Dietary fat intake and blood lipid profiles of South African communities in transition in the North West Province: The PURE study

M. Richter

12418358

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Study leader: Prof. C.M. Smuts

Co-study leader: Prof. E. Wentzel-Viljoen

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Innovation through diversity

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YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
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ABSTRACT

Aim and objectives: This study set out to investigate the diet and blood lipid profiles of subjects in transition in the North West Province in South Africa. It looked specifically at how the diet differed between rural and urban areas, how the blood lipid profiles differed between rural and urban subjects, establishing an association between dietary fat, fatty acid and cholesterol intakes respectively and blood lipid profiles, as well as investigating the differences in blood lipid profiles at different ages, body mass index (BMI) and genders respectively in rural and urban areas.

Design: The present study was a cross-sectional data analysis nested within the Prospective Urban and Rural Epidemiology (PURE) study that is currently undertaken in the North West Province of South Africa amongst other countries.

Methods: Baseline data was obtained in 2005. A randomised paper selection was done of people between 35 – 70 years of age with no reported chronic diseases of lifestyle, TB or HIV of those enrolled into the PURE study if they had provided written consent. Eventually a paper selection was made of 2000 subjects, 500 people in each of the four communities (rural, urban-rural, urban, established urban). For the interpretation purposes of this study, data was stratified for rural (1000 subjects) and urban (1000 subjects) only, with no further sub-division into communities. Physical activity levels and habitual diets were obtained from these subjects. Demographic and dietary intake data in the PURE study was collected using validated, culture sensitive questionnaires. Anthropometric measures and lipid analysis were determined using standardised methodology. Descriptive statistics (means, standard deviations and proportions) were calculated. **One-way analysis of variance (ANOVA)** was used to determine differences between the different levels of urbanisation on blood lipid profiles and dietary intake. When a dietary intake variable proved to be significant for different levels of a factor (urbanisation, blood lipid profile), post-**hoc tests** were calculated to determine which levels for specific variables differed significantly. **Bonferroni-type adjustments** were made for the multiple comparisons. Spearman correlations were calculated to determine associations.

Results: Mean fat intake was significantly higher in urban areas than in rural areas (67.16 ± 33.78 g vs. 32.56 ± 17.66 g, $p<0.001$); and the same was true for the individual fatty acid intakes. Fat and fatty acid intakes were still within recommendations even for urban areas, and low for rural areas. N-3 intake was very low in both rural and urban areas. Serum lipids did not differ significantly between rural and urban areas. Almost half of rural (43%) and urban (47%) subjects presented with elevated total cholesterol (≥5.0 mmol/L). In rural areas 52% and in urban areas 55% of subjects had elevated LDL-C (≥3.0 mmol/L). Amongst 23% of males in rural areas and 18% of males in urban areas HDL-C levels were decreased. Of the females living in rural areas 34.3% had decreased HDL-C levels and 39% of those who

lived in urban areas presented with lowered HDL-C levels. In rural areas 16.3% of subjects and in urban areas 23% of subjects presented with high triglyceride levels. TC, LDL-C and triglyceride levels were higher in higher body mass index (BMI) classes, however, obese subjects did not differ significantly from overweight subjects in terms of blood lipids, suggesting that values stabilise after reaching overweight status. These blood lipids were also higher in higher age groups and higher in women than men, probably due to the high incidence of obesity in women.

Conclusions: Associations between the diet and blood lipid profiles were weak, and diet is not likely to be the only factor responsible for high TC and LDL-C levels. Blood lipid profiles did not differ significantly between rural and urban areas due to the fact that the diet was prudent in terms of fat intake in both rural and urban areas. Higher prevalence of underweight was noted in males (32% in rural areas and 28% in urban areas), while overwieght was a bigger problem amongst women (48% in rural areas and 54% in urban areas). TC, LDL-C and TAG were higher with higher BMI's, while HDL-C levels were lower. TC, LDL-C, and TAG were higher in higher age goups while HDL-C levels were lower. Female subjects presented with higher mean triglycerides than males, probably due to higher prevalence of overweight and obesity.

Key words: Urbanisation; Nutrition transition; Africans; blood lipid profiles; fat intake

OPSOMMING

Doel en doelwitte: Hierdie studie het die dieetinname van vet asook die bloedlipiedprofiele in gemeenskappe in voedingoorgang in die Noordwesprovinsie van Suid-Afrika ondersoek. Die studie het spesifiek gefokus op verskille tussen landelike en stedelike proefpersone ten opsigte van die inname van vet, spesifieke vetsure en cholesterol, hoe bloedlipiedprofiele tussen stedelike en landelike proefpersone verskil asook assosiasies tussen bloellipiedprofiele en die inname van vet, vetsure en cholesterol. Die studie het ook die verskille in bloedlipiedvlakke by verskillende ouderdomme, liggaamsmassaindekse (LMI) en geslagte ondersoek in plaaslike en stedelike areas.

Metodes: Die huidige studie was 'n deursnee-data-analise gesetel binne die Prospektiewe Stedelike en Landelike Epidemiologiese (PURE) studie in die Noordwesprovinsie van Suid-Afrika. Basislyndata is verkry in 2005. ‘n Gerandomiseerde papierseleksie is gedoen van huishoudings met mense tussen 35-70 jaar oud, met geen gerapporteerde chroniese leefstylsiektes, tuberkulose (TB) of menslike immuniteitsgebreksvirus (MIV) wat skriftelike toestemming gegee het en in die PURE studie ingeskryf het. Uiteindelik is 'n papierseleksie gemaak van 2000 proefpersone, 500 mense in elk van die vier gemeenskappe (landelike, stedelik-landelike, stedelike, gevestigde-stedelike). Vir die interpretasie doeleindes van hierdie studie is data slegs gestratifiseer vir landelike gebiede (1000 proefpersone) en stedelike gebiede (1000 proefpersone) met geen verdere verdeling in die gemeenskap nie. Fisiese aktiwiteitvlakke en gebruiklike dieet is verkry vanaf hierdie proefpersone. Demografiese en dieetinnamedata in die PURE-studie is ingesamel met behulp van geldige, kultuursensitiewe vraelyste. Antropometriese data en lipiedanalise is bepaal met behulp van gestandaardiseerde metodiek.

Resultate: Gemiddelde vetinname was aansienlik hoër in stedelike gebiede as in landelike gebiede (67,16 ± 33,78 g teenoor 32,56 ± 17,66 g, p <0,001), en dieselfde was waar vir vetsuurinname. Vet- en vetsuurinname was steeds binne aanbevelings, selfs in stedelike gebiede, en laag in plattelandse gebiede. N-3 inname was baie laag in beide landelike en stedelike gebiede. Bloedlipiede het nie betekenisvol verskil tussen landelike en stedelike gebiede nie. Bykans die helfte van die landelike (43%) en stedelike (47%) proefpersone het verhoogde totale cholesterol (≥ 5,0 mmol/L) gehad, terwyl 52% en 55% van landelike en stedelike proefpersone respektiewelik verhoogde LDL-C (≥ 3,0 mmol/L) gehad het. Onder 23% van die landelike mans en 18% in stedelike gebiede het lae vlakke van HDL-C gehad. Van die vroulike proefpersone in landelike gebiede het 34% en van diegene in stedelike gebiede het 39% verlaagde HDL-C-vlakke gehad. In landelike gebiede het 16,28% van proefpersone en in stedelike gebiede 23% van proefpersone hoë trigliseriedvlakke gehad. TC-, LDL-C- en trigliseriedvlakke was hoër by persone met hoër LMI’s. Vetsugtige proefpersone se bloedlipiedvlakke het nie betekenisvol verskil van oorgewig proefpersone nie, wat daarop dui dat waardes stabiliseer nadat oorgewigstatus bereik is.

Bloedlipiede was ook hoër in hoër ouderdomsgroepe en hoër in vroue as mans, waarskynlik weens die hoë voorkoms van vetsug in die vroue.

Samevatting: Assosiasies tussen die dieet en bloedlipiedprofiele was swak, en dit is onwaarskynlik dat dieet alleen verantwoordelik was vir die hoë TC- en LDL-C-vlakke. Bloedlipiedprofiele het nie betekenisvol verskil tussen landelike en stedelike gebiede nie, moontlik as gevolg van die feit dat die dieet omsigtig was in vetinname in beide landelike en stedelike gebiede. TC, LDL-C en TAG was hoër met hoër LMI's, terwyl die HDL-C-vlakke laer was. TC, LDL-C en TAG was hoër in hoër ouderdomsgroepe, terwyl die HDL-C-vlakke laer was Vroulike proefpersone het hoër gemiddelde trigliseriede as mans gehad, waarskynlik weens die hoër voorkoms van oorgewig en vetsug.

Sleutelwoorde: Verstedeliking; voedingoorgang; Afrikane; bloedlipiedprofiele; vetinname

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LIST OF ABBREVIATIONS

AHA	American Heart Association
AI	Adequate intake
ALA	Alpha-linolenic acid
AMDR	Acceptable macronutrient distribution range
ANOVA	Analysis of variance
Apo	Apo lipoprotein
ATP	Adult treatment panel
BMI	Body mass index
BRISK	Risk factors for coronary heart disease in Cape Peninsula blacks
CAD	Coronary artery disease
CDT	Carbohydrate-deficient transferrin
CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
CHO	Carbohydrate
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EER	Estimated energy requirements
EPA	Eicosapentaenoic acid
FCR	Fractional catabolic rate
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methylglutamyl-coenzyme A
IDL-C	Intermediate density lipoprotein cholesterol
IHD	Ischaemic heart disease
IPAQ	International Physical Activity Questionnaire
KIHD	Kuopio Ischaemic Heart Disease Risk Factor
LA	linoleic acid
LCAT	Lecithin cholesterol acyl transferase

LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LPL	Lipoprotein lipase
MI	Myocardial infarction
MUAC	Mid upper arm circumference
MUFA	Monounsaturated fatty acid
n-3	Omega 3
n-6	Omega 6
NCEP	National Cholesterol Education Program
PAF	Population attributable fraction
PAI	Physical activity index
PUFA	Polyunsaturated fatty acid
PURE	Prospective rural urban epidemiology
RDA	Recommended daily allowance
SD	Standard deviation
SFA	Saturated fatty acid
SMAC	Sequential multiple analyzer computer
TC	Total cholesterol
TAG	Triacylglycerol
THUSA	Transition and Health during Urbanization of South Africans
UAE	United Arabic Emirates
UL	Upper limit
VLDL-C	Very low density lipoprotein cholesterol
QFFQ	Quantitative food frequency questionnaire

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C H A P T E R 1

INTRODUCTION

1.1 Background and problem statement

The nutrition transition addresses a range of socioeconomic and demographic shifts from rural to urban, and is accompanied by rapid changes in diet, lifestyle and patterns of undernourishment and obesity. In developing countries improved socioeconomic conditions and the availability of a wide variety of food associated with transition in the last several decades, results in the increase in the incidence of obesity and non-communicable diseases of lifestyle, among which coronary heart disease (CHD) (Popkin, 2001).

According to Gelderblom and Kok (1994), inequalities in socio-economic conditions in South Africa can mainly be attributed to the previous apartheid policies. Africans were not involved in widespread urbanisation to the same extent as other South African population groups, mainly due to influx control measures, which is still visible in the settlement pattern of the African people which looks different from those of other racial groups in the country. Due to recent political changes however, transition of African people is visible in a rapid influx into urban areas. In line with global predictions by Solomons and Gross (1995), the urban population in South Africa accounts for more than half of the population, a number which is rising, from 55% (re-classified to match demographic classification used in 2001 census) in 1996 to 58% in 2001 (Stats SA, 2003). In the North West Province 44% of the population lived in urban areas in 1996 (re-classified data) and by 2001 the numbers decreased to 41,8%. Statistics South Africa (2003) however, cautioned about interpreting the changes in urbanisation over time without care, since the definition of urban and rural used in the censuses were different (Stats SA, 2003).

Surprisingly, up until now, the African population did not show hypercholesterolaemia at a high level of risk (Oosthuizen *et al*., 2002; Steyn *et al*., 1991) in spite of urbanisation, and in spite of the fact that African women have a very high prevalence of obesity (Kruger *et al*., 2001), considering that urbanisation is accompanied with higher fat intake as well as higher prevalence of obesity and associated diseases of lifestyle (Popkin, 2001). It is however, difficult to make definitive summarised conclusions regarding the process of urbanisation and related effects in South Africa, since studies vary in size, design, methods and outcome measures. Ethnicity of subjects and definitions of rural and urban areas are among the differences in these studies. Vorster and researchers (1997a) cautioned that care should be taken in the integration and interpretation of such results and that conclusions drawn, should take into account that results from small studies may be biased and not representative of the total population.

1.2 Purpose and importance of the study

This study is unique in the sense that it looked specifically at fat and fatty acid intake of subjects with normal blood lipid profiles and those with abnormal blood lipid profiles. Even though a prospective design, as the Prospective Urban Rural Epidemiology (PURE) study intended, would have been more ideal, data collection was not yet finished by the commencement of this study. A large scale cross-sectional design using baseline data from the PURE study was therefore used. The dietary analyses to obtain the nutrient intakes of the subjects in rural and urban areas can give valuable information regarding adequacy of diets and to study the relationship between nutrient intakes and health and disease.

It can also serve as an indicator for the North West Province by providing information regarding the nutritional status of the population, which can be used to identify challenges faced by the health sector in improving the health status of the population and South Africans in general. The results can be used to establish and improve nutrient intake goals, health education programs, interventions and food and nutrition policies, in order to improve the quality of life of the people in the North West province and South Africa.

1.3 Aim and objectives

1.3.1 Overall aim

This study set out to investigate how the diet differed between rural and urban areas of subjects in the North West Province in South Africa and associations between the diet and blood lipid profiles, by means of a cross sectional data analysis of the PURE study conducted in 2005.

1.3.2 Specific objectives

(i) To examine differences in dietary fat, cholesterol and fatty acid intake respectively between rural and urban subjects.
(ii) To determine the differences in blood lipid profiles between rural and urban subjects.
(iii) To establish the association between dietary fat, fatty acid and cholesterol intake respectively and blood lipid profiles in subjects.

(iv) To investigate serum lipid profiles at different ages, body mass index's (BMI) and genders respectively in rural and urban areas.

1.4 Definitions

(i) Urbanisation

The movement of the population from rural to urban areas and the lifestyle changes which accompany this process.

(ii) Transition

In this dissertation, the term nutrition transition refers to changes in the structure and composition of the diet, specifically from a diet high in unrefined carbohydrates (CHOs) and low fat to a diet high in refined CHOs and fat.

(iii) Rural area

Rural areas in this dissertation consisted of areas still under tribal law, urban-rural areas (Ganeysa) and very rural areas (Tklagameng).

(iv) Urban area

Urban areas in this dissertation consisted of established urban areas (Ikageng) and squatter camps (Sonderwater, ext. 7 &11), not under tribal law.

1.5 Structure

The study is divided into six chapters, that summarise the relevant literature (Chapter 2), describe the methodology of the study (Chapter 3), results (Chapter 4), discuss results and compare it to relevant literature (Chapter 5), a conclusion that summarises essential findings of the study and recommendations (Chapter 6).

CHAPTER 2

LITERATURE

2.1 Urbanisation in South Africa

By the year 2000, no national survey on cardiovascular risk factors in a random sample has been conducted as yet in South Africa. Therefore, Norman and research team (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 population attributable fraction (PAF) between groups. The black African population generally had lower total cholesterol (TC) levels than Indian, White or Coloured population groups. Larger differences between population groups were found in older subjects, suggesting that a westernised diet influenced the younger age groups. Even though 28% of black African people over 30 years of age had TC levels above clinical cut-off points (5mmol/l), high density lipoprotein cholesterol (HDL-C) amongst 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. Oelofse and team (1996) found that TC and low density lipoprotein cholesterol (LDL-C) increased with age in the urban African population of the Cape Peninsula. In a report from Statistics South Africa (2008), cerebrovascular disease is the fifth leading cause of natural death in South Africa, taking the lives of 25 246 South Africans in 2006 (**Table 2.1**).

Prior cross-sectional community based analyses in South Africa noted a gradual change in dietary intake patterns with urbanisation. Walker and colleagues (1992) noted amongst other dietary changes, an increase of 6% in total energy and 5% of fat intake in the diet of elderly black women in rural communities of South Africa between 1969 and 1989. The risk factors for CHD in Cape Peninsula blacks (BRISK) study concluded that the urban black African population of the Cape peninsula in South Africa had a saturated fatty acids (SFA) intake of 8.8% of energy in urban areas (Bourne *et al*., 1993). MacIntyre and colleagues (2002) found the percentage energy from fat in rural communities in the North West province of South Africa to be 22.9% and 30.6% for urban communities in the Transition and Health during Urbanisation of South Africans (THUSA) study. Vorster *et al.* (2007) confirmed that percentages of energy from fat increases as the level of urbanisation increases in the THUSA study. They found the highest intakes of total energy, total fat, SFA and dietary cholesterol, to be in middle income categories.

Table 2.1: The ten leading underlying natural causes of death in South Africa, 2006

Causes of death (Based on the Tenth Revision, International Classification of Disease, 1992)	Rank	Number	%
Tuberculosis	1	77 009	12.7
Influenza and pneumonia	2	52 791	8.7
Intestinal infectious diseases	3	39 239	6.5
Other forms of heart disease	4	26 628	4.4
Cerebrovascular diseases	5	25 246	4.2
Diabetes mellitus	6	19 549	3.2
Chronic lower respiratory diseases	7	15 823	2.6
Certain disorders involving the immune mechanism	8	15 736	2.6
Human immunodeficiency virus [HIV] disease	9	14 783	2.4
Ischaemic heart diseases	10	13 025	2.1
Other natural causes		254 741	42.0
Non-natural causes		52 614	8.7
All causes		**607 184**	**100.0**

*Including 604 deaths due to *MDR-TB* and three (3) deaths due to *XDR-TB.*

(STATS SA, 2008)

According to Vorster and fellow researchers (2000), urbanisation could be associated with improvement in some health determinants, but deterioration in others, especially among people in transition. South Africa suffers from the double burden of stunting and underweight, as well as obesity co-existing (Vorster *et al.*, 1997a). Urbanisation also brought on increases in some cardiovascular risk factors like higher TC and LDL-C levels (Vorster *et al*., 2005), as well as increased BMI of men (Vorster *et al*., 2007). In the THUSA study, increase in LDL-C in women was not significant, which was attributed to the presence of overweight and obesity, income and education in women of all levels of urbanisation (Vorster *et al*., 2007). Even though TC was higher in urban areas than in rural areas, low mean values were reported in the same population with low prevalence of CHD (Vorster *et al.,* 2000). Vorster and colleagues (2005) further concluded that HDL-C remained relatively constant between levels of urbanisation in the THUSA study, as did triacylglycerol (TAG). It was suggested that the burden of coronary artery disease (CAD) during the health transition will possibility be heavier in middle income categories, than those with a higher socio-economic position. Mollentze and colleagues (1995), however, found differences in ischaemic heart disease (IHD) risk between rural and urban areas to be the exception rather than the rule, in the Free State black population. Rates of obesity increased with urbanisation, even though high rates were also noted in rural areas (Vorster *et al.,* 2000). Walker and team (1992) also noted an increase in BMI and serum TC in urban African women.

2.2 Dyslipidaemia as risk factor for CVD

Within the blood lipid profiles, raised plasma LDL-C, raised TAG and decreased levels of HDL-C are known independent risk factors for cardiovascular disease (CVD) (Wilson *et al*., 1998; Riccardi *et al*., 2003). High cholesterol was estimated to cause 24 144 deaths (4.6%) of all deaths in South Africa in 2000 (Norman *et al*., 2007).

2.2.1 TC and LDL-C

Results from populations studied in China, Poland and the US, with different risk factors and different socio-political factors and absolute morality figures, concluded that TC was a strong predictor of CHD mortality and all CVD in men (Cai *et al*., 2004). TC is also a major independent predictor of recurrence of myocardial infarction (MI) (De Lorgeril *et al.*, 1999).

The association between LDL-C and risk of CHD has been proven by the fact that interventions that reduce TC and LDL-C have significantly reduced CHD mortality (Baigent *et al.*2005, Riccardi *et al*., 2003). The Hisayama study (Imamura *et al.*, 2009), a long term prospective study of the Japanese population, found LDL-C as risk factor to be comparable to the effect of metabolic syndrome, however, independent of it. Baigent *et al.* (2005) claimed that the relationship between absolute reductions in LDL-C and reductions in coronary events are approximately linear.

The liver synthesizes the majority of cholesterol transported by LDL. The rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methylglutamyl-coenzyme A (HMG-CoA) reductase. Dietary fats and drugs (statins) regulate this coenzyme (Riccardi *et al*., 2003). The pathway of LDL-C metabolism is explained by Riccardi and colleagues (2003) as follows:
The liver secretes very low density lipoprotein cholesterol (VLDL-C) particles. LDL-C particles are formed within the circulation from VLDL-C particles. TAG is progressively removed (and hydrolysed) from the VLDL-C particle, through the action of the enzyme lipoprotein lipase (LPL) in the capillaries of peripheral tissues. Released fatty acids are taken up and used as energy substrate by tissues or stored within adipose tissue. As TAG is removed from the core of the VLDL-C particle, smaller, more cholesterol rich particles, namely intermediate-density lipoprotein cholesterol (IDL-C) are formed. The liver removes IDL-C from circulation or it is converted into LDL-C cholesterol by LPL. LDL-C transports cholesterol to peripheral tissues for the formation of cell membranes and the synthesis of steroid hormones. LDL-C receptors or apolipoprotein B/E (apo B/E) receptors, which can be found on all cell membranes, take up LDL-C. When the levels of cholesterol within the cells are high, LDL-C

receptors are down-regulated to prevent excessive uptake of cholesterol, and LDL-C remains within the circulation.

The factors that determine the LDL-C concentration in the circulation are:

- the rate of formation of VLDL-C and its conversion to LDL-C in the circulation;
- the density of LDL-C receptors on cell membranes; and
- hepatic LDL-C receptor density.

2.2.2 HDL-C

Higher HDL-C and lower non-HDL-C levels are independently associated with lower IHD mortality (Prospective Studies Collaboration, 2007). Increase of cholesterol in peripheral cells can be caused by reduced circulating levels of HDL-C, because of reverse cholesterol transport action, during which cholesterol is transported back from peripheral tissues to the liver for oxidation and removal. This leads to down-regulation of the LDL-C receptors on cell membranes, reducing the rate of uptake of LDL-C from the circulation (Riccardi *et al*., 2003).The pathways entail:

- Cells secrete excess cholesterol in the free unesterified form, which is first taken up by the precursor HDL-C particle. A fatty acid is removed and transferred to cholesterol on HDL-C by the action of lecithin cholesterol acyl transferase (LCAT). A more stable and hydrophobic cholesterol ester is formed, which migrates to the more hydrophobic HDL-C core. The liver can remove the HDL-C molecule that is enriched with a cholesterol ester.

- The cholesterol ester is transferred from HDL-C onto the TAG-rich particles, VLDL-C and chylomicrons and TAG is transferred back onto the HDL-C particles. This transfer is catalysed by cholesterol ester transfer protein (CETP). When hepatic receptors take up VLDL-C and chylmicron particles, the liver rapidly removes the transferred cholesterol ester via high-throughput remnant receptors as well as by LDL-C receptors.

2.2.3 TAG

Initial epidemiological research proved a significant correlation with increased TAG and CHD risk (Gotto *et al*. 1977). Later research like that of Criqui *et al*. (1993) proved TAG to be an independent risk factor for CVD.

The following mechanisms for hypertriglyceridaemia are described by Riccardi and colleagues (2003):

In the fasting state, serum TAG is carried in the VLDL-C fraction, and two factors determine serum levels of VLDL-TAG, specifically rates of hepatic secretion of VLDL-TAG and one's capacity for hydrolysing circulating TAG.

- Hypertriglyceridaemia can be caused by hepatic overproduction of VLDL-TAG which can occur in two ways. First, the total number of VLDL-C particles secreted by the liver can be increased; in this case, amounts of both VLDL-TAG and VLDL-apoB entering the circulation are increased, but the overall composition of VLDL-C particles remain normal. Alternatively the TAG content of each VLDL-C particle is increased, but not the total number of particles synthesised. Diet-induced hypertriglyceridaemia theoretically could occur by either mechanism.

- Another reason for hypertriglyceridaemia is defective lipolysis of TAG-rich lipoproteins. Lipolysis of VLDL-TAG can result from abnormalities or deficiencies in either of lipoprotein lipase and hepatic TAG-lipase: A genetic deficiency of apo C-II (activator of lipoprotein lipase), or possibly abnormalities in TAG-rich lipoproteins that make them poor substrates for lipolytic enzymes. Diet could adversely affect any of these processes in various ways to raise TAG levels.

Higher levels of TAG and chylmicron remnants in the postprandial period are linked with higher risk of CHD. The mechanisms include:

- Direct atherogenic effects of chylmicron and VLDL-C remnant particles: monocytes can take up chylmicron and VLDL-C remnants that are enriched with cholesterol and form foam cells found in the atherosclerotic lesion.

- Reduced levels of HDL-C and increased levels of small, dense LDL-C: TAG levels lead to changes in LDL-C and HDL-C. This happens via excessive transfer of TAG onto HDL-C and LDL-C particles via the CETP-catalysed reaction. TAG undergoes hydrolysis by hepatic lipase when LDL-C and HDL-C obtain large amounts of TAG from the TAG-rich lipoproteins. Small, dense LDL-C and HDL-C are formed when TAG is removed from LDL-C and HDL-C. Smaller and denser HDL-C particles are more rapidly catabolised by the liver, which results in lower levels of circulating HDL-C. Small and dense LDL-C particles on the other hand remains in the circulation much longer due to the fact that it is poorly recognised by the normal LDL-C receptor, it is also more able to penetrate the endothelium and lead to atherogenesis.

- Rapid conversion of the factor VII into its active form (VIIa) is dependent on lipolytic activity and mainly supported by large TAG-rich lipoprotein (Silveira *et al*., 1996).

2.2.4 Current recommendations regarding the blood lipid profile

The National Cholesterol Education Program Expert Panel in its Adult Treatment Panel (ATP) III (NCEP, 2001), guidelines uses Framingham to estimate risk scores and then base LDL-C recommendations on the risk. ATP III recommends more intensive LDL-C lowering therapy in certain groups of people than ATP I and ATP II. Persons with CHD or CHD-risk equivalents have the lowest LDL-C goal (<100 mg/dL, <2.6 mmol/L), where diabetes counts as a CHD risk equivalent because of its high risk of new CHD within 10 years. The second category consists of persons with multiple (2+) risk factors, this group has 10-year risk for CHD of 20%. The LDL-C cholesterol goal for persons with multiple (2+) risk factors is <130 mg/dL (<3.4 mmol/L). The third group has less than two risk factors and, with few exceptions, persons in this category have a 10-year risk <10%. Their LDL cholesterol goal is <160 mg/dL (4.13 mmol/L).

The third joint task force of European and other societies for cardiovascular disease prevention in clinical practice (De Backer *et al*., 2003) recommended that TC should be below 5mmol/l (190mg/dL) and LDL-C should be below 3 mmol/l (115 mg/dL). When patients have clinically established CVD and for patients with diabetes, however, treatment goals for TC should be below 4.5 mmol/l (175 mg/dL) and for LDL-C less than 2.5 mmol/l (100 mg/dL). Fasting TAG should be less than 1.7 mmol/l (150 mg/dL) and HDL-C cholesterol should be higher than 1.0 mmol/l (40 mg/dL) for men and more than 1.2 mmol/l (46mg/dL) for women (**Table 2.4**). According to the South African Medical Association Dyslipidaemia Nutrition working group (2000), dietary intervention should be the first step in the treatment of dyslipidaemia, in order to maintain or achieve desirable body mass, to lower increased TC, LDL-C and TAG levels and raise HDL-C levels.

2.3 Age as risk factor for CHD

It has been suggested that the association between major types of fat and risk of CHD is modified by age (Oh *et al*., 2005), however, in the Prospective Studies Collaboration (2007), continuous positive relations were observed at all ages between TC and IHD mortality. Proportional differences in risk of cholesterol on annual IHD mortality rates decrease with age, however absolute effects are much higher at older than at younger ages. According to the NCEP step III (NCEP, 2001), being older than 45 years is considered a risk factor for men, whereas for women the risk factor is being over 55 years of age.

Table 2.2: **The ten leading underlying causes of natural deaths by age in 2006 in South Africa**

Causes of death on the tenth revision, international Classification of disease, 1992	0-14			15-49			50-64			65+		
	Rank	Number	%	Rank	Number	%	Rank	Number	%	Rank	Number	%
Intestinal infectious diseases	1	14 366	19.6	3	17 810	6.2	8	3 727	3.6	10	3 258	2.3
Influenza and pneumonia	2	10 455	14.2	2	28 232	9.9	2	6 600	6.4	5	7 410	5.2
Respiratory and cardiovascular diseases specific to the perinatal period	3	6 465	8.8	…	…	…	…	…	…	…	…	…
Tuberculosis **	4	2 637	3.6	1	57 660	20.1	1	11 888	11.6	9	4 594	3.2
Malnutrition	5	2 186	3.0	…	…	…	…	…	…	…	…	…
Disorders linked to gestation and foetal growth	6	1 914	2.6	…	…	…	…	…	…	…	…	…
Certain disorders involving the immune mechanism	7	1 524	2.1	4	12 295	4.3	…	…	…	…	…	…
Respiratory and cardiovascular disorders specific to the perinatal period	8	1 407	1.9	…	…	…	…	…	…	…	…	…
Human immunodeficiency [HIV] disease	9	1 235	1.7	5	11 976	4.2	…	…	…	…	…	…
Protozoal diseases	10	1 208	1.6	…	…	…	…	…	…	…	…	…
Other viral diseases	…	…	…	6	8 927	3.1	…	…	…	…	…	…
Inflammatory disease of the central nervous system	…	…	…	7	6 684	2.3	…	…	…	…	…	…
Other forms of heart disease	…	…	…	8	6 610	2.3	5	5623	5.5	2	13 729	9.6
Cerebrovascular diseases	…	…	…	9	4 167	1.5	3	6 215	6.0	1	14 730	10.3
Other acute lower respiratory tract diseases	…	…	…	10	3 678	1.3	…	…	…	…	…	…
Diabetes mellitus	…	…	…	…	…	…	4	6 205	6.0	3	10 440	7.3
Chronic lower respiratory diseases	…	…	…	…	…	…	6	4 536	4.4	6	7 408	5.2
Ischaemic heart disease	…	…	…	…	…	…	7	3 837	3.7	4	7 507	5.2
Hypertensive diseases	…	…	…	…	…	…	9	3 412	3.3	7	7 353	5.1
Malignant neoplasms of the digestive tract	…	…	…	…	…	…	10	3 164	3.1	8	4 706	3.3
	…	…	…	…	…	…	…	…	…	…	…	…
Other natural causes		24655	33.6		90 755	31.7		41 935	40.8		58 439	40.8
Non natural causes		5334	7.3		37 506	13.1		5 654	5.5		3 808	2.7
All causes		73 413			286 300			102 796			143 382	

Excluding 1293 cases with unspecified age. **Including cases with multidrug resistant (MDR) and extremely drug resistant (XDR) tuberculosis (TB). …Category not in top 10 natural underlying causes of death.

(Stats SA, 2008)

In South Africa, IHD was not listed as one of the 10 highest underlying causes of natural death below the age of 49 years in 2006 (**Table 2.2**), however, it is the 7th leading cause for people between 50 and 64

years of age (3.7% of deaths within this age category), and the 4th highest cause of death after the age of 65 years (5.7%of deaths within this age category). Cerebrovascular diseases is listed as the 9th cause for death in 15 – 49 year olds (1.5%) in south Africa, the 3rd leading cause in 50 – 64 year olds (3%) and the number one leading underlying natural cause of death in over 65 year olds(10.3%) (STATS SA, 2008).

2.4 Gender as risk factor for CHD

It has been suggested that the association between major types of fat and risk of CHD is modified by sex (Oh *et al*., 2005). Results of different clinical trials are diverse with respect to the effect of gender on blood lipid profiles. Women have a smaller response to reduced fat diets on TC and LDL-C, but not in HDL-C or TAG than men of similar age and under conditions of stable weight (Obarzanek *et al*., 2001). Kronmal and colleagues (1993) found after analyses that median cholesterol values peaked at about age 50 years for men vs. 60 years for women, yet women had higher TC levels at ages 50 – 80 years, suggesting that men are more prone to hypercholesterolaemia, than women at ages younger than 50 years, after which cholesterol levels in women were higher. According to Mahan and Escott-stump (2000), the incidence of premature CHD risk in men aged 35 – 44 years is three times as high as the incidence in women of the same age. Cerebrovascular diseases was regarded the 5th leading underlying cause of death for both males and females in South Africa in 2006 (**Table 2.3**), accounting for 3.4% of male deaths and 4.9% of female deaths. IHD was the 8th cause for men and not listed as one of the top ten causes for women (STATS SA, 2008).

Table 2.3: Ten leading underlying natural causes of death in 2006 in South Africa by gender

Causes of death (Based on the Tenth Revision, International Classification of Disease, 1992)	Males			Females		
	Rank	Number	%	Rank	Number	%
Tuberculosis **	1	41 985	13.7	1	34 896	11.7
Influenza and pneumonia	2	25 176	8.2	2	27 442	9.2
Intestinal infectious diseases	3	17 827	5.8	3	21 261	7.1
Other forms of heart disease	4	11736	3.8	4	14 835	5.0
Cerebrovascular diseases	5	10474	3.4	5	14 745	4.9
Chronic lower respiratory diseases	6	9254	3.0	10	6 552	2.2
Diabetes mellitus	7	7 620	2.5	6	11 912	4.0
Ischaemic heart disease	8	7 607	2.5	…	…	…
Certain disorders involving the immune mechanism	9	6 967	2.3	7	8 738	2.9
Human Immunodeficiency [HIV] disease	10	6 854	2.2	8	7 893	2.6
Hypertensive diseases	…	...	…	9	7 833	2.6
Other natural causes		121 289	39.5		130 083	43.5
Non-natural causes		39 860	13.0		12 614	4.2

Excluding 1 704 cases with unspecified sex ** Including MDR-TB (353 for males and 251 for females) and XDR-TB (3 for females) … Category not in top ten leading underlying natural causes of death.

(Stats SA, 2008)

2.5 Overweight and obesity as risk factor for CHD

The effects of obesity on blood lipids are believed to be more related to overnutrition, than to obesity itself (Grundy & Denke, 1990). It should be kept in mind that BMI does not distinguish between fat, muscle and bone mass; however BMI can be a good epidemiological marker. Overweight and obesity contributes to higher initial TC and LDL-C (Glaner *et al*., 2010), the condition may stabilise over time, with increased uptake of LDL-C into adipose tissue, through an increase in receptor numbers as proposed mechanism. Obesity also causes low HDL-C (Grundy *et al*., 2004). The absolute effects of cholesterol on annual IHD mortality rates are somewhat greater for obese than for non-obese individuals as obesity is associated with increased risk of CHD (Prospective Studies Collaboration, 2007). Glaner and researchers (2010), however, did not find significant differences between overweight and obesity with regard to blood lipid markers, and therefore concluded that an increase in percentage body fat above normal values

should be regarded as a cause of concern before it reaches obesity. A possible mechanism for higher cholesterol concentrations in overnourished obese individuals is hepatic overproduction of lipoproteins containing apo B-100, reflected by the increased production of VLDL-apo B and LDL-apo B, however it can simply be due to increased intake of SFAs and cholesterol, both of which will suppress LDL-C receptor activity (Sheperd *et al*. 1980). It should therefore be possible for overnourished obese individuals to have both increased production of apo B-containing lipoproteins and reduced activity of LDL-C receptors; as a result they will have two factors acting simultaneously to raise LDL-C levels.

2.6 Effect of fat, fatty acids and cholesterol on the blood lipid profile

Fat, fatty acids and cholesterol influence the blood lipid profile in different ways, and therefore the effect of total fat, fatty acids and cholesterol will be explored in the literature in order to highlight the differences and effects.

Table 2.4: Recommendations regarding the amount of energy from dietary fat (%), fatty acids (%) and cholesterol intake (mg/d)

Variable	American Heart Association (AHA)			NCEP Step III
	General	CHD	Metabolic syndrome	General
Energy from total fat (%)	25 – 35%	30%	±35%	25-35%
Energy from SFA (%)	<10%	<7%	<10%	<7%
Energy from PUFA (%)	10%	10%	10%	≤10%
Energy from MUFA (%)	10%	15 – 20%	15 – 20%	≤20%
Cholesterol mg/dL	<300	<200	<200	<200

Saturated fatty acid (SFA), Polyunsaturated fatty acid (PUFA), Monounsaturated fatty acid (MUFA).

(Lichtenstein *et al*., 2006; NCEP step III, 2001)

2.6.1 Cholesterol

Research disagrees about the effect of dietary cholesterol on the TC and LDL-C. Some researchers like Zanni and colleagues (1987) proved a rise in serum total cholesterol levels with increase in intake of dietary cholesterol, however, most food sources high in saturated fat are also sources of dietary cholesterol. Cholesterol-rich food that are relatively low in saturated fatty acid content namely egg yolks and shrimps have smaller combined adverse effects on cholesterol levels, due to the fact that even though LDL-C increases, HDL-C increases to a greater extent (De Oliveira e Silva *et al*., 1996). Vorster and colleagues (1987) found that the rural black population in South Africa were able to handle high habitual intake of cholesterol from eggs without adverse disturbance of serum cholesterol homeostasis. An inter-individual heterogeneity response to dietary cholesterol does exist (Zanni *et al*., 1987), which tend to go together with responsiveness to saturated fat in people with normal cholesterol intake (Katan *et al*., 1988). Epidemiological studies like the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al*. 1997) found no significant association between the intake of cholesterol and CHD risk. Research by Packard and colleagues (1983) indicated that raising cholesterol intake causes both an increase in production rate for LDL-C and a decrease in fractional catabolic rate (FCR) for LDL-C, through receptor independent mechanisms. According to Grundy and Denke (1990), the proposed mechanism by which LDL-C increase is by suppression of LDL-C receptor activity. VLDL-C is partially removed by LDL-C receptors. Conversion of VLDL-C results into formation of LDL-C (Riccardi *et al.* 2003). Decreased LDL-C receptor activity can therefore cause increased production rate of LDL-C. The quantitative relation between cholesterol intake and serum cholesterol levels is a matter of dispute. Keys and colleagues (1965) reported that Δ Cholesterol. $= 1.5(Z_2 - Z_1)$, where the subscripts refer to the diets compared and Z is the square-root of the dietary cholesterol, measured as mg/1000 Cal, while other researchers, like Hegsted and his team (1965), reported a linear relationship.

The NCEP step III (2001) diet suggested an intake of less than 200 mg cholesterol per day, while the American Heart Association (AHA) diet and lifestyle recommendations (Lichtenstein *et al*., 2006) supports this recommendation for patients with established CHD, however, recommends less than 300mg/d as general guideline (**Table 2.4**).

2.6.2 Total fat

Early research by Hegsted and colleagues (1965) suggested that dietary fat between 22 and 40% of energy does not affect serum cholesterol, suggesting that the type of fatty acids in the dietary fat is of more importance than the amount of dietary fat. Results from Meksawan *et al.* (2004) agree that total fat

does not have an effect on TC and LDL-C. Epidemiological studies reported diverse results regarding this topic. Results were confirmed by a 20 year follow up in the Nurses' Health study which did not find total fat intake as a percentage of energy as a significant risk for CHD due to opposing effects of specific types of fat (Oh *et al*., 2005; Hu *et al*., 1997). The Strong Heart Study (Xu *et al*, 2006), however, concluded that CHD death in middle aged Indian Americans are due to quantity and quality of dietary fat, however, the results were confounded by SFAs. The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study (Laaksonen *et al.*, 2005) found no association between dietary total fat intake, and CVD mortality, and concluded that dietary fat quality seems more important than fat quantity in the reduction of CVD mortality.

In a report from the Institute of Medicine (2005), it was concluded that the acceptable macronutrient distribution range (AMDR) for total fat has been estimated at 20 to 35 percent of energy. Both the NCEP step III (2001) diet and the AHA diet and lifestyle recommendations (Lichtenstein *et al*., 2006) supports this percentage, however, when CHD is present fat intake should be restricted to a maximum of 30% of energy (**Table 2.4**). Neither an adequate intake (AI), nor a recommended daily allowance (RDA) is set for total fat, due to insufficient data to determine a defined level of fat intake at which risk of inadequacy or prevention of chronic disease occurs (Institute of Medicine, 2005).

Low fat, high CHO diets may modify the metabolic profile unfavourably in terms of CHD when compared to higher fat intakes, causing reduction in high density lipoprotein cholesterol concentration and an increase in serum TAG concentration. However, strong evidence that low fat diets predispose to CHD does not exist (Institute of Medicine, 2005), and very low fat diets may be deficient in essential fatty acids (Meksawan *et al*., 2004).

2.6.3 Fatty acids

2.6.3.1 SFA

The majority of dietary SFAs come from animal products such as meat and dairy products. Major dietary SFAs range in chain length from 8 to 18 carbon atoms. These are:

- 8:0 Caprylic acid;
- 10:0 Caproic acid;
- 12:0 Lauric acid;
- 14:0 Myristic acid;
- 16:0 Palmitic acid; and
- 18:0 Stearic acid (Institute of Medicine, 2005).

Intake of SFA's is directly related to plasma cholesterol levels and therefore mortality from CAD (Riccardi *et al.*, 2003). A reduction in SFA intake may be of greater importance than total fat intake, in order to lower TC and LDL-C concentration (McNamara *et al.*, 1987). Epidemiology studies, however, differ in conclusion about the effect of SFA on CHD risk. The Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al*., 1997) found no significant association between the intake of saturated fatty acids and CHD risk, possibly due to random misclassification of dietary exposures or subjects changing their intake after commencement of the study. The Strong Heart study (Xu *et al*., 2006) found a positive correlation between SFA's and CHD and the Nurses' Health Study also associated higher SFA intake with increased CHD risk (Hu *et al*., 1997).There is a positive linear trend between total SFA intake and total and LDL-C concentration and increased risk of CHD (Institute of Medicine, 2005).

Reduction of SFA in the diet, and replacing it with either MUFAs or PUFAs, lead to significant decreases in serum total and LDL-C, however also lowers HDL-C (Wahrburg *et al*., 1992). The degree of the effect of specific SFAs also varies with regards to blood lipid values. A diet high in stearic acid (C18:0) does not raise serum cholesterol levels, however, it lowers LDL-C when compared to diets enriched with palmitic or myristic acid and lauric acid (Tholstrup *et al*., 2004; Grande *et al*., 1970). Rhee and colleagues (1997) concluded that 14% of stearic acid is desaturated and converted to the monounsaturated fatty acid (MUFA), oleic acid, which might explain why dietary stearic acid has metabolic effects closer to those of oleic acid rather than those of other long-chain SFAs (Institute of medicine, 2005). Conversion contributes only a small amount however, with regards to its effect on blood lipids, considering that dietary recommendation strife to reduce consumption of SFA's below 10% of energy (Rhee *et al*., 1997). The hypercholesterolaemic effect of palmitic acid (C16:0) is more than that of lauric acid (C12:0) and myristic acid (C14:0) is more hypercholesterolaemic than palmitic acid, however, part of the effect can be attributed to an increase in HDL-C (Denke & Grundy, 1992). More recent data suggests that palmitic acid is 'conditionally' hypercholesterolaemic if the linoleic acid (LA) intake (C18:2n-6) is not adequate (Clandinin *et al*., 2000). At low levels of dietary LA (2% of energy), increased intake of palmitic acid results in significant increases in TC and LDL-C. Even though further research is needed to establish at what combination of LA and SFA's a beneficial effect occurs, LA at 10% of energy is adequate to promote decreased LDL-C levels. Clandinin and colleagues (2000) further concluded that the endogenous rate of cholesterol synthesis was not affected by either palmitic acid or LA content of the diet, despite an effect of these nutrients on lipoprotein cholesterol levels. The mechanism by which SFA's causes increased LDL-C levels is by suppressing receptor mediated clearance of LDL-C (Sheperd *et al*., 1980).

Neither an AI nor RDA is set for SFAs because of the fact that SFA's are synthesized by the body to provide an adequate level needed for their physiological and structural functions and because they have

no known role in preventing chronic diseases (Institute of Medicine, 2005). No upper limit (UL) is set for saturated fatty acids because any increase in saturated fatty acid intake results in increased CHD risk (Institute of Medicine, 2005). The AHA diet and lifestyle recommendations (Lichtenstein *et al*., 2006) suggests a SFA intake of less than 10% of energy, while the NCEP step III (2001) diet recommends less than 7% of intake from SFA's as part of the therapeutic lifestyle approach to reduce CHD risk (**Table 2.4**).

2.6.3.2 MUFA

Cis MUFA's have one double bond with the hydrogen atoms present on the same side of the double bond and plant sources rich in *cis* MUFA's are canola oil, olive oil, and the high oleic safflower and sunflower oils. MUFA's are present in foods with a double bond located at 7 (*n*-7) or 9 (*n*-9) carbon atoms from the methyl end. MUFA's that are present in the diet include (Institute of Medicine, 2005):

- 18:1*n*-9 Oleic acid;
- 14:1*n*-9 Myristoleic acid;
- 16:1*n*-9 Palmitoleic acid;
- 18:1*n*-7 Vaccenic acid;
- 20:1*n*-11 Eicosenoic acid; and
- 22:1*n*-13 Erucic acid.

Oleic acid (n-9, *cis*18:1) is the most common monounsaturated fatty acid in the diet and accounts for about 92% of dietary MUFA's (Institute of Medicine, 2005).

CVD research became very interested in MUFA's, due to results of the Seven Countries Study which proved that the Mediterranean region, consuming a diet high in fat of which the main source is oleic acid, presented with low CHD incidences (Keys *et al*., 1986). The Lyon Diet Heart study (De Lorgeril *et al*., 1999), a secondary prevention trial, confirms the major protective effect of the Mediterranean diet against MI and cardiovascular complications. Epidemiological studies concluded diverse results regarding the effect of MUFA's on CHD. The Strong Heart study (Xu *et al*., 2006) correlated MUFA's intake with CHD in American Indians, however, the main source of MUFA's documented in the study was animal sources, therefore likely confounded with SFA's. The Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al*. 1997) and Nurses' Health Study (Hu *et al*., 1997) documented an inverse association between the intake of *cis*-MUFA's and LA and coronary death. In the KIHD Study (Laaksonen *et al.,* 2005) MUFA intake was not associated with CVD or overall mortality, which was reasoned to be possibly attributable to SFA intake.

Oleic acid reduces LDL-C levels when substituted for SFA's in the diet. It does not appear to reduce HDL-C like high-LA diets or high-CHO diets, it also does not raise TAG levels when substituted for SFA's like high-carbohydrate diets do (Grundy, 1987). If 10% of the dietary energy derived from SFA were replaced by MUFA, LDL-C would decrease by 0.39 mmol/L (15 mg/dL) (Riccardi *et al.,* 2003). Grundy (1987) speculated that the most likely mechanism for the reduction in LDL-C when MUFA's are substituted for SFA's might be enhancement in activity of LDL-C receptors. It is important to keep in mind that MUFA's do not necessarily stimulate the synthesis of LDL-C receptors, but when SFA's that suppress the activity of LDL-C receptors are removed, receptor activity may increase.

Neither an AI nor an RDA is set for MUFA's, due to the fact that they are synthesized by the body and therefore are not required in the diet, and also because they have no known independent beneficial role in human health. There is insufficient evidence to set an UL for *n*-9 *cis* MUFA's (Institute of Medicine, 2005). The AHA diet and lifestyle recommendations (Lichtenstein *et al*., 2006) propose an intake of 10% of energy as MUFA's, while the NCEP step III (2001) diet recommends no more than 20% of intake as MUFAs (**Table 2.4**).

2.6.3.3 Polyunsaturated fatty acids (PUFA's)

i) Omega 6 (n-6) PUFA

Omega 6 (n-6) PUFAs are characterised by the presence of at least two carbon-carbon double bonds, with the first bond at the sixth carbon from the methyl terminus (Harris *et al*., 2009**).** The major n-6 PUFA's in the diet are:

- 18:2 LA
- 18:3 γ-LA
- 20:3 Dihomo-γ-LA
- 20:4 Arachidonic acid
- 22:4 Adrenic acid
- 22:5 Docosapentaenoic acid (Institute of Medicine., 2005)

Of these fatty acids linoleic acid (C18:2n-6) is the primary dietary n-6 fatty acid in the diet, and accounts for 85% to 90% of the dietary omega-6 PUFA (Harris *et al*., 2009).

The Cross-sectional Family Heart study (Djoussé *et al*., 2001) found an inverse relation between reported intake of LA and CAD, however, the study had several limitations. Epidemiological studies vary in conclusion about the effect of PUFA's on CHD. Cohort studies like 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 LA or n-6 PUFA intake and CHD risk, while in others like the KIHD Study (Laaksonen *et al.*, 2005), and the Nurses' Health Study with 20 years of follow-up (Oh *et al*., 2005; Hu *et al*., 1997), an inverse relationship between PUFA intake and CHD risk was found.

Mattson and Grundy (1985) found oleic and LA equally effective in lowering TC and LDL-C in normotriglyceridaemic patients, before which LA was believed to be more hypocholesterolaemic than oleic acid (Becker *et al.*, 1983). Mensink and Katan (1989) then conducted a larger study using solid food, which also found LA not to be more effective in lowering TC and LDL-C than oleic acid. Howard and colleagues (1995) proposed that higher proportions PUFA's of an NCEP step I diet, resulted in a greater decrease in TC as well as a lowering effect on TAG, than did the inclusion of MUFA's. Vega and colleagues (1982) concluded that the number of LDL-C particles in circulation was reduced by PUFA's, and responses varied from patient to patient. PUFA's, however, lower LDL-C by more than one mechanism. Mostly effects are due to reduction in cholesterol content of LDL-C and an increase in its fractional clearance rate. It could also be due to decreased synthesis of LDL-C or modification in composition of LDL-C particles, which causes reduced capacity for cholesterol transport (Shepherd *et al*. 1980). High intakes of LA reduce HDL-C (Grundy, 1987; Shepherd *et al*., 1980). Lower quantities of LA in the diet (less than 10 – 13% of total energy), does not have the same HDL-C lowering properties, possibly due to the fact that the change is too small to detect in lower quantities (Grundy, 1987). PUFA's also lower TAG and VLDL-C (Shepherd *et al*., 1980). Vega and colleagues (1982) determined that PUFA's reduced every constituent of VLDL-C significantly, without changing the composition.

Dietary recommendations for n-6 PUFAs are aimed at providing optimal intakes to reduce risk for chronic disease, particularly CHD. LA cannot be synthesized by humans, and exact minimum requirements have not been established for healthy adults (Harris *et al*., 2009). However, the Institute of Medicine's (2005) Food and Nutrition Board, in their Dietary Reference Intake Report for Energy and Macronutrients suggested an AI for LA based on the median intake in the United States. The AI is 17 g/d for young men and 12 g/d for young women. There is insufficient evidence to set an UL for n-6 PUFA's. The NCEP step III diet (2001) recommends PUFA consumption of up to 10%, noting that there are no large populations that have consumed large quantities of PUFA's for long periods. However, randomised trials in humans have shown reduced CHD risk with n-6 PUFA intakes of 11% to 21% of energy for up to 11 years with no evidence of harm. The AHA supports an n-6 PUFA intake of at least 5% to 10% of energy in the context of other AHA lifestyle and dietary recommendations, and advises against reducing n-6 PUFA intake with the aim to reduce the ratio of n6:n3 intake (Harris *et al*., 2009). If 10% of the dietary energy derived from SFA's were replaced by n-6 PUFA, LDL-C would decrease by 0.42 mmol/l (18 mg/dl) (Riccardi *et al.*, 2003).

ii) Omega 3 (n-3) PUFA

n-3 PUFA's tend to be highly unsaturated with one of the double bonds located at three carbon atoms from the methyl end. This group includes:

- 18:3 alpha-linolenic acid (ALA);
- 20:5 Eicosapentaenoic acid (EPA);
- 22:5 Docosapentaenoic acid (DHA); and
- 22:6 Docosahexaenoic acid (Institute of Medicine, 2005).

Predominant n-3 fatty acids in fish oils are EPA and DHA, while ALA is the major plant source of n-3 fatty acids. ALA is not synthesized by humans and therefore is an essential fatty acid. Modest increased intakes of long-chain n-3 PUFA results in pronounced cardiovascular benefits, however, a decreased risk in cardiovascular mortality is probably due to the beneficial effect of n-3 PUFA on thrombosis or on cardiac arrhythmias rather than on lipoprotein profile (Riccardi *et al.,* 2003). Results for TC and LDL-C levels vary between studies. Bronsgeest-Schoute and colleagues (1981) suggested an increase in LDL-C, while Sirtori and research team (1992) found a decrease in TC. n-3 fatty acids lowered serum TAG (Sirtori *et al*., 1992) and VLDL-C levels (Bronsgeest-Schoute *et al*., 1981). Bronsgeest-Schoute and colleagues (1981) further indicated that no change in total TC or HDL-C was noted after supplementation with n-3 fatty acids. Omega-3 PUFA lowers postprandial TAG, but has a greater decreasing effect on fasting TAG. ALA may not have an equivalent TAG lowering effect. The mechanism of TAG lowering involves the inhibition of hepatic TAG synthesis and secretion of VLDL from the liver.

The AI for ALA 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 UL for n-3 fatty acids. Approximately 10% of the AMDR for ALA can be consumed as EPA and/or DHA (Institute of Medicine, 2005). Other recommendations for long-chain n-3fatty 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 DRI for the individual long-chain n-3 fatty acids (20 carbons or greater) since the majority of recommendations have been issued on the basis of amount of EPA and DHA together, without recommendations for specific fatty acids (Kris-Etherton *et al*., 2009).

2.6.3.4 *Trans* fatty acids

Tran fatty acids are unsaturated fatty acids and have at least one double bond in the *trans* configuration. The larger bond angle of the *trans* double-bond configuration results in a more extended fatty acid carbon chain more similar to that of saturated fatty acids. Dietary sources include cookies, chips crackers. Pies, margarine and the major *trans* fatty acid is elaidic acid (9-*trans* 18:1). The Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen *et al.*, 1997) and Nurses' Health Study (Oh *et al*., 2005; Hu *et al*., 1997) found significant association between the intake of *trans* fatty acids and CHD risk. Oh and colleagues (2005) attributed it to the adverse effect on blood lipids, including concentrations of LDL-C, HDL-C, TAG, and lipoprotein(a); LDL-C particle size; endothelial function; insulin resistance; and thrombosis. However, the Strong Heart Study (Xu *et al.*, 2006) did not find any association between *trans* fatty acid intake and CHD incidence. *Trans* fatty acids significantly raise LDL-C (Riccardi *et al.*, 2003; Lichtenstein *et al*., 2006), however, to a lesser extent than SFA's (Judd *et al*., 1994). *Trans* fatty acids have an additional negative effect, in the fact that it may result in slight reductions of HDL-C (Judd *et al*., 1994; Riccardi *et al.*, 2003). Judd *et al*. (1994) did not find a significant HDL-C lowering effect with a *trans* fatty acid intake of 3% of energy. However, with a *trans* fatty acid intake of 6.6% of TE, HDL-C was slightly lowered, compared to oleic acid and SFA diets. The total effect of trans fatty acids causes an increase in LDL:HDL ratio, increasing the risk of CHD (Riccardi *et al.*, 2003). The AHA recommends a *trans* fatty acid intake of less than 1% of energy (Lichtenstein *et al*., 2006).

Trans-fatty acids do not provide any known benefit to health and are non-essential. Therefore, no AI or RDA is set. It is recommended that *trans*-fatty acid consumption be as low as possible while consuming a nutritionally adequate diet. An UL is not set for *trans*-fatty acids because any increase in *trans*- fatty acid intake increases CHD risk (Institute of Medicine, 2005)

This chapter summarised the relevant literature regarding the nutrition transition, dietary fat intake and blood lipid values. In the next chapters these factors will be investigated using the PURE data on rural and urban subjects in the North-West Province of South Africa.

C H A P T E R 3

REASEARCH METHODOLOGY

3.1 Design

The present study is a cross-sectional data analysis nested within the PURE baseline study, investigating the effect of transition on blood lipid profiles of the African population in the North West province in South Africa. The PURE study is a large-scale epidemiological study, which aimed to recruit 150,000 adults, initially aged 35 – 70 years from communities in low-middle and high-income regions of the world (Teo *et al*., 2009). The PURE study is an investigator-led study that is funded through a variety of sources including the Canadian Institutes of Health Research, Heart and Stroke Foundation Ontario, grants from several pharmaceutical companies, and grants from various governmental granting bodies in different countries (Appendix B).

3.2 Selection of communities in PURE

As documented by Teo *et al*. (2009), within each country, urban and rural communities have been selected based on broad guidelines.

In South Africa the rural community (A) was identified 450 km west of Potchefstroom on the highway to Botswana. A deep rural community (B), 35 km east from A and only accessible by a gravel road, was also included. Both communities are still under tribal law. The urban communities (C&D) were chosen near to Potchefstroom due to financial constraints. Community C was selected from the established part of the township next to Potchefstroom and D from the informal settlements surrounding community C.

Rural

- Urban rural (A = Ganeysa)
- Very rural (B = Tklagameng)

Urban

- Established urban (C = Ikageng)

- Squatter camps (D = Sonderwater, ext 7 &11)

3. 3 Selection of households and individuals

Selection methods of households and individuals that were used in the PURE international study were published by Teo *et al.* (2009). Baseline data for South Africa were collected during 2005. A randomly selected household census regarding number of individuals, their ages and health profile was done in 6000 houses (1500 in each community) starting from a specific point. The aim of the study and procedures were explained and the head of each household gave written consent to fill out the questionnaire. If a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out.

3.4 Inclusion criteria:

A paper selection was made of all possible subjects who met the inclusion criteria:

- Adults over 35 years of age
- Healthy: not using any medication for chronic disease and/no **known** condition/disease
- Migration stable

3.5 Data collection

During August until the end of November 2005, an appointment with each person who completed the questionnaire was made, and they were voluntarily picked up by taxi and brought to a team of expert researchers, where they again gave informed consent for the measurement of anthropometry and a blood sample to be taken. They were asked to fast for approximately 10 hours. A total of 2000 subjects showed up and were tested (approximately 500 from each community). Data on these 2000 subject's physical activity levels and habitual diets were also obtained by questionnaires. Everyone was tested for human immunodeficiency virus (HIV), but was given the choice whether they wanted to know their status or not. However, everyone received pre-test counselling in groups of 10 persons before the blood sample was taken and post-test counselling was done while giving the results to individuals before going home. Every individual identified with an abnormality regarding tested markers, was referred to the nearest clinic or hospital. All the questionnaires and home visits were done by 16 intensively trained fieldworkers

from the four different communities. Each fieldworker was responsible for 125 subjects for the next 12 years.

For the interpretation purposes of this study, data was stratified for rural and urban only, with no additional sub-division into community A, B C or D.

Standardised interviewer-based questionnaires were used to collect detailed information at the community, family and individual level. In South-Africa the quantitative food frequency questionnaire (QFFQ) that was also used in the Transition and Health during Urbanisation of South Africans (THUSA) study, was used also used in the PURE study. The QFFQ is a culture sensitive questionnaire comprising of 145 food items, developed for the THUSA study (MacIntyre *et al*., 2000a). Respondents were helped to estimate portion sizes by being shown photographs of commonly eaten foods in a validated food portion photograph book, common utensils and containers. The relative validity of the QFFQ was tested by MacIntyre *et al*. (2000b) against a seven-day weighed record, and the reproducibility was proved by MacIntyre and colleagues (2000a). Portion sizes were reported in household measures and were converted to weights using standard tables. The food intake was coded using the new food codes of the South African food composition database of the South African Medical Research Council and expressed as average amounts consumed per day

3.6 Measurements on individual level

3.6.1 Physical measures

The PURE study set out to do physical examination of which anthropometric measures (weight and height) were used in the current study, and BMI was calculated. The formula for BMI :

- BMI (kg/m^2) = weight (kg) ÷.height^2 (m^2).

In accordance with popular literature categories for presentation of CVD risk, subjects for the present study were presented three age groups (35-44; 45-54; ≤50 years).

BMI of subjects were presented in the following BMI-categories: Underweight (BMI<18.5), Normal weight (BMI=18.5-24.9), overweight (BMI= 25-29.9) and obese (BMI≥30).

3.6.2 Blood samples

The PURE study intended to take a 10mL fasting blood sample collected from all consenting participants (Teo *et al*., 2009). In South Africa a 90mL blood sample was taken: Stasis-free fasting (12 hours) blood samples were collected into vacutainer glass tubes from the antecubital vein of a participant using a 21-gauge butterfly in order to analyse the TC, TAG and HDL-C. Blood samples were centrifuged and transferred to centralised long-term storage in secure -70°C freezers or large -180°C liquid nitrogen tanks for future biochemical and genetic testing.

The levels of serum TC, TAG and HDL-C were measured by Sequential Multiple Analyzer Computer (SMAC), using the KonelabTM auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland) (Stojanovic *et al.*, 2005). LDL-C was calculated using the Friedewald-Levy-Fredrickson formula (Roberts, 1988). The following reference values in **Table 3.2** were used as indicators for elevated blood lipid values.

Table 3.2: Reference values for lipoprotein concentrations (mmol/L)

Variable (mmol/L)	Normal value
TC	<5
LDL-C	<3
HDL-C (Male)	≥1
HDL-C (Female)	≥1.2
TAG	<1.7

(De Backer *et al*., 2003)

3.7 Statistical analysis

The data collected was statistically analysed with the SPSS program (SPSS Inc., 2009) and the SAS program (SAS Institute, 2009). Descriptive statistics (means, standard deviations and proportions) were calculated.

One-way analysis of variance (ANOVA) was used to determine differences between the different levels of urbanisation on blood lipid profiles and dietary intake. When a dietary intake variable proved to be significant for different levels of a factor (urbanisation, blood lipid profile), **post-hoc tests** were

calculated to determine which levels for specific variables differed significantly. **Bonferroni-type adjustments** were made for the multiple comparisons.

Spearman correlations were calculated. A p-value of less or equal to 0.05 was regarded as being statistically significant.

Blood lipid profiles were described in terms of age, gender, body mass index (BMI) and dietary intake.

3.8 Ethical aspects and permission

Ethical approval for the PURE study was obtained from the North West University (Project number: 04M10).

CHAPTER 4

RESULTS

4.1 Subjects

The rural population consisted of 47% males and 52% females, while the urban population consisted of 54% males and 49% females. The mean age of subjects in rural areas was 48.78 ± 9.94 years and the mean age in urban areas was 50.22 ± 10.68 years. Mean BMI of rural subjects was 24.17 ± 6.61 kg/m², while mean BMI of subjects in urban areas was 25.17 ± 7.30 kg/m².

4.2 Energy, macronutrient, cholesterol and fatty acid intake

The differences in mean energy, macronutrient, cholesterol and fatty acid intake of subjects living in rural and urban areas are indicated in **Table 4.1**. Mean energy consumption was lower in rural areas than in urban areas (6410.29 ± 2778.22 kJ vs. 9426.73 ± 4036.51kJ $p<0.001$). A higher percentage of energy was derived from fat in the urban areas than in the rural area, with 19.15 ± 6.96 % of energy from fat in the rural diet compared to 26.38 ± 6.46% in the urban diet.

In the rural population, 65.17 ± 9.02 % of energy originated from CHO compared to 55.96 ± 6.99% of energy in the urban areas. Percentage energy from protein was higher in urban areas (10.97 ±1.95 % of energy) than in rural areas (12.54 ± 1.82 % of energy).

Total fat intake was more than double in urban areas (67.16 ± 33.78 g, $p<0.001$), as opposed to in rural areas (32.56 ± 17.66 g) ($p<0.001$). SFA intake was higher amongst urban subjects (16.99 ± 9.29 g) than rural subjects (7.23 ± 4.95g) ($p<0.001$), but so was MUFA (19.24 ± 10.89g vs. 7.63 ± 5.15g) and PUFA (19.44 ± 10.88g vs. 10.83 ± 7.55g).

Intake of all the individual fatty acids, trans-fatty acids, total n-3 and total n-6 fatty acids was also significantly higher in urban areas, compared to rural areas (**Table 4.1**).

Table 4.1: Mean reported energy, macronutrient, cholesterol and fatty acid intake in rural and urban areas

Variable	Rural (n=966)			Urban (n=984)			p-value
Energy (kJ)	6410.29	±	2778.22	9426.73	±	4036.51	< 0.001
Total fat (g)	32.56	±	17.66	67.16	±	33.78	< 0.001
SFA (g)	7.23	±	4.95	16.99	±	9.29	< 0.001
MUFA (g)	7.63	±	5.15	19.24	±	10.89	< 0.001
PUFA (g)	10.83	±	7.55	19.44	±	10.88	< 0.001
Trans fatty acids (g)	0.14	±	0.20	0.68	±	0.74	< 0.001
Cholesterol (mg)	121.64	±	124.01	252.69	±	154.47	< 0.001
Protein (g)	41.22	±	18.22	68.94	±	30.19	< 0.001
Total CHO (g)	243.97	±	101.60	309.92	±	137.26	< 0.001
Energy from total fat (%)	19.15	±	6.96	26.38	±	6.46	< 0.001
Energy from SFA (%)	4.26	±	2.60	6.72	±	2.22	< 0.001
Energy from MUFA (%)	4.43	±	2.39	7.54	±	2.55	< 0.001
Energy from PUFA (%)	6.24	±	3.02	7.60	±	2.49	< 0.001
Energy from total CHO (%)	65.71	±	9.02	55.96	±	6.99	< 0.001
Energy from total protein (%)	10.97	±	1.82	12.54	±	1.95	< 0.001
n-6 (g)	10.44	±	6.51	18.92	±	10.65	< 0.001
n-3 (g)	0.26	±	0.18	0.54	±	0.30	< 0.001
n-6:n-3	83.23	±	45.43	58.47	±	22.94	< 0.001
C14:0 (g)	0.52	±	0.53	1.15	±	0.78	< 0.001
C16:0 (g)	3.47	±	2.32	9.09	±	4.98	< 0.001
C18:0 (g)	2.07	±	1.47	4.96	±	2.80	< 0.001
C18:2 n-6(g)	9.71	±	6.57	18.32	±	10.62	< 0.001
C18:3 n-3(g)	0.13	±	0.12	0.33	±	0.17	< 0.001
C20:4 (g)	0.04	±	0.03	0.11	±	0.07	< 0.001
C20:5 n-3 (g)	0.04	±	0.03	0.07	±	0.06	< 0.001
C22:6 n-3 (g)	0.07	±	0.06	0.12	±	0.11	< 0.001

$p \leq 0.05$ was considered to be significant. 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 (C18:3n-3), Arachidonic acid (C20:4n-6), Eicosapentaenoic acid (C20:5n-3), Docosahexaenoic acid (C22:6n-3).

4.3 Differences in blood lipid profiles between rural and urban areas

Mean TC, LDL-C or HDL-C did not differ significantly (**Table 4.2**) between rural and urban areas. TAG was significantly ($p<0.001$) lower in rural (1.21 ± 0.64 mmol/L) than in urban areas (1.38 ±0.91 mmol/L). More than forty percent of rural (43%) and urban (47%) subjects presented with elevated (≥5.0 mmol/L) total cholesterol. More than half of rural (52%) and of urban (55%) subjects had elevated LDL-C (≥3.0 mmol/L). In rural areas 23% of and 34% of females males had decreased levels of HDL-C (<1 mmol/l for males and <1.2 for females). In urban areas, 18% of males and 39% of females had decreased HDL-C levels. Only 16% of rural subjects and 23% of urban subjects presented with high TAG levels (≥1.7 mmol/L).

Table 4.2: Mean blood lipid profiles of rural and urban subjects

Variables	n	Rural	n	Urban	p-value
Serum TC (mmol/L)	956	4.97 ± 1.36	934	5.05 ± 1.39	0.178
% Elevated TC (≥ 5 mmol/L)		42.78%		47.00%	
Mean LDL (mmol/l)	946	3.22 ±1.23	932	3.25 ±1.23	0.537
% Elevated LDL (≥ 3 mmol/l)		52.22%		55.36%	
Males serum HDL-C (mmol/L)	335	1.55 ± 0.66	376	1.61 ± 0.67	0.220
% Reduced HDL-C (< 1 mmol/L)		23%		18.1%	
Females serum HDL-C (mmol/L)	621	1.50 ± 0.61	558	1.46 ± 0.63	0.255
% Reduced HDL-C (< 1.2 mmol/L)		34.3%		38.5%	
Serum TAG (mmol/L)	946	1.21 ± 0.64	932	1.38 ±0.91	<0.001
% Elevated TAG (≥ 1.7 mmol/L)		16.28%		22.96%	

p≤ 0.05 was considered to be significant. Total Cholesterol (TC), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL-C), Triacylglycerol (TAG).

4.4 Associations between dietary fat intake and blood lipid profiles

As seen in **Table 4.3** TC, LDL-C and TAG were associated positively with higher intakes of SFA, MUFA, cholesterol, n-3, C14:0, C16:0, C18:0, C18:3 and C20:4, however, associations were very weak. Higher TAG was also positively associated with higher intakes of C18:3. Additionally, higher TC and TAG were also associated positively with intake of total fat, PUFA, *trans* fatty acids, n-6 and C18:2, while higher TC-levels and HDL-C were associated with higher C20:5 and C22:6 intakes.

Table 4.3: Associations between dietary fat, fatty acid and cholesterol intake and blood lipid profiles

Variable	TC (n=1853)		LDL –C (n=1823)		HDL-C (n=1853)		TAG (n=1823)	
	r	p-value	R	p-value	r	p-value	r	p-value
Total fat (g)	0.06	0.015	0.04	0.065	0.04	0.133	0.07	0.003
SFA (g)	0.06	0.012	0.06	0.019	0.01	0.720	0.08	<0.001
MUFA (g)	0.06	0.013	0.05	0.040	0.02	0.447	0.08	<0.001
PUFA (g)	0.05	0.048	0.03	0.198	0.05	0.051	0.05	0.021
Trans fatty acids(g)	0.05	0.038	0.04	0.092	<0.01	0.849	0.07	0.001
Cholesterol (mg)	0.08	0.001	0.06	0.014	0.05	0.026	0.07	0.004
n-6 (g)	0.05	0.030	0.04	0.156	0.05	0.028	0.07	0.007
n-3 (g)	0.10	<0.001	0.07	0.002	0.07	0.003	0.08	0.001
C14:0 (g)	0.05	0.024	0.06	0.009	-0.01	0.605	0.07	0.004
C16:0 (g)	0.06	0.007	0.06	0.017	0.01	0.641	0.09	<0.001
C18:0 (g)	0.06	0.010	0.05	0.042	0.02	0.395	0.08	<0.001
C18:2 n-6 (g)	0.05	0.042	0.03	0.162	0.04	0.059	0.06	0.011
C18:3 n-3 (g)	0.07	0.002	0.07	0.002	<-0.01	0.848	0.12	0.000
C20:4 (g)	0.09	<0.001	0.06	0.009	0.07	0.003	0.06	0.007
C20:5 n-3 (g)	0.08	0.001	0.04	0.063	0.11	<0.001	0.01	0.776
C22:6 n-3 (g)	0.07	0.003	0.03	0.206	0.11	<0.001	0.01	0.800

$p \leq 0.05$ was considered to be significant. aturated 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 n-6), Alpha linolenic acid (C18:3 n-3), Arachidonic acid (C20:4), Eicosapentaenoic acid (C20:5 n-3), Docosahexaenoic acid (C22:6 n-6).

Total fat and fatty acid intake did not differ significantly between subjects with elevated LDL-C levels and normal LDL-C levels within the same level of urbanisation (**Table 4.4**). However, with every variable, intake was significantly higher in urban areas than rural areas for subjects with elevated LDL-C levels as well as for those with normal LDL-C levels. The exact same trend was found for TC, TAG and HDL-C in males with regards to fat, fatty acid and cholesterol intake (Data not shown). As noted in **Table 4.5**, HDL-C for females followed the same trend with the exceptions that in urban areas total fat (68.81± 34.05 g vs.61.24 ± 33.05 g, $p<0.0001$), PUFA (20.10 ± 1.05 g vs. 17.77 ± 11.51 g, $p<0.0001$), n3 (0.56 ± 0.30 g vs. 0.47 ± 0.29 g, $p<0.0001$), C20:4 (0.11 ± 0.06 g vs.0.09 ± 0.06, $p<0.0001$), C:20:5 (0.07 ± 0.06 g vs.0.05 ± 0.05, $p<0.0001$), C22:6 (0.12 ± 0.11 g vs.0.09 ± 0.09, $p<0.0001$) were significantly higher in the group that had normal HDL-C compared to those with decreased HDL-C.

Table 4.4: Dietary total fat, cholesterol and fatty acid intake in rural and urban areas, for subjects with normal LDL-C and subjects with elevated LDL-C respectively

Variable	LDL-C ≥ 3 mmol/L			LDL-C < 3 mmol/L		
	Rural (n=474)	Urban (n=509)	p-value	Rural (n=434)	Urban (n=406)	p-value
Total fat (g)	33.35 ± 18.08	66.88 ± 34.20	<0.0001	31.83 ± 17.28	65.52 ± 33.60	<0.0001
SFA (g)	7.45 ± 5.22	17.19 ± 9.46	<0.0001	6.99 ± 4.64	16.29 ± 9.22	<0.0001
MUFA (g)	7.85 ± 5.23	19.25 ± 11.25	<0.0001	7.43 ± 5.05	18.64 ± 10.54	<0.0001
PUFA (g)	11.03 ± 7.36	19.11 ± 10.95	<0.0001	10.67 ± 7.88	19.19 ± 10.76	<0.0001
Trans fat (g)	0.14 ± 0.21	0.69 ± 0.75	<0.0001	0.14 ± 0.19	0.63 ± 0.71	<0.0001
Cholesterol (mg)	125.69 ± 109.72	249.13 ± 146.27	<0.0001	118.99 ± 141.86	251.54 ± 167.30	<0.0001
n-6 (g)	10.78 ± 7.07	18.52 ± 10.73	<0.0001	10.06 ± 5.87	18.73 ± 10.50	<0.0001
n-3 (g)	0.29 ± 0.17	0.56 ± 0.31	<0.0001	0.27 ± 0.19	0.53 ± 0.31	<0.001
C14:0 (g)	0.54 ± 0.59	1.19 ± 0.79	<0.0001	0.49 ± 0.44	1.09 ± 0.77	<0.0001
C16:0 (g)	3.60 ± 2.39	9.13 ± 5.06	<0.0001	3.35 ± 2.25	8.79 ± 4.97	<0.0001
C18:0 (g)	2.12 ± 1.49	4.99 ± 2.86	<0.0001	2.01 ± 1.45	4.77 ± 2.76	<0.0001
C18:2 n-6 (g)	10.07 ± 7.09	17.98 ± 10.69	<0.0001	9.37 ± 5.99	18.07 ± 10.47	<0.0001
C18:3 n-3 (g)	0.14 ± 0.10	0.33 ± 0.17	<0.0001	0.13 ± 0.14	0.31 ± 0.17	<0.0001
C20:4 (g)	0.04 ± 0.03	0.11 ± 0.07	<0.0001	0.04 ± 0.04	0.11 ± 0.07	<0.0001
C20:5 n-3 (g)	0.04 ± 0.04	0.07 ± 0.06	<0.0001	0.04 ± 0.03	0.06 ± 0.06	<0.0001
C22:6 n-3 (g)	0.07 ± 0.06	0.12 ± 0.11	<0.0001	0.07 ± 0.06	0.12 ± 0.11	<0.0001

p≤ 0.05 was considered to be significant. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega 6 (n-6), omega 3 (n-3), polyunsaturated: saturated fatty acid ratio (PS ratio), Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Linoleic acid (C18:2 n-6), Alpha linolenic acid (C18:3 n-3), Arachidonic acid (C20:4), Eicosapentaenoic acid (C20:5 n-3), Docosahexaenoic acid (C22:6 n-3).

Table 4.5: Dietary total fat, cholesterol and fatty acid intake in rural and urban areas, for females with normal HDL-C and females with elevated HDL-C respectively

Variable	HDL-C ≥ 1.2 mmol/L			HDL-C < 1.2 mmol/L		
	Rural(n=397)	Urban (n=341)	p-value	Rural (n=200)	Urban (n=206)	p-value
Total fat (g)	32.95 ± 18.28	68.81± 34.05[a]	<0.0001	32.46 ± 17.80	61.24 ± 33.05[a]	<0.0001
SFA (g)	7.25 ± 4.72	17.36 ± 9.33	<0.0001	7.33 ± 5.57	16.03 ± 8.88	<0.0001
MUFA (g)	7.59 ± 5.05	19.62 ± 10.78	<0.0001	7.70 ± 5.04	17.89 ± 10.50	<0.0001
PUFA (g)	11.24 ± 8.66	20.10 ± 1.05[b]	<0.0001	10.78 ± 7.14	17.77 ± 11.51[b]	<0.0001
Trans fat (g)	0.15 ± 0.23	0.75 ± 0.84	<0.0001	0.14 ± 0.20	0.68 ± 0.70	<0.0001
Cholesterol (mg)	122.29 ± 129.70	248.76 ± 138.67	<0.0001	119.15 ± 93.22	219.88 ± 144.19	<0.0001
n-6 (g)	10.84 ± 6.79	19.62 ± 10.69	<0.0001	10.03 ± 6.73	17.56 ± 11.38	<0.0001
n-3 (g)	0.27 ± 0.15	0.56 ± 0.30[c]	<0.0001	0.29 ± 0.26	0.47 ± 0.29[c]	<0.0001
C14:0 (g)	0.52 ± 0.48	1.18 ± 0.78	<0.0001	0.54 ± 0.65	1.12 ± 0.72	<0.0001
C16:0 (g)	3.45 ± 2.28	9.23 ± 4.96	<0.0001	3.47 ± 2.28	8.51 ± 4.78	<0.0001
C18:0 (g)	2.07 ± 1.39	5.01 ± 2.77	<0.0001	2.06 ± 1.61	4.66 ± 2.64	<0.0001
C18:2 n-6 (g)	10.15 ± 6.86	18.93 ± 10.76	<0.0001	9.44 ± 6.72	16.92 ± 11.23	<0.0001
C18:3 n-3 (g)	0.13 ± 0.08	0.33 ± 0.17	<0.0001	0.15 ± 0.19	0.30 ± 0.17	<0.0001
C20:4 (g)	0.04 ± 0.03	0.11 ± 0.06[d]	<0.0001	0.04 ± 0.03	0.09 ± 0.06[d]	<0.0001
C20:5 n-3 (g)	0.04 ± 0.04	0.07 ± 0.06[e]	<0.0001	0.04 ± 0.04	0.05 ± 0.05[e]	0.34
C22:6 n-3 (g)	0.07 ± 0.06	0.12 ± 0.11[f]	<0.0001	0.07 ± 0.06	0.09 ± 0.09[f]	0.05

$p \leq 0.05$ was considered to be significant. Means with the same symbol between groups differed significantly, a=0.0082, b=0.0412, c=0.0003, d=0.0003, e=0.0005, f=0.0006. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega 6 (n-6), omega 3 (n-3), polyunsaturated: saturated fatty acid ratio (PS ratio), Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Linoleic acid (C18:2 n-6), Alpha linolenic acid (C18:3 n-3), Arachidonic acid (C20:4), Eicosapentaenoic acid (C20:5 n-3), Docosahexaenoic acid (C22:6 n-3).

4.5 Blood lipid profiles at different ages, BMI's and genders in rural and urban areas

4.5.1 Age and blood lipid profiles

Age correlated positively with TC (r=0.16, p<0.001), LDL-C (r=0.14, p<0.001), HDL-C (r=0.06, p=0.010) and TAG (r=0.20, p<0.001) (**Table 4.6**).

Table 4.6: Associations between age and BMI respectively and blood lipid profile

Variable	TC			LDL-C			HDL-C			TAG		
	n	r	p-value	n	r	p-value	n	r	p-value	n	r	p-value
Age	1890	0.16	<0.001	1878	0.14	<0.001	1890	0.06	0.010	1878	0.20	<0.001
BMI	1783	0.19	<0.001	1771	0.31	<0.001	1783	-0.24	<0.001	1771	0.29	<0.001

p≤ 0.05 was considered to be significant

TC (p<0.01) **(Figure 4.1)**, LDL-C (p<0.01) **(Figure 4.2)** and TAG (p<0.01) **(Figure 4.4)** differed significantly between age groups (ANOVA). Post Hoc tests proved that TC of rural subjects in the 35-44 years category was significantly lower than TC of subjects in the older category (45-54 years) (4.83 ± 1.37 mmol/L vs. 5.14 ± 1.34 mmol/L, p=0.0162) and the oldest category (≥55 years) (4.83 ± 1.37 mmol/L vs. 5.37 ± 1.25 mmol/L, p=0.0407) **(Table 4.7)**. In urban areas TC of subjects in the youngest group (35-44 years) was significantly lower than those in the oldest group (4.93 ± 1.40 mmol/L vs. 5.74 ± 1.34 mmol/L, p<0.0037). Between the age categories 35-44 years and 45-54 years, rural subjects differed significantly from each other with regards to LDL-C (3.08 ± 1.2 mmol/L vs. 3.41 ± 1.25 mmol/L, p<0.0023). LDL-C for urban subjects in the ≥55 years category, was significantly higher than the 44-54 years (3.68 ± 1.19 mmol/L vs. 3.22 ±1.21 mmol/L, p=0.0136) and 35-44 years (3.68 ± 1.19 mmol/L vs. 3.19 ± 1.24 mmol/L, p<0.0036) categories respectively. Within urban areas TAG levels were higher in older subjects. The combination of age and urbanisation has an effect onTAG (p<0.01) **(Figure 4.4)** (ANOVA). Post Hoc tests proved a significant difference in TAG **(Table 4.7)** between rural subjects of the 35-44 years and the 45-54 years groups (1.11 ± 0.54 mmol/L vs. 1.4 ± 0.77 mmol/L, p<0.0001). In urban areas TAG of the subjects in the age group ≥55 years was significantly higher than those in the 45-54 years group (1.68 ± 0.98 mmol/L vs. 1.39 ± 0.78 mmol/L, p=0.0161) and the 35-44 years group (1.68 ± 0.98 mmol/L vs. 1.31 ± 0.97 mmol/L, p<0.0002). ANOVA analysis further illustrated a combined effect of urbanisation and age on LDL-C (p=0.04) **(Figure 4.2)**, HDL cholesterol (p=0.01) **(Figure 4.3)**.

In the age group 33-45 years the percentage of subjects with increased TC were 33% of males and 37% of females in rural areas, and 43% of males and 38% of females in the urban areas. The percentage presenting with elevated LDL-

C was 35% of males and 51% females in rural areas and 48% of males and 54% of females in urban areas. Twenty four percent of males and 36% of females in rural areas and 21% of males and 45% of females in urban areas had decreased HDL-C. In rural areas, 15% of males and 14% of females presented with elevated TAG levels, while 17% of male and 19% of females in urban areas had elevated TAG levels.

In the age group 45-54 years, 32% of males and 50% of females in rural areas and 45% of males and 56% of females in urban areas presented with elevated TC levels. Forty four percent of males and 58% of females in rural areas and, 48% of males and 61% of females in urban areas had elevated LDL-C. Twenty four percent of males and 32% of females in rural areas and 19% of males and 36% of females in urban areas had decreased HDL-C. In rural areas, 7% of males and 20% of females presented with elevated TAG levels, while 19% of males and 34% of females in urban areas had elevated TAG levels (Data not shown).

In the age group >55 years, 49% of male- and 58% of female subjects in rural areas and 38% of male- and 59% of female subjects in urban areas presented with elevated TC. In rural areas, 59% of males and 69% of females had elevated LDL-C levels, while 40% of males and 72% of females in urban areas had elevated LDL-C. Nineteen percent of male- and 34% of female subjects in rural areas and 13% of males and 32% of females in urban areas presented with decreased levels of HDL-C, while 19% of males and 30% of females in rural areas and 15% of males and 37% of females in urban areas had increased TAG levels.

Urbanisation did not seem to have a significant effect on TC (ANOVA **Figures 4.1, 4.6 and 4.11**), LDL-C (ANOVA, **Figures 4.2, 4.7 and 4.12**), HDL-C (ANOVA **Figures 4.3, 4.8 and 4.13**). However, ANOVA analysis showed a difference in TAG ($p<0.01$) between rural and urban subjects **(Figure 4.4)**. TAG was significantly higher in urban areas than in rural areas **(Table 4.7)** for the age groups 35 – 44 years ($p<0.01$) and the age group ≥55 years ($p<0.01$). The only significant differences between rural and urban subjects within specific age categories were found for TAG in the 35-44 years category (1.11 ± 0.54 mmol/L vs. 1.31 ± 0.97 mmol/L, $p<0.0004$) and within the ≥55 years group (1.22 ± 0.56 mmol/L vs. 1.68 ± 0.98 mmol/L, $p<0.009$) **(Table 4.7)**.

Table 4.7: Mean blood lipid values for different ages, in rural and urban areas

Variable	35-44 years			45-54 years			≥55 years		
	Rural (n=578)	Urban (n=504)	p-value	Rural (n=314)	Urban (n=327)	p-value	Rural (n=64)	Urban (n=103)	p-value
TC (mmol/L)	4.83 ±1.37[ab]	4.93 ± 1.40[c]	1.00	5.14 ± 1.34[a]	5.10 ± 1.35	1.00	5.37 ± 1.25[b]	5.74 ± 1.34[c]	1.00
LDL-C (mmol/L)	3.08 ± 1.2[a]	3.19 ± 1.24[b]	1.00	3.41 ± 1.25[a]	3.22 ±1.21[c]	0.7290	3.54 ± 1.16	3.68 ± 1.19[bc]	1.00
HDL-C (mmol/L)	1.53 ± 0.63	1.48 ± 0.66	1.00	1.48 ± 0.61	1.60 ± 0.65	0.2075	1.59 ± 0.6	1.46 ± 0.54	1.00
TAG (mmol/L)	1.11 ± 0.54[a]	1.31 ± 0.97[b]	0.0004	1.4 ± 0.77[a]	1.39 ± 0.78[c]	1.00	1.22 ± 0.56	1.68 ± 0.98[bc]	0.0029

$p \leq 0.05$ was considered to be significant. Means with the same symbol between groups differed significantly. Total cholesterol (TC), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL-C), Triacylglycerol (TAG).

Table 4.8: Mean blood lipid values for different BMI categories, in rural and urban areas

Variable	Underweight (BMI <18.5 kg/m²)			Normal weight (BMI 18.5 – 24.9 kg/m²)			Overweight (BMI 25 – 29.9 kg/m²)			Obese (BMI ≥30 kg/m²)		
	Rural (n=185)	Urban (n= 140)	p-value	Rural (n=412)	Urban (n=342)	p-value	Rural (n=155)	Urban (n=154)	p-value	Rural (n= 179)	Urban (n=189)	p-value
TC (mmol/L)	4.47 ± 1.18[abc]	4.81 ± 1.33[e]	0.563	4.93 ± 1.35[ad]	4.94 ± 1.31	1.00	5.38 ± 1.4[bd]	5.29 ± 1.44	1.00	5.19 ± 1.35[c]	5.32 ± 1.41[e]	1.00
LDL-C (mmol/L)	2.62 ± 1.03[abc]	2.76 ± 1.19[fg]	1.00	3.15 ± 1.17[ade]	3.07 ± 1.06[hi]	1.00	3.64 ± 1.23[bd]	3.65 ± 1.23[fh]	1.00	3.62 ± 1.26[ce]	3.7 ± 1.[25gi]	1.00
HDL-C (mmol/L)	1.66 ± 0.69[a]	1.82 ± 0.76[cde]	0.621	1.56 ± 0.67[b]	1.61 ± 0.68[cfg]	1.00	1.47 ± 0.53	1.34 ± 0.53[df]	1.00	1.29 ± 0.44[ab]	1.3 ± 0.48[eg]	1.00
TG (mmol/L)	0.96 ± 0.43[ab]	1.08 ± 0.52[def]	1.00	1.16 ± 0.6[c]	1.31 ± 0.92[dgh]	0.157	1.37 ± 0.67[a]	1.54 ± 0.92[eg]	1.00	1.41 ± 0.7[bc]	1.55 ± 0.77[fh]	1.00

$p \leq 0.05$ was considered to be significant. Means with the same symbol between groups differed significantly. Total Cholesterol (TC), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL-C), Triacylglycerol (TAG).

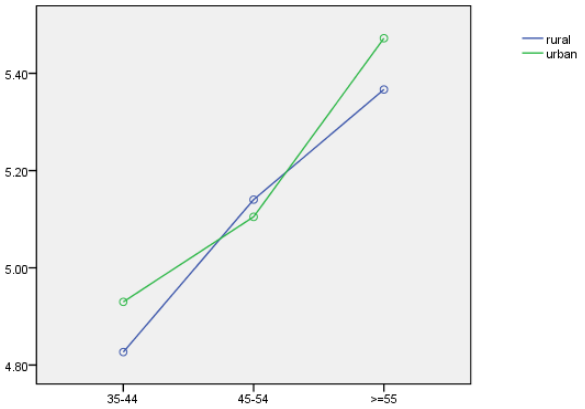

Figure 4.1: **The effect of age and urbanisation on total cholesterol**

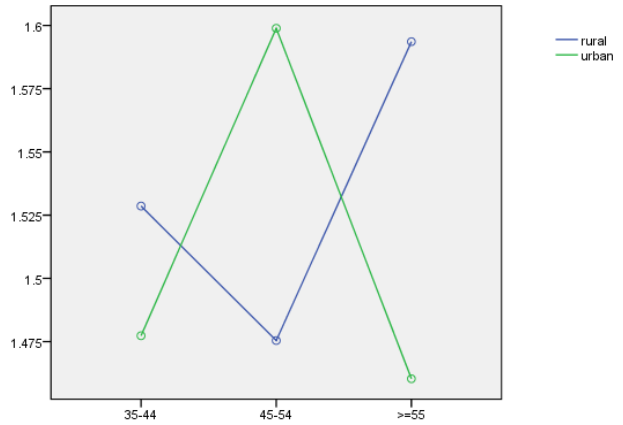

Figure 4.3: The effect of age and urbanisation on HDL-C

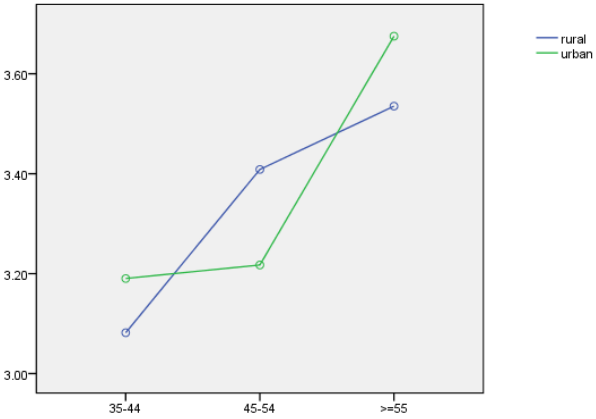

Figure 4.2: The effect of age and urbanisation on LDL-C

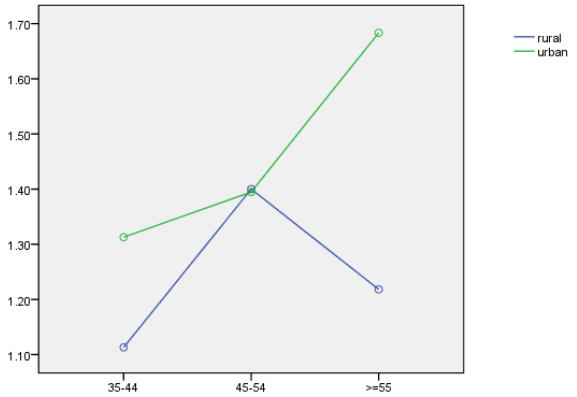

Figure 4.4: The effect of age and urbanisation on TAG

4.5.2 BMI and blood lipid profiles

In rural areas 20% population were underweight (32% of males and 14% of females), 44% fell within normal ranges (56% of males and 38% of females), while 17% were overweight (9% of males and 20% of females) and 20% obese (4% of males and 28% of females). In urban areas 16% of subjects were underweight (28% of males and 9% of females), 42% were normal (58% of males and 30% of females), 19% overweight (12% of males and 24% of females) and 23% and obese (3% of males and 37% of females) (Data not shown).

BMI correlated positively with TC ($r=0.188$, $p<0.001$), LDL-C ($r=0.308$, $p<0.001$) and TAG ($r=0.288$, $p<0.001$), and negatively with HDL-C ($r=-0.241$, $p<0.001$) (**Table 4.6**).

TC and LDL-C levels were higher with each higher BMI-category, however levels were slightly and non-significantly lower in the obese (BMI>30 kg/m²) category, than the overweight (BMI: 25-30 kg/m²) category. ANOVA analyses recognized this influence of BMI on cholesterol (**Figure 4.5**) (<0.01) and LDL-C (**Figure 4.6**) ($p<0.01$). No significant differences between rural and urban subjects within each category of BMI **(Table 4.8)** were observed for either total cholesterol, LDL-C or TAG **(Table 4.8)**. Post Hoc tests on total cholesterol levels found that subjects in rural areas in the underweight group, differed significantly from subjects with a normal weight (4.47 ± 1.18 mmol/L vs. 4.93 ± 1.35 mmol/L, $p<0.0028$) and from those who were overweight (4.47 ± 1.18 mmol/L vs. 5.38 ± 1.4 mmol/L, $p<0.0001$) and obese (4.47 ± 1.18 mmol/L vs. 5.19 ± 1.35 mmol/L, $p<0.0001$), and the normal weight and overweight groups also differed from each other (4.93 ± 1.35 mmol/L vs. 5.38 ± 1.4 mmol/L, $p=0.0001$). Amongst urban subjects total cholesterol differed between underweight and obese groups (4.81 ± 1.33 mmol/L vs. 5.32 ± 1.41 mmol/L, $p=0.0221$). For LDL-C, subjects in rural areas who were underweight had lower values than those who had a normal weight (2.62 ± 1.03 mmol/L vs. 3.15 ± 1.17 mmol/L, $p<0.0001$) or those who were overweight (2.62 ± 1.03 mmol/L vs. 3.64 ± 1.23 mmol/L, $p<0.0001$) and obese (2.62 ± 1.03 mmol/L vs. 3.62 ± 1.26 mmol/L, $p<0.0001$). The normal weight group also differed significantly from the overweight (3.15 ± 1.17 mmol/L vs. 3.64 ± 1.23 mmol/L, $p<0.0002$) and obese (3.15 ± 1.17 mmol/L vs. 3.62 ± 1.26 mmol/L, $p<0.0002$) groups. Amongst urban subjects the underweight group differed significantly from the overweight (2.76 ± 1.9 mmol/L vs. 3.65 ± 1.23 mmol/L, $p<0.0001$) and obese groups (2.76 ± 1.9 mmol/L vs. 3.7 ± 1.25 mmol/L, $p<0.0001$).

Obese subjects in rural areas had significantly lower HDL-C levels than underweight (1.29 ± 0.44 mmol/L vs. 1.66 ± 0.69 mmol/L, $p<0.0001$) and normal weight subjects (1.29 ± 0.44 mmol/L vs.1.56 ± 0.67 mmol/L, $p<0.0001$). HDL-C of the underweight subjects in the urban groups was significantly higher than that of the normal weight (1.82 ± 0.76 mmol/L vs. 1.61 ± 0.68 mmol/L, $p=0.0310$), overweight (1.82 ± 0.76

mmol/L vs. 1.34 ± 0.53 mmol/L, p<0.0001) and obese subjects (1.82 ± 0.76 mmol/L vs. 1.30 ± 0.48 mmol/L, p<0.0001), normal weight subjects in urban areas also proved to have higher HDL-C levels than those who's BMI fell in the overweight (1.61 ± 0.68 mmol/L vs. 1.34 ± 0.53 mmol/L, p<0.0001) and obese (1.61 ± 0.68 mmol/L vs. 1.30 ± 0.48 mmol/L, p<0.0001) categories. ANOVA analysis proved that BMI had a significant inverse effect on HDL-C (p<0.01) **(Figure 4.7)**, with no significant differences between rural and urban subjects within each category of BMI **(Table 4.8)**. The combination of the effect (ANOVA) of urbanisation and BMI was only significant for HDL-C (p=0.02).

With each higher BMI-category triglycerydes increased (p<0.01) **(Figure 4.8).** Post Hoc tests proved that within rural areas, underweight subjects had significantly lower TAG than overweight (0.96 ± 0.43 mmol/L vs. 1.37 ± 0.67 mmol/L, p<0.0001) and obese (0.96 ± 0.43 mmol/L vs. 1.41 ± 0.70 mmol/L, p<0.0001) subjects, while subjects with a normal weight had lower TAG levels than obese (1.16 ± 0.6 mmol/L vs. 1.41 ± 0.7 mmol/L, p=0.0032) subjects. Amongst urban subjects, those falling into the underweight category had significantly lower TAG than those falling into the normal weight (1.08 ± 0.52 mmol/L vs. 1.31 ± 0.92 mmol/L, p=0.0492), overweight (1.08 ± 0.52 mmol/L vs. 1.54 ± 0.92 mmol/L, p<0.0001) or obese (1.08 ± 0.52 mmol/L vs.1.55 ± 0.77 mmol/L, p<0.0001) categories, while normal weight subjects also had significantly lower TAG than overweight (1.31 ± 0.92 mmol/L vs. 1.54 ± 0.92 mmol/L, p=0.0321) and obese (1.31 ± 0.92 mmol/L vs. 1.55 ± 0.77 mmol/L, p=0.0065) subjects.

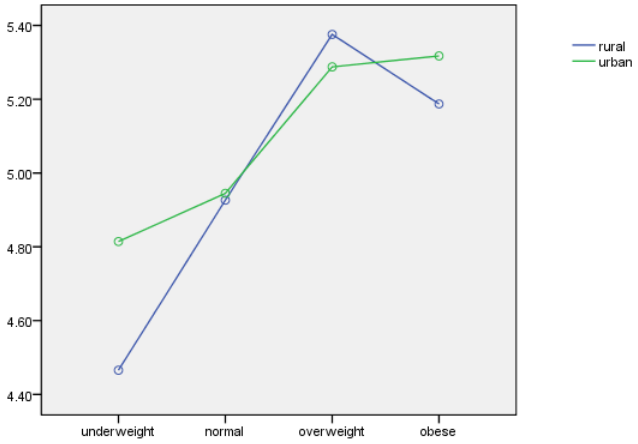

Figure 4.5: The effect of BMI and urbanisation on TC

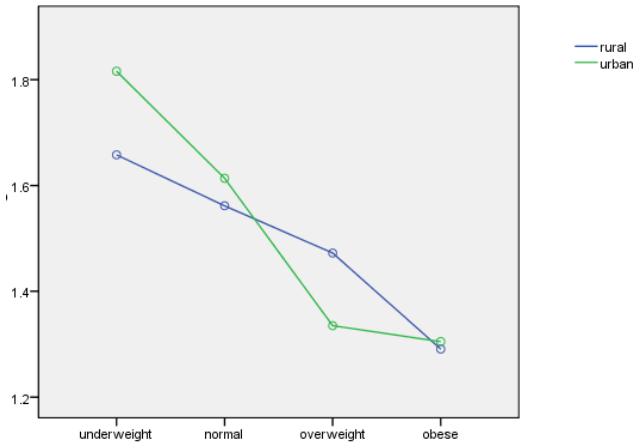

Figure 4.7: The effect of BMI and urbanisation on HDL

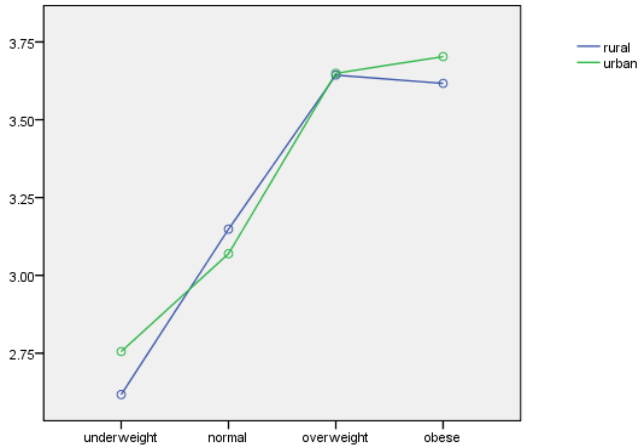

Figure 4.6: The effect of BMI and urbanisation on LDL-C

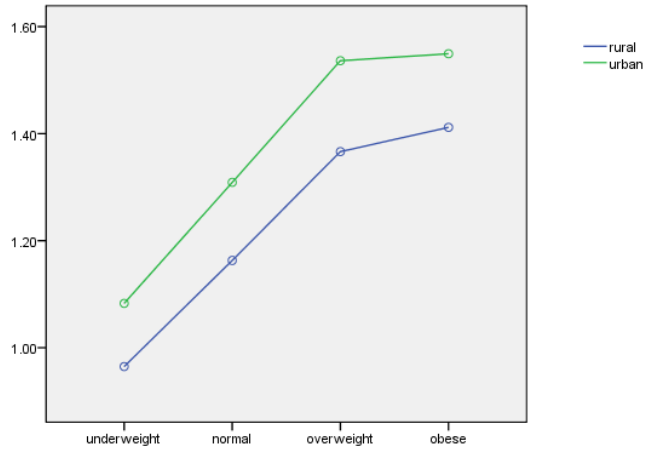

Figure 4.8: The effect of BMI and urbanisation on TAG

4.5.3 Gender and blood lipid profiles

Gender has an effect on TC ($p<0.01$) **(Figure 4.9)**, LDL-C ($p<0.01$) **(Figure 4.10)**, HDL-C ($p<0.01$) **(Figure 4.11)**, TAG ($p<0.01$) **(Figure 4.12).** Male subjects had lower cholesterol, LDL-C and TAG levels than female subjects, whereas females had lower HDL-C than males. There were no significant difference between rural and urban subjects amongst males for any of the blood lipid values. Amongst female subjects, TAG were the only paramater where a significant difference ($p<0.0001$) were noted between rural and urban areas **(Table 4.9)**. Females in rural areas had lower TAG (1.24 ± 0.62) levels than those in urban areas (1.45 ± 0.86). In rural areas, male subjects had significantly lower TC (4.73± 1.33 mmol/L vs. 5.09 ± 1.37 mmol/L, $p<0.0005$) and LDL-C (2.96 ± 1.18 mmol/L vs. 3.36 ± 1.23 mmol/L, $p<0.0001$) than female subjects. In urban areas, 41.8% of males and 50.5% of females had elevated TC, 45.5% of males and 62% of urban females had elevated LDL-C values, 17.4% of males and 30.6% of females had elevated TAG (Data not shown). In urban areas the TC (5.16 ± 1.41 mmol/L vs. 4.88 ± 1.34 mmol/L, $p=0.0120$), LDL-C (3.42 ±1.21 mmol/L vs. 3.01 ± 1.23 mmol/L, $p<0.0001$), TAG (1.45 ± 0.86 mmol/L vs. 1.27 ± 0.98 mmol/L, $p<0.0037$) were significantly higher for females than males, while HDL-C were significantly lower in female subjects than in male subjects (1.46 ± 0.63 mmol/L vs. 1.61 ± 0.67 mmol/L ,$p<0.01$) (**Table 4.9**). In rural areas 37.3% of males, and 45.9% of females had elevated TC. Elevated LDL-C were found in 44.6% of rural males and 45.5% females. Above normal TAG levels, documented in rural areas were 19.7% for females and 13.6% for males (Data not shown). Results for HDL-C stratified by gender are given in paragraph 4.3 (**Table 4.2**) because HDL-C has different cutt-off values for males and females. No significant combination effect of urbanisation and gender on any of the blood lipid values was noted either.

Table 4.9: The effect of gender on blood lipid values between rural and urban areas

Variable	Male			Female		
	Rural (n=335)	Urban (n=376)	p-value	Rural (n=621)	Urban (n=558)	p-value
TC (mmol/L)	4.73± 1.33[a]	4.88 ± 1.34[b]	0.8244	5.09 ± 1.37[a]	5.16 ± 1.41[b]	1.00
LDL-C (mmol/L)	2.96 ± 1.18[a]	3.01 ± 1.23[b]	1.00	3.36 ± 1.23[a]	3.42 ±1.21[b]	1.00
HDL-C (mmol/L)	1.55 ± 0.66	1.61 ± 0.67[a]	1.00	1.50 ± 0.61	1.46 ± 0.63[a]	1.00
TAG (mmol/L)	1.16 ± 0.67	1.27 ± 0.98[a]	0.2790	1.24 ± 0.62	1.45 ± 0.86[a]	<0.0001

$p \leq 0.05$ was considered to be significant. Means with the same symbol between groups differed significantly. Total cholesterol (TC), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL-C), Triacylglycerol (TAG).

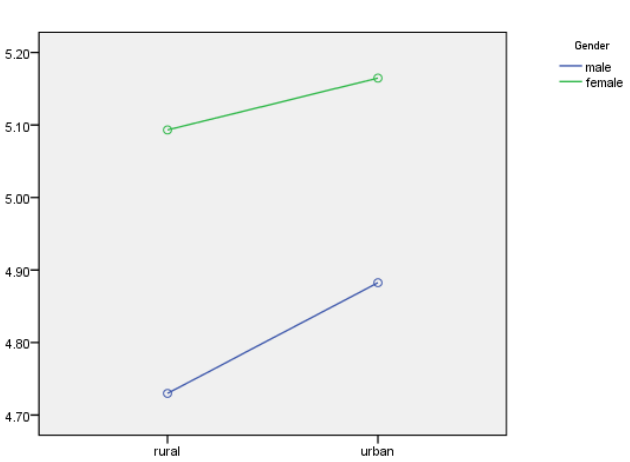

Figure 4.9: The effect of gender and urbanisation on TC

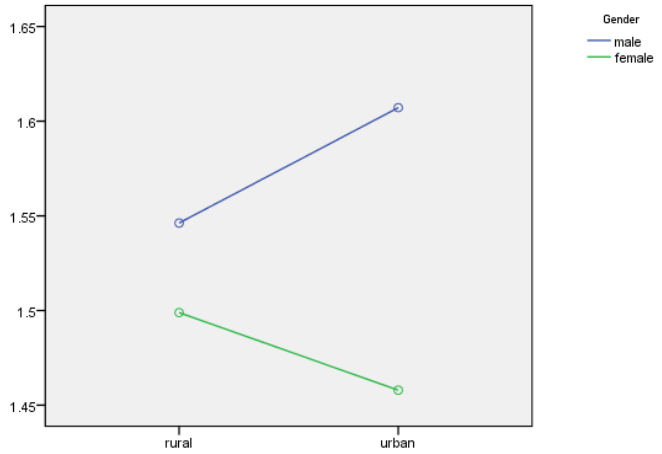

Figure 4.11 : The effect of gender and urbanisation HDL-C

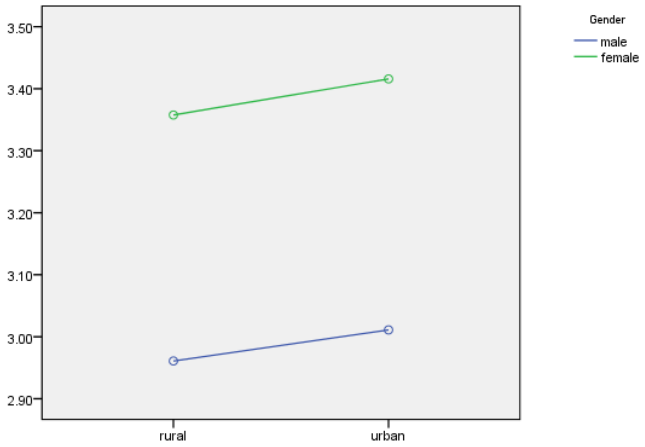

Figure 4.10: The effect of gender and urbanisation on LDL-C

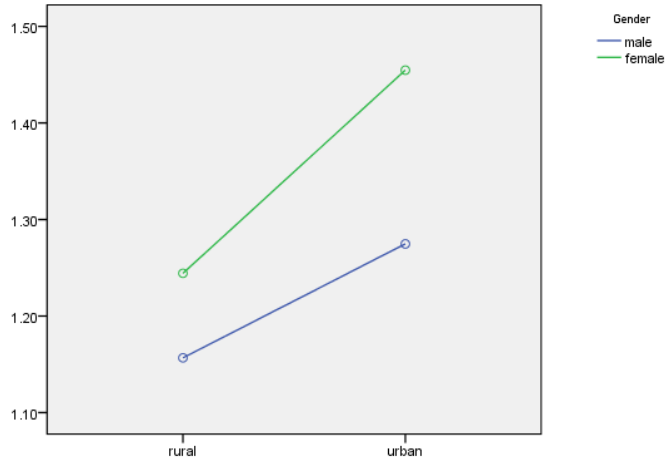

Figure 4.12: The effect of gender and urbanisation TAG

CHAPTER 5

DISCUSSION

5.1 Background

The transition from rural to urban lifestyle has been shown to increase fat intake (Popkin, 2001), and the blood lipid profile of communities undergoing such change are also influenced negatively (Vorster *et al*., 2007). An unfavourable lipid profile (increased TC, LDL-C, TAG and decreased HDL-C) is one of the known independent risk factors for CHD (Wilson *et al*., 1998; Criqui *et al*., 1993), which causes concern regarding the effect of transition. The aim of the study was to investigate how urbanisation influenced the diet of subjects in the North West Province in South Africa and to investigate the subsequent effect on blood lipid profiles. What was unique about the present study was that fat intake of subjects with normal lipid profiles was compared to intake of those with abnormal profiles in both rural and urban areas. An interesting finding of this study was that even though the fat intake differed significantly between rural and urban areas (**Table 4.1**), the blood lipid profile did not (with the exception of TAG) (**Table 4.2**), nor did intakes differ between subjects with normal lipid profiles from those with abnormal profiles in both rural and urban areas. Prior South African studies documented differences in the blood lipid profile between rural and urban areas (Vorster *et al*., 2007). Cultural influences could explain some of the differences observed when comparing data with other South African studies, however, it is suspected that poverty, household food insecurity and other factors caused by socio-economic realities, might play a more important role in nutrient intakes (Vorster *et al*., 1997a).

A prospective design, as the PURE study intended, would have been ideal for the study. However, at commencement of this study, follow up of patients in the PURE study were not completed and therefore only baseline data could be used in a cross sectional design.

5.2 Energy, macronutrient, fatty acid and cholesterol intake

Mean energy intakes (intake stratified by gender not tabled) in the current study in rural (6976.63 ± 3204.69 kJ/d for males and 6112.33 ± 2476.37 kJ/d for females) and urban (10048.83 ± 4159.76 kJ/d for males and 9013.03 ± 3901.37 kJ/d for females) areas are well below the estimated energy requirements (EER) for total energy intake (males: 12881 kJ/d, and females: 10 093 kJ/d). A comparison of earlier South African research by Vorster *et al*. (1997b) found diets of different South African ethnic groups to be mostly adequate in energy intake, with the exception of rural African women from Kwa-Zulu Natal

who had a documented lower energy intake. Energy intakes of the urban African population in the Cape Peninsula were much lower (8500 kJ for males and 6400 kJ for females) than reported in the present study (Bourne *et al*., 1993). Reported urban energy intakes in the current study compared well with more recent South African results obtained in the North West province, however, rural energy intakes were much lower (MacIntyre *et al*., 2002; Vorster *et al*., 2005). The lower reported energy intake may, in part, have been due to underreporting by respondents. The validation study (MacIntyre *et al.*, 2000b) showed that the QFFQ tended to under report energy intakes in comparison with a seven-day weighed record. Subjects from the rural areas might not have been as educated as the urban subjects and that could have caused them to respond less accurately to the QFFQ. It cannot be concluded that low values were solely the result of underreporting, however, and the possibility should be explored that the intakes in the current study was just exceptionally low, especially in rural areas, and if in future, this study is interpreted prospectively instead of cross-sectionally, it might be possible to make more definitive conclusions. Percentage energy from protein was higher in urban areas (12.54 ± 1.82%) than rural areas (10.97 ± 1.95%). The same trend was reported in previous South African research studies (Vorster *et al*., 2005).

Even though mean CHO intake was higher in urban areas than rural areas, a higher proportion of the rural diet (65.2% of energy) originated from CHO compared to the urban areas (56.0% of energy). This finding is in contrast with prior South African research by Vorster *et al*. (2005) that documented lower intake of CHO rich food in urban areas than in rural areas (48%vs. 67%). This occurrence could be linked to the lower total energy intake, since CHOs are usually the main contributor to energy intake.

International studies also confirmed that transition is accompanied by eating pattern shifts, which resulted in diets higher in fat (Popkin, 2001). Fat intake in the present study was higher in urban than in rural areas, however, mean total fat intake was still low. The same conclusion was made by Vorster and research team (2000). Currently no AI or RDA for fat exists because of insufficient data to determine a level of intake at which the risk of inadequacy or prevention of chronic disease occurs (Institute of Medicine, 2005). Even though fat intake was more than double the amount in urban (67.16 ± 33.78 g) areas than in rural (32.56 ± 17.66 g) areas (**Table 4.1**), the percentage energy from fat (26.38%) in urban areas was still within the European guidelines for cardiovascular risk prevention (De Backer *et al*., 2003) of less than 30% of energy, and the recommend 25-35% of energy by the AHA (Lichtenstein *et al*., 2006) and NCEP step III (2001). In rural areas, however, percentage energy from fat (19.15%) was below the recommended minimum. Fat and energy restricted diets can make it difficult to reach optimum intake for essential fatty acids and low fat, high CHO diets may modify the metabolic profile unfavourably in terms of CHD when compared to higher fat intakes, causing a reduction in HDL-C concentration (Meksawan *et al*., 2004). However, strong evidence that low fat diets predispose to CHD does not exist (Institute of Medicine, 2005). Higher intake of fat in urban areas (30.6 to 31.8%) than in rural areas (22.6 to 23.6% of

energy) was documented in prior South African research on the black population in South Africa (MacIntyre *et al*., 2002; Vorster *et al*., 2005). Values reflected in the present study are much lower for rural areas, but mirrored results from the BRISK study (27%) amongst the African urban population in the Cape Peninsula (Bourne *et al*., 1993). Much higher fat intakes were documented in the rural areas of the Western Cape (35% of energy) and Free State areas (36% of energy in higher socio economical groups and 40% of energy in lower socio economical groups) (Vorster *et al.*, 1997b).

SFA intake in urban areas (16.99 ± 9.29 g) was more than double that of rural areas (7.23 ± 4.95g) coinciding with the higher total fat intake and TE intake in urban areas. The % energy from SFA for both rural (4.26 ± 2.60%) and urban (6.72 ± 2.22%) groups were within the AHA recommendations (Lichtenstein *et al*., 2006), NCEP step III recommendations (2001:10) of less than 7% of energy and the European guidelines of less than a third of fat intake (De Backer *et al*., 2003). Prior South African results show a higher SFA intake of 8.8% of energy in urban areas (Bourne *et al*., 1993), however, total energy in this population was also lower than in our study and therefore increasing the percentage value attributed to fat. The absolute values for intake of SFA's, however, in the latter study were also higher (20 – 22g for males and 15 – 16g for females) in comparison with the present study. A higher intake of MUFA (4.43 ± 2.39 vs. 7.54 ± 2.55% of energy) and PUFA (6.24 ± 3.02 vs. 7.60 ± 2.49% of energy) was noticed in urban areas in comparison to rural areas in the present study. This can partially be attributed to higher total fat intake in urban areas. Neither an AI nor an RDA exists for MUFA's due to the fact that MUFA's are synthesized by the body and therefore are not required in the diet (Institute of Medicine, 2005). MUFA intake in urban and rural areas fell well within the recommendations of NCEP step III (2001) (up to 20%) and AHA (10% TE) (Lichtenstein *et al*., 2006), and urban MUFA intake was slightly lower than prior South African results (Bourne *et al.,* 1993). PUFA intake in rural and urban areas was in line with current recommendations by the National Cholesterol Education Program (2001) (up to 10% of TE), and urban PUFA intake was slightly higher in comparison with prior reported data on the South African population (Bourne *et al*., 1993).

Even though intake of all the individual fatty acids, *trans* fatty acids, total n-3 and n-6 fatty acids were significantly higher in urban areas than rural areas (**Table 4.1**), ALA, EPA and DHA intake were very low. The intake of ALA was substantially below the AI of 1.6 and 1.1 g/d for men and women respectively, in both rural and urban areas. Intake of LA was more in line with the AI, which is 17 g/d for young men and 12 g/d for young women in urban areas, but lower in rural areas. EPA and DHA intakes fell far short of the recommended combined intake of 250mg – 500mg/d (Deckelbaum *et al*., 2008). It can be difficult to consume required amounts of essential fatty acids on a low fat diet or energy restricted diet (Meksawan *et al.,* 2004). The low intake of ALA, EPA and DHA is of concern since ALA specifically is considered an essential fatty acid and is therefore not synthesized by the body.

Cholesterol intake was more than double the amount in urban areas (252.69 ± 154.47mmol/l) than in rural areas (121.64 ± 124.01mmol/l). Both rural and urban mean cholesterol intake fell within the AHA's diet and lifestyle recommendations of less than 300 mg/d (Lichtenstein *et al*., 2006), however urban cholesterol intake was higher than the recommended 200 mg by the NCEP step III guidelines (2001). Urban cholesterol intake compared well to intake of cholesterol by males of all age groups and intake of females from the younger age groups in the BRISK study (Bourne *et al*., 1993).

5.3 Differences in blood lipid profiles between rural and urban areas

The major finding in this study was that blood lipid profiles did not differ significantly between rural and urban areas, probably due to the fact that even though fat and fatty acid intake were significanly higher in urban than in rural areas, it was still within recommendations.

This study's results differed from results from the THUSA study with regards to the fact that the THUSA study found significant increases in LDL-C and TC (Vorster *et al*., 2005, Vorster *et al*., 2007) with urbanisation and in this study mean TC and LDL-C did not differ significantly (**Table 4.2**) between rural and urban areas. Another finding that sets this study apart from other studies is that, up until now, blood lipid levels of other African populations in transition were found to be within recommendations (Oosthuizen *et al*., 2002). Even though rural and urban areas did not differ significantly with regards to TC, mean TC for rural areas (4.97 ± 1.36) was still within the recommended <5 mmol/l, while in urban areas (5.05 ± 1.39) it was slightly above the recommendation. Mean LDL-C levels for both rural (3.22 ±1.23 mmol/l) and urban areas (3.25 ±1.23 mmol/l) were above the recommendation of <3 mmol/l. When the mean values are taken into context it showed that 42.8% of rural and 47.0% of urban subjects presented with elevated total cholesterol (≥5.0 mmol/L) and 52.2% of rural and 55.4% of urban subjects had elevated LDL-C (≥3.0 mmol/l). This is a cause for concern, since TC is considered to be a major independent predictor of recurrence of MI (De Lorgeril *et al*., 1999), and LDL-C is also considered an independent risk factor for coronary events (Baigent *et al.,* 2005). The high incidence of elevated TC and LDL-C could not be linked to fat and fatty acid intake in the present study since the intakes were within recommendations.

In the THUSA study HDL-C remained relatively constant between groups (Vorster *et al*., 2005) and our study also did not detect significant differences between rural and urban areas for HDL-C. For males, mean HDL-C in both rural (1.55 ± 0.66 mmol/l) and urban (1.61 ± 0.67mmol/l) areas were well above the recommended minimum of 1 mmol/l (**Table 4.2**). Female mean HDL-C levels were also above the recommended minimum of 1.2 mmol/l in both rural (1.50 ± 0.61) and urban (1.46 ± 0.63) settings, an occurrence which was also documented in the THUSA study (Oosthuizen *et al*., 2002). Reduced HDL-C proved to be a bigger problem amongst females than males in both rural and urban areas, with 23% of

rural males presenting with decreased levels of HDL-C (<1 mmol/l) and 18.1% in urban areas, while 34.3% of females had decreased HDL-C (<1.2 mmol/l) levels in rural areas and 38.5% in urban areas. Decreased HDL-C is a known independent risk factor for CVD (Wilson *et al*., 1998). Very low fat diets have proven to reduce HDL-C (Meksawan, *et al*., 2004), which could possibly explain why males presented with a higher percentage of reduced HDL-C in rural areas where fat intake was lower than in urban areas. The opposite was true for females, which could be due to high rates of obesity among urban females, which was also linked in the literature to reduced HDL-C levels (Grundy *et al*., 2004). TAG was significantly ($p<0.001$) lower in rural (1.21 ± 0.64 mmol/L) than in urban areas (1.38 ±0.91 mmol/L), which also differed from results of the THUSA study (Vorster *et al*., 2005) that did not find significant differences for TAG between rural and urban groups. Mean TAG levels were within recommendations although 16.28% of rural subjects and 22.96% of urban subjects presented with high TAG levels (≥1.7 mmol/L). Raised TAG is a known independent risk factor for CVD (Wilson *et al*., 1998).

5.4 Association between dietary fat intake (SFA, MUFA, and PUFA) and blood lipid profiles

Associations between blood lipids and dietary fat intake were extremely weak (**Table 4.3**), though highly significant. Due to the fact that higher associations were seen when data was not stratified for urbanisation, data was presented in this manner. Higher TC, LDL-C and TAG were associated with higher intakes of SFA, MUFA, cholesterol, N-3, C14:0, C16:0, C18:0, C18:3 and C20:4. Additionally higher TC and TAG were also associated with higher intake of total fat, PUFA, *trans*-fatty acids, n-6, C18:2, while higher TC-levels were associated C20:5 and C22:6. Literature links SFA (Institute of Medicine, 2005), C14:0 and C16:0 (Tholstrup *et al*., 2004; Grande *et al*., 1970) to higher TC and LDL-C levels, however, results are in contrast with results that prove C18:0 is not associated with increased TC and LDL-C (Tholstrup *et al*., 2004; Grande *et al*., 1970), nor MUFA (Grundy, 1987), PUFA (Howard *et al*., 1995) or total fat (Meksawan *et al*., 2004). Literature documented diverging results regarding the effect of dietary n-3 fatty acids (Sirtori *et al*., 1992; Bronsgeest-Schoute *et al*., 1981) and cholesterol (Zanni *et al*., 1987), and an inverse relationship between TAG and n-3 fatty acids (Sirtori *et al*., 1992). In the present study, n-6 and n-3, cholesterol, C20:4, C20:5, C22:6 were significantly and positively associated with higher HDL-C, while literature does not link n-3 fatty acids to any changes in HDL-C (Bronsgeest-Schoute *et al*., 1981).

An earlier South African study did not find significant associations between TC and risk factors like percentage energy from fat, -SFA, -PUFA, -MUFA or *trans* fatty acids (Oosthuizen *et al*., 2002). Low or weak associations between fat and blood lipids can possibly be explained by fat intakes that were still within recommendations. Vorster *et al.* (1997a) mentioned that correlations of biochemical variables with other measures of nutritional status are often poor. Kruger *et al*. (2001) asked to be cautious when

interpreting cross-sectional studies carelessly, and added that associations between variables do not necessarily imply causality as was seen in the results from our study.

The present study was unique to our knowledge, in the sense that it compared fat and fatty acid intake of subjects with abnormal blood lipid profiles to intakes of subjects with normal blood lipid profiles in both urban and rural settings. To simplify the tables only results from LDL-C and HDL-C for females were tabled in **Table 4.4** and **Table 4.5** respectively, since results for TC and TAG followed the same trend as LDL-C. Fat and fatty acid intakes from subjects with elevated TC, LDL-C, TAG and decreased HDL-C did not differ from those with normal blood lipids, within the same level of urbanisation, even though the nutrient composition of the diets did differ between rural and urban areas. It can therefore be concluded that the diet is not the deciding factor in whether or not TC, LDL-C, TAG or HDL-C in males will be normal or not in this population. This makes sense when considered that the population in this study consumed fat and fatty acids within recommendations. Urban females were the exception, because total fat, PUFA, n-3, C20:4, C20:5 and C22:6 were significantly lower in urban females with decreased HDL-C than in urban females with normal HDL-C. This could not fully be explained by the finding of Meksawan and colleagues (2004), which states that very low fat diets affects HDL-C negatively, because even though fatty acid intakes in females with normal HDL-C were higher, it was still low in comparison with recommendations, and fat intake of rural females were even lower at normal HDL-C levels. When the fat and fatty acid intake was compared between rural and urban areas, the same conclusion can be made as by Oosthuizen and colleagues (2002) in that more nutritionally adequate diets were probably eaten by urban subjects than rural subjects.

5.5 Blood lipid profiles at different ages, BMI's and genders in rural and urban areas

5.5.1 Age

Even though a higher age was significantly positively associated with TC, LDL-C, HDL-C and TAG (**Table 4.6**), the associations were weak. Total cholesterol and LDL-C increased with age, in both rural and urban areas (**Table 4.7**), a trend which was also documented for urban Africans in prior South African research (Oelofse *et al*., 1996). No significant differences were found between different levels of urbanisation within the same age groups for TC, LDL-C and HDL-C in the latter study, indicating that urbanisation does not have an effect on these blood lipids within specific age groups, as was confirmed by the current study. TAG was the exception, being significantly higher in urban areas than in rural areas for both the age groups 35-44 years and ≥55years. Possible reasons for the higher TAG could be due to higher CHO intake in urban areas, higher incidence of overweight and obesity in urban areas and possibly higher stress levels in urban areas (Meksawan *et al*., 2004). Alcohol consumption was not likely to be the cause, because no significant difference was found for alcohol consumption between rural and urban

areas (data not shown). Oelofse and team (1996) found TAG to increase dramatically with age. We could only confirm this in urban areas. In the present study, TAG peaked between 45 – 54 years of age in rural areas, and were slightly lower in the age group ≥55 years. HDL-C additionally did not differ significantly between age groups in both rural and urban areas. Literature states that age becomes a risk factor for CVD when men are older than 45 years, whereas women are considered to be at increased risk over 55 years of age (NCEP, 2001)

In both rural and urban areas the percentage of subjects with elevated TC and LDL-C were higher in each higher age category, as was to be expected, since it is clearly documented in the literature that age negatively affects cholesterol levels and CHD risk (Oh *et al*., 2005; Prospective studies collaborations, 2007). However, each time urban males older than 55 years were the exception, with had a lower percentage of subjects presenting with elevated TC and LDL-C than the age group 45-54 years. It could be possible that this age group, even though urbanised was so set in their cultural ways and therefore not so susceptable to changes in diet and lifestyle as younger age groups. There were no progressive increase or decrease with age in the percentage subjects presenting with decreased HDL-C, although the percentages did seem to decrease in older subjects. The highest percentage of decreased HDL-C occurred in urban females in the younger age category and the lowest percentage of subjects with decreased HDL-C occurred in urban males older than 55 years. In each category the percentage of rural subjects with elevated TAG were less than the percentage urban subjects with elevated TAG. Higher percentages of subjects presenting with elevated TAG tended to occur at higher age groups, with the exception of males in the age category 45-54 years of which only 7.4% presented with elevated TAG.

5.5.2 BMI

Over- and underweight both seemed to be a problem in the current study population, since less than half of the population fell within the normal BMI range (18.5 – 24.9 kg/m²) in both the rural (44%) and urban (42%) population groups. Amongst males underweight seems to be a bigger problem than overweight, with 32% of rural males and 28% of urban males presenting with a low BMI (<18.5), while overweight and obesity presented as a much bigger problem in females, with 20% being overweight and 28% obese in rural areas and 24% overweight and 37% obese in urban areas. This illustrates to a certain extend the double burden of underweight as well as obesity occurring alongside each other in South Africa (Vorster *et al*., 1997a). It is possible that factors not explored in the present study, like culture, education level, genetics, hormones, stress levels, etc., could have played a role. Culturally, for example, it is more acceptable for women to be overweight in the black population in South Africa (Mciza et al., 2005). Possible causes of obesity described by prior research on the North West population group included household income, total energy, fat intake and low physical activity (Kruger *et al*., 2001). However, fat and fatty acid intake most likely did not play a big role in the present study, due to the fact that fat and

fatty acid intake not only fell within recommendations, but was not significantly different from males in both rural and urban areas (Data not shown). It can be argued that females are supposed to have a lower fat intake than males, although in the present study it is much rather a case of male intake being too low, than female intake being too high. Another possible reason for higher incidence of obesity in women could be due to inactivity (Kruger et al., 2002) or to childhood stunting (**Figure 5.1**) which is linked to excess weight gain later in life (Sawaya *et al*., 2004), since the National Food consumption survey found stunting to be one of the greatest nutritional concerns in South Africa, with almost one in five children being stunted, and affecting nearly one in three children on commercial farms (rural areas) (Labadarios *et al.*, 2005).

BMI correlated positively and highly significantly with TC, LDL-C and TAG, and negatively with HDL-C (**Table 4.6**), however, associations were not very strong. The association between BMI and blood lipid risk factors for CVD in the subjects confirms the results of other studies in black African populations in South Africa (Kruger *et al*., 2001). International data proved that the result of nutrition transition in China was a decline in under nutrition accompanied by a rapid increase in obesity (Popkin, 2001). The current study did not find a significant difference between rural and urban areas for TC, LDL-C, HDL-C and TAG in underweight, normal weight, overweight and obese subjects respectively (**Table 4.8**). Total cholesterol and LDL-C levels showed an increase with higher BMI until it reached an overweight status (BMI: 25-30 kg/m²). It did not increase significantly between overweight and obese subjects (BMI>30 kg/m²) in both rural and urban areas. Glaner and researchers (2010) documented the same trend, and suggested that values stabilise over time, with increase in uptake of LDL-C into adipose tissue, through an increase in receptor numbers as a proposed mechanism. Like previous research (Grundy *et al*., 2004), the present study found HDL-C to decrease progressively as BMI increased in both rural and urban areas. It is unlikely that high intake of saturated fatty acids and resulting LDL receptor inhibition, can be the reason for higher LDL-C, since SFA intakes were generally within recommendations. The same goes for the mechanism suggesting higher cholesterol concentrations in overnourished obese individuals is hepatic overproduction of apoB-containing lipoproteins (Shepherd *et al*. 1980) since energy intakes were below recommendations. Overweight and obesity were listed in the literature as contributors to higher TC and LDL-C (Glaner *et al*., 2010). The rate of formation of VLDL-C and its conversion to LDL-C in the circulation, and hepatic LDL-C receptor density could also be a cause of high LDL-C levels (Riccardi *et al*., 2003).

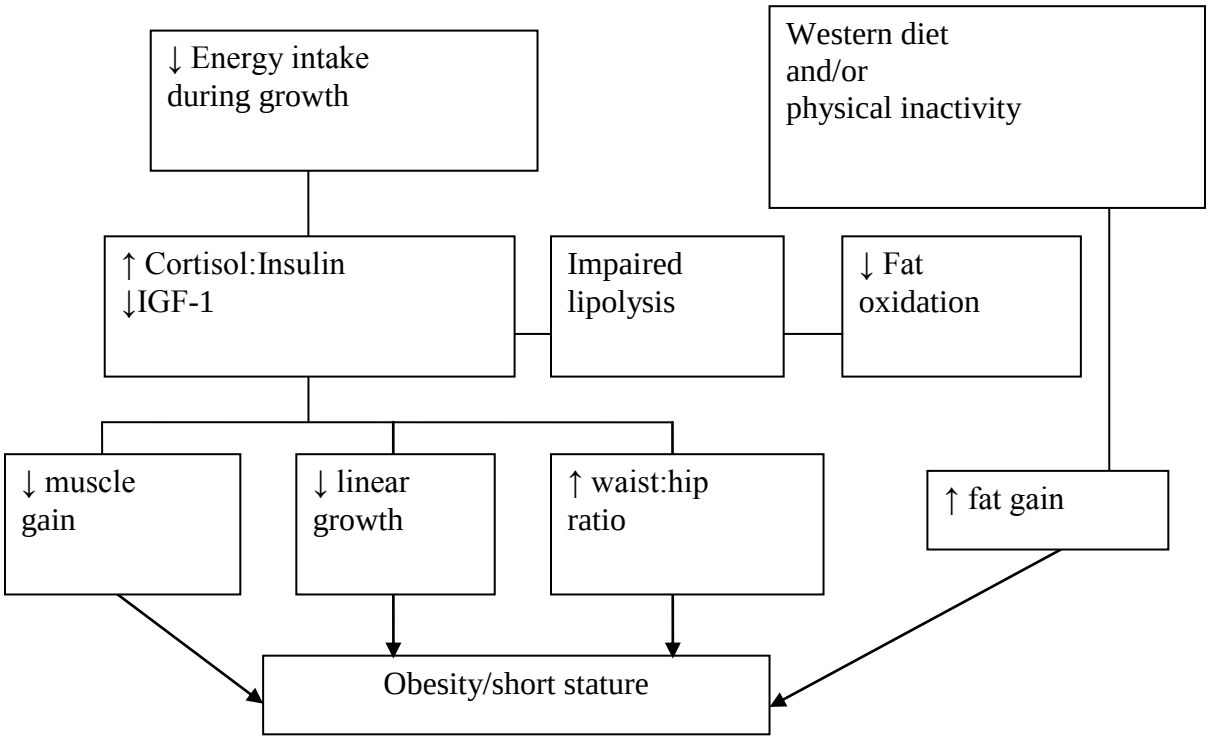

Figure 5.1 Proposed mechanisms for the explanation of stunting and western diet causing obesity later in life (adapted from Sawaya *et al*., 2004)

5.5.3 Gender

No significant differences were detected between rural and urban areas for TC, LDL-C and HDL-C of males and females respectively, with the exception of TAG which was significantly higher in urban female subjects than rural female subjects (**Table 4.9**). Mean TC was lower in the BRISK study for men and women than in the present study (Oelofse *et al*., 1996). In urban areas female subjects had significantly higher TC, LDL-C, and TAG and lower HDL-C than male subjects. In rural areas, however, only LDL-C and TC were significantly higher for females than males. One would think the opposite to be true, because according to literature males are proven to be more prone to hypercholesterolaemia (Kronmal *et al*., 1993), but clearly factors come to play in the present study that sets this population apart from typical gender lipid profile studies. Higher TC, LDL-C, TAG and lower HDL-C in females, can probably be explained by a high prevalence of obesity in females, since obesity is linked to increased TC and LDL-C (Glaner et al., 2010) and decreased HDL-C (Grundy *et al*., 2004). Another possible reason for higher TC and LDL-C in women could be a smaller response with regards to TC and LDL-C to reduced fat diets by women than by men of similar age (Obarzanek *et al*., 2001). Oosthuizen *et al*. (2002) agreed that women in urban areas were at higher risk for increased TC, LDL-C and TAG levels. Documented research in South Africa confirmed a higher age specific TC and LDL-C for females than for males in the urban black communities of the Cape Peninsula (Oelofse *et al*., 1996).

In the present study the percentage subjects with increased TC (45.9% - 50.5% in females and 37.3%-41.8% in males) are much higher in both rural and urban areas compared to the BRISK study, which found 15.4% of men and 23.5% of females had elevated TC (Oelofse *et al*., 1996). Contrary to the current study that presented with very high percentages of subjects with elevated LDL-C (45.5% - 62% of females and 44.6% - 45.5% of males), few men and women in the BRISK study exceeded the cut-off levels for LDL-C (Oelofse *et al*., 1996). In the present study higer percentages of females presented with decreased HDL-C levels than males. BRISK study (Oelofse *et al*., 1996) found the opposite to be true with lower percentages in both males (16.2%) and females (15.6%). It should be noted, however, that a cut-off point of 1 mmol/l was used for HDL-C in both males and females by Oelofse and team (1996), as opposed to 1.2 mmol/l as cut-off for females in the present study. Not only did our study find a much higher percentage of females (30.6% in urban and 19.7% for rural areas) than males (17.4% in urban areas and 13.6% in rural areas) to present with elevated TAG, but also a higher percentage of females in urban areas presented with elevated TAG than in rural areas, compared to the much lower percentage (12.9% of men and 5.2% of women) documented in the BRISK study. It should be noted that Oelofse and colleages (1996) used 2.3 mmol/l as indicator for elevated TAG in their study, compared with the 1.7 mmol/l of our study.

In conclusion, this study indicated that even though fat intake were significanly higher in urban than in rural areas, it was still within recommendations, and probably as a consequence thereof, blood lipid profiles did not differ significantly between rural and urban areas. Blood lipid profiles were more negative with higher ages, with higher BMI's and with the female gender. Blood lipid profiles were not affected by urbanisation.

C H A P T E R 6

CONCLUSIONS AND RECOMMENDATIONS

This study set out to investigate how urbanisation influenced the diet of subjects in the North West Province in South Africa and to investigate the subsequent effect on blood lipid profiles, by means of a cross sectional data analysis of the PURE study. In order to achieve the overall aim of the study, specific objectives were set and investigated. This chapter will summarise the findings and conclusions drawn from these objectives.

6.1 Differences in dietary fat, cholesterol and fatty acid intake between rural and urban subjects

Results from analysis of the data indicated that the intake of energy, fat, specific fatty acids and cholesterol were significanly higher in urban than in rural areas, but still within recommendations. It can be concluded that more adequate diets were probably eaten by urban subjects than rural subjects. In turn, exceptionally low intake in rural areas could result in difficulty to reach recommendations with regards to essential fatty acids and fat soluble vitamins and it could possibly even lead to deficiencies. Remarkably low intake of specific fatty acids ALA, EPA and DHA were of particular concern in both rural and urban areas, because of the known beneficial factors associated with adequate essential fatty acid intakes. The QFFQ, however, have shown underreporting, therefore whether the reported low intake of energy and specific fatty acids were a true reflection of the situation in these communities could be further explored in future studies

6.2 Differences in blood lipid profiles between urban and rural subjects

Probably as a consequence of the fact that fat and fatty acid intake were within recommendations in both rural and urban areas, the major finding of this study was that blood lipid profiles did not differ significantly between rural and urban areas in contrast with prior South African studies done on other African populations.

It was interesting to find that, in contrast with the above mentioned prior South African studies, prevalence of elevated TC was nearly half of the study population while prevalence of elevated

LDL-C levels were more than half of the subjects in both rural and urban areas in the present study. Mean HDL-C was high in both men and women in rural and urban areas, which should account for a protecting factor, however, the percentage of the female population that presented with decreased HDL levels accounted for more than a third in both rural and urban areas which is worrysome. While mean TAG was within the recommendations, 16.28% of rural subjects and 22.96% of urban subjects presented with high TAG. The blood lipid profile of this population is therefore a cause of concern.

Whether the total fat, fatty acid and cholesterol intake differed between the proportion of the population that presented with normal blood lipid profiles and those that presented with abnormal profiles, were explored in more detail in the next objective.

6.3 Associations between dietary fat intake (SFA, MUFA and PUFA) and blood lipid profiles in subjects residing in rural areas and urban areas

Associations between fat, fatty acids and cholesterol and blood lipid profiles were weak. Even though these associations are known to be poor, in this population it is most likely that factors besides fat, fatty acid and cholesterol intake are responsible for abnormal lipid levels, since these dietary factors were still within recommendations. It also could not be proved that the fat, fatty acid and cholesterol intake of subjects with abnormal lipid profiles differed from intake of subjects with normal blood lipid profiles in both rural and urban areas, which only confirmed the unlikeliness of the diet as a contributing factor in the unfavourable blood lipid profiles of subjects in the present study. Further investigation regarding the contributing factors is therefore warrented.

6.4 Blood lipid profiles at different ages, BMI's and genders in rural and urban areas

Age

Higher age was associated with higher TC, LDL-C, HDL-C and TAG. With the exception of urban males between 35-44 and over 55 who presented with a higher TAG levels than rural males, no other significant differences were detected between blood lipid profiles of rural and urban subjects in each age category.

BMI

BMI was not affected by urbanisation in the present study. Both overweight and underweight proved to be a cause of concern in the present study population. Less than half the population fell within normal BMI ranges in both rural and urban areas Underweight was a bigger problem in males (31.5% in rural areas and 27.5% in urban areas), while overweight was a bigger problem amongst women (47.8% in rural areas and 54.2% in urban areas). TC, LDL-C and TAG were higher with higher BMI's, while HDL-C levels were lower. The specific causes of increases in BMI in this population should be investigated, since it did not form part of the current study.

Gender

Urbanisation did not have an effect on blood lipid values within the genders. Females presented with higher TC, LDL-C and TAG and lower HDL-C than males in rural and urban areas. Blood lipid profiles of women in this study population, were therefore more unfavourable than those of men, independent of urbanisation.

This specific study did not investigate how the diet differed at different ages in this population. This study also did not examine differences in diet between different categories of weight in this population, nor BMI at different ages. Future research on the PURE data can be directed at these areas. A prospective design as the PURE study intended for future studies, might bring more clarity regarding the dietary intake as well as anthropometry and blood lipid profiles, in this population in transition, than the present cross-sectional design.

The present study highlighted the fact that obesity and under nutrition co-exist in this population, as well as the need for nutritional advice. This creates a difficult challenge for the health care system. Our strategies, policies and programmes to address this problem should take these differences into account and be flexible enough to adapt to needs in different populations. Great care should be taken when promoting adequacy, in order not to magnify overnourishment. Education on healthier food choices should be an important focus point, with specific focus on essential fatty acids.

ANNEXURE A

PURE Quantitative Food Frequency Questionnaire

Subject ID Initials **Subject**

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

Today's date: | | | | | | | | |

year *month* *day*

1. Name: _____

_____

2. Not applicable in South Africa

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

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

5. Sex: ☐ Female ☐ Male

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

- If you eat the food
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

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

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Bought: Specify topping _____ _____						3353 (base+ch)	
You are being very helpful. Can I now ask you about meat? **CHICKEN, MEAT, FISH** **How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?** _____								
Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							
Do you eat chicken skin? Always **1** Sometimes **2** Never **3**								
Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____ _____								
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit **VEGETABLES AND FRUIT**								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo / beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat ……...							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……...							
	Without fat						3925	
	Canned						4192	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat ……							
	Boiled, little sugar and fat Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat ……							
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat ……							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							
Do you like fruit?		Yes 1		No 2				
Apples							3592	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit?			Yes	**1**	No	**2**		
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
BREAD AND BREAD SPREADS								
Bread / Bread rolls	White						3210	
	Brown						3211	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Whole wheat						3212	
Do you spread anything on the bread?		Always 1	Sometimes 2	Never 3				
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	
	Skimmed milk powder Brand: _____						2825 (powder)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks	Sweetened						3981	
Coke, fanta, etc	Diet							
Maueu/Motogo							4056	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	

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

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS **Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat**								
Specify								

Thank you very much for your cooperation and patience.

Good-bye!

ANNEXURE B

Sponsorship for PURE study (international)

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- Brazil—Unilever Health Institute, Brazil;
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- Malaysia—Ministry of Science, Technology and Innovation, UniversitiTeknologi MARA;
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CHAPTER 7

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