baseline data collected in 2005 were included in this dissertation in a cross-sectional analysis. Dietary intakes of the participants assessed by means of a quantitative food frequency questionnaire were used to calculate an energy-adjusted Dietary Inflammatory Index (E-DII) (a positive score indicates a more pro-inflammatory diet and negative score a more anti-inflammatory diet). Biochemical variables included blood lipids, glucose, liver enzymes and inflammatory markers – C-reactive protein (CRP), albumin, soluble urokinase-type plasminogen activator receptor (SuPAR) and interleukin-6 (IL-6) – as well as fibrinogen concentration and fibrin clot properties.

Results:

The PURE population consumed, on average, a pro-inflammatory diet with a mean E-DII of $+0.37 \pm 1.25$. Urban women consumed the most anti-inflammatory diet, although their mean E-DII was still reflective of a pro-inflammatory diet (0.25 ± 0.14), followed by urban men (0.31 ± 0.16). Rural

men consumed the most pro-inflammatory diet (0.70 ± 0.66). In addition, the E-DII range of the PURE participants spanned only 61% of the theoretical E-DII range, reflecting a limited variety in the PURE diets. On average, urban women consumed higher amounts of 19 of the 28 anti-inflammatory food components and urban men 5 of the 8 pro-inflammatory components. Age decreased across E-DII quartiles whereas the number of alcohol consumers ($p < 0.001$) and tobacco users ($p = 0.001$) increased. Most of the biochemical variables did not differ across E-DII quartiles, including the blood lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride), the inflammatory markers (CRP, SuPAR, IL-6 and albumin), body mass index, fibrinogen and the clot properties. Only fasting glucose and the liver enzymes increased across the E-DII quartiles.

Conclusion:

This study described for the first time the inflammatory potential of a continental African study population. The inflammatory potential of the participants' diets differed significantly between men and women and those of rural/urban status, with urban women following the most anti-inflammatory and rural men the most pro-inflammatory diet. The lack of variety within the PURE population's diet likely contributed to a lack of association between the E-DII and inflammatory markers, fibrinogen concentration and fibrin clot properties. This relationship should be investigated in study populations with a more varied diet with more overt anti- and pro-inflammatory properties. The results of this study highlight the need to better understand the role of the inflammatory potential of the diet in the context of food insecurity, unhealthy lifestyle practices and increased CVD incidence, which are highly prevalent in developing countries such as South Africa.

Keywords: E-DII, Inflammatory potential, fibrinogen concentration, urban and rural, African

LIST OF ABBREVIATIONS AND SYMBOLS

au	absorbance units
au/s	absorbance units per second
ALT	alanine transaminase
α	alfa
ANOVA	analyses of variance
ANCOVA	analyses of covariance
AIDI	Anti-Inflammatory Diet Index
AST	aspartate transaminase
β	beta
BMI	body mass index
CVD	cardiovascular disease
C-DII	Children's Dietary Inflammatory Index
CLT	clot lysis time
COSM	Cohort of Swedish Men
CRP	C-reactive protein
$^{\circ}\text{C}$	degree Celsius
DII	dietary inflammatory index
DBP	diastolic blood pressure
DRI	dietary reference intake
EDIP	Empirical Dietary Inflammatory Pattern
E-DII	energy-adjusted dietary inflammatory index

ELISA	enzyme-linked immunosorbent assay
F	factor
FPA	fibrinopeptide A
FPB	fibrinopeptide B
FCT	food composition table
FFQ	food frequency questionnaire
GGT	gamma-glutamyl transferase
γ'	gamma prime
GLM	generalised linear models
HbA1c	glycated haemoglobin
g	gram
g/d	gram per day
g/L	gram per litre
HDL-C	high-density lipoprotein cholesterol
hsCRP	high-sensitivity C-reactive protein
IL-1 β	interleukin-1 beta
IL-4	interleukin-4
IL-6	interleukin-6
IL-6REs	interleukin-6 response elements
IL-10	interleukin-10
ISAK	International Society for the Advancement of Kinanthropometry
IU/L	international units per litre
Fe	iron

kcal	kilocalories
kg/m ²	kilogram per square meter of body height
kJ	kilojoule
KNHANES	Korean National Health and Nutrition Examination Survey
LDL-C	low-density lipoprotein cholesterol
Mg	magnesium
MSc	Master of Science
MRC	Medical Research Council
MEDIS	MEDiterranean ISlands
µg	microgram
µmol/L	micromole per litre
mg	milligram
mg/L	milligram per litre
mmHg	millimetre of mercury
mL/min	millimetre per minute
mmol/L	millimoles per litre
MUFA	mono-unsaturated fatty acid
ng/mL	nanogram per millilitre
nm	nanometre
NHANES	National Health and Nutrition Examination Survey
NWU	North-West University
NHS	Nurses' Health Study
NAHSIT	Nutrition and Health Survey in Taiwan

NAI	Nutrition anti-inflammatory score
ω -3	omega-3
ω -6	omega-6
p	p-value
%	percentage
pg/mL	picogram per millilitre
PAI-1	plasminogen activator inhibitor-1 activity
PUFA	poly-unsaturated fatty acid
PURE	Prospective Urban and Rural Epidemiology study
PURE-SA-NW	Prospective Urban and Rural Epidemiology study, South Africa, North West
QFFQ	quantitative food frequency questionnaire
RBC	red blood cell
RRR	reduced rank regression
RE	retinol equivalents
SFA	saturated fatty acids
Se	Selenium
STAT-3	signal transducer and activator of transcription-3
suPAR	soluble urokinase-type plasminogen activator receptor
SD	standard deviation
SPSS	Statistical Package for the Social Sciences
SMC	Swedish Mammography Cohort
SBP	systolic blood pressure
TC	total cholesterol

TE	total energy
TF	tissue factor
tPA	tissue plasminogen activator
tHcy	total homocysteine
TG	triglyceride
TNF- α	Tumour necrosis factor-alfa
U/mL	units per millilitre
vWF	von Willebrand Factor
WHO	World Health Organization
WHR	waist-to-hip ratio

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CHAPTER 1 INTRODUCTION

1.1 Background

Cardiovascular disease (CVD) is a leading cause of mortality worldwide, causing around 17 million deaths per year (Naghavi *et al.*, 2017). In recent years the prevalence of CVD has been declining in high-income countries while increasing in low- and middle-income countries (Lim *et al.*, 2007; Roth *et al.*, 2017).

It has now been well established that CVD is strongly linked to inflammation (Guarner & Rubio-Ruiz, 2015; Libby *et al.*, 2002; Rupprecht *et al.*, 2001). During inflammatory conditions, platelet adhesion and aggregation are increased, resulting in coagulation activation, thrombus formation and ischaemic events (Willoughby *et al.*, 2002). Markers of inflammation are currently used as screening tools to identify patients who are at risk of CVD (DeBoer, 2013). Inflammation and coagulation are often studied independently, but these two pathways are connected to form a unique defence response (Cicala & Cirino, 1998) and should not be considered as two separate pathways. For example, fibrinogen is an acute-phase protein that plays an essential role in both the inflammatory and blood coagulation processes (Clark *et al.*, 1982; Donaldson *et al.*, 1989; Kamath & Lip, 2003; Sahni & Francis, 2000; Sahni *et al.*, 1998). A large body of evidence also shows that increased fibrinogen is a recognised predictor of CVD (Ajjan & Grant, 2006; Lefevre *et al.*, 2004; Thompson *et al.*, 1995).

Fibrinogen plays a critical role in blood clotting, not only as the main structural component of a thrombus but also owing to its ability to influence blood viscosity, platelet and red blood cell aggregation and endothelial function (De Moerloose *et al.*, 2010; Dunn *et al.*, 2004; Ernst & Resch, 1993; Pulanić & Rudan, 2005; Undas, 2016). Through these properties, increased fibrinogen concentration results in more dense clots with increased stability that are more difficult to break down (lyse) (Gerth *et al.*, 1974; Glover *et al.*, 1975; Roberts *et al.*, 1974; Scrutton *et al.*, 1994; Shen *et al.*, 1975). Clots that are not broken down timeously increase the risk of vessel occlusion, which could cause, for instance, a stroke or heart attack. Various clot properties known to result from increased fibrinogen concentrations, such as more dense clots with thinner fibres and increased mechanical stiffness, have been associated with CVDs such as deep vein thrombosis, arteriosclerosis, stroke and coronary heart disease (Undas & Ariëns, 2011).

An overwhelming body of evidence shows that diet plays a central role in chronic inflammation as the cornerstone of CVD and many other chronic diseases (Cavicchia *et al.*, 2009; Cui *et al.*, 2012; Giugliano *et al.*, 2006). An increased number of people are suffering from inflammation-based chronic diseases such as cancer (Keibel *et al.*, 2009; Thun *et al.*, 2004), diabetes (Galassetti,

2012) and CVD (Hotamisligil, 2006; Pearson *et al.*, 2003). This necessitated the development of dietary inflammatory indices, in order to determine the anti-/pro-inflammatory potential of whole diets (Cavicchia *et al.*, 2009) and thereby establish to what degree diet contributes to the overall inflammatory state. Two types of dietary indices have been developed to measure the inflammatory potential of whole diets: an empirically-based and a literature-based index.

The empirically-based index incorporates the relationship between nutrient intakes of a specific study population and inflammatory markers measured in the same population. Three population-specific empirical inflammatory indices have been developed thus far (Kaluza *et al.*, 2018; Tabung *et al.*, 2016; Tyrovolas *et al.*, 2018).

In contrast to the empirically-based indices, the literature-based index is based on available literature investigating the effect of nutrients and/or food components on inflammation in different populations throughout the world. In 2009, the original literature-based score was developed and named the dietary inflammatory index (DII). A total of 1943 peer-reviewed research articles published from 1950 to 2010 on the effect of dietary factors on six inflammatory markers (interleukin-1 beta, interleukin-4, interleukin-6 (IL-6), interleukin-10, tumour necrosis factor and C-reactive protein (CRP)) were identified and scored by the original developers of the DII to derive a component-specific inflammatory effect score for 45 dietary factors. These dietary factors comprise macronutrients, micronutrients and a number of bioactive components (Shivappa *et al.*, 2014a). Soon after, this score was updated by the original developers to improve its usability and is now referred to as the new, updated DII (Shivappa *et al.*, 2014a) and an energy-adjusted dietary inflammatory index (E-DII).

These inflammatory indices discriminate between the consumption of anti-/pro-inflammatory diets. The DII, specifically, has been used to demonstrate the relationship between diets of varying inflammatory potential and blood-based inflammatory markers, such as CRP and IL-6 (Julia *et al.*, 2017; Mayr *et al.*, 2018; Shin *et al.*, 2019; Shivappa *et al.*, 2017a; Shivappa *et al.*, 2014c), CVD risk factors (Pimenta *et al.*, 2015; Ruiz-Canela *et al.*, 2015; Sokol *et al.*, 2016; Wirth *et al.*, 2014), incidence of CVD (Garcia-Arellano *et al.*, 2015; Ramallal *et al.*, 2015), cancer (Alkerwi *et al.*, 2015; Ge *et al.*, 2015; Li *et al.*, 2018; Maisonneuve *et al.*, 2016; Shivappa *et al.*, 2014b; Tabung *et al.*, 2015; Zamora-Ros *et al.*, 2015) and mortality (Deng *et al.*, 2017; Graffouillere *et al.*, 2016; Hodge *et al.*, 2018).

Although these indices have been used successfully to determine the relationship between an individual's diet and several health outcomes, less is known regarding the dietary inflammatory potential of African population groups whose dietary patterns and intakes differ from populations included in the development of the DII. Traditional African diets consist mainly of indigenous foods

and need to be explored in relation to their inflammatory characteristics. In South Africa, the consumption of traditional African diets is varied, with more traditional intakes found in rural environments with a transition into Westernised dietary patterns in urban populations. Participants investigated in this study were recruited from both urban and rural areas in the North West Province of South Africa. Participants had a predominantly low socio-economic status, which affected their dietary intake (Dolman *et al.*, 2014). Additionally, rural participants experienced drought that affected the availability and affordability of fresh food such as fruit and vegetables (Wentzel-Viljoen *et al.*, 2018b) during the time of data collection. Overall, rural participants tend to follow a diet that is low in energy and fat whereas urban participants follow a more high-energy, high-fat diet (Vorster, 2002), likely leading to differences in the dietary inflammatory potential of rural and urban diets.

While it is clear that the DII is associated with several CVD risk factors, its potential to alter coagulation and, specifically, fibrinogen concentration and the concomitant fibrin clot structure as a potential mechanistic pathway, has not been investigated. Furthermore, the inflammatory potential of diets regularly consumed by Black South Africans has not yet been studied and these studies are necessary to increase our knowledge regarding possible pathways through which diet increases an individual's CVD risk. This dissertation therefore aims to describe the inflammatory potential of the diet of Black South Africans who participated in the South-African, North-West Province arm of the international Prospective Urban and Rural Epidemiology (SA-NW-PURE) study, based on the E-DII, according to rural-urban status and sex. Next, it investigated whether the inflammatory index of the diet is associated with fibrinogen concentration and the concomitant blood clot structure as a possible pathway through which diet can be related to increased CVD risk in this population.

1.2 Aims and objectives

The aims of this study were, firstly, to describe the inflammatory potential of the diet of Black South Africans who participated in (SA-NW-PURE) study. Next, it determined the relationship between the E-DII and inflammatory markers, fibrinogen concentration and fibrin clot properties. These relationships were investigated cross-sectionally.

1.2.1 Objectives

- To adapt the published E-DII for the participants who took part in the PURE-SA-NW study in 2005, based on their dietary intakes;
- To determine the relationship between the E-DII and inflammatory markers (CRP, albumin, soluble urokinase-type plasminogen activator receptor and IL-6);
- To determine the relationship between the E-DII and fibrinogen concentration and plasma clot properties obtained using turbidimetry;
- To determine whether the relationship between the E-DII and clot properties is mediated by inflammation and/or fibrinogen concentration.

1.3 Research team

Table 1-1: Research team

Team member	Qualification	Professional registration	Role in study
Ms Maylene Ferreira	B.Sc. Dietetics	HPCSA- Dietitian	M.Sc. student: Critical evaluation of the literature, protocol drafting and editing. Development of an E-DII for the PURE study participants, which included compiling a list of nutrients and food components consumed by the PURE population, identification and exclusion of under- and over-reporters. Additionally, calculating the summed intake of food components such as onions by combining intakes from different meal items consumed, based on their recipes. Calculation and analyses of the flavonoid content of raw, cooked and dried food components. Determination of the fatty acids to be included in the compilation of the omega-3 and -6 fatty acid intake,

			calculation of the omega-3 and-6 intake for each individual in the PURE population. Calculate an E-DII for each PURE participant. Statistical analysis, interpretation of results, writing of dissertation.
Prof. Marlien Pieters	Ph.D. Dietetics	HPCSA- Dietitian	Supervisor of M.Sc. student: study conceptualisation, guidance regarding writing of protocol, literature review, training student in score development methodology, application and statistical analysis, guidance in interpretation of results, guidance in writing of mini-dissertation.
Dr Tertia van Zyl	Ph.D. Dietetics	HPCSA- Dietitian	Co-supervisor of M.Sc. student: Guidance regarding writing of protocol, literature review and dissertation, guiding student in dietary analyses, application and statistical analysis.

1.4 Structure of dissertation

This dissertation is written in chapter format and includes five chapters. All five chapters were edited by a qualified language editor and technical requirements were met in accordance with the North-West University (NWU), with referencing adhering to the Harvard style as stipulated by the NWU.

Chapter 2 is a literature review that provides the background information necessary for the interpretation of the study results. The chapter first describes haemostasis, the coagulation cascade, the structure and physiology of fibrinogen, and fibrinolysis. In addition, it describes how inflammation affects fibrinogen and fibrin clot properties. Thereafter, the dietary inflammatory index is described, including the difference between empirically-based and literature-based indices. The literature review also includes a detailed description of how to calculate the dietary

inflammatory index. Lastly, an overview of the PURE participants' diet and how it differs between urban and rural participants is presented.

Chapter 3 describes the study design and characteristics of the PURE study population. An overview of methods used for anthropometrical and biochemical analyses is included. A detailed description of the Quantitative Food Frequency Questionnaire used, and the analyses of the flavonoid and fatty acid content are included. Additionally, a table that indicates which food items were included in the PURE E-DII and a step-by-step description on how the PURE E-DII was calculated is included in this Chapter. The statistical analysis performed in this study is also reported in Chapter 3.

Chapter 4 provides the results. The chapter provides results in table format that includes the characteristics of food components (pro- and anti-inflammatory food components) and study variables (anthropometrical, biochemical and demographic variables) of men and of women and of participant rural/urban status. Additionally, these study variables are reported across E-DII quartiles and associations between E-DII quartiles and study variables are also reported.

Chapter 5 discusses the findings of this study and motivations as to why certain associations were found and others not. Limitations as well as recommendations for future research are also included in Chapter 5. This chapter is concluded with a brief summary of the entire dissertation and current literature. This dissertation is completed with a reference list and sources that are cited throughout all five chapters as well as addenda providing supporting documentation.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide and is expected to account for more than 23.6 million deaths by 2030 (Mathers & Loncar, 2006; Murphy *et al.*, 2018; Naghavi *et al.*, 2016). According to the South African Statistical Bureau (Stats SA), CVD held the third position of the top 10 underlying causes of death in South Africa in 2016 (Statistics South Africa, 2016:37).

It is hypothesised that inflammation is a major underlying cause of CVD (Guarner & Rubio-Ruiz, 2015; Libby, 2006). In the presence of inflammation, platelets respond rapidly to alterations in endothelial cells, resulting in thrombus formation (Willoughby *et al.*, 2002). To identify patients at risk of cardiovascular events, several markers of inflammation are used for screening, one being fibrinogen concentration (Kannel, 1997; Ridker, 1999). Fibrinogen is an important acute-phase and coagulation protein, synthesised mostly by hepatocytes (Herrick *et al.*, 1999). The expression of fibrinogen can be significantly upregulated during inflammatory stress. Stimulation of the pro-inflammatory cytokine, interleukin-6 (IL-6) during inflammatory stress is associated with a significant increase in expression of fibrinogen (Dandona *et al.*, 2004; Otto *et al.*, 1987).

With fibrinogen being an important factor in both coagulation and inflammation, these two complex pathways are often studied in relation to each other (Choi & Levi, 2006; Cicala & Cirino, 1998; Davalos & Akassoglou, 2012; Verhamme & Hoylaerts, 2009). The increase in fibrinogen concentration leads to increased red blood cell (RBC) aggregation, platelet aggregation and plasma viscosity, which are all associated with thrombus formation (De Moerloose *et al.*, 2010; Ernst & Resch, 1993; Pulanić & Rudan, 2005). Additionally, increased fibrinogen concentration is known to be the most important modulator of the fibrin network structure, which is considered a novel risk factor in thrombotic CVD events (Ajjan & Grant, 2005; Ariëns, 2011; Undas *et al.*, 2011).

With the increasing number of people suffering from inflammation-based chronic diseases, measures to manage these are critically important. One such measure is diet, which is considered to be a strong modulator of chronic inflammation (Ahluwalia *et al.*, 2013). Based on the overwhelming body of evidence reporting on the relationship of individual dietary components with inflammation, dietary indices have been developed in order to reflect the overall anti-/pro-inflammatory potential of the total diet instead of focussing on the effects of individual nutrients only (Shivappa *et al.*, 2014a). These dietary inflammatory indices have been used successfully to demonstrate the relationship between the consumption of anti-/pro-inflammatory diets and a variety of CVDs and cancers (Hébert *et al.*, 2019). They have also been validated against

inflammatory markers in the blood (Shivappa *et al.*, 2017a; Shivappa *et al.*, 2015). The potential of the diet to alter coagulation specifically, fibrinogen concentration and the concomitant fibrin clot structure as a potential mechanistic pathway, has, however, not been investigated.

The following literature review chapter will focus on the introduction of haemostasis, highlighting clot structure and how inflammation affects fibrinogen concentration and clot properties. The next section of the review outlines two different types of indices created to assess the inflammatory potential of whole diets. Lastly, a description of the dietary intake of the study population will be given to provide context for the use of dietary inflammatory indices in an African study population consuming African cuisine.

2.2 Introduction to haemostasis

Haemostasis can be defined as the termination of bleeding (Friedewald *et al.*, 1972). Haemostasis includes several mechanisms, namely vascular constriction, platelet plug formation, blood clot formation through blood coagulation, lysis of the blood clot and fibrous tissue growth (Davie *et al.*, 1991).

Under normal circumstances, the presence of anticoagulants prevents coagulation of circulating blood but, if injury occurs, procoagulants within the surrounding tissue are successively converted to active enzymes, resulting in blood clot formation (Davie & Ratnoff, 1964).

Haemostasis has been described in two different ways. Firstly, the haemostatic process has been described as a waterfall model (Davie & Ratnoff, 1964). Activation of this model is triggered by tissue factor and/or collagen and continues through an extrinsic and intrinsic pathway to a combined common pathway (Adams *et al.*, 2007).

Secondly, a cell-based conceptual model for haemostasis has been developed (Hoffman & Monroe III, 2001; Monroe *et al.*, 1996; Monroe *et al.*, 1994). This model was developed based on the fact that there is significant evidence from many researchers showing that different cell surfaces have different properties necessary for the coagulation process (Hoffman & Monroe III, 2001). This led to the development of a model that explains coagulation on the basis of cellular control and suggests that haemostasis occurs in three different, but overlapping steps: initiation, amplification and propagation (Hoffman & Monroe III, 2001; McMichael, 2005). The following section will describe the cell-based model of haemostasis.

2.2.1 Initiation phase

Tissue factor (TF), also called thromboplastin or factor (F) III, is expressed on the surface of cells such as fibroblast (Kalter *et al.*, 1983). Clotting occurs when TF comes into contact with blood and results in the binding of TF with Factor (F) VII, a vitamin K-dependent protein (McMichael, 2012; Wildgoose *et al.*, 1992). This action leads to the formation of enzymatically active TF/ FVIIa complexes. These complexes activate factor (F) IX and factor (F) X to factor (F) IXa and factor (F) Xa (Bom & Bertina, 1990; Komiyama *et al.*, 1990; Silverberg *et al.*, 1977). Factor (F) V is then activated by factor (F) Xa to factor (F) Va and these two activated factors associate to form the prothrombinase complex on TF-bearing cells (Monroe *et al.*, 1996). This complex converts the precursor, prothrombin, into limited amounts of thrombin (Monroe & Hoffman, 2006). The initiation phase can be seen in Figure 2.1

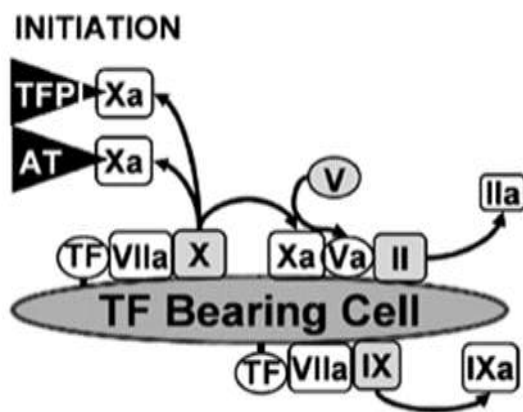


Figure 2-1: Initiation phase (Adapted from Smith & Care, 2009)

TF: Tissue Factor, II: prothrombin; IIa: thrombin; TFPI: tissue factor pathway inhibitor; AT: antithrombin

2.2.2 Amplification phase

The small amount of thrombin generated in the initiation phase is vital to the activation of platelets (McMichael, 2012). This constitutes the amplification phase and takes place on the platelet surface (Figure 2.1). The platelets are partially activated when they adhere at the site of injury but the addition of the formed thrombin induces a higher level of procoagulant activity (Alberio & Dale, 1999). This leads to platelet degranulation and some contents of the alpha-granules, such as fibrinogen, von Willebrand Factor (vWF) and factor (F) V, are released into the extracellular space (Mannila *et al.*, 2006). Another function of thrombin, activated during the initiation phase, is the activation of co-factors (F) V, factor (F) VIII and factor (F) XI on the surface of the activated platelet (Baglia & Walsh, 1998; Oliver *et al.*, 2002). By the end of the amplification phase, conditions have been set for large-scale thrombin generation during the propagation phase (Monroe & Hoffman, 2006).

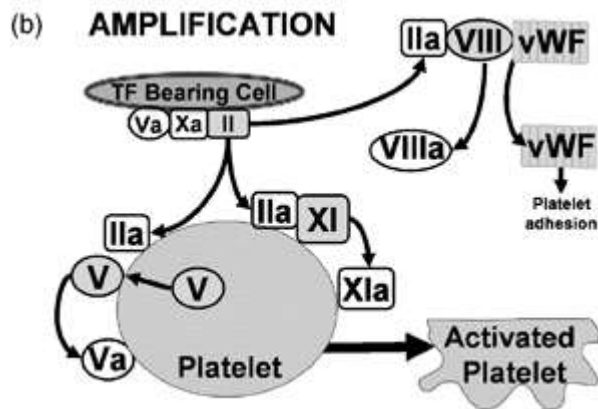


Figure 2-2: Amplification phase (Adapted from Smith & Care, 2009)

TF: Tissue factor; II: prothrombin; IIa: thrombin; vWF: von Willebrand Factor

2.2.3 Propagation phase

The propagation phase also occurs on the surface of activated platelets (Figure 2.3) (Riddel Jr et al., 2007). Factor (F) IXa, which is activated during the initiation phase, can now bind to factor (F) VIIIa on the surface of the activated platelets and form the factor (F) IXa/ factor (F) VIIIa complex (Hoffman, 2003b; Monroe & Hoffman, 2006). This complex then activates Factor (F) X to factor (F) Xa (Hoffman, 2003b). Factor (F) Xa cannot move effectively from the TF-bearing cells to activated platelets. However, Factor (F) Xa can be provided directly on the platelet surface by the factor (F) IXa/VIIIa complex; therefore, additional factor (F) IXa is supplied by platelet-bound factor (F) XIa in order for this to happen. Factor (F) Xa then associates rapidly with factor (F) Va to form a prothrombinase complex (Hoffman, 2003a; Monroe & Hoffman, 2006). This complex produces sufficient thrombin to activate fibrinogen to form the fibrin network. This process will be described in the next section.

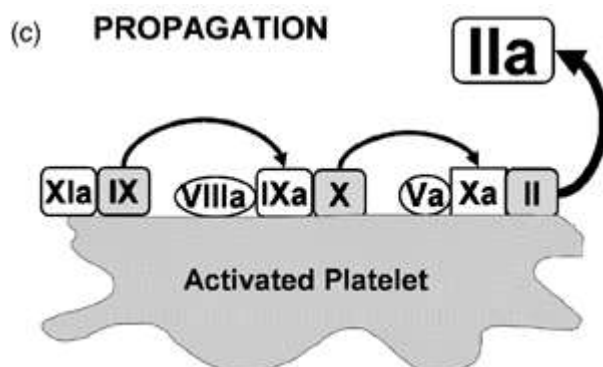


Figure 2-3: Propagation phase (Adapted from Smith & Care, 2009)

II: prothrombin; IIa: thrombin

2.2.4 Fibrinogen structure and physiology

Fibrinogen is a soluble plasma protein synthesised in the liver. It is essential for wound healing, fibrinolysis, inflammation, angiogenesis, cellular and matrix interactions, and neoplasia (Pulanić & Rudan, 2005). Fibrinogen mostly circulates in the plasma, but is also present in platelets, lymph nodes and interstitial fluid. The normal circulating plasma concentration of fibrinogen varies between 1.5 and 4.5 g/L while only 0.5 to 1.0 g/L is required for haemostasis (Kamath & Lip, 2003). The fibrinogen molecule consists of 2964 amino acids (Weisel & Litvinov, 2013).

Fibrinogen contains six polypeptides, two each of an A α , B β and γ chain, linked by 29 disulphide bonds (Gorkun *et al.*, 1997). The fibrinogen molecule is 45 to 46 nm in length (Blombäck, 1996; Lim *et al.*, 2008; Weisel, 2005) and has a width of 9 nm (Blombäck, 1996). Fibrinogen consists of an E-region, the centre part of fibrinogen that contains the N-termini of all six chains (Standeven *et al.*, 2005) and two identical D-regions at the distal parts of the fibrinogen molecule (Figure 2.4) (Weisel & Litvinov, 2017). The three polypeptides protrude from the E-region to form α -helical coiled-coil rods until they reach the D-regions. The D-region consists of the C-terminal ends of the B β , and γ chains. The C-terminal ends of the A α chains protrude from the D-region, interacting with each other and extending back to the E-region, where they remain associated until fibrin formation occurs (Blombäck, 1996; Mosesson, 2005; Standeven *et al.*, 2005; Weisel, 2005). The E-region contains the N-terminus of the fibrinogen molecule, which is important for clot formation. In order for coagulation to start, thrombin must first cleave the N-terminal regions of both the A α - and B β -chain polypeptides of fibrinogen, where they are then known as fibrinopeptide A (FPA) and fibrinopeptide B (FPB) respectively (Mosesson, 2005; Weisel, 2005).

2.2.5 Fibrin formation

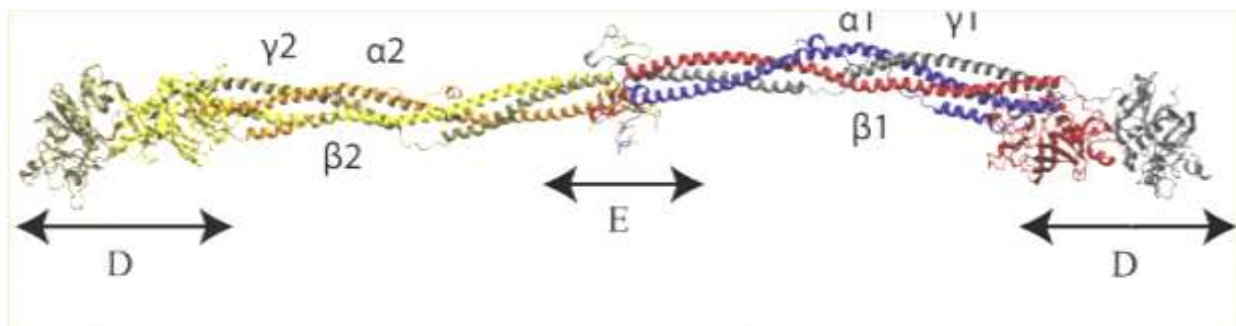


Figure 2-4: Fibrinogen structure: (Adapted from Weisel & Litvinov, 2013)

The thrombin-induced conversion of fibrinogen to fibrin is essential in the final stage of blood coagulation (Scheraga & Laskowski Jr, 1957). The conversion from fibrinogen to fibrin includes

cleavage of four fibrinopeptides (A and B) from the central region of each fibrinogen molecule to form one fibrin monomer with exposed knobs and holes (Figure 2.5) (Davie *et al.*, 1991; Weisel, 2005). These knobs on fibrin monomers bind with corresponding holes on other monomers that result in the formation of protofibrils. Protofibrils aggregate laterally to form fibrin fibres that finally form a three-dimensional structure, trapping cells, platelets and plasma (Mosesson, 2005; Weisel & Litvinov, 2013; Wolberg, 2007). Another function of thrombin is the activation of factor (F) XIII. This results in the formation of cross-links between fibrin molecules by means of transglutaminase reactions (Mosesson *et al.*, 2001) resulting in a more stable clot (Weisel & Litvinov, 2013). Cross-linking plays an important role in fibrin clot structure by increasing clot rigidity, decreasing inelastic deformation (Weisel, 2007) and forming a clot that is more resistant to lysis.

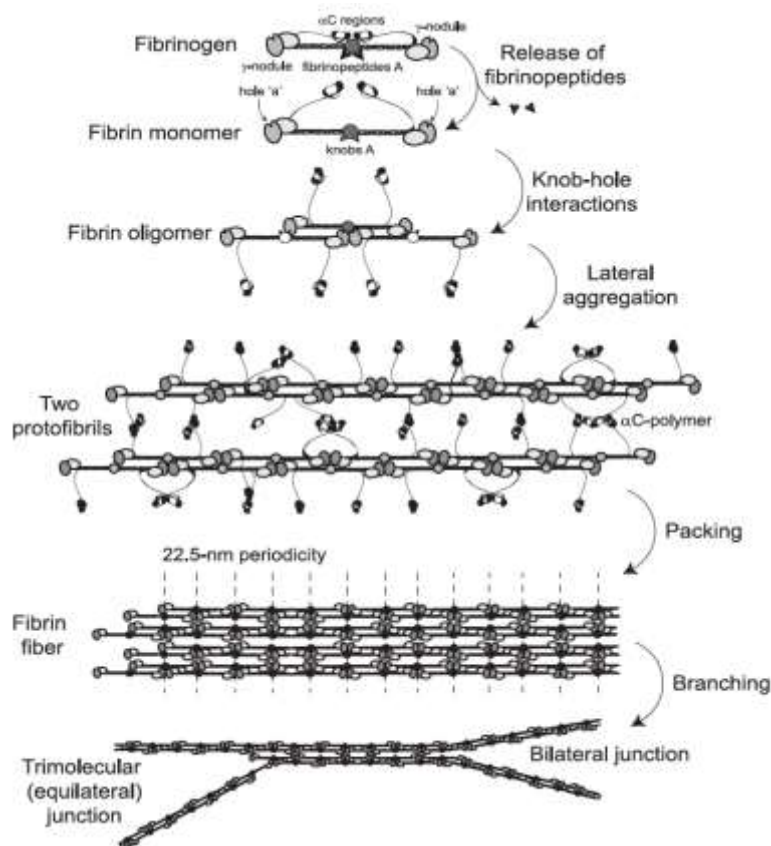


Figure 2-5: Illustration of the formation of fibrin (Adapted from Weisel & Litvinov, 2013)

2.2.6 Fibrinolysis

Fibrinolysis is the dissolution of fibrin clots in the blood vessels (Collen & Lijnen, 1991). If a clot is not optimally lysed, the risk of complete or partial occlusion of the blood vessel is increased. Reduced fibrinolytic activity has been associated with cardiovascular events such as stroke (Undas *et al.*, 2009a), myocardial infarction (Fatahl *et al.*, 1996) acute coronary syndrome (Undas *et al.*, 2008) and venous thromboembolism (Undas *et al.*, 2009b).

Plasmin is the major fibrinolytic protease that circulates in the blood in its inactive form, plasminogen. Plasminogen can be converted to plasmin by tissue plasminogen activator (tPA) and urokinase (uPA) (Ajjan & Grant, 2006; Cesarman-Maus & Hajjar, 2005). Plasmin digests fibrin by cleaving lysine bonds in the polypeptide chain of the α C-domain, followed by cleavage of an N-terminal peptide of the β -chain and the coiled-coil connector of the D- and E-regions (Longstaff & Kolev, 2015).

Several factors can affect fibrinolysis, one of them being clot properties. Many studies have reported the relationship between fibrin's structural properties and rates of fibrinolysis and it is suggested that fibrin structure directly affects the rate of fibrinolysis (Weisel, 2007). Plasma fibrin clots with a dense fibre arrangement made of thin fibres are lysed at a slower rate when compared with those made of thicker fibres (Weisel, 2007). Individual thin fibres may, however, digest faster than thicker fibres but the action of plasmin in whole clots is slower because of the slow diffusion of plasmin through these clots (Bridge *et al.*, 2014; Longstaff & Kolev, 2015).

The architecture of a fibrin network is important as it has been demonstrated to be a novel risk factor for the development of arterial and venous thrombotic events (Undas & Ariëns, 2011). Low-grade inflammation has also been associated with increased cardiovascular risk (Davi *et al.*, 2004). Inflammatory markers such as IL-6, C-reactive protein (CRP), and tumour necrosis factor- α (TNF- α) are some key markers that have been linked to the future occurrence of cardiovascular events (Davi & Falco, 2005). With fibrinogen being considered an acute-phase protein (Thompson *et al.*, 1992), there is a potentially strong link between inflammation and fibrin clot properties. The following section will describe this in more detail.

2.3 Inflammation and fibrinogen clot properties

Fibrinogen is known to play an important role in both haemostasis (Dalmon *et al.*, 1993) and inflammation (Pulanić & Rudan, 2005) and there have been comprehensive discussions on its role in linking these two systems (Levi, 2010; Levi *et al.*, 2004; Lindsay, 2007).

The expression of fibrinogen by the liver is regulated in two ways: during basal expression and as part of the acute-phase response (Fish & Neerman-Arbez, 2012; Fuller & Zhang, 2001). Under basal conditions, fibrinogen is synthesised and assembled mainly by hepatic parenchymal cells (Caspary & Kekwick, 1957) and is present in the plasma at a concentration of 1.5 to 4.5 g/L (Kamath & Lip, 2003; Weinstock *et al.*, 2006) with a biological half-life of nearly 100 hours (Pulanić & Rudan, 2005). Under inflammatory conditions, fibrinogen levels increase, mainly as a result of stimulation by IL-6. IL-6 is a circulating cytokine that is secreted from activated macrophages and lymphocytes during inflammation (Bataille & Klein, 1992; Heinrich *et al.*, 1990). IL-6 response

elements (IL-6REs) are observed on all three of the fibrinogen genes and they interact with signal transducer and activator of transcription-3 (STAT-3) to upregulate the production of fibrinogen (Fish & Neerman-Arbez, 2012). In addition, polymorphic variation within the fibrinogen gene can alter IL-6-induced fibrinogen expression (Morozumi *et al.*, 2009; Verschuur *et al.*, 2005). The increase in fibrinogen concentration during inflammatory situations is associated with thrombotic disease (Esmon, 2005). The inflammatory response is, furthermore, a complex defence mechanism (Yudkin *et al.*, 2000) that should be kept under strict homeostatic control since its chronic disturbance can lead to tissue injury (Dahlbäck, 2012).

Since fibrinogen is the precursor of fibrin, its concentration is seen as the main contributor to the clot's structure (Dunn *et al.*, 2004; Undas, 2016). An increase in fibrinogen concentration typically results in the formation of a stiffer clot with increased fibre density which is more difficult to lyse (Gerth *et al.*, 1974; Glover *et al.*, 1975).

Thus, inflammation can affect a clot's structure in part through the accompanying increase in fibrinogen concentration. Although limited, there is some evidence in the literature for such an association. Cronjé *et al.* (2017) stated that fibrinogen concentration is associated with IL-6, and that it mediates the relationship between IL-6 and clot formation and structure (Cronje *et al.*, 2017). Additionally, Undas *et al.* (2008) reported that clot permeability and fibrinolysis are determined by the degree of inflammation present (Undas & Ariëns, 2011; Undas *et al.*, 2008).

As an increasing number of people are suffering from inflammation-based chronic diseases such as CVD, diabetes, respiratory diseases, mental disorders, autoimmune diseases (AID) and cancers (Bosma-den Boer *et al.*, 2012), measures to manage the presence of low-grade inflammation are critically important. Diet is considered to be a strong modulator of chronic inflammation (Ahluwalia *et al.*, 2013). There is, in fact, such an overwhelming body of evidence reporting on the relationship of individual dietary components with inflammation that dietary inflammatory indices have been developed in an attempt to reflect the overall anti-/pro-inflammatory potential of whole diets rather than focussing on effects of individual nutrients (Shivappa *et al.*, 2014a). Two types of dietary indices have been developed to measure the inflammatory potential of the diet. The following section will discuss these in more detail.

2.4 Dietary inflammatory scores

The two types of dietary indices that have been developed to measure the inflammatory potential of the diet differ in the sense that one type is based on available literature investigating the effect of nutrients and/or food components on inflammation in different populations all over the world, called the literature-based index. The other, namely the empirically-based index, is based on an empirical approach relating the nutrient intakes of a specific study population to the inflammatory markers measured in the same population.

The original literature-based index was developed in 2009 and was called the dietary inflammatory index (DII) (Cavicchia *et al.*, 2009). This was updated in subsequent years and referred to as the new, updated dietary inflammatory index (DII) (Shivappa *et al.*, 2014b), the energy-adjusted dietary inflammatory index (E-DII) (Hébert *et al.*, 2019) and a Children's dietary inflammatory index (C-DII) (Khan *et al.*, 2018), which was developed specifically for the use in children. Three empirical inflammatory indices have thus far been developed. These include an index developed specifically in a U.S. population (Tabung *et al.*, 2016), named the empirical dietary inflammatory pattern (EDIP) (Tabung *et al.*, 2016). Two others were developed in different target populations, including the anti-inflammatory dietary index (AIDI), which was developed in Sweden (Kaluza *et al.*, 2018) and the nutrition anti-inflammatory (NAI) score, which was developed in a Greek population (Tyrovolas *et al.*, 2018). This section will discuss and explain the construction and validation of the different inflammatory scores.

2.4.1 Empirically-based scores

2.4.1.1 Empirical Dietary Inflammatory Pattern (EDIP)

The Empirical Dietary Inflammatory Pattern (EDIP) was created to provide a score based on food groups (Tabung *et al.*, 2016) in an American study population, thus assessing the inflammatory potential of whole diets. The EDIP was developed from data obtained in the Nurses' Health Study (NHS), an ongoing prospective cohort study established in 1976 (Tabung *et al.*, 2016). The intake of 39 food groups was calculated and applied in reduced rank regression (RRR) models to derive a dietary pattern predictive of three inflammatory markers: IL-6, CRP, and TNF- α . The first factor that was obtained from the RRR underwent further data reduction by means of stepwise linear regression to identify the most important component food groups contributing to the RRR dietary pattern. Regression coefficients from the final stepwise linear regression analyses were used as component weights. The stepwise linear regression identified 18 food groups and/or components to be included in the EDIP score. Of the 18 food groups and/or components 9 were considered anti-inflammatory and 9 pro-inflammatory. The pro-inflammatory food groups included in the EDIP

scores are: processed meat, red meat, organ meat, fish (other than dark-meat fish), other vegetables (i.e., vegetables other than green-leafy vegetables and dark-yellow vegetables), refined grains, high-energy beverages (cola and other carbonated beverages with sugar, fruit drinks), low-energy beverages (low-energy cola and other low-energy carbonated beverages), and tomatoes. In contrast, beer, wine, tea, coffee, dark-yellow vegetables (comprising carrots, yellow squash, and sweet potatoes), green-leafy vegetables, snacks (1 small bag or 28.3 g potato chips, corn chips, or popcorn; 1 cracker), fruit juice (1 small glass of apple juice or cider, orange juice, grapefruit juice, or other fruit juice), and pizza showed an inverse association with plasma inflammatory markers (Tabung *et al.*, 2016).

2.4.1.2 Anti-Inflammatory Diet Index (AIDI)

The AIDI was developed in 3503 women from the Swedish Mammography Cohort (SMC) with the aim of predicting chronic low-grade systemic inflammation in a non-U.S. population (Kaluza *et al.*, 2018). The AIDI has three different versions: AIDI-20, AIDI-25 and AIDI-16 and was created from a 123-item food frequency questionnaire. Of the foods in the 123-item FFQ, 20 food groups were significantly correlated with CRP concentrations and were included in the AIDI-20 (Kaluza *et al.*, 2018). The AIDI-20 score therefore ranged from 0-20. Pro-inflammatory potential was indicated with a lower score of “0” and “20” indicated a more anti-inflammatory potential (Kaluza *et al.*, 2018), which is a scoring system opposite to the scoring direction of the DII (Zhong *et al.*, 2017). Of the 20 food groups, 15 had anti-inflammatory potential and 5 pro-inflammatory potential (Kaluza *et al.*, 2018).

For each food group, a cut-off consumption value was identified to determine what amount of food needs to be consumed for it to have sufficient anti- or pro-inflammatory potential to be included in the index calculation. Consumption cut-off values were based on an empirical approach, where each food group was tested in association with CRP concentration at different cut-off values. The cut-off values for each food group that demonstrated the strongest association between the AIDI-20 and CRP concentration were selected. Table 2.1 shows the different food products, divided in two groups: foods with anti-inflammatory potential and pro-inflammatory potential, with each product's consumption cut-off (Kaluza *et al.*, 2018). A “1” was assigned for each food item on the anti-inflammatory list that was consumed above the cut-off. The scoring system for the pro-inflammatory food items included a “1” if the consumption of processed and unprocessed red meat was below the cut-off, and for organ meats, chips, as well as soft-drink beverages, a “1” was assigned for no consumption. For the AIDI-25, 5 additional foods were included, based on literature that reported these foods as having an association with inflammation. To the anti-inflammatory groups 1) low-fat yogurt and 2) fish (herring/mackerel,

salmon, cod/saithe/plaice/grenadier, tuna, and other fish) were added; and 3) white bread (white crisp bread and white bread/loaf), 4) baked goods (buns/pastries, biscuits/wafers/cookies, and cake/desserts) and 5) sugar/honey were added to the pro-inflammatory group (Kaluza *et al.*, 2018). The AIDI-20 was also adapted to be used with a 96-item food frequency questionnaire (FFQ), which was used in the SMC and another large population-based Cohort of Swedish Men (COSM) (Kaluza *et al.*, 2018). Dried fruit, beans/lentils, linseeds and herbal tea were removed from the AIDI-20 (Kaluza *et al.*, 2018) owing to a lack of questions in the FFQ regarding the consumption of these items and consequently, the AIDI-16 was created (Kaluza *et al.*, 2019).

Table 2-1: Food included in the anti-inflammatory diet index-20 and consumption cut-off values

Products	Consumption cut-off
Foods with anti-inflammatory potential	
Serving/day	
Total fruits and vegetables	≥ 6
Tea	≥ 3
Coffee	≥ 2
Whole-grain bread	≥ 2
Breakfast cereal	≥ 1
Low-fat cheese	≥ 1
Chocolate	≥ 1
Dry fruits	≥ 0.5
Herbal Tea	≥ 0.5
Olive oil/ Canola oil	> 0
Servings/ week	
Legumes	≥ 2
Nuts	≥ 2
Linseeds	≥ 2
Red wine	2-7
Beer	2-14
Foods with pro-inflammatory potential	

(Servings/ day)	
Unprocessed red meat	≤0.5
Processed meat	≤0.5
Organ meats	0
Chips	0
Soft-drink beverages	0

[Adapted from Kaluza (2018)]

2.4.1.3 Nutrition anti-inflammatory (NAI) score

A research group in Spain also developed an empirical dietary inflammatory score for use in Mediterranean populations. The MEDIS (MEDiterranean ISlands) study included 24 Mediterranean islands and the rural Mani region (Peloponnesus) of Greece. A validated MEDIS FFQ was used to identify a total of seven food components to develop the NAI score. The seven food components included: energy balance, proteins, carbohydrates, total fats as percentage of energy intake, tea, coffee and alcohol consumption (Tyrovolas *et al.*, 2018). The calculation of the NAI score is described by Tyrovolas *et al.*, 2018. Briefly, each of the food components received a rating of “+1” or “-1” according to their anti-inflammatory effects. A “+1” was assigned to all food components which had a low anti-inflammatory effect, and a “-1” to all food components which had a high anti-inflammatory effect. With the help of the NAI score, nutrients and foods were categorised as high, medium and low anti-inflammatory (Tyrovolas *et al.*, 2018). A higher NAI score indicated lower anti-inflammatory potential (Tyrovolas *et al.*, 2018). The NAI score was created in such a way to avoid collinearity between the aforementioned components to ensure that the statistical analysis is correctly performed (Tyrovolas *et al.*, 2018). The following table shows how each food component received its rating.

Table 2-2: Foods included in the Nutrition Anti-inflammatory Score and their individual rating based on consumption

Food component	+1	-1
ENERGY	Energy balance > 0	Energy balance < 0
PROTEIN	Higher or lower than recommended range	Within recommended range

Table 2.2 *continued*

CARBOHYDRATES	Higher or lower than recommended range	Within recommended range
TOTAL FAT	Higher or lower than recommended range	Within recommended range
TEA	Consumed	Not consumed
COFFEE	Consumed	Not consumed
ALCOHOL	Consumed	Not consumed

[Adapted from Tyrovolas (2018)]

Disadvantages of empirical scores

The strength of empirical scores lies in the fact that the dietary intake and inflammatory marker data used to construct these scores were obtained within the same study population, directly relating the dietary intakes to the degree of inflammation. There are, however, some disadvantages to the use of such empirically-based scores, as highlighted by Hébert *et al.* (2019).

Food groups that were included did not represent the intake from different parts of world. Food items like processed meat, organ meat, and pizza are not necessarily consumed in all parts of the world, therefore limiting the use of these scores in populations with diets that differ from those investigated in the empirical score studies.

- The origin of empirical scores requires data on the inflammatory markers (IL-6, CRP, and TNF- α). Thus, if a study did not collect any of the inflammatory markers, the pattern cannot be computed.
- An FFQ was used to calculate the score, which means that there is a limited set of dietary questions, while in the literature-based approach, a food list-unconstrained approach is used, and thus there is no restriction or limitation on the foods that can be included in the score.
- If a pattern is originally created to predict CRP, it may differ from a pattern that is originated to predict IL-6, interleukin-1 beta (IL-1 β) or TNF- α , because the score is extremely dependent on the inflammatory markers being calculated.

2.4.2 Literature-based score – Dietary Inflammatory Index (DII)

The DII represents a new index for assessing the inflammatory potential of the diet. The development of the DII began in 2004 (Hébert *et al.*, 2019) with the original goal of quantifying the overall effect of diet on inflammation. In 2009 the first version of the DII was created to categorise individual diets from maximally anti-inflammatory to pro-inflammatory (Cavicchia *et al.*, 2009). The DII has been updated twice since it was first developed, with the later versions named the updated DII and the E-DII – which includes adjustment for energy. A DII was also developed for specific use in children, called the C-DII. The original DII and the adaptations made in the later versions will now be discussed.

- Original DII

The approach of the researchers who developed the DII was to identify nutrients and/or foods that have been demonstrated in the literature as being associated with inflammatory markers. All literature published between 1950 and 2007 (n=2700 articles) reporting the effect of whole foods or dietary constituents on specific inflammatory markers: IL-1 β , interleukin-4 (IL-4), IL-6, interleukin-10 (IL-10), TNF- α , and CRP, were considered for inclusion. Data from 927 articles were finally included in the original DII. The scoring algorithm included three probable values according to the effect the food parameter has on inflammation. A “+1” was assigned if the effects were pro-inflammatory; “-1” if the effects were anti-inflammatory and “0” if no significant change in the inflammatory marker was produced (Shivappa *et al.*, 2014a). In some cases, specific nutrients were reported in the literature to have both a pro- and anti-inflammatory effect. To manage this discrepancy, the mean overall effect on inflammation was used (Cavicchia *et al.*, 2009). Scoring the inflammatory index included several steps: firstly, articles were weighted by study type and design. Table 2.3 shows the contribution of the articles based on the study design. Secondly, the weighted values were used to calculate a score for each food and constituent. To calculate the score the researchers (i) divided the weighted number of pro- and anti-inflammatory articles by the total number of articles found for each specific nutrient / food component; (ii) then they subtracted the pro-inflammatory fraction from the anti-inflammatory fraction (Shivappa *et al.*, 2014a). Thirdly, the scores were adjusted for each food and constituents by the total weighted number of articles. A cut point of 100, that is the median of the total weighted articles, was chosen to indicate an optimal literature pool (Cavicchia *et al.*, 2009). Foods and food components with a weighted number of articles greater than 100 were assigned the full value of the score. If there were fewer than 100 articles, the adjustment was as follows: the total number of articles was divided by 100; that fraction was then multiplied by the score for that constituent or food, which provided the new adjusted score (Table 2.4) (Cavicchia *et al.*, 2009).

The original DII excluded eugenol and four flavonoids because these food components provided fewer than 10 total weighted articles (Cavicchia *et al.*, 2009). Next, specific scores for food and food components were multiplied by the intake for each participant. Vitamin A and beta-carotene, which are recorded in thousands of micrograms, were divided by 100 to compare the range of intake to other micronutrients. Omega-3 and -6 fatty acids were multiplied by 10 because they are recorded in gram amounts and consumption is generally in low quantities (Cavicchia *et al.*, 2009). Each food and constituent score product was summed to create the overall Inflammatory Index Score for each participant. Last, the Inflammatory Index Score was divided by 100 to help with the interpretation of statistical analyses.

Table 2-3: Study design weights based on the type of study

Type of study	Study design	Value
Human	Experimental	10
	Prospective cohort	8
	Case-control	7
	Cross-sectional	6
Animal	Experimental	5
Cell culture	Experimental	3

[Adapted from Cavicchia(2009)]

Following the publication of the original DII, some limitations were identified, as reported by Hebert *et al.* (2019). As mentioned previously, the intakes of certain nutrients such as vitamin A and beta-carotene had to be divided by 100, whereas omega-3 and omega-6 fatty acid intakes had to be multiplied by 10 to ensure that their effect on the overall inflammatory score was not biased.

- During the analysis of data, right skewing of the dietary parameters stood to be a potential problem considering that some statistical tools require normally distributed data.
- The original DII included literature only up until 2007 and therefore did not consider new findings published later on.
- Flavonoids were originally not included in the DII, based on the limited number of articles available, but they should in fact be included when calculating the DII score because they have since been identified as important modulators of systemic inflammation.

- The scoring system assigning positive scores to anti-inflammatory foods and negative scores to pro-inflammatory foods was found to be confusing and needed to be reversed.

This led to the development of a new, updated version of the DII, which was created to overcome these limitations.

- New, updated DII

Although the calculation of the new, updated DII is similar to the original score, it includes three additional years of evidence up until 2010 to provide a total of 1943 included articles (Hébert *et al.*, 2019). Since a larger literature pool was used, a cut-off point of 236 (the median of the total weighted articles) was chosen to assign the full value of the score (instead of 100, which was used in the original DII) (Shivappa *et al.*, 2014a). Table 2.5 presents the food parameters included in the new, updated DII as well as their respective inflammatory effects scores.

Table 2-4: Constituents included in the original Dietary Inflammatory Index (DII) and their adjusted scores

Constituent	Adjusted score
Energy (kJ/d)	-0.0549
Energy (kcal/d)	-0.23
Garlic (g/d)	0.27
Ginger (g/d)	0.18
Saffron (g/d)	0.18
Turmeric (g/d)	0.774
Tea (g/d)	0.552
Caffeine (g/d)	0.035
Wine (g/d)	0.48
Beer (g/d)	0.2
Liquor (g/d)	0.1
Alcohol (g/d)	0.534

Table 2.4 *continued*

Carbohydrate (g/d)	-0.346
Fibre (g/d)	0.52
Fat (g/d)	-0.323
ω -3 Fatty acids (g/d x 10)	0.384
ω -6 Fatty acids (g/d x 10)	-0.016
MUFA (g/d)	-0.05
Saturated Fat (g/d)	-0.25
Protein (g/d)	0.05
Cholesterol (mg/d)	-0.21
Vitamin A ($\mu\text{g/d} \div 100$)	0.58
Thiamine (mg/d)	0.05
Riboflavin (mg/d)	0.16
Niacin (mg/d)	0.26
Vitamin B6 (mg/d)	0.286
Folic Acid ($\mu\text{g/d}$)	0.214
Vitamin B12 ($\mu\text{g/d}$)	-0.09
Vitamin C (mg/d)	0.367
Vitamin D ($\mu\text{g/d}$)	0.342
Vitamin E (mg/d)	0.401
Beta-carotene ($\mu\text{g/d} \div 100$)	0.725
Magnesium (mg/d)	0.905
Zinc (mg/d)	0.316

Iron (mg/d)	0.029
Selenium (mg/d)	0.021
Quercetin (mg/d)	0.49
Luteolin (mg/d)	0.43
Genistein (mg/d)	0.68
Daidzein (mg/d)	0.17
Cyanidin (mg/d)	0.13
Epicatechin (mg/d)	0.12

[Adapted from Cavicchia (2009)]

Next, a reference database was developed in an attempt to standardise intakes to avoid the arbitrariness that might arise from using only raw consumption amounts. This database was compiled by including a wide range of diets across diverse populations. The authors of articles reporting on nutrition surveys in eleven different countries were contacted to request access to the complete data sets (Hébert *et al.*, 2019). These data sets included: USA- the National Health and Nutrition Examination Survey (NHANES) (2007-2008), Australia- National Nutrition Survey (1999), Bahrain- National Nutrition Survey for Adult Bahrain aged 19 years and above (2002), Denmark- Danish National Survey of Diet and Physical Activity (2002), India- Indian Health and the Mumbai cohort data set Study data set, Japan- National Nutrition Survey Report (2002), New Zealand- National Nutrition Survey Report (1997), Taiwan- Nutrition and Health Survey in Taiwan (NAHSIT) (1993-1996), South Korea- Reanalysis of 2007 Korean National Health and Nutrition Examination Survey (2007 KNHANES), Mexico- Mexican National Health and Nutrition Survey (2006) and, UK- The National Diet and Nutrition Survey: adults aged 19 – 64 years (2004) (Shivappa *et al.*, 2014a). The data set contains means and standard deviation (SDs) for the intake of 45 food parameters. If data on specific food parameters were missing, means were calculated from other data sets or articles that published mean values for the respective populations (Hébert *et al.*, 2019).

A z-score for each individual's intake of a specific food parameter could thus be computed by subtracting the standard global mean, obtained from the reference database, from the reported dietary intake and then dividing it by the global standard deviation reported in the reference database (Peres *et al.*, 2017). To reduce the effect of right skewing, these values are expressed as cumulative proportions by multiplying the data by 2 and subtracting 1 (Hébert *et al.*, 2019). The

centred values for each food parameter are then multiplied by its overall food parameter-specific inflammatory effect score to obtain the food parameter-specific DII score. To create the overall DII score for an individual, all the food parameter-specific DII scores are summed (Harmon *et al.*, 2017).

Table 2-5: Food parameters included in the new, updated Dietary Inflammatory Index (DII) and each inflammatory effect score

Food parameter	Overall inflammatory effect score
Alcohol (g)	-0.278
Vitamin B12 (µg)	0.106
Vitamin B6 (mg)	-0.365
β-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)	0.298
Fibre (g)	-0.663
Folic acid (mg)	-0.190
Garlic (g)	-0.412
Ginger (g)	-0.453
Fe (mg)	0.032
Mg (mg)	-0.484
MUFA (g)	-0.009
Niacin (mg)	-0.246

Table 2.5 *continued*

Ω-3 Fatty acids (g)	-0.436
Ω-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
Thiamin (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

Table 2.5 *continued*

Anthocyanidins (mg)	-0.131
Isoflavones (mg)	-0.593
Pepper (g)	-0.131
Thyme/oregano (mg)	0.102
Rosemary (mg)	-0.013

[Adapted from Shivappa (2014)]

The development of the new, updated DII led to several articles assessing the inflammatory potential of the diet. During this process, it was observed that there seems to be a relationship between total calorie intake and the DII score. In most populations, the DII score decreased with an increase in calorie intake (Ge *et al.*, 2015; Harmon *et al.*, 2017; Peres *et al.*, 2017). The DII was consequently updated once again to account for this association with energy intake, now called the E-DII.

- Energy-adjusted Dietary Inflammatory Index (E-DII)

The energy-adjusted dietary inflammatory index (E-DII) controls for energy intake by standardising intakes to a total energy intake of 1000 kcal (Mazidi *et al.*, 2017), equivalent to 4180 kJ (Shivappa *et al.*, 2017a) energy content of food consumed (Shivappa *et al.*, 2017a). The E-DII is calculated by dividing food parameters by the total energy consumed and then multiplying by 1000 kcal (4180 kJ) (Shivappa *et al.*, 2017a). Recent published articles using the E-DII improved prediction in comparison with unadjusted DII scores and can be used to compare DII scores across populations (Hébert *et al.*, 2019)

- Children's Dietary Inflammatory Index (C-DII)

A DII was also developed for children, namely the C-DII. The C-DII is similar to the current E-DII method (Khan *et al.*, 2018). For the C-DII, a world reference database (16 sets of data) (Hébert *et al.*, 2019) with food parameters that could be used to calculate the C-DII among children was also created (Khan *et al.*, 2018). The food parameters used to calculate C-DII scores are: vitamin A, thiamine, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, vitamin D, vitamin C, vitamin E, beta carotene, energy, carbohydrates, fibre, total fat, saturated fat, mono-unsaturated fatty acid (MUFA), poly-unsaturated fatty acid (PUFA), cholesterol, protein, alcohol, iron (Fe), magnesium (Mg), selenium (Se), and zinc (Zn) (Khan *et al.*, 2018). The data of different published papers, for

children aged 6-14 years with a sample size of more than 200, were used (Khan *et al.*, 2018). The C-DII differs slightly from the DII in that the C-DII identified only 25 food parameters compared with the DII, which identified 45 food parameters (Khan *et al.*, 2018).

Since the publication of the DII, several research groups have used it to investigate the inflammatory potential of the diet in different populations. Some of these authors, however, made a number of adjustments to the DII. In particular, certain food components were excluded to avoid overestimating their inflammatory effect. These components include total energy, total fat, and alcohol. These authors argued that the inflammatory effect of total energy will be represented by the energy provided by the macronutrients, which are already included in the index and should therefore not be additionally included in the index calculation (Agudo *et al.*, 2017; van Woudenberg *et al.*, 2013; Harmon *et al.*, 2017; Solans *et al.*, 2020). The same is seen for total fat. The inflammatory effect of total fat is the sum of the inflammatory effect of the separate fat components (Agudo *et al.*, 2017; van Woudenberg *et al.*, 2013; Harmon *et al.*, 2017; Solans *et al.*, 2020). Other methodology adaptations included the modification of the inflammatory effect that alcohol holds. The DII considered alcohol to be anti-inflammatory, but this was true only for literature that reported low/moderate alcohol intake (less than 30 – 40 g/d) (Avellone *et al.*, 2006; Sierksma *et al.*, 2002). Therefore, these researchers weighted alcohol to “0” if a participant consumed more than 40g of alcohol per day and considered only intakes less than 40g as anti-inflammatory (van Woudenberg *et al.*, 2013; Harmon *et al.*, 2017; Solans *et al.*, 2020). Other groups removed individual alcoholic beverages if total ethanol intake was included or *vice versa* (Calder *et al.*, 2011).

In conclusion, the following table reports the main differences between the empirical and literature-based approaches discussed above (Tabung *et al.*, 2017). Clearly understanding the difference between the literature-based and the empirically-based approach is necessary when deciding which tool to use for calculating the dietary inflammatory potential of individuals in a specific population.

Table 2-6: Methodological differences between the Dietary Inflammatory Index (DII) and Empirical Dietary Inflammatory Pattern (EDIP)

Main elements	DII	EDIP
Approach	Literature-based	Empirically-based
Statistical methods	Normalisation and standardisation	Reduced rank regression and stepwise linear regression

Table 2.6 *continued*

Predictors	Mainly nutrients	Food and food groups
Outcomes	6 inflammatory markers: IL-1 β , IL-4, IL-6, IL-10, TNF- α and CRP	3 inflammatory markers: IL-6, TNF- α and CRP
Number of components	45 components 36 anti-inflammatory; 9 pro-inflammatory	18 components 9 anti-inflammatory; 9 pro-inflammatory
Weights	Derived from literature	Derived from regression analyses
Influenced by supplements	Yes	No
Score interpretation	Higher score indicates pro-inflammatory diet; lower score indicates anti-inflammatory diet	Higher score indicates pro-inflammatory diet; lower score indicates anti-inflammatory diet

Adapted from [Tabung et al. (2017)]

DII: Dietary Inflammatory Index; EDIP: Empirical Dietary Inflammatory Pattern; IL-1 β : interleukin-1 beta; IL-4: interleukin 4; IL-6: interleukin-6; IL-10: interleukin-10; TNF- α : tumour necrosis factor- α ; CRP: C-reactive protein

These dietary inflammatory indices have successfully demonstrated relationships between the consumption of anti-/pro-inflammatory diets and inflammatory markers (Julia *et al.*, 2017; Mayr *et al.*, 2018; Shivappa *et al.*, 2017a; Shivappa *et al.*, 2017b), CVD risk factors (O'Neil *et al.*, 2015; Pimenta *et al.*, 2015; Ruiz-Canela *et al.*, 2015; Sokol *et al.*, 2016; Wirth *et al.*, 2014), incidence of CVD (Garcia-Arellano *et al.*, 2015; Ramallal *et al.*, 2015), cancer (Accardi *et al.*, 2019; Ge *et al.*, 2015; Li *et al.*, 2018; Maisonneuve *et al.*, 2016; Tabung *et al.*, 2015; Zamora-Ros *et al.*, 2015) and mortality (Deng *et al.*, 2017; Graffouillere *et al.*, 2016).

2.5 The DII in the context of African diets

While dietary inflammatory indices have successfully been linked to inflammation and CVD, much less is known regarding these indices in the context of dietary patterns of African populations. Traditional African diets, which consist mainly of indigenous foods, are different from both Western and Mediterranean diets (for which these indices were developed) and need to be explored in relation to their inflammatory characteristics. Within the context of South Africa, the consumption of traditional African diets is varied, with more traditional intakes found in rural

environments with a transition into Westernised dietary patterns in urban populations. Participants investigated in this study formed part of the Prospective Urban and Rural Epidemiology (PURE) study, which specifically recruited and compared Black South Africans in rural and urban study sites in the North West Province of South Africa. This section provides background information on the dietary intakes of the PURE study participants.

Based on previous analysis, participants within the PURE study had a low socio-economic status that affected their dietary intake (Dolman *et al.*, 2014). Rural participants generally followed a low-energy, low-fat diet whereas urban participants followed a more Westernised high-energy, high-fat diet (Vorster, 2002), which evidence suggests can increase an individual's risk of CVD (Dolman *et al.*, 2014; Pillay-van Wyk *et al.*, 2016). Additionally, it was reported that the rural areas experienced a drought in 2005 that affected availability of food and income in rural compared with urban settings (Wentzel-Viljoen *et al.*, 2018b). In comparison with rural participants, the urban group consumed more of almost all the nutrients and foods in 2005 (Mchiza *et al.*, 2015). According to the Dietary Reference Intake (DRI), the acceptable macronutrient distribution range for fat, protein and carbohydrates is 20-35%, 10-35% and 45-65% respectively (Escott-Stump & Mahan, 2008). Mchiza *et al.* (2015) investigated the percentage energy derived from macronutrients for PURE participants in 2005. While most macronutrient intakes were within the recommended ranges, there were, however, differences in intake between those of rural/urban status and men and women. Urban participants consumed higher amounts of fat and protein. Urban women had the highest intake of fat and urban men the highest intake of protein. Compared with urban participants, rural women, however, consumed higher amounts of carbohydrates, followed by rural men. The World Health Organization (WHO) recommends added sugar intake of less than 10% of total energy or 25 g/d (WHO, 2015). Urban participants had higher intakes of added sugar (56.5 g) in comparison with their rural counterparts (32.5 g). The mean intake for fibre was below the Recommended Dietary Allowance (RDA) for both men (38 g) and women (25 g) (Escott-Stump & Mahan, 2008) but higher amounts were consumed in the urban group. Furthermore, both rural and urban participants had very low intakes of fruit and vegetables, below the recommended intake of 400 g/d (Dolman *et al.*, 2014), which may account for the low fibre intake. Fruit and vegetables are also important sources of other anti-inflammatory components.

In an effort to demonstrate the differences in dietary intakes between men and women in rural and urban settings, Wentzel-Viljoen *et al.* (2018) compared which food items were consumed by 80% or more of the participants in the respective groups (Table 2.7) (Wentzel-Viljoen *et al.*, 2018a). From this list it is clear that although a number of the same food items were consumed by more than 80% of both rural and urban men and women, the urban participants consumed more food (a wider variety) than did their rural counterparts (both men and women).

Table 2-7: Table of more than 80% of foods consumed by rural/urban and men and women, listed from most consumed

Urban women	Urban men	Rural women	Rural men
Cooked porridge (e.g. maize meal, oats)	Cooked porridge (e.g. maize meal, oats)	Cooked porridge (e.g. maize meal, oats)	Cooked porridge (e.g. maize meal, oats)
Sunflower oil	Sunflower oil	Sunflower oil	Sunflower oil
Samp, maize rice, wheat rice, rice, macaroni, spaghetti	Samp, maize rice, wheat rice, rice, macaroni, spaghetti	Samp, maize rice, wheat rice, rice, macaroni, spaghetti	Samp, maize rice, wheat rice, rice, macaroni, spaghetti
Sugar, white and brown	Sugar, white and brown	Sugar, white and brown	Sugar, white and brown
Bread, rolls, vetkoek	Bread, rolls, vetkoek	Bread, rolls, vetkoek	Bread, rolls, vetkoek
Chicken, turkey, goose, duck, cooked, including Kentucky	Chicken, turkey, goose, duck, cooked, including Kentucky	Chicken, turkey, goose, duck, cooked, including Kentucky	Chicken, turkey, goose, duck, cooked, including Kentucky
Yellow and red vegetables (e.g. red peppers, carrots, pumpkin, beetroot)	Yellow and red vegetables (e.g. red peppers, carrots, pumpkin, beetroot)	Yellow and red vegetables (e.g. red peppers, carrots, pumpkin, beetroot)	Yellow and red vegetables (e.g. red peppers, carrots, pumpkin, beetroot)
Coffee and tea	Coffee and tea	Coffee and tea	Coffee and tea
Other cooked vegetables (e.g. parsnips, green beans, peas)	Other cooked vegetables (e.g. parsnips, green beans, peas)	Other cooked vegetables (e.g. parsnips, green beans, peas)	Other cooked vegetables (e.g. parsnips, green beans, peas)
Green vegetables (e.g. spinach, cabbage, coleslaw, broccoli)	Green vegetables (e.g. spinach, cabbage, coleslaw, broccoli)	Green vegetables (e.g. spinach, cabbage, coleslaw, broccoli)	Green vegetables (e.g. spinach, cabbage, coleslaw, broccoli)
Tomato, cooked and raw	Tomato, cooked and raw	Tomato, cooked and raw	Tomato, cooked and raw
White-flesh fruit (e.g. apples, bananas, pears)	White-flesh fruit (e.g. apples, bananas, pears)		

Table 2.7 continued

Starchy vegetables (e.g. potato, sweet potato, mealies)	Starchy vegetables (e.g. potato, sweet potato, mealies)
Beef, cooked + meatballs	Beef, cooked + meatballs
Milk, full fat	Milk, full fat
Meat products (e.g. boerewors, biltong, ham, corned meat, Viennas, frankfurter, salami, patties)	Meat products (e.g. boerewors, biltong, ham, corned meat, Viennas, frankfurter, salami, patties)
Organ meats (e.g. liver, kidney, tripe, offal, heart, giblets, lung, tongue, etc.)	

[Adapted from Wentzel-Viljoen (2018)]

The above is evidence that the dietary intakes of the PURE participants are very different from the dietary patterns that were used to derive the DII. It is therefore of great importance to understand the dietary intake and pattern of urban and rural participants when considering the inflammatory index of the diet of the PURE study population.

2.6 Conclusion

Recently, CVD mortality has increased significantly in South Africa and from this literature review it is evident that inflammation is intricately linked to CVD development. Evidence points, furthermore, to a potential causal role for fibrinogen concentration and the ensuing clot structure in CVD aetiology. Less is known however, about how the relationship between inflammation and fibrinogen concentration can affect clot properties, specifically in Black South Africans. Additionally, studies report a strong relationship between dietary components and inflammation that led to the development of dietary indices to reflect the overall inflammatory potential of individual diets. However, the inflammatory potential of diets consumed by Black South Africans has not yet been studied and these studies are necessary to increase our knowledge regarding possible pathways through which diet increases continental Africans' risk of CVD, such as through

altering fibrinogen concentration and the concomitant blood clot structure. The following chapter (Chapter 3) provides the methodology and study design that will be used to investigate the dietary inflammatory potential of a Black South African study population and its association with inflammatory markers, fibrinogen concentration and plasma clot properties.

CHAPTER 3 METHODS

3.1 Introduction

Cardiovascular disease (CVD) is increasing at an alarming rate in South Africa and the literature reports that inflammation plays a major role in CVD. It is also known that diet contributes to inflammation and that inflammation increases the expression of fibrinogen concentration and alters the fibrin clot structure. However, the relationship between diet, inflammation and fibrinogen concentration and fibrin clot structure in Black South Africans has not been investigated. This study therefore aimed to determine whether the inflammatory potential of the diet is associated with plasma levels of inflammatory markers, fibrinogen concentration and fibrin clot structure in 2010 Black South Africans who participated in the Prospective Urban and Rural Epidemiology (PURE) study in 2005.

The objectives of the study were:

- To adapt the published energy-adjusted dietary inflammatory index (E-DII) for the participants who took part in the PURE, South Africa, North West (PURE-SA-NW) study in 2005, based on their dietary intakes;
- To determine the relationship between the E-DII and inflammatory markers (C-reactive protein (CRP), albumin, soluble urokinase-type plasminogen activator receptor (SuPAR) and interleukin-6 (IL-6));
- To determine the relationship between the E-DII and fibrinogen concentration and plasma clot properties obtained using turbidimetry;
- To determine whether the relationship between the E-DII and clot properties is mediated by inflammation and/or fibrinogen concentration.

3.2 Ethics approval

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

3.3 Study design

The international PURE study is a large-scale, prospective, epidemiological study aimed at 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 Western Cape and North West province are currently the only two study sites in South Africa. For this MSc, only the data collected in the North West province (PURE SA-NW) will be used. Baseline data were collected in 2005 and follow-up data collected in 2010 and 2015. This MSc dissertation is a cross-sectional study that investigated the 2010 participants who participated in the 2005 PURE-SA-NW study.

3.3.1 Study population

Permission to access and recruit individuals within the community was granted on a judicial (national) and goodwill (tribal/community and household) basis, by the South African Department of Health, Potchefstroom and Ganyesa city mayors, tribal chiefs, community leaders (acting as gatekeepers) and household heads respectively. Tswana-speaking individuals residing in Ikageng, Zonderwater, Extension 7 or Extension 11 (urban settlements); Ganyesa (urban-rural town); or Tlaskgameng (rural area) were recruited for the PURE-SA-NW study. These areas were chosen in an effort to represent the urban, urban-rural (transitional) and rural areas of the North West province equally.

Upon approval by all the above-mentioned stakeholders, possible participants were approached; if their verbal consent was given, a trained fieldworker then explained the study to possible participants in their home language (English, Afrikaans, Tswana). Individuals then had one week to discuss their participation with their family members.

Fieldworkers returned to the individual's place of residence after a week to obtain written voluntary informed consent (Annexure B). Illiterate participants gave verbal consent in the presence of an independent witness who signed on the participants' behalf. Participants had the option to decide whether or not they would show up on the data collection day, regardless of their initial decision to be involved or not. Individuals who made the decision not to be involved in the PURE study and did not show up for baseline measurements were consequently not approached with any

further requests for involvement. Participants were constantly informed that they could withdraw from the study at any stage.

3.3.2 Recruitment

A door-to-door approach was used to recruit individuals in the community. Trained fieldworkers selected an address randomly in each community and spoke to all possible participants individually. Fieldworkers were selected from the communities and were able to communicate in the home language of the study participants. The fieldworkers had no conflict of interest.

Based on a power calculation published by Vorster (2002), a participant group of 2000 or more was required for the PURE study (Vorster, 2002). Therefore, a total of 6000 households was approached during recruitment. The selected communities and the households included were considered to be stable regarding movement in and out of the area.

3.3.3 Inclusion and exclusion criteria

Individuals were eligible to participate in the PURE study if they were apparently healthy, older than 30 years, and not using any acute or chronic medication (apart from blood pressure medication). Individuals also had to be willing to take part in follow-up data collection for the subsequent 10 years. Finally, individuals were eligible to participate if they were voluntarily willing to be involved in the study, and if the individual was able to fully comprehend the information given prior to signing voluntary written informed consent. Consequently, individuals who were inebriated or not capable of being fully informed at the time of consent, were not allowed to take part. The head of the households approached had to approve the participation of household members. If participation was denied, a non-complier questionnaire was completed, and the house as a whole was excluded.

After being fully informed, participants had the opportunity to sign the informed consent form. Participants were also asked to complete a validated questionnaire regarding their health (physical and psychological), lifestyle, socio-economic status and background. Participants were informed of the dates and travel arrangements regarding the applicable data collection and were given all the necessary information relating to their participation. Participants had the option of showing up for data collection or not, and in case of the latter, no further participation was required. Out of the 3750 participants that filled out the questionnaires upon the initial informed consent, 2010 decided to participate further, and took part in the baseline data collection. Participants had the opportunity to question the researchers directly during data collection.

3.4 Anthropometrical measurements

Body composition is related to diet, inflammation and haemostasis, and was consequently considered as a possible confounder or mediator when investigating the relationship between dietary inflammation and cardiovascular outcomes.

For all anthropometrical measurements, participants had to remove their shoes and wear minimal clothing to ensure accuracy. Privacy was ensured by performing measurements in closed, separate rooms. Standard procedures were used (0.1 cm or 0.01 kg) and anthropometric assessments were performed in accordance with guidelines of the International Society for the Advancement of Kinanthropometry (ISAK).

3.4.1 Height

Height was measured to the nearest 0.1 cm on a calibrated Invicta Stadiometer (IP 1465, Invicta, London, UK, Stadiometer). Participants stood upright with their arms hanging freely at their sides, shoulders relaxed, legs straight and heels together. Participants' shoulder blades, buttocks and heels had to touch the stadiometer. The participant's head was held in the Frankfurt position (lowest point of the eye orbit in line with the tragon of the ear) and the participant had to inhale fully and maintain the position before the measurement was taken.

3.4.2 Weight

Weight was measured to the nearest 0.01 kg (Electronic scale, Precision Health Scale, A&D Company, Tokyo, Japan). Scales were placed on an even, stable horizontal floor and calibrated daily with standard weights. Apart from measuring participants with minimal clothing and barefoot, participants were also instructed to keep their arms freely at their sides.

3.4.3 Body Mass Index (BMI)

BMI represents an attempt to "standardise" the weight in terms of height - a single measure to compare individuals with different heights. BMI is calculated by an equation:

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

The World Health Organization (WHO) defined the following categories commonly used in nutritional epidemiology studies (WHO, 2000).

- Underweight when BMI ≤ 18.5 (kg/m²)

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

3.4.4 Waist and hip circumference

Waist and hip circumference were measured with a Holtain non-stretchable metal tape. The WHO states that a waist circumference measurement must be made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest (WHO, 2011). A hip circumference measurement should be taken around the widest portion of the buttocks.

Waist-to-hip ratio (WHR) is used as an indicator or measure of health, and the risk of developing certain health conditions. WHR is calculated as waist circumference divided by hip circumference (Waist Circumference $\div$ Hip Circumference). According to the WHO, a healthy WHR is (WHO, 2011):

- 0.9 or less in men
- 0.85 or less for women

In both men and women, a WHR of 1.0 or higher increases the risk of CVD and other conditions that are all related to being overweight or obese (WHO, 2011).

3.5 Biochemical analysis

Fasting blood samples (90 ml) were collected from the antecubital vein by a registered nurse, using sterile winged infusion sets and syringes with disposable needles. Blood collection took place from 7:00 am to 11:00 am. Upon collection, samples were carried on ice to the on-site laboratory for centrifugation and storage. Samples were centrifuged at $2,000 \times g$ for 15 minutes within 30 minutes after collection and stored at -80°C until analysis.

3.5.1 Plasma glucose

Plasma glucose was quantified by Vitros DT6011 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, USA) and sodium fluoride tubes were used for the storage of plasma glucose.

3.5.2 Glycated haemoglobin

HbA1c was determined with the D-10 Haemoglobin testing system (Biorad, Hercules, California, USA) and stored in ethylenediamine tetra acetic acid (EDTA) tubes.

3.5.3 Uric acid

Uric acid was quantified using the Konelab20i™ auto-analyser (Thermo Fischer Scientific, Vantaa, Finland)

3.5.4 Plasma Homocysteine (Hcy)

Plasma homocysteine was stored in EDTA tubes. The Abbott automated immunoassay analyser (AxSYM) was used to measure plasma Hcy concentrations.

3.5.5 Plasma clot properties

Blood intended for the analysis of haemostatic variables was collected in 3.2% sodium citrate tubes. Markers of plasma clot formation, structure and lysis were measured using the global fibrinolytic assay. Plasma was activated with tissue factor (TF) to form plasma clots and lysed with tissue plasminogen activator (tPA), as described in the method of Lisman *et al.* (2005) (Lisman *et al.*, 2005). Tissue factor and tPA concentrations were modified slightly for the purpose of obtaining comparable clot lysis times (CLTs) of about 60–100 minutes. The final concentrations in the plasma clots were 125 x diluted TF (Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany), 17 mmol/L CaCl₂, 100 ng/mL tPA (Actilyse, Boehringer Ingelheim, Ingelheim, Germany) and 10 mmol/L phospholipid vesicles (Rossix, Mölndal, Sweden) to provide a lipid surface on which the reactions could take place. Turbidity curves were obtained from a spectrophotometer at an absorbance of 405 nm, and were analysed using Origin® software, version 8.5 (Origin lab®, 2010) (Figures 3.1 and 3.2). Lag time (minutes) was calculated as a representation of the time required for the activation of the coagulation cascade and for fibrin protofibrils to reach length sufficient for lateral aggregation. The rate of lateral aggregation was represented by the slope of the turbidity curve on the side of clot formation. The difference between absorbance at the peak of the curve and the starting point was used to determine maximum absorbance, an indicator of clot density. CLT is defined as the time from clot formation (time from the midpoint of the transition from baseline to maximum turbidity) to clot lysis (to the midpoint in transition from maximum turbidity back to baseline).

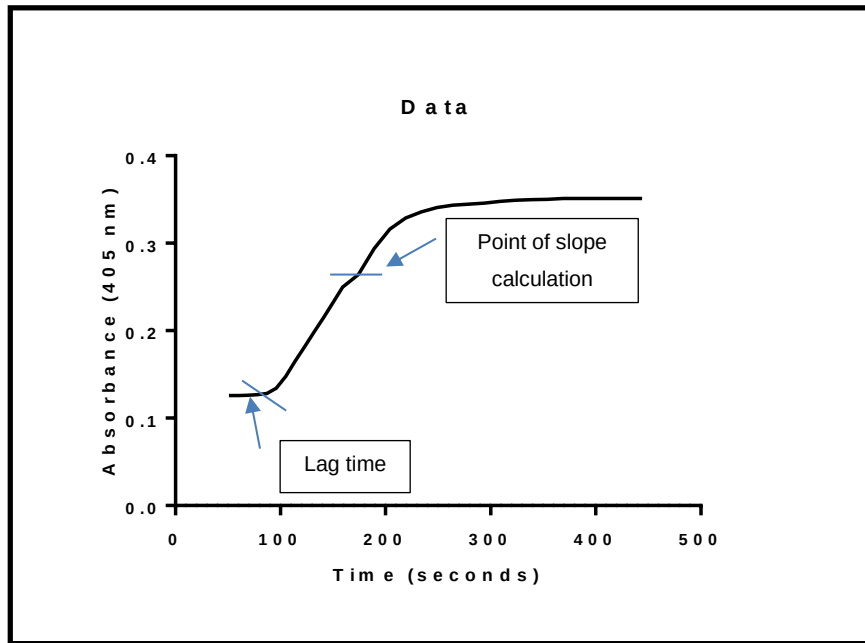


Figure 3-1: Positions on curve to be used for determination of slope and lag time (Adapted from Pieters et al., 2018).

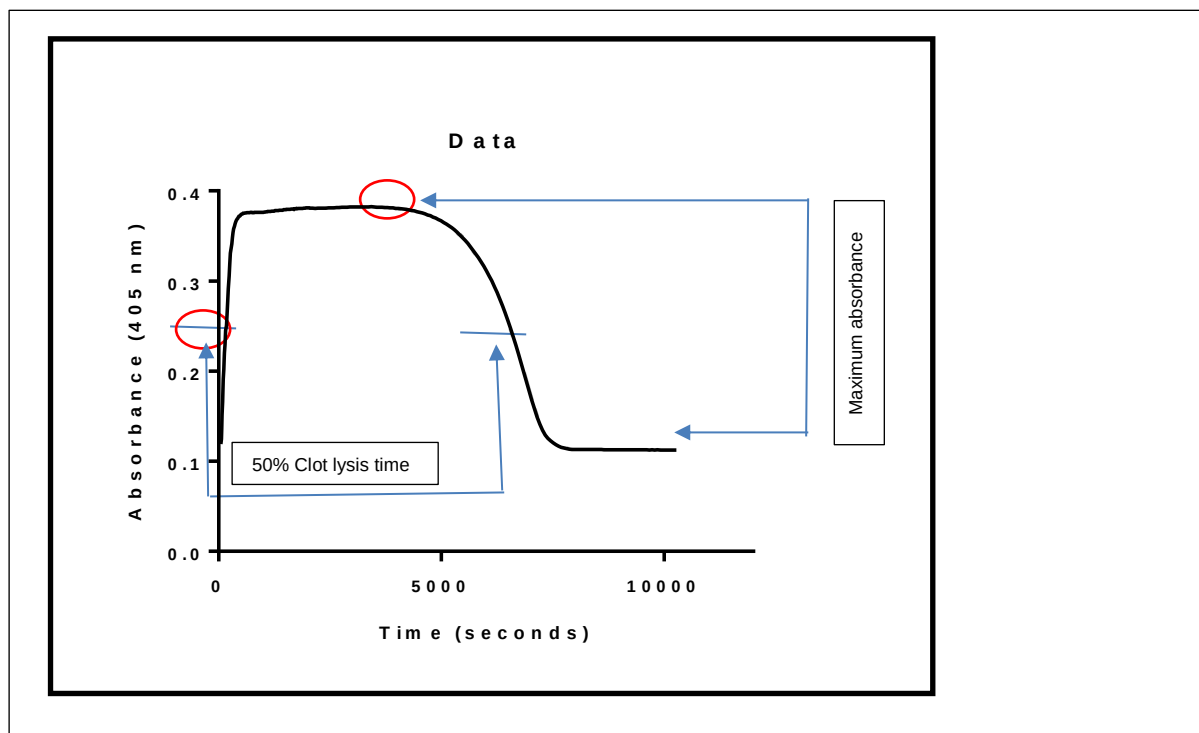


Figure 3-2: Positions on curve to be used for determination of maximum absorbance and 50% clot lysis time (Adapted from Pieters et al., 2018).

3.5.6 Total fibrinogen concentration

Total fibrinogen concentrations were quantified using the modified Clauss method on the Dade Behring BCS coagulation analyser (Multifibrin U-test Dade Behring, Deerfield, IL, USA). This analysis focuses on the prothrombin time, where rapid thrombin production and formation of fibrin fibres occur. A standard curve was prepared, using a series of diluted calibration plasma at 25% and 50%, to calculate the quantity of fibrinogen concentration in the sample plasma, expressed as g/L.

3.5.7 Fibrinogen γ' concentration

An enzyme-linked immunosorbent assay (ELISA) using a Santa Cruz Biotechnology (Santa Cruz, USA) 2.G2.H9 mouse monoclonal coating antibody against human γ' , and an Abcam (Cambridge, USA) goat polyclonal HRP-conjugated antibody against human fibrinogen, was used to determine fibrinogen γ' concentrations. This was done according to the method of Uitte de Willige *et al.* (2005). Fibrinogen γ' was reported as a relative concentration (%) of total fibrinogen concentration (reported as γ' ratio).

3.5.8 Inflammatory markers

Blood used to quantify inflammatory marker concentrations was collected in tubes not containing anti-coagulants.

High-sensitivity CRP (hsCRP) and albumin was quantified using the Konelab20i™ auto-analyser (Thermo Fischer Scientific Oy, Vantaa, Finland). Serum IL-6 was analysed through enzyme immunoassays (Elecsys 2010, Roche, Basel, Switzerland). Soluble urokinase-type plasminogen activator receptor was quantified using the suPARnostic® ELISA kit (ViroGates, Copenhagen, Denmark).

3.5.9 Blood lipids

Blood lipids (triglyceride, high-density lipoprotein [HDL] and total cholesterol [TC]) were quantified using the Konelab20i™ auto-analyser (Thermo Fischer Scientific, Vantaa, Finland), whereas low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald–Levy–Fredrickson formula (Roberts, 1988). However, this equation does have some limitations and was not used under the following circumstances:

- when chylomicrons were present
- when plasma triglyceride concentration exceeded 400 mg/dL (4.52 mmol/L)

- in patients with dysbetalipoproteinaemia (type III hyperlipoproteinaemia)

3.5.10 Blood pressure

The Omron HEM-757 (Omron Healthcare, Kyoto, Japan) was used to determine brachial systolic and diastolic blood pressure (bSBP, bDBP), and heart rate (HR). Blood pressure was measured in duplicate upon a 10-minute resting period. Pulse pressure (PP) was calculated as the difference between SBP and DBP.

3.6 Quantitative food frequency questionnaire

Data regarding food consumption and dietary habits were captured by means of a country-specific, validated quantitative food frequency questionnaire (QFFQ) (MacIntyre *et al.*, 2002) (Annexure C). Fieldworkers were extensively trained in administering the QFFQ and how to quantify the participants' food intake. Fieldworkers had all the appropriate equipment (household measures, sponge food replicas, utensils, food picture atlas) needed for an accurate estimate of general food consumption. The QFFQ was checked for completeness and if there were any missing data the fieldworkers could correct this while the participant was still on site. The coding and capturing of the data was done by the researchers as well as trained research assistants. The foods were coded according to the Medical Research Council's (MRC) Food Composition Tables (FCT) (Wolmarans, 2010) and the amounts were determined in grams, using the Medical Research Council Food Quantities Manual. Household measures of food were converted to grams by using standard tables (Langenhoven *et al.*, 1991). Food intake and nutrient analyses were performed by the South African MRC at their Biostatistics Unit based on the South African FCT (Wolmarans, 2010).

The FCT are continuously being updated to increase the nutrient coverage in these tables and also to address any fortification legislation that will alter the nutrient content of the fortified food items. The FCT originally used to calculate the PURE dietary intakes of 2005, have therefore since been updated. However, it was decided to use the original nutrient analyses rather than re-analysing the intakes with the latest FCT, in order to prevent inaccurate information resulting from any food fortification that became mandatory after 2005. We did however, compare the amount of missing data in the FCT originally used, and the data available in the most recent version for β -carotene (as included in the original DII); we noticed a significant decrease in the number of food items with missing β -carotene data. We therefore imported the updated β -carotene data to the original FCT and re-analysed the β -carotene data to provide a more accurate reflection of the β -carotene intake.

The E-DII furthermore included three food components not specifically addressed in the FCT, omega-3 and -6 fatty acids and flavonoids. The following steps were taken to address this in order to ensure an accurate representation of these nutrients in the E-DII calculation of the PURE participants.

3.7 Flavonoid content

The following flavonoids were included in the original DII as set out by Shivappa *et al.* (2014): Anthocyanidins, Flavan-3-ol, Flavonones, Flavones, Flavonols and Isoflavones. Food included in the South African FCT, have, however, not yet been analysed for flavonoid content. We therefore determined the flavonoid content of the foods consumed by the PURE participants, using the Phenol Explorer programme, a comprehensive database of the polyphenol content in foods. Phenol Explorer provides the flavonoid content for raw food, expressed as mg/100 g fresh weight. Therefore, for all raw, fresh foods, the amount of flavonoid is used as is. To calculate the flavonoid content for cooked or dried food the following steps were followed:

- The FCT (Wolmarans, 2010) provides the moisture content for all the food as well as recipes used in the QFFQ.
- Each of the different flavonoids (Anthocyanidins, Flavan-3-ol, Flavonones, Flavones, Flavonols and Isoflavones), is reported in the Phenol Explorer according to a number of sub-categories of these compounds. The amounts of the sub-categories were summed to provide the total amount of the flavonoids specified in the DII, for each food item.
- This amount provided the total flavonoid content (mg) in 100 g of fresh food
- To calculate the flavonoid content of dried or cooked food, the moisture content of the same fresh food is subtracted from 100.
- This amount provided the remaining solid content for each food.
- The following equation was used to determine the exact flavonoid content for cooked or dried food:

Example: Flavonoid content of dried apple

1. There is 135.98582 mg flavonoid in 100 g fresh apple with a moisture content of 83.9
2. $100 - 83.9$
=16.1 solid apple
3. There is thus 135.98582 mg flavonoid in 16.1 solid apple
4. Dried apple has a moisture content of 31.8
5. $100 - 31.8$
=68.2 solid apple

6. Thus, if there is 135.98582 in 16.1 g solid (fresh) apple, how much is in 68.2 g (dried) solid apple?

$$= 135.98582 \times 68.2 / 16.1$$

$$= 576.04 \text{ mg flavonoid in 100 g dried apple}$$

3.8 Fatty acid content

Although data are missing for fatty acids in the FCT, it was decided that the available fatty acid data would give an accurate reflection of the fatty acid content of the foods consumed based on the following:

- A list was compiled that reported food groups and food products that 80% or more of the population consumed
- This list ensured that consumptions amounts were calculated for food that were consumed by 80% or more of the population
- It was noticed that food groups and food rich in omega-3 and -6 had fewer missing values than food groups and food that had a lower omega-3 and -6 content
- Foods that were lower in omega-3 and omega-6 content and had more missing values, were not consumed by 80% or more of the population
- Thus, if there were missing values for certain foods or food products, we first determined if our population actually consumed this product, then decided whether the reported intake was sufficient to be included in the DII
- Since food consumed by at least 80% of the PURE study population had sufficient fatty acid data, the data as reported in the FCT were deemed an accurate reflection of the actual intakes. Omega-3 fatty acids were calculated by summing the individual intakes of the following carbon bonds: C18_3 C18_4 C20_5 C22_5 and C22_6. Furthermore, omega-6 intake was calculated by summing carbon bonds C18_2 and C20_4.

3.9 Dietary Inflammatory Index

The published E-DII was used (Shivappa *et al.*, 2014a), and adapted to the PURE-SA-NW participants' diets. In particular, any nutrients and/or food components that were not included in the PURE-SA-NWU-2005 data set were excluded from the analyses, as is commonly done by researchers using the E-DII (Garcia-Arellano *et al.*, 2019; Hodge *et al.*, 2018; Phillips *et al.*, 2018; van Woudenberg *et al.*, 2013; Yang *et al.*, 2019). The E-DII additionally adjusts all intakes to 4180 kJ to exclude the potential confounding effect that differences in energy intake may have on the outcome of the score.

Several steps were followed prior to creating an E-DII for each PURE-SA-NW participant:

- Firstly, participants who reported consuming less than 3000 kJ or more than 30000 kJ were removed from the dataset in order to exclude under- or over-reporters.
- Thereafter, any nutrients that were not included in the E-DII list were removed from the data set and a separate data sheet was created that included only the foods according to the E-DII list.
- Within this data set, the total consumption amounts for, tea, caffeine and onions were calculated. The consumption of caffeine for each participant was calculated by adding the total intake of coffee then dividing the coffee consumption by 100 and then multiplying the answer by 68, since there are 68 mg of caffeine in 100 ml of the type of coffee mostly consumed by PURE participants. Additionally, the contribution of caffeine from black tea (brewed) was also considered. The total intake was determined for each participant, divided by 100 and then multiplied by 20, since there are 20 mg of caffeine in 100 ml of the type of tea that is mostly consumed by PURE participants. The final answers derived from the coffee and tea calculations were summed to obtain the total caffeine intake.
- To determine the overall tea intake, the intake of both rooibos and black tea (brewed) was summed for each participant.
- In order to calculate the onion intake for each participant, the weighted onion intake within each recipe was determined. Thus, the total amount of a specific onion-containing recipe consumed was divided by 100 and then multiplied by the amount of onion(g) per 100 g within each recipe, as seen below. Thereafter, the different onion intakes were summed to determine the total onion intake for each participant:
 - Spinach (Swiss Chard, cooked with potato, onion, sunflower oil): 10 g of onion (boiled)/ 100 g
 - Green beans (cooked with potato, onion and sunflower oil): 10 g of onion (boiled)/ 100 g
 - Carrot (cooked with potato, onion and sunflower oil): 10 g of onion (boiled)/ 100 g
 - Cabbage (cooked with potato, onion and sunflower oil): 10 g of onion (boiled)/ 100 g
 - Chicken (with skin, stew, tomato and onion): 14 g of onion (boiled)/ 100 g
 - Fish (casserole low fat fish, tomato and onion sauce): 25 g of onion (boiled)/ 100 g
 - Tomato and onion (stewed no sugar): 25 g of onion (boiled)/ 100 g
 - Tomato and onion (stewed with sugar): 25 g of onion (boiled)/ 100 g
 - Tomato and onion (canned): 25 g of onion (boiled)/ 100 g
 - Beef (mince lean, savoury tomato, onion): 25 g of onion (boiled)/ 100 g
 - Beef (corned, savoury, potato, onion): 15 g of onion (boiled)/ 100 g

After the data set was cleaned and the consumption amounts for the specific foods were determined, the population-specific E-DII could be created. The steps detailed below were followed to calculate the E-DII of the diet for the PURE study participants:

Step 1: Normalise the total energy consumed by each participant to 1000 kcal (4180 kJ). For each participant, multiply the nutrient intake 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) for nutrients and food components consumed in the PURE-SA-NW population.

Step 3: Standardise intakes 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 dividing the difference by the SD of the PURE study 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 is subtracted.

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

Step 6: The energy-adjusted standardised dietary intake (Steps 1 to 4) of each food component or nutrient will be added for each individual 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 will be. Negative scores indicate an anti-inflammatory diet.

Table 3.1 below presents the foods and nutrients included in the published E-DII and their inflammatory effects scores.

Table 3-1: Food parameters included in the DII and each inflammatory effect score

Food parameter	Overall inflammatory effect score
Alcohol (g)	-0.278
Vitamin B12 (µg)	0.106
Vitamin B6 (mg)	-0.365
β-Carotene (µg)	-0.584
Caffeine (g)	-0.110
Carbohydrate (g)	0.097

Table 3.1 *continued*

Cholesterol (mg)	0.110
Energy (kcal)	0.180
Eugenol (mg)	-0.140
Total fat (g)	0.298
Fibre (g)	-0.663
Folic acid (mg)	-0.190
Garlic (g)	-0.412
Ginger (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
Thiamin (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:	
7.98 (more pro-inflammatory) to -8.87 (more anti-inflammatory)	
<i>[Adapted from Shivappa et al. (2014)]</i>	

Some adaptations were made to the original index based on recommendations by other researchers who have used the E-DII (van Woudenberg *et al.*, 2013; Hodge *et al.*, 2018; Yang *et al.*, 2019). These included 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 fats and saturated fat summed provides the value of total fat consumed by an individual. Lastly, alcohol provides an anti-inflammatory effect only if less than 40 g/d is consumed (Calder *et al.*, 2011). Thus, alcohol was given a score of zero when the intake was more than 40 g/d since alcohol intake of more than 40 g/d is no longer considered to be anti-inflammatory.

The table below shows the final list of nutrients and food components included in the DII for the pure population, considering the adaptations mentioned above and whether the nutrients/ food components were a) consumed by the PURE-SA-NW participants and b) whether the data are available in the South African FCT.

Table 3-2: 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 (ug)	Yes
Vitamin B6 (mg)	Yes
β-Carotene (ug)	Yes
*Caffeine (g)	Yes
Carbohydrate (g)	Yes
Cholesterol (mg)	Yes
Energy (kcal)	Yes
Eugenol (mg)	No
Total fat (g)	Yes
Fibre (g)	Yes
Folic acid (ug)	Yes
Garlic (g)	Not reported
Ginger (g)	Not reported
Fe (mg)	Yes
Mg (mg)	Yes
MUFA (g)	Yes
Niacin (mg)	Yes
° n-3 Fatty acids (g)	Yes
τ n-6 Fatty acids (g)	Yes
* Onion (g)	Yes
Protein (g)	Yes
PUFA (g)	Yes
Riboflavin (mg)	Yes
Saffron (g)	No
Saturated fat (g)	Yes
Se (ug)	Yes
Thiamin (mg)	Yes
Trans fat (g)	Yes
Turmeric (mg)	No
Vitamin A (RE)	Yes
Vitamin C (mg)	Yes
Vitamin D (ug)	Yes
Vitamin E (mg)	Yes
Zn (mg)	Yes

Table 3.2 continued

§ Black tea/ Rooibos (g)	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:

6.67 (pro-inflammatory) to -7.65 (anti-inflammatory)

** caffeine 68mg / 100ml coffee; caffeine 20mg / 100ml tea, brewed

° n-3 fatty acids compiled by summing C18_3, C18_4, C20_5, C22_5 and C22_6

τ n-6 fatty acids compiled by summing C18_2 and C20_4

⌘ 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.

§ compiled by adding intake of both rooibos and black tea

3.10 Statistical analyses

Statistical analyses were performed with SPSS® version 23 (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 $\pm$ standard deviation or median and interquartile ranges based on their distribution. Data that were not normally distributed were log transformed to improve normality and used in further analyses. Counts and percentages will be used for categories. Independent t-tests were used to compare data between sex, rural/urban, smoking and alcohol consumption groups and analysis of variance (ANOVA) when comparing data split according to both sex and rural/urban status and also when dividing the population into E-DII quartiles. This is followed by two consecutive multivariate-adjusted generalised linear models (GLMs). The first model adjusts for demographic differences between the quartiles (sex, rural/urban status, age, alcohol consumption and smoking) to determine whether the differences observed were related to differences in the demographic profile between the E-DII quartiles or to the E-DII itself. This analysis was also adjusted for anti-inflammatory and aspirin use and was applied to all biochemical variables. The second GLM was performed for the fibrin clot properties only and additionally adjusted for biochemical variables that differed across the quartiles. Marginal

least-squares means were derived by groups and compared following the Tukey-Kramer method to take into account groups of unequal sizes (Kramer, 1956). Model assumptions of linearity and variance homogeneity were evaluated by visual inspection of scatter plot of regression residuals and by Levene's test.

CHAPTER 4 RESULTS

4.1 Results

The first aim of this study was to investigate and describe the inflammatory index of the diet of the Prospective Rural and Urban Epidemiology (PURE) study participants in 2005. The second was to determine whether the E-DII was associated with fibrin clot properties. The inflammatory index of the diet was calculated making use of the literature-based, energy-adjusted dietary inflammatory index (E-DII) developed by Shivappa *et al.*, (2014) and adapted for the PURE study as detailed in the Methods chapter.

First, the E-DII range of the total PURE population is compared with the theoretical E-DII range, to give perspective regarding the overall anti- or pro-inflammatory status of the diet of the participants. Next, a comparison of the E-DII in the rural and urban groups, as well as between men and women, are presented, together with the intakes of the E-DII food components in the respective groups. This is followed by a characterisation of the biochemical and anthropometric profile of the 4 groups. We also report on whether the E-DII differs between smokers and non-smokers and alcohol consumers and non-consumers. Lastly, the E-DII is divided into quartiles, and demographic, anthropometric and biochemical variables are compared across the quartiles. This is followed by two consecutive analyses of covariance (ANCOVAs). The first model adjusts for demographic differences between the quartiles (sex, rural/urban status, age, alcohol consumption and smoking) to determine whether the differences observed were related to differences in the demographic profile between the E-DII quartiles or to the E-DII itself. This analysis was also adjusted for anti-inflammatory and aspirin use and was applied to all biochemical variables. The second ANCOVA model was performed for the fibrin clot properties only and additionally adjusted for biochemical variables that differed across the quartiles (glucose and gamma-glutamyl transferase (GGT)).

The original E-DII published by Shivappa *et al.* (2014) consists of 45 food components, with most of the components having anti-inflammatory properties (Table 2.6). The goal of the E-DII is to determine the inflammatory potential of the diet, with a negative score being more anti-inflammatory and a positive score more pro-inflammatory. The PURE population had a mean E-DII of $+0.37 \pm 1.25$, which indicates an overall mean pro-inflammatory diet, and a minimum and maximum score of -3.8 and +5.0 respectively. Within our population, we studied a total of 37 food components that, in theory, should provide a minimum range of -7.67 and a maximum range of +6.67. This indicates that the PURE dietary intakes spanned 61% of the theoretical range, with the most anti-inflammatory index being only 50% of the theoretical maximum anti-inflammatory potential and the most pro-inflammatory diet reaching 74% of the theoretical maximum pro-

inflammatory potential. Food components that were excluded from the original 45 were not reported by the PURE population; these included components such as turmeric, garlic, ginger, rosemary, pepper, saffron, oregano and eugenol.

Table 4.1 reports the intake of the E-DII food components for men and women and for rural/urban status. Urban women consumed the most anti-inflammatory diet, although their mean E-DII (0.25 ± 0.14) was still reflective of a pro-inflammatory diet, followed by urban men (0.31 ± 0.16). Rural men consumed the most pro-inflammatory diet (0.70 ± 0.66). The food components presented in Table 4.1 are divided into two groups according to their pro- or anti-inflammatory potential. Differences were observed in the intake of the E-DII components between men and women and their rural or urban status and are therefore presented in the 4 sub-groups. Urban men consumed the highest energy, with a median energy consumption of 9244 kJ, followed by urban women, consuming on average 8445 kJ. Rural men consumed an average of 6545kJ and the rural women an average of 5926 kJ. Participants with an urban status had a higher consumption of almost all food components compared with their rural counterparts. Urban women consumed higher amounts of 19 of the 28 food components with anti-inflammatory properties such as fibre, zinc, selenium, vitamin A, thiamine, riboflavin, vitamin B6, mono-unsaturated fat, vitamin C, D, E, omega-3, omega-6, beta-carotene, anthocyanidins, flavan-3-ol, flavonones, flavones and flavonols, while urban men consumed the highest amount of 7 of the 28 anti-inflammatory food-components, including alcohol, magnesium, niacin, folate, PUFA, isoflavones and onions. Rural men (and women) had the lowest intake of all the afore-mentioned food components, except for caffeine and tea. Concerning the pro-inflammatory E-DII components, urban men consumed the highest amounts of 5 of the 8 components. These food components include macronutrients (carbohydrates and protein), cholesterol and vitamin B12, while urban women had the highest intake of the other 3 components (saturated fatty acids (SFA), trans fat and iron). However, when considering macronutrient intake expressed as % of total energy, rural women's diets had the highest carbohydrate content (66%), followed by rural men (63%) and then urban men and women (56% each).

Table 4-1: Comparison of characteristics of the food components in diets of men and women and of people with rural or urban status

Food components	Women		Men	
	Urban (n=389)	Rural (n=457)	Urban (n=290)	Rural (n=258)
E-DII	0.25 ±0.14	0.41 ±0.38	0.31 ±0.16	0.70 ±0.66
Pro-inflammatory				
Energy (kJ)	8445 (5884; 11226)	5926 (4666; 7420)	9244 (7045; 12060)	6545 (5055; 8223)
Carbohydrates (g)	279 (190; 363)	233 (176; 290)	306 (223; 403)	241 (190; 312)
Carbohydrates % of TE	56.2 (38.2; 73.1)	66.8 (50.4; 83.2)	56.3 (41.1; 74.1)	62.6 (49.4; 81.0)
Protein (g)	60.1 (43.6; 84.4)	38.3 (29.2; 47.9)	67.9 (50.6; 87.5)	41.4 (31.3; 55.7)
Protein % of TE	12.1 (8.78; 17.0)	11.0 (8.38; 13.7)	12.5 (9.31; 16.1)	10.8 (8.13; 14.5)
SFA (g)	15.0 (9.8; 21.7)	6.29 (3.86; 9.27)	14.4 (10.0; 21.0)	6.28 (3.80; 9.13)
Trans fat (g)	0.48 (0.20; 0.98)	0.08 (0.03; 0.18)	0.38 (0.19; 0.78)	0.09 (0.03; 0.17)
Cholesterol (g)	210 (132; 315)	94.4 (49.4; 148)	238 (153; 332)	101 (53.4; 146)
Iron (mg)	16.0 ±7.30	12.4 ±5.66	11.2 ±4.76	12.4 ±5.66
Vitamin B12 (µg)	4.16 (2.18; 6.98)	1.50(0.65; 2.76)	4.68 (2.56; 7.72)	1.49 (0.81; 2.74)
Anti-inflammatory				
Alcohol (g)	0.00 (0.00; 11.4)	0.00 (0.00; 0.00)	14.2 (0.00; 28.6)	1.93 (0.00; 24.9)
Fibre (g)	25.7 ±12.4	18.7 ±8.62	17.3 ±7.01	18.7 ±8.61
Magnesium (mg)	312 (219; 403)	224 (171; 295)	381 (283; 511)	274 (194; 397)
Zinc (mg)	12.7 ±5.83	8.76 ±4.03	7.71 ±3.20	8.76 ±4.03
Selenium (µg)	29.1 (17.8; 43.6)	14.8 (8.65; 24.3)	28.4 (19.3; 41.9)	15.0 (7.29; 25.8)
Vitamin A (RE)	831 (474; 1402)	452 (288; 675)	809 (531; 1347)	403 (247; 617)
Thiamine (mg)	1.97 ±0.94	1.61 ±0.76	1.45 ±0.61	1.61 ±0.76
Riboflavin (mg)	1.63 ±0.80	1.05 ±0.73	0.82 ±0.53	1.05 ±0.73
Niacin (mg)	14.9 (10.6; 20.6)	9.84 (7.52; 12.6)	17.5 (13.3; 23.3)	11.3 (8.59; 15.8)

Table 4.1 continued

Vitamin B6 (mg)	1.84 ±0.90	1.24 ±0.58	1.16 ±0.49	1.24 ±0.58
Folate (µg)	356 (241; 500)	323 (236; 422)	430 (307; 605)	363 (237; 460)
MUFA (g)	16.4 (10.9; 24.9)	6.61 (4.15; 9.93)	16.1(11.6; 24.3)	6.69 (4.08; 9.74)
PUFA (g)	17.1 (11.4; 24.3)	9.61 (6.43; 14)	17.2 (12.2; 24.4)	9.43 (6.39; 13.6)
Vitamin C (mg)	33.9 (17.5; 55.9)	12.0 (7.3; 17.6)	29.3 (18.6; 53.8)	11.2 (6.21; 15.6)
Vitamin D (µg)	3.59 ±2.58	1.93 ±2.63	1.99 ±2.34	1.94 ±2.63
Vitamin E (mg)	11.9 (7.42; 16.2)	7.80 (5.07; 10.9)	11.9 (7.92; 16.7)	7.09 (4.38; 10.7)
ω-3 PUFA (g)	0.57 ±0.32	0.26 ±0.16	0.26 ±0.16	0.26 ±0.16
ω-6 PUFA (g)	16.4 (10.5; 22.7)	8.79 (5.71; 13.2)	15.9 (11.1; 23)	8.56 (4.84; 12.8)
β Carotene (mcg)	1490 (524; 2885)	806 (129; 1395)	1361 (440; 2411)	348 (75.5; 1048)
Anthocyanidins (mg)	0.31 (0.00; 1.22)	0.07 (0.00; 0.21)	0.21 (0.00 ;0.61)	0.05 (0.00; 0.16)
Flavan-3-ol (mg)	158 (91.1; 248)	23.8 (3.80; 83.7)	154 (88.9; 228)	28.7 (6.45; 90.4)
Flavonones (mg)	4.33 (0.47; 19.1)	0.14 (0.05; 1.31)	3.65 (0.59; 15.0)	0.27 (0.08; 1.47)
Flavones (mg)	3.09 (0.63; 6.90)	2.61 (0.92; 5.39)	2.71 (0.43; 6.95)	2.78 (0.76; 5.65)
Flavonols (mg)	33.2 (19.6; 49.7)	5.9 (2.00; 17.9)	32.2 (19.1; 43.0)	6.49 (2.95; 19.0)
Isoflavones (mg)	0.01 (0.00; 0.03)	0.01 (0.00; 0.03)	0.02 (0.01; 0.04)	0.02 (0.00; 0.07)
Onion (g)	86.4 (59.9; 128)	43.4 (25.0; 70.5)	92.5 (69.0; 128)	40.0 (18.0; 61.0)
Caffeine (mg)	420 (252; 840)	1428 (816; 1516)	372 (240; 820)	1428 (453; 1428)
Tea (g)	1540 (900; 3080)	1950 (900; 2100)	1540 (900; 2100)	2100 (1260; 2100)

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 expressed as mean value (standard deviation)

Not normally distributed variables are expressed as median (25–75th percentiles)

Table 4-2: Comparison of characteristics of the study variables between men and women and people of rural and urban status

Study Variables	Women		Men		p-value ANOVA
	Urban (n=389)	Rural (n=457)	Urban (n=290)	Rural (n=258)	
Alcohol use (n/%)	147/38	88/19	200/68	133/50	<0.0001
Smokers (n/%)	196/50	244/52	209/72	159/60	<0.0001
Alcohol & Smoker (n/%)	108/28	75/16	174/60	115/45	
Age (y)	48.2 (42.1; 57.0) ^a	46.6 (41.1; 53.8) ^{ab}	48.6 (41.9; 56.6)	48.8 (42.3; 57.2) ^b	0.002
DBP (mmHg)	89.2 ±14.7 ^{ac}	86.5 ±14.3 ^{abc}	88.9 ±14.9 ^{bc}	86.1 ±15.1 ^{ab}	0.01*
SBP (mmHg)	136 ±26.6 ^{ac}	128 ±22.2 ^{abc}	139 ±24.6 ^{bc}	134 ±24.3 ^c	<0.0001
BMI (kg/m ²)	25.9 (21.9; 31.0) ^a	24.1 (20.6; 28.7) ^a	20.1 (18.4; 22.6) ^a	19.9 (18.2; 22.3) ^a	<0.0001
Fasting glucose (mmol/L)	4.90 (4.30; 5.40)	4.70 (4.40; 5.20)	4.80 (4.25; 5.40)	4.70 (4.40; 5.10)	0.20
HbA1c (%)	5.60 (5.20; 5.90) ^a	5.55 (5.30; 5.80)	5.50 (5.20; 5.70)	5.50 (5.20; 5.70) ^a	0.005
hsCRP (mg/L)	2.37 (0.87; 4.89) ^a	2.25 (0.68; 4.79)	4.53 (0.63; 3.39) ^a	1.73 (0.53; 4.35) ^a	0.001
suPAR (ng/mL)	3.40 (2.87; 4.32)	3.35 (2.79; 4.11)	3.34 (2.73; 4.19)	3.36 (2.76; 4.26)	0.39
IL-6 (pg/mL)	2.67 (0.75; 5.81) ^a	1.98 (0.75; 3.69) ^a	2.29 (0.75; 4.17)	2.27 (0.75; 5.23)	0.02
tHcy (μmol/L)	8.32 (6.90; 10.5) ^{ac}	8.91 (7.19; 11.2) ^{bc}	9.60 (7.96; 12.2) ^{ac}	11.2 (8.77; 14.1) ^{abc}	<0.0001
Creatinine (μmol/L)	70.9 (59.1; 92.9) ^{ab}	64.3 (55.7; 76.8) ^{ac}	70.0 (59.6; 91.5) ^c	64.9 (57.6; 73.6) ^{bc}	<0.0001
Creatinine clearance (mL/min)	0.90 (0.58; 1.19) ^{ac}	0.98 (0.77; 1.26) ^{abc}	1.00 (0.70; 1.26) ^{bc}	1.12 (0.91; 1.33) ^{ab}	<0.0001
Albumin (g/L)	43.5 (39.3; 58.2)	43.5 (40.6; 57.1)	43.0 (38.4; 58.0) ^a	44.4 (41.5; 57.7) ^a	0.01
Uric acid (mmol/L)	0.37 (0.26; 0.58) ^a	0.32 (0.25; 0.42) ^{ab}	0.46 (0.35; 0.76) ^{ab}	0.36 (0.30; 0.48) ^{ab}	<0.0001
Urea (nmol/L)	3.85 (3.00; 5.20) ^a	3.63 (3.00; 4.83) ^{abc}	3.83 (3.00; 5.40) ^b	4.12 (3.17; 5.35) ^c	<0.0001
TC (mmol/L)	5.16 ±1.41	5.09 ±1.37	4.88 ±1.33 ^a	4.73 ±1.33 ^a	0.01
HDL-C (mmol/L)	1.46 ±0.63 ^a	1.50±0.61	1.61 ±0.66 ^a	1.55 ±0.66	0.01
LDL-C (mmol/L)	3.04 ±1.18 ^{ab}	3.03 ±1.17 ^b	2.68 ±1.19 ^{ab}	2.67 ±1.13 ^b	<0.0001

Table 4.2 continued

TG (mmol/L)	1.15 (0.85; 1.72) ^{ab}	1.08 (0.80; 1.47) ^{ab}	1.01 (0.79; 1.49)	0.97 (0.77; 1.40) ^a	0.001
ALT (IU/L)	16.1 (12.0; 24.2) ^{ab}	16.0 (12.0; 21.4) ^{bc}	19.9 (14.0; 30.0) ^{abc}	19.9 (14.2; 29.4) ^{ac}	<0.0001
AST (IU/L)	25.0 (19.7; 35.0) ^a	24.2 (18.0; 34.1) ^b	31.9 (24.2; 49.5) ^{ab}	28.8 (20.0; 44.8) ^b	<0.0001
GGT (IU/L)	41.0 (27.0; 82.0) ^a	35.0 (24.6; 55.3) ^a	66.2 (41.0; 133) ^a	48.2 (31.4; 103) ^a	<0.0001
Lag time (minutes)	5.67 ±1.97 ^{ab}	4.38 ±1.67 ^{ab}	5.73 ±2.01 ^b	4.05 ±1.70 ^{ab}	<0.0001
Slope (au/s)	5.25 ±3.31 ^{ac}	8.02 ±4.16 ^{abc}	5.02 ±3.07 ^{bc}	8.21 ±4.20 ^{ab}	<0.0001
Maximum absorbance (au)	0.43 ±0.34 ^{ab}	0.44 ±0.13 ^{bc}	0.40 ±0.13 ^{abc}	0.40 ±0.13 ^{ac}	<0.001
Clot lysis time (minutes)	59.6 ±10.3 ^{ab}	58.0 ±9.18 ^{bc}	52.2 ±11.5 ^{abc}	52.6 ±10.3 ^{ac}	<0.0001
Fibrinogen (g/L)	2.60 (2.10; 3.70) ^{ac}	3.00 (2.30; 4.80) ^{abc}	2.40 (2.00; 3.10) ^{ac}	2.50 (2.10; 3.20) ^{bc}	<0.0001
y' fibrinogen (%)	11.4 (8.56; 16.2) ^{ab}	10.3 (6.66; 14.5) ^{ab}	10.3 (7.90; 13.9)	9.61 (6.59; 13.6) ^a	<0.0001
PAI-1 _{act} (U/mL)	6.72 (3.59; 10.4) ^a	5.52 (2.77; 8.36) ^a	3.96 (1.51; 7.70) ^a	2.90 (1.00; 5.78) ^a	<0.0001

E-DII: energy-adjusted dietary inflammatory index, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, HbA1c: glycated haemoglobin, hs-CRP: high-sensitivity C-reactive protein, suPAR: soluble urokinase-type plasminogen activator receptor, IL-6: interleukin 6, tHcy: total homocysteine, TC: total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: triglyceride, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyl transferase, PAI: plasminogen activator inhibitor-1 activity; au – absorbance units

Normally distributed variables are expressed as mean value ± standard deviation

Not normally distributed variables are expressed as median (25–75th percentiles)

^{a,b,c} Means with the same symbol differ significantly between groups as indicated by post-hoc tests

*Fisher

Table 4.2 reports the demographic and biochemical variables for the men and women split for rural/urban status. Most variables showed a significant difference between the 4 groups. Compared with the women, more men (both rural and urban) consumed alcohol and smoked. The rural women's mean age was on average two years younger than the other groups (46 vs 48 years). Mean diastolic blood pressure (DBP) and systolic blood pressure (SBP) was higher in the urban group than the rural group for both men and women. Systolic blood pressure was lowest in rural women (128 mmHg). Urban women had a significantly higher mean body mass index (BMI) (25.9 kg/m²) compared with the other groups ($p < 0.0001$). When looking at inflammatory markers C-reactive protein (CRP), soluble urokinase-type plasminogen activator receptor (suPAR), interleukin-6 (IL-6) and albumin, urban men presented with the highest CRP (4.53 mg/L) and lowest albumin level (43 g/L) while urban women had the highest IL-6 level (2.67 pg/mL). There was no significant difference in suPAR levels among the four groups. All liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT)) differed significantly between the groups, with men generally having higher levels than women. Blood lipids were, however, more favourable in men, who generally had lower total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) and higher high-density lipoprotein cholesterol (HDL-C) than the women. Coagulation markers also differed between the groups. The mean lag times were longer in the urban men and women compared with their rural counterparts. Slope was, however, higher in men than women while maximum absorbance was higher and clot lysis time (CLT) longer in the women than the men. Women also had higher fibrinogen and plasminogen activator inhibitor-1 activity (PAI-1_{act}) levels than men.

Table 4-3: Significance of E-DII between groups; sex, rural/urban status, alcohol use, HIV status and tobacco use.

Category	E-DII	p-value
Men (n=525)	0.49 ±1.19	0.03
Women (n=798)	0.33 ±1.30	
Rural (n=657)	0.52 ±1.31	<0.001
Urban (n=666)	0.27 ±1.19	
Alcohol use (n=727)	0.58 ±1.19	<0.001
Non-alcohol use (n=529)	0.29 ±1.31	
Smoker (n=752)	0.49 ±1.24	<0.001
Non-smoker (n=563)	0.27 ±1.27	
HIV (n= 219)	0.51 ± 1.26	0.15
Non-HIV (n= 1209)	0.37 ± 1.24	

E-DII: energy-adjusted dietary inflammatory index; HIV: human immunodeficiency virus

Significant p-value <0.05

Table 4.3 reports the significance of E-DII between sex (without splitting for rural/urban status), rural/urban status (without splitting for sex), alcohol consumers and non-consumers, HIV status as well as ever and never smokers. The E-DII was significantly higher in men than women ($p=0.03$), in rural participants ($p<0.001$), smokers ($p<0.001$) and alcohol users ($p<0.001$) and there was no significant difference for HIV status.

Table 4-4: Characteristics of the study variables across E-DII quartiles

E-DII categories	1 (-3.70 - <-0.45)	2 (-0.45 - <0.32)	3 (0.32 - <1.20)	4 (1.20 - 5.08)	p-value ANOVA	p-value ANCOVA
Age (y)	49.3 (43.1; 57.9) ^a	48.4 (42.3; 56.8)	47.5 (41.2; 54.6) ^b	46.5 (41.3; 55.4) ^{ab}	0.005	
Women (%)	67	58	59	58	0.059	
Alcohol use (%)	30	41	43	48	<0.0001	0.03* (0.46)**
Alcohol consumption (g/day) [#]	12.4 ±16.9	15.7 ±16.4	19.1 ±22.7	43.9 ±43.2	<0.0001	
Tobacco use (%)	49	58	56	65	0.001	
Rural (%)	46	43	50	60	<0.0001	
DBP (mmHg)	87.4 ±15.4	88.9 ±14.7	88.5 ±15.0	86.4 ±14.1	0.13	0.13
SBP (mmHg)	134 ±25.3	137 ±25.4 ^a	134 ±24.7	132 ±23.7 ^a	0.04	0.15
BMI (kg/m ²)	22.4 (19.5; 27.7)	23.0 (19.3; 27.0)	22.6 (19.1; 27.1)	22.0 (19.1; 26.5)	0.18	0.15
Fasting glucose (mmol/L)	4.70 (4.40; 5.20) ^{ab}	4.80 (4.30; 5.30)	4.80 (4.30; 5.20) ^a	4.90 (4.30; 5.40) ^b	0.005	0.08
HbA1c (%)	5.50 (5.30; 5.80)	5.50 (5.20; 5.80)	5.50 (5.20; 5.80)	5.50 (5.20; 5.80)	0.42	0.62
hsCRP (mg/L)	2.12 (0.64; 5.10)	2.00(0.62; 4.35)	2.04 (0.82; 4.29)	2.10 (0.76; 4.70)	0.86	0.63
suPAR (ng/mL)	3.30 (2.83; 4.09)	3.32 (2.77; 4.14)	3.47 (2.76; 4.34)	3.46 (2.84; 4.32)	0.35	0.32
IL-6 (pg/mL)	2.28 (0.75; 3.92)	2.09 (0.75; 4.30)	2.20 (0.75; 4.23)	2.49 (0.75; 4.44)	0.64	0.49
tHcy (μmol/L)	9.17 (7.52; 12.0)	9.26 (7.36; 12.2)	9.17 (7.36; 11.9)	9.21 (7.46; 12.3)	0.48	0.67
Creatinine (μmol/L)	66.2 (57.6; 77.7)	68.6 (57.0; 82.5)	68.0 (58.0; 84.4)	67.9 (58.7; 81.0)	0.48	0.27
Creatinine clearance (mL/min)	0.98 (0.73; 1.24)	0.99 (0.75; 1.26)	1.01 (0.74; 1.30)	0.99 (0.76; 1.24)	0.93	0.87
Albumin (g/L)	44.3 (41.0; 60.0)	43.9 (39.9; 58.0)	43.1 (39.1; 57.1)	43.3 (40.0; 57.0)	0.11	0.11
Uric acid (mmol/L)	0.40 (0.29; 0.57)	0.37 (0.28; 0.51)	0.36 (0.27; 0.55)	0.35 (0.27; 0.48)	0.10	0.06
Urea (nmol/L)	4.00 (3.04; 5.40) ^a	3.84 (3.0; 5.14)	3.80 (3.00; 5.13)	3.65 (3.00; 5.02) ^a	0.05	0.27
TC (mmol/L)	5.15 ±1.27	5.02 ±1.32	4.96 ±1.43	5.04 ±1.27	0.36	0.61
HDL-C (mmol/L)	1.59 ±0.58	1.56 ±0.62	1.57 ±0.69	1.57 ±0.65	0.97	0.3

Table 4.4 continued

LDL-C (mmol/L)	2.96 ±1.11	2.86 ±1.15	2.86 ±1.14	2.89 ±1.13	0.64	0.67
TG (mmol/L)	1.08 (0.82; 1.55)	1.11 (0.82; 1.62)	1.00 (0.76; 1.42)	1.04 (0.80; 1.49)	0.09	0.37
ALT (IU/L)	17.0 (12.9; 24.0)	17.0 (12.0; 23.3)	17.0 (12.0; 25.8)	19.0 (13.3; 26.0)	0.03	0.07
AST (IU/L)	26.0 (20.0; 37.3)	25.8 (19.6; 37.0) ^a	26.0 (19.7; 39.0)	28.9 (20.6; 45.0) ^a	0.01	0.09
GGT (IU/L)	42.0 (27.7; 68.5) ^a	45.4 (30.3; 84.7)	41.0 (28.0; 87.7) ^b	49.1 (30.3; 119) ^{ab}	0.01	0.05
Lag time (minutes)	5.22 ±1.97 ^a	5.02 ±1.97 ^b	4.98 ±1.88	4.61 ±2.00 ^{ab}	0.001	0.34 (0.70)
Slope (au/s)	6.60 ±4.00	6.91 ±3.94	6.60 ±3.97	6.95 ±4.28	0.55	0.42 (0.71)
Maximum absorbance (au)	0.44 ±0.13 ^a	0.43 ±0.14	0.41 ±0.13	0.41 ±0.14 ^a	0.01	0.17 (0.41)
Clot lysis time (minutes)	57.6 ±10.4 ^a	55.9 ±10.6	56.6 ±10.6	54.7 ±10.8 ^a	0.01	0.31 (0.11)
Fibrinogen (g/L)	2.60 (2.10; 3.70)	2.70 (2.20; 4.30)	2.60 (2.10; 3.70)	2.60 (2.00; 3.40)	0.18	0.17
y' fibrinogen (%)	10.5 (7.9; 14.3)	9.90 (6.74; 13.8)	10.7 (7.18; 14.8)	10.6 (7.59; 15.2)	0.13	0.46
PAI-1 _{act} (U/mL)	4.78 (2.29; 7.78)	5.44 (2.29; 8.50)	5.19 (2.43; 8.70)	4.88 (2.04; 8.98)	0.81	0.35

E-DII: energy-adjusted dietary inflammatory index, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, HbA1c: glycated haemoglobin, hs-CRP: high-sensitivity C-reactive protein, suPAR: soluble urokinase-type plasminogen activator receptor, IL-6: interleukin 6, tHcy: fasting total homocysteine, TC: total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: triglyceride, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyl transferase, PAI: plasminogen activator inhibitor-1 activity.

Normally distributed variables are expressed as mean value ±standard deviation

Not normally distributed variables are expressed as median (25–75th percentiles)

[#] Mean intake of alcohol consumers only

^{*} adjusted for: sex and age

^{**} adjusted for: sex, age and smoking

ANCOVA – adjusted for: sex, rural/urban status, smoking, alcohol and age, anti-inflammatory medication use, aspirin use

ANCOVA – p in brackets additionally adjusted for: GGT and fasting glucose

^{a,b,c} Means with the same symbol differ significantly between groups as indicated by post-hoc tests

In order to determine the association of the E-DII with fibrin clot properties and other biochemical variables, the E-DII was divided into quartiles and the means of the variables were compared across the quartiles (Table 4.4). Age decreased across the quartiles, suggesting that older participants followed a more anti-inflammatory diet. There were more women in each quartile than men, with this proportion tending to differ across the quartiles and being highest in quartile 1. The number of rural participants increased across the E-DII categories ($p<0.001$). Alcohol ($p<0.001$) and tobacco ($p=0.001$) use also increased across the E-DII quartiles despite alcohol consumption being considered an anti-inflammatory component of the E-DII. The significant difference in number of alcohol consumers across the quartiles remained after adjustment for sex and age ($p=0.03$) but disappeared after additional adjustment for smoking ($p=0.46$). Systolic blood pressure furthermore demonstrated a moderate decrease across the quartiles ($p=0.04$). Most of the biochemical variables did not differ across the E-DII quartiles, including the blood lipids (TC, HDL-C, LDL-C and TG), the inflammatory markers (CRP, SuPAR, IL-6 and albumin), BMI and total and γ' fibrinogen. The only variables that increased across the quartiles were fasting glucose and the liver enzymes (ALT, AST and GGT), which increased, particularly in the most pro-inflammatory E-DII category (quartile 4). The time required for fibrin clot formation to start (lag time), the fibrin clot density (maximum absorbance) and clot lysis time all decreased across the E-DII quartiles in the unadjusted model, suggesting the formation of less dense clots that lysed faster in the more pro-inflammatory E-DII category. However, after adjustment for the covariates (both model 1 and 2), these differences were no longer significant.

To determine whether the differences in biochemical variables observed between the quartiles were due to differences in the demographic variables (age, sex, rural/urban status, alcohol consumption, smoking), an ANCOVA adjusting for these covariates was conducted. Adjustments were also made for anti-inflammatory and aspirin use. Following the adjustment, fasting glucose no longer differed across the quartiles and differences in liver enzymes became of borderline significance only. All differences observed for clot properties also disappeared. Furthermore, we conducted a second ANCOVA (p -value in brackets) for the clot properties as outcome variables, additionally adjusting for GGT and fasting blood glucose (which initially differed across the quartiles), but this additional adjustment did not significantly alter the results. The covariate that significantly contributed to lag time as outcome was rural/urban status ($\beta=-1.59$ [-1.86; -1.32], $p<0.0001$ – rural compared with urban). For maximum absorbance as outcome the covariates that significantly contributed to the model were sex ($\beta=-0.04$ [-0.06; -0.02], $p<0.0001$ – men compared with women) and age ($\beta=0.002$ [0.001; 0.003], $p<0.0001$). For CLT, the covariates were sex ($\beta=-4.67$ [-6.10; -3.24], $p<0.0001$ – men compared with women), smoking ($\beta=2.4$ [0.9; 3.9], $p=0.002$ – never compared with ever smokers), GGT ($\beta=-0.01$ [-0.02; -0.005], $p<0.0001$) and fasting glucose ($\beta=0.97$ [0.62; 1.33], $p<0.0001$). All of these covariates, except fasting glucose,

increased or decreased respectively across the E-DII quartiles in accordance with the direction of their contribution in the regression models, thus explaining the apparent differences in clot properties across the quartiles.

CHAPTER 5 DISCUSSION

5.1 Introduction

Cardiovascular diseases (CVD) remain a leading cause of death worldwide, and a complex pathway exists between chronic inflammation and CVD (Bordoni *et al.*, 2017; Pearson *et al.*, 2003; Santos *et al.*, 2013). Fibrinogen concentration and fibrin clot properties are known predictors of CVD and are strongly linked to inflammation. An individual's diet can contribute significantly to chronic inflammation, providing a modifiable pathway through which to influence CVD outcome (Ahmadi-Abhari *et al.*, 2013; Kantor *et al.*, 2013; Libby, 2007). To our knowledge, this is the first study to investigate the association between the inflammatory potential of the diet and levels of inflammatory markers, fibrinogen concentration and fibrin clot properties in Black South Africans. We analysed the consumption of the 45 food components included in the original literature-based inflammatory index (DII) developed by Shivappa and co-workers (Shivappa *et al.*, 2014b) in the Prospective Urban and Rural Epidemiology (PURE) study participants in order to calculate the energy-adjusted dietary inflammatory index (E-DII) of their diets.

This study aimed to determine the relationship between the E-DII and markers of inflammation, fibrinogen concentration and fibrin clot properties in 2010 Black South Africans who participated in Prospective Urban and Rural Epidemiology study, South Africa, North West (PURE-SA-NW) study. The objectives of this study were to:

- adapt the published E-DII for the participants who took part in the PURE-SA-NW study in 2005, based on their dietary intakes;
- determine the relationship between the E-DII and inflammatory markers (C-reactive protein (CRP), albumin, soluble urokinase-type plasminogen activator receptor (SuPAR) and interleukin-6 (IL-6));
- determine the relationship between the E-DII and fibrinogen concentration and plasma clot properties obtained using turbidimetry;
- determine whether the relationship between the E-DII and clot properties is mediated by inflammation and/or fibrinogen concentration.

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

5.2 Creating and adapting the E-DII for the PURE population

Shivappa and colleagues published the original DII in 2014 (Shivappa *et al.*, 2014b), which describes the inflammatory potential of an individual's overall diet. The E-DII is an updated version of the DII, standardising all intakes to 4180 kJ to account for the effect of different total energy intakes on the inflammatory potential of the diet (van Woudenberg *et al.*, 2013). The E-DII is calculated by making use of the intake of 45 selected food components and nutrients. Hence an important step in accurately calculating the E-DII is ensuring the correctness of the food and nutrient intakes. While food intakes can be directly calculated from the quantitative food frequency questionnaire (QFFQ), nutrient intakes are determined by making use of Food Composition Table (FCT) that rely on, amongst others, availability of nutrient analysis data. For this study, we analysed the nutrient composition of the participants' dietary intakes using the Medical Research Council's (MRC) FCT (Wolmarans *et al.*, 2010). This database is continuously updated as more nutrient analysis data become available and it is also updated whenever the nutrient composition of foods changes, as happens for example, as a result of national food fortification programmes.

Since the dietary intakes in this study were collected in 2005, the nutrient analysis was originally performed using the version of the FCT available in 2005. A caveat, however, of using a FCT is that it relies on availability of nutrient data and at any given point in time it can contain missing data either in the form of nutrients not yet analysed e.g. flavonoid content of foods, or nutrient data being available for some but not all foods e.g. beta-carotene. It is therefore important to first determine the completeness of the nutrient data in the FCT before analysing dietary intakes in order to prevent incorrect reporting of low intakes of a specific nutrient resulting from missing data in the FCT. Each version of the FCT, therefore, contains a list of such missing data that should be considered when interpreting the nutrient profile.

Based on the above, the following steps were taken to ensure accurate information for nutrient analysis in this study:

1. A list of foods consumed by at least 80% of the population was compiled to determine whether any nutrients were missing for these foods in the FCT.
2. This list was checked against the FCT and it was found that the only nutrient included in the E-DII with missing information in the 2005 version of the FCT for these foods was beta-carotene.
3. The beta-carotene content in the latest version of the FCT was found to be much more complete, and therefore the beta-carotene content of the PURE dietary intakes were determined using the latest version of the FCT.

4. It was decided not to use the latest version of the FCT for all nutrients as South Africa introduced legislation for the fortification of bread flour (including bread) and maize meal with nutrients such as vitamin A, thiamine, riboflavin, niacin, pyridoxine, vitamin B12, folic acid, iron and zinc (Department of Health, 2003:6). This fortification commenced after the 2005 data collection, hence the nutrient content of these products in the latest FCT version would not reflect the correct content of the bread flour, bread and maize meal consumed in 2005.
5. Lastly, Anthocyanidins, Flavan-3-ol, Flavonones, Flavones, Flavonols and Isoflavones are all flavonoids that are included in the E-DII. However, the FCT does not provide the flavonoid content of foods. The Phenol Explorer program, Phenol-Explorer® version 1, United States Department of Agriculture (Neveu *et al.*, 2010) was therefore used to determine the flavonoid intake of each PURE participant. As mentioned in Chapter 3, this program is a comprehensive database that provides the polyphenol content of food.

The above-mentioned steps emphasise our detailed preparation in ensuring accurate nutrient intakes and show the importance of comprehensive preparation and careful selection of the FCT to be used when analysing a population's dietary intake.

The original DII included 45 food components and/or nutrients, each of which either contributes to or inhibits chronic inflammation within the body. These 45 food components result in an inflammatory score range of -8.87 to +7.98 and a positive score is indicative of a more pro-inflammatory diet. If food components and/or nutrients are not consumed by a population, these components can be excluded from the analysis, as has been done by several other researchers (Ramallal *et al.*, 2015; Shivappa *et al.*, 2015; Shivappa *et al.*, 2014a; Shivappa *et al.*, 2014c; van Woudenberg *et al.*, 2013), resulting in an adjusted score range. The PURE population consumed only 37 of the 45 E-DII components. Food components not captured, by the PURE population were turmeric, garlic, ginger, rosemary, pepper, saffron, oregano and eugenol. As a result, a theoretical score range of -7.65 to 6.67 was possible within the PURE population. However, the E-DII of the PURE population ranged only from -3.85 to 5.0 with a mean E-DII of +0.37, indicating an overall pro-inflammatory diet. Several studies reported that a more pro-inflammatory diet could contribute to chronic low-grade inflammation, which can affect an individual's risk of different CVD outcomes (Bodén *et al.*, 2017; Chai *et al.*, 2017). Additionally, the use of the E-DII for accurately determining an individual or population's dietary inflammatory potential is a fast-growing field and has been used in several studies (Cavicchia *et al.*, 2009; Garcia-Arellano *et al.*, 2019; Li *et al.*, 2018; O'Neil *et al.*, 2015; Shivappa *et al.*, 2017a; Shivappa *et al.*, 2014c; van Woudenberg *et al.*, 2013). One of the objectives of our study was to adapt the original DII as published by Shivappa and colleagues in order to accurately determine the PURE population's dietary potential. The next section will discuss the dietary inflammatory potential of the PURE population

and how it differs between men and women and rural and urban participants, with an additional focus on the consumption patterns of the food and nutrient components of the E-DII in these sub-groups.

5.3 The E-DII within the PURE population

As indicated above, the PURE population had, on average, a more pro-inflammatory diet with a mean E-DII of $+0.37 \pm 1.25$. This suggests that, overall, more pro-inflammatory food components and nutrients were consumed in proportionally higher quantities than anti-inflammatory components, contributing to the average E-DII value being pro-inflammatory. In addition, the actual E-DII range spanned only 61% of the theoretical E-DII range, reflecting the limited variety in the PURE diets.

Dietary intakes between men and women and urban and rural participants differed, with urban participants generally consuming higher amounts of all nutrients than rural participants did. Urban women consumed the most anti-inflammatory diet (although their mean index was still pro-inflammatory), followed by urban men. Rural participants followed the most pro-inflammatory diet. This may be partly attributed to limited food availability in rural areas as well as affordability of certain food groups like fruit and vegetables that have anti-inflammatory properties (Mchiza *et al.*, 2015). Additionally, rural areas experienced a drought in 2005 that affected their dietary intake and the availability of fresh products such as fruit and vegetables (Wentzel-Viljoen *et al.*, 2018). Median energy intakes ranged from 5926 kJ in rural women to 9224 kJ in urban men; this is, however, within the dietary reference range for men and women. The mean percentage carbohydrate and protein intakes (contributing to total energy) in both rural and urban participants were, however, within the dietary reference range for men and women. This is likely the result of the high consumption of maize, bread and sugar (Labadarios *et al.*, 2011; Shisana *et al.*, 2014), which are the most affordable staple food products in these communities (Ronquest-Ross *et al.*, 2015).

Anti-inflammatory micronutrient consumption was highest among urban participants, specifically urban women. Although higher consumption was observed in urban groups, both urban and rural participants' consumption was below the dietary reference intake for micronutrients such as zinc, selenium, magnesium, niacin, vitamin A, riboflavin, vitamin B6, folate, vitamin C, vitamin D and vitamin E. While our study did not focus on the consumption of specific food groups such as fruit and vegetables, urban women were found to consume higher amounts of fruit and vegetables than any of the other three groups (Dolman *et al.*, 2014). Fruit and vegetables are typically considered good sources of a number of the above-mentioned nutrients, including a variety of vitamins, minerals and fibre (Schneider *et al.*, 2007). It is also known that fruit and vegetables

may be protective against CVDs and certain cancers (Willett *et al.*, 2006). Dolman *et al.* (2014) reported, however, that although intakes in urban groups were higher than in rural groups, the PURE population consumed less than half the recommended fruit and vegetable intake (Dolman *et al.*, 2014). Additionally, fibre intake was highest among urban women and was in line with the dietary reference intake for women, while intakes among rural men and women were below the dietary reference range. This comparatively low intake of fruit and vegetables very likely contributes to the fact that the most anti-inflammatory diet consumed in the PURE study population had an E-DII score that was only 50% of the theoretical maximum anti-inflammatory potential.

On the other hand, urban women also consumed the highest amount of the pro-inflammatory component, fat (trans fat and saturated fat) followed by urban men and, lastly, rural participants. Dolman *et al.* (2014) reported, however, that when expressed as percentage of total energy, the total fat intake was still below recommended guidelines, therefore probably contributing only modestly as a pro-inflammatory component to the E-DII. Fat intake should, however, be monitored because a large increase in dietary fat intake may increase an individual's risk of NCDs (Shisana *et al.*, 2014). Although urban men consumed the highest amounts of five of the eight pro-inflammatory components, in comparison with their rural counterparts they also consumed higher amounts of most of the anti-inflammatory components, resulting in their average E-DII still being more anti-inflammatory than those of the rural participants. Conversely, rural men, who had on average the most pro-inflammatory diet, did not have the highest intakes of any of the pro-inflammatory components, suggesting that their intake of the anti-inflammatory components was proportionally even lower than the intakes in the other three groups.

The fact that the E-DII of the PURE population spanned only 61% of the theoretical E-DII range suggests an overall lack of diversity in their diets, together with low total consumption of a number of the E-DII components, as explained above. Additionally, this is of great concern since it is not in line with one of the South African Food Based Dietary Guidelines that states, “enjoy a variety of food” (Love *et al.*, 2001) since dietary diversity is an important prerequisite for achieving all nutrient requirements (Steyn *et al.*, 2006).

A nutrient with a particularly interesting finding in this population is alcohol. While alcohol intake is considered an anti-inflammatory component in the E-DII, in the PURE population, there were more individuals who consumed alcohol in the most pro-inflammatory E-DII quartile. Furthermore, the mean alcohol intake (g/d) also increased across the quartiles. It should, however, be kept in mind that only intakes of <40 g/d are considered anti-inflammatory. The mean consumption in the 4th quartile was 43.9 g/d which is higher than the anti-inflammatory cut-off. These high intakes partly explain the apparently contradictory association of alcohol consumption with the E-DII in

this study population, as intakes of >40 g/d were omitted from the E-DII calculation and therefore considered as neither anti- nor pro-inflammatory. Furthermore, after additional adjustment for smoking (which was also highest in the 4th E-DII quartile), alcohol consumption no longer differed across the 4 quartiles. This suggests that the positive association between alcohol intake and the E-DII is partly explained by the concomitant use of tobacco products. A study conducted by Hodge *et al.* (2016) found that a high E-DII was associated with increased risk of lung cancer among current smokers (Hodge *et al.*, 2016), suggesting that current smokers likely have a more pro-inflammatory diet.

While moderate alcohol consumption is often associated with a healthy lifestyle, such as observed in the Mediterranean diet, in this population it is clear that a number of participants consume excessive, rather than moderate, amounts of alcohol and that these individuals are also often smokers. These are more indicative of an unhealthy lifestyle which can contribute to a more pro-inflammatory diet and finally increase the likelihood of developing CVDs.

5.4 The association of the E-DII with biochemical markers

5.4.1 Glucose and liver enzymes

The only biochemical variables that differed across the E-DII categories were fasting blood glucose and the liver enzymes gamma-glutamyl transferase (GGT) and aspartate transaminase (AST) (apart from the clot properties, which will be discussed in the next section). All three increased over the E-DII categories. This positive association between increasing fasting blood glucose and an increase in pro-inflammatory diet components has also been reported by other researchers (Phillips *et al.*, 2018; Wirth *et al.*, 2014). Insulin resistance has been postulated as the main mechanism behind this association as low-grade chronic inflammation has been shown to be associated with insulin resistance (Lopez-Candales *et al.*, 2017) through intracellular signalling pathways that are involved in the regulation of insulin (White, 1997). Inhibition of these signalling pathways through inflammation leads to insulin resistance (Hotamisligil *et al.*, 1996), which may ultimately result in increased fasting blood glucose. The significance for glucose was however adjusted for covariates.

There was also a positive association between GGT and AST and the E-DII in the PURE study population. This finding is in agreement with other studies that also found that a pro-inflammatory diet is associated with elevated liver enzymes, but this was additionally accompanied by obesity and fatty liver disease (Cantero *et al.*, 2018; Mazidi *et al.*, 2019). Researchers identified a possible pathway linking a pro-inflammatory diet to obesity and fatty liver disease (Cantero *et al.*, 2018; Mazidi *et al.*, 2019), with inflammation suggested as the underlying cause linking obesity to liver

damage (Cantero *et al.*, 2018). Cantero *et al.* (2018) reported, furthermore, that participants with a higher body mass index (BMI) consumed a more pro-inflammatory diet and also had higher liver enzymes such as AST, alanine transaminase (ALT) and GGT. Although the PURE study population's GGT and AST increased across the E-DII quartiles, the levels were still within normal ranges. Furthermore, an increase in E-DII was not associated with an increase in BMI; in fact, the lowest mean BMI value was reported in the highest E-DII category. Urban women had the highest intake of anti-inflammatory food components and nutrients and also presented with the highest BMI value. The higher liver enzymes in this population may be related to the higher alcohol consumption that was also observed in the 4th E-DII quartile, as discussed above. Alcohol affects liver enzymes and GGT is most sensitive to alcohol consumption (Salaspuro, 1999; Sillanauke, 1996). GGT increases with an increase in alcohol intake (Teschke & Koch, 1986) and can be used to verify reported alcohol intake (Anttila *et al.*, 2003; Jeppsson *et al.*, 1993; Keating *et al.*, 1998; Viitala *et al.*, 1998). GGT significantly increased with an increase in E-DII quartiles ($p=0.01$). After adjusting for sex, rural/urban status, smoking, alcohol, age, anti-inflammatory medication and aspirin use, the significance for GGT across E-DII quartiles remained. GGT was the highest in the 4th E-DII quartile, which also had the highest number of alcohol consumers and tobacco users.

These findings suggest that, while the final calculated inflammatory index of the diet is positively associated with liver enzymes, the factors contributing to the final E-DII score of a participant in this population probably differ from those in more affluent Western populations. These differences are likely anchored in the overall low intakes of food components and total energy as reflected by intakes of a number of components being lower than the recommended ranges, as well as the monotonous nature of the diet consumed and the observed high use of alcohol and tobacco. While a more pro-inflammatory diet contributed to an increase in GGT and AST in the PURE population, it does not appear to do so through the known pathway of obesity, necessitating further study into the role of the inflammatory potential of the diet in the context of food insecurity and unhealthy lifestyle practices which are highly prevalent in developing countries such as South Africa.

5.4.2 Inflammatory markers

Our study did not show a relationship between the E-DII and inflammatory markers, specifically CRP and IL-6. Of the previous studies investigating the association between E-DII and several different inflammatory markers (CRP, IL-6, homocysteine, fibrinogen) (Phillips *et al.*, 2018; Ren *et al.*, 2018; Shivappa *et al.*, 2017a; Shivappa *et al.*, 2014c; Shivappa *et al.*, 2019), six found a positive association with either CRP, IL-6 or homocysteine (Ren *et al.*, 2018; Shivappa *et al.*, 2015; Shivappa *et al.*, 2014c; Shivappa *et al.*, 2017b; Shivappa *et al.*, 2019) and only two studies reported no association with CRP or fibrinogen (Shivappa *et al.*, 2017a; Shivappa *et al.*, 2015). The majority of these studies used fewer food components/nutrients than were included in this study. The present study included 37 of the 45 food components/nutrients as set out by Shivappa *et al.* (2014), which potentially may have contributed to the lack of association between the E-DII and inflammatory markers. However, previous studies that did show an association between the E-DII and inflammatory markers contained even fewer food components (Shivappa *et al.*, 2017b; Shivappa *et al.*, 2019), suggesting that this is unlikely to be the reason behind the lack of association.

The lack of association between the E-DII and inflammatory markers in the PURE study population may be attributed 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 population was $+0.37 \pm 1.25$, which is indicative of a pro-inflammatory diet, but it is also close to the middle of the theoretical E-DII range, hence not strongly pro- or anti-inflammatory. Furthermore, the fact that the E-DII range of the PURE participants spanned only 61% of the theoretical range also indicates that the study participants consumed a relatively monotonous diet lacking in variety, with most diets being only modestly pro- or anti-inflammatory. This limited variation in inflammatory potential may explain the lack of association observed in the inflammatory markers as the diets were not sufficiently pro- or anti-inflammatory to alter serum inflammatory marker levels. Furthermore, a number of other factors that contribute significantly to CRP level in the PURE study population have previously been identified and may have had a more pronounced effect on CRP levels than the diet did (Myburg, 2018:30). Myburg (2018:30) reported that age, sex, ethnicity and socio-economic status all significantly influenced CRP concentration. Moreover, CRP is an acute-phase protein that increases when inflammation is present. Although individuals with a CRP level >10 mg/L were excluded from our analysis, South Africa has high rates of HIV/AIDS and tuberculosis infections. Individuals infected with HIV/AIDS, tuberculosis or other inflammatory conditions and also individuals with chronic low-grade inflammatory conditions, such as obesity, may present with elevated CRP levels. All these factors may contribute to the absence of an association

between the inflammatory potential of the diet and the inflammatory markers measured in serum and should be considered for future studies.

5.4.3 Fibrinogen and fibrin clot properties

There was a significant difference in lag time (time to clot formation), maximum absorbance (clot density) and CLT (time required to lyse the *ex vivo* formed plasma clots) across E-DII quartiles. Lag time, maximum absorbance and CLT all decreased across E-DII quartiles, suggesting that less dense clots that lyse faster are formed in participants with a more pro-inflammatory diet. This finding was somewhat unexpected. However, after adjustment for the covariates (sex, rural/urban status, smoking, alcohol, age, anti-inflammatory medication and aspirin use), which also differed across the E-DII quartiles and significantly contributed to the regression models, this difference disappeared. This finding suggests that the observed negative association between plasma clot properties and the E-DII probably does not reflect a real relationship between the inflammatory potential of the diet and the clot properties, and that the association is explained rather by association with co-variables that differed between the E-DII quartiles. All of these covariates, except fasting glucose, increased or decreased respectively across the E-DII quartiles in accordance with the direction of their contribution in the regression models, thus explaining the apparent differences in clot properties across the quartiles.

To our knowledge there have been, until now, no studies that investigated the relationship between the E-DII and fibrinogen and fibrin clot properties. The lack of association observed can probably also be attributed to the monotonous diet of the PURE study participants and fits with the lack of association found between the E-DII and the inflammatory markers. Being an acute-phase protein, fibrinogen concentration is increased in inflammatory conditions and a complex pathway exists that links inflammation and haemostasis (Levi & van der Poll, 2005; Pulanić & Rudan, 2005). During inflammatory conditions, fibrinogen levels increase as a result of stimulation by IL-6. This increase is due mainly to IL-6 response elements present in fibrinogen genes (Bataille & Klein, 1992; Fish & Neerman-Arbez, 2012). With an increase in fibrinogen concentration a stiffer, more dense clot is formed, which is more difficult to lyse (Gerth *et al.*, 1974; Roberts *et al.*, 1974; Shen *et al.*, 1975). Ultimately, research suggests that clot structure and lysis are strongly determined by the degree of inflammation present (Undas *et al.*, 2008). Since the E-DII did not relate to inflammatory markers (IL-6 or CRP), the expected causal pathway was absent, and consequently no association was seen with fibrinogen concentration or fibrin clot properties.

5.5 Limitations and direction for future studies

While this data significantly contributed to the body of evidence pertaining to the inflammatory potential of the diet, the study had a number of limitations:

- The cross-sectional nature of the study design does not allow the determination of causality. Future research should employ intervention studies to determine the effect of following a pro/anti-inflammatory diet on fibrinogen concentration and fibrin clot properties.
- Limited availability of inflammatory markers did not allow the development of an empirical dietary inflammatory score within this study population and a literature-based score, therefore, had to be used. It might be necessary to investigate this research question by developing an empirical score designed with the dietary intake data and additional inflammatory markers measured in this population since a number of differences were observed in the results compared with previously published reports in European and American populations.
- The limited variety and narrow E-DII range of the PURE participants' diet prohibited the drawing of firm conclusions regarding the association of the inflammatory index of the diet with inflammatory markers, fibrinogen and fibrin clot properties. This should be investigated in a population with a more varied diet.
- Drinking patterns could not be investigated in this study. Although only daily average alcohol consumption is required for the E-DII calculation, alcohol patterns e.g. binge drinking vs daily moderate alcohol consumption, have different health effects and should be considered in future research. This is of particular importance in any population known for binge drinking consumption patterns with high alcohol intakes over weekends and low consumption throughout the week.
- Dietary recall/ food frequency questionnaires are not accurate representations of dietary intake, as some participants may lie about their consumption (food and/or food components or alcohol) or some participants can't remember what they consumed and the exact amount of what they consumed.

5.6 Conclusion

This study described for the first time the inflammatory potential of a continental African study population consuming a typical South African diet within the constraints of food insecurity and poverty. It furthermore investigated the cross-sectional association of the inflammatory potential of the diet with inflammatory markers as well as fibrinogen concentration and fibrin clot properties. The inflammatory potential of the participants' diets differed significantly between men and women and rural and urban status, with urban women following the most anti-inflammatory diet and rural men the most pro-inflammatory diet. While micronutrient and total fat intakes were

highest in urban groups, these intakes were still lower than the dietary reference ranges. Overall, urban participants consumed higher amounts of both the anti- and pro-inflammatory food components/nutrients, whereas their rural counterparts consumed fewer food components/nutrients from both categories. This finding is supported by the higher kilojoule intake in urban compared with rural individuals. Additionally, alcohol did not demonstrate the expected anti-inflammatory contribution to the E-DII, as seen in Europeans. This is probably the result of higher intakes and the concomitant use of tobacco products. While the positive association between the E-DII and liver enzymes was confirmed, in this population, the association is likely to be mediated by excessive alcohol intake rather than obesity. The lack of variety within the study populations' diet may, furthermore, have contributed to a lack of association between the E-DII and inflammatory markers, fibrinogen concentration and fibrin clot properties. Although these associations were not present in this study, it does not mean that the inflammatory potential of the diet cannot influence fibrinogen concentration and fibrin clot properties. This relationship should be investigated in study populations with a more varied diet with more overt anti- and pro-inflammatory properties. The results of this study highlight the need to better understand the role of the inflammatory potential of the diet in the context of food insecurity, unhealthy lifestyle practices and increased CVD incidence, all of which are highly prevalent in developing countries such as South Africa.

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ANNEXURE A: ETHICS CERTIFICATE OF STUDY



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-HRECAppl@nwu.ac.za (for human studies)

18 November 2019

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Health Research Ethics Committee (NWU-HREC) on 18/11/2019, 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 association of the inflammatory potential of the diet with fibrinogen concentration and fibrin clot properties in black South Africans																	
Principal Investigator/Study Supervisor/Researcher: Prof M Pieters																	
Student: M Ferreira - 26043424																	
Ethics number:																	
N	W	U	-	0	0	0	3	4	-	1	7	-	A	1	-	0	3
Institution			Study Number					Year		Status		Sub-study					
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation																	
Application Type: Sub-study																	
Commencement date: 18/11/2019																	
Expiry date: 30/11/2020																	
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 November annually until completion of the study.																	

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-HRECAppl@nwu.ac.za or 018 299 1206

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: 2019.11.20
13:36:14 +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: INFORMED CONSENT FORM



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:

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

.....
(Signature of the subject)

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

Witnesses

1. 2.

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



PART 1

1. **School/Institute:**
Faculty of Health Sciences, North-West University

2. **Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study

3. **Full names, surname and qualifications of project leader:** Dr. Annamarie Kruger, Ph.D. (Nutrition)

4. **Rank/position of project leader:** Research Manager

5.. **Aim of this project**

PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.

6. **Explanation of the nature of all procedures, including identification of new procedures:**

Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire) with the help of field workers. A blood and urine sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and an ECG will be performed.

7. **Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**

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

8. **Precautions taken to protect the subjects:**

The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.

9. **Description of the benefits which may be expected from this project:**

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

10. **Alternative procedures which may be beneficial to the subjects:**

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



PART 2

To the subject signing the consent:

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

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

Dr ANNAMARIE KRUGER

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



ANNEXURE C: QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

PURE

Quantitative Food Frequency Questionnaire

Subject ID

Centre #

Community #

Household #

Subject #

Subject Initials

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

6 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		
<u>PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH</u>								
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

VEGETABLES AND FRUIT

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		
<u>DRINKS</u>								
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: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	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								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Chocolates	Name: _____ _____ _____							
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: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Jelly							3983	
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								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
<u>MISCELLANEOUS:</u> Please mention any other foods used more than once/two times a week which we have talked about:								
<u>INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS</u> 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!