Associations between plasma fatty acids, dietary fatty acids and cardiovascular risk factors: The PURE study

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Abstract

Background: Cardiovascular disease (CVD) is the leading global cause of death. CVD risk factors are considered intermediaries for the association between dietary fatty acids and CVD. Raised plasma total cholesterol, low density lipoprotein (LDL) cholesterol, raised triglycerides and decreased levels of high density lipoprotein (HDL) cholesterol, as well as reduced fibrinolytic potential (measured as increased clot lysis time) are known risk factors for CVD. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of the fibrinolytic process and an elevated PAI-1 level is therefore considered to be a potential risk factor for CVD. The growing number of controversies around the role that fat intake (more specifically the type of dietary fat) plays in CVD risk, is making it increasingly difficult for consumers and practitioners alike to form conclusions, and make recommendations and decisions regarding fat intake. Knowledge of the intake of individual fatty acids, fatty acid status (as opposed to subgroups of fat such as polyunsaturated fatty acids) and their associations with blood lipids, PAI-1_{act} and fibrinolytic potential is lacking in black South Africans and other populations. Therefore we aimed to investigate dietary fatty acid intake, as well as plasma phospholipid fatty acid status and their associations with blood lipids, PAI-1_{act} and clot lysis time, as a marker for fibrinolytic potential.

Methods: Cross-sectional data analysis within the Prospective Rural Urban Epidemiology (PURE) baseline study of apparently healthy black South African men and women (n=1950, 35–70 years) from rural and urban areas in the North West Province, from whom dietary data were collected. Blood lipid analyses, as well as laboratory analyses of fibrinolysis markers such as PAI-1_{act} and clot lysis time were also performed. Plasma phospholipid fatty acid extraction and isolation were performed on a random subsample (n = 716).

Results: The intake of individual fatty acids was significantly higher in urban than rural dwellers. However, the intake of omega-3 polyunsaturated fatty acids was below recommendations in all groups (rural and urban males, and rural and urban females). Total cholesterol and LDL cholesterol were higher in females than in males, with no rural–urban differences. Intake of alpha-linolenic acid was positively associated with total cholesterol (β=0.143) and triglycerides (β=0.256) in males. The risk of having elevated LDL cholesterol also increased with increased intake of alpha-linolenic acid (OR 1.49, 95% CI 1.04, 2.14). In females, dietary arachidonic acid and eicosapentaenoic acid (EPA) were positively associated with total cholesterol and LDL cholesterol, whereas docosahexaenoic acid (DHA) was negatively associated with total cholesterol and LDL cholesterol. Dietary alpha-linolenic acid was positively correlated with plasma EPA (males $r = 0.19$, $p = 0.002$, females $r = 0.25$, $p < 0.001$) and DHA (males $r = 0.33$, $p < 0.001$, females $r = 0.30$, $p < 0.001$). Plasma DHA was positively associated with triglycerides in males ($\beta = 0.410$, $p< 0.001$) and in females ($\beta = 0.379$, $p< 0.001$). PAI-1_{act} was positively associated with clot lysis time, and plasma myristic acid and DHA were positively associated

with PAI-1_{act} in females. However, these fatty acids were not associated with clot lysis time. Different types of plasma fatty acids were associated with PAI-1_{act} than with clot lysis time. Plasma alpha-linolenic acid (β = 0.123, P = 0.037), mead acid (β = 0.176, P = 0.019), arachidonic acid (β = 0.253, 0.025) and omega-3 docosapentaenoic acid (omega-3 DPA) (β = 0.224, P = 0.002) were positively associated with clot lysis time, while both myristic acid (β = -0.130, P = 0.016) and EPA (β = -0.131, P = 0.021) were negatively associated with clot lysis time in male subjects. Plasma oleic acid (C18:1n9) (β = -0.411, P = 0.001) and omega-6 DPA (C22:5n6) (β = -0.285, P = 0.001) were negatively associated with clot lysis time, while dihomo-gamma-liolenic acid (DGLA) (C20:3n6) were positively associated (β = 0.178, P = 0.001) with clot lysis time in females.

Conclusions: These results suggest that specific individual dietary fatty acids might be associated with blood lipids in males differently than in females, irrespective of rural or urban dwelling. It is not known however, if associations would still be present under conditions of greater intake of alpha-linolenic acid. Our results further suggest that a higher percentage of alpha-linolenic acid might be converted to DHA in this population with low intake of essential and long-chain polyunsaturated fatty acids compared to populations with a high intake of these fatty acids. These results suggest that plasma phospholipid fatty acids should not be used in isolation as biomarkers for intake of fat, without taking dietary intake data into consideration also. Associations between fatty acids and clot lysis time might be independent from PAI-1_{act}. The association between mead acid and clot lysis time indicates that clot lysis time might increase with an essential fatty acid deficiency. This may be of particular concern in this population with a documented lower fat intake. Because the study design of this study is cross-sectional, it is not able to determine cause-and-effect, and results should therefore be verified with a randomised controlled trial.

Key terms: Dietary fatty acids, plasma phospholipid fatty acids, blood lipids, PAI-1, fibrinolysis

Uittreksel

Agtergrond: Kardiovaskulêre siektes (KVS) is die hoofoorsaak van mortaliteit in die wêreld. KVS risikofaktore word oorweeg as tussengangers vir die assosiasie tussen vetsure in die dieet en KVS. Verhoogde plasma vlakke van totale cholesterol, lae-digtheid lipoproteïen (LDL) cholesterol, trigliseriede, verlaagde plasma vlakke van hoë-digtheid lipoproteïen (HDL) cholesterol sowel as verminderde fibrinolitiese potensiaal (aangedui deur verhoogde klontlisetyd) is bekend as risikofaktore vir KVS. Plasminogeen aktiveerder inhibeerder-1 (PAI-1) is 'n belangrike inhibeerder van die fibrinolitiese proses en ‘n verhoogde PAI-1 vlak word dus beskou as ‘n moontlike risikofaktor vir KVS. Groeiende kontroversie rondom die rol wat die inname van vet (meer spesifiek die tipe vetsure in die dieet) in KVS risiko speel, maak dit toenemend moeilik vir verbruikers en praktisyns om gevolgtrekkings, aanbevelings en besluite ten opsigte van die inname van vet te maak. Kennis van die inname van individuele vetsure, vetsuurstatus (in teenstelling met subgroepe van vet soos poli-onversadigde vetsure) en hul assosiasies met bloedlipiede, PAI-1_{akt} en fibrinolitiese potensiaal in die swart Suid-Afrikaanse bevolking en in ander bevolkings ontbreek. Daarom het ons dit ten doel gehad om dieetvetsuurinname sowel as plasma fosfolipied vetsuurstatus en hul assosiasies met bloed lipiede, PAI-1_{akt} en klontlisetyd (as 'n merker van fibrinolitiese potensiaal), te ondersoek.

Metodes: ‘n Dwarsdeursnit data analise binne die Prospektiewe Stedelike Landelike Epidemiologie (PURE) basislynstudie van skynbaar gesonde swart Suid-Afrikaanse mans en vroue (n = 1950, 35-70 jaar) van landelike en stedelike gebiede van die Noord-Wes Provinsie, van wie dieetdata ingesamel is, is uitgevoer. Bloedlipied analises, asook laboratoriumontleding van merkers van fibrinolise, PAI-1_{act} en klontlisetyd is ook uitgevoer. Die ekstraksie en isolasie van plasma fosfolipied vetsure is uitgevoer op 'n ewekansige substeekproef (n = 716).

Resultate: Die inname van individuele vetsure was aansienlik hoër in stedelike as landelike inwoners, hoewel die inname van omega-3 poli-onversadigde vetsure laer was as die aanbevelings in alle groepe (landelike mans en vrouens en stedelike mans en vrouens). Totale cholesterol en LDL cholesterol was hoër in vrouens as mans, met geen landelik-stedelike verskille. Die inname van alfa-linoleensuur het positief geassosieer met totale cholesterol (β = 0.143) en trigliseriede (β = 0.256) in die mans. Die risiko vir verhoogde LDL cholesterol het toegeneem met 'n verhoogde inname van alfa-linoleensuur (OR 1.49, 95 % CI 1.04, 2.14). In die vrouens het die inname van beide aragidoonsuur en eikosapentaenöesuur (EPS) positief geassosieer met totale cholesterol en LDL cholesterol, terwyl dokosaheksaenöesuur (DHS) negatief met die totale cholesterol en LDL cholesterol gekorreleer het. Dieet alfa-linoleensuur positief gekorreleer met plasma EPS (mans r = 0.19, p = 0.002, vrouens r = 0.25, p <0.001) en plasma DHS (mans r = 0.33, p < 0.001, vrouens r = 0.30, p < 0.001). Plasma DHS het positief geassosieer met trigliseriede in die mans (β = 0.410, p <0.001) en in die vrouens (β = 0.379, p <

0.001). PAI-1_{act} het positief geassosieer met klontlisetyd, en plasma miristiensuur en DHS het positief geassosieer met PAI-1_{act}. Tog het hierdie vetsure nie geassosieer met klontlisetyd nie. Die plasma vetsure wat met PAI-1_{act} geassosieer het, het verskil van die vetsure wat met klontlisetyd geassosieer het. Plasma alfa-linoleensuur (β = 0.123, P = 0.037), heuningsuur (β = 0.176, P = 0.019), aragidoonsuur (β = 0.253, 0.025) en omega-3 dokosapentaenoïeksuur (omega-3 DPS; C22:5n3) (β = 0.224, P = 0.002) het positief geassosieer met klontlisetyd, terwyl miristiensuur (β = -0.130, P = 0.016) en EPA (β = -0.131, P = 0.021) negatief geassosieer het met klontlisetyd in die mans. Beide plasma oleïensuur (C18:1n9) (β = -0.411, P = 0.001) en omega-6 DPS (C22:5n6) (β = -0.285, P = 0.001) het negatief geassosieer met klontlisetyd, terwyl dihomo-gamma-linoleïen suur (DGLS) (C20:3n6) positief geassosieer het (β = 0.178, P = 0.001) met klontlisetyd in die vrouens.

Gevolgtrekkings: Hierdie resultate dui daarop dat die spesifieke individuele dieetvetsure bloedlipiede in mans anders kan beïnvloed as in vrouens, ongeag van landelike of stedelike bewoning. Dit is egter nie bekend of assosiasies steeds teenwoordig sou wees onder toestande van groter inname van alfa-linoleensuur nie. Ons resultate dui verder daarop dat ‘n hoër persentasie van alfa-linoleensuur moontlik omgeskakel kan word na DHS in hierdie populasie met 'n lae inname van essensiële en lang-ketting poli-onversadigde vetsure, in teenstelling met populasies met 'n hoë inname van hierdie vetsure. Hierdie resultate wys verder daarop dat plasma fosfolipied vetsure nie gebruik moet word in isolasie as biomerkers vir die inname van vet, sonder om dieetinname data ook in ag te neem nie. Assosiasies tussen vetsure en klontlisetyd mag onafhanklik wees van PAI-1_{akt}. Die assosiasie tussen heuningsuur en klontlisetyd dui daarop dat klontlisetyd met 'n essensiële vetsuur tekort kan verleng. Dit kan van besondere belang wees in hierdie populasie met ‘n gedokumenteerde laer vetinname. Aangesien die studie-ontwerp van hierdie studie ‘n deursnee studie is, is dit nie moontlik om oorsaak en effek te bepaal nie en die resultate moet deur ‘n gerandomiseerde studie geverifieer word.

Sleutel woorde: Dieetvetsure, plasma fosfolipied vetsure, bloedlipiede, PAI-1, fibrinolise

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Abbreviation list

AMDR	Acceptable macronutrient distribution range
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CVD	Cardiovascular disease
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
ELOVL	Elongation of very long-chain fatty acids
EPA	Eicosapentaenoic acid
FADS	Fatty acid desaturase gene clusters
FAO	Food and Agricultural Orginization
GLA	Gamma-linolenic acid
HDL	High density lipoprotein
HIV	Human Immunodeficiency virus
HMG-CoA	3-hydroxy-3methyl-glutaryl coenzyme A reductase
IDL	Intermediate density lipoprotein
JELIS	Japan Eicosapentaenoic acid Lipid Intervention Study
KIHD	Kuopio Ischaemic Heart Disease Risk Factor
LCAT	Lecithin—cholesterol acyltransferase
LDL	Low density lipoprotein
NCD	Non-communicable diseases
NCEP	National education cholesterol program
$PAI\text{-}1_{act}$	Plasminogen activator inhibitor-1 (activated)
PPARα	Peroxisome proliferator activating receptor α
PURE	Prospective Urban and Rural Epidemiology study
RCT	Randomised controlled trial
SERBP-1	Sterol regulatory element-binding protein 1

TAFI	Thrombin-activatable fibrinolysis inhibitor
t-PA	Tissue-plasminogen activator
THUSA	Transition in Health and Disease
u-PA	urinary plasminogen activator
VLDL	Very low density lipoprotein
WHO	World Health Organization

Chapter 1 Introduction

1.1 Background

Cardiovascular disease (CVD) is the leading global cause of death (WHO, 2011). In 2011, ischaemic heart diseases were among the top 10 causes of death in men in South Africa and were responsible for 2.6% of deaths, while cerebrovascular diseases moved to third place among the top 10 causes of death in South Africa, accounting for 4.5% of death (Statistics SA, 2014). Improved socio-economic conditions and the availability of a variety of foods are associated with the nutrition transition. These conditions have resulted in changes in diet, lifestyle and patterns of undernourishment, obesity and lifestyle diseases (such as CVD) in developing countries (Popkin, 2001). South Africa is experiencing a continuous urbanisation of Africans (Statistics SA, 2003). In line with global predictions (Solomons and Gross, 1995), the urban population is growing in relation to the rural population in South Africa and since 1996 has increased from 55% of the population (data were reclassified to match the classification of the demographics used in the census of 2001) to 58% in 2001 (Statistics SA, 2003). The Transition and Health during Urbanisation of South Africans (THUSA) study showed a reduction in intake of carbohydrate-rich food and an increase in the intake of animal-derived foods and added fats with urbanisation (Vorster et al., 2005). Despite this, the preliminary data from the Prospective Urban Rural Epidemiology (PURE) study still showed prudent fat intake in both rural and urban areas (Smuts and Wolmarans, 2013).

CVD risk factors are considered intermediaries for the association between dietary fatty acids and CVD (Wilson et al., 1998; Arab, 2003). Raised plasma total cholesterol, low density lipoprotein (LDL) cholesterol, raised triglycerides and decreased levels of high density lipoprotein (HDL) cholesterol are known risk factors for CVD (Wilson et al., 1998; Perk et al., 2012). Reduced fibrinolytic potential (measured as increased clot lysis time) have also been shown to be a risk factor for CVD (Meltzer et al., 2010). Plasminogen activator inhibitor (PAI-1) is a major inhibitor of the fibrinolytic process (Lee et al., 2012a) and PAI-1 levels were found to be the main contributing factor to clot lysis time by Meltzer and colleagues (Meltzer et al., 2010). Therefore increased PAI-1 levels are considered to be potential risk factors for CVD (Meltzer et al., 2010).

Scientific interest in and public awareness of the role of fatty acids in human health have increased in the past few years and fatty acids have been shown to have an effect on CVD and CVD risk factors (Nestel et al., 2002; Serrano-Martinez et al., 2004). A high omega-3 (n-3) fatty acid status is thought to be cardioprotective through the reduction of risk factors such as dyslipidaemia, hypertension, thrombosis, arrhythmia and the improvement of arterial

compliance, endothelial vasodilator functioning and heart rate variability (Pase et al., 2011) . Convincing evidence exists for the protective effect of the polyunsaturated fatty acids linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on CVD (Horrobin and Huang, 1987; Anderson et al., 2009). Increased risk of CVD is also associated with the intake of the saturated fatty acids myristic and palmitic acid (Anderson et al., 2009).

Traditionally, research focussed more on groups of fat (saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids), and increasingly differentiated between omega-3 polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids. Recently, it has become clear that even within subtypes of fat such as omega-3 fatty acids, specific individual fatty acids can cause different effects with regard to blood lipids and other risk factors such as PAI-1. However, directions of the effects were inconsistent (Mori et al., 2000; Egert et al., 2009; Wei and Jacobson, 2011).

The growing number of controversies around the role fat intake (more specifically the type of dietary fat) plays in CVD is making it increasingly difficult for consumers and practitioners alike to form conclusions, and make recommendations and decisions regarding fat intake. It is becoming clear that more research is needed on this topic and that the answers are not as straightforward as previously believed. Furthermore, this topic is challenging to researchers since fat intake is one of the most difficult dietary components to measure (Arab, 2003). Studies investigating dietary fat intake are criticised for limitations surrounding dietary data collection and interpretation. It can be very difficult for subjects to recognise and quantify fat, particularly when it comes to food preparation (Arab, 2003). In addition, accuracy during recording and coding is continuously questioned (Arab, 2003; Wolmarans et al., 2009). Furthermore, when looking at associations between fatty acid biomarkers and biomarkers of dyslipidaemia only, investigators are faced with the question of how intake and metabolism affects biomarkers.

Knowledge of the intake of individual fatty acids, fatty acid status (as opposed to subgroups of fat such as polyunsaturated fatty acids) and their associations with blood lipids, PAI-1 and fibrinolytic potential is lacking in this population of healthy black South Africans in rural and urban areaas as well as in other populations. The role that individual fatty acids play in rising levels of dyslipidaemia, as well as their role in PAI-1 and fibrinolytic potential in this population needs to be better understood. Former studies have not provided conclusive evidence regarding the relation between dietary fat intake and the haemostatic system and were mostly focussed on examining Caucasian individuals. While a limited number of studies have investigated the association between omega-3 fatty acids and PAI-1, no studies to our knowledge have been performed on the association between omega-3 fatty acids and global fibrinolytic potential. PAI-1 is, however, is the major protein involved in fibrinolysis and the use of the global fibrinolytic assay provides a better reflection of the true fibrinolysis rate of an

individual. No studies have been performed on the association between omega-3 fatty acids and global fibrinolytic potential. This necessitates an investigation into the possible effects of omega-3 fatty acids on global fibrinolytic potential in addition to PAI-1.

The results of our study will be useful in South Africa, not only by providing information regarding the nutritional status and phospholipid fatty acid status of a population in South Africa, but also by giving insight regarding associations with CVD risk factors in this population group. This in turn can be used to identify strategies for improving the health status of South Africans. The results can be used to establish and improve nutrient intake goals, specifically the quality of fat in the diet. Health education programmes, interventions, and food and nutrition policies can make use of the fatty acid intake and status data of a population in order to improve the quality of life of the people of the North West Province in particular, which is where this study was conducted, and South Africa in general.

1.2 Aim and objectives

1.2.1 Aim

The aim of this study was to investigate associations between dietary fatty acid intake (as measured by Quantified Food Frequency Questionnaire), plasma fatty acid composition and specific CVD risk factors of healthy black participants in the rural and urban areas of the North West Province of South Africa by means of cross-sectional data analyses of the PURE study.

1.2.2 Objectives

- To investigate associations between dietary fatty acid intake and blood lipids in relation to urbanisation and gender.
- To determine the plasma phospholipid fatty acid status in this population.
- To investigate associations between dietary fatty acid intake and plasma phospholipid fatty acid composition.
- To investigate associations between plasma phospholipid fatty acids and blood lipids in the PURE study.
- To investigate associations between dietary fatty acids, phospholipid fatty acids and PAI-1 in the PURE study.
- To investigate associations between dietary fatty acids, phospholipid fatty acids and fibrinolytic potential by means of global clot lysis time in the PURE study.

1.3 Structure

The structure of this thesis is in article format and is divided into six chapters.

- **Chapter 1** provides the background information, aim and objectives, structure of the thesis and information about the research team.
- **Chapter 2** summarises the relevant literature, while the methods used and results are given in three manuscripts (chapters 3, 4 and 5).
- **Chapter 3** Manuscript 1 was published in the *International Journal of Cardiology*. The title of the article is **'Different dietary fatty acids are associated with blood lipids in healthy South African men and women: the PURE study'**. This article described the dietary fatty acid intake of the population and investigated associations between dietary fatty acids and blood lipids in the PURE study.
- **Chapter 4** Manuscript 2 has been submitted to the *International Journal of Cardiology*. The title reads '**Associations between dietary fatty acids, plasma phospholipid fatty acids and blood lipids in healthy South Africans from the PURE study'.** In this manuscript the phospholipid fatty acid status of this population was described. The associations between dietary fatty acid intake and plasma phospholipid fatty acid composition were also investigated. Additionally, associations between dietary plasma phospholipid fatty acids and blood lipids were explored.
- **Chapter 5** The title of manuscript 3 is **'Associations of plasma phospholipid fatty acid status and dietary fatty acid intake with PAI-1 and clot lysis time in healthy South African men and women from the PURE study'.** In this manuscript the associations between dietary fatty acids, phospholipid fatty acids and PAI-1, and fibrinolytic potential were investigated.
- **Chapter 6** comprises a conclusion that summarises the essential findings of the study and provides recommendations.

Chapters 1, 2, 5 and 6 will be presented in South African English, while chapters 3 and 4 will be in U.S. English, due to the preference of the journal.

1.4 Research team

Table 1-1: Research team, affiliation and role

Members of the research team	Affiliation	Role
M. Richter	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Ph.D. student, protocol writing, analysis of samples, statistical analysis, interpretation of results and writing of the literature and manuscripts
Prof Marius Smuts	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Promoter of PhD thesis. Guidance regarding protocol, writing of the literature review, interpretation of results and co-author of all papers
Dr Jeannine Baumgartner	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Assistant promoter of PhD thesis. Guidance regarding statistics, writing of the literature review, interpretation of results and co-author of all papers.
Prof Marlien Pieters	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Co-promoter of PhD thesis. Guidance regarding protocol and interpretation of results related to PAI-1 and fibrinolytic potential and co-author of manuscript 3.
Prof Edelweiss Wentzel-viljoen	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Guidance regarding dietary methodology and co-author of all manuscripts

Prof Annemarie Kruger	Africa Unit for Transdisciplinary Health Research, North-West University, Potchefstroom Campus, South Africa	South African PURE study coordinator
Ellenor Rossouw	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Guidance with laboratory processes
Walter Dreyer	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Guidance with laboratory processes and quantification
Linda Malan	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Guidance with laboratory processes and quantification
Rebaone Rammutla	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Assisted with laboratory analyses

1.5 Bibliography

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Chapter 2: Literature review

In South Africa a gradual change in dietary intake patterns has been observed with urbanisation. Despite urbanisation, fat intake in the black South African population has remained within the 25-35% of energy from fat, recommended by the World Health Organization (WHO) (FAO/WHO, 2009) and targets set for South Africa (Smuts and Wolmarans, 2013). Traditionally the nutrition transition is accompanied by a westernised lifestyle (which includes higher fat intake – specifically saturated fat intake and lower activity levels), which in turn is associated with cardiovascular disease (CVD) and other diseases of lifestyle (Popkin, 2001). Recently, CVD has been on the increase worldwide (WHO, 2011), and there have been some controversies surrounding the role that fat and fatty acids play in CVD. Risk factors of CVD include among others unfavourable blood lipid levels (increased total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides, and decreased high density lipoprotein (HDL) cholesterol), as well as haemostatic factors such as plasminogen activator inhibitor-1 (PAI-1) and reduced fibrinolytic potential. Additionally fat intake is not only challenging to measure, but it is also accompanied by many limitations. Fatty acid status on the other hand is considered a biomarker of intake for some of the fatty acids (Arab, 2003).

2.1 Dietary fatty acids

A fatty acid consists of a chain of carbon atoms surrounded by hydrogen atoms. It is characterised by a carboxyl (acid) group located at the acid (“delta”) end and a methyl group at the opposite omega (ω or n) end. The carboxyl group comprises two oxygen molecules and dissociable hydrogen (proton). This carbon chain can be between four to 28 carbons in length and is mostly even numbered (IUPAC, 1997). The main fatty acid classes are: saturated, monounsaturated and polyunsaturated. Polyunsaturated fatty acids can further be subdivided into omega-3 polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids. Polyunsaturated fatty acid s (omega-3 or omega-6) and their structures contain at least two double bonds. They are referred to as long-chain polyunsaturated fatty acids when the chain lengths are at least 20 carbon atoms or more. Their nomenclature is derived from the position of the first double bond, as well as the total number of double bonds on the carbon chain (Sala-Vila et al., 2008).

Saturated fatty acids contain no double bonds and are therefore saturated with hydrogen atoms. Saturated fatty acids that are of relevance in the diet are: lauric acid (C12:0); myristic acid (C14:0); palmitic acid (C16:0); and stearic acid (C18:0). The most common sources of saturated fat are animal products such as dairy and meat products (Institute of Medicine, 2005). Common

dietary sources of saturated fatty acids in this population are discussed under 2.1.2 Dietary fat and fatty acid intake pattern of South Africans.

Monounsaturated fatty acids have one double bond. The hydrogen atoms of monounsaturated fatty acids are on the same side of the double bond and located at nine carbon atoms (omega-9) from the methyl end. The main dietary monounsaturated fatty acids include: oleic acid (C18:1 omega-9); myrisoleic acid (C14:1 omega-9); palmitoleic acid (C16:1omega-9); vaccenic acid (C18:1 omega-7); eicosenoic acid (C20:1omega-11); and erucic acid (C22:1 omega-13) (Institute of Medicine, 2005), of which oleic acid is the most commonly occurring dietary monounsaturated fatty acid, accounting for about 92% of dietary monounsaturated fatty acids (Institute of Medicine, 2005).Common dietary sources of monounsaturated fatty acids in this population are discussed under 2.1.2 Dietary fat and fatty acid intake pattern of South Africans.

Omega-3 polyunsaturated fatty acids in the diet include: the alpha-linolenic acid (C18:3n3); eicosapentaenoic acid (EPA, C20:5n3); docosapentaenoic acid (DPA, C22:5n3); and docosahexaenoic acid (C22:6, DHAn3) (Institute of Medicine, 2005). The omega-3 fatty acids from animal sources are EPA and DHA occurring in fish and fish oils, while alpha-linolenic acid is the predominant plant source of omega-3 polyunsaturated fatty acids. Alpha-linolenic acid is called an essential fatty acid because it is not synthesised endogenously by humans. Common dietary sources of omega-3 fatty acids in this population are discussed under 2.1.2 Dietary fat and fatty acid intake pattern of South Africans.

Omega-6 polyunsaturated fatty acids contain at least two carbon-to-carbon double bonds, with the first double bond at the sixth carbon from the methyl end (Harris et al., 2009). Omega-3 polyunsaturated fatty acids are usually highly unsaturated fatty acids and one of the double bonds are positioned three carbon atoms from the methyl end (Institute of Medicine, 2005). The major dietary omega-6 polyunsaturated fatty acids are: the essential fatty acid linoleic acid (C18:2n6); γ- linoleic acid (C18:3n6); dihomo-gamma- linoleic acid (C20:3n6); arachidonic acid (C20:4n6); adrenic acid (C22:4n6); DPA (C22:5n6) (Institute of Medicine, 2005), of which linoleic acid (C18:2omega-6) is by far the primary dietary omega-6 fatty acid, accounting for 85% to 90% of the omega-6 polyunsaturated fatty acids in the diet (Harris et al., 2009). Linoleic acid cannot be synthesised by humans and exact minimum requirements have not been established for healthy adults (Harris et al., 2009). Common dietary sources of omega-6 polyunsaturated fatty acids in this population are discussed under 2.1.2 Dietary fat and fatty acid intake pattern of South Africans.

Trans fatty acids are unsaturated fatty acids and their configuration of the bond to the trans position causes the carbon chain to become less curved, similar in shape to that of saturated fatty acids (Riccardi et al., 2003). The negative effects of industrially produced trans fatty acids

on coronary heart disease (CHD) are well accepted and are mostly attributed to adverse effects on blood lipids, insulin resistance, endothelial function and thrombosis (Hu et al., 1997; Pietinen et al., 1997; Oh et al., 2005). Due to the structure of trans fatty acids, which is similar to that of saturated fatty acids, they reduce membrane fluidity (Elmadfa and Kornsteiner, 2009). Trans fatty acids increase LDL cholesterol significantly (Riccardi et al., 2003; Lichtenstein et al., 2006) and might also cause slight reductions in HDL cholesterol (Judd et al., 1994) which in turn result in an increase in LDL:HDL ratio (Riccardi et al., 2003). Trans fatty acids might additionally have a negative effect on Lipoprotein(a) and LDL cholesterol particle size negatively (Riccardi et al., 2003). An intake of less than 1% of total energy is recommended for the intake of trans fatty acids (Lichtenstein et al., 2006). No adequate intake value or recommended daily allowance is set for trans fatty acids because they have no known health benefit. No upper limit is set for the intake of trans fatty acids either, because any increase in trans fatty acid intake result in increased CHD risk (Institute of Medicine, 2005).

2.1.1 Dietary recommendations

Recommendations for dietary fat intake are based on essential fatty acid needs, neurodevelopmental support, CVD health, and prevention of degenerative diseases (Uauy et al., 1999).

Neither an adequate intake, nor a recommended daily allowance is set for total fat, saturated fatty acids, and monounsaturated fatty acids, due to insufficient data to determine a defined level of fat intake at which risk of inadequacy or prevention of chronic disease occurs and because saturated fatty acids can be synthesised by the body and have no known role in the prevention of chronic diseases (Institute of Medicine, 2005).

No upper limit is set for intake of saturated fatty acids because any increase in saturated fatty acid intake results in increased CHD risk (Institute of Medicine, 2005). The WHO recommends less than 10% of total energy intake to come from saturated fatty acids. The American Heart Association diet and lifestyle recommendations (Lichtenstein et al., 2006) suggests a saturated fatty acid intake of less than 10% of energy, while the National cholesterol education program (NCEP) step III diet recommends less than 7% of energy intake from saturated fatty acids as part of the therapeutic lifestyle approach to reduce CHD risk (Cleeman, 2001).

There is insufficient evidence to set an upper limit for dietary omega-9 cis monounsaturated fatty acid intake (Institute of Medicine, 2005). The American Heart Association diet and lifestyle recommendations (Lichtenstein et al., 2006) propose an intake of 10% of energy as monounsaturated fatty acids, while the NCEP step III (2001) diet recommends no more than 20% of intake as monounsaturated fatty acids (Cleeman, 2001).

Dietary recommendations for omega-6 polyunsaturated fatty acids are aimed at providing optimal intakes to reduce risk for chronic disease, particularly coronary heart disease. An adequate intake for linoleic acid based on the median intake in the United States is 12 g/d for young women and 17 g/d for young men. There is insufficient evidence to set an upper limit for intake of omega-6 polyunsaturated fatty acids. The NCEP step III diet recommends polyunsaturated fatty acid consumption of up to 10% (Cleeman, 2001). The American Heart Association supports an omega-6 polyunsaturated fatty acid intake of at least 5% to 10% of energy, and advises against reducing omega-6 polyunsaturated fatty acid intake with the aim of reducing the ratio of omega-6:omega-3 intake (Harris et al., 2009).

The adequate intake for alpha-linolenic acid is 1.6 and 1.1 g/d for men and women respectively and is based on median intakes in the United States. There is insufficient evidence to set an upper limit for omega-3 fatty acid intake. Approximately 10% of the acceptable micronutrient distribution range (AMDR) for alpha-linolenic acid can be consumed as EPA and/or DHA (Institute of Medicine, 2005). Other recommendations for long-chain omega-3 fatty acids and fish for primary prevention of CHD death and after a coronary event is 250–500mg/day of EPA+DHA. However, it is an estimate with no evidence of harm at higher intakes (Deckelbaum et al., 2008). There is a need to establish a dietary reference intake for the individual long-chain omega-3 fatty acids (20 carbons or greater) since the majority of recommendations have been issued on the basis of the amount of EPA and DHA together, without recommendations specifically for EPA or DHA (Kris-Etherton et al., 2009).

Many professional bodies and societies have set recommendations for omega-3 polyunsaturated fatty acids in the general population. These range between 200mg to 2000mg for EPA and DHA combined, between 0.6 – 1.3 of total energy for alpha linolenic acid and 0.5 to 2% of total energy for total omega-3 intake (Table 2-1). South Africa, however, recommends between 2 to 3% of total energy from omega-3 intake, of which 250 to 500mg per day for EPA and DHA combined constitutes 0.6 to 1.2% of total energy from alpha-linolenic acid (Smuts and Wolmarans, 2013).

In 2009 in South Africa, an expert group who met regarding the ‘health significance of fat quality in the diet’ recommended a fat intake between 20-35% of energy (Smuts and Wolmarans, 2013). They concluded that saturated fatty acid intake should be less than 10% of energy intake, and less than 7% of energy in those at risk for CVD, while polyunsaturated fatty acids should provide 6-10% of energy (5-8% from omega-6 and 1-2% from omega-3 polyunsaturated fatty acids); Monounsaturated fatty acid intake should be the remainder of energy and trans fatty acids should be limited to less than 1% of energy (Smuts and Wolmarans, 2013).

Table 2-1:International recommendations for dietary omega 3 fatty acid intake from different professional bodies. Adapted from Mozaffarian & Wu (2011).

Recommended by	Year	EPA+DHA	Alpha-linolenic acid	Total omega-3
European Commission Euro diet Core report	2000	≥ 200mg EPA+DHA /d	Target: 2g/d	
Health Council of Netherlands	2001	≥ 200mg EPA+DHA /d	Adequate: 1% of energy	
U.S. National Academy of Sciences	2002	AMDR 0.06 – 0.12% of energy	AMDR: 06. – 1.2% of energy	
French agency of Food Environmental and Occupational Health and Safety Omega-3 Report	2003	400 – 500 mg EPA+DHA /d, (100 – 120mg/d DHA)	Target: 1.6 – 2g/d	Target: 1% of e
European Society of Cardiology	2003	Recommendation: ~1g/d		
Joint UN FAO/WHO Expert Consultation.	2003	1-2 fish servings/week 400 – 1000 mg EPA+DHA /d		Target: 1 – 2%
International Society for the Study of Fatty Acids and Lipids: Policy Statement	2004	≥ 500 mg/d	Target: 0.7% of energy	
UK Scientific Advisory Committee on Nutrition	2004	(≥2 fish servings/week) ≥ 450mg EPA+DHA /d		
AHA	2010	Minimum 2 servings fish/week ~1g EPA+DHA /d		
National Health and Medical Research Council (Australia and New Zealand)	2006	Adequate: 90 – 160 mg EPA+DHA /d Target: 430 – 610 mg EPA+DHA /d	Adequate: 0.8 – 1.3 g/d Target: 2.7 g/d	
UN FAO Report on Fats and Fatty Acids in Human Nutrition	2008	AMDR: 250 – 2000 mg EPA+DHA /d	Minimum: 0.5% of energy	AMDR: 0.5 – 2
US Department of Agriculture, 2010 Dietary Guidelines for Americans	2010	≥ 2 servings of fish on average ≥250 mg EPA+DHA /day	0.6 – 1.2 % energy	

Acceptable Micronutrient Distribution Range (AMDR). Food and Agriculture Organization (FAO), world health organisation (WHO), United Nations (UN), American Heart Association (AHA), Medical research council (MRC), United Kingdom (UK), United States (US)

2.1.2 Dietary fat and fatty acid intake pattern of South Africans

In South Africa the major dietary saturated fat sources include: palm kernel oil, coconut oil, palm oil, animal and dairy fat, including butter, and products made with these products (Smuts and Wolmarans, 2013). Sources of monounsaturated fatty acids are mainly of plant origin, but can also be found in meat products. Major sources of monounsaturated fat in the diet are canola and olive oil, and products made with these oils (Smuts and Wolmarans, 2013). Additional sources include sunflower oil, soya oil, avocado, nuts and peanut butter. The main contributors of omega-6 polyunsaturated fatty acids to the diet are sunflower and soybean oil, and margarines made from these oils (Smuts and Wolmarans, 2013). Major omega-3 fatty acids in the diet include: canola oil, green leafy vegetables, soya and pilchards (Smuts and Wolmarans, 2013), while other sources include walnuts, mackerel, herring and tuna. Trans fatty acids can occur naturally in very low quantities in milk and meat or it can be formed through partial hydrogenation by the food industry in order to improve shelf-life and increase the melting point (Eckel et al., 2007). Margarines are regulated in South Africa and does not contain more than 1% trans fatty acids (Smuts and Wolmarans, 2013). Other dietary sources include processed food, such as margarine, cookies, crackers and pies. The quality of dietary lipids has previously been speculated to cause negative effects due to the use of cast-iron pots and re-use of oils that may cause peroxidation. These practices might result in modified proteins and nucleotides which may modify the epithelium to initiate vascular disease, due to aldehyde emanation from specifically polyunsaturated fatty acids (Haywood et al., 1995). A study by Stonehouse et al. (2010), however, found that the use of omega-6 polyunsaturated fatty acid-rich vegetable oils and the way they were used did not cause safety concerns in a previous study on the PURE population on human immunodeficiency virus (HIV) positive and HIV negative participants.

South Africa is a culturally diverse country, resulting in diverse food intake in addition to the changes associated with the nutrition transition (Steyn and Nel, 2006). A fat intake of more than 30% of energy has been documented in some in the South African populations, particularly the of the Cape Peninsula (Langenhoven et al., 1988) and South African men from European descent (Faber et al., 1992). When Walker and colleagues (1992) investigated the diets of elderly black women in rural and urban communities in South Africa between 1969 and 1989, increases of 6% in energy intake and 5% in fat intake were found in that timeframe. Later a review of the nutritional status of South Africans between 1975 and 1996 (Vorster, 1997) summarised the intake of fat for black South Africans as follows: urban women 22.3% and rural women 16.3% to 20.8%. The mean dietary fat intake of children between the ages of 1-9 was 23% of energy and ranged between 20% and 30% of total energy in 1999 (Labadarios et al., 2005). Even though the intake in children may differ from the intake in adults, this data could be indicative of the macronutrient composition of the household diet to some degree (Smuts and Wolmarans, 2013). The 2 more recent epidemiological studies investigating dietary intake in the

black South African population are the Transition and Health during Urbanisation of South Africans (THUSA) study and the Prospective Urban Rural Epidemiology (PURE) studies. The THUSA study investigated dietary intake from 1996 to 1998 in adult black South African men and women aged 16 to 65 years, in urban and rural areas of the North West province in South Africa (MacIntyre et al., 2002). Fat intake in men in rural areas was 23.3% of energy, while in urban areas intake was 27.2% of energy. In females intake was 23.9% in rural areas and 28.8% in urban areas. Data from the PURE study indicated that dietary fat intake was also low in black South Africans between the ages of 35 and 65 years from the North West province. A dietary fat intake of 17.6% of energy for males and 20.3% of energy for females was found in rural areas, while in urban areas intake from fat was 24.1% of energy for women and 22.6% of energy for men (Smuts and Wolmarans, 2013). A comparison of intake data from 1975 to more recent intake data in 2005 showed that total fat intake increased from 21% to 30% of energy in women living in urban areas in South Africa, while intake increased from 15% to just over 20% of energy in those living in rural areas (Vorster et al., 2011). All recent reports of fat intake adapted from a summary by Smuts and Wolmarans and reproduced with permission from the authors (Table 2-2, Addendum D), indicated saturated, monounsaturated and polyunsaturated fatty acid intake below 10% of energy, except Macintyre et al (2002) who found monounsaturated fatty acid intake in urban women 10.4% of energy.

The urban black African population of the Cape peninsula in South Africa had a saturated fatty acid intake of 8.8% of energy in urban areas in the Coronary Heart Disease Risk Factor study in the African population of the Cape Peninsula (BRISK) study (Bourne et al., 1993).

Dietary fatty acid intake might influence plasma and cellular fatty acid status, both of which can have an effect on the health of an individual (Arab, 2003).

In the past, even though quantitative goals of the South African food based dietary guidelines were to reduce saturated fatty acids and replace it with monounsaturated fatty acids and polyunsaturated fatty acids, a big emphasis was placed on the reduction of the total amount of fat in the diet by recommending to ‘Eat fats sparingly’. In an attempt to place more emphasis on the quality of fat, the new South African food based dietary guidelines now recommend ‘Eat fats sparingly, and choose vegetable fats rather than hard fats’ (Smuts and Wolmarans, 2013). After consideration of the lower fat intake of parts of the population and the possible negative consequences thereof, as well as the fact that other countries have increased fat intake recommendations to 30-35% of intake, Smuts and Wolmarans (2013) suggested an alternative guideline that reads ‘Eat and use the right type of fats and oils in moderation.

Table 2-2: South African studies reporting dietary fat intake. Adapted from Smuts & Wolmarans (2013), with permission from the authors.

Reference	Gender	Sample size	Age (years)	Total fat	SFAs	MUFAs	PUFAs
				% of energy			
MacIntyre *et al* 2002 (rural)*	Male	431	15-65	23.3	6.6	7.2	5.5
MacIntyre *et al* 2002 (rural)*	Female	610	15-65	23.9	7.2	7.7	6.0
Faber *et al* 2005 (rural)	Female	187	25-55	23.0	No data	No data	No data
Wentzel-Viljoen[a] 2012(rural)**	Male	332	35-65	17.6	3.9	4.2	5.7
Wentzel-Viljoen[a] 2012 (rural) **	Female	634	35-65	20.3	4.5	4.7	6.9
Wentzel-Viljoen[b] 2012 (rural) **	Male	212	35-65	22.6	6.3	6.9	6.8
Wentzel-Viljoen[b] 2012 (rural)**	Female	469	35-65	24.1	6.7	7.0	7.7
MacIntyre *et al* 2002 (urban)*	Male	312	15-65	27.2	7.7	9.4	6.3
MacIntyre *et al* 2002 (urban)*	Female	398	15-65	28.8	9.0	10.4	6.7
Wentzel-Viljoen[a] 2012(rural)**	Male	392	35-65	25.3	9.1	7.2	7.2
Wentzel-Viljoen[a] 2012 (rural) **	Female	592	35-65	28.3	7.3	8.2	8.3
Wentzel-Viljoen[b] 2012 (rural) **	Male	205	35-65	26.2	7.1	8.3	7.6
Wentzel-Viljoen[b] 2012 (rural)**	Female	367	35-65	27.0	7.4	8.8	8.1
Steyn *et al* 2006	Female	1726	15-49	23.8	6.7	8.1	5.7

MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids, SFAs: saturated fatty acids
"Rural" is represented by people living in traditional African villages, farm dwellers and those in informal settlements. "Urban" is represented by both middle- and upper-class individuals (black South Africans)
* Transition and Health during Urbanisation of South Africans (THUSA) study. Weighted calculations were carried out
** Prospective Urban Rural Epidemiology (PURE) study. Black South Africans. (Wentzel-Viljoen E. Personal communication, November 2012)
[a] Data from 2005 survey; b Data from 2010 survey

2.1.3 Measurement of fat intake

Fat is one of the most difficult dietary components to measure (Arab, 2003). The accuracy of participants' reporting is prone to bias. It can be very difficult for study participants to recognise and quantify fat, especially fats used for food preparation. In general, a food frequency questionnaire tends to overestimate the intake of a population, especially for food items that are eaten regularly but not daily (Wolmarans et al., 2009). (MacIntyre et al., 2001b) found that the quantitative food frequency questionnaire used in the THUSA study, which was also used for the PURE study, tended to underreport energy and fat intakes in comparison with a seven-day weighed record. Better agreement was, however, found at lower intake levels, which is the case in the population group being studied. Usually, underreporting of fat is more of a concern among overweight individuals because of the social implications (Arab, 2003). It has been shown in a metabolic-unit study of 33 women that high fat foods were not prone to bias in reporting per se (Poppitt et al., 1998). Failure to report between-meal snack foods, however, was the major cause of underreporting in both obese and non-obese participants (Poppitt et al., 1998). However, the attitude to obesity of the population group being studied must be kept in mind. It is, for example, more culturally acceptable for a black South African women to be overweight than for women from other cultures in South Africa (McIza et al., 2006). On the other hand, coding is also subject to human error. Additionally, the completeness of food composition tables is also questioned. Yet, despite the limitations in dietary assessment in epidemiology, the food frequency questionnaire still remains the method of choice (Gibson, 2005; Wentzel-Viljoen et al., 2011).

2.2 Fatty acid status

Non-esterified fatty acids circulate the blood on hydrophobic sites of albumin and at concentrations below 1mmol/L. Fatty acids, however, are seldom available as unesterified fatty acids (free fatty acids) in the circulation. They form part of the triglycerides and phospholipids, which are transported by lipoproteins. Triglycerides, phospholipids and cholesterol esters provide 3, 2 and 1 mmol/L of fatty acid per mmol measured in plasma. When fatty acids are metabolised to individual fatty acids, they have important functions, such as storage units for energy, structural units in membranes such as red blood cell membranes (by forming phospholipid bilayers) and precursors to eicosanoids, and they can therefore be found in serum, membranes and adipocytes (Arab, 2003). Biomarkers of fat intake have been proposed to quantify consumption of essential and exogenously produced fatty acids (Arab, 2003). Fatty acids exchange between different mediums such as serum, red blood cells and adipose tissue (Reed, 1968). While plasma phospholipid fatty acid statuses are said to reflect short to medium-term intake to a certain degree, red blood cells reflect longer term (~4 monts) intake, while adipose tissue reflect long term intake, except during fasting (Reed, 1968; Baur et al., 2000;

Arab, 2003). However, many of the fatty acids can be lengthened, synthesised or desaturated endogenously, which can affect the use of fatty acid measures as biomarkers of consumption. Additionally, a large proportion (<15-35%) of alpha-linolenic acid is oxidized for energy (Arterburn et al., 2006). Therefore, interpretation requires an understanding of fatty acid metabolism, exogenous factors and the contributions of various body pools (Arab, 2003). As far as biomarkers are concerned, this literature review will focus on plasma phospholipid fatty acids, since that was the analysis used for fatty acid composition analysis in this study.

Phospholipids each contain a hydrophilic polar head, adjacent to a phosphate group and two hydrophobic tails consisting of fatty acids esterified to the glycerol backbone. Phospholipids are primarily components of cell membranes in the form of a lipid bilayer, which is a thin polar membrane made up of two layers of phospholipid molecules. These membranes form a continuous barrier around cells that allows cells to regulate salt concentrations and pH through ion pumps. Fatty acids within the phospholipids influence many biochemical and physiological functional properties of membranes. These functions include eicosanoid signalling, ion channel modulation pinocytosis and gene expression regulation, and are determined by a combination of the length of the carbon chain, the number of double bonds and their placement, as well as isomerism around these bonds (Psota et al., 2006). Other phospholipids such as sphingomyelin, including sphingomyelin (phosphate) were not examined but they also influence cellular metabolism. Sphingomyelin are more prone to intermolecular hydrogen bonding than other phospholipids, and undergoes significant interactions with cholesterol which has the ability to eliminate the liquid to solid phase transition in phospholipids (Massey, 2001).

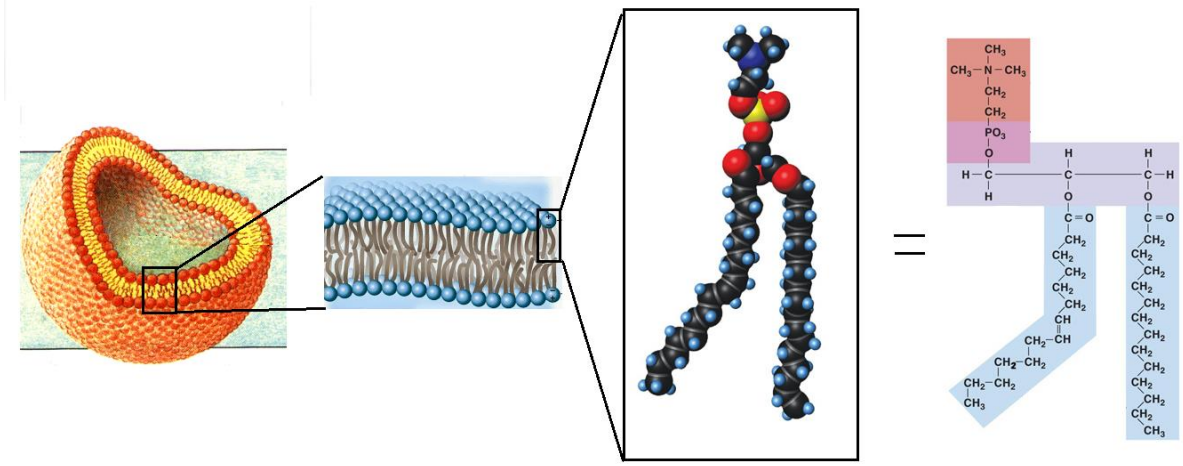


Figure 2-1: Structure of tetradecanol phosphatidycholine as part of a lipid bilayer cell membrane. Figure adapted from (http://academic.brooklyn.cuny.edu/biology/bio4fv/page/phosphb.htm, https://sites.google.com/site/davidbirdprovidencehigh/Home/courses/summer-biology/day-6, and http://wps.aw.com/bc_martini_eap_5/105/27046/6923957.cw/index.html)

2.1.1 Metabolic pathways of fatty acids

Desaturase enzymes remove two hydrogen atoms from the hydrocarbon chain of a fatty acid, creating a carbon-to-carbon double bond, while elongation enzymes add two carbon atoms to the carboxyl end of the fatty acid, increasing the chain length (Arab, 2003). Elongation is usually a faster process than desaturation and these two processes usually (but not exclusively) alternate (de Alaniz and Marra, 2003). Desaturase and elongase enzymes are located in the endoplasmic reticulum and peroxisomes of the liver. The following desaturases are present in humans: Δ9-desaturase, Δ 6-desaturase and Δ5-desaturase – and there is still some controversy about the existence of a Δ4-desaturase which presumably can synthesize DHA from omega-3 DPA, and omega-6 DPA from adrenic acid (C22:4n6) (Martinez et al., 2010).

Plasma saturated fat content is usually not a good indication of dietary saturated fat intake, because it can be synthesised endogenously from acetyl CoA from either carbohydrates or by elongation of shorter chain fatty acids two carbons at a time (Arab, 2003). Additionally Δ9-desaturase is used to produce oleic acid by desaturation of stearic acid, while high intakes of saturated fatty acids combined with low linoleic acid intake result in increases in the proportions of palmitic, palmitoleic, and oleic acids in plasma (Anderson et al., 2009). Rhee and colleagues (1997) found in their study that 14% of the stearic acid was desaturated and converted to oleic acid. Conversion of stearic acid (a saturated fatty acid) to the oleic acid (a monounsaturated fatty acid) might explain why dietary stearic acid has metabolic effects closer to those of oleic acid rather than those of saturated fatty acids (Institute of Medicine, 2005). When consuming more than 25% of energy from fat sources, synthesis of saturated fatty acids is proposed to be uncommon (Hellerstein, 1999). Thus, if dietary recommendations succeed in reducing the consumption of saturated fatty acids to below 10% of energy, conversion of saturated fatty acids should contribute only a small amount to its effect on blood lipids (Rhee et al., 1997).

The plasma phospholipid status of monounsaturated fatty acids and polyunsaturated fatty acids are usually regarded as good indicators of dietary intake (Hodge et al., 2007). In contrast with plants, mammals lack Δ12-desaturase, causing an inability to convert oleic acid into linoleic acid (omega-9 to omega-6 conversion), and a lack of Δ15-desaturase causes the inability to convert linoleic into alpha-linoleic acid or the inter-conversion of omega-6 and omega-3 fatty acids de novo in humans (Arab, 2003). Because linoleic acid and alpha-linolenic acid cannot be synthesised endogenously, they can only be obtained from dietary sources and therefore they are considered essential. However, they can be metabolised to longer chain fatty acids, through desaturation and elongation. The generally accepted pathway for the conversion of precursors LA and alpha-linolenic into long chain polyunsaturated fatty acids (Sprecher pathway) is illustrated in Figure 2–2. Omega-3 and omega-6 fatty acids compete for the same enzyme systems involved in desaturation and elongation in order to produce longer-chain fatty acids,

which are more biologically active (Psota et al., 2006). Additionally, omega-3 and omega-6 fatty acids also compete for the lipoxygenases and cyclooxygenases involved in the production of leukotrines and prostagladins that mediate cell functions relevant to CVD, including inflammatory responses, platelet aggregation, and vasoconstriction and vasodilation cellular adhesion processes (Psota et al., 2006). The conversion of alpha-linolenic acid to DHA is not known to be a major determinant of variations in the proportion of DHA in plasma lipids and it is affected by diet and gender (Pawlosky et al., 2003b; Arterburn et al., 2006). Approximately 5% to 10% of dietary alpha-linolenic is thought to be converted to EPA. The conversion of alpha-linolenic to DHA is typically less than 5% and might be as low as 1%. The rate limiting step is the conversion from alpha-linolenic acid to 18:4 omega-3. (Burdge and Calder, 2005). The efficiency of the conversion of alpha-linolenic acid to EPA was shown to be 0.2% by Pawlosky and colleagues (2001) in 8 participants in a metabolic unit stable isotope controlled feeding trial in the United States of America. Of the fatty converted EPA, 63% have been shown to be converted to omega-3 DPA acid, and 37% of omega-3 DPA to DHA (Pawlosky et al., 2003b).

Therefore, proportions of EPA and DHA in plasma lipids are determined mainly by intake of preformed EPA and DHA and negatively influenced by the intake of linoleic acid (Anderson et al., 2009). Excessively high intake of linoleic acid compared to alpha-linolenic acid increases the production of arachidonic acid relative to that of EPA and DHA. In addition, large reservoirs of linoleic acid in adipose tissue slow down conversion of alpha-linolenic acid to EPA and DHA (Psota et al., 2006).

Arachidonic acid is synthesised from linoleic acid more efficiently but might also be obtained from meat and fish (Anderson et al., 2009). The metabolic utilisation of omega-3 fatty acids differs from omega-6 fatty acid metabolism. Even though both groups are incorporated into membranes and utilised as substrates for lipid mediators (Psota et al., 2006), the metabolite fatty acids of linoleic acid and alpha-linolenic acid, specifically arachidonic acid and docosahexaenoic acid (DHA), are preferentially incorporated into the lipid bilayers of cell membranes and serve there as important structural components of membranes, determining and influencing the behaviour of membrane-bound enzymes and receptors (Das, 2008). EPA, on the other hand, is predominantly utilised as a substrate for eicosanoid synthesis (Psota et al., 2006). Long-chain fatty acids (arachidonic acid, EPA and DHA) are more biologically active than the 18 carbon fatty acids (linoleic acid and alpha-linolenic acid), which serve primarily as substrates for synthesis of longer-chain counterparts (Psota et al., 2006).

As mentioned in **section 2.9**, phospholipids can influence functional membrane properties such as eicosanoid signalling, ion channel modulation pinocytosis and gene expression regulation, and are determined by the combination of the length of the carbon chain, number of double bonds and their placement, as well as isomerism around these bonds (Das, 2008). Increased

amounts of saturated fatty acids in the membrane structure decrease the membrane fluidity. When more polyunsaturated fatty acids are incorporated into the membrane, on the other hand, they result in a more fluid membrane structure (Psota et al., 2006). There is competition between omega-3 and omega-6 fatty acids for enzymes involved in desaturation and elongation of the carbon chain (to form longer chain fatty acids), as well as for cyclooxygenases and lipoxygenases. The production of prostaglandin, prostacyclin and thromboxane result from the cyclooxygenase pathways, while leukotrienes, hepoxylins and lipoxins that are 20-carbon cyclised metabolites of dihomo-gamma-linolenate, arachidonate or eicosapentaenoate, originate from the lipoxygenase pathways (Arab, 2003). Therefore, the ratio of intake of these two will to a certain degree determine the physiological effects that will dominate. Eicosanoids from the 2- and 4-series are produced from arachidonic acid and they are pro-inflammatory and prothrombotic. The 3- and 5-series eicosanoids produced from EPA, on the other hand, either result in lower levels of inflammation and thrombosis, or have the opposite effects (Psota et al., 2006). Increasing consumption of EPA and DHA can improve the balance (Wijendran and Hayes, 2004).

Factors that can influence fatty acid status include genetic variation, disease status, lifestyle differences, micronutrient status, circulation apolipoprotein levels, the hormonal balance of the individual, sex, age and body mass index (BMI) (Arab, 2003; Hodge et al., 2007). Enzyme activities for production of long-chain polyunsaturated fatty acids are believed to be influenced by polymorphisms within the fatty acid desaturase (FADS) gene clusters and the elongation of very long-chain fatty acids (ELOVL) gene family (Cormier et al., 2014). Positive selection in Africans are hypothesised to have increased alleles in the FADS region, which is associated with enhanced metabolism of medium-chain polyunsaturated fatty acids to long-chain polyunsaturated fatty acids (Mathias et al., 2012). FADS enzymes coded by FADS1 and FADS2, and hypothetically by FADS3, are non-heme, iron-containing, oxygen-dependent endoplasmic reticulum proteins introducing double bonds between two carbon atoms in long-chain polyunsaturated fatty acid biosynthesis and are positioned on chromosome 11 (Cho et al., 1999; Park et al., 2012). FADS gene coding for Δ5-desaturase and Δ6-desaturase production could influence the rate of conversion, (Cho et al., 1999; Park et al., 2012) and their expression might be regulated by sterol regulatory element-binding protein 1 (SERBP-1) and peroxisome proliferator activating receptor α (PPARα) (Matsuzaka et al., 2002).

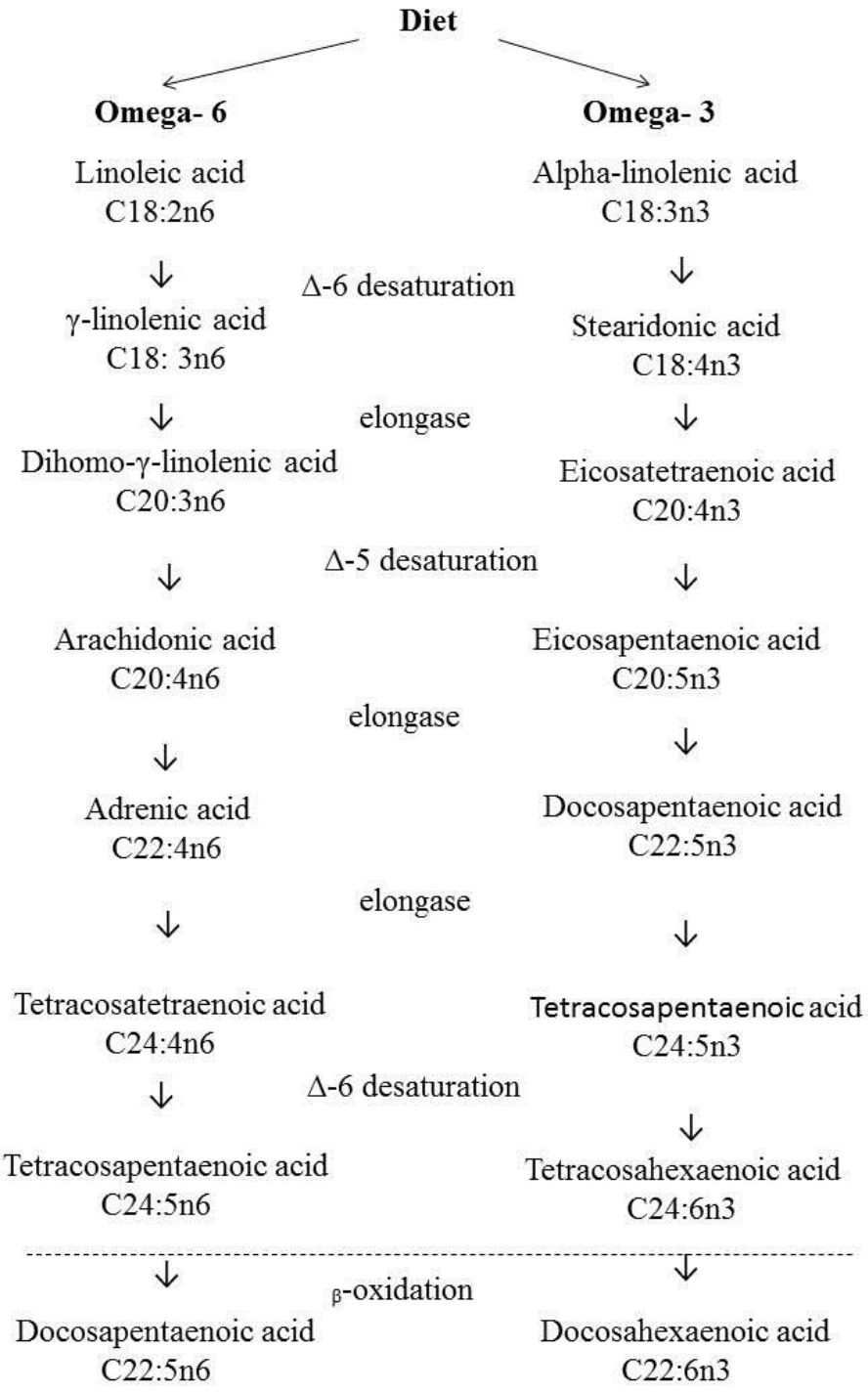


Figure 2-2: Biochemical pathways (Sprecher pathway) for desaturation and elongation of omega-6 and omega-3 polyunsaturated fatty acids to their longer chain counterparts. Adapted from Arterburn *et al* (2006).

2.2.2 Fatty acid status in South Africans

The only published study available for fatty acid status in adults in South Africa was a case-control study within the PURE population, comparing HIV infected and non-infected participants' status by Stonehouse and colleagues (2010). For the 301 uninfected participants, this study only reported total saturated fatty acids (34.3%), monounsaturated fatty acids (24.5%) omega-6 polyunsaturated fatty acids (42.1%), linoleic acid (26.3%) and omega-3 polyunsaturated fatty acids (3.17%), without distinguishing the composition of individual fatty acids further (Stonehouse et al., 2010). Other investigations in South Africa that reported fatty acid status are limited to children. Baseline fatty acid composition of preschool children in a rural community in South Africa found the major contributors to plasma phosphatidylcholine (a class of phospholipid identified by choline) composition to be palmitic acid (26.9 ± 3.1 %), stearic acid (17.0 ± 2.0%), oleic acid (12.1 ± 2.4%), linoleic acid (21.8 ± 4.4%), dihomo-gama-linolenic acid (2.8 ± 0.7%), arachidonic acid (12.5 ± 2.3%) and DHA (4.11 ± 1.5%) (Tichelaar et al., 1999). It has been shown that dietary fat intake and red blood cell fatty acid status of caregivers in South Africa followed the same trend as their children's dietary fatty acid intake and status (Ford, 2013). It is clear from available fatty acid status data in South Africa that this field requires more research in order to accurately describe the population's fatty acid status.

2.3 Transport of fat

The physiological role of lipoproteins includes transporting lipids to cells for energy, storage or use as a substrate for the synthesis of prostaglandins, thromboxanes and leukotrienes (Mahan et al., 2012). Lipoproteins are water-soluble complexes consisting of lipids (triglycerides, cholesterol, cholesterol ester and phospholipids) and apolipoproteins. Hydrophobic triglycerides and cholesteryl esters are found in the centre of the lipoprotein particles, surrounded by more hydrophilic phospholipids, cholesterol and apolipoproteins (Assmann, 1982). Apolipoproteins target the delivery of lipids to specific cells. Lipoproteins are divided into categories and distinguished from each other by their densities, which are determined by ultracentrifugation. They are defined further by their particle size, apolipoprotein content and electrophoretic mobility (Assmann, 1982; Riccardi et al., 2003).

Very low density lipoprotein (VLDL) particles are synthesised in the liver and their function is to transport triglycerides and cholesterol (Riccardi et al., 2003). They can be divided into large triglyceride-rich VLDL and small cholesteryl ester-rich VLDL (Assmann, 1982).

Intermediate density lipoproteins (IDLs) are formed when VLDL particles are hydrolysed by lipoprotein lipase (Mahan et al., 2012). IDLs are precursor particles of LDL and metabolic products of VLDL (Assmann, 1982).

LDL's primary role is to transport cholesterol for the formation of cell membranes and the synthesis of steroid hormones in the body (Riccardi et al., 2003). The liver removes IDL from the circulation or it is converted into LDL (Mahan et al., 2012). Subclasses IDL appear to be precursors of LDL subclasses (i.e. large IDL → to large LDL) (Krauss, 1994). Triglyceride enrichment in these two pathways might then result in even smaller and denser LDL3 and LDL4. While at lower triglyceride levels there is a shift towards less dense LDL (LDL1 and LDL2) and higher metabolic turnover (Griffin and Packard, 1994).

The main function of HDL is the transport of cholesterol from the peripheral tissues back to the liver in the process of reverse cholesterol transport (see section 2.3.3) (Riccardi et al., 2003).

Lipoprotein metabolism is a combination of complex processes, which include the degradation, synthesis and exchange of different classes of lipoproteins (Assmann, 1982). Factors that can affect lipoprotein metabolism and concentration include, among others, age, gender, hormones, genetics and dietary intake (Zhang et al., 2010). The main lipoprotein metabolic systems are summarised under the following sub-headings: exogenous fat transport, endogenous fat transport and reverse cholesterol transport.

2.3.1 Exogenous fat transport

Chylomicrons are formed in the mucosal cells of the duodenum and jejunum and their function is to transport exogenous triglycerides (Riccardi et al., 2003).

Breakdown products of fat digestion, namely free fatty acids and cholesteryl esters, are repackaged into chylomicrons in the form of triglycerides and cholesteryl esters for transportation. These chylomicrons, which contain the triglycerides and cholesterol, and also apo B-48, apo A1 apo AII and AIV on the surface, enter the lacteals in the intestinal villi through the lymphatic system and through the thoracic duct into the circulatory system. Chylomicrons are then rapidly hydrolysed into core remnants, enriched with cholesteryl esters, apolipoprotein and surface material, enriched with phospholipids, cholesterol and apolipoproteins (Assmann, 1982). The free fatty acids and glycerol that were hydrolysed from chylomicrons are used either as sources of energy or, if unused, reassembled into triglycerides to be stored in adipocytes (Berg et al., 2002). Phospholipids are used mainly for cell membrane synthesis (Lodish et al., 2005). Surface material is transferred to HDL (at least partially) and apo E is in turn transferred from HDL cholesterol to the chylomicron remnants. Chylomicron remnants are removed from the circulation by hepatic receptors that recognise surface apo E (Chait, 1983). Cholesterol is used by the liver to form bile, either as free cholesterol or as bile acids. Some of the cholesterol and bile acids are recycled by the liver after reabsorption in the intestine, while a portion of the cholesterol and bile acids are excreted with the faeces (Brown et al., 1981).

2.3.2 Endogenous fat transport

VLDL is produced and secreted by the liver. The liver converts carbohydrates and fatty acids into triglycerides. Cholesterol can be obtained from either dietary sources or from synthesis in the liver. The rate limiting enzymes in cholesterol synthesis is 3-hydroxy-3methyl-glutaryl coenzyme A reductase (HMG-CoA) (Riccardi et al., 2003). Triglycerides and cholesterol assemble with VLDL particles containing apo B-100, apo E and apo C, which is secreted into the blood (Assmann, 1982). VLDL also feeds triglycerides to cells and adipose tissue when hydrolysed, and then forms IDL (Riccardi et al., 2003) that retains apo B-100 and apo E on its surface (Assmann, 1982). Excess surface molecules, phospholipids, free cholesterol and apo Cs are displaced from the lipolysed VLDL (to new forming HDLs). Hepatic LDL receptors again recognise and bind apo E, this time on the IDL's surface in order to remove IDL from plasma (Chait, 1983). IDL particles that were not cleared are further lipolysed by hepatic lipase, forming LDL cholesterol. Cholesterol in HDL is esterified via Lecithin—cholesterol acyltransferase (LCAT) (see previous section) and Cholesteryl ester transfer protein (CETP) transfers the cholesteryl esters to IDL. Through continued lipolysis, cholesterol ester-rich LDL particles that contain only apo B are formed. These are recognised and removed by hepatic and extra-hepatic receptors. LDL proceeds to the lysosomes after endocytosis, where apo B and cholesterol esters are hydrolysed, releasing amino acids and free cholesterol respectively.

The free cholesterol migrates into the cytoplasm, where it causes HMG-CoA reductase suppression and subsequently inhibits the synthesis of cholesterol. It also causes the expression of LDL receptors to be down regulated, as well as the activation of acyl coenzyme A: cholesteryl acyltransferase, which serves to esterify cholesterol and therefore enables storage of excess cholesterol as cholesterol esters.

After several days in plasma and after hydrolysis, LDLs might become modified (smaller and denser) and are not recognised to the same degree by LDL receptors. They therefore remain in the circulation longer (Riccardi et al., 2003) and are eventually removed from circulation by scavenger receptors on endothelial cells and macrophages. This is one of the initial triggers for the formation of atherosclerotic plaques.

Factors determining the concentration of LDL in the circulation are: the rate of VLDL formation and conversion thereof to LDL, hepatic LDL receptor density and the LDL receptor density of cell membranes (Riccardi et al., 2003).

2.3.3 Reverse cholesterol transport

Reverse cholesterol transport is the transport of cholesterol by HDL from peripheral tissues to the liver for oxidation and removal, which plays an important role in regulating cholesterol

homeostasis. Cells secrete excess cholesterol in a free unesterified form (Riccardi et al., 2003), after which free cholesterol is first taken up by the precursor HDL particle and is incorporated into HDL. Interaction of HDL with a specific surface receptor can also promote the translocation of excess cholesterol from intracellular to membrane pools (Assmann, 1982).

After free cholesterol has been incorporated, it is esterified by the action of lecithin cholesterol acyltransferase (Riccardi et al., 2003). These cholesterol esters in HDL3 are transferred by cholesterol ester transfer protein to apo B-containing VLDL and IDL. Cholesterol ester transfer protein exchanges cholesterol esters for triglycerides between HDL and VLDL, causing larger HDL2 particles to form, which are cholesterol ester poor and triglyceride rich. When a triglyceride threshold has been reached, HDL2 is then converted back into small triglyceride- and cholesterol ester-poor HDL3 through hydrolysis of triglycerides and phospholipids by the action of hepatic lipase. HDL particles that are smaller and more dense are catabolised more rapidly by the liver, resulting in lower circulating HDL levels (Riccardi et al., 2003).The majority of cholesterol esters are delivered to the liver via LDL receptors, but can also be delivered to the liver via direct binding to apo E receptors. HDL can also directly deliver cholesterol esters to hepatocytes through independent particle uptake or retro-endocytosis. Reduced circulating HDL levels result in increased cholesterol in peripheral cells due to decreased reverse cholesterol transport. Subsequently, this leads to down-regulation of the LDL receptors on cell membranes, causing reduced cellular LDL uptake from the circulation (Riccardi et al., 2003).

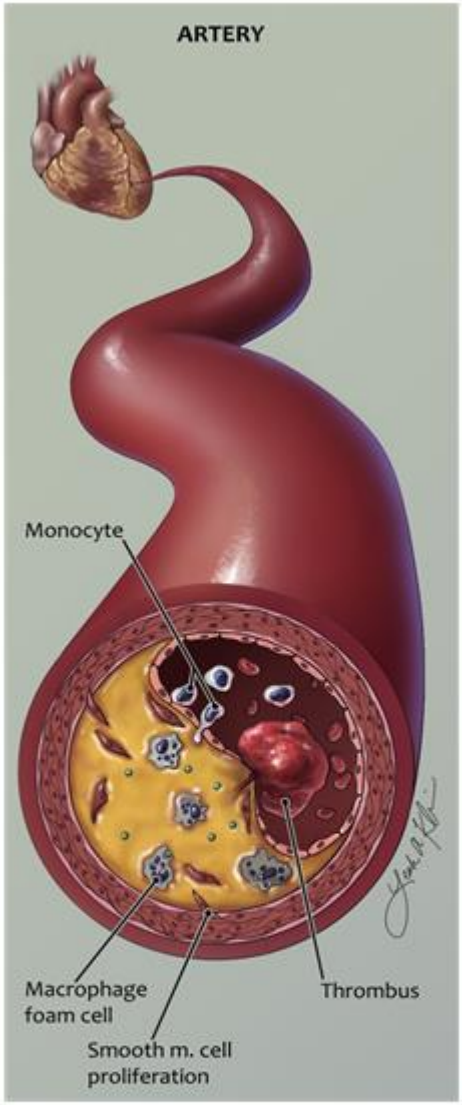
2.4 Cardiovascular disease

CVDs are a set of diseases involving the heart and blood vessels which include: coronary heart disease; cerebrovascular disease (including stroke); deep vein thrombosis and pulmonary embolism; congenital heart disease; rheumatic heart disease; peripheral arterial disease (WHO, 2011). Many risk factors have been established for CVD, however for the purpose of this thesis the focus will be on blood lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglcerides), as well as PAI-1 and fibrinolytic potential.

2.4.1 Pathogenesis of atherosclerosis and thrombosis: the role of lipoproteins and the haemostatic system

Atherosclerosis develops through a series of pathological stages, as illustrated in Figure 2-3. Previously, chronic coronary artery disease was considered a cholesterol storage disease (Libby and Theroux, 2005). Plaque formation happens when the immune system responds to

inflammation (caused by oxidised LDL, chylomicron and VLDL remnants) by releasing circulating monocytes, which turn into macrophages within the sub-endothelial space (Riccardi et al., 2003; Mahan et al., 2012). Native LDL is practically devoid of lipid peroxidation products and oxidative damages moves along a spectrum of becoming minimally modified and then oxidised. The first stage does not much affect the ligand activity of LDL receptors but the later stage has lost this capacity and instead becomes a ligand for scavenger receptors (along with chylomicron and VLDL remnants) which abound on macrophages and such uptake results in the formation of foam cells as well as cytokines which cluster in the sub-endothelial space to become a fatty streak/plaque. . More recently the pathogenesis of atherosclerosis has been proposed to be much more complex, brought on by a variety of risk factors as diverse as dyslipidaemia, products of glycoxidation associated with hyperglycaemia, vasoconstrictor hormones inculpated in hypertension and proinflammatory cytokines derived from excess adipose tissue or certain bacterial products.



Figrue 2-3: Pathogenesies of atherosclerosis and thrombosis. Adapted from (Rask-Madsen and King, 2013)

These risk factors increase the expression of adhesion molecules that promote the adhesion of leukocytes to the inner surface of the arterial wall (Libby and Theroux, 2005). The expression of cytokines, which regulate the migration of leukocytes into the intima, is associated with risk factors for atherosclerosis. In the intima, communication between leukocytes and smooth muscle cells are dependent on mediators of inflammation and immunity, including lipid mediators such as prostanoids and other derivatives of arachidonic acid, such as leukotrienes (Libby and Theroux, 2005).

A protective collagen layer (atheroma) forms between the deposits and the artery lining and smooth muscle cells migrate from the tunica media into the intima to form a fibrous cap over the plaque. (Libby and Theroux, 2005; Mahan et al., 2012). Over time, cells proliferate and more cholesterol, connective tissue and cell debris accumulate, turning the streak into plaque (Ajjan and Ariens, 2009). An atheroma can rupture and tissue factor is subsequently exposed, this attracts platelets and activates the coagulation cascade, resulting in the formation of a

thrombus. Rapid conversion of factor VII into its active form (FVIIa) is dependent on lipolytic activity and is mainly supported by large triglyceride-rich lipoproteins. The triglyceride-rich lipoproteins, chylomicrons and VLDL remnants are known to be atherogenic because they activate platelets, the coagulation cascade and clot formation. This response can result in the occlusion of a blood vessel or restricted blood flow, which can lead to ischaemic symptoms or an ischaemic event such as a stroke, myocardial infarction or deep vein thrombosis due to loss of blood flow and subsequent oxygen delivery to vital organs (Ajjan and Ariens, 2009; Mahan et al., 2012). The incomplete dissolution of clots might also lead to smaller pieces of the clot breaking off and travelling down the vasculature, which can result in an embolism when a smaller blood vessel downstream is blocked. Clot formation triggers the release of PAI-1. Binding to tissue-plasminogen activator (t-PA) initially acts to stabilise the thrombus and prevent premature fibrinolysis (Kohler & Grant, 2000). Elevated PAI-1 levels, however, will inhibit fibrinolysis through the mechanisms explained in section 2.4.3. Therefore increased PAI-1 levels and decreased fibrinolytic activity have been considered potential risk factors for CVD, and are also associated with the development of myocardial infarction and enhanced progression to thrombosis (Hoekstra et al., 2004; Lisman et al., 2005).

A positive association exists between triglycerides and PAI-1, and this association has been proposed to be the explanation for decreased fibrinolytic activity associated with hypertriglyceridaemia (Hamsten et al., 1985; Hansen et al., 2000). It has been reported that hypertriglyceridaemic VLDL is a more potent stimulus than normotriglyceridaemic VLDL, due to the fact that hypertriglyceridaemic VLDL were found to bind more effectively to human endothelial cells than normotriglyceridaemic VLDL. The mechanism involves the binding of VLDL to the apo B and E receptors, which is more effective in hypertriglyceridaemia (Stiko-Rahm et al., 1990). A VLDL induced response element in the PAI-1 promoter region is shown to be influenced by the 4/5 guanosine (4G/5G) polymorphism. This has been shown to influence plasma PAI-1 activity and to be involved in an allele-specific response to triglycerides (Eriksson et al., 1998).

2.4.2 Blood lipids as risk factors for CVD

Elevated plasma total cholesterol, LDL, elevated triglycerides and decreased levels of HDL are known risk factors for CVD. The ratio of total cholesterol to LDL cholesterol or LDL cholesterol to HDL cholesterol is a summary of two trends that is a convenient marker of risk in persons without severe genetic defects in metabolism (Wilson et al., 1998; Perk et al., 2012).

Total cholesterol is the measurement of LDL cholesterol and HDL cholesterol and VLDL cholesterol, while LDL cholesterol is usually calculated from the Friedewald-Levy-Fredrickson formula (Roberts, 1988). Triglycerides are esters derived from glycerol and three fatty acids (Mahan et al., 2012). Triglycerides are transported in the circulation within the core of

lipoproteins (Riccardi et al., 2003). The total triglyceride level is a measurement of the triglycerides carried on both the VLDL and IDL remnants. Approximately 60% of the VLDL particle consists of triglycerides (Mahan et al., 2012).

The latest recommended target levels for blood lipids are used for normal healthy population, according to the Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and invited experts) as cut-off values for blood lipids. The cut off values are as follows: total cholesterol < 5mmol/l (190mg/dL) and LDL cholesterol < 3 mmol/l (115 mg/dL) (Perk et al., 2012). The South African dyslipidaemia guideline supports a target of <5mmol/l for screening purposes. When patients have clinically-established CVD and or diabetes, however, treatment goals change and different recommendations exist for the desired blood lipid levels. In the case of clinically established CVD, the South African dyslipidaemia guideline suggests LDL targets based on the Framingham total CVD risk score as seen in table 2-3 (Klug, 2012). According to the third joint task force, total cholesterol should be <4.5 mmol/l (175 mg/dL) and for LDL <2.5 mmol/l (100 mg/dL) when patients have CVD or diabetes. Fasting triglyceride levels greater than 1.7 mmol/l (150 mg/dL) continue to be considered a risk marker for CVD, even though levels less than 1.7 mmol/l are not considered evidence-based target levels for treatment (Perk et al., 2012). HDL cholesterol levels <1.0 mmol/l (40 mg/dL) for men and < 1.2 mmol/l (46mg/dL) for women are regarded as markers of increased CHD risk. However, there is not sufficient evidence for these HDL cholesterol values to be considered as goals for therapy (Perk et al., 2012). Other cut-off values exist, such as the Adult Treatment Panel (ATP), the NCEP Expert Panel exist (NCEP step III guidelines), which uses Framingham to estimate risk scores and then bases LDL recommendations (Cleeman, 2001).

Evidence for the association between total cholesterol, as well as LDL cholesterol, and risk of developing CVD is well established, both in people with established CVD and those without (Perk et al., 2012). Results from populations studied in China, Poland and the US, with different risk factors and different socio-political factors and absolute morality figures, demonstrated that total cholesterol was a strong predictor of CHD mortality and all CVD in men (Cai et al., 2004). Total cholesterol is also a major independent predictor of recurrence of myocardial infarction (MI) (de Lorgeril et al., 1999). The association between LDL and the risk of CHD has been proven by the fact that interventions that reduce total cholesterol and LDL have significantly reduced CHD mortality (Riccardi et al., 2003; Baigent et al., 2005). The Hisayama study (Imamura et al., 2009), a long-term prospective study of the Japanese population, found LDL as a risk factor to be comparable to the effect of metabolic syndrome, however, independently of it. Baigent et al. (2005) claimed that the relationship between absolute reductions in LDL and reductions in coronary events are approximately linear. Smaller and more dense LDL particles are associated with higher CVD risk (Krauss, 1994).

Table 2-3. Intervention strategies as a function of Framingham total CVD risk score and LDL-C levels according to the South African dyslipidaemia guideline consensus statement (Klug, 2012).

Total CVD risk score	LDL-C levels			
	< 1.8 mmol/l	1.8 - <2.5 mmol/l	2.5 – 4.9 mmol/l	>4.9 mmol/l
<3% **Low risk**	No lipid intervention	No lipid intervention	Lifestyle intervention	Lifestyle intervention, consider drug if uncontrolled
3-15% **Moderate risk**	Lifestyle intervention	Lifestyle intervention	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention, consider drug if uncontrolled
15 – 30% **High risk**	Lifestyle intervention, consider drug	Lifestyle intervention, consider drug	Lifestyle intervention, and immediate drug intervention	Lifestyle intervention, and immediate drug intervention
>30% **Very high risk**	Lifestyle intervention, consider drug	Lifestyle intervention, and immediate drug intervention	Lifestyle intervention, and immediate drug intervention	Lifestyle intervention, and immediate drug intervention

Even though triglycerides are considered an independent CVD risk factor for CVD (Criqui et al., 1993; Sarwar et al., 2007), it has also been shown that when controlling for HDL cholesterol, the association between triglycerides and CVD no longer exists (Di Angelantonio et al., 2009). Evidence suggests that non-fasting triglycerides might predict CHD risk better (Nordestgaard et al., 2007). However, it is not recommended for use due to the lack of standardisation for measurement (Perk et al., 2012). Low concentrations of HDL cholesterol are independently associated with higher risk of CVD (Chapman et al., 2011). Higher HDL and lower non-HDL levels are independently associated with lower IHD mortality (Lewington et al., 2007).

2.4.3 Reduced fibrinolytic potential as risk factor for CVD

The process of haemostasis involves the endothelium, activation of the coagulation cascade, the formation of a fibrin clot and then the lysis of the clot once the damaged endothelium has been healed. A complex haemostatic system controls the balance between coagulation and fibrinolysis. Fibrinolysis is the process whereby a fibrin clot is broken down. It plays a role in maintaining haemostasis by preventing naturally occurring blood clots to cause an obstruction or thrombotic occlusion and thereby sustain blood flow. and can be measured using proxy markers such as concentration and activation of proteins involved in the fibrinolytic pathway or through the use of global assays which measure clot lysis time (time from formation of a blood clot to its dissolution) (Gorog, 2010). For the purpose of this literature review, coagulation will only briefly be touched on as an introduction to fibrinolysis, which will be described in detail below.

The coagulation cascade is a series of reactions, in which coagulation factors (generally enzymes, but also glycoproteins and transglutaminase) circulate as inactive enzyme precursors, which are activated and cleaved to downstream functional proteins. As summarised by Medved & Nieuwenhuizen (2003), activation of the coagulation cascade results in the generation of thrombin, which converts fibrinogen into fibrin (Medved and Nieuwenhuizen, 2003) that forms a network that traps platelets, red and white blood cells at the site of endothelial injury. White clots with platelets are more lysable than red clots with red cells and thinner fibrils are less lysable than thicker ones. The process of coagulation is a cascade consisting of three overlapping but consecutive steps: the initiation, amplification and propagation phases (Ajjan and Ariens, 2009; Gale, 2011). The coagulation cascade is maintained in a prothrombotic state until it is down-regulated by the anticoagulant pathways. The formation of fibrin triggers the activation of fibrinolysis on the surface of the fibres of the clot (Medved and Nieuwenhuizen, 2003).

Fibrinolysis is the process by which a cross-linked fibrin clot is broken down in order to maintain haemostasis. Fibrin clot lysis is initiated by the binding of plasminogen and tPA on the fibrin surface (Medved and Nieuwenhuizen, 2003).

The zymogen (pro-enzyme) plasminogen is produced in the liver as a single-chain glycoprotein (Gale, 2011). Two protease plasminogen activators, namely t-PA and urinary plasminogen activator (u-PA), of which t-PA is the most important, are found in the circulation and their function is the conversion of plasminogen into plasmin (Gale, 2011). While t-PA is active as a single chain protein, u-PA needs to be cleaved by FXIIa, plasmin or kallikrein in order to become active (Rijken and Sakharov, 2001).

Plasminogen and t-PA bind to lysine residues (binding sites) on the surface of a fibrin clot. As seen in figure 2-4, tPA then cleaves plasminogen to form active plasmin. The presence of fibrin

increases the activation rate of plasminogen to plasmin by t-PA (Medved and Nieuwenhuizen, 2003). Plasmin causes degradation of the fibrin polypeptide chain by binding two lysine groups on the surface of the fibrin fibre and then cleaving the fibre at the lysine, exposing new C-terminal lysine groups for subsequent cleavage (Rijken and Sakharov, 2001). Continuous binding and degradation eventually cause the degradation of the clot into soluble fibrin fragments (Medved and Nieuwenhuizen, 2003). Plasminogen can also be activated by u-PA in the presence of the u-PA receptor found on various cells (Gale, 2011).

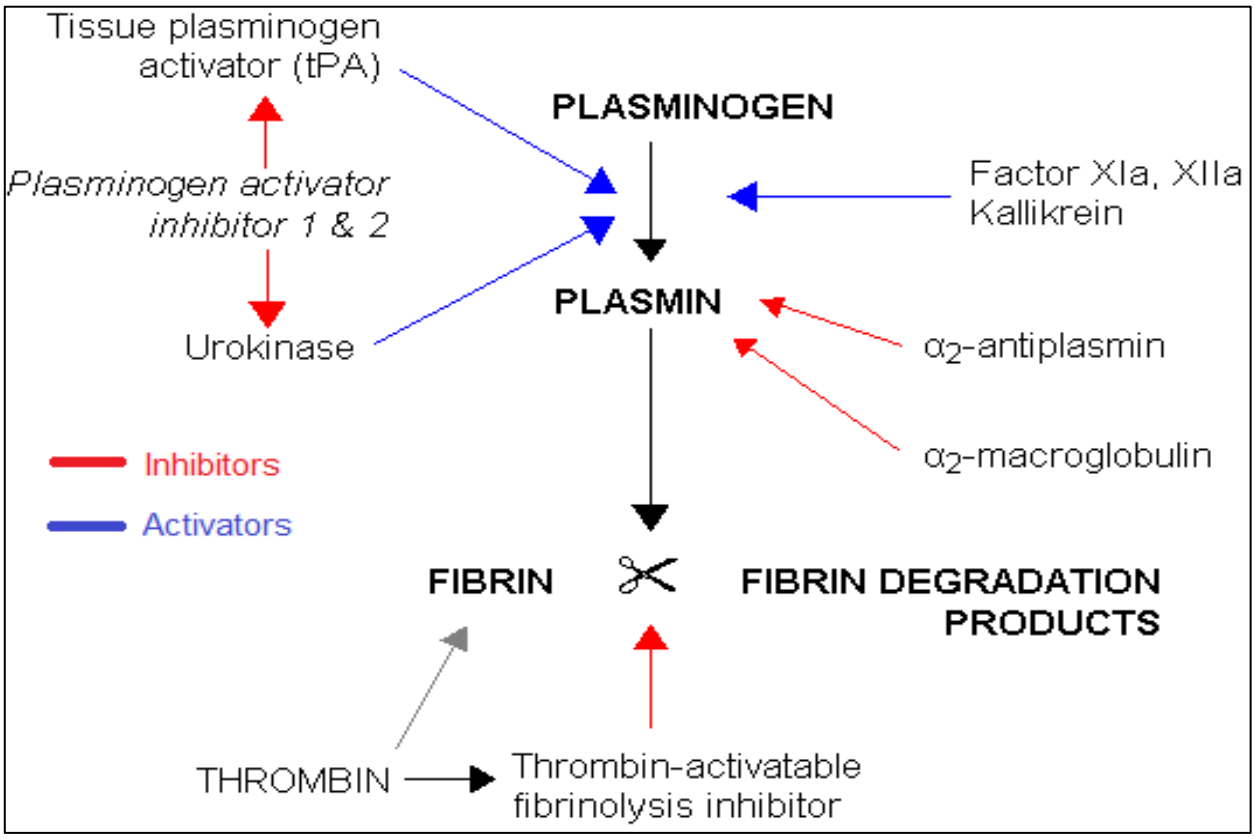


Figure 2-4: The role of PAI-1 in fibrinolysis. Figure adapted from: http://en.wikipedia.org/wiki/Plasminogen_activator_inhibitor-1#mediaviewer/File:Fibrinolysis.png

The main inhibitor of the fibirnolytic pathway is called PAI-1. It is a single-chain 379 serine protease inhibitor, it has a mass of 50 KDa (Rau et al., 2007); (Pannekoek et al., 1986) and is synthesised by platelets, endothelial cells, hepatocytes, vascular smooth muscle cells (Sprengers and Kluft, 1987; Rau et al., 2007) and also adipose tissue (Stiko-Rahm et al., 1990). Members of this family include PAI-1, PAI-2, PAI-3 (protein C inactivator) and protease nexin 1, and their main function is being inhibitors of the plasminogen activators t-PA and u-PA, as well as thrombin, and as such are considered potential risk factors for CVD (Rau et al., 2007). Active

PAI-1 has a half-life of 1 – 2 hours in the circulation. However, in plasma it is mainly bound to the extracellular matrix protein vitronectin, which forms the PAI-1-vitronectin complex which has a two to fourfold increased half-life (4 – 6 hours) (Lawrence et al., 1997). PAI-1 is mainly removed from the circulation by the liver (Rau et al., 2007). PAI-1 inhibits t-PA and u-PA, while other fibrinolysis inhibitors such as α2-antiplasmin and, to a lesser degree, α2-macroglobulin inhibit plasmin (Rijken and Sakharov, 2001; Medved and Nieuwenhuizen, 2003). The mechanistic pathway through which PAI-1 inhibits fibrinolysis (Meltzer et al., 2010) is through binding of t-PA to form the inactive PAI/tPA complex, which prevents the binding of plasminogen to fibrin and results in impaired fibrinolytic function (Lee and Lip, 2003). Plasminogen requires the presence of tPA to bind to fibrin. Two-thirds of plasma t-PA is bound to PAI-1 (Rau et al., 2007).Thrombin-activatable fibrinolysis inhibitor (TAFI) is another inhibitor of fibrinolysis. It is activated by thrombin in the presence of thrombomodulin and acts by removing C-terminal lysine (through competitive binding) on partially degraded fibrin and subsequently inhibits the activation of plasminogen to plasmin (Colucci et al., 2004).

Ex vivo studies of clots can be problematic because they do not incude the same blend of platelets, white cells and red cells. An additional problem in ex-vivo studies is that flow and permeation may differ. The body's ability to lyse clots can be measured in different ways, namely the measurement of activity and/or concentration of proteins involved in fibrinolysis regulation, such as PAI-1 or through, global fibrinolytic assays which report the body's global ability to lyse clots in the plasma as clot lysis time (Lisman et al., 2001). A number of global assays exist; however, not all of them give an accurate reflection of plasma clot lysis time, due to differences in methodology. Reduced fibrinolytic potential has been shown to be associated with CVD (Kannel, 2005). Decreased clot lysis could increase the risk of embolism formation or occlusion of blood vessels (Gale, 2011). An increase in non-communicable diseases (NCDs) associated with urbanisation might also result in a modest increase in PAI-1 (Pieters and Vorster, 2008) and therefore have a negative effect on fibrinolysis. Historically, the prevalence of CVD was relatively rare among black South Africans (Seedat et al., 1992), but an increase in the prevalence of CVD, and the accompanying morbidity and mortality in the black South African population has also been documented (Steyn et al., 2005), especially among urban dwellers (Sliwa et al., 2008). However, lower PAI-1 levels for black South Africans compared to Caucasians have been documented (Greyling et al., 2007). Pieters and Vorster (2008) reviewed the limited data available and found that PAI-1 levels in black South Africans were low in general, but may be on the increase as a result of large scale urbanisation of black South Africans and the accompanying increased CVD risk associated with it.

Even though an official cut-off value for elevated PAI-1 levels does not exist, normal plasma levels for PAI-1 can have distinct 24-hourly variations (Kluft et al., 1988) and have been reported to range between 5 – 20 ng/ml (Binder et al., 2002). PAI-1 concentrations peak in the

morning, after which it declines over the course of the day (Scheer and Shea 2014). Higher PAI-1 levels have been detected in various diseases, as well as in patients with characteristics that increase their risk for CVD, including increased body weight and central fat distribution (Juhan-Vague and Alessi, 1993). No official cut off value for clot lysis time exist either.

2.4.4 Fatty acids and CVD

2.4.4.1 Total fat intake and CVD

Early research by Hegsted and colleagues (1965) suggested that the type of fatty acids in the dietary fat is of more importance than the amount of dietary fat (in the range between 22 – 40% energy from fat). Since then, results from several studies have confirmed that total fat is not a significant risk for CHD and CVD mortality and that dietary fat quality seems more important than fat quantity in the reduction of CVD mortality (Hu et al., 1997; Laaksonen et al., 2005; Oh et al., 2005). While The Strong Heart Study (Xu et al., 2006) concluded that CHD in Americans is due to the quantity and quality of dietary fat, these results were confounded by saturated fatty acids. With regard to risk factors, the results from Meksawan et al (2004) indicate that total fat does not have an effect on total cholesterol and LDL cholesterol. The effect of fat on PAI-1 is most likely dependent on the type of fat instead of the amount (Hoekstra et al., 2004).

Some studies suggest that low fat, high carbohydrate diets might have unfavourable effects in terms of CHD when compared to higher fat intakes, because they might cause a reduction in HDL cholesterol concentration and an increase in serum triglyceride concentration (Parks, 2001). In many of these studies, however, the results could have been due to the use of a liquid form of refined carbohydrates without fibre as a source of carbohydrates in these studies (Mozaffarian, 2005). Moreover, strong evidence that low fat diets predispose to CHD does not exist (Institute of Medicine, 2005). Additionally, very low fat diets might cause an essential fatty acid deficiency (Meksawan et al., 2004).

2.4.4.2 Saturated fatty acids and CVD risk factors

In the 1960s and 1970s, consistent reports from ecological studies, animal studies and short-term metabolic trials in healthy adults, (evaluating total cholesterol and LDL cholesterol) provided support for the argument that saturated fatty acids increase the risk factor for CHD (Micha and Mozaffarian, 2010). Already in 1987 McNamara and fellow researchers concluded that a reduction in saturated fatty acid intake might be of greater significance to lower total cholesterol and LDL cholesterol concentration than total fat intake (McNamara et al., 1987).

There is a considerable body of evidence on saturated fatty acids, including randomised controlled trials of saturated fatty acid nutrient substitutions and multiple risk pathways as endpoints, including lipid and also non-lipid risk factors. There are also large prospective cohort

studies and meta-analyses of randomised controlled trials of saturated fatty acid consumption and clinical disease endpoints that provide direct evidence of the effects on disease (Micha and Mozaffarian, 2010). More recent epidemiological studies, however, have shown positive (Hu et al., 1997; Xu et al., 2006), inverse (Mozaffarian et al., 2004) or no associations (Pietinen et al., 1997) of dietary saturated fat with CHD mortality and/or morbidity. The possible reasons for the differences in results include random misclassification of dietary exposures or subjects changing their intake after commencement of a study (Pietinen et al., 1997). In northern Sweden it was observed that harmful changes in blood cholesterol levels paralleled higher intake of fat (specifically a butter-based spread and butter for cooking), in a study following more than 14,000 participants' dietary patterns for 25 years (Johansson et al., 2012). A recent meta-analysis of prospective cohort studies that evaluated the association of saturated fat with CVD found no significant evidence for concluding that dietary saturated fat is associated with increased risk of CHD (Siri-Tarino et al., 2010). This study further suggested that the historically assumed beneficial effects of diets with reduced saturated fat on CVD risk might be dependent on a significant increase in polyunsaturated fat in these diets due to substitution to keep energy constant between groups in the studies (Siri-Tarino et al., 2010). An evaluation of recovered data from the Sydney Diet Heart Study, however, found that substituting dietary saturated fat with linoleic acid increased the rates of death from CHD and CVD (Ramsden et al., 2013). Additionally, a recent systematic review and meta-analysis of 72 unique studies, of which 45 were prospective observational cohorts and 27 were randomised controlled trials, found no association between total saturated fatty acids and coronary risk in studies using dietary intake and in those using circulating biomarkers (Chowdhury et al., 2014). Another meta-analyses and meta-regression, published in 2014, provides no evidence of a beneficial secondary preventive effect of either reduced and/or modified fat diets or replacement of saturated fatty acids by polyunsaturated fatty acids in participants with established CVD (Schwingshackl and Hoffmann, 2014).

However, these recent studies created confusion. Their results need to be interpreted, bearing the limitations in mind, which include incorrect data handling and inclusion of studies not appropriate for the analyses, and that only a few studies reported on all fatty circulating fatty acids. Additionally selective reporting in observational studies might have played a role in the results of meta-analyses.

Randomised controlled trials have established multiple effects of saturated fatty acids consumption on circulating lipids and lipoproteins, depending on the comparison nutrient (the nutrient isocalorically replaced for saturated fatty acids). Micha and Mozaffarian (2010) summarised the results of these randomised controlled trials as follows: Compared to carbohydrates, saturated fatty acid intake increases total and LDL cholesterol, but also lowers triglycerides and raises HDL cholesterol. However, it does not have a significant effect on total

cholesterol-to-HDL cholesterol ratio. Total cholesterol, LDL cholesterol, HDL cholesterol (slightly) and the total cholesterol-to-HDL cholesterol ratios are lowered, and few effects were seen on triglycerides when replacing saturated fatty acids with monounsaturated or polyunsaturated fatty acids. Compared to trans fatty acids, saturated fatty acids have a minimal effect on LDL cholesterol, raise HDL cholesterol and lower triglycerides, with improvement in total cholesterol-to-HDL cholesterol ratio. Therefore overall changes in lipoprotein levels predict minimal effects on CHD risk when carbohydrates replace saturated fatty acids, but benefits when polyunsaturated or monounsaturated fatty acids replace saturated fatty acids and harm when trans fatty acids replace saturated fatty acids. A reduction of saturated fatty acids in the diet and replacing it with either monounsaturated fatty acids or polyunsaturated fatty acids leads to significant decreases in serum total and LDL cholesterol, but also lowers HDL cholesterol (Wahrburg et al., 1992). The WHO/FAO report of an expert consultation also concluded that there is convincing evidence that replacing saturated with polyunsaturated fat decreases the risk of CHD (FAO/WHO, 2009).

Studies often only measure the total amount of saturated fat, monounsaturated fat or polyunsaturated fat in the diet. However, even within classification groups of fat such as polyunsaturated fatty acids and subgroups (i.e. omega-3 or omega-6 polyunsaturated fatty acids), specific individual fatty acids or even ratios can cause different effects with regards to coronary heart disease. It should be noted, however, that individual dietary saturated fatty acids do not necessarily affect CHD risk factors equally and that the food sources of these fatty acids could also play a role. For example, a diet high in stearic acid (C18:0) does not raise serum cholesterol levels (Bonanome and Grundy, 1988), but it lowers LDL cholesterol when compared to diets enriched with palmitic (C16:0) or myristic acid (C14:0) and lauric acid (C12:0) (Grande et al., 1970; Tholstrup et al., 2004). The hypercholesterolaemic effect of palmitic acid is more than that of lauric acid (Denke and Grundy, 1992). Myristic acid is more hypercholesterolaemic than palmitic acid, but part of the effect can be attributed to an increase in HDL cholesterol (Denke and Grundy, 1992). Additionally, data from Clandinin et al., 2000 suggests that, when linoleic acid intake is not adequate, palmitic acid can become ‘conditionally’ hypercholesterolaemic (Clandinin et al., 2000). Further research is needed to establish at what combination of linoleic acid and saturated fatty acids a beneficial effect occurs, but linoleic acid at 10% of energy was adequate to promote decreased LDL cholesterol levels. The mechanism by which saturated fatty acids cause increases in total cholesterol is mainly through increases in LDL cholesterol levels, which is caused by suppressing receptor mediated clearance of LDL cholesterol (Shepherd et al., 1980). Another possible mechanism is through enhanced synthesis of apo-B containing lipoproteins. It is generally accepted that saturated fatty acids do not decrease HDL cholesterol levels (Grundy and Denke, 1990). The mechanism behind stearic acid’s neutral effect might be that it is rapidly converted into oleic acid (Bonanome and Grundy, 1988).

Positive correlations have been found between saturated fatty acids in phospholipids and PAI-1 (Hansen et al., 2000). Oil containing red palm olein and palm olein have no effects on PAI-1 levels, according to (Scholtz et al., 2004). Intervention with a diet low in saturated fat (in order to lower serum triglycerides) proved to accompany improvement of fibrinolytic activity (Hansen et al., 2000).

2.4.4.3 Monounsaturated fatty acids

Research on monounsaturated fatty acids was initially inspired by results from the Seven Countries Study. This study reported that the Mediterranean region, which was characterised by a diet high in total fat, of which the main source was oleic acid, had a low incidence of CHD mortality (Keys et al., 1986). After that, epidemiological studies produced mixed results regarding the effect of monounsaturated fatty acids on CHD. Positive associations (Xu et al., 2006), inverse associations (Hu et al., 1997; Pietinen et al., 1997) and no associations (Laaksonen et al., 2005) between M monounsaturated fatty acid intake and CHD were found. When the main source of monounsaturated fatty acids in studies were from animal sources they were likely to have been confounded by saturated fatty acid intake, which could be a reason for the difference in results (Laaksonen et al., 2005; Xu et al., 2006). The Lyon Diet Heart study (de Lorgeril et al., 1999), a secondary prevention trial, confirmed the major protective effect of the Mediterranean diet against myocardial infarction and cardiovascular complications. The systematic review and meta-analysis of Chowdhury et. al. (2014), however, found no associations of total and individual monounsaturated fatty acids with coronary risk in studies using both dietary intake and circulating fatty acid composition.

Oleic acid reduces LDL cholesterol levels when substituted for saturated fatty acids in the diet (Mattson and Grundy, 1985). It does not appear to reduce HDL cholesterol such as high-linoleic acid diets or high cholesterol. It also does not raise triglyceride levels when substituted for saturated fatty acids as high-carbohydrate diets do (Grundy, 1987). The most likely mechanism for the reduction in LDL cholesterol when monounsaturated fatty acid are substituted for saturated fatty acids is that when saturated fatty acids that suppress the activity of LDL cholesterol receptors are removed from the diet, receptor activity might increase (Grundy, 1987; Grundy and Denke, 1990).

While Hansen and colleagues did not find correlations between monounsaturated fatty acids and PAI-1, Lopez-Segura (1996) reported a decrease in PAI-1 activity with consumption of a diet high in monounsaturated fatty acids. It was hypothesised in this study that a decrease in insulin resistance on the high monounsaturated fatty diet might have been the cause of the decrease in PAI-1 activity (Lopez-Segura et al., 1996).

2.4.4.4 Omega 6 polyunsaturated fatty acids

Whereas omega-6 fatty acids generally exert their cardioprotective effects through changes in lipid and lipoproteins, omega-3 fatty acids contribute benefits through their antiarrhythmic, anti-inflammatory and anti-thrombotic effects.

Epidemiological studies have reported various outcomes with regard to the effect of polyunsaturated fatty acids on CHD. An inverse association between the intake of linoleic acid and coronary death was reported by The Nurses' Health Study (Hu et al., 1997), and the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The Cross-sectional Family Heart study (Djousse et al., 2001) found an inverse relation between reported intake of linoleic acid and coronary artery disease, although the study had several limitations. Cohort studies such as the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen et al., 1997), Lipid Research Clinics study (Esrey et al., 1996) and the Strong Heart study (Xu et al., 2006) found no significant associations between linoleic acid or omega-6 polyunsaturated fatty acids intake and CHD risk, while in others, such as the KIHD Study (Laaksonen et al., 2005) and the Nurses' Health Study with 20 years of follow-up (Oh et al., 2005), an inverse relationship between polyunsaturated fatty acids intake and CHD risk was found.

Originally oleic acid was believed to be less hypocholesterolaemic than linoleic acid (Becker et al., 1983). However, Mattson and Grundy (1985) found that linoleic acid was just as effective as oleic acid in lowering total cholesterol and LDL cholesterol in normotriglyceridaemic patients. Mensink and Katan (1989) reported similar results on total and LDL cholesterol. Howard et al. (1995), on the other hand, found that a higher intake of polyunsaturated fatty acids in an NCEP step I diet caused a greater reduction in total cholesterol than the inclusion of monounsaturated fatty acids and additionally found lowering effects on triglycerides (Howard et al., 1995). Vega and colleagues (1982) concluded that the number of LDL cholesterol particles in circulation was reduced by polyunsaturated fatty acids and responses varied from patient to patient. polyunsaturated fatty acids, however, lower LDL cholesterol by more than one mechanism (Vega et al., 1982). These effects are mostly due to modification in composition of LDL particles (reduction of cholesterol content), which causes a reduced capacity for cholesterol transport and an increase in its fractional clearance rate, but it could also be due to decreased synthesis of LDL cholesterol (Shepherd et al., 1980). High intakes of linoleic acid reduce HDL cholesterol (Shepherd et al., 1980; Grundy, 1987). Horrobin & Huang (1987) speculated that a metabolite of linoleic acid, rather than the fatty acid itself, might be responsible for the cholesterol-lowering effect (Horrobin and Huang, 1987). Interactions between certain prostaglandins and the lipoprotein surface can induce modifications, which might play a role in cholesterol homeostasis. Quantities of less than 10 – 13% of total energy from linoleic acid in the diet does not have the same HDL cholesterol lowering properties, possibly due to the fact that the change is too small to

detect in lower quantities (Grundy, 1987). Polyunsaturated fatty acids also lower triglyceride and VLDL cholesterol (Shepherd et al., 1980). Vega and colleagues (1982) determined that polyunsaturated fatty acids reduced every constituent of VLDL cholesterol significantly, without changing the composition. (Hansen et al., 2000) indicated positive correlations between the major omega-6 fatty acids in total phospholipids and PAI-1. Sunflower oil (containing mainly linoleic acid) had no effect on PAI-1 levels, according to a study done by Scholtz and colleagues (2004).

2.4.4.5 Omega 3 polyunsaturated fatty acids

According to Mozaffarian and Wu (2011), there is enough evidence from experimental studies, controlled trials of physiological risk factors, prospective observational studies of clinical endpoints and adequately powered RCTs of clinical endpoints that modest omega-3 polyunsaturated fatty acid consumption, compared with little or no consumption, reduces CHD mortality in populations without established CVD. Systematic reviews and meta-analyses of Studies of Fish or Long-Chain n-3 PUFA Consumption and Risk of CVD Outcomes indicate that fish and n-3 PUFA consumption reduce the risk of CHD events, primarily due to prevention of CHD death (Mente et al., 2009; Wang et al., 2006). The controversial systematic review and meta-analysis of Chowdhury and fellow researchers (2014), which included observational studies of fatty acid biomarkers, and randomised controlled trials (RCTs), found no significant associations in prospective studies of coronary disease that involved assessment of dietary intake of long-chain omega-3 polyunsaturated fatty acids. Additionally they provided evidence that circulating levels of EPA, DHA and arachidonic acid are each associated with lower CHD risk, while supplementation with these fatty acids, however, did not show statistically significant reduction of risk (Chowdhury et al., 2014). A previous meta-analysis also reported a lack of effect of omega-3 supplementation on CVD (Rizos et al., 2012). It was seen in meta-analyses of observational studies of alpha-linolenic acid consumption and risk of CVD outcomes, showed risk of fatal CHD (Brouwer et al., 2004).

Red blood cell concentrations of omega-3 fatty acids (EPA and DHA) are a strong reflection of dietary intake, therefore it has been proposed that an omega-3 fatty acid biomarker, the omega-3 index (erythrocyte eicosapentaenoic acid plus DHA), should be considered as a potential risk factor for CHD mortality, especially sudden cardiac death (Harris, 2008). The calculation of the omega-3 index has been adapted to be able to calculate the index in plasma phospholipid fatty acids also (Harris and Von Schacky, 2004). The proposed omega-3 index risk zones are (in percentages of erythrocyte FAs): high risk, <4%; intermediate risk, 4-8%; and low risk, >8%. The omega-3 index is an independent risk factor, which compares well with other risk factors for CHD. (Harris and Von Schacky, 2004).

The lower risk of CVD mortality with increased intake of long-chain omega-3 polyunsaturated fatty acids is most likely due to the favourable effect of omega-3 polyunsaturated fatty acids on cardiac

arrhythmias or thrombosis, rather than on the lipoprotein profile (Riccardi et al., 2003). Recent meta-analyses, however, warns that benefits are not as great as previously thought (Kotwal 2012). Results vary between studies with regard to the effect of omega-3 polyunsaturated fatty acids on total cholesterol and LDL cholesterol levels (Bronsgeest-Schoute et al., 1981; Mori et al., 2000; Egert et al., 2009). Bronsgeest-Schoute and research team (1981) suggested an increase in LDL cholesterol, while Sirtori et al. (1992) found a decrease in total cholesterol. Omega-3 fatty acids lowered serum triglyceride (Sirtori et al., 1992) and VLDL cholesterol levels (Bronsgeest-Schoute et al., 1981). Bronsgeest-Schoute and team (1981) further indicated that no change in total cholesterol or HDL cholesterol was noted after supplementation with omega-3 fatty acids. Omega-3 polyunsaturated fatty acids lower postprandial triglyceride, but have a greater decreasing effect on fasting triglyceride. Alpha-linolenic acid might not have an equivalent triglyceride lowering effect. Even though dietary alpha-linolenoic acid fed at 6.3% of intake for 56 days in a small cross-over study in 10 men caused a significant increase in serum alpha linolenic acid, it did not have an effect on plasma triglycerides, LDL- ,HDL- or total cholesterol (Kelley et al., 1993). The mechanism of triglyceride lowering involves the inhibition of hepatic triglyceride synthesis and secretion of VLDL from the liver. Omega-3 polyunsaturated fatty acids directly regulate hepatic genes, suppressing triglyceride production by means of decreasing de novo lipogenesis (DNL), as well as other possible effects. The evidence suggests that long-chain omega-3 polyunsaturated fatty acids affect all four metabolic nuclear receptors (LXR, FXR, HNF-4α and PPAR- α, -β and -γ) that reduce triglyceride levels (Davidson, 2006). Other mechanisms might include reduced LDL synthesis, increased conversion of VLDL to LDL, increased HDL receptor numbers and turnover of HDL, and reduced CETP activity, and hence the retention of HDL of cholesterol esters within HDL (Bronsgeest-Schoute et al., 1981).

Conflicting results exist for the effect of omega-3 fatty acids on PAI-1 activity levels. Various studies have observed an increase in PAI-1 with the intake of omega-3 polyunsaturated fatty acids (Hansen et al., 2000), whereas other studies indicated no substantial association (Scarabin et al., 2001) or an inverse association with PAI-1 activity (Lee et al., 2012a). Because dietary intervention with long-chain omega -3 fatty acids from marine sources is known to decrease serum triglycerides (Bronsgeest-Schoute et al., 1981; Davidson, 2006), it was thought that there might be a causal relationship between omega-3 fatty acids and PAI-1 through its lowering properties on triglycerides. The association between PAI-1 and triglycerides has been proposed to be the explanation for decreased fibrinolytic activity associated with hypertriglyceridemia (Hansen et al., 2000).

A causal relationship between PAI-1 and triglycerides could not be proved conclusively, due to the conflicting results of clinical trials. Research initially suggested that omega-3 fatty acids might enhance plasma fibrinolysis, but further studies done on this topic indicated no effect or worsening of fibrinolysis (Barcelli et al., 1985). The fact that many studies find an increase in PAI-1 activity

with the supplementation of omega-3 might explain the worsened effect on fibrinolysis. It is not yet known why the triglyceride lowering effect of omega-3 fatty acids is not accompanied by a decrease in PAI-1 activity. The problem might very well be, as it is with research on omega-3 fatty acids, that factors such as study design, time of supplementation, combined or separate effects of EPA and DHA or whether it is given as purified oil or in fish form might have implications for the outcome of trials. In the study by Hansen and colleagues (2000), for example, normotriglyceridaemic men were used. It could therefore have been the case that the decrease in serum triglycerides with omega-3 supplementation was not big enough to induce PAI-1 activity change. Hansen and colleagues (2000) also noted that most fish oil studies that caused a significant increase in PAI-1 activity contained more EPA than DHA, hinting at a differential effect between EPA and DHA.

RCTs that did not find reductions in CHD mortality include the alpha-omega, omega, JELIS, DART and SU.FOL.OM3 trials. Among these, only the DART 2 trial was sufficiently powered to detect such effects. However, it was a dietary advice trial with several limitations, including lack of participant blinding, inadequate funding that interrupted recruitment, little follow-up to reinforce dietary advice or evaluate long-term compliance and no evaluation of changes in medications or other behaviours. Overly optimistic estimates of benefits of omega-3 polyunsaturated fatty acid could continue to foster implementation of small underpowered RCTs, which would contribute to further confusion regarding CVD risk. Expectations that omega-3 PIFA should have much larger benefits are unrealistic, especially in patients receiving modern medical and interventional therapies (Mozaffarian and Wu, 2011).

Problems surrounding research on omega-3 fatty acids were summarised by (Mozaffarian and Wu, 2011). These problems include that most studies of CHD death in generally healthy populations have evaluated fish consumption, not fish oil supplementation. Fish contains several potentially beneficial nutrients not contained in fish oil supplements. At least some of the benefits of omega-3 diminish as consumption increases and an omega-3 threshold is estimated at approximately 250mg/d. Observational studies require higher numbers of subjects in trials due to differences in comparison groups (high intake vs low intake). Therefore, investigating fish vs fish oil supplementation and the threshold of benefit for CHD mortality are important factors that can influence the results of RCTs. Other factors might have an effect include, trial design and statistical power among others.

2.4.5 Cardiovascular disease in South Africa

CVD is responsible for an estimated 17.3 million deaths from CVD (30% of all deaths globally) in 2008 (WHO, 2011). It is projected that CVD will remain the leading cause of death and that numbers will increase to 23.3 million deaths by 2030 (Mathers and Loncar, 2006).

Cerebrovascular disease was the fifth leading underlying cause of natural death in South Africa in 2009 and ischemic heart disease ninth (Statistics SA, 2011). In the 2011 report, released in 2014, ischaemic heart diseases (2.6% of deaths) were recorded as being among the 10 leading underlying causes of death for males (but not for females) and cerebrovascular diseases moved to third place, accounting for 4.5% of deaths (Statistics SA, 2014). Between 1997 and 2004 an average of 195 people per day died of CVD in South Africa (Steyn, 2007). Statistics show that twice as many men die from heart attacks as women (Steyn, 2007) and that more than a third of deaths can be attributed to coronary artery disease, hypertensive heart disease and stroke (Bradshaw et al., 2003). More than half of the deaths caused by chronic diseases, including heart disease, occur before the age of 65 years (Bradshaw et al., 2003). Although most heart attacks, heart failure and other chronic diseases occur only in middle age, the influences of risk factors can start before birth and can have an impact throughout life. Premature deaths can have severe effects on the workforce and economy of a country (Steyn, 2007). Statistics on the economic impact of diseases in South Africa are rare and outdated; in 1991 it was shown that the total costs of heart disease and stroke accounted for 2 – 3% of the gross domestic product (GDP) per capita of South Africa (i.e. between R4,135 billion and R5,035 billion) (Pestana et al., 1996). Considering the increase in CVD in South Africa and the rate of inflation since 1991, those figures are a gross underestimation of the costs today (Steyn, 2007).

CVD risk factors are considered intermediaries for the association between dietary fatty acids and CVD (Arab, 2003). High cholesterol was estimated to cause 24,144 deaths (4.6%) of all deaths in South Africa in 2000 (Norman et al., 2007). Due to a lack of national representative data at the time, Norman and research team (Norman et al., 2007) estimated rates of high blood cholesterol by collecting data from nine available studies conducted in different settings across South Africa between 1980 and 2000. They concluded that 59% of ischaemic heart disease (IHD) was attributable to raised cholesterol, with considerable differences among different population groups. The black African population generally had lower total cholesterol levels than Indian, White or Coloured population groups. Because there were bigger differences between population groups among older subjects, they suspected that a Westernised diet influenced the younger age groups. South Africa is experiencing rapid urbanisation and an accompanying nutrition transition (figure 2-5). A shift towards a higher caloric content of diets (often with higher fat and convenience food) and lower physical activity usually accompanies urbanisation, resulting in a higher prevalence of obesity (Popkin, 2001). Urbanisation is also accompanied by additional stress and therefore a higher risk of CVD. In 1992, Walker and fellow researchers noted higher serum total cholesterol in urban than in rural African women (Walker et al., 1992). Additionally, South Africa suffers from the double burden of stunting and underweight, as well as co-existing obesity (Vorster, 1997).

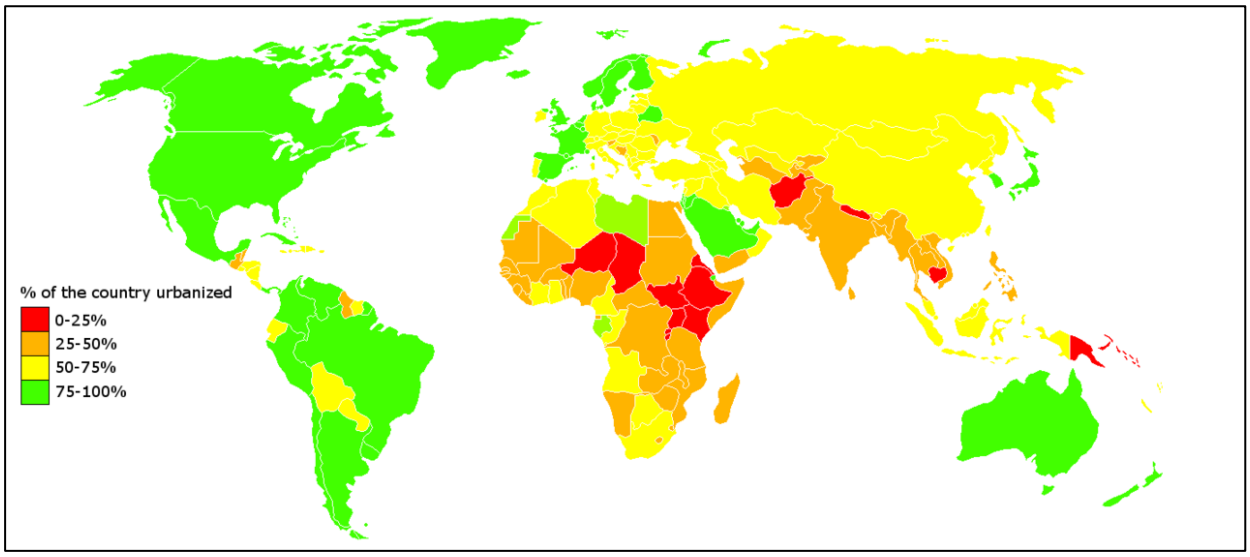


Figure 2-5: Global urbanisation map. From Wikimedia Commons, the free media repository (http://commons.wikimedia.org/wiki/File:Global_urbanization.png).

Urbanisation was also associated with increases in some cardiovascular risk factors such as higher total cholesterol and LDL levels (Vorster et al., 2005), as well as an increased BMI in men (Vorster et al., 2007). In the THUSA study, higher LDL levels in women was not found to be significant, which was attributed to the presence of being overweight and obesity among women at all levels of urbanisation (Vorster et al., 2007). Vorster and colleagues (2005) further concluded that HDL remained relatively constant between levels of urbanisation in the THUSA study, as did triglycerides.

Even though total cholesterol was higher in urban areas than in rural areas, low mean values were reported in the same population, with a low prevalence of CHD (Vorster et al., 2000). Even though 28% of black African people over 30 years of age had total cholesterol levels above clinical cut-off points (5mmol/l), HDL among the black African population was also higher. Older women in this ethnic group were found to be more hypercholesterolaemic than men and the effect was attributed to high obesity rates in older black women. It was confirmed that total cholesterol and LDL increases with age this in the urban African population of the Cape Peninsula (Oelofse et al., 1996).

The black South African population did not present with a high prevalence of dyslipidaemia in previous studies (Steyn et al., 1991; Mollentze et al., 1995; Oelofse et al., 1996). And up until now it seemed as if an increase in CVD was only attributed to stroke rather than ischaemic heart disease (Vorster, 2002). It was only recently, however, that a higher mean LDL cholesterol level was reported in black South Africans (Pieters et al., 2011).

2.1 Conclusion:

Because of the limitations in methodology for the collection and processing of dietary intake data, pairing it with fatty acid status would complete the picture. From the current literature available it is clear that fatty acid status data is lacking and studies are needed in South Africa to describe the status of the population and to explain the associations between fatty acid status and CVD in South Africa.

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Chapter 3: Manuscript 1

Different dietary fatty acids are associated with blood lipids in healthy South African men and women: the PURE

This chapter includes:

- The article titled "**Different dietary fatty acids are associated with blood lipids in healthy South African men and women: the PURE**", first published online 22 January 2014 and presented in the technical style specified by the journal.
- The instructions given to authors by the International Journal of Cardiology in Addendum B.
- The published article, which is provided as Addendum D.

Different dietary fatty acids are associated with blood lipids in healthy South African men and women: the PURE study

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Key words: nutrition transition, blood lipids, total cholesterol, low-density lipoprotein, dietary fatty acids, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid.

Abstract

Background: Preliminary data from the baseline Prospective Urban and Rural Epidemiology (PURE) study in South Africa indicated a higher prevalence of dyslipidaemia than previous South African studies. The intake of specific individual dietary fatty acids may affect blood lipids differently than sub-groups of fat (i.e. polyunsaturated fatty acids). We investigated the dietary

intake of different individual fatty acids and their associations with blood lipids, in relation to urbanisation and gender.

Methods: Cross-sectional data analysis within the PURE baseline study of healthy subjects (n=1950, 35–70 years) from rural and urban areas. Dietary data were collected and blood lipid analysis performed.

Results: Intake of individual fatty acids was significantly higher in urban than rural dwellers. However, the intake of n-3 PUFAs was below recommendations in all groups. Total cholesterol and LDL were higher in females than males, with no rural–urban differences. Intake of alpha-linolenic acid (ALA) was positively associated with total cholesterol (β=0.143) and triglycerides (β=0.256) in males. The risk for having elevated LDL also increased with increased intake of ALA (OR 1.49, 95% CI 1.04, 2.14). In females, arachidonic acid and eicosapentaenoic acid (EPA) were positively associated with total cholesterol and LDL, whereas docosahexaenoic acid was negatively associated with total cholesterol and LDL.

Conclusions: These results suggest that specific individual dietary fatty acids may affect blood lipids in males differently than in females irrespective of rural or urban dwelling. The positive association between ALA and total cholesterol and triglycerides in males is a concern, because current advice aims to improve the dietary linoleic acid to ALA ratio by increasing ALA intake.

1. Introduction

The nutrition transition describes a range of socio-economic and demographic shifts caused by the migration from rural to urban environments, and is accompanied by rapid changes in diet, lifestyle and patterns of undernourishment and obesity[1]. In developing countries, improved socio-economic conditions and the availability of a variety of food associated with the nutrition transition, has resulted in increased incidence of obesity and non-communicable diseases of lifestyle, such as coronary heart disease[1]. South Africa is experiencing continuous urbanisation of Africans[2]. In line with global predictions[3], the urban population in South Africa accounts for

more than half of the population, the proportion of which is rising, from 55% (re-classified data to match demographic classification used in 2001 census) in 1996 to 58% in 20012.

Scientific interest and public awareness of the role of fatty acids in human health has increased in the past years and dietary fatty acids have been shown to have an effect on coronary heart disease[4]. Total fat intake cannot be linked definitively to cardiovascular disease risk, in part due to opposing effects of specific types of fat[5]. The fatty acid metabolites of linoleic acid and alpha-linolenic acid (ALA), arachidonic acid and docosahexaenoic acid (DHA), are important constituents of all cell membranes, determining and influencing the behavior of membrane-bound enzymes and receptors[6]. Recently it has become clear that even within subtypes of fat such as omega-3 fatty acids, specific individual fatty acids can cause different effects with regard to blood lipids. Several studies have shown that these fatty acids can affect blood lipid profiles, but the directions of the effects were inconsistent[7-9]. Convincing evidence exists for a protective effect of the polyunsaturated fatty acids, linoleic acid, eicosapentaenoic acid (EPA), and DHA on cardiovascular disease[10, 11]. Increased risk of cardiovascular disease is also associated with intake of the saturated fatty acids (SFAs), myristic and palmitic acid[10].

Surprisingly, until recently, the African population did not show hypercholesterolaemia at a high level of risk[12, 13]. This is in spite of urbanisation, which is accompanied with higher fat intake[1], and the fact that African women have a very high prevalence of obesity[14], which is also associated with dyslipidemia[15, 16]. However, results from the baseline Prospective Urban Rural epidemiology (PURE) study in South Africa conducted in 2005 indicated high median total cholesterol levels18 and preliminary data analysis indicated a high prevalence of dyslipidaemia, in contrast with prior studies in the South African population that found lipid levels of South African populations to be within recommendations[12, 17]. It is well known that total cholesterol and low-density lipoprotein (LDL) cholesterol predicts coronary heart disease risk18. Knowledge of the intake of individual fatty acids (as opposed to subgroups of fat like polyunsaturated fatty acids) and their associations with blood lipids is lacking in this population undergoing the nutrition transition and other populations. Because higher fat intake is associated with the nutrition transition, the role that individual fatty acids play in rising levels of dyslipidaemia in this

population needs to be better understood. Thus, we investigated dietary intake of individual fatty acids and their associations with blood lipids in rural and urban subjects and found some unexpected associations, especially considering the generally lower fat intakes of this population.

2. Methods

2.1. Study population

This cross-sectional study forms part of the international PURE study, which is a large-scale multinational cohort study that tracks societal influences, risk factors and chronic non-communicable diseases in urban and rural areas of 17 countries in transition[19]. The baseline data collection of the South African leg in 2005 included just over 2000 apparently healthy, migration stable subjects (aged >35 years) from a sample of 6000 randomly selected households in rural and urban areas in the North West Province of South Africa. The rural communities were still under tribal law. Exclusion criteria were the use of chronic medication for non-communicable diseases and/or any self-reported acute illness[20]. Trained field workers, with the same background as participants, conveyed all information about the objectives and procedures of the study, before recruitment in the participants' home language. All participants gave written informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Data were treated confidentially and analyses were performed with coded data. For the purpose of the analyses in this paper, subjects from whom no dietary data were available were excluded, leaving 333 male subjects from rural areas, 393 males from urban areas, 633 female subjects from rural areas and 591 females from urban areas (Figure 1).

2.2. Data collection

Participants were interviewed in the language of their choice. Structured demographic, socio-economic and lifestyle questionnaires that were developed and standardized for the international PURE study were used[19]. Interviewer-based quantitative food frequency

questionnaires (QFFQ) were used to assess dietary intake of volunteers, which were validated for the population in the Transition and Health during Urbanisation of South Africans (THUSA) study[21] and for which reproducibility was proven[22, 23]. Dietary methodology of the PURE study was explained in detail previously[23]. In SA the physical activity index (PAI) questionnaire as developed and tested in the THUSA-study was used for the PURE study, as described previously by Kruger and colleagues[24].

Height, weight and waist circumference were measured using standardized methods, with instruments calibrated by the International Society for the Advancement of Kinanthropometry accredited anthropometrists (Precision Health Scale, A& D Company, Japan; Leicester Height Measure, Seca, Birmingham UK).

Fasting blood samples, with minimal stasis were collected by a registered nurse obtained with a sterile winged infusion set from the antecubital vein and stored at -80ºC. In the rural areas, samples were rapidly frozen and stored at -18ºC (no longer than 5 days) until they could be transported to the laboratory facility and were then stored at -80ºC[20].

Serum lipids were measured by using Sequential Multiple Analyzer Computer (SMAC), using the KonelabTM auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland)25. LDL was calculated using the Friedewald-Levy-Fredrickson formula[26]. Reference values used as indicators for elevated blood lipid values were total cholesterol <5 mmol/l, LDL < 3 mmol/l, HDL ≥1mmol/l for males and ≥1.2mmol/l for females, triglycerides > 1.7 mmol/l[27].

2.3. Statistical analyses

Statistical analysis was done by using IBM SPSS Statistics Version 19 (IBM Company, Armonk, NY, USA). Not normally distributed data were log transformed for data analysis. Differences in serum lipids, dietary fat and fatty acid intake were stratified by urbanisation (rural vs. urban) and gender (male vs. female), and their interactions were explored by using 2-way ANCOVA, adjusting for age. Differences between groups (rural male, rural female, urban male, urban female) were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Three

separate hierarchical multiple linear regression models were used for both males and females to determine associations between specific dietary fatty acids and blood lipid concentrations, adjusting for energy intake, BMI, physical activity, waist circumference, age and urbanisation. The first hierarchical multiple linear regression model was a simple model only including the individual fatty acids and energy intake was controlled for. The second model additionally controlled for alcohol intake, soluble fibre intake, BMI, physical activity, waist circumference and age, while the third fully adjusted model also controlled for potential differences between rural and urban areas. A residual of energy intake was calculated for each of the fatty acids and alcohol to account for differences in energy intake. A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference. Logistic regression analysis, using the fully adjusted model, was used to determine whether individual dietary fatty acids increased or decreased the odds ratios (ORs) for having unfavorable blood lipid profiles. A P-value < 0.05 was considered significant.

3. Results

Dietary intake data were obtained from 1950 participants. Characteristics and differences in energy, fat and fatty acid intake of male and female subjects living in rural and urban areas are indicated in Table 1.

For all dietary variables, intake was significantly higher in urban than in rural areas. Absolute intake of total fat, SFA, MUFA, PUFA, linoleic acid and ALA did not differ significantly between male and female subjects. However, when calculated as percentage of energy (%E) intake, additional to the significant urbanisation effects, females had significantly higher intakes than male subjects in rural and urban areas, with the exception of %E from MUFA, which was only significantly higher in females than males within the urban area.

We found significant effects of gender on the intake of trans fatty acids (absolute and in %E), EPA and DHA, as well as significant gender x urbanisation interactions, indicating that intakes of these fatty acids were significantly higher in males than females in urban areas only, with no significant differences between gender in rural areas.

A significant gender effect was present for all serum lipid variables. Total cholesterol, LDL, and total cholesterol to LDL ratio and LDL: HDL (high-density lipoprotein) ratios were higher in female than male subjects from both, rural and urban areas (Table 2). We found a gender effect ($P < 0.001$), as well as a gender x urbanisation interaction, on HDL ($P = 0.041$), indicating significantly higher HDL in urban males than urban females, with no significant differences between genders in rural areas. We found a significant effect of gender and of urbanisation on triglycerides, indicating that in urban areas, triglycerides were significantly higher in females than in males, but at the same time, the urban females had significantly higher triglyceride levels than the rural females.

Prevalence of unfavorable blood lipid levels is shown in figure 2.

In females, the intake of arachidonic acid and EPA was positively and significantly associated, while DHA intake was negatively associated with total cholesterol (Table 3) even after controlling for energy-, alcohol- and soluble fibre intake, as well as BMI, physical activity and waist circumference. The association between LDL and arachidonic acid became significant in the fully adjusted model, and there tended to be an association between LDL and EPA ($P = 0.051$) and between LDL and DHA ($P = 0.052$), while the associations were significant in the second model which did not include urbanisation. Higher arachidonic acid intake also tended to increase the risk for having elevated total cholesterol in females (OR=1.22; 95% CI 1.00, 1.49, $P = 0.052$) (Figure 2). In male subjects, ALA was the only fatty acid that was significantly and positively associated with total cholesterol in the fully adjusted model (table 4), and ALA also tended to be positively associated with LDL ($P = 0.061$). A higher intake of myristic acid (OR= 1.63, 95% CI 1.17, 2.27, $P = 0.004$) and ALA (OR=1.49, 95% CI 1.04, 2.14, $P = 0.029$) also increased the risk of having elevated LDL in male subjects. In male subjects, palmitic acid was negatively associated ($P = 0.031$), while ALA was positively associated with triglyceride levels ($P < 0.001$). ALA intake also tended to increase the risk of having elevated triglyceride levels in males (OR = 1.47, 95% CI 0.992, 2.19, $P = 0.055$).

4. Discussion

This is the first study, to our knowledge, that investigated associations between the intake of individual fatty acids and blood lipid profiles in the South African population in relation to gender and urbanisation. Our results indicated that even though the intake of energy, fat and specific fatty acids were significanly higher in people living in urban than in rural areas, no differences in blood lipid profiles were found beetween subjects from the two groups. This could in part have been because intake of fat and fatty acids was mostly still within WHO28 and American Heart Association (AHA)[29] recommendations even in urban areas. However, intake of the essential fatty acid ALA was below the recommended 0.5% of intake in both areas, and thus could lead to deficiencies of this fatty acid [28].

The serum lipid profiles of females in this population were found to be significantly more unfavorable than those of males, particularly in urban areas. BMI could be one of the most important causes of this finding. Females had significantly higher BMI than males, especially in urban areas. Higher BMI remained independently and positively associated with total cholesterol, LDL, and triglycerides, as well as negatively associated with HDL in both genders. Higher BMI also increased the risk for having elevated total cholesterol in males, elevated LDL in females, decreased HDL in males and females and increased triglycerides in males and females (Figure 2). Obesity has previously been shown to be an important determinant of serum lipid levels30. According to Grundy and Denke[31], the effects of obesity on blood lipids could be related to the overnutrition that causes obesity. However, the subjects in our study generally had low energy and fat intake. Other possible reasons for obesity in women could be physical inactivity24 or childhood stunting, which is linked to excess weight gain later in life[32]. Data from the National Food Consumption Survey indicated that stunting affected nearly one in five children in South Africa33 and 15% of children in the North West Province[34]. The double burden of malnutrition, which describes the presence of undernutrition along with a rapid rise in overweight and obesity, is a known problem in South Africa[35]. However, we found that physical activity did not play a major role in the serum lipid profiles of this population, which is evident by the lack of associations found in the applied multiple linear regression models or logistic

regression models. The only exception was the increased risk for having elevated LDL with decreasing physical activity found in females (Figure 2).

To our surprise, we found dietary ALA intake to be positively associated with total cholesterol and triglycerides in male subjects, but not in females. In contrast with our results, an inverse relationship or no associations between dietary ALA and total cholesterol, LDL and triglycerides have been reported previously[8,36,37]. A recent parallel intervention study in 81 subjects, providing either a diet high or low in ALA for 6 month, found that serum triglycerides were significantly lower in the group receiving the high ALA diet compared to the low ALA diet group[38]. In the present study, mean ALA intake was below the recommended 0.5% of energy in all groups[28]. Findings from Patenaude et al.[39] indicated that the provision of ALA in the form of ground flaxseed or flaxseed oil for 4 weeks lowered triglycerides in younger subjects (18–29 years) but not in older subjects (45–69 years). In prior South African studies, total cholesterol and LDL levels were shown to increase with age[17]. In our study, age was associated positively with total cholesterol, LDL and triglycerides in the fully adjusted model, however, only in females and not in males. The age group of this study population started at 35 years, which could have contributed to higher baseline triglyceride levels. It was also interesting to find such a strong inverse association between palmitic acid and triglycerides in males, which to our knowledge has not been described in the current literature before.

To date, not much information is available on the relationship between dietary arachidonic acid and blood lipids. In this study, dietary arachidonic acid was positively associated with LDL and also tended to be associated with total cholesterol in females ($P = 0.051$). A small cross-over intervention study in 10 men found that the provision of a diet rich in arachidonic acid (210 mg/d) for 50 days did not change blood lipids or lipoproteins[40].

Another interesting finding of our study is that in female subjects, EPA was positively associated, whereas DHA was negatively associated with total cholesterol. The associations were in the same directions for LDL, but did not reach statistical significance (EPA: $P = 0.051$, DHA: $P = 0.052$). The existing literature is inconclusive about the effects of EPA and DHA on

total cholesterol and LDL. Either no effect, decreases or increases have been reported in previous intervention trials[8,9,41]. Fewer studies focused on EPA and DHA individually than on the combination effect of long-chain n-3 fatty acids. A meta-analysis of randomized controlled trials that investigated the nett effects of purified EPA or DHA as monotherapy on serum lipids found that EPA had no effect on LDL, while DHA raised LDL[7]. Our results were in contrast with the effect reported in this meta-analysis. However, it should be noted that some studies reported increases while others no associations. Researchers linked the increasing effects of DHA on LDL to an increase in particle size[42], however, a decrease in the number of small dense LDL particles is also associated with DHA[43]. Even though we do not have a record of the LDL particle size in the current study, a recent study in South Africa comparing different ethnicities found black South African women to have the highest proportion of the smallest, most dense LDL particles[44].

Limitations of this study are that, regardless of the level of detail obtained in food frequency questionnaires (FFQs), dietary assessments are limited by available food composition databases[45,46] and accuracy during recording and coding[47]. In general, a QFFQ tends to overestimate intake of a population, especially for food items that are eaten regularly but not daily[47]. MacIntyre and colleagues[21] found that the quantitative food frequency questionnaire (QFFQ) used in the THUSA study tended to under-report energy and fat intakes in comparison with a seven-day weighed record, with better agreement at lower intake levels. However, in epidemiology the food frequency questionnaire is the method of choice to assess relationships between dietary intake and other indices of health status or disease [23,48].

Only part of the variance in blood lipids were explained by the fully adjusted models (Tables 3 and 4). Thus, other factors not explored in the present study, like culture, educational level, genetics, hormones and stress levels, could have also played a direct or indirect role on blood lipids. According to the thrifty phenotype hypothesis, for example, poor fetal growth (possibly due to fetal malnutrition) can lead to increased insulin resistance[49]. Insulin resistance in turn leads to elevated nonesterified fatty acids in the plasma, which can overload the liver and lead

to atherogenic dyslipidaemia[50]. Therefore, future studies should investigate and include these factors.

In conclusion, the results from this study give insightful information about novel associations between dietary fat and fatty acid intake and blood lipids in a population in transition. Furthermore, it was interesting to find that different dietary fatty acids and lifestyle factors were associated with unfavorable blood lipid levels in males and females. A possible reason why blood lipid levels did not differ between rural and urban dwellers is that median fat and fatty acid intake was generally within recommendations in both groups, except from intake of specific n-3 PUFAs that was below recommendation in both groups. Low intake of long chain n-3 PUFAs may also have detrimental effects on mental health of a population. Irrespective of the low intake, however, DHA was inversely associated with total cholesterol. LDL tended to be positively associated with DHA in females, while ALA was positively associated with total cholesterol and triglycerides in males and LDL tended to be positively associated with ALA. EPA was positively associated with total cholesterol and LDL in females. This raises the question about possible detrimental effects of ALA and EPA intake on blood lipid levels. It may be that in this black African population dietary ALA exerts different effects on blood lipids in males than in other populations with higher intake.

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Figure Legends

Figure 1

Outline of study population.

Figure 2

Prevalence of unfavorable serum lipid profiles by urbanisation and gender. Body mass index (BMI), waist circumference (WC), myristic acid (C14:0), alpha-linolenic acid (C18:3), physical activity (PA), alcohol (Alc), urbanisation (Urb).

Logistic regression analysis was used to determine whether individual dietary FAs increased or decreased the odds ratios (ORs) for having unfavorable blood lipid profiles, adjusting for total energy intake, age, BMI, physical activity and waist circumference. A *P*-value < 0.05 was considered significant.

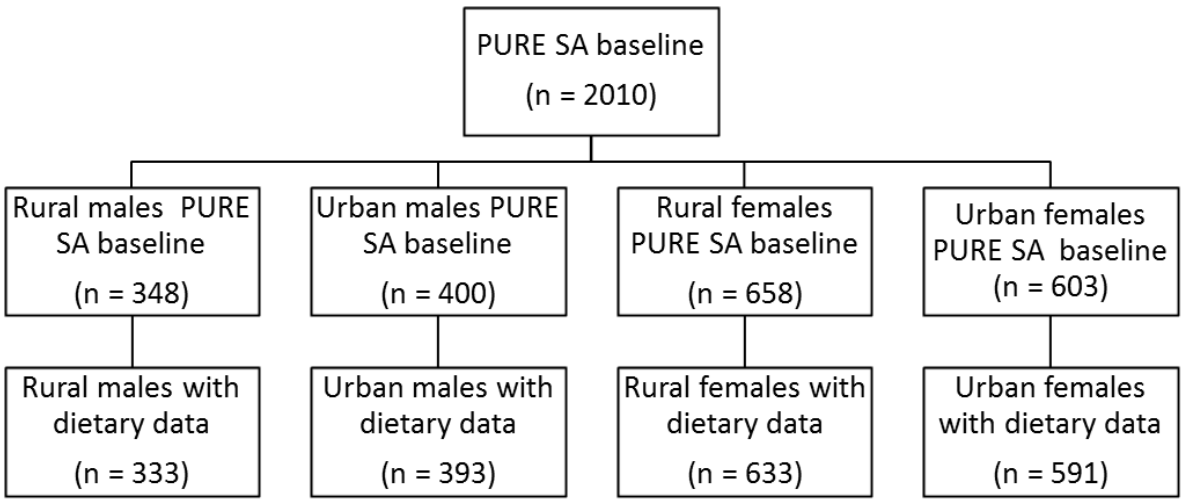

Figure 1

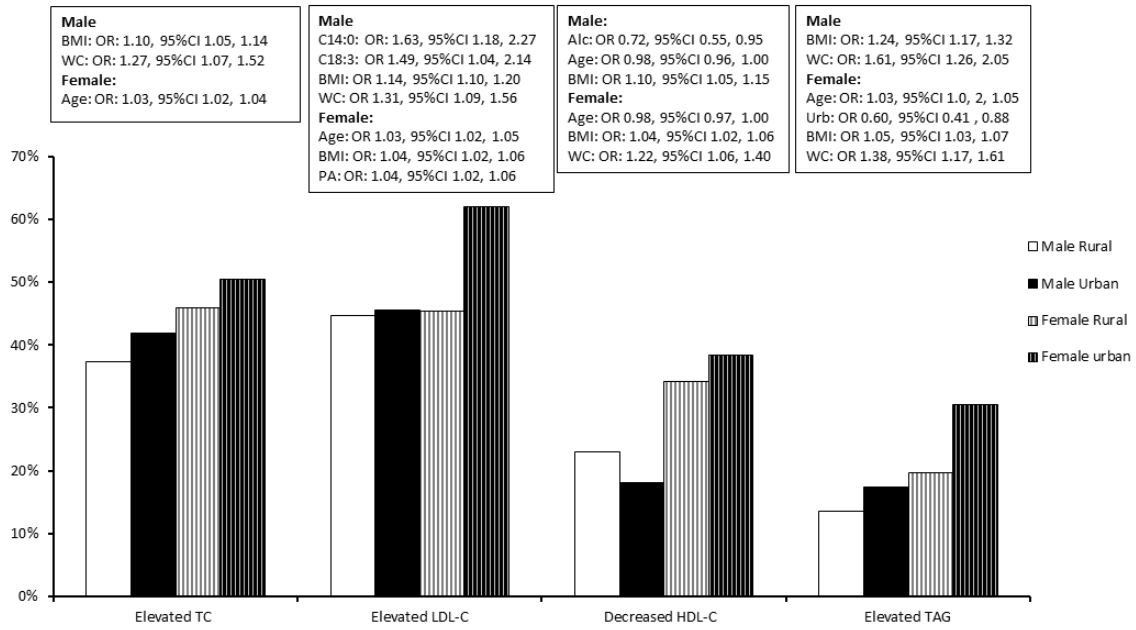
Male
BMI: OR: 1.10, 95%CI 1.05, 1.14
WC: OR: 1.27, 95%CI 1.07, 1.52
Female:
Age: OR: 1.03, 95%CI 1.02, 1.04
Male
C14:0: OR: 1.63, 95%CI 1.18, 2.27
C18:3: OR 1.49, 95%CI 1.04, 2.14
BMI: OR 1.14, 95%CI 1.10, 1.20
WC: OR 1.31, 95%CI 1.09, 1.56
Female:
Age: OR 1.03, 95%CI 1.02, 1.05
BMI: OR: 1.04, 95%CI 1.02, 1.06
PA: OR: 1.04, 95%CI 1.02, 1.06
Male:
Alc: OR 0.72, 95%CI 0.55, 0.95
Age: OR 0.98, 95%CI 0.96, 1.00
BMI: OR 1.10, 95%CI 1.05, 1.15
Female:
Age: OR 0.98, 95%CI 0.97, 1.00
BMI: OR: 1.04, 95%CI 1.02, 1.06
WC: OR: 1.22, 95%CI 1.06, 1.40
Male
BMI: OR: 1.24, 95%CI 1.17, 1.32
WC: OR: 1.61, 95%CI 1.26, 2.05
Female:
Age: OR: 1.03, 95%CI 1.0, 2, 1.05
Urb: OR 0.60, 95%CI 0.41 , 0.88
BMI: OR 1.05, 95%CI 1.03, 1.07
WC: OR 1.38, 95%CI 1.17, 1.61
70%
60%
50%
40%
30%
20%
10%
0%
Elevated TC
Elevated LDL-C
Decreased HDL-C
Elevated TAG
Male Rural
Male Urban
Female Rural
Female urban

Figure 2

Tables:

Table 1. Characteristics and differences in energy, fat and fatty acid intake by gender and urbanization[*]

Variable	Male		Female		Significance[§]		
	Rural (n=333)	Urban (n=393)	Rural (n=633)	Urban(n=591)	Gender	Urbanization	Gender x Urbanization
Age (years) [†]	49[48-51][ab]	50[49-51][a]	48[47-49][b]	50[49-51][a]	0.220	0.002	0.081
Physical activity index[‡]	3.00(2.26-3.41)[ab]	2.66(2.39-3.00)[b]	3.08(2.70-3.41)[a]	2.71(2.48-2.95)[c]	0.065	<0.001	0.005
BM[‡l]	19.71(17.99-2.25)[c]	19.96(18.26-22.78)[c]	24.89(20.77-30.70)[b]	27.21(22.30-32.59)[a]	<0.001	0.001	0.004
WC (cm) [‡]	74.50(70.15-80.50)[c]	74.30(69.65-81.75)[c]	78.48(69.30-89.25)[b]	82.80(73.20-92.70)[a]	<0.001	0.020	0.001
Energy (kJ) [‡]	6,548(4931-8341)[c]	9,440(7174-12384)[a]	5,889(4519-7422)[d]	8,539(6009-11457)[b]	<0.001	<0.001	0.732
Total fat (%E) [†]	17.83[17.09-18.56][d]	24.68[24.07-25.29][b]	19.84[19.30-20.38][b]	27.51[26.99-28.03][a]	<0.001	<0.001	0.192
SFA (%E) [†]	3.96[3.71-4.22][d]	6.14[5.94-6.35][b]	4.42[4.21-4.63][c]	7.11[6.92-7.29[a]]	<0.001	<0.001	0.184

MUFA (%E) †	8.13[7.13-8.43]^{c}	11.24[10.99-11.50]^{b}	8.78[8.59-9.96]^{c}	12.16[11.94-12.38]^{a}	<0.001	<0.001	0.258
PUFA (%E) †	5.66[5.37-5.95]^{d}	7.09[6.85-7.33]^{b}	6.55[6.31-6.80]^{b}	7.94[7.74-8.14]^{a}	<0.001	<0.001	0.876
LA (%E) †	5.21[4.92-5.50]^{d}	6.80[6.56-7.04]^{c}	6.05[5.81-6.28]^{b}	7.690[7.48-7.90]^{a}	<0.001	<0.001	0.878
ALA (%E) †	0.07[0.07-0.08]^{d}	0.12[0.12-.013]^{b}	0.08[0.08-0.09]^{c}	0.14[0.14-0.14]^{a}	<0.001	<0.001	0.100
Trans fat (%E) †	0.08[0.07-0.09]^{c}	0.20[0.18-0.22]^{b}	0.09[0.08-0.10]^{c}	0.30[0.28-0.32]^{a}	<0.001	<0.001	<0.001
Total fat (g) ‡	29.66(21.41-41.19)^{b}	60.76(44.31-81.63)^{a}	30.21(20.95-40.64)^{b}	61.47(42.49-85.84)^{a}	0.779	<0.001	0.588
SFA (g) ‡	6.32(3.77-9.17)^{b}	14.70(10.33-20.80)^{a}	6.31(3.87-9.49)^{b}	15.53(10.61-22.14)^{a}	0.374	<0.001	0.830
MUFA (g) ‡	6.55(4.09-9.76)^{b}	16.35(11.69-24.21)^{a}	6.60(4.08-9.91)^{b}	17.51(11.56-25.30)^{a}	0.645	<0.001	0.988
PUFA (g) ‡	9.34(6.26-13.63)^{b}	17.23(12.05-24.44)^{a}	9.75(6.35-14.21)^{b}	17.54(11.88-25.61)^{a}	0.399	<0.001	0.526
C14:0 (mg) ‡	400 (181-674)^{b}	876(617-1402)^{a}	400(186-733)^{b}	991(562-1546)^{a}	0.330	<0.001	0.530
C16:0 (mg) ‡	3,070(1897-4459)^{b}	7,877(5560-11212)^{a}	3,084(1876-4476)^{b}	8,466(5550-11744)^{a}	0.668	<0.001	0.885
C18:0 (mg) ‡	1742(1049-2747)^{b}	4230.74(2884-6186)^{a}	1779(1053-2692)^{b}	4358(3021-6547)^{a}	0.671	<0.001	0.972
C18:1 (mg) ‡	5,866(3661-9047)^{b}	15 081(10732-21990)^{a}	6,139(3767-8902)^{b}	15 691(10547-23140)^{a}	0.585	<0.001	0.688

C18:2 n-6(mg) ‡	8,478(4950-12735)[b]	15 944(10859-23195)[a]	8,590(5591-12981)[b]	16 565(10827-24071)[a]	0.216	<0.001	0.463
C20:4 n-6 (mg) ‡	34(19-56)[b]	102(68-150)[a]	33(18-60)[b]	94(63-140)[a]	0.138	<0.001	0.087
C18:3 n-3(mg) ‡	111(71-170)[b]	274(207-391)[a]	115(77-165)[b]	293(208-423)[a]	0.197	<0.001	0.386
C20:5 n-3 (mg) ‡	38(15-62)[c]	61(31-108)[a]	33(14-57)[c]	46(21-84)[b]	0.002	<0.001	0.006
C22:6 n-3 (mg) ‡	62(21-104)[c]	109(53-187)[a]	52(17-96)[c]	83(37-156)[b]	0.005	<0.001	0.029
Alcohol (g)	2.21(0.00-25.71)[b]	11.57(0.00-26.65)[a]	0.00(0.00-0.00)[d]	0.00(0.00-11.43)[c]	<0.001	<0.001	0.763
Soluble fibre (g)	0.93(0.54-1.43)[c]	1.94(1.28-3.21)[b]	1.11(0.66-1.62)[c]	2.27(1.34-3.43)[a]	<0.001	<0.001	0.851

Body mass index (BMI), waist circumference (WC), kilojoules (kJ), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3).

*Differences between groups were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Means/medians in a row with different superscripts (^{abcd}) differ significantly, $P<0.05$.

†Normally distributed values are reported as mean [95% CI].

‡Not normally distributed data are reported as median (25^{th} -75^{th} percentile), data were log transformed to perform analysis.

§2 way ANCOVA was used to test effects of gender and urbanization adjusting for age with all dietary intake variables, BMI and physical activity.

Table 2. Serum lipid levels by urbanization and gender*

Variable	Male		Female		Significance ‡		
	Rural (n=335)	Urban (n=376)	Rural (n=621)	Urban (n=558)	Gender	Urbanization	Gender x urbanization
TC (mmol/L) †	4.50(3.81-5.53)[b]	4.68(3.84-5.71)[b]	4.85(4.11-5.95)[a]	5.02(4.18-6.09)[a]	<0.001	0.171	0.200
LDL-C (mmol/L) †	2.85(2. 9-3.62)[b]	2.86(2.17-3.73)[b]	3.15(2.50-4.09)[a]	3.35(2.55-4.12)[a]	<0.001	0.602	0.766
HDL-C (mmol/L) †	1.45(1.02-1.94)[ab]	1.50(1.12-2.04)[a]	1.39(1.09-1.84)[ab]	1.36(1.03-1.78)[b]	<0.010	0.955	0.041
Triglycerides (mmol/L) †	0.97(0.76-1.35)[c]	1.00(0.78-1.46)[c]	1.09(0.81-1.49)[bc]	1.18(0.87-1.78)[a]	<0.001	<0.001	0.326
TC:HDL †	3.28(2.38-4.27)[b]	3.09(2.36-4.16)[b]	3.51(2.72-4.49)[a]	3.68(2.86-4.77)[a]	<0.001	0.322	0.174
LDL:HDL †	2.09(1.30-2.99)[b]	1.95(1.26-2.90)[b]	2.38(1.62-3.27)[a]	2.46(1.72-3.47)[a]	<0.001	0.653	0.187

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C).

*Differences between groups were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Means/medians in a row with different superscripts ([abcd]) differ significantly, $P<0.05$.

† Not normally distributed data are reported as median (25^{th} -75^{th} percentile), data were log transformed to perform analysis.

‡ 2 way ANCOVA was used to test effects of gender and urbanization adjusting for age with all dietary intake variables, BMI and physical activity.

Table 3. Associations between individual fatty acids and blood lipids of female subjects using multiple linear regression

Models		TC		LDL		HDL		Triglycerides	
		β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
1	Energy	0.068	0.029	0.019	0.541	0.090	0.004	0.084	0.008
	C14:0*	-0.011	0.831	0.037	0.488	-0.059	0.262	-0.117	0.028
	C16:0*	0.098	0.553	0.036	0.827	0.097	0.558	0.142	0.395
	C18:0*	0.040	0.645	0.059	0.501	-0.056	0.519	0.148	0.091
	C18:1*	-0.159	0.213	-0.157	0.220	-0.009	0.945	-0.178	0.164
	C18:2*	0.017	0.710	0.044	0.336	-0.042	0.356	-0.006	0.899
	C18:3*	0.000	0.997	0.043	0.462	-0.095	0.100	0.049	0.399
	C20:4*	0.123	0.009	0.113	0.016	0.059	0.208	-0.014	0.759
	C20:5*	0.335	0.004	0.302	0.009	0.148	0.201	0.046	0.689
	C22:6*	-0.330	0.005	-0.321	0.007	-0.088	0.455	-0.075	0.525
	R^2	0.028		0.024		0.026		0.019	
2	Energy	0.055	0.080	-0.009	0.758	0.119	0.000	0.079	0.010
	C14:0*	-0.009	0.863	0.021	0.691	-0.026	0.623	-0.086	0.095
	C16:0*	0.132	0.419	0.079	0.625	0.080	0.619	0.162	0.309
	C18:0*	0.023	0.786	0.059	0.488	-0.085	0.317	0.114	0.174

C18:1*	-0.189	0.131	-0.201	0.105	0.008	0.948	-0.193	0.115
C18:2*	0.035	0.448	0.046	0.321	-0.008	0.859	0.035	0.447
C18:3*	-0.023	0.695	-0.005	0.936	-0.047	0.416	0.022	0.699
C20:4*	0.106	0.021	0.089	0.051	0.071	0.118	-0.029	0.525
C20:5*	0.267	0.019	0.228	0.043	0.153	0.177	-0.019	0.862
C22:6*	-0.248	0.033	-0.229	0.047	-0.105	0.367	0.013	0.906
Alcohol*	0.000	0.993	-0.058	0.098	0.090	0.011	0.082	0.019
Soluble fibre	-0.034	0.330	-0.040	0.244	0.000	0.993	-0.003	0.923
BMI	0.076	0.016	0.172	<0.001	-0.208	<0.001	0.159	<0.001
Physical activity	-0.021	0.498	-0.043	0.161	0.040	0.197	0.016	0.593
WC†	0.028	0.370	0.039	0.208	-0.050	0.104	0.144	<0.001
Age	0.194	0.000	0.172	0.000	0.062	0.047	0.201	<0.001
R^2	0.075		0.094		0.084		0.117	
Energy (kJ)	0.074	0.033	0.008	0.809	0.141	<0.001	0.031	0.352
C14:0 (mg)*	-0.012	0.815	0.018	0.732	-0.029	0.575	-0.079	0.126
C16:0 (mg)*	0.158	0.336	0.102	0.528	0.110	0.499	0.099	0.535

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C18:0 (mg) *	0.016	0.852	0.052	0.539	-0.094	0.273	0.132	0.116
C18:1 (mg) *	-0.191	0.127	-0.203	0.101	0.005	0.965	-0.188	0.124
C18:2 (mg) *	0.031	0.509	0.042	0.365	-0.013	0.774	0.046	0.322
C18:3 (mg) *	-0.015	0.796	0.002	0.967	-0.038	0.511	0.003	0.954
C20:4 (mg) *	0.113	0.014	0.096	0.037	0.080	0.082	-0.047	0.295
C20:5 (mg) *	0.260	0.023	0.221	0.051	0.144	0.205	0.000	0.999
C22:6 (mg) *	-0.243	0.037	-0.225	0.052	-0.099	0.393	0.002	0.989
Alcohol*	0.007	0.853	-0.053	0.140	0.097	0.007	0.067	0.057
Soluble fibre	-0.030	0.389	-0.037	0.289	0.005	0.893	-0.013	0.714
BMI	0.078	0.012	0.175	0.000	-0.205	<0.001	0.152	<0.001
Physical activity	-0.029	0.358	-0.051	0.108	0.031	0.333	0.036	0.246
WC†	0.028	0.370	0.038	0.208	-0.050	0.104	0.144	<0.001
Age	0.196	0.000	0.174	0.000	0.065	0.039	0.196	<0.001
Urbanisati	-0.051	0.206	-0.047	0.244	-0.059	0.146	0.125	0.002

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R^2	0.076	0.095	0.086	0.126

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3), body mass index (BMI), waist circumference (WC).

*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

†A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Table 4. Associations between individual fatty acids and blood lipids of male subjects using multiple linear regression

Models		TC		LDL		HDL		Triglycerides	
		β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
1	Energy	-0.009	0.816	-0.082	0.038	0.116	0.003	-0.005	0.908
	C14:0*	0.081	0.266	0.122	0.091	-0.081	0.262	0.093	0.202
	C16:0*	-0.329	0.194	-0.159	0.528	-0.175	0.484	-0.489	0.053
	C18:0*	-0.035	0.763	-0.140	0.230	0.131	0.257	0.153	0.190
	C18:1*	0.278	0.092	0.182	0.269	0.105	0.518	0.249	0.129
	C18:2*	-0.058	0.347	0.014	0.814	-0.124	0.041	-0.024	0.692
	C18:3*	0.146	0.029	0.178	0.008	-0.118	0.075	0.272	<0.001
	C20:4*	0.023	0.741	-0.001	0.992	0.034	0.611	0.033	0.625
	C20:5*	0.222	0.205	0.264	0.129	0.065	0.705	-0.210	0.227
	C22:6*	-0.105	0.559	-0.175	0.328	0.039	0.824	0.162	0.363
	R^2	0.032		0.056		0.061		0.055	0.884
2	Energy	-0.021	0.593	-0.088	0.019	0.107	0.005	0-.029	0.446
	C14:0*	0.084	0.255	0.092	0.191	-0.022	0.760	0.121	0.086
	C16:0*	-0.309	0.215	-0.145	0.542	-0.166	0.489	-0.473	0.048
	C18:0*	-0.050	0.670	-0.117	0.294	0.066	0.558	0.102	0.360

C18:1*	0.270	0.096	0.159	0.305	0.130	0.406	0.265	0.088
C18:2*	-0.053	0.399	-0.029	0.634	-0.032	0.597	-0.032	0.594
C18:3*	0.149	0.030	0.124	0.058	-0.012	0.855	0.260	<0.001
C20:4*	0.014	0.835	-0.008	0.900	0.032	0.619	0.036	0.578
C20:5*	0.166	0.336	0.200	0.224	0.087	0.600	-0.261	0.114
C22:6*	-0.042	0.812	-0.096	0.571	0.005	0.978	0.213	0.209
Alcohol*	0.020	0.696	-0.101	0.037	0.221	<0.001	0.058	0.230
Soluble fibre	-0.033	0.451	-0.023	0.589	-0.028	0.516	0.047	0.268
BMI	0.201	<0.001	0.318	<0.001	-0.225	<0.001	0.286	<0.001
Physical activity	-0.022	0.581	-0.025	0.514	-0.001	0.973	0.006	0.880
WC†	0.102	0.012	0.094	0.015	-0.010	0.800	0.192	<0.001
Age	0.023	0.571	-0.001	0.973	0.044	0.263	0.007	0.852
R^2	0.085		0.176		0.151		0.172	0.787
Energy (kJ)	-0.046	0.295	-0.094	0.024	0.078	0.064	-0.049	0.238
C14:0 (mg) *	0.089	0.227	0.093	0.186	-0.015	0.828	0.125	0.076
C16:0 (mg) *	-0.368	0.146	-0.160	0.510	-0.235	0.335	-0.524	0.031

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C18:0 (mg) *	-0.029	0.803	-0.112	0.320	0.089	0.429	0.119	0.290
C18:1 (mg) *	0.291	0.074	0.164	0.292	0.154	0.324	0.284	0.069
C18:2 (mg) *	-0.057	0.368	-0.030	0.623	-0.037	0.548	-0.035	0.558
C18:3 (mg) *	0.143	0.038	0.123	0.061	-0.019	0.775	0.256	<0.001
C20:4 (mg) *	0.004	0.949	-0.010	0.872	0.021	0.747	0.028	0.669
C20:5 (mg) *	0.169	0.328	0.201	0.223	0.090	0.587	-0.259	0.117
C22:6 (mg) *	-0.046	0.794	-0.097	0.568	0.000	1.000	0.210	0.216
Alcohol*	0.017	0.742	-0.101	0.036	0.217	<0.001	0.055	0.253
Soluble fibre	-0.038	0.394	-0.024	0.573	-0.033	0.441	0.043	0.309
BMI	0.205	<0.001	0.318	<0.001	-0.221	<0.001	0.289	<0.001
Physical activity	-0.018	0.664	-0.024	0.535	0.004	0.914	0.010	0.800
WC†	0.108	0.008	0.095	0.014	-0.002	0.954	0.198	<0.001
Age	0.019	0.643	-0.002	0.953	0.039	0.321	0.004	0.923
Urbanisati	0.064	0.197	0.015	0.745	0.076	0.116	0.054	0.256

on				
R^2	0.088	0.176	0.155	0.174

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3), body mass index (BMI), waist circumference (WC).

*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

†A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Chapter 4: Manuscript 2

Associations between dietary fatty acids, plasma phospholipid fatty acids and blood lipids in healthy South Africans from the PURE study

This chapter includes:

The article titled "**Associations between dietary fatty acids, plasma phospholipid fatty acids and blood lipids in healthy South Africans from the PURE study**", prepared for the following journal: Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA).

- The instructions given to authors by the International Journal of Prostagladins, Leukotrienes and Essential Fatty Acids Addendum (PLEFA) Addendum A.

Chapter 4: Manuscript 2

Associations between dietary fatty acids, plasma phospholipid fatty acids and blood lipids in healthy South Africans from the PURE study

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Disclosures

We have no conflicts to disclose.

Key words: blood lipids, triglycerides, dietary fatty acids, plasma phospholipid fatty acids, alpha-linolenic acid, docosahexaenoic acid.

Abstract

Introduction: Previously, we found that alpha-linolenic acid (ALA) intake was positively associated with total cholesterol and triglycerides in males. Therefore, we explored associations between dietary FA intake and plasma FAs, and associations between plasma FAs and blood lipids in this population.

Methods: Cross-sectional analysis of healthy black South Africans (35-70 years) in transition. Dietary data were collected, blood lipids analysed and plasma total phospholipid FAs extracted in a subsample (n=716).

Results: We found a positive correlation between dietary ALA and plasma docosahexaenoic acid (DHA) (males r=0.33, females r=0.30), stronger than the association between dietary and plasma DHA (males r=0.21, P<0.001; females non-significant). Plasma DHA was positively associated with triglycerides (males β=0.410, P<0.001; females β=0.379, P<0.001). Along with our previous results, this suggests an efficient conversion of ALA to DHA in this unique population, and highlights that plasma phospholipid FAs should not be used in isolation as biomarkers for intake.

Introduction

Cardiovascular disease is on the increase worldwide and ischemic heart diseases are among the top 10 causes of death for men in South Africa [1]. Biomarkers of dyslipidemia are considered potential intermediaries for the association between dietary fatty acids and cardiovascular disease [2]. Considering the controversy surrounding the role of fat intake (more specifically the type of dietary fat) in cardiovascular disease, it is becoming clear that more research is needed on this topic. Furthermore, this topic is challenging to researchers since fat intake is one of the most difficult dietary components to measure [2]. Studies that look only at intake are criticized for limitations surrounding dietary data collection and interpretation. It can be very difficult for subjects to recognize and quantify fat, particularly when it comes to food preparation [2]. In addition, accuracy during reporting and coding is continuously questioned [2, 3]. Moreover, when looking

only at associations between fatty acid biomarkers and biomarkers of dyslipidemia, investigators are faced with the question of how intake and metabolism affects biomarkers. The absolute amount of fat consumed cannot be determined by biomarkers yet [2] and the level to which biomarkers can be used as indicators for dietary intake is still disputed. Plasma phospholipid fatty acids are thought to reflect, to some extent, medium-term fatty acid intake, but endogenous synthesis, elongation and desaturation all affect the use of fatty acid measures as biomarkers of consumption [2]. Biomarkers are, however, considered useful to quantify circulation change in fatty acid intake and have been used to quantify the consumption of essential and nonessential exogenously produced fatty acids [2]. Interpretation, however, requires an understanding of fatty acid metabolism, exogenous factors and the contributions of various body pools [2].

In a previous study, we found different associations between dietary fatty acids and blood lipids in males to those in females. Interestingly we also found that alpha-linolenic acid was positively associated with total cholesterol and triglycerides in males, and increased the risk of having elevated low density lipoprotein (LDL) cholesterol in males but not in females [4]. In the current study we therefore investigated the associations between dietary fatty acid intake and plasma phospholipid fatty acid composition, as well as associations with plasma fatty acids and blood lipids, to determine if the previous results could be explained further by biomarker analyses and to describe plasma phospholipid fatty acid status in this population.

Methods

Study population

This cross-sectional study forms part of the international Prospective Urban Rural Epidemiology (PURE) study, which is a large-scale multinational cohort study that tracks societal influences, risk factors and chronic non-communicable diseases in urban and rural areas of 17 countries in transition [5]. The baseline data collection of the South African leg in 2005 included just over 2,000 apparently healthy, migration stable subjects (aged >35 years) from a sample of 6,000 randomly selected black South African households in rural and urban areas in the North West Province. Exclusion criteria were the use of chronic medication for non-communicable diseases

and/or any self-reported acute illness [6]. Trained field workers, with the same background as the participants, conveyed all the information about the objectives and procedures of the study before recruitment in the participants' home languages. All participants gave written, informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research ethics committee. Data were treated confidentially and analyses were performed with coded data. For the purpose of the analyses in this paper, a random sub-sample was selected from the original 2000 volunteers (with both dietary data and plasma samples available) after exclusion of 305 participants with human immunodeficiency virus (HIV) and 305 matched controls due to previous use of plasma for analyses [7]. The number of participants that was randomly selected from stratified groups (rural male, rural female, urban male and urban female) the remaining was 125 male subjects from rural areas, 151 males from urban areas, 219 female subjects from rural areas and 221 females from urban areas (Figure 1).

Data collection

The participants were interviewed in the language of their choice. Structured demographic, socio-economic and lifestyle questionnaires that were developed and standardized for the international PURE study were used [5]. Interviewer-based quantitative food frequency questionnaires (QFFQ) were used to assess the dietary intake of volunteers, which were validated for the population in the Transition and Health during Urbanization of South Africans (THUSA) study [8] and for which reproducibility was proven [9, 10]. The dietary methodology of the PURE study was explained in detail previously [10]. In SA the physical activity index questionnaire, as developed and tested in the THUSA study, was used for the PURE study, as described previously by Kruger and colleagues [11]. The dietary intake data and associations between dietary fatty acids and blood lipids were published recently [4] and the descriptive statistics for dietary data of the subsample are accessible as supplementary material (Supplementary table 1). No significant differences were found between the published dietary data and the subsample within each group (rural male, rural female, urban male, urban female). The significance of between-group differences in the dietary data of the subsample also resembled the published dietary data (Supplementary table 1).

Therefore the subsample used in this study for comparisons with plasma phospholipid composition was considered representative of the previously published data [4].

Height, weight and waist circumference were measured using standardized methods, with instruments calibrated by the International Society for the Advancement of Kinanthropometry-accredited anthropometrists (Precision Health Scale, A& D Company, Japan; Leicester Height Measure, Seca, Birmingham UK).

Fasting blood samples with minimal stasis were collected by a registered nurse, who obtained them with a sterile winged infusion set from the antecubital vein, and stored at -80ºC. In the rural areas, samples were rapidly frozen and stored at -18ºC (no longer than five days) until they could be transported to the laboratory facility and were then stored at -80ºCv [6].

Serum lipids were measured on a Sequential Multiple Analyzer Computer (SMAC), using the KonelabTM auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland [12]. LDL was calculated using the Friedewald-Levy-Fredrickson formula [13]. Reference values used as indicators for elevated blood lipid values were total cholesterol <5 mmol/l, LDL cholesterol < 3 mmol/l, high density lipoprotein (HDL) cholesterol ≥1mmol/l for males and ≥1.2mmol/l for females, triglycerides > 1.7 mmol/l [14].

For plasma fatty acid analysis, lipids were extracted with chloroform: methanol (2:1 v/v) by modification of the method of Folch et al. (1957) [15]. Phospholipid fatty acid fractions were isolated by thin layer chromatography, transmethylated to yield fatty acid methyl esters (FAME) and analyzed by quadrupole gas chromatography electron ionization mass spectrometry (GC-EI-MS) on Agilent Technologies 7890 A GC system equipped with an Agilent Technologies 5975C VL mass selective detector, as previously described by Baumgartner et al. (2012) [16].

Statistical analyses

Statistical analysis was done by using IBM SPSS Statistics Version 22 (IBM Company, Armonk, NY, USA). Data were expressed as mean [95% CI].Differences in serum lipids, dietary fat and fatty acid intake were stratified by urbanization (rural vs urban) and gender (male vs female), and

their interactions were explored by using two-way ANCOVA, adjusting for age. Differences between groups (rural male, rural female, urban male, urban female) were analyzed with one-way ANOVA and Tukey's post-hoc tests were performed. Two separate hierarchical multiple linear regression models were used for both males and females to determine associations between plasma phospholipid fatty acid composition and blood lipid concentrations, adjusting for energy intake, BMI, physical activity, waist circumference, age and urbanization. Because sugar intake and carbohydrate intake and soluble fibre can have an effect on blood lipids it was included into the model, but removed again because it did not change the associations between fatty acids and blood lipids and were not found to be significantly associated with blood lipids in these models. The first hierarchical multiple linear regression model included only the individual fatty acids and energy intake was controlled for. The second fully adjusted model additionally controlled for alcohol intake, BMI, physical activity, waist circumference and age. A residual of BMI was calculated for waist circumference to avoid co-linearity between BMI and waist circumference. Logistic regression analysis, using the fully adjusted model, was used to determine whether individual dietary fatty acids increased or decreased the odds ratios (ORs) for having unfavorable blood lipid profiles. A P-value < 0.05 was considered significant.

Results

Plasma phospholipid data were obtained from 716 participants and characteristics are presented in table 1. Differences in plasma phospholipid fatty acid composition by gender and urbanization are shown in table 2. Dietary intake data for the complete sample were published previously [4] and dietary intake data for the subsample is presented in supplementary table 1.

A gender effect (females higher than males) was found for plasma total phospholipid fatty acid composition levels of myristic acid (C14:0), stearic acid (C18:0), dihomo-gamma-linolenic acid (DGLA, C20:3n6), mead acid (C20:3n9), arachidonic acid (C20:4n6), omega-3 docosapentaenoic acid (DPA, C22:5n3) and docosahexaenoic acid (DHA, C22:6n3). Male subjects had higher plasma fatty acid levels than females of palmitic acid (C16:0), oleic acid (C18:1n9), gamma-linolenic acid (GLA, C18:3n6), alpha-linolenic acid (alpha-linolenic acid C18:3n3), eicosapentaenoic acid (EPA, C20:5n3), adrenic acid (C22:4n6) and osbond acid (C22:5n6). An

urbanization effect (urban higher than rural) was found for myristic acid, C18:1n9, C20:3n9, EPA and DHA. For oleic acid, C22:4n6 and omega-3 DPA plasma fatty acid levels were higher in rural than urban dwellers. A gender x urbanization interaction was found for alpha-linolenic acid, indicating that the gender effect was found only in rural areas. For mead acid the gender x urbanization interaction indicated that the urbanization effect was found only in male participants.

Associations were found between dietary intake and plasma total phospholipid fatty acid composition levels for the following fatty acids: myristic acid (males, $r = 0.16$, $P = 0.008$; females $r = 0.13$, $P = 0.007$), palmitic acid (females $r = 0.18$, $P < 0.001$), stearic acid (males, $r = -0.14$, $P = 0.022$, females $r = -0.19$, $P < 0.001$), arachidonic acid (females $r = -0.14$, $P = 0.004$), eicosapentaenoic acid (EPA) (males $r = 0.20$, $P = 0.001$, females $r = 0.10$, $P = 0.047$) and docosahexaenoic acid (DHA) (males $r = 0.20$, $P = 0.001$). Dietary alpha-linolenic acid was positively correlated with plasma EPA (males $r = 0.21$, $P < 0.001$, females $r = 0.24$, $P < 0.001$) and DHA (males $r = 0.33$, $P < 0.001$, females $r = 0.30$, $P < 0.001$) (Figure 2 & 3).

In the fully adjusted multiple linear regression models investigating the associations between plasma total phospholipid fatty acids and blood lipids, we controlled for energy intake, alcohol intake, physical activity, BMI and a residual of waist circumference for the part which was not explained by BMI (Tables 3 & 4). There was a positive association between oleic acid and total cholesterol ($\beta = 0.322$, $P = 0.029$), as well as DGLA (C20:3n6) ($\beta = 0.207$, $P = 0.002$) and total cholesterol, while alpha-linolenic acid was inversely associated ($\beta = -0.118$, $P = 0.041$) with total cholesterol in females. In males there was a negative association between EPA and LDL ($\beta = -0.178$, $P = 0.017$). In females DGLA was positively associated with LDL ($\beta = 0.261$, $P < 0.001$). GLA was positively associated ($\beta = 0.245$, $P = 0.001$) and DGLA inversely associated ($\beta = -0.246$, $P = 0.001$) with HDL in males. In females oleic acid ($\beta = 0.297$, $P = 0.025$) and EPA ($\beta = 0.204$, $P = 0.003$) was positively associated with HDL, while DGLA ($\beta = -0.133$, $P = 0.025$) and DHA ($\beta = -0.220$, $P = 0.007$) were inversely associated with HDL. Palmitic acid, stearic acid, oleic acid, linoleic acid, DGLA, arachidonic acid, osbond acid and DHA were positively associated with triglycerides in males and females.

Discussion and conclusion

This is the first study, to our knowledge, that investigated associations between the plasma phospholipid fatty acid composition and blood lipid profiles in the black South African population and compared it with dietary fatty acid intake data. One of the purposes of this study was to determine the plasma phospholipid composition of this population with lower total fat intake and low omega-3 fatty acid intake [4].

It was interesting to find that even though previously reported median dietary EPA+DHA intake (33 to 61 mg EPA + 52 to 109mg DHA) [4] in this population was far below recommendations, including those made by the International Society for the Study of Fatty Acids and Lipids (500mg per day) [17] and the Joint United Nations FAO/WHO Expert Consultation (400 – 1000mg per day) [18], mean plasma phospholipid DHA (3.45 – 5.43% of plasma phospholipids) was relatively high compared to the mean DHA percentage of either plasma phospholipid or red blood cells (2.5% to 3.4% of fatty acids) reported for healthy individuals in other studies [19-21]. This was also evident when compared to a Canadian population group with a relatively low intake of DHA from fish sources (1.19% of plasma phospholipids) [22]. Mean plasma phospholipid arachidonic acid (13.04% to 14.01%) and omega-3 DPA (1.33% to 1.45%) were also higher in our study compared to healthy individuals in other studies – arachidonic acid: 6.24% to 11.5%[19, 21, 22]; DPA: 0.77% to 1.1% [19, 21]. A previous study looking at differences in fatty acid profiles between population groups, concluded that metabolism of Africans might be in favour of producing higher amounts of arachidonic acid in plasma phospholipid fatty acids [23]. More recently it has been shown that 81% of African Americans as opposed to 46% of European Americans in the Diabetes Heart study had the homozygous GG allele associated with high arachidonic acid levels [24]. In our study, plasma phospholipid oleic acid (8.80% to 10.68%), linoleic acid (15.76% to 17.18%) and EPA (0.61% to 0.86%) were lower than in other populations – oleic acid: 11.06% to 12.4% [19, 21], linoleic acid: 18.55% to 21%[19, 21] and EPA: 0.96% to 1.22% [21]. Mean plasma phospholipid palmitic acid and DGLA compared well to other studies [19, 21].

In the literature, the plasma phospholipid status of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) appears to be a good indicator of dietary intake [25], which

does not seem to be the case in this population for oleic acid, linoleic acid and arachidonic acid. Humans lack delta-12 desaturase; therefore conversion of oleic acid into linoleic acid (omega-9 to omega-6 conversion) is not possible. Additionally, the lack of delta 15 desaturase in humans prevents the conversion of linoleic into alpha-linolenic acid or the interconversion of omega-6 and omega-3 fatty acids in humans. Therefore dietary sources of these families of omega-6 and omega-3 fatty acids are the sole contributors to stores [2]. However, it has been shown previously that a low fat diet (20% of energy) promotes increases in long chain omega-3 content and decreases in the omega-6 content of plasma phospholipid fatty acids in comparison to a high fat diet (45% of energy) when fatty acid composition is kept constant [26]. Previous studies showed that the conversion of the essential n-3 fatty acid alpha-linolenic acid to EPA and DHA is limited in humans and affected by diet and gender [27, 28]. Proportions of EPA and DHA in plasma lipids are known to be determined mainly by intake of preformed DHA and negatively influenced by a high intake of linoleic acid [2, 28]. The linoleic acid intake of this population has been reported to be sufficient but not high and even low in rural areas among male subjects [4], and is therefore not expected to have a negative impact on the DHA formation in plasma phospholipids in this population. A stronger correlation was found in our study between dietary alpha-linolenic acid and plasma phospholipid DHA (males $r = 0.33$, $p < 0.001$, females $r = 0.30$, $p < 0.001$) than between dietary DHA and plasma DHA (males $r = 0.21$, $P < 0.001$; non-significant in females), while linoleic acid was not positively associated with arachidonic acid. This gives an indication that there might be a higher percentage of dietary alpha-linolenic acid converted to DHA in this population, with its low intake of alpha-linolenic acid and preformed long-chain omega-3 fatty acids, than in populations with a higher dietary intake of both. The conversion of omega-3 and omega-6 compete for enzymes, and the conversion favours the omega-3 fatty acids. Higher intakes of alpha-linolenic acid have not been shown to influence the conversion of alpha-linolenic acid to EPA and DHA. A number of small isotope studies found that high intake of EPA and DHA inhibits the conversion of alpha-linolenic acid to EPA or DHA [28-30]. It could be possible that alpha-linolenic acid is first utilized for conversion before beta-oxidation occurs, if EPA and DHA from dietary sources are not sufficient. Therefore, if the level is not yet reached whereby excess alpha-linolenic acid is beta-oxidized instead of converted, alpha-linolenic acid might be more strongly

associated with DHA. Additionally, it has been theorized that positive selection acted to increase alleles in the fatty acid desaturase (FADS) region in the African population, which is associated with enhanced metabolism of medium-chain PUFAs to long-chain PUFAs [31].

The dietary saturated fatty acids myristic and palmitic acids were positively associated with their plasma counterparts, but the associations were weak. Saturated fatty acids can be synthesized endogenously from acetyl CoA units by carbohydrate feeding, which is just one of the reasons why plasma saturated fat content is usually not a good indication of dietary saturated fat intake. Oleic acid can also be retro-converted into saturated fatty acids. Additionally, elongating the existing saturated fatty acids two carbons at a time creates new fatty acids [2]. Synthesis of fat is reported to be relatively rare in people who consume more than 25% of their energy as fat [32]. In this population, however, this would certainly be a possibility when looking at previously published intake data for this population, since only urban females had a median fat intake of more than 25% [4]. It is also suggested in the literature that when essential fatty acid deficiencies occur, palmitic acid could be higher.

Differences between male and female subjects are again evident in associations between the plasma phospholipid fatty acids and blood lipids of the present study, as was seen in associations between the dietary fatty acids and blood lipids previously published for this population [4]. A possible reason for which different plasma phospholipid fatty acids than dietary fatty acids were associated with blood lipids might be an altered plasma phospholipid composition due to the low fat intake of this population. The most interesting finding was that plasma phospholipid DHA was positively associated with triglycerides in both male and female participants in our study. This is interesting because dietary DHA is well known for its triglyceride-lowering properties [33, 34]. This might partially be explained by the association between dietary alpha-linolenic acid and plasma phospholipid DHA, as well as the previously published positive association between dietary alpha-linolenic acid and triglycerides in male participants [4]. Since high amounts of refined carbohydrates can influence HDL cholesterol and triglycerides [35], this might be of particular concern in this low fat intake population (as carbohydrates replace fats). We therefore ran the models again, additionally controlling for added sugar as a residual of energy, soluble fibre and

HIV status. However, this did not change the association between fatty acids and triglycerides in male or female participants. Additionally none of these were significantly associated with HDL or triglycerides (apart from added sugar in the model for HDL, but did not change associations between fatty acids and HDL which were the focus of this paper) and were therefore removed from the models. These models were also run adding carbohydrate intake as a residual of energy instead of added sugar and again the associations did not change between fatty acids and triglycerides in female participants. In male participants the only additional associations were that DGLA was now significantly associated with higher triglycerides ($\beta = 0.230$, $P = 0.005$) and DHA was now significantly and inversely associated with HDL ($\beta = -0.233$, $P = 0.023$) (data not shown). The evidence suggests that long-chain omega-3 polyunsaturated fatty acids affect all four metabolic nuclear receptors (LXR, FXR, HNF-4α and PPAR- α, -β and -γ) that reduce triglyceride levels [34]. Therefore there might be a genetic link or an error in the mechanism of hepatic inhibition of triglyceride synthesis in this population, resulting in the positive associations seen between dietary alpha-linolenic acid and plasma phospholipid DHA and triglycerides. Another possibility could be an inverted U-shaped curve in the association between plasma DHA and triglycerides, as well as dietary alpha-linolenic acid and triglycerides. Unfortunately our study population did not have participants with high intakes of alpha-linolenic acid, so this could not be investigated. As in our study, DGLA was also positively associated with triglycerides in a cross-sectional study of 177 heart failure patients [36].

The positive association between plasma phospholipid EPA and HDL and inverse association between DHA and HDL found in females in our study resembled the results of a cross-sectional study in Quebec consisting of 1,460 participants (who also had a low fish intake) done by Dewailly and colleagues [22]. In male participants in our study, however, it was GLA that was positively associated with HDL and DGLA that was inversely associated with HDL.

The limitations of our study include the fact that additional factors such as genetic variation, disease status, circulation apo-lipoprotein levels, micronutrient status and the hormonal balance of the individuals, which can influence fatty acid status and conversion [2, 25] were not determined.

These factors should therefore be investigated in future in this population. HIV was not investigated in this study, due to exclusion of the majority of HIV positive participants, who's plasma was used in a previous case-control study [7]. Strengths of this study include the large sample size and that confounders such as sex, age and body mass index (BMI) were controlled for. Another one of the strengths is that dietary as well as plasma phospholipid fatty acids were investigated in this unique group for which limited information on the role of fatty acids and CVD is available.

In conclusion, despite the reported low intake of EPA and DHA of our study population, we found that mean plasma phospholipid DHA was relatively high compared to other studies. Additionally, a stronger correlation was found in our study between dietary alpha-linolenic acid and plasma phospholipid DHA than between dietary DHA and plasma DHA. This large, cross-sectional study provide valuable information about the fatty acid status of this unique population in the process of nutrition transition with omega-3 fatty acid intake below recommended levels and gives direction for further research in this population for whom there is a shortage of information on the role of fat on CVD. Our data supports the theory that dietary alpha-linolenic acid might be an important contributor to plasma EPA and DHA in populations with low preformed EPA and DHA intake (e.g. from fish). Even though the positive association found between plasma DHA and triglycerides in this population is surprising, the finding is in line with the finding from our previous study, when considering that our current results suggest an efficient conversion of alpha-linolenic acid to DHA in this population. This article highlights that plasma phospholipid fatty acids should not be used in isolation as biomarkers for intake, without looking at dietary intake data in combination.

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Figure Legend:

Figure 1

Outline of study population.

Figure 2

Bivariate correlation between dietary alpha-linolenic acid (ALA, C18:3) and plasma phospholipid docosahexaenoic acid (DHA, C22:6n3) in male participants

$r = 0.33$, $p < 0.001$

Figure 3

Bivariate correlation between dietary alpha-linolenic acid (ALA, C18:3) and plasma phospholipid docosahexaenoic acid (DHA, C22:6n3) in female participants

$r = 0.30$, $p < 0.001$

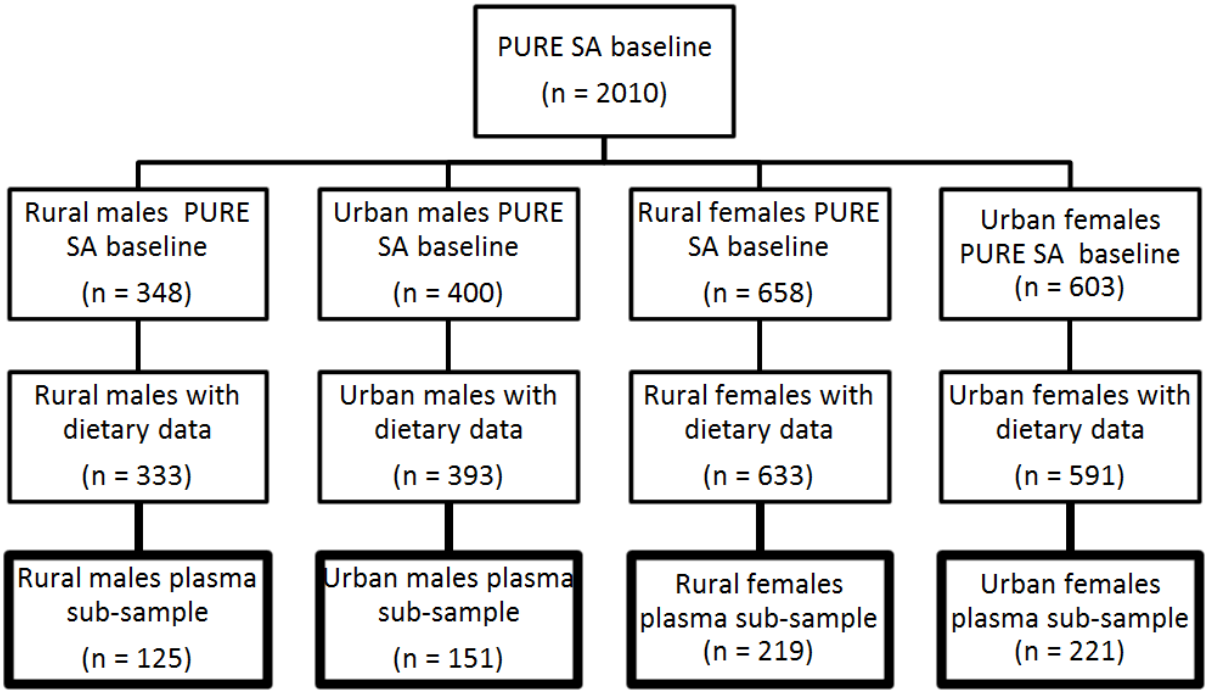

Figure 1

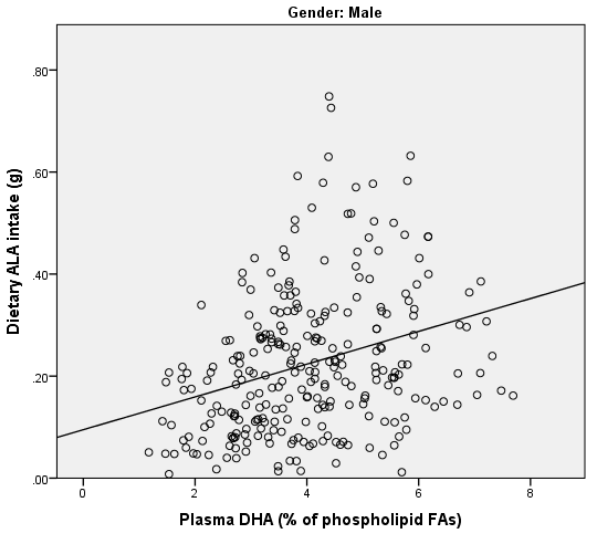

Figure 2

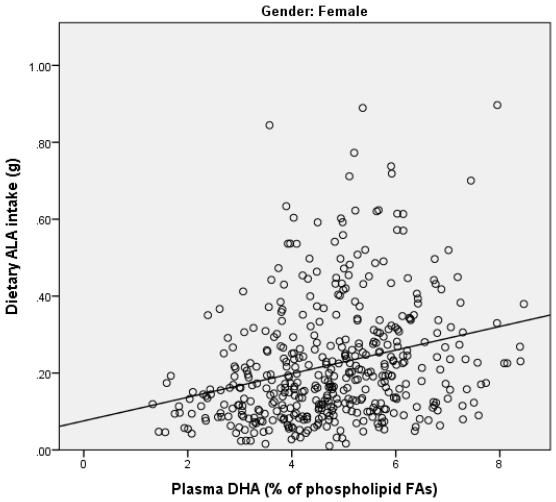

Figure 3

Table 1: Characteristics of the population by gender and urbanization

	Male		Female	
Variable	Rural (n= 122)	Urban (n=144)	Rural (n=209)	Urban (n= 220)
Age	53 (51, 55)	52 (51, 54)	51 (50, 52)	54 (53, 56)
Total cholesterol	5.08 (4.85, 5.30)	5.08(4.86, 5.28)	5.18(5.00, 5.36)	5.47(5.29, 5.66)
LDL	3.24(3.02, 3.45)	3.42(3.26, 3.59)	3.23(3.02, 3.45)	3.56(3.39, 3.72)
HDL	1.60(1.49, 1.72)	1.70(1.59, 1.81)	1.51(1.42, 1.60)	1.59(1.51, 1.67)
Triglycerides	1.21(1.11, 1.31)	1.27(1.11, 1.43)	1.28(1.19, 1.37)	1.63(1.48, 1.77)
Physical activity index	2.96 (2.85, 3.06)	2.94 (2.71, 3.17)	3.08 (3.00, 3.15)	2.71 (2.61, 2.80)
BMI	21.16 (20.32, 22.01)	21.03 (20.36, 21.69)	26.16 (25.22, 27.11)	28.16 (27.09, 29.23)
Waist Circumference	77.68 (75.58, 79.78)	76.69 (75.07, 78.32)	80.30 (78.46, 82.14)	84.30 (82.53, 86.08)
Energy	7310 (6721, 7900)	9521 (8914, 10128)	6093 (5760, 6426)	8729 (8227, 9231)
Alcohol(g)	20.47 (14.46, 26.49)	15.06 (12.36, 17.77)	6.89 (3.86, 9.92)	7.66 (5.68, 9.65)
HIV positive (%)	8.0%	8.8%	9.6%	11.8%

Body mass index (BMI), waist circumference (WC), kilojoules (kJ), human immunodeficiency virus (HIV).

Data were presented as mean (95% CI)

Supplementary table 1: Dietary intake of individual fatty acids (subsample)

Variable	Male		Female		Significance[§]		
	Rural (n=333)[*]	Urban (n=393)[*]	Rural (n=633)[*]	Urban(n=591)[*]	Gender	Urbanization	Gender x Urbanization
Energy (kJ)	7310 (6721 – 7900)[b]	9521 (8914 – 10128)[a]	6093 (5760 – 6426)[c]	8729 (8227 – 9231)[a]	<0.001	<0.001	0.428
Total fat (%E) [†]	18.04 (16.83 – 19.25)[d]	24.72 (23.73 – 25.72)[b]	20.04 (19.10 – 20.98)[c]	27.24 (26.31 – 28.16)[a]	<0.001	<0.001	0.647
C14:0 (mg)	558 (460 – 655)[b]	1036 (926 – 1146)[a]	507 (444 – 569)[b]	1154 (1054 – 1254)[a]	0.490	<0.001	0.088
C16:0 (mg)	3734 (3299 – 4170) [b]	8328 (7669 – 8987) [a]	3429 (3140 – 3719) [b]	8868 (8216 – 9519) [a]	0.677	<0.001	0.157
C18:0 (mg)	2195 (1919 – 2471) [b]	4613 (4230 – 4997) [a]	2037 (1852 – 2223) [b]	4817 (4448 – 5187) [a]	0.884	<0.001	0.332
C18:1 (mg)	7501 (6567 – 8435) [b]	16251 (14821 – 17682) [a]	6971 (6386 – 7556) [b]	17148 (15829 – 18467) [a]	0.753	<0.001	0.253
C18:2 n-6(mg)	10167 (8935 – 11398) [b]	16858 (15549 – 18167) [a]	10020 (9189 – 10851) [b]	17710 (166286 -19135) [a]	0.583	<0.001	0.466
C18:3 n-3(mg)	132 (117 – 147) [b]	301 (280 – 323) [a]	129 (119 – 138) [b]	314 (292 – 335) [a]	0.655	<0.001	0.438
C20:4 n-6 (mg)	39 (34 – 45) [b]	110 (100 – 120) [a]	40.56 (36 – 45) [b]	102 (93 – 111) [a]	0.397	<0.001	0.237
C20:5 n-3 (mg)	38 (32 – 43)[c]	77 (67 – 87) [a]	40 (35 – 45) [c]	57 (49 – 63) [b]	0.014	<0.001	0.002
C22:6 n-3 (mg)	60 (50 -70)[c]	134 (117 – 152) [a]	65 (57 -74) [c]	102 (89 – 115) [b]	0.039	<0.001	0.005

Kilojoules (kJ), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3).

[§]2 way ANCOVA was used to test effects of gender and urbanization, adjusting for age with plasma phospholipid fatty acid variables.

*Differences between groups were analyzed with 1 way ANOVA and Tukey's post-hoc test were performed. Means in a row with different superscripts (^{abcd}) differ significantly, $P<0.05$.

Table 2: Plasma phospholipid fatty acids (% of plasma phospholipid fatty acid composition) by gender and urbanization

	MALE		FEMALE		Significance[§]		
Variable	RURAL (n= 125)[*]	URBAN (n=148)[*]	RURAL (n=218)[*]	URBAN (n= 221)[*]	Gender	Urbanisation	Interaction
C14:0	0.24 (0.22, 0.25)[a]	0.26 (0.25, 0.28)[b]	0.26 (0.25, 0.27)[ab]	0.28 (0.27, 0.29)[b]	0.007	<0.001	0.411
C16:0	27.19 (26.55, 27.83)[ab]	27.52 (27.04, 27.99)[b]	26.40 (25.88, 26.92)[a]	26.76 (26.33, 27.19)[ab]	0.002	0.131	0.957
C18:0	15.36 (14.90, 15.83)[b]	13.49 (13.19, 13.80)[a]	16.60 (16.21, 16.99)[c]	14.70 (14.43, 14.97)[b]	<0.001	<0.001	0.763
C18:1n9	9.42 (8.88, 9.97)[b]	10.68 (10.08, 11.28)[c]	7.72 (7.42, 8.03)[a]	8.80 (8.41, 9.20)[b]	<0.001	<0.001	0.945
C18:2n6	16.04 (15.41, 16.67)[a]	16.18 (15.66, 16.71)[a]	15.76 (15.25, 16.26)[a]	15.97 (15.54, 16.39)[a]	0.349	0.398	0.742
C18:3n6	0.12 (0.11, 0.13)[b]	0.14 (13, 0.56)[c]	0.10 (0.09, 0.11)[a]	0.12 (0.11, 0.13)[b]	<0.001	<0.001	0.726
C20:3n6	2.90 (2.77, 3.02)[a]	2.86 (2.75, 2.96)[a]	3.00 (2.89, 2.09)[a]	3.05 (2.96, 3.15)[a]	0.008	0.921	0.528
C18:3:n3	0.10 (0.09, 0.12)[b]	0.09 (0.09, 0.10)[ab]	0.08 (0.08, 0.09)[a]	0.09 (0.08, 0.09)[a]	0.001	0.352	0.007
C20:3n9	0.28 (0.26, 0.32)[b]	0.37 (0.32, 0.42)[c]	0.22 (0.20, 0.24)[a]	0.24 (0.22 0.26)[ab]	<0.001	0.002	0.032
C20:4:n6	13.39 (12.92, 13.87)[ab]	13.04 (12.67, 13.42)[a]	14.01 (13.63, 14.39)[b]	13.68 (13.39, 13.97)[ab]	0.001	0.072	0.987
C20:5:n3	0.61 (0.55, 0.66)[a]	0.86 (0.77, 0.95)[b]	0.51 (0.47, 0.55)[a]	0.81 (0.76, 0.86)[b]	0.014	<0.001	0.411

C22:4n6	0.68 (0.65, 0.71)[b]	0.65 (0.63, 0.68)[a]	0.56 (0.54, 0.58)[b]	0.54 (0.52, 0.56)[a]	0.051	<0.001	1.000
C22:5n6	0.62 (0.57, 0.67)[a]	0.71 (0.67, 0.74)[a]	0.56 (0.52, 0.60)[b]	0.58 (0.55, 0.62)[a]	0.013	<0.001	0.146
C22:5n3	1.45 (1.38, 0.53)[b]	1.33 (1.28, 1.38)[b]	1.44(1.39, 1.49)[a]	1.44 (1.39, 1.49)[ab]	0.006	0.264	0.125
C22:6:n3	3.45 (3.24, 3.66)[a]	4.50 (4.29, 4.71)[c]	4.08 (3.92, 4.24)[b]	5.42 (5.26, 5.58)[d]	<0.001	<0.001	0.239
Omega-3 index	7.36 (7.14, 7.58)[a]	8.64 (8.40, 8.87)[c]	7.89 (7.72, 8.06)[b]	9.48 (9.31, 9.65)[d]	<0.001	<0.001	0.115

Myristic acid (C14:0), palmitic acid (C16:0) stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3:n3), mead acid (C20:3n9), arachidonic acid (C20:4:n6), eicosapentaenoic acid (EPA, C20:5:n3), adrenic acid (C22:4n6), omega-6 docosapentaenoic acid (DPA C22:5n6), omega-3 docosapentaenoic acid (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6:n3). Omega-3 Index (%) = Whole blood EPA + DPA + DHA (%) x 0.95 + 0.35.

Data were presented as mean (95% CI)

[§]2 way ANCOVA was used to test effects of gender and urbanization, adjusting for age with plasma phospholipid fatty acid variables.

*Differences between groups were analyzed with 1 way ANOVA and Tukey's post-hoc test were performed. Means in a row with different superscripts ([abcd]) differ significantly, $P<0.05$.

Table 3: Associations between plasma phospholipid fatty acids and blood lipids in male participants

Model		TC		LDL		HDL		Trig	
		Beta	Sig.	Beta	Sig.	Beta	Sig.	Beta	Sig.
1	Energy	-0.015	0.818	-0.042	0.511	0.045	0.404	-0.050	0.414
	C14:0	-0.118	0.119	-0.099	0.171	-0.071	0.241	0.041	0.547
	C16:0	0.258	0.252	0.234	0.276	-0.068	0.706	0.648	0.002
	C18:0	0.085	0.656	0.068	0.711	-0.102	0.503	0.716	<0.001
	C18:1n9	0.207	0.418	-0.025	0.919	0.315	0.123	0.713	0.002
	C18:2n6	0.155	0.434	0.155	0.413	-0.092	0.563	0.445	0.014
	C18:3n3	0.135	0.113	0.105	0.195	0.057	0.404	0.115	0.138
	C18:3n6	0.014	0.884	-0.117	0.201	0.240	0.002	-0.012	0.889
	C20:3n6	0.099	0.259	0.230	0.006	-0.276	<0.001	0.326	<0.001
	C20:3n9	0.095	0.376	0.166	0.105	-0.175	0.041	0.065	0.502
	C20:4n6	0.269	0.094	0.177	0.247	0.162	0.204	0.366	0.012
	C20:5n3	-0.106	0.196	-0.179	0.023	0.104	0.115	-0.015	0.844
	C22:4n6	-0.166	0.190	-0.172	0.154	0.015	0.882	-0.121	0.289
	C22:5n6	0.046	0.701	0.002	0.985	-0.088	0.356	0.326	0.003
	C22:5n3	0.166	0.101	0.122	0.204	0.068	0.395	0.062	0.493
	C22:6n3	0.025	0.839	0.083	0.488	-0.221	0.028	0.484	<0.001
	Age	-0.006	0.931	-0.009	0.887	0.012	0.827	0.032	0.591
	R^2	0.061		0.15		0.40		0.23	

2	Energy	-0.038	0.558	-0.069	0.260	0.052	0.331	-0.075	0.203
	C14:0	-0.136	0.068	-0.129	0.065	-0.045	0.458	0.020	0.763
	C16:0	0.223	0.313	0.226	0.274	-0.112	0.535	0.625	0.002
	C18:0	-0.002	0.992	-0.019	0.914	-0.093	0.548	0.621	<0.001
	C18:1n9	0.242	0.336	0.042	0.856	0.248	0.230	0.733	0.001
	C18:2n6	0.133	0.495	0.155	0.396	-0.126	0.434	0.452	0.011
	C18:3n3	0.142	0.085	0.116	0.132	0.049	0.471	0.117	0.114
	C18:3n6	0.007	0.939	-0.126	0.146	0.245	0.001	-0.011	0.894
	C20:3n6	0.020	0.818	0.139	0.093	-0.246	0.001	0.230	0.004
	C20:3n9	0.073	0.490	0.137	0.162	-0.158	0.068	0.056	0.551
	C20:4n6	0.231	0.139	0.142	0.331	0.158	0.217	0.318	0.024
	C20:5n3	-0.110	0.167	-0.178	0.017	0.095	0.149	-0.016	0.820
	C22:4n6	-0.160	0.191	-0.163	0.155	0.008	0.939	-0.109	0.318
	C22:5n6	0.065	0.575	0.032	0.765	-0.110	0.245	0.357	0.001
	C22:5n3	0.194	0.051	0.163	0.079	0.046	0.574	0.102	0.252
	C22:6n3	-0.050	0.685	0.001	0.996	-0.197	0.054	0.410	<0.001
	Age	-0.066	0.322	-0.084	0.177	0.043	0.434	-0.032	0.587
	Alcohol	-0.153	0.032	-0.178	0.007	0.069	0.235	-0.096	0.132
	Physical activity	0.018	0.787	-0.028	0.649	0.078	0.148	-0.009	0.879
	Waist circumference	0.169	0.010	0.170	0.006	-0.002	0.973	0.141	0.017

BMI	0.203	0.006	0.251	<0.001	-0.117	0.052	0.257	<0.001
R^2	0.14		0.25		0.42		0.31	

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n7), stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6),alpha linolenic acid (C18:3,n3), arachidonic acid (C20:4n6), mead acid (C20:3n9), eicosapentaenoic acid (C20:5n3), adrenic acid (C22:4n6), osbond acid(C22:5n6), docosapentanoic acid (C22:5n3) docosahexaenoic acid (C22:6n3), body mass index (BMI), waist circumference (WC).
[†]A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Table 4: Associations between plasma phospholipid fatty acids and blood lipids in female participants.

		TC		LDL		HDL		Trig	
Model		Beta	Sig.	Beta	Sig.	Beta	Sig.	Beta	Sig.
1	Energy	0.087	0.097	0.066	0.207	0.033	0.504	0.110	0.015
	C14:0	-0.038	0.559	-0.064	0.313	0.016	0.783	0.075	0.175
	C16:0	0.250	0.122	0.191	0.230	0.006	0.970	0.478	0.001
	C18:0	0.146	0.286	0.104	0.441	-0.128	0.312	0.733	<0.001
	C18:1n9	0.310	0.033	0.102	0.477	0.298	0.027	0.534	<0.001
	C18:2n6	0.186	0.177	0.056	0.681	0.156	0.223	0.430	<0.001
	C18:3n3	-0.118	0.040	-0.090	0.112	-0.067	0.206	-0.063	0.201
	C18:3n6	-0.058	0.398	-0.060	0.379	-0.022	0.732	0.046	0.438
	C20:3n6	0.190	0.003	0.272	<0.001	-0.198	0.001	0.195	<0.001
	C20:3n9	0.006	0.940	0.002	0.983	-0.028	0.714	0.126	0.075
	C20:4n6	0.204	0.076	0.178	0.117	-0.012	0.911	0.282	0.004
	C20:5n3	0.035	0.642	-0.052	0.483	0.162	0.020	0.056	0.381
	C22:4n6	-0.104	0.343	-0.091	0.400	0.008	0.934	-0.161	0.087
	C22:5n6	-0.115	0.229	-0.191	0.043	-0.018	0.840	0.481	<0.001
	C22:5n3	0.047	0.531	0.057	0.446	-0.023	0.744	0.053	0.412
	C22:6n3	-0.002	0.985	0.055	0.529	-0.243	0.004	0.403	<0.001
	Age2005	0.158	0.002	0.158	0.002	0.003	0.952	0.137	0.002
	R^2	0.12		0.14		0.24		0.32	

2	Energy	0.087	0.100	0.063	0.227	0.038	0.420	0.103	0.023
	C14:0	-0.026	0.694	-0.067	0.305	0.049	0.407	0.050	0.369
	C16:0	0.239	0.147	0.219	0.179	-0.078	0.596	0.532	<0.001
	C18:0	0.172	0.216	0.123	0.369	-0.109	0.381	0.718	<0.001
	C18:1n9	0.322	0.029	0.116	0.427	0.297	0.025	0.545	<0.001
	C18:2n6	0.180	0.200	0.090	0.517	0.069	0.584	0.477	<0.001
	C18:3n3	-0.118	0.041	-0.092	0.106	-0.063	0.226	-0.066	0.178
	C18:3n6	-0.066	0.337	-0.058	0.393	-0.044	0.473	0.060	0.310
	C20:3n6	0.207	0.002	0.261	<0.001	-0.133	0.025	0.153	0.007
	C20:3n9	-0.004	0.961	0.002	0.983	-0.052	0.489	0.138	0.052
	C20:4n6	0.220	0.059	0.180	0.117	0.021	0.843	0.268	0.007
	C20:5n3	0.047	0.532	-0.058	0.442	0.204	0.003	0.035	0.584
	C22:4n6	-0.104	0.345	-0.088	0.418	0.002	0.985	-0.161	0.085
	C22:5n6	-0.118	0.224	-0.173	0.070	-0.065	0.454	0.515	<0.001
	C22:5n3	0.029	0.710	0.075	0.326	-0.106	0.129	0.102	0.123
	C22:6n3	0.002	0.985	0.049	0.583	-0.220	0.007	0.379	<0.001
	Age	0.163	0.002	0.148	0.004	0.036	0.435	0.117	0.008
	Alcohol	-0.005	0.921	0.036	0.495	-0.087	0.068	0.004	0.933
	Physical activity	-0.036	0.503	-0.060	0.254	0.052	0.278	-0.029	0.519
	Waist circumference	-0.057	0.262	0.002	0.969	-0.140	0.002	0.086	0.049

BMI	-0.038	0.518	0.060	0.296	-0.217	<0.001	0.130	0.009
R^2	0.12		0.15		0.29		0.33	

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n7), stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6),alpha linolenic acid (C18:3,n3), arachidonic acid (C20:4,n6), mead acid (C20:3n9), eicosapentaenoic acid (C20:5,n3), adrenic acid (C22:4n6), osbond acid(C22:5n6), docosapentanoic acid (C22:5n3) docosahexaenoic acid (C22:6,n3), body mass index (BMI), waist circumference (WC).
[†]A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Chapter 5: Manuscript 3

Associations of plasma phospholipid fatty acid status and dietary fatty acid intake with PAI-1 and clot lysis time in healthy South African men and women from the PURE study

This chapter includes:

The article titled "**Associations of plasma phospholipid fatty acid status and dietary fatty acid intake with PAI-1 and clot lysis time in healthy South African men and women from the PURE study**", prepared for the following journal: Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA).

- The instructions given to authors by PLEFA in Addendum A.

Associations of plasma phospholipid fatty acid status and dietary fatty acid intake with PAI-1 and clot lysis time in healthy South African men and women from the PURE study

Associations between dietary fatty acids, plasma phospholipid fatty acids and blood lipids in healthy South Africans from the PURE study

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Disclosures

We have no conflicts to disclose.

Key words: clot lysis time, plasminogen activator inhibitor-1, triglycerides, dietary fatty acids, plasma phospholipid fatty acids.

Summary

Data on the association between omega-3 fatty acids and PAI-1 and fibrinolysis are limited and lacking in the black South African population. Therefore we investigated the associations

between dietary fatty acid intake, plasma phospholipid fatty acid status and PAI-1act and fibrinolytic potential by means of a random cross-sectional subsample (n=716) analysis within the PURE baseline study of healthy subjects (35-70 years) in South Africa. Dietary data were collected and plasma total phospholipid fatty acid extraction and isolation were performed on a subsample. PAI-1act and fibrinolitic potential were measured. Our results indicate that the associations between fatty acids and clot lysis time might be independent of PAI-1act. This may be of particular concern in this population with documented lower fat intake.

Introduction

Ischaemic heart diseases are among the top 10 causes of death in South Africa for men, but not for women, while hypertensive diseases are among the top 10 causes of death in women but not men [1]. Between 1997 and 2004 an average of 195 people died per day from cardiovascular disease [2].

Reduced plasma fibrinolytic potential, measured as increased clot lysis time, is a risk factor for venous and arterial thrombosis [3-5], which increases the risk for ischaemic disease. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of the fibrinolytic process [6] and PAI-1 levels were found to be the main contributing factor to clot lysis time by Meltzer and colleagues (2010) [5]. Therefore increased PAI-1 levels and reduced fibrinolytic potential are considered to be potential risk factors for CVD and venous and arterial thrombosis [3-5, 7]. A positive association exists between triglycerides and PAI-1, and this association has been proposed to be the explanation for decreased fibrinolytic activity associated with hypertriglyceridaemia [8, 9]. An inverse association was recently reported between PAI-1 and serum n-6 fatty acids by Lee and fellow researchers (2012) [6]. Because dietary intervention with long-chain omega-3 fatty acids from marine sources has been shown to decrease serum triglycerides [10], a causal relationship between omega-3 fatty acids and PAI-1 via its lowering properties on triglycerides has been proposed by other researchers. Former studies have not

provided conclusive evidence for the relation between dietary fat intake and the haemostatic system, and focussed mostly on examining Caucasian individuals.

While a limited number of studies have investigated the association between omega-3 fatty acids and PAI-1, no studies have been performed on the association between n-3 fatty acids and global fibrinolytic potential. PAI-1 is, however, considered to be the main inhibitor of fibrinolysis and the use of the global fibrinolytic assay provides a better reflection of the true fibrinolytic potential of an individual.

Additionally, the black South Africa population is undergoing a process of urbanisation, which is associated with changes in diet. Thus studies are needed that provide information about the association between PAI-1, fibrinolytic potential, dietary and plasma fatty acid levels in black South Africans. Being an important inhibitor of fibrinolysis, PAI-1 is largely used as a proxy marker of fibrinolytic capacity. Therefore we performed a cross-sectional study, investigating associations between dietary fatty acids, plasma phospholipid fatty acids, and PAI-1act and clot lysis time.

Materials and Methods

Study population

This cross-sectional study forms part of the international PURE study, which is a large-scale multinational cohort study that tracks societal influences, risk factors and chronic non-communicable diseases in the urban and rural areas of 17 countries in transition [11]. The baseline data collection of the South African leg in 2005 included just over 2,000 apparently healthy, migration stable subjects (aged >35 years) from a sample of 6,000 randomly selected households in rural and urban areas in the North West Province of South Africa. The rural communities were still under tribal law. Exclusion criteria were the use of chronic medication for non-communicable diseases and/or any self-reported acute illness [12]. Trained field workers with the same background as the participants conveyed all the information about the objectives and procedures of the study before recruitment. This was done in the participants' home

languages. All participants gave written, informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Data were treated confidentially and analyses were performed with coded data. For the purpose of the analyses in this paper, subjects from whom no plasma fatty acid composition and dietary data were available were excluded, leaving a subsample of 125 male subjects from rural areas, 151 males from urban areas, 219 female subjects from rural areas and 221 females from urban areas (Figure 1).

Data collection

Participants were interviewed in the language of their choice. Structured demographic, socio-economic and lifestyle questionnaires that were developed and standardised for the international PURE study were used [11]. Interviewer-based quantitative food frequency questionnaires (QFFQ) were used to assess the dietary intake of volunteers, which was validated for the population in the Transition and Health during Urbanisation of South Africans (THUSA) study [13] and for which reproducibility was proven [14, 15]. The dietary methodology of the PURE study was explained in detail previously [15]. In SA the physical activity index questionnaire, as developed and tested in the THUSA study, was used for the PURE study, as described previously by Kruger and colleagues (2002) [16].

The dietary intake data of the complete study population was published recently [17]. The descriptive statistics for dietary data and the plasma phospholipid fatty acid status of the subsample were presented in another article [18] and are accessible as supplementary material (Supplementary Table 1). No significant differences were found between the published dietary data and the subsample within each group (rural male, rural female, urban male, urban female) [18]. The significance of between-group differences in the dietary data of the subsample also resembled the published dietary data. Therefore the subsample used in this study for comparisons with plasma phospholipid composition was considered representative of the published data.

Height, weight and waist circumference were measured using standardised methods, with instruments calibrated by the International Society for the Advancement of Kinanthropometry-accredited anthropometrists (Precision Health Scale, A& D Company, Japan; Leicester Height Measure, Seca, Birmingham UK).

Fasting blood samples with minimal stasis were collected by a registered nurse, who obtained these using a sterile winged infusion set from the antecubital vein, and stored at -80ºC. In the rural areas, samples were rapidly frozen and stored at -18ºC (no longer than five days) until they could be transported to the laboratory facility and then stored at -80ºC [12].

For the determination of PAI-1act, blood was collected in citrate tubes. The collection tube was placed on ice until processing. Samples were centrifuged at 2000 x g for 15 minutes at 10oC within two hours of collection and stored in bio-freezers at -80oC until further analysis.

A chromogenic assay kit was used for the determination of PAI-1act by the PURE SA study group. This was done by adding a fixed amount of t-PA to a citrate plasma sample, which was then allowed to react with the PAI-1act present in the sample. The sample was then acidified to destroy potential plasmin inhibitors and was subsequently diluted. A mixture of Glu-plasminogen, poly-D-lysine and chromogenic substrate were added. The amount of colour that developed was proportional to the amount of active t-PA in the sample. The PAI-1act content in the sample was therefore determined by the difference between the amount of t-PA added to the sample and the amount of t-PA recovered (Spectrolyse pL PAI-1, Biopool, Trinity Biotech, Ireland).

Plasma fibrinolytic potential of tissue factor-induced clots, lysed by exogenous t-PA, was measured using the method of Lisman et al.(2005) [7], with a slightly modified tissue factor and t-PA concentrations in order to obtain comparable clot lysis times of about 60 min (CV = 6.6%). Final concentrations were: tissue factor (125 x diluted; Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany), CaCl2 (17 mmol/l), t-PA (100 ng/ml; Actilyse, Boehringer Ingelheim, Ingelheim, Germany) and phospholipid vesicles (10 µmol/l; Rossix, Mölndal, Sweden).

Clot lysis time is defined as the time from the midpoint of clear turbidity to maximum turbidity, which is representative of clot formation, to the midpoint of maximum turbidity to clear turbidity, which represents the lysis of the clot.

Plasma phospholipid fatty acid analysis

For plasma fatty acid analyses, lipids were extracted with chloroform: methanol (2:1 v/v) by modification of the method of Folch et al. (1957)[19]. Phospholipid fatty acid fractions were isolated by thin layer chromatography, trans-methylated to yield fatty acid methyl esters (FAME) and analysed by quadrupole gas chromatography electron ionisation mass spectrometry (GC-EI-MS) on Agilent Technologies 7890 A GC system equipped with an Agilent Technologies 5975C VL mass selective detector, as previously described by Baumgartner et al. (2012) [20].

Statistics:

Statistical analysis was done using the IBM SPSS Statistics Version 19 (IBM Company, Armonk, NY, USA). Mean dietary fat and fatty acid intake, plasma phospholipid fatty acid status and cardiovascular risk markers were stratified by urbanisation (rural vs urban) and gender (male vs female). Three separate hierarchical multiple linear regression models were used for both males and females to determine associations between specific dietary FAs and PAI-1act and clot lysis time in relation, adjusting for energy intake, age, alcohol intake, BMI, physical activity, waist circumference and triglycerides. The first hierarchical multiple linear regression model included the individual FAs and controlled for energy and alcohol intake, BMI, physical activity, waist circumference (residual from BMI to avoid co-linearity between BMI and waist circumference) and age. The second model additionally controlled for triglycerides. For clot lysis time, a third model additionally controlled for PAI-1act. A residual of energy intake was calculated for alcohol to account for differences in energy intake. A P-value < 0.05 was considered significant.

Results

Data were obtained from 716 participants. Characteristics of the population are shown in Table 1. In model 1, investigating associations between dietary fatty acids and PAI-1act, no fatty acids were associated with PAI-1act in male or female participants. In the fully adjusted model (Model 2, Table 2) dietary alpha-linolenic acid (C18:3n3) was negatively associated with PAI-1act (β = -0.268, P = 0.049) in females. R2 was 0.23 for males and 0.17 for females. In the multiple linear regression model investigating dietary fatty acids and clot lysis time, no dietary fatty acids were associated with clot lysis time in the basic model (Table 3, Model 1), as well as in the model additionally controlling for triglycerides (Model 2). However, there was a non-significant trend for a negative association between alpha-linolenic acid and clot lysis time (β = -0.296, P = 0.079) in males and a non-significant trend for a positive association between myristic acid and clot lysis time in females (β = 0.193, P = 0.079). In Model 3 (additionally controlling for PAI-1), alpha-linolenic acid was negatively associated with clot lysis time (β = -0.356, P = 0.024) in males and myristic acid (C14:0) was positively associated with clot lysis time in females (β = 0.208, P = 0.041).

In Table 4, in the basic model investigating associations between plasma phospholipid fatty acids and PAI-1act, myristic acid (β = 0.185, P = 0.003), stearic acid (C18:0) (β = 0.306, P = 0.020) oleic acid (C18:1n9) (β = 0.290, P = 0.038) and DHA (C22:6n3) (β = 0.258, P = 0.003) were positively associated with PAI-1 in females. In male subjects adrenic acid (C22:4n6) was negatively associated (β = -0.237, P = 0.036) and osbond acid (C22:5n6) positively associated with PAI-1 (β = 0.400, P < 0.001). In the fully adjusted model (Model 2), myristic acid remained positively associated with PAI-1 in both males (β = 0.145, P = 0.034) and females (β = 0.183, P = 0.004). In male subjects, adrenic acid was negatively associated (β = -0.222, P = 0.048) and osbond acid positively associated with PAI-1 (β = 0.351, P = 0.001), while DHA remained positively associated with PAI-1 in females (β = 0.236, P = 0.008).

In Model 1, shown in Table 5, investigating associations between plasma phospholipid fatty acids and clot lysis time, palmitic acid, stearic acid, GLA (C18:3n6), alpha-linolenic acid, mead

acid (C20:3n9), arachidonic acid (C20:4n6), omega-3 DPA (C22:5n3) and DHA were significantly and positively associated, while EPA (C20:5n3) was inversely associated with clot lysis time in males. In female participants, alpha-linolenic acid and arachidonic acid were positively associated, while osbond acid was negatively associated with clot lysis time. In Model 2 (additionally controlling for triglycerides), GLA, alpha-linolenic acid, mead acid, arachidonic acid and C22:5n3 (omega-3 DPA) remained positively associated, and EPA remained negatively associated with clot lysis time in males. In female participants, oleic acid was negatively associated, while alpha-linolenic acid and osbond acid were positively associated with clot lysis time in this model. In the fully adjusted model (model 3), we investigated the associations between phospholipid fatty acids and clot lysis time independent of their associations with triglycerides and PAI-1. Alpha-linolenic acid (β = 0.123, P = 0.037), mead acid (β = 0.176, P = 0.019), ARA (β = 0.253, 0.025) and omega-3 DPA (β = 0.224, P = 0.002) were positively associated, while myristic acid (β = -0.130, P = 0.016) and EPA (β = -0.131, P = 0.021) were negatively associated with clot lysis time in male subjects. Oleic acid (C18:1n9) (β = -0.411, P = 0.001) and osbond acid (C22:5n6) (β = -0.285, P = 0.001) was negatively associated, while alpha-linolenic acid (C18:3n3) were positively associated (β = 0.237, P = 0.001) with clot lysis time in females.

Discussion

The novelty of this study lies in using the global fibrinolytic assay to investigate the associations between plasma phospholipid fatty acid status, dietary fatty acids and fibrinolysis. The literature is inconclusive regarding the effect of omega-3 fatty acids on PAI-1. In the present study, DHA is significantly and positively associated with PAI-1act in females. Two studies have observed an increase in PAI-1 with the intake of n-3 polyunsaturated fatty acids [9], whereas other studies indicated no significant association [21, 22] in PAI-1act. In a randomised controlled trial in South Africa conducted by Scholtz and colleagues (2004) [23], red palm olein, palm olein (consisting mainly of palmitic and oleic acid), and sunflower oil (consisting mainly of linoleic acid) had no effect on PAI-1 levels, which is consistent with our findings. Oleic acid was positively associated with PAI-1act in female participants in the present study, but not independently of the effect of

triglycerides. In male participants there was a significant positive association between DPA (C22:5n6) and PAI-1act in our study. This is in contrast with a cross-sectional study of 915 men (aged 40 – 49 years), in whom serum omega-6 fatty acids were significantly and inversely associated with PAI-1 [6].

It was interesting to find that more individual plasma phospholipid fatty acids were associated with clot lysis time than with PAI-1act, considering that PAI-1 is regarded as the main contributing factor to clot lysis time [5]. Also, different phospholipid fatty acids were associated with clot lysis time than with PAI-1act. We therefore controlled for the associations between fatty acids with PAI-1act and with triglycerides in the fully adjusted model investigating associations between plasma phospholipid fatty acids and clot lysis time. We found that myristic acid and EPA remained negatively associated and mead acid, ARA and DPA remained positively associated with clot lysis time, independently of their associations with PAI-1act and triglycerides in males. It was also interesting that, even though PAI-1act was positively associated with clot lysis time, and myristic acid and DHA was positively associated with PAI-1act, these fatty acids were not associated with clot lysis time in either the model adjusting for PAI-1act or the one that does not adjust for PAI-1act. Additionally, the fully adjusted model determining associations between PAI-1act and plasma phospholipid fatty acids only explained 29% of the variance in male participants and 22% of the variance in female participants, while the fully adjusted model investigating associations between clot lysis time and plasma phospholipid fatty acids explained 59% of the variance in males and 44% of the variance in females. These results indicate that the associations between fatty acids and clot lysis time might be via different mechanisms than PAI-1. A randomised controlled, double-blind, crossover study of 20 men with coronary heart disease concluded that omega-3 fatty acids (EPA and DHA) did not alter endothelial vasomotor function, endothelial t-PA release, or platelet and monocyte activation [24] and are therefore unlikely to be the reasons for the associations we found between C22:5n3 (DPA) (which is believed to be primarily obtained from endogenous elongation of EPA) and clot lysis time[25, 26].

The limitations of our study include the fact that additional factors such as genetic variation and the hormonal balance of the individuals, which can influence fatty acid status and conversion [28, 29] were not determined. These factors should therefore be investigated in future in this population. One of the strengths of this study is that confounders such as sex, age and body mass index (BMI) were controlled for.

In conclusion, even though PAI-1act was positively associated with clot lysis time, and myristic acid and DHA was positively associated with PAI-1act, these fatty acids were not associated with clot lysis time in both the model adjusting for PAI-1act and the one that does not adjust for PAI-1act. Additionally the different types of fatty acids that were associated with PAI-1act and clot lysis time, as well as the degree of variance between the results, indicates that the associations between fatty acids and clot lysis time might be independent of PAI-1. The association between mead acid and clot lysis time indicates that fibrinolysis might be reduced with an essential fatty acid deficiency. This may be of particular concern in this population with documented lower fat intake.

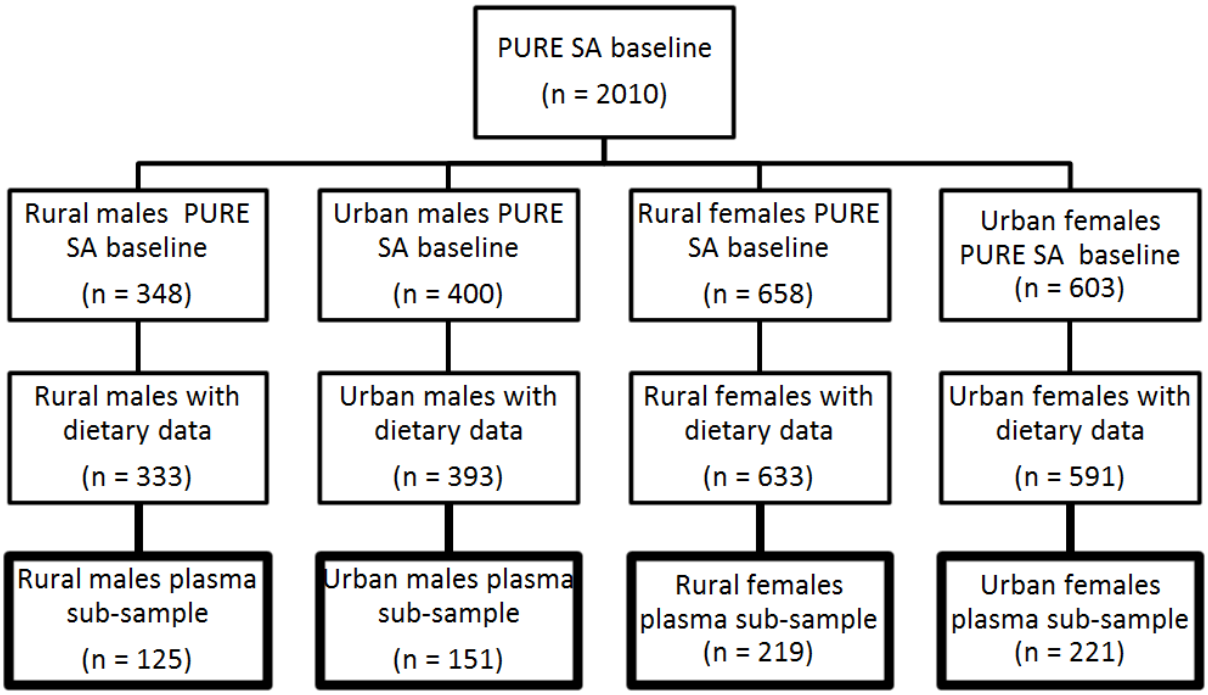

Figure 1. Outline of study population

Table 1. Characteristics of the population by gender and urbanization

Variable	Male			Female		
	Rural (n=125)	Urban (n=147)	Total	Rural (n=218)	Urban(n=218)	Total
Age (years)	53 (51, 55)	52 (51, 54)	52 (51, 54)	51 (50, 52)	54 (53, 56)	53 (52, 54)
Physical activity index	2.96 (2.85, 3.06)	2.94 (2.71, 3.17)	2.95 (2.82, 3.08)	3.08 (3.00, 3.15)	2.71 (2.61, 2.80)	2.89 (2.83 – 2.95)
BMI (kg/m^2)	21.16 (20.32, 22.01)	21.03 (20.36, 21.69)	21.09 (20.56, 21.62)	26.16 (25.22, 27.11)	28.16 (27.09, 29.23)	27.11 (26.40 – 27.83)
Waist Circumference (cm)	77.68 (75.58, 79.78)	76.69 (75.07, 78.32)	77.14 (75.85, 78.44)	80.30 (78.46, 82.14)	84.30 (82.53, 86.08)	82.31 (81.02 – 83.60)
Alcohol (g)	20.47 (14.46, 26.49)	15.06 (12.36, 17.77)	17.71 (14.59, 20.83)	6.89 (3.86, 9.92)	7.66 (5.68, 9.65)	7.29 (5.49 – 9.10)
PAI-1act (IU/ml)	3.48(2.65 – 4.31)	5.16 (3.79 – 6.54)	4.39 (3.55, 5.23)	5.71(4.96 – 6.47)	7.72 (6.76 – 8.68)	6.72 (6.10 – 7.33)
Clot lysis time (min)	53.18(51.28-55.08)	51.62 (49.61 – 53.63)	52.37 (50.98 – 53.75)	59.66 (58.27-61.04)	59.85 (58.60 - 61.08)	59.75 (58.82 – 60.68)
Triglycerides (mmol/l)	1.21(1.11, 1.31)	1.27(1.11, 1.43)	1.24 (1.14, 1.34)	1.28(1.19, 1.37)	1.63(1.48, 1.77)	1.46 (1.37 – 1.55)

Plasminogen activator inhibitor 1 (PAI-1), body mass index (BMI), waist circumference (WC)

Values are reported as mean (95% CI).

Supplementary table 1: Dietary fatty acid intake and plasma phospholipid fatty acid status of subsample

	Male			Female		
Variable	Rural (n=125)	Urban (n=147)	Total	Rural (n=218)	Urban(n=218)	Total
Dietary data						
Energy (kJ)	7310 (6721 – 7900)	9521 (8914 – 10128)	8509 (8066 – 8952)	6093 (5760 – 6426)	8729 (8227 – 9231)	7420 (7095 – 7745)
Total fat (%E)	18.04 (16.83 – 19.25)	24.72 (23.73 – 25.72)	21.66 (20.90 – 22.53)	20.04 (19.10 – 20.98)	27.24 (26.31 – 28.16)	23.67 (22.93 – 24.40)
C14:0 (mg)	558 (460 – 655)	1036 (926 – 1146)	827 (776 – 877)	507 (444 – 569)	1154 (1054 – 1254)	817 (738 – 896)
C16:0 (mg)	3734 (3299 – 4170)	8328 (7669 – 8987)	6189 (5861 – 6518)	3429 (3140 – 3719)	8868 (8216 – 9519)	6225 (5735 – 6715)
C18:0 (mg)	2195 (1919 – 2471)	4613 (4230 – 4997)	3463 (3278 – 3648)	2037 (1852 – 2223)	4817 (4448 – 5187)	3506 (3225 – 3787)
C18:1 (mg)	7501 (6567 – 8435)	16251 (14821 – 17682)	12152 (11491 – 12813)	6971 (6386 – 7556)	17148 (15829 – 18467)	12245 (11222 – 13268)
C18:2 n-6(mg)	10167 (8935 – 11398)	16858 (15549 – 18167)	13854 (13185 – 14524)	10020 (9189 – 10851)	17710 (166286 -19135)	13794 (12809 – 14779)
C18:3 n-3(mg)	132 (117 – 147)	301 (280 – 323)	222 (221 – 234)	129 (119 – 138)	314 (292 – 335)	224 (207 – 240)
C20:4n-6 (mg)	39 (34 – 45)	110 (100 – 120)	74 (69 – 78)	40.56 (36 – 45)	102 (93 – 111)	78 (70 – 85)
C20:5n-3 (mg)	38 (32 – 43)	77 (67 – 87)	52 (49 – 56)	40 (35 – 45)	57 (49 – 63)	59 (53 – 65)
C22:6n-3 (mg)	60 (50 -70)	134 (117 – 152)	90 (84 – 97)	65 (57 -74)	102 (89 – 115)	100 (89 – 112)

Plasma phospholipid fatty acids (% of total fatty acids)						
C14:0 (%)	0.24 (0.22, 0.25)	0.26 (0.25, 0.28)	0.25 (0.24 – 0.26)	0.26 (0.25, 0.27)	0.28 (0.27, 0.29)	0.27 (0.26 – 0.28)
C16:0 (%)	27.19 (26.55, 27.83)	27.52 (27.04, 27.99)	27.49 (27.01 – 27.78)	26.40 (25.88, 26.92)	26.76 (26.33, 27.19)	26.54 (26.20 – 26.88)
C18:0 (%)	15.36 (14.90, 15.83)	13.49 (13.19, 13.80)	14.33 (14.04 – 14.62)	16.60 (16.21, 16.99)	14.70 (14.43, 14.97)	15.63 (15.38 – 15.88)
C18:1n9 (%)	9.42 (8.88, 9.97)	10.68 (10.08, 11.28)	10.14 (9.73 – 10.56)	7.72 (7.42, 8.03)	8.80 (8.41, 9.20)	8.28 (8.02 – 8.53)
C18:2n6 (%)	16.04 (15.41, 16.67)	16.18 (15.66, 16.71)	16.11 (15.71 – 16.51)	15.76 (15.25, 16.26)	15.97 (15.54, 16.39)	15.87 (15.55 – 16.20)
C18:3n6 (%)	0.12 (0.11, 0.13)	0.14 (13, 0.56)	0.13 (0.13 – 0.14)	0.10 (0.09, 0.11)	0.12 (0.11, 0.13)	0.12 (0.11 – 0.12)
C20:3n6 (%)	2.90 (2.77, 3.02)	2.86 (2.75, 2.96)	2.88 (2.80 – 2.96)	3.00 (2.89, 2.09)	3.05 (2.96, 3.15)	3.02 (2.95 – 3.09)
C18:3n3 (%)	0.10 (0.09, 0.12)	0.09 (0.09, 0.10)	0.10 (0.9 – 0.10)	0.08 (0.08, 0.09)	0.09 (0.08, 0.09)	0.09 (0.08 – 0.09)
C20:3n9 (%)	0.28 (0.26, 0.32)	0.37 (0.32, 0.42)	0.33 (0.30 – 0.36)	0.22 (0.20, 0.24)	0.24 (0.22 0.26)	0.23 (0.22 – 0.24)
C20:4n6 (%)	13.39 (12.92, 13.87)	13.04 (12.67, 13.42)	13.15 (12.86 – 13.45)	14.01 (13.63, 14.39)	13.68 (13.39, 13.97)	13.86 (13.63 -14.10)
C20:5n3 (%)	0.61 (0.55, 0.66)	0.86 (0.77, 0.95)	0.75 (0.69 – 0.80)	0.51 (0.47, 0.55)	0.81 (0.76, 0.86)	0.66 (0.63 – 0.70)
C44:4n6 (%)	0.68 (0.65, 0.71)	0.65 (0.63, 0.68)	0.61 (0.59 – 0.63)	0.56 (0.54, 0.58)	0.54 (0.52, 0.56)	0.60 (0.58 – 0.61)
C22:5n6 (%)	0.62 (0.57, 0.67)	0.71 (0.67, 0.74)	0.59 (0.56 – 0.62)	0.56 (0.52, 0.60)	0.58 (0.55, 0.62)	0.65 (0.62 – 0.67)
C22:5n3 (%)	1.45 (1.38, 0.53)	1.33 (1.28, 1.38)	1.45 (1.40 – 1.49)	1.44(1.39, 1.49)	1.44 (1.39, 1.49)	1.37 (1.34 – 1.41)

C22:6n3 (%)	3.45 (3.24, 3.66	4.50 (4.29, 4.71)	4.02 (3.86 – 4.18)	4.08 (3.92, 4.24)	5.42 (5.26, 5.58)	4.76 (4.63 – 4.89)

Myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3n3), mead acid (C20:3n9), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, C20:5,n3), adrenic acid (C22:4n6), osbond acid (C22:5n6), docosapentaenoic omega 3 (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6n3).

Values are reported as mean (95% CI).

Table 2. Associations between dietary fatty acids and PAI-1_{act} using multiple linear regression models

Model		Male (n = 272)		Female (n = 436)	
		β	P	β	P
1	Energy (kJ)	0.101	0.336	0.180	0.054
	C14:0 (mg) *	0.013	0.924	-0.033	0.763
	C16:0 (mg) *	0.590	0.368	0.668	0.112
	C18:0 (mg) *	-0.349	0.181	0.135	0.531
	C18:1 (mg) *	-0.284	0.464	-0.555	0.084
	C18:2 n-6(mg) *	0.056	0.690	0.005	0.969
	C18:3 n-3(mg) *	-0.038	0.833	-0.248	0.072
	C20:4 n-6 (mg) *	0.050	0.691	0.031	0.719
	C20:5 n-3 (mg) *	-0.468	0.124	-0.078	0.641
	C22:6 n-3 (mg) *	0.401	0.193	0.011	0.950
	Age	-0.140	0.028	-0.020	0.698
	Alcohol (g) *	-0.005	0.949	0.039	0.523
	BMI	0.333	<0.001	0.196	<0.001
	Physical activity	-0.042	0.518	-0.064	0.204
	Waist circumference (cm)†	0.149	0.017	0.229	<0.001
	R2	0.19		0.14	

2	Energy (kJ)	0.141	0.173	0.192	0.037
	C14:0 (mg) *	-0.029	0.832	-0.017	0.878
	C16:0 (mg) *	0.446	0.488	0.636	0.126
	C18:0 (mg) *	-0.188	0.468	0.095	0.655
	C18:1 (mg) *	-0.222	0.558	-0.505	0.112
	C18:2 n-6 (mg) *	0.024	0.861	-0.008	0.947
	C18:3 n-3 (mg) *	-0.060	0.733	-0.268	0.049
	C20:4 n-6 (mg) *	0.011	0.931	0.025	0.769
	C20:5 n-3 (mg) *	-0.328	0.274	-0.078	0.639
	C22:6 n-3 (mg) *	0.265	0.384	0.018	0.917
	Age	-0.138	0.028	-0.043	0.406
	Alcohol (g) *	-0.005	0.942	0.016	0.790
	BMI	0.260	<0.001	0.181	<0.001
	Physical activity	-0.045	0.474	-0.065	0.190
	Waist circumference (cm)†	0.111	0.074	0.207	<0.001
	Triglycerides	0.225	0.001	0.164	0.001
	R2	0.23		0.17	

Kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3n3), mead acid (C20:3n9), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, C20:5,n3), adrenic acid (C22:4n6), osbond acid (C22:5n6), docosapentaenoic omega 3 (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6n3).
body mass index (BMI), waist circumference (WC).
*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

†A Residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Table 3. Associations between dietary fatty acids and clot lysis time using multiple linear regression models

Model		Male (n = 272)		Female (n = 436)	
		β	P	B	P
1	Energy intake	0.028	0.779	0.036	0.707
	C14:0	0.044	0.740	0.179	0.106
	C16:0	0.705	0.240	-0.252	0.581
	C18:0	-0.357	0.140	-0.188	0.399
	C18:1	-0.243	0.496	0.235	0.466
	C18:2 n-6	-0.012	0.925	-0.094	0.432
	C18:3 n-3	-0.290	0.092	0.060	0.689
	C20:4 n-6	0.119	0.306	0.093	0.279
	C20:5 n-3	-0.007	0.981	0.273	0.188
	C22:6 n-3	0.046	0.882	-0.321	0.133
	Age	-0.180	0.002	0.162	0.002
	Alcohol *	-0.213	0.004	-0.099	0.110
	BMI	0.500	<0.001	0.324	<0.001
	Physical activity	0.003	0.966	0.005	0.923

	Waist circumference†	0.091	0.112	0.147	0.003
	R2	0.36		0.20	
2	Energy intake	0.034	0.729	0.042	0.660
	C14:0	0.003	0.985	0.193	0.079
	C16:0	0.545	0.356	-0.231	0.611
	C18:0	-0.177	0.467	-0.228	0.304
	C18:1	-0.203	0.562	0.249	0.438
	C18:2 n-6	-0.004	0.978	-0.098	0.410
	C18:3 n-3	-0.296	0.079	0.032	0.831
	C20:4 n-6	0.071	0.535	0.087	0.308
	C20:5 n-3	0.167	0.580	0.286	0.165
	C22:6 n-3	-0.132	0.666	-0.326	0.125
	Age	-0.192	0.001	0.144	0.005
	Alcohol*	-0.204	0.005	-0.114	0.065
	BMI	0.412	<0.001	0.312	<0.001
	Physical activity	-0.004	0.942	0.002	0.965
	Waist circumference†	0.065	0.253	0.131	0.009
	Triglycerides	0.210	0.003	0.124	0.015
	R2	0.39		0.21	
3	Energy intake	0.024	0.791	0-.028	0.752

C14:0	-0.017	0.889	0.208	0.041
C16:0	0.726	0.188	-0.488	0.246
C18:0	-0.236	0.301	-0.288	0.159
C18:1	-0.266	0.415	0.461	0.120
C18:2 n-6	-0.016	0.894	-0.102	0.349
C18:3 n-3	-0.356	0.024	0.137	0.324
C20:4 n-6	0.042	0.698	0.082	0.297
C20:5 n-3	0.279	0.322	0.323	0.089
C22:6 n-3	-0.222	0.436	-0.339	0.083
Age	-0.146	0.008	0.158	0.001
Alcohol *	-0.218	0.001	-0.128	0.024
BMI	0.303	<0.001	0.248	<0.001
Physical activity	0.019	0.731	0.028	0.543
Waist circumference†	0.029	0.590	0.055	0.243
Triglycerides	0.161	0.014	0.066	0.167
PAI-1	0.327	<0.001	0.380	<0.001
R2	0.47		0.33	

Kilojoules (kJ), Myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), Kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3n3), mead acid (C20:3n9), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, C20:5,n3), adrenic acid (C22:4n6), osbond acid (C22:5n6), docosapentaenoic omega 3 (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6n3).
body mass index (BMI), waist circumference (WC).

*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.
†A Residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Table 4: Associations between plasma phospholipid fatty acids and PAIact using multiple linear regression models

Model		Male (n = 272)		Female (n = 436)	
		β	P	B	P
1	Energy intake	0.046	0.440	0.135	0.007
	C14:0	0.148	0.032	0.185	0.003
	C16:0	0.207	0.308	0.224	0.151
	C18:0	0.209	0.227	0.306	0.020
	C18:1n9	0.237	0.305	0.290	0.038
	C18:2n6	0.125	0.488	0.046	0.729
	C18:3n6	0.049	0.517	0.035	0.526
	C20:3n6	-0.029	0.736	-0.026	0.691
	C18:3n3	0.099	0.225	0.104	0.096
	C20:3n9	-0.013	0.889	-0.101	0.200
	C20:4n6	0.231	0.109	0.116	0.290
	C20:5n3	-0.014	0.850	0.052	0.465
	C22:4n6	-0.237	0.036	0.063	0.545
	C22:5n6	0.400	<0.001	0.027	0.767
	C22:5n3	-0.001	0.991	0.087	0.235
	C22:6n3	0.127	0.266	0.258	0.003

	Age	-0.163	0.008	-0.068	0.167
	Alcohol*	-0.018	0.783	0.035	0.481
	Physical activity	-0.041	0.496	-0.052	0.302
	Waist circumference†	0.119	0.048	0.184	<0.001
	BMI	0.245	<0.001	0.183	0.001
	R2	0.27		0.22	
2	Energy intake	0.057	0.345	0.130	0.011
	C14:0	0.145	0.034	0.183	0.003
	C16:0	0.122	0.553	0.193	0.223
	C18:0	0.125	0.481	0.265	0.054
	C18:1n9	0.138	0.558	0.259	0.070
	C18:2n6	0.063	0.728	0.019	0.890
	C18:3n6	0.033	0.662	0.038	0.483
	C20:3n6	-0.027	0.748	-0.029	0.653
	C18:3n3	0.068	0.411	0.096	0.131
	C20:3n9	-0.021	0.826	-0.108	0.169
	C20:4n6	0.187	0.194	0.101	0.362
	C20:5n3	-0.012	0.873	0.050	0.483
	C22:4n6	-0.222	0.048	0.072	0.489
	C22:5n6	0.351	0.001	-0.002	0.982

C22:5n3	-0.015	0.869	0.081	0.269
C22:6n3	0.071	0.543	0.236	0.008
Age	-0.158	0.010	-0.074	0.133
Alcohol *	-0.005	0.940	0.035	0.484
Physical activity	-0.040	0.506	-0.050	0.317
Waist circumference†	0.100	0.099	0.179	<0.001
BMI	0.210	0.003	0.175	0.002
Triglycerides	0.136	0.044	0.057	0.324
R2	0.29		0.22	

Kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3n3), mead acid (C20:3n9), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, C20:5,n3), adrenic acid (C22:4n6), osbond acid (C22:5n6), docosapentaenoic omega 3 (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6n3), body mass index (BMI), waist circumference (WC).
*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

†A Residual of BMI was calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

Table 5: Associations between plasma phospholipid fatty acids and clot lysis time using multiple linear regression models

		Male (n = 272)		Female (n =436)	
		β	P	B	P
1	Energy intake	0.000	0.996	0.048	0.321
	C14:0	-0.070	0.247	-0.018	0.765
	C16:0	0.474	0.009	0.265	0.075
	C18:0	0.379	0.014	0.078	0.532
	C18:1n9	0.137	0.504	-0.231	0.083
	C18:2n6	0.281	0.079	0.001	0.997
	C18:3n6	0.162	0.017	0.024	0.639
	C20:3n6	-0.034	0.654	-0.009	0.890
	C18:3n3	0.188	0.010	0.237	<0.001
	C20:3n9	0.181	0.036	-0.018	0.811
	C20:4n6	0.394	0.002	0.207	0.048
	C20:5n3	-0.139	0.033	-0.009	0.896
	C22:4n6	-0.191	0.056	0.141	0.153
	C22:5n6	0.086	0.364	-0.210	0.016
	C22:5n3	0.241	0.003	0.051	0.466
	C22:6n3	0.185	0.067	0.117	0.154

	Age	-0.177	0.001	0.082	0.078
	Alcohol *	-0.119	0.040	0.003	0.951
	Physical activity	-0.009	0.860	-0.045	0.349
	Waist circumference†	0.093	0.083	0.146	0.002
	BMI	0.324	<0.001	0.203	<0.001
	R2	0.45		0.31	
2	Energy intake	0.017	0.746	0.032	0.497
	C14:0	-0.075	0.205	-0.025	0.670
	C16:0	0.336	0.061	0.187	0.214
	C18:0	0.242	0.116	-0.027	0.835
	C18:1n9	-0.025	0.901	-0.311	0.022
	C18:2n6	0.181	0.249	-0.069	0.588
	C18:3n6	0.136	0.040	0.034	0.510
	C20:3n6	-0.031	0.669	-0.017	0.779
	C18:3n3	0.138	0.055	0.215	<0.001
	C20:3n9	0.168	0.044	-0.038	0.610
	C20:4n6	0.323	0.010	0.168	0.109
	C20:5n3	-0.136	0.032	-0.014	0.836
	C22:4n6	-0.167	0.086	0.165	0.094
	C22:5n6	0.006	0.945	-0.286	0.002

	C22:5n3	0.219	0.006	0.036	0.604
	C22:6n3	0.094	0.350	0.061	0.463
	Age	-0.169	0.002	0.065	0.162
	Alcohol*	-0.098	0.083	0.002	0.960
	Physical activity	-0.007	0.886	-0.041	0.393
	Waist circumference†	0.061	0.242	0.133	0.004
	BMI	0.267	<0.001	0.184	0.001
	Triglycerides	0.222	<0.001	0.146	0.008
	R2	0.49		0.32	
3	Energy intake	-0.005	0.921	-0.018	0.690
	C14:0	-0.130	0.016	-0.095	0.079
	C16:0	0.289	0.072	0.112	0.414
	C18:0	0.194	0.159	-0.129	0.279
	C18:1n9	-0.077	0.672	-0.411	0.001
	C18:2n6	0.157	0.264	-0.077	0.512
	C18:3n6	0.123	0.037	0.019	0.682
	C20:3n6	-0.021	0.749	-0.006	0.915
	C18:3n3	0.112	0.082	0.178	0.001
	C20:3n9	0.176	0.019	0.004	0.956
	C20:4n6	0.253	0.025	0.129	0.177

C20:5n3	-0.131	0.021	-0.033	0.589
C22:4n6	-0.083	0.345	0.137	0.127
C22:5n6	-0.127	0.141	-0.285	0.001
C22:5n3	0.224	0.002	0.005	0.940
C22:6n3	0.068	0.455	-0.030	0.701
Age	-0.109	0.024	0.094	0.028
Alcohol*	-0.096	0.058	-0.011	0.797
Physical activity	0.008	0.869	-0.021	0.625
Waist circumference†	0.023	0.620	0.064	0.133
BMI	0.188	0.001	0.116	0.017
Triglycerides	0.170	0.001	0.124	0.013
PAI-1	0.379	<0.001	0.386	<0.001
R2	0.59		0.44	

Kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0stearic acid (C18:0), Oleic acid (C18:1n9), linoleic acid (C18:2n6), gamma linolenic acid (GLA, C18:3n6), dihomo-gamma-linolenic acid (DGLA C20:3n6), alpha linolenic acid (C18:3n3), mead acid (C20:3n9), arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA, C20:5,n3), adrenic acid (C22:4n6), osbond acid (C22:5n6), docosapentaenoic omega 3 (DPA, C22:5n3) docosahexaenoic acid (DHA, C22:6n3), body mass index (BMI), waist circumference (WC).
*Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

†A Residual of BMI was calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

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Chapter 6: Conclusions and recommendations

This study set out to investigate the associations between dietary fatty aicd intake, plasma fatty acid composition and specific cardiovascular disease risk factors in subjects in the North West Province of South Africa by means of a cross-sectional data analysis of the Prospective Urban and Rural Epidemiology (PURE) study. This chapter will summarise the main findings and conclusions drawn from these findings.

In the first article, we reported results from the aim of **investigating the associations between dietary fatty acids and blood lipids in relation to gender and urbanisation** (Richter *et al.*, 2014). A possible reason why blood lipid levels did not differ between rural and urban dwellers is that median fat and fatty acid intake was generally within recommendations in both groups, except for the intake of specific omega-3 polyunsaturated fatty acids, which was below recommended levels in both groups. We found different associations between dietary fatty acids and blood lipids in males to those in females. Irrespective of the low intake, docosahexaenoic acid (DHA) was inversely associated with total cholesterol. Interestingly, DHA tended to be positively associated with low density lipoprotein (LDL) in females, while alpha-linolenic acid was positively associated with total cholesterol and triglycerides in males. Furthermore, alpha-linoleic acid tended to be positively associated with LDL and significantly increased the risk of having elevated LDL. Even though the odds ratio increased the risk with less than two fold, a lifetime exposure of many people in the community could contribute to a public health problem. Eicosapentaenoic acid (EPA) was positively associated with total cholesterol and LDL in females. This raised the question about the possible detrimental effects of alpha-linolenic acid and EPA intake on blood lipid levels. From the results it seemed possible that in this black African population dietary alpha-linolenic acid might exert different effects on blood lipids in males than on other populations with a higher intake. Because of the cross-sectional nature of the study however, it could also be possible that other factors such as hormones or metabolic syndrome, which might not have been considered in this study could have confounded the results. Additionally dietary intake data is notorious for the limitations surrounding data collection and processing. The quantified food frequency questionnaire (QFFQ) might underestimate intake, however, it might be less likely to have had a big effect in this population, since MacIntyre and colleagues found better agreement with fat intake at lower intake levels (which was the case with our study) when testing the validity and reproducibility of the same questionnaire used in this study in the same population group within the Transition and Health during Urbanisation of South Africa (THUSA) study (MacIntyre *et al.*, 2001b).

In light of these surprising and interesting findings, the lack of fatty acid status data in South Africa, as well as the possible limitations of dietary intake data, in the second article we therefore set out to **describe the plasma phospholipid fatty acid status of this population**. Additionally, we **investigated the associations between dietary fatty acid intake and plasma phospholipid fatty acid composition**, as well as **associations between plasma fatty acids and blood lipids**, in order to determine if the previous results could be explained further by biomarker analyses. Even though the primary aim in this article was not to report gender and urbanisation differences, the models were kept consistent with the first article in order to draw better conclusions from it.

After **describing the plasma phospholipid fatty acid composition of this population**, we found that despite the low intake of EPA and DHA reported in the first article (Richter *et al.*, 2014), mean plasma phospholipid DHA (3.45 – 5.43% of total plasma phospholipids) was relatively high compared to other studies in westernised countries, and compared well to the status of preschool children from a study done in South Africa (Tichelaar *et al.*, 1999). Mean plasma phospholipid arachidonic acid and omega-3 docosapentaenoic acid (DPA) were also higher than in other studies done on western populations. These results are in support of studies that found Americans of African descent to be better converters of alpha-linolenic acid to DHA by means of Fatty acid desaturase gene clusters (FADS) polymorphisms and that more African Americans as opposed to European Americans had the homozygous GG allele associated with high arachidonic acid levels (Sergeant *et al.*, 2012). Mean plasma phospholipid palmitic acid and Dihomo-gamma-linolenic acid (DGLA) compared well to other studies (Conquer *et al.*, 1999; Laidlaw and Holub, 2003). High intake of preformed EPA and DHA has previously been shown to decrease the conversion of alpha-linolenic acid to EPA and DHA (Arterburn *et al.*, 2006). Therefore, conversion of alpha-linolenic acid to EPA and DHA might be even more efficient when preformed EPA and DHA intake is low, as it was in our study population.

When the objective to **investigate the associations between dietary fatty acid intake and plasma phospholipid fatty acid composition** was explored, monounsaturated fatty acid and polyunsaturated fatty acid status in this population did not appear to be good indicators of dietary fatty acids, due to lack of associations between dietary intake and fatty acid status. Conversion of oleic acid to mead acid might be one of the reasons why plasma phospholipid status of monounsaturated fatty acid was not associated with dietary intake. Other reasons might include that stearic acid is converted to oleic acid, while linoleic acid is converted to gamma-linolenic acid (GLA), DGLA and arachidonic acid. Alpha-linolenic acid and EPA are converted to DHA, which might be even more efficient when preformed EPA and DHA intake is low. Conversion also happens during de novo synthesis of saturated fatty acids from acetyl CoA from carbohydrate feeding when fat intake is low (notably below 25% of energy) and

carbohydrate intake is high, which is likely the case in this population when considering its fat intake. The major finding resulting from this objective was that alpha-linolenic acid was better correlated with plasma phospholipid DHA than dietary DHA correlated with plasma phospholipid DHA.

The **main finding from the aim to investigate associations between plasma phospholipid fatty acid status and blood lipids was** the surprising association between plasma DHA and triglycerides, because because dietary DHA is well known for its triglyceride-lowering properties (Bronsgeest-Schoute *et al.*, 1981; Davidson, 2006). This finding was however in line with the finding from our first article (which found a positive association between dietary alpha-linolenic acid and triglycerides in men), considering that our results suggest an efficient conversion of alpha-linolenic acid to DHA in this population. Possible reasons for the association between plasma DHA and triglycerides (besides the cross-sectional design that might allow for confounding factors not tested for), might include an error in the mechanism of hepatic inhibition of triglyceride synthesis in this population or an inverted U-shaped curve in the association between plasma DHA and triglycerides, as well as between dietary alpha-linolenic acid and triglycerides. Unfortunately our study population did not have participants with high intakes of alpha-linolenic acid, so this could not be investigated. Future clinical trials should explore associations between dietary alpha-linolenic acid and triglycerides at different levels of intake.

Even though elevated triglycerides are considered one of the risk factors for CVD, it has been shown recently in other trials that this effect is not independent of HDL, and that LDL has a bigger impact on CVD risk. If this is put into context, the positive association between triglycerides and plasma phospholipid DHA might be of slightly less relevance. However, what remains interesting and relevant is that these results support the theory that dietary alpha-linolenic acid might be an important contributor to plasma EPA and DHA in this population. Not only should the effective conversion of alpha-linolenic acid to DHA be confirmed with randomised controlled clinical feeding trials in this population, but it should also be investigated whether it might be due to genetic reasons or due to the low intake of preformed EPA and DHA in this population or possibly a combination. Additionally the randomised controlled trials should investigate further if alpha-linolenic acid might have possible negative effects on this population, because it is not known however, if associations would still be present under conditions of greater intake of alpha-linolenic acid.

This study suggests that it is not advisable in epidemiology to use plasma phospholipid fatty acids as biomarkers for intake in isolation, without considering the dietary intake data of a population in combination.

In the third article we **investigated the associations between dietary and plasma phospholipid fatty acids and more CVD risk factors, namely plasminogen activator inhibitor-1 (PAI-1_{act}) and clot lysis time, as markers for fibrinolytic potential**. Again we found different associations when looking at the associations of the dietary fatty acids with PAI-1 and clot lysis time than when looking at associations between plasma phospholipid fatty acids and PAI-1 and clot lysis time. Fewer fatty acids were associated with PAI-1 and clot lysis time in the dietary models than in the plasma models. These differences could again be due to an altered plasma phospholipid composition as a result of the low fat intake in this population. The most interesting finding from this article was that even though PAI-1_{act} was positively associated with clot lysis time, and phospholipid myristic acid and DHA were positively associated with PAI-1_{act}, these fatty acids were not associated with clot lysis time in both the model adjusting for PAI-1_{act} and the one that did not adjust for PAI-1_{act}. Together, the different types of fatty acids that were associated with PAI-1_{act} and clot lysis time, as well as the degree of variance between results, gave an indication that the associations between fatty acids and clot lysis time might be independent of PAI-1_{act}. The positive association between mead acid (which is an indicator of essential fatty acid deficiency) and clot lysis time suggests that fibrinolysis might be impaired when an essential fatty acid deficiency exists. This might be of particular concern in this population, considering its documented lower fat intake.

The limitations of the study include the fact that factors such as genetic variation, disease status, lifestyle differences, micronutrient status, apolipoprotein levels and the hormonal balance of the individuals, which could influence fatty acid metabolism and associations between fatty acids and cardiovascular disease (CVD) risk factors (Arab, 2003; Hodge *et al.*, 2007), were not explored and attention should be given to these factors in future studies, such as PURE follow ups. Another limitation of our study is the cross-sectional design, from which causality cannot be assumed. The study sample was, however, very large and therefore provided useful information about the dietary fatty acid intake and fatty acid status of this population. The associations found are very interesting and give an indication of more effective conversion in Africans, giving direction to future research which should be directed in this area.

Although our study did control for gender, age, body mass index (BMI), alcohol intake and added sugar intake, a prospective longitudinal design for the future, making use of the follow-up data from PURE participants, might bring more clarity regarding how fatty acid intake and metabolism could influence associations with plasma phospholipid fatty acid status and predict risk for CVD over time in this population. The interesting associations that suggest better conversion of alpha-linolenic acid to DHA and possibly linoleic acid to arachidonic acid in this population should also be explored further. The study design should make use of a randomised

controlled trial, in which different doses of dietary linoleic acid, alpha-linolenic acid, EPA and DHA are tested against a control diet.

If a better fatty acid conversion in this population can indeed be confirmed, it would be extremely relevant to health in communities in South Africa, which are continuously faced with problems such as cost implications, sustainability and the environmental impact of advising intake of long chain omega-3 fatty acids from fish sources. This would enable directing advice to improve DHA status to intake of a lower cost plant omega-3 oil blend. Plant oils are more sustainable and would be far more feasible in the South African context, in which unemployment and under-nutrition and higher cost of fatty fish are particularly relevant problems. Additionally, it could have a positive effect on the environmental impact if less fatty fish is needed. The quality of dietary lipids has previously been speculated to cause negative effects due to the use of cast-iron pots and re-use of oils that may cause peroxidation. These practices might result in modified proteins and nucleotides which may modify the epithelium to initiate vascular disease, due to aldehyde emanation from specifically polyunsaturated fatty acids. A study by Stonehouse et al. (2010), however, found that the use of omega-6 polyunsaturated fatty acid-rich vegetable oils and the way they were used did not cause safety concerns in a previous study on the PURE population.

In conclusion this large, cross-sectional study provide valuable information about the fatty acid status of this unique population in the process of nutrition transition with omega-3 fatty acid intake below recommended levels and gives direction for further research in this population for whom there is a shortage of information on the role of fat on CVD. Because the study design of this study is cross-sectional, it is not able to determine cause-and-effect, and results should therefore be verified with a randomised controlled trial. Our data supports the theory that dietary alpha-linolenic acid might be an important contributor to plasma EPA and DHA in populations with low preformed EPA and DHA intake (e.g. from fish), and highlights that plasma phospholipid fatty acids should not be used in isolation as biomarkers for intake, without looking at dietary intake data in combination.

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Addendum A: Instructions to authors for sumission to the Journal of Prostagladins, Leukotrienes & Essential Fatty Acids (PLEFA)

PROSTAGLANDINS, LEUKOTRIENES & ESSENTIAL FATTY ACIDS (PLEFA)

Official Journal of the International Society for the Study of Fatty Acids and Lipids

AUTHOR INFORMATION PACK

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DESCRIPTION

The role of **lipids**, including **essential fatty acids** and their **prostaglandin**, **leukotriene** and other derivatives, is now evident in almost all areas of biomedical science. **Cell membrane** behaviour and **cell signalling** in all tissues are highly dependent on the lipid constituents of cells. *Prostaglandins, Leukotrienes & Essential Fatty Acids* aims to cover all aspects of the roles of lipids in cellular, organ and whole organism function, and places a particular emphasis on human studies. Papers concerning all medical specialties are published. Much of the material is particularly relevant to the development of novel treatments for disease.

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Addendum B: Instructions to authors for the International Journal of Cardiology

INTERNATIONAL JOURNAL OF CARDIOLOGY

Affiliated with the International Society for Adult Congenital Heart Disease

AUTHOR INFORMATION PACK

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30 October 2014

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New Zealand

Re: Marilize Richter

To whom it may concern

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Yours sincerely,

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Different dietary fatty acids are associated with blood lipids in healthy South African men and women: The PURE study

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ABSTRACT

Background: Preliminary data from the baseline Prospective Urban Rural Epidemiology (PURE) study in South Africa indicated a higher prevalence of dyslipidemia than previous South African studies. The intake of specific individual dietary fatty acids may affect blood lipids differently than sub-groups of fat (i.e. polyunsaturated fatty acids). We investigated the dietary intake of different individual fatty acids and their associations with blood lipids, in relation to urbanization and gender.

Methods: Cross-sectional data analysis within the PURE baseline study of healthy subjects (n = 1950, 35–70 years) from rural and urban areas. Dietary data were collected and blood lipid analysis performed.

Results: Intake of individual fatty acids was significantly higher in urban than rural dwellers. However, the intake of n-3 PUFAs was below recommendations in all groups. Total cholesterol and LDL were higher in females than in males, with no rural urban differences. Intake of alpha-linolenic acid (ALA) was positively associated with total cholesterol ($\beta = 0.143$) and triglycerides ($\beta = 0.256$) in males. The risk for having elevated LDL also increased with increased intake of ALA (OR 1.49, 95% CI 1.04, 2.14) in males. In females, arachidonic acid and eicosapentaenoic acid (EPA) were positively associated with total cholesterol and arachidonic acid was also positively associated with LDL, whereas docosahexaenoic acid was negatively associated with total cholesterol and LDL.

Conclusions: These results suggest that specific individual dietary fatty acids may affect blood lipids in males differently than in females irrespective of rural or urban dwelling. The positive association between ALA and total cholesterol and triglycerides in males is a concern, because current advice aims to improve the dietary linoleic acid to ALA ratio by increasing ALA intake.

1. Introduction

The nutrition transition describes a range of socio-economic and demographic shifts caused by the migration from rural to urban environments, and is accompanied by rapid changes in diet, lifestyle and patterns of undernourishment and obesity [1]. In developing countries, improved socio-economic conditions and the availability of a variety of food associated with the nutrition transition, has resulted in increased incidence of obesity and non-communicable diseases of lifestyle, such as coronary heart disease [1]. South Africa is experiencing continuous urbanization of Africans [2]. In line with global predictions [3], the urban population in South Africa accounts for more than half of the population, the proportion of which is rising, from 55% (re-classified data to match demographic classification used in 2001 census) in 1996 to 58% in 2001 [2].

Scientific interest and public awareness of the role of fatty acids in human health has increased in the past years and dietary fatty acids have been shown to have an effect on coronary heart disease [4]. Total fat intake cannot be linked definitively to cardiovascular disease risk, in part due to opposing effects of specific types of fat [5]. The fatty acid metabolites of linoleic acid and alpha-linolenic acid (ALA), arachidonic acid and docosahexaenoic acid (DHA), are important constituents of all cell membranes, determining and influencing the behavior of membrane-bound enzymes and receptors [6]. Recently it has become clear that even within subtypes of fat such as omega-3 fatty acids, specific individual fatty acids can cause different effects with regard to blood lipids. Several studies have shown that these fatty acids can affect blood lipid profiles, but the directions of the effects were inconsistent [7–9]. Convincing evidence exists for a protective effect of the polyunsaturated fatty acids, linoleic acid, eicosapentaenoic acid (EPA), and DHA on cardiovascular disease [10,11]. Increased risk of cardiovascular disease is also associated with intake of the saturated fatty acids (SFAs), myristic and palmitic acid [10].

Surprisingly, until recently, the African population did not show hypercholesterolemia at a high level of risk [12,13]. This is in spite of urbanization, which is accompanied with higher fat intake [1], and the fact that African women have a very high prevalence of obesity [14],

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which is also associated with dyslipidemia [15,16]. However, results from the baseline Prospective Urban Rural Epidemiology (PURE) study in South Africa conducted in 2005 indicated high median total cholesterol levels [18] and preliminary data analysis indicated a high prevalence of dyslipidemia, in contrast with prior studies in the South African population that found lipid levels of South African populations to be within recommendations [12,17]. It is well known that total cholesterol and low-density lipoprotein (LDL) cholesterol predict coronary heart disease risk [18]. Knowledge of the intake of individual fatty acids (as opposed to subgroups of fat like polyunsaturated fatty acids) and their associations with blood lipids is lacking in this population undergoing the nutrition transition and other populations. Because higher fat intake is associated with the nutrition transition, the role that individual fatty acids play in rising levels of dyslipidemia in this population needs to be better understood. Thus, we investigated dietary intake of individual fatty acids and their associations with blood lipids in rural and urban subjects and found some unexpected associations, especially considering the generally lower fat intake of this population.

2. Methods

2.1. Study population

This cross-sectional study forms part of the international PURE study, which is a large-scale multinational cohort study that tracks societal influences, risk factors and chronic non-communicable diseases in urban and rural areas of 17 countries in transition [19]. The baseline data collection of the South African leg in 2005 included just over 2000 apparently healthy, migration stable subjects (aged >35 years) from a sample of 6000 randomly selected households in rural and urban areas in the North West Province of South Africa. The rural communities were still under tribal law. Exclusion criteria were the use of chronic medication for non-communicable diseases and/or any self-reported acute illness [20]. Trained field workers, with the same background as participants, conveyed all information about the objectives and procedures of the study, before recruitment in the participants' home language. All participants gave written informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Data were treated confidentially and analyses were performed with coded data. For the purpose of the analyses in this paper, subjects from whom no dietary data were available were excluded, leaving 333 male subjects from rural areas, 393 males from urban areas, 633 female subjects from rural areas and 591 females from urban areas (Fig. 1).

2.2. Data collection

Participants were interviewed in the language of their choice. Structured demographic, socio-economic and lifestyle questionnaires that were developed and standardized for the international PURE study were used [19]. Interviewer-based quantitative food frequency questionnaires (QFFQ) were used to assess dietary intake of volunteers, which were validated for the population in the Transition and Health during Urbanisation of South Africans (THUSA) study [21] and for which reproducibility was proven [22,23]. Dietary methodology of the PURE study was explained in detail previously [23]. In SA the physical activity index (PAI) questionnaire as developed and tested in the THUSA-study was used for the PURE study, as described previously by Kruger and colleagues [24].

Height, weight and waist circumference were measured using standardized methods, with instruments calibrated by the International Society for the Advancement of Kinanthropometry accredited anthropometrists (Precision Health Scale, A& D Company, Japan; Leicester Height Measure, Seca, Birmingham UK).

Fasting blood samples, with minimal stasis were collected by a registered nurse obtained with a sterile winged infusion set from the antecubital vein and stored at −80 °C. In the rural areas, samples were rapidly frozen and stored at −18 °C (no longer than 5 days) until they could be transported to the laboratory facility and were then stored at −80 °C [20].

Serum lipids were measured by using Sequential Multiple Analyzer Computer (SMAC), using the KonelabTM auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland) [25]. LDL was calculated using the Friedewald–Levy–Fredrickson formula [26]. Reference values used as indicators for elevated blood lipid values were total cholesterol < 5 mmol/l, LDL < 3 mmol/l, HDL ≥ 1 mmol/l for males and ≥ 1.2mmol/l for females, triglycerides > 1.7 mmol/l [27].

2.3. Statistical analyses

Statistical analysis was done by using IBM SPSS Statistics Version 21 (IBM Company, Armonk, NY, USA). Not normally distributed data were log transformed for data analysis. Differences in serum lipids, dietary fat and fatty acid intake were stratified by urbanization (rural vs. urban) and gender (male vs. female), and their interactions were explored by using 2-way ANCOVA, adjusting for age. Differences between groups (rural male, rural female, urban male, urban female) were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Three separate hierarchical multiple linear regression models were used for both males and females to determine associations between specific dietary fatty acids and blood lipid concentrations, adjusting for energy intake, BMI, physical activity, waist circumference, age and urbanization. The first hierarchical multiple linear regression model was a simple model only including the individual fatty acids and energy intake was controlled for. The second model additionally controlled for alcohol intake, soluble fiber intake, BMI, physical activity, waist circumference and age, while the third fully adjusted model also controlled for potential differences between rural and urban areas. A residual of energy intake was calculated for each of the fatty acids and alcohol to account for differences in energy intake. A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference. Logistic regression analysis, using the fully adjusted model, was used to determine whether individual dietary fatty acids increased or decreased the odds ratios (ORs) for having unfavorable blood lipid profiles. A P-value < 0.05 was considered significant.

3. Results

Dietary intake data were obtained from 1950 participants. Characteristics and differences in energy, fat and fatty acid intake of male and female subjects living in rural and urban areas are indicated in Table 1.

For all dietary variables, intake was significantly higher in urban than in rural areas. Absolute intake of total fat, SFA, MUFA, PUFA, linoleic acid and ALA did not differ significantly between male and female subjects. However, when calculated as percentage of energy (%E) intake, additional to the significant urbanization effects, females had significantly higher intakes than male subjects in rural and urban areas, with the exception of %E from MUFA, which was only significantly higher in females than males within urban areas.

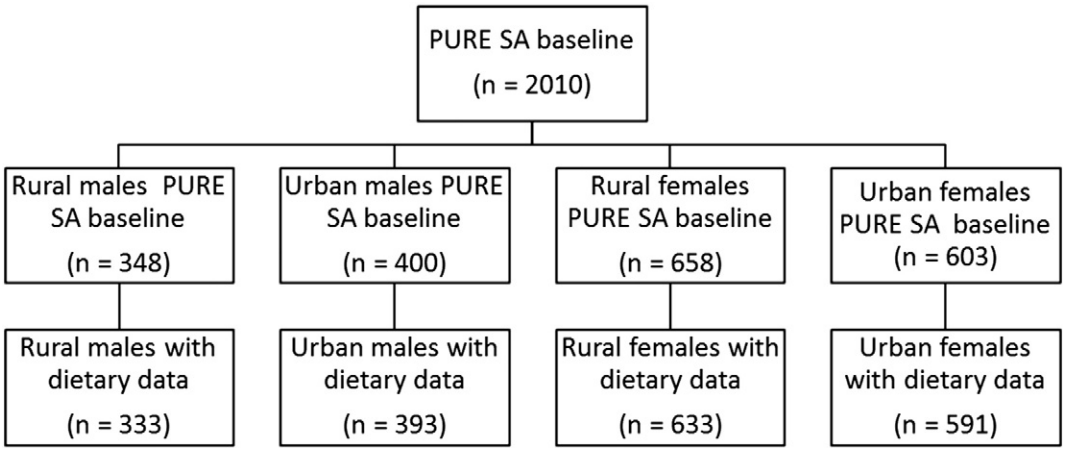

Fig. 1. Outline of study population.

Table 1
Characteristics and differences in energy, fat and fatty acid intake by gender and urbanization*.

Variable	Male		Female		Significance§		
	Rural (n = 333)	Urban (n = 393)	Rural (n = 633)	Urban(n = 591)	Gender	Urbanization	Gender x Urbanization
Age (years)†	49[48–51]^{ab}	50[49–51]^{a}	48[47–49]^{b}	50[49–51]^{a}	0.220	0.002	0.081
Physical activity index‡	3.00(2.26–3.41)^{ab}	2.66(2.39–3.00)^{b}	3.08(2.70–3.41)^{a}	2.71(2.48–2.95)^{c}	0.065	<0.001	0.005
BM‡,I	19.71(17.99–2.25)^{c}	19.96(18.26–22.78)^{c}	24.89(20.77–30.70)^{b}	27.21(22.30–32.59)^{a}	<0.001	0.001	0.004
WC (cm)‡	74.50(70.15–80.50)^{c}	74.30(69.65–81.75)^{c}	78.48(69.30–89.25)^{b}	82.80(73.20–92.70)^{a}	<0.001	0.020	0.001
Energy (kJ)‡	6,548(4931–8341)^{c}	9,440(7174–12384)^{a}	5,889(4519–7422)^{d}	8,539(6009–11457)^{b}	<0.001	<0.001	0.732
Total fat (%E)†	17.83[17.09–18.56]^{d}	24.68[24.07–25.29]^{b}	19.84[19.30–20.38]^{b}	27.51[26.99–28.03]^{a}	<0.001	<0.001	0.192
SFA (%E)†	3.96[3.71–4.22]^{d}	6.14[5.94–6.35]^{b}	4.42[4.21–4.63]^{c}	7.11[6.92–7.29^{a}]	<0.001	<0.001	0.184
MUFA (%E)†	8.13[7.13–8.43]^{c}	11.24[10.99–11.50]^{b}	8.78[8.59–9.96]^{c}	12.16[11.94–12.38]^{a}	<0.001	<0.001	0.258
PUFA (%E)†	5.66[5.37–5.95]^{d}	7.09[6.85–7.33]^{b}	6.55[6.31–6.80]^{b}	7.94[7.74–8.14]^{a}	<0.001	<0.001	0.876
LA (%E)†	5.21[4.92–5.50]^{d}	6.80[6.56–7.04]^{c}	6.05[5.81–6.28]^{b}	7.690[7.48–7.90]^{a}	<0.001	<0.001	0.878
ALA (%E)†	0.07[0.07–0.08]^{d}	0.12[0.12–.013]^{b}	0.08[0.08–0.09]^{c}	0.14[0.14–0.14]^{a}	<0.001	<0.001	0.100
Trans fat (%E)†	0.08[0.07–0.09]^{c}	0.20[0.18–0.22]^{b}	0.09[0.08–0.10]^{c}	0.30[0.28–0.32]^{a}	<0.001	<0.001	<0.001
Total fat (g)‡	29.66(21.41–41.19)^{b}	60.76(44.31–81.63)^{a}	30.21(20.95–40.64)^{b}	61.47(42.49–85.84)^{a}	0.779	<0.001	0.588
SFA (g)‡	6.32(3.77–9.17)^{b}	14.70(10.33–20.80)^{a}	6.31(3.87–9.49)^{b}	15.53(10.61–22.14)^{a}	0.374	<0.001	0.830
MUFA (g)‡	6.55(4.09–9.76)^{b}	16.35(11.69–24.21)^{a}	6.60(4.08–9.91)^{b}	17.51(11.56–25.30)^{a}	0.645	<0.001	0.988
PUFA (g)‡	9.34(6.26–13.63)^{b}	17.23(12.05–24.44)^{a}	9.75(6.35–14.21)^{b}	17.54(11.88–25.61)^{a}	0.399	<0.001	0.526
C14:0 (mg)‡	400 (181–674)^{b}	876(617–1402)^{a}	400(186–733)^{b}	991(562–1546)^{a}	0.330	<0.001	0.530
C16:0 (mg)‡	3,070(1897–4459)^{b}	7,877(5560–11212)^{a}	3,084(1876–4476)^{b}	8,466(5550–11744)^{a}	0.668	<0.001	0.885
C18:0 (mg)‡	1742(1049–2747)^{b}	4230.74(2884–6186)^{a}	1779(1053–2692)^{b}	4358(3021–6547)^{a}	0.671	<0.001	0.972
C18:1 (mg)‡	5,866(3661–9047)^{b}	15 081(10732–21990)^{a}	6,139(3767–8902)^{b}	15 691(10547–23140)^{a}	0.585	<0.001	0.688
C18:2 n-6(mg)‡	8,478(4950–12735)^{b}	15 944(10859–23195)^{a}	8,590(5591–12981)^{b}	16 565(10827–24071)^{a}	0.216	<0.001	0.463
C20:4 n-6 (mg)‡	34(19–56)^{b}	102(68–150)^{a}	33(18–60)^{b}	94(63–140)^{a}	0.138	<0.001	0.087
C18:3 n-3(mg)‡	111(71–170)^{b}	274(207–391)^{a}	115(77–165)^{b}	293(208–423)^{a}	0.197	<0.001	0.386
C20:5 n-3 (mg)‡	38(15–62)^{c}	61(31–108)^{a}	33(14–57)^{c}	46(21–84)^{b}	0.002	<0.001	0.006
C22:6 n-3 (mg)‡	62(21–104)^{c}	109(53–187)^{a}	52(17–96)^{c}	83(37–156)^{b}	0.005	<0.001	0.029
Alcohol (g)	2.21(0.00–25.71)^{b}	11.57(0.00–26.65)^{a}	0.00(0.00–0.00)^{d}	0.00(0.00–11.43)^{c}	<0.001	<0.001	0.763
Soluble fiber (g)	0.93(0.54–1.43)^{c}	1.94(1.28–3.21)^{b}	1.11(0.66–1.62)^{c}	2.27(1.34–3.43)^{a}	<0.001	<0.001	0.851

Body mass index (BMI), waist circumference (WC), kilojoules (kJ), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), and docosahexaenoic acid (C22:6,n-3).

*Differences between groups were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Means/medians in a row with different superscripts (^{abcd}) differ significantly, $P<0.05$.

†Normally distributed values are reported as mean [95% CI].

‡Not normally distributed data are reported as median (25th–75th percentile), data were log transformed to perform analysis.

§2 way ANCOVA was used to test effects of gender and urbanization adjusting for age with all dietary intake variables, BMI and physical activity.

We found significant effects of gender on the intake of trans fatty acids (absolute and in %E), EPA and DHA, as well as significant gender x urbanization interactions, indicating that intakes of these fatty acids were significantly higher in males than females in urban areas only, with no significant differences between gender in rural areas.

A significant gender effect was present for all serum lipid variables. Total cholesterol, LDL, and total cholesterol to LDL ratio and LDL: HDL (high-density lipoprotein) ratios were higher in female than in male subjects from both, rural and urban areas (Table 2). We found a gender effect ($P< 0.001$), as well as a gender x urbanization interaction, on HDL ($P = 0.041$), indicating significantly higher HDL in urban males than urban females, with no significant differences between genders in rural areas. We found a significant effect of gender and of urbanization on triglycerides, indicating that in urban areas, triglycerides were significantly higher in females than in males, but at the same time, the urban females had significantly higher triglyceride levels than the rural females.

Prevalence of unfavorable blood lipid levels is shown in Fig. 2.

In females, the intake of arachidonic acid and EPA was positively and significantly associated, while DHA intake was negatively associated with total cholesterol (Table 3) even after controlling for energy-, alcohol- and soluble fiber intake, as well as BMI, physical activity and waist circumference. The association between LDL and arachidonic acid became significant in the fully adjusted model, and there tended to be an association between LDL and EPA ($P = 0.051$) and between LDL and DHA ($P = 0.052$), while the associations were significant in the second model which did not include urbanization. Higher arachidonic acid intake also tended to increase the risk for having elevated total cholesterol in females (OR = 1.22; 95% CI 1.00, 1.49, $P = 0.052$) (Fig. 2). In male subjects, ALA was the only fatty acid that was

Table 2
Serum lipid levels by urbanization and gender*.

Variable	Male		Female		Significance‡		
	Rural (n = 335)	Urban (n = 376)	Rural (n = 621)	Urban (n = 558)	Gender	Urbanization	Gender x urbanization
TC (mmol/L)†	4.50(3.81–5.53)^{b}	4.68(3.84–5.71)^{b}	4.85(4.11–5.95)^{a}	5.02(4.18–6.09)^{a}	<0.001	0.171	0.200
LDL-C (mmol/L)†	2.85(2. 9–3.62)^{b}	2.86(2.17–3.73)^{b}	3.15(2.50–4.09)^{a}	3.35(2.55–4.12)^{a}	<0.001	0.602	0.766
HDL-C (mmol/L)†	1.45(1.02–1.94)^{ab}	1.50(1.12–2.04)^{a}	1.39(1.09–1.84)^{ab}	1.36(1.03–1.78)^{b}	<0.010	0.955	0.041
Triglycerides (mmol/L)†	0.97(0.76–1.35)^{c}	1.00(0.78–1.46)^{c}	1.09(0.81–1.49)^{bc}	1.18(0.87–1.78)^{a}	<0.001	<0.001	0.326
TC:HDL†	3.28(2.38–4.27)^{b}	3.09(2.36–4.16)^{b}	3.51(2.72–4.49)^{a}	3.68(2.86–4.77)^{a}	<0.001	0.322	0.174
LDL:HDL†	2.09(1.30–2.99)^{b}	1.95(1.26–2.90)^{b}	2.38(1.62–3.27)^{a}	2.46(1.72–3.47)^{a}	<0.001	0.653	0.187

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL-C).

*Differences between groups were analyzed with 1 way ANOVA and Bonferroni post-hoc test were performed. Means/medians in a row with different superscripts (^{abcd}) differ significantly, $P<0.05$.

†Not normally distributed data are reported as median (25th–75th percentile), data were log transformed to perform analysis.

‡2 way ANCOVA was used to test effects of gender and urbanization adjusting for age with all dietary intake variables, BMI and physical activity.

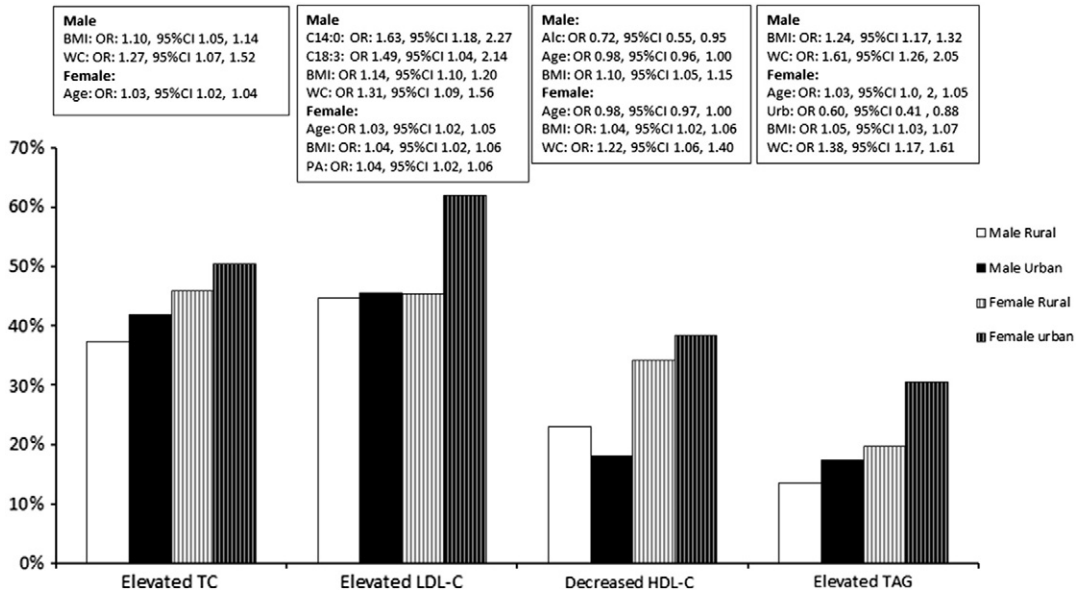

Fig. 2. Prevalence of unfavorable serum lipid profiles by urbanization and gender. Body mass index (BMI), waist circumference (WC), myristic acid (C14:0), alpha-linolenic acid (C18:3), physical activity (PA), alcohol (Alc), and urbanization (Urb). Logistic regression analysis was used to determine whether individual dietary FAs increased or decreased the odds ratios (ORs) for having unfavorable blood lipid profiles, adjusting for total energy intake, age, BMI, physical activity and waist circumference. A P-value < 0.05 was considered significant.

significantly and positively associated with total cholesterol in the fully adjusted model (Table 4), and ALA also tended to be positively associated with LDL ($P = 0.061$). A higher intake of myristic acid (OR = 1.63, 95% CI 1.17, 2.27, P = 0.004) and ALA (OR = 1.49, 95% CI 1.04, 2.14, $P = 0.029$) also increased the risk of having elevated LDL in male subjects. In male subjects, palmitic acid was negatively associated ($P = 0.031$), while ALA was positively associated with triglyceride levels ($P < 0.001$). ALA intake also tended to increase the risk of having elevated triglyceride levels in males (OR = 1.47, 95% CI 0.992, 2.19, $P = 0.055$).

4. Discussion

This is the first study, to our knowledge, that investigated associations between the intake of individual fatty acids and blood lipid profiles in the South African population in relation to gender and urbanization. Our results indicated that even though the intake of energy, fat and specific fatty acids were significantly higher in people living in urban than in rural areas, no differences in blood lipid profiles were found between subjects from the two groups. This could in part have been because intake of fat and fatty acids was mostly still within WHO [28] and American Heart Association (AHA) [29] recommendations even in urban areas. However, intake of the essential fatty acid ALA was below the recommended 0.5% of intake in both areas, and thus could lead to deficiencies of this fatty acid [28].

The blood lipid profiles of females in this population were found to be significantly more unfavorable than those of males, particularly in urban areas. BMI could be one of the most important causes of this finding. Females had significantly higher BMI than males, especially in urban areas. Higher BMI remained independently and positively associated with total cholesterol, LDL, and triglycerides, as well as negatively associated with HDL in both genders. Higher BMI also increased the risk for having elevated total cholesterol in males, elevated LDL in females, decreased HDL in males and females and increased triglycerides in males and females (Fig. 2). Obesity has previously been shown to be an important determinant of serum lipid levels [30]. According to Grundy and Denke [31], the effects of obesity on blood lipids could be related to the overnutrition that causes obesity. However, the subjects in our study generally had low energy and fat intake. Other possible reasons for obesity in women could be physical inactivity [24] or childhood stunting, which is linked to excess weight gain later in life [32]. Data from the National Food Consumption Survey indicated that stunting affected nearly one in five children in South Africa [33] and 15% of children in the North West Province [34]. The double burden of malnutrition, which describes the presence of undernutrition along with a rapid rise in overweight and obesity, is a known problem in South Africa [35]. However, we found that physical activity did not play a major role in the serum lipid profiles of this population, which is evident by the lack of associations found in the applied multiple linear regression models or logistic regression models. The only exception was the increased risk for having elevated LDL with decreasing physical activity found in females (Fig. 2).

To our surprise, we found dietary ALA intake to be positively associated with total cholesterol and triglycerides in male subjects, but not in females. In contrast with our results, an inverse relationship or no associations between dietary ALA and total cholesterol, LDL and triglycerides have been reported previously [8,36,37]. A recent parallel intervention study in 81 subjects, providing a diet either high or low in ALA for 6 months, found that serum triglycerides were significantly lower in the group receiving the high ALA diet compared to that of the low ALA diet group [38]. In the present study, mean ALA intake was below the recommended 0.5% of energy in all groups [28]. Findings from Patenaude et al. [39] indicated that the provision of ALA in the form of ground flaxseed or flaxseed oil for 4 weeks lowered triglycerides in younger subjects (18 29 years) but not in older subjects (45 69 years). In prior South African studies, total cholesterol and LDL levels were shown to increase with age [17]. In our study, age was associated positively with total cholesterol, LDL and triglycerides in the fully adjusted model, however, only in females and not in males. The age group of this study population started at 35 years, which could have contributed to higher baseline triglyceride levels. It was also interesting to find such a strong inverse association between palmitic acid and triglycerides in males, which to our knowledge has not been described in the current literature before.

To date, not much information is available on the relationship between dietary arachidonic acid and blood lipids. In this study, dietary arachidonic acid was positively associated with LDL and also tended to be associated with total cholesterol in females ($P = 0.051$). A small cross-

Table 3
Associations between individual fatty acids and blood lipids of female subjects using multiple linear regression.

		TC		LDL		HDL		Triglycerides	
Models		β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
1	Energy	0.068	0.029	0.019	0.541	0.090	0.004	0.084	0.008
	C14:0*	−0.011	0.831	0.037	0.488	−0.059	0.262	−0.117	0.028
	C16:0*	0.098	0.553	0.036	0.827	0.097	0.558	0.142	0.395
	C18:0*	0.040	0.645	0.059	0.501	−0.056	0.519	0.148	0.091
	C18:1*	−0.159	0.213	−0.157	0.220	−0.009	0.945	−0.178	0.164
	C18:2*	0.017	0.710	0.044	0.336	−0.042	0.356	−0.006	0.899
	C18:3*	0.000	0.997	0.043	0.462	−0.095	0.100	0.049	0.399
	C20:4*	0.123	0.009	0.113	0.016	0.059	0.208	−0.014	0.759
	C20:5*	0.335	0.004	0.302	0.009	0.148	0.201	0.046	0.689
	C22:6*	−0.330	0.005	−0.321	0.007	−0.088	0.455	−0.075	0.525
	R^2	0.028		0.024		0.026		0.019	
2	Energy	0.055	0.080	−0.009	0.758	0.119	0.000	0.079	0.010
	C14:0*	−0.009	0.863	0.021	0.691	−0.026	0.623	−0.086	0.095
	C16:0*	0.132	0.419	0.079	0.625	0.080	0.619	0.162	0.309
	C18:0*	0.023	0.786	0.059	0.488	−0.085	0.317	0.114	0.174
	C18:1*	−0.189	0.131	−0.201	0.105	0.008	0.948	−0.193	0.115
	C18:2*	0.035	0.448	0.046	0.321	−0.008	0.859	0.035	0.447
	C18:3*	−0.023	0.695	−0.005	0.936	−0.047	0.416	0.022	0.699
	C20:4*	0.106	0.021	0.089	0.051	0.071	0.118	−0.029	0.525
	C20:5*	0.267	0.019	0.228	0.043	0.153	0.177	−0.019	0.862
	C22:6*	−0.248	0.033	−0.229	0.047	−0.105	0.367	0.013	0.906
	Alcohol*	0.000	0.993	−0.058	0.098	0.090	0.011	0.082	0.019
	Soluble fiber	−0.034	0.330	−0.040	0.244	0.000	0.993	−0.003	0.923
	BMI	0.076	0.016	0.172	<0.001	−0.208	<0.001	0.159	<0.001
	Physical activity	−0.021	0.498	−0.043	0.161	0.040	0.197	0.016	0.593
	WC†	0.028	0.370	0.039	0.208	−0.050	0.104	0.144	<0.001
	Age	0.194	0.000	0.172	0.000	0.062	0.047	0.201	<0.001
	R^2	0.075		0.094		0.084		0.117	
3	Energy (kJ)	0.074	0.033	0.008	0.809	0.141	<0.001	0.031	0.352
	C14:0 (mg)*	−0.012	0.815	0.018	0.732	−0.029	0.575	−0.079	0.126
	C16:0 (mg)*	0.158	0.336	0.102	0.528	0.110	0.499	0.099	0.535
	C18:0 (mg)*	0.016	0.852	0.052	0.539	−0.094	0.273	0.132	0.116
	C18:1 (mg)*	−0.191	0.127	−0.203	0.101	0.005	0.965	−0.188	0.124
	C18:2 (mg)*	0.031	0.509	0.042	0.365	−0.013	0.774	0.046	0.322
	C18:3 (mg)*	−0.015	0.796	0.002	0.967	−0.038	0.511	0.003	0.954
	C20:4 (mg)*	0.113	0.014	0.096	0.037	0.080	0.082	−0.047	0.295
	C20:5 (mg)*	0.260	0.023	0.221	0.051	0.144	0.205	0.000	0.999
	C22:6 (mg)*	−0.243	0.037	−0.225	0.052	−0.099	0.393	0.002	0.989
	Alcohol*	0.007	0.853	−0.053	0.140	0.097	0.007	0.067	0.057
	Soluble fiber	−0.030	0.389	−0.037	0.289	0.005	0.893	−0.013	0.714
	BMI	0.078	0.012	0.175	0.000	−0.205	<0.001	0.152	<0.001
	Physical activity	−0.029	0.358	−0.051	0.108	0.031	0.333	0.036	0.246
	WC†	0.028	0.370	0.038	0.208	−0.050	0.104	0.144	<0.001
	Age	0.196	0.000	0.174	0.000	0.065	0.039	0.196	<0.001
	Urbanization	−0.051	0.206	−0.047	0.244	−0.059	0.146	0.125	0.002
	R^2	0.076			0.095		0.086	0.126	

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3), body mass index (BMI), and waist circumference (WC).

* Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

† A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

over intervention study in 10 men found that the provision of a diet rich in arachidonic acid (210 mg/day) for 50 days did not change blood lipids or lipoproteins [40].

Another interesting finding of our study is that in female subjects, EPA was positively associated, whereas DHA was negatively associated with total cholesterol. The associations were in the same directions for LDL, but did not reach statistical significance (EPA: $P = 0.051$, DHA: $P = 0.052$). The existing literature is inconclusive about the effects of EPA and DHA on total cholesterol and LDL. Either no effect, decreases or increases have been reported in previous intervention trials [8,9,41]. Fewer studies focused on EPA and DHA individually than on the combination effect of long-chain n-3 fatty acids. A meta-analysis of randomized controlled trials that investigated the nett effects of purified EPA or DHA as monotherapy on serum lipids found that EPA had no effect on LDL, while DHA raised LDL [7]. Our results were in contrast with the effect reported in this meta-analysis. However, it should be noted that some studies reported increases while others no associations. Researchers linked the increasing effects of DHA on LDL to an increase in particle size [42], however, a decrease in the number of small dense LDL particles is also associated with DHA [43]. Even though we do not have a record of the LDL particle size in the current study, a recent study in South Africa comparing different ethnicities found black South African women to have the highest proportion of the smallest, most dense LDL particles [44].

Limitations of this study are that, regardless of the level of detail obtained in food frequency questionnaires (FFQs), dietary assessments are limited by available food composition databases [45,46] and accuracy during recording and coding [47]. In general, a FFQ tends to overestimate intake of a population, especially for food items that are eaten regularly but not daily [47]. MacIntyre and colleagues [21] found that the quantitative food frequency questionnaire (QFFQ) used in the THUSA study tended to under-report energy and fat intakes in comparison with a seven-day weighed record, with better agreement at lower intake levels. However, in epidemiology the food frequency questionnaire

Table 4
Associations between individual fatty acids and blood lipids of male subjects using multiple linear regression.

		TC		LDL		HDL		Triglycerides	
Models		β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value
1	Energy	−0.009	0.816	−0.082	0.038	0.116	0.003	−0.005	0.908
	C14:0*	0.081	0.266	0.122	0.091	−0.081	0.262	0.093	0.202
	C16:0*	−0.329	0.194	−0.159	0.528	−0.175	0.484	−0.489	0.053
	C18:0*	−0.035	0.763	−0.140	0.230	0.131	0.257	0.153	0.190
	C18:1*	0.278	0.092	0.182	0.269	0.105	0.518	0.249	0.129
	C18:2*	−0.058	0.347	0.014	0.814	−0.124	0.041	−0.024	0.692
	C18:3*	0.146	0.029	0.178	0.008	−0.118	0.075	0.272	<0.001
	C20:4*	0.023	0.741	−0.001	0.992	0.034	0.611	0.033	0.625
	C20:5*	0.222	0.205	0.264	0.129	0.065	0.705	−0.210	0.227
	C22:6*	−0.105	0.559	−0.175	0.328	0.039	0.824	0.162	0.363
	R^2	0.032		0.056		0.061		0.055	0.884
2	Energy	−0.021	0.593	−0.088	0.019	0.107	0.005	0−.029	0.446
	C14:0*	0.084	0.255	0.092	0.191	−0.022	0.760	0.121	0.086
	C16:0*	−0.309	0.215	−0.145	0.542	−0.166	0.489	−0.473	0.048
	C18:0*	−0.050	0.670	−0.117	0.294	0.066	0.558	0.102	0.360
	C18:1*	0.270	0.096	0.159	0.305	0.130	0.406	0.265	0.088
	C18:2*	−0.053	0.399	−0.029	0.634	−0.032	0.597	−0.032	0.594
	C18:3*	0.149	0.030	0.124	0.058	−0.012	0.855	0.260	<0.001
	C20:4*	0.014	0.835	−0.008	0.900	0.032	0.619	0.036	0.578
	C20:5*	0.166	0.336	0.200	0.224	0.087	0.600	−0.261	0.114
	C22:6*	−0.042	0.812	−0.096	0.571	0.005	0.978	0.213	0.209
	Alcohol*	0.020	0.696	−0.101	0.037	0.221	<0.001	0.058	0.230
	Soluble fiber	−0.033	0.451	−0.023	0.589	−0.028	0.516	0.047	0.268
	BMI	0.201	<0.001	0.318	<0.001	−0.225	<0.001	0.286	<0.001
	Physical activity	−0.022	0.581	−0.025	0.514	−0.001	0.973	0.006	0.880
	WC†	0.102	0.012	0.094	0.015	−0.010	0.800	0.192	<0.001
	Age	0.023	0.571	−0.001	0.973	0.044	0.263	0.007	0.852
	R^2	0.085		0.176		0.151		0.172	0.787
3	Energy (kJ)	−0.046	0.295	−0.094	0.024	0.078	0.064	−0.049	0.238
	C14:0 (mg)*	0.089	0.227	0.093	0.186	−0.015	0.828	0.125	0.076
	C16:0 (mg)*	−0.368	0.146	−0.160	0.510	−0.235	0.335	−0.524	0.031
	C18:0 (mg)*	−0.029	0.803	−0.112	0.320	0.089	0.429	0.119	0.290
	C18:1 (mg)*	0.291	0.074	0.164	0.292	0.154	0.324	0.284	0.069
	C18:2 (mg)*	−0.057	0.368	−0.030	0.623	−0.037	0.548	−0.035	0.558
	C18:3 (mg)*	0.143	0.038	0.123	0.061	−0.019	0.775	0.256	<0.001
	C20:4 (mg)*	0.004	0.949	−0.010	0.872	0.021	0.747	0.028	0.669
	C20:5 (mg)*	0.169	0.328	0.201	0.223	0.090	0.587	−0.259	0.117
	C22:6 (mg)*	−0.046	0.794	−0.097	0.568	0.000	1.000	0.210	0.216
	Alcohol*	0.017	0.742	−0.101	0.036	0.217	<0.001	0.055	0.253
	Soluble fiber	−0.038	0.394	−0.024	0.573	−0.033	0.441	0.043	0.309
	BMI	0.205	<0.001	0.318	<0.001	−0.221	<0.001	0.289	<0.001
	Physical activity	−0.018	0.664	−0.024	0.535	0.004	0.914	0.010	0.800
	WC†	0.108	0.008	0.095	0.014	−0.002	0.954	0.198	<0.001
	Age	0.019	0.643	−0.002	0.953	0.039	0.321	0.004	0.923
	Urbanization	0.064	0.197	0.015	0.745	0.076	0.116	0.054	0.256
	R^2	0.088		0.176			0.155		0.174

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), kilojoules (kJ), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), alpha linolenic acid (C18:3,n-3), arachidonic acid (C20:4,n-6), eicosapentaenoic acid (C20:5,n-3), docosahexaenoic acid (C22:6,n-3), body mass index (BMI), and waist circumference (WC).

* Regression coefficients are presented for the residual value predicted by total energy intake for all dietary fatty acids.

† A residual of BMI was also calculated for waist circumference to avoid co-linearity between BMI and waist circumference.

is the method of choice to assess relationships between dietary intake and other indices of health status or disease [23, 48].

Only part of the variance in blood lipids were explained by the fully adjusted models (Tables 3 and 4). Thus, other factors not explored in the present study, like culture, educational level, genetics, hormones and stress levels, could have also played a direct or indirect role on blood lipids. According to the thrifty phenotype hypothesis, for example, poor fetal growth (possibly due to fetal malnutrition) can lead to increased insulin resistance [49]. Insulin resistance in turn leads to elevated nonesterified fatty acids in the plasma, which can overload the liver and lead to atherogenic dyslipidemia [50]. Therefore, future studies should investigate and include these factors.

In conclusion, the results from this study give insightful information about novel associations between dietary fat and fatty acid intake and blood lipids in a population in transition. Furthermore, it was interesting to find that different dietary fatty acids and lifestyle factors were associated with unfavorable blood lipid levels in males than in females. A possible reason why blood lipid levels did not differ between rural and urban dwellers is that median fat and fatty acid intake was generally within recommendations in both groups, except from intake of specific n-3 PUFAs that was below recommendation in both groups. Low intake of long chain n-3 PUFAs may also have detrimental effects on mental health of a population. Irrespective of the low intake, however, DHA was inversely associated with total cholesterol. LDL tended to be positively associated with DHA in females, while ALA was positively associated with total cholesterol and triglycerides in males and LDL tended to be positively associated with ALA. EPA was positively associated with total cholesterol and tended to be positively associated with LDL in females. This raises the question about possible detrimental effects of ALA and EPA intake on blood lipid levels. It may be that in this black African population dietary ALA exerts different effects on blood lipids in males than in other populations with higher intake.

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