DIETARY FAT MODULATES THE RELATIONSHIP BETWEEN POLYMORPHISMS IN THE *TNFA* AND *IL-6* GENES, AND OBESITY AND SERUM LIPID CONCENTRATIONS IN BLACK AND WHITE SOUTH AFRICAN WOMEN

YAEL JOFFE

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By

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‘A journey of a thousand miles begins with a single step’.

Chinese philosopher Laozi

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DECLARATION

I, **Yael Tracey Joffe,** do hereby declare that the experiments presented in this thesis were conceived and executed by myself except where otherwise indicated.

Neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in the University or any other University.

This thesis is presented in fulfilment of the requirements for the degree of PhD.

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LIST OF PUBLICATONS

Publications directly related to this thesis

1. **Joffe YT**, van der Merwe L, Carstens M, Collins M, Jennings C, Levitt NS, Lambert EV, Goedecke JH: Tumor necrosis factor-alpha gene -308 G/A polymorphism modulates the relationship between dietary fat intake, serum lipids, and obesity risk in black South African women. *The Journal of nutrition* 2010, 140(5):901-907.
2. **Joffe YT**, van der Merwe L, Collins M, Carstens M, Evans J, Lambert EV, Goedecke JH: The -308 G/A polymorphism of the tumour necrosis factor-alpha gene modifies the association between saturated fat intake and serum total cholesterol levels in white South African women. *Genes & Nutrition* 2011, 6(4):353-359.
3. **Joffe YT**, van der Merwe L, Evans J, Collins M, Lambert EV, September A, Goedecke JH: The tumor necrosis factor-alpha gene -238 G>A polymorphism, dietary fat intake, obesity risk and serum lipid concentrations in black and white South African women. *Eur J Clin Nutr* 2012, 66(12):1295-1302.
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1. Berman P, Collins M, Baumgarten I, Seoighe C, Jennings CL, **Joffe Y**, Lambert EV, Levitt NS, Faulenbach MV, Kahn SE *et al*: Association between the 4 bp proinsulin gene insertion polymorphism (IVS-69) and body composition in black South African women. *Obesity (Silver Spring)* 2009, 17(6):1298-1300.
2. Chantler S, Dickie K, Goedecke JH, Levitt NS, Lambert EV, Evans J, **Joffe Y**, Micklesfield LK: Site-specific differences in bone mineral density in black and white premenopausal South African women. *Osteoporos Int* 2011.
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4. Micklesfield LK, Evans J, Norris SA, Lambert EV, Jennings C, **Joffe Y**, Levitt NS, Goedecke JH: Dual-energy X-ray absorptiometry and anthropometric estimates of visceral fat in Black and White South African Women. Obesity (Silver Spring) 2010, 18(3):619-624.

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2. The Society for Endocrinology, Metabolism and diabetes of South Africa (SEMSDA) Conference, April 2012 - Associations between Interleukin-6 gene polymorphisms and obesity and serum lipids, and their interaction with dietary fat intake in black and South African white women.
3. Nutrition Congress, October 2010 - Tumor Necrosis Factor-! Gene -308 G/A Polymorphism Modulates the Relationship between Dietary Fat Intake, Serum Lipids, and Obesity Risk in Black South African Women
4. Nutrition Congress Africa, October 2012 - Associations between interleukin-6 gene polymorphisms and obesity and serum lipids, and their interaction with dietary fat intake.

ABBREVIATIONS

AA, arachidonic acid

ALA, !-linolenic acid

AT, adipose tissue

ANOVA, analysis of variance

APOA1, apolipoprotein A1

APOE, apolipoprotein E

BMI, body mass index

BRISK, Coronary Risk Factor Study in blacks

CRP, C-reactive protein

CHO, carbohydrate

DNA, deoxyribonucleic acid

CCL2, chemokine (C-C motif) ligand 2 and its receptor

CCR2, C-C chemokine receptor type 2

CD68, cluster of differentiation 68

CSF-1, colony stimulating factor-1

CAD, coronary artery disease*

CHD, coronary heart disease*

CVD, cardiovascular disease*

DLEU7, deleted in lymphocytic leukemia

DSAT, deep SAT

DHA, docosahexaenoic acid

DXA, dual-energy X-ray absorptiometry

ABBREVIATIONS

EDTA, ethylenediaminetertaacetic acid

EPA, eicosapentaenoic acid

FADS, fatty acid desaturase gene

FFA, free fatty acid

GCP, good clinical practice

GWAS, genome-wide association studies

HWE, Hardy Weinberg Equilibrium

HDL-C, high-density lipoprotein cholesterol

IL-1, interleukin one

IL-6, interleukin six

IL-8, interleukin eight

IHD, ischaemic heart disease

IQR, interquartile range

kJ, kilojoule

LA, linoleic acid

LD, linkage disequilibrium

LDL-C, low-density lipoprotein cholesterol

LTA, lymphotoxin-!

MIF, macrophage inhibitory factor

MetS, metabolic syndrome

MCP-1, monocyte chemotactic protein-1

MC4R, Melanocortin 4 Receptor

mRNA, messenger ribonucleic acid

MUFA, monounsaturated fat

NCBI, National Centre for Biotechnology Information

ABBREVIATIONS

NO, nitric oxide

OR, odds ratio

%E, percentage energy

n-3 PUFA, omega-3 polyunsaturated fatty acid

n-6 PUFA, omega-6 polyunsaturated fatty acids

n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio

NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells

PAI-1, plasminogen activator inhibitor-1

PPARγ, peroxisome proliferator-activated receptor gamma

PCR, polymerase chain reaction

PUFA, polyunsaturated fatty acids

P:S ratio, polyunsaturated : saturated fat ratio

PGE_2, prostaglandin E2

ROS, reactive oxygen species

RFLP, restriction fragment length polymorphism analysis

SA, South Africa

SFA, saturated fat

SNP, single nucleotide polymorphism

SREBP, sterol regulatory element-binding protein

SVF, stroma vascular fraction

SAT, subcutaneous adipose tissue

SSAT, superficial SAT

TLR4, toll-like receptor 4

TAG, triglyceride

T-C, total cholesterol

ABBREVIATIONS

T-C:HDL-C ratio, total cholesterol : high-density lipoprotein cholesterol ratio

TAG, triacylglycerol

TCF7L2, transcription factor 7-like 2

TNF!, tumour necrosis factor-!

TNFA, tumour necrosis factor-! gene

USA, United States of America

VLDL-C, very low-density lipoprotein cholesterol

VIGHOR, Vanderbijlpark Information Project on Health, Obesity and Risk Factors

VAT, visceral adipose tissue

WTCCC, Wellcome Trust Case Control Consortium

WHR, waist hip ratio

WHO, world health organisation

* CVD, CHD, and CAD are used throughout the thesis at various points. They are not meant to be used interchangeably; they are used as they appear in the literature cited.

ABSTRACT

The overall prevalence of overweight and obesity in South African (SA) women is high, with black women being more affected than white women (58.5% vs. 52.9%). Furthermore, black SA women, similar to African Americans have been shown to have a less atherogenic lipid profile than white women. Obesity is a condition characterised by chronic low-grade inflammation, which also mediates all stages of atherosclerosis. DNA sequence variants in the tumour necrosis factor (*TNFA)* and interleukin-6 *(IL*-6) genes have been shown to impact molecular processes of the inflammatory pathways, serum lipids and the obese phenotype, and to interact with dietary fatty acids, modulating these relationships.

The primary aim of the thesis was to investigate associations between *TNFA* (*TNFA* -308 G>A and -238 G>A) and *IL-6* (-174 G>C, IVS3+281 G>T, IVS4+869 A>G) sequence variants and obesity and serum lipid concentrations in black and white SA women. This included identifying sequence variants in the *IL-6* gene with a reported high heterozygosity in both the white and black SA populations (rs1554606 and rs2069845). Dietary intake data of adequate reporters was then included in the analysis to investigate whether dietary fatty acid intake modulated the interactions between the *TNFA* and *IL-6* SNPs (*TNFA* -308 G>A and -238 G>A & *IL-6* -174 G>C, IVS3+281 G>T, IVS4+869 A>G) and obesity, measures of adiposity and serum lipid concentrations, and whether interactions identified differed between black and white women.

ABSTRACT

Normal-weight (body mass index (BMI) ! 25 kg/m^2, N=107) and obese (BMI " 30 kg/m^2, N=120) black and normal-weight (N=89) and obese (N=62) white urban SA women underwent measurements of body composition, fasting lipids and dietary intake and were genotyped for the *TNFA* -308 G>A and -238 G>A polymorphisms, and *IL-6* -174 G>C, IVS3+281 G>T, IVS4+869 A>G polymorphisms. Dietary intake was estimated using a food frequency questionnaire. Under-reporting and over-reporting were detected by applying the Goldberg cut-offs for energy intake:basal metabolic rate. Of the total sample, 42 under-reporters, 268 adequate reporters, and 73 over-reporters were identified. Only adequate reporters were included in the diet analyses, including 73 normal-weight and 74 obese black, and 73 normal-weight and 48 obese white adequate reporters.

The objectives of the first study of the thesis were to characterise the body composition, serum lipid concentrations and dietary intake of the black and white SA women. Associations between the *TNFA* -308 G>A polymorphism and obesity and serum lipid concentrations, as well as interactions with dietary fatty acid intake, were then explored. White women were taller and heavier than the black women, but because of their shorter stature the black women had a higher BMI than the white women (P=0.002). The white women had greater waist, waist hip ratio (WHR), and visceral adipose tissue (VAT) than the black women, whereas the black women had higher body fat % (P=0.004), and subcutaneous adipose tissue (SAT) (P=0.028) than the white women. As expected, triglycerides (TAG), total cholesterol (T-C), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were higher in the white women compared to the black women (P<0.001). In terms of dietary intake, the black women consumed more energy (kJ),

carbohydrate (CHO) percentage total energy intake (%E)(P< 0.001) and total fat (%E)(P=0.002) than the white women. When individual dietary fatty acid intake was analysed, the black women consumed more total polyunsaturated fatty acids (PUFAs) (%E) and omega-6 (n-6) PUFAs (%E), including greater amounts of linoleic acid (LA) and arachidonic acid (AA) (%E)(P=0.001) than the white women. Notable was the higher omega-6:omega-3 polyunsaturated fatty acid (n-6:n-3 PUFA) ratio in the black women compared to the white women (P<0.001).

When exploring genotype-phenotype associations in the black and white women, there were no differences in the genotype or allele frequency of the *TNFA* -308 G>A polymorphism between the BMI groups. Nor were any genotype associations observed for body composition or serum lipid concentrations. However, a diet-gene interaction was observed in the black women. When dietary fat intake was 30% of total energy intake (%E), the odds of being obese with the *TNFA* GA+AA genotype was only 12% of that with GG, but increasing intake of dietary fat intake (%E) was associated with a significantly greater rate of increase in obesity risk in women with the *TNFA* GA+AA genotype compared to those with the GG genotype (P=0.036). In the black women, diet-gene interactions were also identified between !-linolenic acid (ALA) (%E) intake and total cholesterol : HDL-cholesterol (T-C:HDL-C) ratio (P=0.036), and PUFA (%E) intake and LDL-C levels (P=0.026), with subjects with the A allele being more responsive to changes in relative fat intake. In the white women, an interaction effect between dietary saturated fat (SFA) intake (%E) and *TNFA* -308 genotypes on serum total cholesterol (T-C) concentrations (P=0.047) was identified. With increasing SFA intake (%E), T-C levels decreased for the GG genotype and increased for the GA + AA genotypes. It was concluded that although the *TNFA* -308 G>A polymorphism was not independently associated with obesity or

serum lipid concentrations in this study, dietary fat intake modified the relationship between the *TNFA* -308 G>A polymorphism, obesity risk and serum lipid concentrations.

While a number of studies have investigated the *TNFA* -308 G>A polymorphism, only a few have reported on the *TNFA* –238 G>A polymorphism and obesity and serum lipids. Therefore, the aim of the second study was to explore whether the *TNFA* -238 G>A polymorphism is independently associated with adiposity and serum lipid concentrations, and whether these associations are impacted by dietary fat intake. The black women had a higher *TNFA* -238 GA genotype frequency than the white women (P<0.001), but there were no differences between BMI groups. Black women with the *TNFA* -238 A allele had a greater body fat % than those with the GG genotype (P<0.001). Further, when dietary intake data was included in the analyses, it was observed that with increasing polyunsaturated : saturated fat ratio (P:S ratio) and n-6:n-3 PUFA ratio, HDL-C concentrations decreased and T-C:HDL-C ratio increased in black women with the GA genotype but not the GG genotype. In addition, with increasing omega-3 polyunsaturated fatty acid intake (n-3 PUFA) (%E), T-C:HDL-C ratio decreased in black women with the GA genotype, but not in those with the GG genotype. In the white women, with increasing eicosapentaenoic acid (EPA) (%E) intake, LDL-C concentrations decreased in those with the GG genotype but not the GA genotype. It was concluded that the *TNFA* -238 G>A polymorphism was associated with body fat %, and that the -238 A allele was responsive to increasing dietary fat intake on measures of adiposity in black SA women. This study also showed that the relationship between the *TNFA* -238 G>A

polymorphism and serum lipid concentrations differed depending on PUFA intake, in both black and white SA women, but that these interactions were ethnic-specific.

The cytokine IL-6 is known to regulate inflammation, and within the *IL-6* gene, the functional and well studied polymorphism *IL-6* -174 G>C (rs1800795) has been associated with obesity and dyslipidaemia. However, the -174 G>C polymorphism has been shown to be rare in persons of African descent. Therefore, the third study of this thesis identified two additional informative (more common) polymorphisms in both black and white SA populations, and investigated associations between these *IL-6* polymorphisms (*IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G) and obesity and dyslipidaemia in black and white women. The *IL-6* IVS4+869 G allele was associated with greater waist (P=0.014) and fat mass (P=0.034) in black women. In the white women, those with the *IL-6* IVS3+281 T allele had lower TAG concentrations than the IVS3+281 GG genotype (P=0.008). It was concluded that *IL-6* polymorphisms in this study were associated with obesity in the black women and serum lipid concentrations in the white women. These findings are novel in that no other studies have reported on the association between the IVS4+869 A>G polymorphism and adiposity.

It is not known whether these genotype-phenotype associations are impacted by dietary fat intake, as was observed for the *TNFA* -308 G> and -238 G>A polymorphisms in the first two studies of this thesis. The aim of this fourth and final study of this thesis was therefore to investigate the relationship between the *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms and dietary fat intake on obesity and serum lipids in black and white women. A number of diet-gene

interactions were observed for both the white and black women. In the white women; with increasing n-3 PUFA intake and decreasing n-6:n-3 PUFA ratio, BMI decreased in those with the *IL-6* -174 C allele, *IL-6* IVS3+281 T allele and *IL-6* IVS4+869 AG genotype. In the black women, with increasing dietary fat intake, adiposity decreased in those with the *IL-6* IVS3+281 TT and *IL-6* IVS4+869 GG genotypes and increased in the *IL-6* IVS3+281 GT+GG and *IL-6* IVS4+869 AA or AG genotypes. In white women, with increasing n-3 PUFA intake; TAG and T-C:HDL-C ratio decreased and HDL-C increased in those with the *IL-6* -174 C allele, and T-C:HDL-C ratio decreased in the *IL-6* IVS3+281 TT compared to the GG and GT genotype, with HDL-C increasing with each T allele. In contrast, HDL-C decreased with each *IL-6* IVS4+869 G allele. In black women, with increasing total fat intake, TAG and T-C:HDL-C ratio increased in those with the *IL-6* IVS4+869 G allele and decreased in the AA genotype. It was concluded that dietary fat intake, and the quality of the dietary fatty acids, modulated the relationship between the *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms, on measures of obesity and serum lipids, with different effects in black and white women

In summary, this thesis is the first to show that dietary fat intake modulates the relationship between *TNFA* and *IL-6* polymorphisms on obesity and serum lipids, and that these relationships differ between black and white SA women. These findings suggest that diet-gene interactions and ethnicity contribute to the complexity and heterogeneity observed within the obese phenotype and serum lipid profiles in different populations. The thesis results also highlight the importance of understanding the inflammatory impact of different dietary fatty acids and how they may differentially interact with genes. The future study of nutrigenomics offers the

opportunity to clarify the underlying molecular mechanisms governing the interactions between dietary fatty acids and the inflammatory and obese phenotype, potentially elucidating the observed differences between ethnic groups and enabling the development of population-based targeted interventions to reduce risk associated with obesity, serum lipid profiles and coronary artery disease.

CHAPTER ONE

LITERATURE REVIEW

The data presented in this chapter has been published, in part, in the following peer-reviewed article:

Joffe YT, Collins M, Goedecke JH: The Relationship between Dietary Fatty Acids and Inflammatory Genes on the Obese Phenotype and Serum Lipids. *Nutrients* 2013, 5(5):1672-1705.

Statement of contribution to manuscript and chapter:

Y.T.J, J.H.G and M. Collins conceived the structure and content of the review; **Y.T.J** wrote the paper with editorial input from J.H.G and M.C; **Y.T.J** had final primary responsibility for final content.

CHAPTER ONE

1.1. Introduction to obesity

1.1.1. Overview of global obesity

An estimated 1.3 billion people are overweight or obese world-wide, of these approximately 300 million are obese [1]. Traditionally, obesity has been viewed as a disease of developed countries, however, there is an alarming increase in obesity in developing countries such as South Africa (SA), Mexico and South American countries, likely due to epidemiological and nutrition transition. Epidemiological transition is defined as changing patterns of population age distributions, mortality, fertility, life expectancy, and causes of death [2-4].

Obesity is a chronic disorder with a multi-factorial aetiology, which includes a strong genetic component [5]. When more energy is consumed compared to energy expended, excess energy is stored in adipocytes that enlarge. It is thought by some to be adipocyte hypertrophy that generates the metabolic aberrations that result in obesity-associated comorbidities [6]. These include cardiovascular disease (CVD), coronary heart disease (CHD), hypertension, insulin resistance, type 2 diabetes, and certain cancers [6, 7]. The SA prevalence and presentation of these conditions have been discussed extensively in the technical report; Chronic Diseases of Lifestyle in SA: 1995 – 2005 [8]. Since this thesis investigates associations and interactions between dietary fatty acids and tumour necrosis factor alpha (*TNFA)* and interleukin-6 (*IL-6)* polymorphisms on obesity and serum lipid concentrations in urbanized black and white SA women, this literature review focused on obesity and CHD, specifically dyslipidaemia in black and white South Africans.

1.1.2. Obesity in South Africa

SA presents with a 'quadruple burden' of disease, characterised by: i) under-nutrition and under-development, found predominantly in children, ii) emerging chronic diseases associated with ever-increasing overweight and obesity, iii) HIV/AIDs and iv) injuries [3, 8-10]. Despite the need to address under-nutrition, poverty and infectious diseases such as HIV/AIDS and tuberculosis, obesity and its co-morbidities contribute significantly to the burden on the SA health care system [11].

The first SA Demographic Health Survey (SADHS), including 8,162 SA women, and 5,665 men, aged 15 to 95 years old, reported differences in obesity prevalence between SA ethnic groups and between men and women. The overall prevalence of overweight (body mass index (BMI) >25 kg/m^2) and obesity (BMI >30 kg/m^2) in SA is high with 29% of men and 56% of women being classified as overweight or obese [3]. This is higher than that reported in other African countries [7]. Black women had the highest prevalence of overweight and obesity (58.5%), followed by white women (52.9%) and coloured women (52.0%) [3]. The ethnic group within the Western Cape region of SA self-identified as coloured is ancestrally derived from admixtures of one or more of the indigenous African populations (Khoe- and San-speaking or Bantu-speaking), immigrants from Western Europe, or slave labourers from West Africa, Indonesia, Madagascar, Java, India and Malaysia [12].

Black urban women had higher BMI's than their rural counterparts. A different pattern was seen in men, the prevalence for overweight and obesity was highest in white men (54.5%), followed by Indian men (32.7%) and coloured men (31%), with the lowest prevalence in black men (25%)[3].

1.2. Coronary artery disease and serum lipids profiles in SA

Coronary artery disease (CAD) is a major health problem in all SA population groups. In 2000, CAD contributed 23% for black, 41% for white, 31% for coloured, and 52% for Indian population groups of the total age-standardised death rates in SA [8, 13]. Despite CAD prevalence being the lowest in blacks, several studies have shown that increasing urbanization and the nutrition transition has been accompanied by an increase in CAD risk factors in this population group [14, 15]. Significant risk factors for CAD are obesity and weight gain, with the latter increasing CAD risk irrespective of the initial BMI [16]. In the Nurses' Health Study, CAD risk was 3.3-fold higher when BMI was greater than 29 kg/m^2 compared with women with a BMI less than 21 kg/m^2 [17].

Dyslipidaemia is associated with an increased risk for atherosclerosis and CVD [18], and is characterized by reduced high-density lipoprotein cholesterol (HDL-C) levels, raised triglycerides (TAG), and increased small low-density lipoprotein (LDL) particles [19]. Raised serum lipid levels are an important CAD risk factor in all population groups in SA. In SA adult men and women (30+ years), approximately 59% of ischaemic heart disease (IHD) and 29% of ischaemic stroke burden were attributable to a high total-cholesterol (T-C) (≥ 3.8 mmol/l). However, the burden varied by population group, with cholesterol-attributable mortality estimates being significantly lower in black than white SA women (47 vs. 152 deaths per 100 000) [20]. Black SA women and African Americans have been shown to have a less atherogenic lipid profile than white women, characterized by low TAG, T-C and LDL-C [21-23]. Studies in SA women have generally reported no ethnic difference in

HDL-C levels [22], whereas African Americans have been shown to have higher HDL-C levels than their white counterparts [23]. In contrast, Goedecke *et al.* found HDL-C to be lower in a cohort of black Xhosa women compared to white women, although HDL-C levels were still within the reference range [19, 24].

The more favourable serum lipid profiles observed in black versus white women may be attributed to relatively low levels of visceral adipose tissue (VAT) [21]. For the same level of body fatness, black women have less VAT than white women [22, 25]. Nieves *et al.* showed that increased VAT was a more significant determinant of an atherogenic lipid profile than insulin resistance in apparently healthy individuals [26]. Despite less VAT and less atherogenic lipids, black women are more insulin resistant than their white counterparts [27-29]. In SA women, Goedecke *et al.* reported that lipid levels and LDL particle size correlated with insulin sensitivity and body fatness in white women, but not in black women, in whom lipid levels were more closely associated with modifiable lifestyle factors [19]. Similarly, Sumner *et al.* demonstrated that triglyceride levels, triglyceride to HDL ratio, and LDL particle size were not associated with insulin sensitivity in African Americans and for a given triglyceride level, African Americans were more insulin resistant than their white counterparts [29]. Although the reasons for the ethnic differences in these associations are not known, inflammation has been known to regulate both and to differ by ethnicity [23].

1.3. The relationship between inflammation, obesity and lipid metabolism

White adipose tissue constitutes the majority of adipose tissue (AT) [30] and can be divided into two fractions; the adipocyte fraction composed of mature adipocytes, and the stroma vascular fraction (SVF) composed of many other cell types, including preadipocytes, macrophages, endothelial cells and fibroblasts [31]. Although AT is considered a metabolically active endocrine organ, the macrophages present in the SVF are the primary source of obesity-induced inflammation [32-34]. The number of macrophages present in the SVF is directly correlated with the level of adiposity and adipocyte size [33, 35]. Adipocyte hypertrophy, results in increased chemokine secretion e.g. transforming growth factor beta 1, soluble ICAM, and monocyte chemotactic protein-1 (MCP-1)[33]. There is a subsequent increase in the infiltration of macrophages, which in turn secrete cytokines such as IL-6 and TNF!. Since AT expansion is characterized by increased macrophage infiltration, these cells are responsible for almost all cytokines, for example TNF!, and significant amounts of IL-6 secreted by AT [33].

Low-grade chronic inflammation mediates all stages of atherosclerosis from initiation through to the complications of thrombosis. So much so, that elevated inflammatory markers are often used to define risk of atherosclerotic complications, independent of myocardial damage [36]. One of the proposed mechanisms linking inflammatory processes to the development of atheroma, is the LDL oxidation hypothesis [37]. LDL in the intima, the innermost layer of an artery or vein, undergoes oxidative modification, inducing expression of pro-inflammatory cytokines, chemokines and

other mediators of inflammation. Very-low density lipoprotein (VLDL) and intermediate-density molecules are also believed to undergo oxidative modification and may themselves activate the inflammatory processes of the vascular endothelial cells [38], through activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)[39].

1.4. A nutrigenetic approach to inflammation, obesity and serum lipids

Genetic variation, as well as lifestyle factors such as diet and exercise, plays an important role in the development and progression of obesity and dyslipidaemia. Dietary components such as fatty acids interact with genetic variants to regulate the development and progression of obesity and its co-morbidities. These complex nutrigenetic interactions may explain differences observed in the obese phenotype and its comorbidities that vary both within and across populations [40]. The aim of this review is to illustrate the current state of knowledge with regards:

- The impact of diet and genetic variation on inflammation
- The associations between genetic variation in cytokine genes and obesity and serum lipids
- The interactions between dietary fatty acids and polymorphisms in cytokine genes and their impact on obesity and serum lipids, and
- The role of ethnicity as a confounder in these interactions.

Specifically, dietary fatty acids modulate the regulation and production of cytokines such as TNF ! and IL-6, thereby influencing inflammatory status. Furthermore, emerging research suggests that diet–gene interactions play an important role in the regulation of these inflammatory cytokines, impacting the obese phenotype and

serum lipids, and these interactions may differ by ethnic group. This review focuses on interactions between polymorphisms in the inflammatory genes *TNFA* and *IL-6*, and dietary fatty acids, and their relationship with obesity and serum lipid levels as proof-of-principle examples. Figure 1.1. illustrates the development of obesity-associated low-grade inflammation and the impact of diet-gene interactions on obesity and dyslipidaemia.

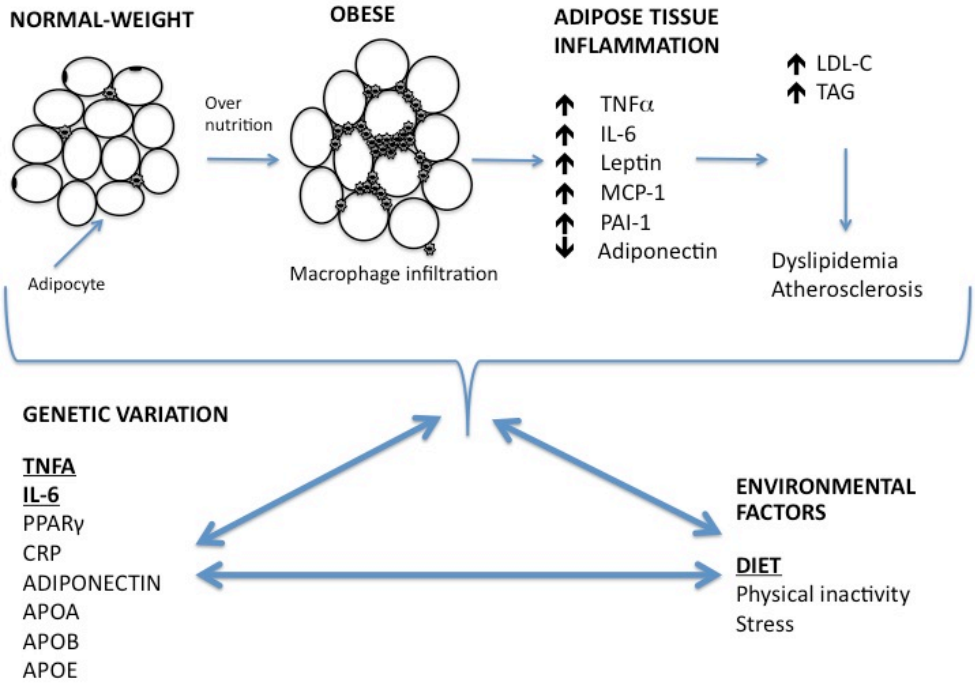

Figure 1.1. A proposed schematic diagram for obesity-associated low-grade inflammation, and the relationship of diet-gene interactions on obesity and dyslipidaemia. Adipocytes become hypertrophic through over-nutrition. Expansion of adipose tissue in obesity leads to a subsequent increase in the production of chemokines by the adipocytes, resulting in increasing macrophage infiltration and enhanced production of pro-inflammatory cytokines such as TNF ! and IL-6. Together, these adipocyte- and macrophage-derived cytokines act in a paracrine and autocrine manner contributing to adipose tissue inflammation. Obesity-associated low-grade inflammation results in an increase in serum triglycerides, and LDL-C concentrations and is associated with dyslipidaemia. Environmental factors, especially diet, and deoxyribonucleic acid (DNA) sequence variations in inflammatory genes, interact to impact molecular processes of the inflammatory pathway, serum lipids and the obese phenotype. Abbreviations: *APOA*, Apolipoprotein A; *APOB*, Apolipoprotein B; *APOE*, Apolipoprotein E; *CRP*, C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemotactic protein-1; PAI, plasminogen activated inhibitor; *PPARγ*, peroxisome proliferator-activated receptor gamma; TAG, triglycerides; TNF ! , tumour necrosis factor

1.5. The impact of diet on inflammation

1.5.1. Dietary intake, physical activity and inflammation

For the purpose of this thesis it is important to understand dietary intake and lifestyle choices with regards their impact on low-grade inflammation. The effects of different diets, dietary components and exercise on low-grade inflammation have been extensively investigated through cross-sectional, prospective and intervention studies. A thorough review by Calder *et al.* provides detail on this topic [41]. However, the impact of diet, dietary components and exercise on low-grade chronic inflammation may be summarized as follows:

- Following the consumption of a meal, there is a transient postprandial inflammatory response that plays a role in the development of both insulin resistance and atherosclerosis [42, 43]. In particular, hyperglycaemia or a high-fat meal promotes postprandial inflammation [44, 45].
- During hypo-energetic diets or energy restriction, metabolic efficiency is improved and inflammatory processes are reduced by decreasing the activation of NFkB [46, 47]. A reduction in energy intake appears to be effective independent of the macronutrient composition of the low-energy diet [48].
- Observational and intervention studies strongly suggest that the Mediterranean diet [49-51], vegetarian diet [52, 53], and the ‘prudent’ diet; synonymous with the US dietary guidelines [54-56] , reduces chronic low-grade inflammation and improves endothelial function.
- The review by Calder *et al.* details individual components of the diet and micronutrients associated with lower inflammatory markers. These include whole

grains, fibre, vegetables, fruit, fish, vitamin C, vitamin E and carotenoids. Moderate consumption of wine and beer also decreases low-grade inflammation. It is not clear whether such effects are due to the alcohol content or to other components, such as phenolic compounds in these fermented beverages [41, 57].

- A number of excellent reviews have addressed the positive influence of physical activity and fitness on low-grade inflammation [58-60]. It is unclear whether the anti-inflammatory benefits of a physically active lifestyle are due to exercise per se or changes in body composition. It has been hypothesized that these anti-inflammatory effects may be mediated by increased insulin sensitivity and/or improved concentrations of HDL-C, reactive oxygen species (ROS) or endothelial function [61].

1.5.2. Dietary fatty acids and inflammation

Dietary fatty acids have received considerable attention for their ability to regulate cytokine gene expression and secretion [41, 62, 63]. It has been proposed that dietary fatty acids affect inflammatory processes through the modulation of transcription factors such as NFκB and peroxisome proliferator-activated receptor gamma (PPARγ) [62, 64]. PPARγ inhibits NFκB, and both transcription factors are sensitive to dietary fatty acids [65]. Polyunsaturated fatty acids (PUFA), especially marine omega-3 (n-3) PUFA protects against inflammation, whereas oxidized lipids, saturated fatty acids (SFA) and *trans*-fatty acids promote inflammation [41].

1.5.3. Saturated fat

There is general agreement that increasing dietary SFA intake, especially in overweight or obese individuals, is associated with raised inflammatory markers [41], predominately by activating the toll-like receptor 4 (TLR4) pathway. The TLR4 pathway is expressed in both subcutaneous adipose tissue (SAT) and VAT. SFAs serve as ligands for TLR4, inducing inflammatory changes in both adipocytes and macrophages through NFκB activation [66], increasing adipocytokine gene expression and production [67, 68]. It has been shown that when adipocytes are exposed to the SFA, palmitic acid, IL-6 messenger ribonucleic acid (mRNA) expression and protein production increased, most likely through activation of NFκB [69, 70]. Similarly, monocytes were directly activated when exposed to SFA, especially lauric acid [68, 71].

1.5.4. N-6 PUFAs: linoleic acid and arachidonic acid

The omega-6 (n-6) PUFA, linoleic acid (LA), constitutes the majority of PUFA intake in the western diet. LA is the precursor of the n-6 PUFA arachidonic acid (AA). A high LA intake has on occasion been considered pro-inflammatory, however the evidence to support this is contradictory and not conclusive [41, 72-74]. AA intake in the diet is low relative to LA intake, its metabolic precursor. AA is the most prevalent n-6 PUFA in inflammatory cell membranes and is the substrate for the synthesis of the pro-inflammatory eicosanoids, including prostaglandin E2 (PGE_2) and 4-series leukotrienes, associated with inflammatory processes [41]. In addition, AA regulates cellular receptors such as NFkB, PPAR and sterol regulatory element-binding protein

(SREBP), thereby modulating the expression of inflammatory proteins, including IL-6 and TNF ! [75]. Despite this, studies investigating the impact of AA on inflammatory markers are inconclusive, and few human intervention studies have reported on the effect of dietary intake of AA on low-grade inflammation [76-78].

1.5.5. N-3 PUFAs: !-linolenic acid, eicosapentanoic acid and decosahexanoic acid

The n-3 PUFA, alpha-linolenic acid (ALA) is an 18-carbon n-3 essential fatty acid common in canola, soybean oil and some nuts, but in greatest concentrations in flaxseed and flaxseed oil [79]. ALA is elongated and desaturated to eicosapentanoic acid (EPA) and further to decosahexanoic acid (DHA), however the efficiency of this conversion has been debated [80]. Association studies between dietary intake of ALA and inflammatory markers suggest a modest anti-inflammatory effect of ALA [41, 72, 74, 81-83].

The long chain n-3 PUFAs, EPA and DHA are found in seafood, especially oily fish and in some algal oils. Is it proposed that n-3 PUFAs affect inflammation mainly through altered eicosanoid production, but potentially also impacting cell signalling and gene expression [41, 81]. When EPA and DHA are incorporated into human inflammatory cells, this is partly at the expense of AA, providing less substrate for eicosanoid production [41]. Culture systems, animal models and human intervention studies are generally consistent in recognising the anti-inflammatory actions of marine n-3 PUFAs [62-64, 81, 84-86].

1.6. Ethnicity as a confounder

As discussed in sections 1.1.2. and 1.2. for SA populations, and described here in African Americans [25, 87], the prevalence of obesity may be high in a given population, but the prevalence of obesity-associated co-morbidities may differ between ethnic groups [25, 87-90]. As an example; while both African American and black SA women have a higher prevalence of obesity [7, 25, 91, 92], they have less atherogenic lipid profiles than their white counterparts; characterized by low TAG, T-C, and LDL-C concentrations [21-23, 88]. Furthermore, the association established between insulin sensitivity and serum lipids, which has seen the TAG:HDL-C ratio recommended as a tool to predict insulin resistance in overweight women, appears to differ by ethnicity [23, 93]. While the ratio predicts insulin resistance in white women, it is ineffective in African American, black SA and West African women [23, 29, 93, 94].

Differences between ethnicities comprise complex aetiologies. Ethnicity incorporates a variety of different components including; genetic variation, diet and lifestyle, as well as cultural, behavioural and socio-demographic conditions [87]. In the example of ethnic differences in lipid profiles described above, ethnic variability may be observed in dietary intake, body fat distribution (VAT *vs.* SAT)[19, 95], distribution of polymorphisms and genotype frequencies in genes such as apolipoprotein E *(APOE*) [96]*,* and inflammatory gene expression (*TNFA* and *IL-6)*[97, 98]. In addition, dietary intake and physical activity [99], as well as diet-gene interactions may differ between ethnic groups [96]. In considering differences between ethnic groups resident in developed and developing countries, attitudes to food as well as the quality of the urban environment will also pay a role [87].

1.6.1. Body fat distribution

There is considerable heterogeneity among the various AT depots as both the physical and physiological properties of VAT and SAT differ [100-104]. VAT is associated with increased risk for metabolic syndrome, insulin resistance, diabetes, dyslipidaemia and atherosclerosis compared to SAT depots [100, 105, 106]. While the exact mechanisms are not clearly known, even the anatomic location of the different fat depots affects endocrine function [107]. Endocrine hormones from VAT are secreted into the portal system, affecting hepatic metabolic function, whereas those derived from SAT are secreted into the systemic circulation [100, 107]. VAT also exhibits greater expression of the receptors; β3-adrenergic, glucocorticoid, and androgen receptors relative to SAT [100, 107]. Furthermore, these fat depots differ in cytokine expression and secretion. For example, VAT has greater expression and secretion of TNF !, IL-6 and plasminogen activator inhibitor-1 (PAI-1), whereas leptin and adiponectin secretion are greater in SAT. It has also been shown that deep SAT (DSAT), a sub-compartment within the abdominal SAT depot, has a greater inflammatory gene expression profile than superficial SAT (SSAT), and in the case of TNF ! expression, similar to that of VAT [98, 108]. These differences in AT depots are particularly relevant in countries with ethnic diversity such as the United States of America (USA) and SA. Both African American women and black SA women present with less VAT for the same waist or body fat than do their respective white counterparts [22, 88, 91, 102, 103, 109, 110].

1.6.2. Adipose tissue and systemic inflammation

Both obesity [30, 111-113] and CAD [113-117] have been described as a state of low grade inflammation, attributed to excess adipose tissue, adipose tissue inflammation, increased release of free fatty acids and increased secretion of adipokines from enlarged fat cells [6, 7]. It is interesting to note that even though VAT is regarded as more inflammatory than SAT, black SA women have greater SAT [102], and a higher SAT inflammatory gene expression profile compared to white SA women, despite showing no ethnic differences in circulating inflammatory markers [118]. In contrast, Albert *et al.* found significantly higher levels of C-reactive protein (CRP) in African American women compared to Caucasian women [119]. It has also been reported that the levels of leptin, CRP and fibrinogen were higher in black compared to white South Africans, after adjusting for central obesity [120]. These variations in findings may be partly due to the fact that cytokines act in a paracrine and autocrine manner making it difficult to capture an accurate measurement [88, 98].

1.6.3. Inflammatory gene expression

Only a few studies have examined the effect of ethnicity on cytokine gene and protein expression. In morbidly obese African American and white American women undergoing bariatric surgery, there were no differences in mRNA levels or *in vitro* release of IL-6, interleukin-8 (IL-8) and PGE_2 from VAT between ethnic groups [121]. In contrast, when comparing black and white SA women, black women had higher abdominal and gluteal SAT inflammatory gene expression then white women, which

was independent of age, total adiposity and VAT. This was characterized by increased chemokine (C-C motif) ligand 2 (CCL2), and its receptor, C-C chemokine receptor type 2 (CCR2), cluster of differentiation 68 (CD68), TNF!, colony stimulating factor-1 (CSF-1), and macrophage inhibitory factor (MIF) [98].

1.6.4. Genotype frequency

Functional polymorphisms have been reported in the promoter regions of the *TNFA, IL-1*, *IL-6* and lymphotoxin-! *(LTA)* inflammatory genes, altering cytokine production [122-125]. Several of these polymorphisms have been shown to interact with dietary fatty acids to regulate production and secretion of cytokines, predisposing an individual to inflammation and altering obesity and associated comorbidity risk [40, 126].

In a recent review article by Phillips [127], it has been suggested that inconsistencies in previous studies on the influence of cytokine polymorphisms on the risk for obesity, diabetes and the metabolic syndrome (MetS), may be, in part, explained by the fact that cytokines act in a complex network, and that studying single genes may not provide full insight [127]. The recent LIPGENE-SU.VI.MAX MetS case control study reported by Phillips *et al.* examined the relationship between *LTA, IL-6* and *TNFA* gene variants and MetS. They reported that the G allele of the *TNFA* -308 G>A polymorphism and the minor A allele of the *LTA* rs915654 polymorphism were associated with a 20-40% higher MetS risk [128], but the combined effect of carrying both risk genotypes further increased MetS risk. It was also shown that total plasma PUFA:SFA levels exacerbated the observed additive genetic effects of *IL-6, TNFA*

and *LTA* [128], highlighting the importance of studying nutrigenetic single nucleotide polymorphism (SNP)-SNP or gene-gene interactions.

A number of studies have investigated the relationship between cytokine polymorphism frequencies and ethnicity. The consensus from these studies is that African Americans have a greater frequency of alleles associated with high production of pro-inflammatory cytokines and a low production of anti-inflammatory cytokines [97]. Specifically, interleukin-1 (*IL-1) A* -889 T, *IL-1 B*, -3957 C and -511A, *IL-6* -174 G, interleukin-18 (*IL-18)* -137 G, and *TNFA* -308 A alleles have been associated with an increase in cytokine production and are found more frequently among African Americans [97, 129-131]. There is a scarcity of cytokine genotype studies in Sub-Saharan and Southern African populations [132], and none examining differences in genotype frequencies between black and white South Africans. Genotype and allele frequencies of polymorphisms included in this thesis have been compared in Table 1.1 for European, British in England and Scotland, African, and African American populations (Ensemble public database; http://browser.1000genomes.org). These polymorphisms are polymorphic in all these populations, however there is a very low frequency of the *IL-6* -174 C allele in populations of African descent [133]. Furthermore, it is not yet known if these ethnic differences in polymorphism frequencies are associated with functional differences in inflammatory pathways.

Table 1.1. *TNFA* and *IL-6* genotype and minor allele frequencies

	Ensemble 1000 Genomes: phase 1			
	EUR	GBR	AFR	ASW
***TNFA* -308 G>A rs1800629**				
GG	0.75	0.80	0.81	0.87
GA	0.24	0.17	0.18	0.13
AA	0.02	0.03	0.00	0.00
A allele	0.14	0.12	0.10	0.10
***TNFA* -238 G>A rs361525**				
GG	0.87	0.81	0.93	0.90
GA	0.13	0.19	0.07	0.10
AA	0.00	0.00	0.00	0.00
A allele	0.07	0.10	0.03	0.05
***IL-6* 174 G>C rs1800795**				
GG	0.36	0.39	0.95	0.78
GC	0.44	0.42	0.05	0.21
CC	0.20	0.19	0.0	0.0
C allele	0.41	0.40	0.02	0.11
***IL-6* IVS3+281 G>T rs1554606**				
GG	0.34	0.36	0.48	0.39
GT	0.45	0.44	0.46	0.57
TT	0.20	0.19	0.05	0.03
T allele	0.43	0.41	0.28	0.32
***IL-6* IVS4+869 A>Grs2069845**				
AA	0.34	0.36	0.46	0.37
GA	0.45	0.44	0.47	0.57
GG	0.20	0.19	0.05	0.04
G allele	0.43	0.41	0.29	0.33

Population frequencies are from the Ensemble public database (http://browser.1000genomes.org) [134]. Abbreviations: EUR, European; GBR, British in England and Scotland; AFR, African; ASW, Americans of African Ancestry in SW USA; *TNFA*, tumour necrosis alpha; *IL-6*, interleukin-6

1.6.5. Dietary intake in black and white South Africans

In evaluating dietary intake in SA, it is important to consider the health and nutrition transition caused by growing urbanisation [9, 15, 135, 136]. The health transition is characterised by a decrease in physical activity, changes in diet and eating patterns, tobacco use and increased alcohol use [9]. The changes in dietary intake are largely due to urban exposure and availability of fast foods and drinks replacing leisurely meal times of home grown and indigenous foods [15, 135-137]. Many of the food items in urban areas are high in fat and sugar, having a high energy density, but a low nutrient density [9]. Specifically, rural dwellers have a higher intake of cereals and vegetables, however for all other food groups, urban intake is greater. This is particularly true for sugar, meat, vegetable oil, dairy, fruit, roots, tubers and alcohol consumption [9, 138].

This thesis focuses on dietary intake of SA urban whites and urban blacks residing in townships. Figure 1.2 compares macronutrient distribution between the Coronary Risk Factor Study in blacks (BRISK) (black urban) and Vanderbijlpark Information Project on Health, Obesity and Risk Factors (VIGHOR) (white urban) dietary studies in SA [139, 140]. In summary, black and white urban populations appear to consume similar amounts of sugar and protein, however total fat and SFA intake is higher in white urban compared to black urban diets, and carbohydrate (CHO) intake is greater in the black urban diet compared to the white [139, 140].

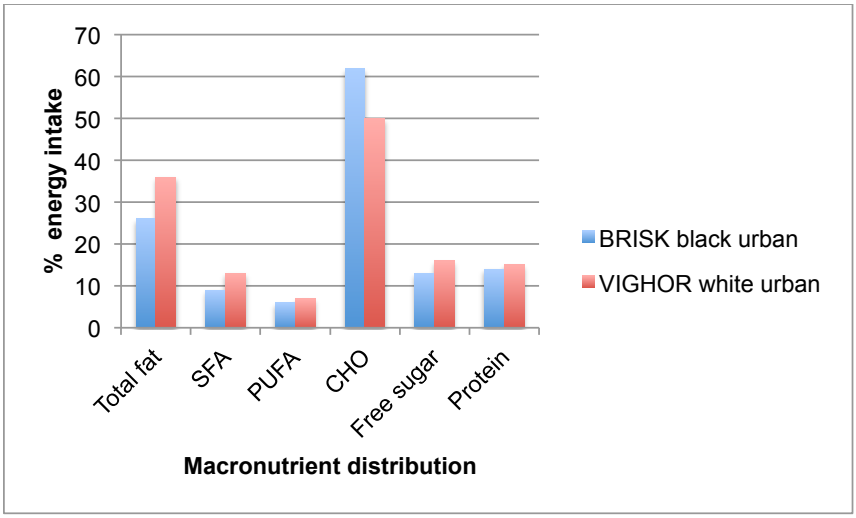

Figure 1.2. Mean macronutrient intake as a % of total energy intake from BRISK (black urban) and VIGHOR (white urban) studies [139, 140]. Abbreviations: CHO, carbohydrate; PUFA, polyunsaturated fatty acid; SFA; saturated fat; BRISK, Coronary Risk Factor Study in blacks; VIGHOR Vanderbijlpark Information Project on Health, Obesity and Risk Factors

While it is known that a high and increasing dietary fat (PUFA and SFA) intake is cause for concern in both black and white South Africans, these studies are 20 years old and have not been replicated recently, nor have new studies been conducted. Furthermore, in these studies only total fat intake and groupings of dietary fatty acids such as PUFA, monounsaturated fatty acids (MUFA) and SFA were analysed, while individual dietary fatty acids being consumed were not identified. Knowing the intake of individual fatty acids in different populations is valuable in order to investigate and understand their impact on inflammation, and the aetiology of dietary fatty acids in lipid metabolism.

1.6.6. Diet-gene interactions

In a systematic review, Patel *et al.* investigated the influence of ethnicity on the relationship between n-3 PUFA intake and CVD in six ethnic groups (African and

African American, Inuit and Eskimo, Italian, South Asian, Eastern Asian, and indigenous white populations of Europe, the USA and Australia)[99]. They concluded that studies have not shown consistency in the effects of n-3 PUFA on CVD risk and that ethnicity is a factor that can account for some of this variation. This includes an ethnic groups actual dietary intake of n-3 PUFAs, as well as their ability to absorb and utilise n-3 PUFAs from the diet [99]. Another key consideration in reviewing dietary intake and diet-gene interactions is the impact that certain genes have on dietary fatty acid metabolism, and how these may differ between ethnic groups. Of interest is the fatty acid desaturase gene (*FADS*), which codes for enzymes in PUFA metabolism. Lu *et al.* reported how genetic variation in the *FADS1* gene interacted with dietary intake of both n-3 and n-6 PUFA to affect T-C and HDL-C concentrations [141]. Furthermore, it has recently been shown that *FADS* polymorphisms altered the capacity of different ethnic groups to synthesize long-chain fatty acids [75, 142]. Specifically, Sergeant *et al.* found that *FADS* genotype frequencies differed significantly between African Americans and European Americans, and *FADS* polymorphisms in African Americans were associated with the ability to more efficiently convert the n-6 fatty acid LA to the pro-inflammatory fatty acid AA, resulting in higher circulating AA levels, and potentially a more deleterious impact of a diet high in LA in this ethnic group [75].

1.7. Gender as a confounder

Like ethnicity, it is likely that the gender of study participants may impact genotype-phenotype interactions. A number of studies have observed

gene–diet–gender interactions, whereby an interaction was identified in one sex, but not another. For example, the FINGEN study, which examined the effect of long chain n-3 PUFA supplementation and *APOE* genotype on plasma lipids, reported greater TAG lowering effects following dietary intervention in *APOE4* males than in females [143]. Further, results from the Framingham Heart Study showed that dietary PUFA intake modulates the effect of the apolipoprotein A1 (*APOA1*) −75 G>A polymorphism (rs670) on plasma HDL-C concentrations in women but not in men [144, 145]. In addition, Phillips *et al.* has shown that the transcription factor 7-like 2 *(TCF7L2)* rs7903146 polymorphism influences MetS risk, and is impacted by both gender and dietary SFA intake [145]. Phillips *et al.* have also identified associations between genetic variants of the apolipoprotein B and *APOA1* gene and MetS risk, however the modulation of MetS risk by dietary fat intake observed in the entire cohort was observed in the male high-fat consumers only [146].

For the remainder of this review genetic variation in the cytokine genes *TNFA* and *IL-6* will be discussed in detail, including their association with obesity and serum lipids, and their interaction with dietary fatty acids.

1.8. Tumour necrosis factor-!

1.8.1. TNF! and obesity

TNF! was the first cytokine associated with inflammation [125]. TNF! is overexpressed in the AT of obese individuals, with greater expression in VAT than in SAT [113, 147]. TNF! acts in a paracrine manner, suggesting that circulating TNF!

levels may not be indicative of actual TNF ! levels [148]. Despite this caution, elevated circulating levels of TNF ! have been observed in the obese phenotype, which decrease with weight loss [149]. It was originally proposed that adipocytes were the principle source of raised TNF ! levels in obesity, however, it is now well recognized that TNF ! is abundantly produced by macrophages in the SVF [33, 113, 147, 148]. TNF ! has numerous effects in AT including regulating adipogenesis, lipid metabolism and insulin signaling [34, 40]. In addition to its ability to increase other pro-inflammatory cytokines [147, 150], TNF ! has also been associated with a reduction in anti-inflammatory adipokines such as adiponectin [151, 152]. Conclusively, TNF ! plays a powerful role in regulating inflammatory pathways, favouring an overall inflammatory state [40].

1.8.2. TNF ! and serum lipids

The influence of TNF ! on lipid metabolism is complex with the underlying mechanisms still unclear. However, Chen *et al.* in his review describes four signal pathways involved in TNF !-mediated lipid metabolism [153]. The effects of TNF ! on lipid metabolism occurs in different cells, tissues, and organs and includes a number of metabolic processes. TNF ! induces lipolysis, increasing free fatty acid (FFA) production. In addition, TNF ! regulates cholesterol metabolism and other adipocyte-derived adipokines such as leptin, adiponectin, etc. which may also alter lipid metabolism [153].

A number of studies, in cell culture, rodent and human clinical models have

confirmed the relationship between TNF ! and lipid metabolism [153]. In clinical patients with dyslipidaemia, TNF ! levels were altered. Compared with healthy subjects, patients with hyperlipoproteinaemia showed higher TNF ! levels and raised T-C, TAG, and LDL-C concentrations. Furthermore, after treatment with fenofibrate, T-C, TAG and VLDL-C decreased, which correlated with decreasing TNF ! concentrations [154]. It has also been shown that cholesterol-lowering drugs such as simvastatin and atorvastatin decrease TNF ! concentrations [155, 156], and that blockading TNF ! production improves lipid metabolism [157]. Lastly, administration of TNF ! interferes with plasma lipid levels [153, 158, 159]. Feingold *et al.* has shown that an increase in hepatic VLDL-TAG secretion induced by TNF ! is due to both the stimulation of hepatic *de novo* fatty acid synthesis and an increase in lipolysis [158].

1.8.3. TNF ! and dietary fatty acids

Several studies have investigated the effect of dietary fatty acids on TNF ! concentrations and *TNFA* gene expression in cell, animal and human models (Table 1.2.). Plasma TNF ! levels and *TNFA* gene expression increased in 3T3-L1 adipocytes incubated with the SFA palmitic acid, whereas incubation with the MUFA, oleic acid and the n-3 PUFA, DHA had no effect [160]. In rodent studies, supplementing the diet with n-3 PUFA decreased *TNFA* gene expression in mice [161]. In another study, rats were fed a standard diet (18% energy from protein, 76% as CHO and 6% of energy as lipid) or a high-fat cafeteria diet (9% energy as protein, 29% as CHO and 62% as lipid). The high fat diet increased both body weight and fat mass, however when these rats were supplemented with EPA they gained less

weight, decreased their food intake and increased leptin production. *TNFA* gene expression was also increased by the high fat diet, but not in the rats supplemented with EPA [162].

Similar results were reported in human studies; Caucasians supplemented with ALA in the form of flaxseed oil in domestic food preparation for four weeks, experienced a 30% reduction in TNF ! production. When these subjects were exposed to further supplementation with fish oil (9g/d) for an additional four weeks, TNF ! production was reduced by up to 70%. There was a significant inverse exponential relationship between TNF ! synthesis and mononuclear cell content of EPA [163]. Similarly, Endres *et al.* used a radioimmunoassay to measure TNF ! produced in vitro by stimulated peripheral-blood mononuclear cells. They reported that supplementation with n-3 PUFA (18g/d) for six weeks decreased TNF ! levels but these levels returned to baseline levels once supplementation was stopped [164]. However, Grimble *et al.* demonstrated that the change in TNF ! production in response to n-3 PUFA supplementation depended on the subjects' plasma TNF ! levels prior to supplementation, with subjects with lower pre-supplementation TNF ! levels showing the greatest decrease in TNF ! levels post-supplementation [165]. It is possible that the inherent inflammatory status, potentially due to a pre-existing condition such as obesity, could determine the extent of the inflammatory response to different dietary fatty acids. In addition to the independent influence of dietary fatty acids on TNF ! production, variation in the *TNFA* gene may also contribute to the individual variability observed in TNF ! production and *TNFA* gene expression.

Table 1.2. Dietary fatty acids and TNF ! production and gene expression

	Study type	Dietary fatty acid	Effect on gene expression	Effect on plasma levels	Reference
3T3-L1 adipocytes. Incubation at 24 and 48h with 50 or 500μM fatty acid	Cell culture	SFA (PA) MUFA (OA)	Increase No effect	Increase No effect	[160]
Human macrophages treated with n-3 PUFA.	Cell culture	EPA & DHA	Decrease	Decrease	[166]
Male Wistar rats, high fat diet, 1g/kg per day EPA, 5 weeks	Rodent	n-3 PUFA (EPA)	Prevent over expression	Not examined	[162]
NZB/NZW F1 Lupus-prone female mice, 10% fat, fed *ad lib* for lifespan	Rodent	n-3 PUFA	Decrease	Not examined	[161]
Caucasians. Supplemented normal diet with 18g fish oil daily for 6 weeks.	Human intervention (9)	n-3 PUFA		Decrease	[164]
Caucasians. Supplemented normal diet with 6g fish oil daily for 12 weeks.	Human intervention (111)	n-3 PUFA		Decrease in subjects with lower levels of TNF ! before supplementation.	[165]
Caucasians. Supplemented normal diet with flaxseed oil, and flaxseed oil and butter spread for 8 weeks. At week 4, diets were supplemented with fish oil, (1.62g EPA, 1.08g DHA) /day.	Human intervention (28)	n-3 PUFA (EPA & DHA)		Decrease	[163]

The number of subjects (n) is in parentheses. Abbreviations: TNF; tumour necrosis factor, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; (n-3) PUFA, omega-3 polyunsaturated fatty acid; (n-6) PUFA, ALA, !-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. AA, arachidonic acid; DA, lauric acid

1.8.4. TNFA gene variants, obesity and serum lipids

Several polymorphisms have been identified in the promoter region of the *TNFA* gene, however the *TNFA* -308 G>A (rs1800629) and -238 G>A (rs361525) polymorphisms are most commonly associated with measures of adiposity, obesity risk and serum lipids (Table 1.3). The A allele of the functional -308 G>A polymorphism results in a 2-fold increase in *TNFA* transcription, with a subsequent increase in TNF ! production [125]. Several studies have reported that carriers of the pro-inflammatory -308 A allele (AA and GA genotypes) reported a higher BMI and/or percent body fat than those with the GG genotype [167-172]. In a recent meta-analysis by Yu *et al.* including 48 eligible studies, the -308 GA+AA genotypes were associated with an increased risk of obesity (odds ratio (OR), 1.19; 95% CI, 1.02–1.39) [126]. This result was consistent with the study by Sookoian *et al.*, who performed a meta-analysis of 31 observational studies with a total of 3562 individuals and showed that individuals with the GA+AA genotypes had a 23% elevated risk of obesity compared with the GG genotype (OR, 1.23; 95% CI, 1.05–1.45) [173]. Not all studies have however shown an association between the -308 G>A polymorphism and obesity [173-177]. In comparison to the -308 G>A polymorphism, only two studies have investigated the association between the -238 G>A polymorphism and obesity, and neither found an association between the -238 G>A polymorphism and BMI [177, 178].

To our knowledge only two papers have found an independent association between the -308 G>A polymorphism and serum lipid concentrations. In Caucasian men, the -308 A allele was associated with increased TAG [179], and in Polish Caucasian

men and women the AA genotype was associated with lower HDL-C concentrations compared to the GG genotype [180]. Furthermore, to our knowledge, no independent associations have been reported between the -238 G>A polymorphism and serum lipid concentrations.

No studies have reported on associations between *TNFA* polymorphisms, obesity and serum lipids in African populations. While the A allele of the -308 G>A and -238 G>A polymorphisms appear to be associated with the obese phenotype and serum lipid concentrations, it is highly likely that genetic variation in the *TNFA* gene may provide only a partial explanation with regards the inter-individual variability observed and the heterogeneity of the results in these studies. Other variables such as ethnicity, gender, diet, lifestyle and environmental factors may modulate these associations and contribute to the different results observed.

Table 1.3. Studies investigating associations between *TNFA* polymorphisms and obesity and serum lipids.

SNP	Study cohort	Genotype frequency	Result	Ref.
Obesity				
TNFA -308 G>A	Caucasian N-W (154) and obese (154)	N-W, GG: 75.8%; GA+AA: 24.2% Obese, GG: 70.8%; GA+AA: 29.2%	• G>A SNP by itself indicates only minor effect on obesity risk.	[170]
	Caucasian women (378)	GG: 72.2%; GA+AA: 27.7%	• AA genotype more obese than GA and GG • Body fat of AA genotype increased by 1/3 compared with GA and GG genotype. • In obese females BMI and body fat of AA genotype higher than GA and GG.	[169]
	Caucasian BMI < 27.3 (44), BMI 27.3 – 31.9 (44), BMI 31.9 – 36.5 (44) and BMI > 36.5 (44)	BMI < 27.3, GG: 75%; GA+AA: 25%. BMI 27.3 – 31.9, GG: 68.2%; GA+AA: 31.8%. BMI 31.9 – 36.5, GG: 77%; GA+AA: 33%. BMI >36.5, GG: 47.7%; GA+AA: 52.3%	• Difference in allele frequencies of the SNP across BMI quartiles. • Higher A allele frequency in highest BMI group • A allele carriers had higher BMI than G carriers.	[167]
	Caucasian (1392)	GG: 67.6%; GA+AA: 32.3%	• Carriers of the A allele were more frequently obese than non-carriers (OR = 1.52).	[168]
	Caucasian normal wt. (79) and obese (115)	N-W, GG: 73.4%; GA+AA: 24.2% Obese, GG: 75.6%; GA+AA: 26%	• No genotype difference between N-W and obese groups.	[174]
	Korean normal wt. (82) and obese (153)	N-W, GG: 82.9%; GA+AA: 17% Obese, GG: 84.3%; GA+AA: 15.7%	• No difference in genotype between N-W and obese subjects. • WHR was significantly lower in those with GA and AA genotype in obese women.	[176]
	Caucasian normotensive (113) and hypertensive (62)	Normotensive, GG: 84.8%; GA+AA: 15% Hypertensive, GG: 67.7%; GA+AA: 32.3%	• No BMI difference for genotypes.	[173]
	Caucasian normal weight (64) and overweight (65)	Not shown	• A allele associated with efficient lipid storage in overweight subjects.	[171]
	Caucasian men (262)	GG: 56.4%; GA+AA: 43.5%	• A allele tendency to higher BMI value, WHR and abdominal diameter.	[172]
	Meta-analysis (48 eligible studies)		• A allele associated with an increased risk of obesity (OR, 1.19; 95% CI, 1.02–1.39)	[126]
TNFA -308 G>A & -238 G>A	Iranian men and women (BMI <25, 25 ! BMI >30, BMI " 30.) (239)	Under 18 years, BMI < 85%, GG: 80.8%; GA: 19.2%. BMI > 85%, GG: 81.2%; GA: 12.5%; AA: 6.2%. Above 18 years, BMI <25, GG: 89.3%; GA: 10.7%, ! BMI < 30, GG: 87.7%; GA: 12.3%, BMI " 30, GG: 80.4%; 15.2%; 4.3%.	• A allele had no association with BMI	[181]
	Caucasian and African-American non-diabetics (424)	-308 G>A BMI < 25, GG: 73.2%; GA: 22.5%, AA: 4.3%. BMI 25 – 29.9, GG: 66.7%; GA: 27%, AA: 6.3%. BMI 30 – 40, GG: 67.2%; GA: 30.2%; AA: 2.6%. BMI >40, GG: 73.4%; GA: 24.3%; AA: 2.4% -238 G>A BMI < 25, GG: 90.4%; GA: 9.1%, AA: 0.5%. BMI 25 – 29.9, GG: 90.5%; GA: 7.9%, AA: 1.6%. BMI 30 – 40, GG: 85.9%; GA: 14.1%. BMI >40, GG: 91.1%; GA: 8.9%.	• A allele had no association with BMI	[177]
Serum lipids				
TNFA -308 G>A	Caucasian obese women (136) and obese men (34)	Obese women, GG: 57.3%; GA+AA: 42.6% Obese men, GG: 64.7%; GA+AA: 35.3%	• AA genotype had lower HDL cholesterol than the GG genotype.	[179]
	Caucasian obese men (38) and obese women (83)	Obese men, GG: 50%; GA+AA: 50% Obese women, GG: 54.2%; GA+AA: 45.7%	• A allele carriers in men had significantly increased levels of TG, FFAs and fasting glucose.	[180]

Genotype frequency is expressed as a percentage. The number of subjects (n) is in parentheses. Abbreviations: SFA, *TNFA*, tumour necrosis factor alpha; N-W; Normal-weight; WHR, waist-hip ratio; TAG, triglycerides; T-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-C:HDL-C ratio, total cholesterol: high-density lipoprotein cholesterol ratio.

1.8.5. TNFA gene and diet interactions on obesity and serum lipids

The *TNFA* -308 G>A and -238 G>A polymorphisms have been shown to modulate the relationship between dietary fat intake on obesity and serum lipid profiles (Table 1.4). Despite studies showing independent associations between the *TNFA* polymorphisms and obesity (Table 1.3.), to our knowledge only one study has investigated interactions between diet and the *TNFA* -308 G>A polymorphism. Nieters *et al.* found that German Caucasian men and women with the -308 A allele, who were in the highest tertile for intake of the n-6 PUFAs LA and AA (%E), had an increased obesity risk [170]. To date, no studies have reported on diet-gene interaction between the -238 G>A polymorphism, dietary intake and obesity (Table 1.4.).

In contrast, Fontaine-Bisson *et al.* have shown interactions between the -308 G>A and -238 G>A polymorphisms and dietary fat intake on serum lipid profiles (Table 1.4.). In ethnically diverse diabetic Canadians, PUFA intake was inversely associated with HDL-C concentrations in -308 A allele carriers, but positively associated with HDL-C concentrations in -238 A allele carriers [182]. In a combined analysis in healthy non-diabetic Canadians, they also reported that in individuals with the -308 GA + AA & -238 GG genotypes, an inverse relationship was observed between HDL-C concentrations and n-3, n-6 and total PUFA intake [183]. These diet-gene interactions have not been investigated in African populations, and would be of interest due to the higher inflammatory genotype observed, as well as differences in dietary intake between black and white populations.

Table 1.4 Diet-gene interactions between *TNFA* & *IL-6* polymorphisms and dietary fat intake on obesity and serum lipids**.**

SNP	Study cohort	Genotype frequency	Diet assessment	Diet-gene association	Reference
TNFA					
Obesity					
TNFA -308 G>A	Caucasian N-W (154) and obese (154)	Normal weight, GG: 75.8%; GA+AA: 24.2% Obese, GG: 70.8%; GA+AA: 29.2%	Food frequency questionnaire measured energy and dietary fatty acid intake.	• A allele carriers had increased obesity risk with increasing intake of linoleic and arachidonic acid.	[170]
Serum lipids					
TNFA -308 G>A & -238 G>A	Ethnoracially diverse Canadian diabetic men (53) and women (56)	-308 G>A GG: 63.3%; GA: 32%; AA: 0.05%. -238 G>A GG: 75.2%; GA: 21.1%; AA: 0.04%.	Three-day food record measured dietary fat intake.	• PUFA intake was inversely associated with HDL-C in carriers of the -308 A allele, but not in those with the GG genotype. • PUFA intake was positively associated with serum HDL-C in *-238 A* allele carriers but negatively associated in the *GG* genotype.	[182]
TNFA -308 G>A & -238 G>A	Ethnoracially diverse Canadian healthy men (202) and women (393)	-308 G>A 11% for A allele -238 G>A 5% for A allele	Food frequency questionnaire measured dietary intake.	• In individuals with the -308 GG & -238 GG genotypes, n-3, n-6 and total PUFA intake was positively associated with HDL-C in men and women, and an inverse relationship was observed among men carrying the -308 A & -238 GG genotypes.	[183]
IL-6					
Obesity					
IL-6 -174 G>C	Obese Caucasians men (181) and women (541)	GG: 28.8%; GC: 50.2%, CC: 18%.	Test meal consisted of 95 %E from fat, of which 60% SFA.	• The ability to increase fat oxidation after a high fat load was increased in subjects with ! 174 C allele.	[184]
	737 Spanish men and women	GG: 37.6%; GC: 46.8%; CC: 15.6%	Three years diet intervention assigned to low-fat diet; Mediterranean diet supplemented with virgin olive oil or with nuts.	• At baseline, the CC genotype was associated with higher measures of adiposity. • After 3 years, CC subjects following the Mediterranean diet supplemented with virgin olive oil had the lowest weight gain.	[185]
Serum lipids					
IL-6 -174 G>C	Spanish Caucasian men and women (32)	GG: 25%; GC: 40.6%; CC: 34.4%	Measured fasting and post-glucose load plasma lipids.	• G allele carriers showed higher TAG and VLDL-C, and higher fasting and post-glucose load free fatty acids levels than C allele carriers.	[186]

Genotype frequency is expressed as a percentage. The number of subjects (n) is in parentheses. Abbreviations: *IL*, interleukin; SFA, *TNAF*, tumour necrosis factor alpha; N-W; Normal-weight; TAG, triglycerides; T-C, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; T-C:HDL-C ratio, total cholesterol: high density lipoprotein cholesterol ratio, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P:S ratio, polyunsaturated fatty acid : saturated fatty acid ratio; (n-3) PUFA, omega-3 polyunsaturated fatty acid; (n-6) PUFA, omega-6 polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, omega-6:omega-3 polyunsaturated fatty acid ratio; ALA, !-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

1.9. Interleukin-6

1.9.1. IL-6 and obesity

During an acute incident, the cytokine IL-6 acts as an anti-inflammatory cytokine, but in a chronic inflammatory condition, IL-6 is pro-inflammatory, as well as being a key regulator of hepatic CRP production [187, 188]. IL-6 is secreted by a variety of cells, however approximately 30% of total IL-6 is produced by AT, and macrophages that have infiltrated WAT produce approximately 50% of AT-derived IL-6 [187-189]. Higher circulating concentrations of IL-6 have been associated with obesity, especially VAT deposition [190-192], and decrease in response to weight loss [190, 193]. One should however cautiously interpret circulating IL-6 levels as both adipokines and myokines contribute to IL-6 concentrations. A number of studies have reported that contracting muscle fibers secrete IL-6, exerting a local effect within the muscle, as well as releasing IL-6 into the circulation [194].

1.9.2. IL-6 and serum lipids

IL-6 is associated with lipid metabolism and plays a role in the development of atherosclerosis through a number of different mechanisms causing metabolic and endothelial dysfunction. [36, 189]. IL-6 impairs insulin action, elevating lipolysis, and increasing FFA release [195]. The increase in FFAs reduces nitric oxide (NO) bioavailability and impairs vasodilation [196]. Insulin resistance and subsequent hyperglycaemia also increases lipoprotein oxidation and subsequent increased expression of adipocytokines [115]. In rats administered IL-6, serum TAG, T-C levels

and hepatic TAG secretion increased [197]. Similarly, in human studies, increased IL-6 levels have been associated with an increase in serum TAG and FFAs, and low HDL-C concentrations [198, 199].

1.9.3. IL-6 and dietary fatty acids

Several studies have investigated the effect of different dietary fatty acids on IL-6 production and *IL-6* gene expression in cell, animal and human models, with all studies showing similar results (Table 1.5). An *in vitro* study reported that IL-6 plasma levels and *IL-6* gene expression increased when 3T3-L1 adipocytes were incubated with the SFA, palmitic acid, whereas no effect was observed for lauric acid (C12:0) and the n-3 PUFA, DHA [69]. Similarly, macrophages treated with EPA or DHA showed a decrease in lipopolysaccharide-stimulated (LPS) *IL-6* mRNA and IL-6 production. In agreement with these findings, rats fed a diet high in n-6 PUFA-rich sunflower oil showed moderate IL-6 release from adipocytes, which was less than when fed a SFA-rich diet, but greater than when fed a MUFA-rich diet [200]. In addition, in mice fed a low fat diet or one of two high-fat diets, consisting of either unsaturated soybean oil or saturated palmitic acid for 16 weeks, MCP-1 expression in AT tissue increased for both high-fat diets compared to the low-fat diet. However, the high saturated fat diet also showed a three-fold increase in *IL-6* expression in AT not observed in the soybean oil diet [201].

In human studies (Table 1.5.), increased long-chain n-3 PUFA intake and fish consumption were associated with decreased plasma IL-6 concentrations and other inflammatory markers (matrix metalloproteinase-3, CRP, soluble intercellular

adhesion molecule-1) in men from the Multi-Ethnic Study of Atherosclerosis cohort [202]. Furthermore, Ferrucci *et al.* reported that plasma levels of PUFAs, especially n-3 PUFAs, were independently associated with lower levels of pro-inflammatory markers and higher levels of anti-inflammatory markers [203]. A controlled feeding trial investigating the effects of SFA and MUFA–rich diets on serum lipid concentrations and whole-genome microarray gene expression profiles of AT, found that consuming a SFA-enriched diet for eight weeks resulted in increased expression of genes involved in inflammatory processes in AT including *IL-6,* and NF!B signaling, whereas the MUFA-enriched diet led to a more anti-inflammatory gene expression profile, accompanied by a decrease in serum LDL-C concentration [204].

Table 1.5. Dietary fatty acids and IL-6 production and gene expression

	Study type	Dietary fatty acid	Effect on gene expression	Effect on plasma levels	Reference
3T3-L1 adipocytes. Incubation at 24h with 250μM fatty acid	Cell culture	SFA (PA) SFA (DA) n-3 PUFA (DHA)	Increase No effect No effect	Increase No effect No effect	[69]
Human macrophages treated with n-3 PUFA.	Cell culture	EPA & DHA	Decrease	Decrease	[166]
Male c57bl/10sCn mice fed ad lib either high fat control diet (soybean oil) or a high PA diet for 16 weeks.	Rodent	SFA (PA)	Increase	Not examined	[205]
Male Sprague-Dawley rats fed ad lib one of 3 diets: SFA, MUFA, or PUFA for 4 weeks.	Rodent	SFA (coconut oil) MUFA (olive oil) PUFA (sunflower oil)	Not examined Not examined Not examined	Increase IL-6 release from adipocytes Decrease IL-6 release from adipocytes Moderate IL-6 release from adipocytes	[200]
Abdominally overweight Caucasians. Fed either SFA-rich diet (19% SFA and 11% MUFA) or MUFA-rich diet (20% MUFA and 11% SFA) for 8 weeks.	Human intervention (20)	SFA MUFA	Increase IL-6 gene expression Decrease *IL-6* gene expression		[204]
African Americans, Caucasians, Chinese and Hispanics men and women. Relationship between dietary intake (food frequency questionnaire) and biomarkers of inflammation and endothelial activation.	Human (5677)	n-3 PUFA		Decrease in IL-6 levels	[202]
Relationship between plasma fatty acids and inflammatory marker levels.	Human (1123)	n-3 PUFA (DHA) n-6 PUFA (AA)		Low plasma levels of DHA associated with increased IL-6 levels. Low plasma levels of AA associated with increased IL-6 levels.	[203]

The number of subjects (n) is in parentheses. Abbreviations: *IL*, interleukin; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; (n-3) PUFA, omega-3 polyunsaturated fatty acid; (n-6) PUFA, ALA, !-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. AA, arachidonic acid; DA, lauric acid

1.9.4. IL-6 gene variants, obesity and serum lipids

There is growing scientific evidence reporting associations between deoxyribonucleic acid (DNA) sequence variants within the *IL-6* gene and increased risk of obesity and dyslipidaemia (Table 1.6) [187, 189, 206-208]. The most frequently studied *IL-6* polymorphism is -174 G>C (rs1800795). This is a functional polymorphism, with most studies showing the -174 C allele to be associated with raised IL-6 and CRP concentrations in mostly Caucasian populations [126, 209-212]. Association studies between the -174 G>C polymorphism, obesity and dyslipidaemia have yielded conflicting results. A recent meta-analysis found the -174 G>C polymorphism was associated with obesity [126]. However, this finding was not reproduced in two other meta-analyses [210, 213]. The relationship between the -174 G>C polymorphism appears to be complex in that while the -174 C allele appears to be the risk allele associated with obesity [126], it is the -174 G allele that is associated with raised serum lipids concentrations [206, 208, 214], despite the -174 C allele being shown to be associated with raised IL-6 and CRP levels [126, 209-212]. The lack of consistent results in these association studies may be due to interactions between multiple polymorphisms on the *IL-6* gene that may modulate these relationships. This was illustrated by Qi *et al.* who found no independent association between the -174 G>C polymorphism and obesity, but did identify an association between an *IL-6* haplotype containing the -174 G>C polymorphism and adiposity in healthy American men and women [210].

A number of studies have reported associations between the -174 G>C polymorphism and serum lipid profiles (Table 1.6.). Though not consistent for all the

studies [214], for most, in Caucasian men and women the -174 G allele was associated with higher T-C, LDL-C, VLDL-C and TAG concentrations compared to the C allele [186, 206, 208]. Riikola *et al.* suggested several possible reasons that may explain the inconclusiveness of study results, including differences in body mass and body composition, as well as metabolic and pharmacological interference in the different study cohorts. They also suggested that the effect of IL-6 on serum lipids might differ depending on the phase of development of atherosclerosis. Age might also impact these associations as subtle allelic effects observable in young populations may be masked by stronger life-long diet and lifestyle covariates in older populations [208].

Table 1.6 Studies investigating associations between *IL-6* polymorphisms and obesity and serum lipids.

SNP	Study cohort	Genotype frequency	Result	Reference
IL-6				
Obesity				
IL-6 -174 G>C	Finnish men and women (1334)	GG: 19.3%; GC: 51.3%; CC: 29.3%	In men BMI was higher in the -174 CC genotype compared to GC and GG	[208]
	Meta-analysis (48 eligible studies)		• The C allele was associated obesity when using allelic comparisons, the recessive genetic model and the dominant genetic model with OR (95% CI) of 1.95 (1.37-2.77), 1.44 (1.15-1.80), and 1.36 (1.16-1.59), respectively.	[126]
	Meta-analysis Caucasians, diabetic and non-diabetic (25635)		• No evidence for association between -174 G>C SNP and BMI.	[213]
IL-6 -174 G>C & IVS3+281 G>T and IVS4+869 A>G	Health men (980) and women (2255) and Meta-analysis (26944)		• No association between -174 G>C SNP and adiposity. • T allele of the IVS3+281 G>T SNP associated with adiposity when part of a haplotype.	[210]
Serum lipids				
IL-6 -174 G>C	Caucasian men (245) and women (252)	Women, GG: 28%; GC: 47%; CC: 24% Men, GG: 30%; GC: 46%; CC: 24%	• The CC genotype was associated with lower levels of T-C and LDL-C in women.	[206]
	Finnish men and women (1334)	GG: 19.3%; GC: 51.3%; CC: 29.3%	• In men, serum T-C and LDL-C was higher in !174 GG than in the GC or CC genotype	[208]
	Spanish Caucasian men (15) and women (17)	GG: 25%; GC: 40.6%; CC: 34.4%	• G allele associated with high carriers TAG, VLDL-C and slightly lower HDL-C compared to the C allele.	[186]
	Finnish men and women (2228)	GG: 20.8%; GC: 50.4%; CC: 28.8%	• In men for HDL cholesterol was higher for !174 GG compared to GC or CC	[214]

Genotype frequency is expressed as a percentage. The number of subjects (n) is in parentheses. Abbreviations: *IL*, interleukin; SFA; N-W; Normal-weight; WHR, waist-hip ratio; TAG, triglycerides; T-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-C:HDL-C ratio

1.9.5. IL-6 gene and diet interactions on obesity and serum lipids

As previously described, studies have shown that dietary fat intake modulates the relationship between *TNFA* gene variants and obesity and serum lipid profiles (Table 1.4.). However, to our knowledge, only two published studies have reported on the relationship between any *IL-6* polymorphism and dietary intake, and both studies investigated *IL-6* -174 G>C. Corpeleijn *et al.* reported that the ability to increase fat

oxidation after a high fat load was increased in obese European Caucasians with the -174 C allele [184]. In Spanish men and women with a high CVD risk, the -174 CC genotype was associated with higher levels of adiposity at baseline, however after three years of nutritional intervention, those with the -174 CC genotype following a Mediterranean-style diet, had the greatest reduction in body weight [185].

To our knowledge only a single study has reported an interaction between the -174 G>C polymorphism and dietary intake on serum lipids concentrations in a Caucasian population. In this study, those individuals with the G allele had higher post-glucose load TAG and VLDL-C concentrations, and higher post-glucose load FFA levels than C allele carriers [186].

No studies have reported on *IL-6* associations, or on diet-*IL-6* gene interactions with obesity and serum lipids in African populations. The -174 G>C polymorphism is rare in individuals of African ancestry, is not informative, and has therefore not been studied in African populations. However, it would be valuable to identify and investigate *IL-6* polymorphisms that are representative in these populations.

1.10. Literature summary and conclusions

Both black and white SA women present with a high prevalence of overweight and obesity, however the co-morbidities associated with excess AT in these two groups appear to be different. Despite black SA women being more obese than white SA women, they have a less atherogenic lipid profile than the white women. Obesity and its associated co-morbidities are known to have a strong genetic contribution and a

multifactorial aetiology. Obesity is considered a chronic low-grade inflammatory condition. Hypertrophic AT is associated with macrophage infiltration and subsequent raised inflammatory markers. Elevated inflammatory markers are associated with oxidative modification of serum lipids and all stages of atherosclerosis. Therefore, low-grade inflammation is a plausible mechanism underlying both obesity and dyslipidaemia.

Different dietary fatty acids, as well as genetic sequence variants of inflammatory genes have been shown to alter inflammatory gene expression and protein production. This review suggests that polymorphisms involved in inflammation may interact with environmental exposures such as dietary intake, to modulate an individual's susceptibility to developing obesity and dyslipidaemia.

Two key and well-researched inflammatory cytokines are TNF ! and IL-6, which have been discussed in detail in this review. In summary, dietary fatty acids, in particular SFAs and the n-3 and n-6 PUFAs impact the expression of the cytokine genes *TNFA* and *IL-6,* and alter TNF ! and IL-6 production. In addition, polymorphisms in these genes have also been shown to alter their gene expression and plasma levels, and are associated with obesity, measures of adiposity and serum lipid concentrations.

To our knowledge, only a few studies have examined interactions between dietary fatty acids and the *TNFA* -308 G>A and -238 G>A polymorphisms on obesity [170], and serum lipid concentrations [182, 183], and interactions between dietary fatty acids and the *IL-6* -174 G>C polymorphism on obesity [184, 185], and serum lipids [186]. These studies have only been conducted in Caucasian and ethnically-diverse

populations.

In summary, Nieters *et al.* found that German Caucasians with the *TNFA* -308 A allele, who were in the highest tertile for intake of the n-6 PUFAs had an increased obesity risk [170]. In ethnically-diverse diabetic and non-diabetic Canadians, Fontaine-Bisson *et al.* reported interactions between n-3, n-6 and total PUFA intake and the *TNFA* -308 G>A and -238 G>A polymorphisms on HDL-C concentrations [182, 183].

Corpeleijn *et al.* reported that obese European Caucasians with the *IL-6* -174 C allele exhibited an ability to increase fat oxidation after a high fat load [184]. In a Spanish population, the *IL-6* -174 CC genotype was associated with higher levels of adiposity at baseline, but these individuals had the greatest reduction in body weight after following a Mediterranean-style diet [185]. In another Spanish Caucasian population, individuals with the *IL-6* -174 G allele had higher post-glucose load TAG and VLDL-C concentrations, and higher post-glucose load FFA levels than C allele carriers [186].

To our knowledge, there are no studies that have examined these polymorphisms, and their association with obesity and serum lipids, nor the impact of diet-gene interactions on obesity and serum lipids in African populations. This is of interest due to the higher inflammatory genotype observed in black populations, as well as differences previously reported in dietary intake between black and white populations.

The inter-individual and ethnic variability in the obese phenotype and serum lipid profiles, and inconsistencies in study results may be better understood by researching diet-gene interactions more extensively, and understanding further the role of ethnicity as a confounder. Understanding the complexity of obesity and its co-morbidities in SA will require a greater understanding of dietary intake, especially intake of individual dietary fatty acids, the frequency of polymorphisms in different populations and how these factors may interact in different ethnic groups in SA.

1.11. Aims and objectives

In a convenience sample of urbanized black and white SA women, the primary aim of the thesis was to investigate associations between *TNFA* and *IL-6* sequence variants and obesity and serum lipid concentrations. Only women were included because of the high prevalence of obesity reported in black and white SA women [3, 7]. Only black and white SA populations were compared, due to the disparities in obesity risk and risk for dyslipidaemia and CAD observed in these two groups[8].

The candidate genes (*TNFA* and *IL-6*) were selected based on the biological function of their encoded proteins (TNF ! and IL-6), and the role they play in both systemic and adipose tissue inflammation. The objectives of the specific gene association studies that addressed the primary aim of this thesis were as follows:

- Characterise ethnic differences in measures of adiposity and serum lipid levels between black and white SA women (Chapter 3).
- Investigate associations between the *TNFA* -308 G>A and -238 G>A

polymorphisms and obesity, measures of adiposity and serum lipid concentrations in both black and white SA women (Chapters 3 & 4).

- In addition to the commonly researched polymorphism *IL-6* -174 G>C, which has a low frequency in black populations; identify sequence variants in the *IL-6* gene with a reported high heterozygosity in both the white and black SA populations (rs1554606: IVS3+281 G>T, intron 3 and rs2069845: IVS4+869 A>G, intron 4) (Chapter 5).
- Determine if the *IL-6* polymorphisms (-174 G>C, IVS3+281 G>T, IVS4+869 A>G), were associated with obesity, measures of adiposity and serum lipid concentrations in black and white SA women (Chapter 5).
- A secondary objective of these studies was to investigate if these associations differed between black and white SA ethnic groups (Chapters 3,4 & 5).

The secondary aim of this thesis was to investigate interactions between *TNFA* and *IL-6* polymorphisms (*TNFA* -308 G>A and -238 G>A & *IL-6* -174 G>C, IVS3+281 G>T, IVS4+869 A>G) and dietary fatty acids on obesity, measures of adiposity and serum lipid concentrations. The objectives of the interaction studies that addressed the secondary aim of this thesis were as follows:

- Determine dietary intake, in particular dietary fatty acid intake of black and white SA women (Chapter 3).
- Investigate interactions between the *TNFA* -308 G>A polymorphism and dietary fatty acids on obesity and serum lipids in black and white SA women (Chapter 3).
- Investigate interactions between the *TNFA* -238 G>A polymorphism and

dietary fatty acids on obesity and serum lipids in black and white SA women (Chapter 4).

- Investigate interactions between the *IL-6* -174 G>C, IVS3+281 G>T, IVS4+869 A>G polymorphisms and dietary fatty acids on obesity and serum lipids in black and white SA women (Chapter 6).
- A secondary objective of these studies was to investigate if interactions identified differed between black and white SA ethnic groups (Chapters 3, 4 and 6).

CHAPTER TWO

METHODS

CHAPTER TWO

2.1. Subject selection

A convenience sample of 107 normal-weight (BMI ≤ 18.5- 24.9 kg/m²) and 120 obese (BMI ≥ 30 kg/m²) urban black and 89 normal-weight and 62 obese urban white SA women between the ages of 18 and 45 years were recruited in the greater Cape Town area, from 2004 to 2006. White and black subjects were recruited from church groups, community centres, and universities. Racial identity was self-reported. Subjects who reported both parents as black, or both parents as white, where included in the study. The majority of the black participants reported being of Xhosa ancestry (n=186, 78%), the remaining women were either of Zulu (n=4, 1.6%), Pedi (n=4, 1.6%), Tswana (n=3,1.2%), or mixed SA tribal ancestry (n=25, 10%), other black ancestries each represented less than 1%. Other inclusion criteria included: no previous diagnosis or undergoing therapy for diabetes, hypertension, dyslipidaemia, HIV or other metabolic diseases, and not currently pregnant or lactating. Only women were included because of the high prevalence of overweight and obesity seen in both black and white SA women, and because of the differences in serum lipids between black and white women previously described [19]. Only 73 normal-weight and 74 obese black, and 73 normal-weight and 48 obese white adequate reporters, according to the Goldberg cut-offs, were included in the analysis, as described below [215]. Specific numbers are given in each chapter as study group numbers differ for each chapter according to the number of subjects that were successfully genotyped for the polymorphism being investigated.

2.2. Ethics approval

Approval was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (REC REF: 101/2004) (Chapter 10, appendix), and written informed consent was obtained from all participants prior to participation. The study was performed in accordance with the principles of the Declaration of Helsinki, ICH Good Clinical Practice (GCP).

2.3. Assessment Methods

2.3.1. Body Composition

Basic anthropometric measurements included weight (in light-weight clothing without shoes), height, waist circumference (at the level of umbilicus) and hip circumference (largest gluteal area). Whole body composition was measured using dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500 Discovery-W dual-energy X-ray absorptiometer, software version 4.40, Hologic Inc., Bedford, MA, USA) according to standard procedures. *In vivo* precision (%CV) was determined for fat-free tissue mass (0.7%) and fat mass (1.7%) by measuring thirty individuals twice on the same day with re-positioning. The arm-replacement method was used to determine body composition in individuals who exceeded the scanning area (n=16) [216]. This method requires the radiographer to use the data from one arm for both arms. This method has been shown to be more accurate for the measurement of fat mass and fat-free tissue mass than the

half-body method proposed by Tataranni *et al.* [217]. A single slice computerised tomography (CT, Toshiba Xpress Helical Scanner, Japan) scan was taken at the level of the L4-L5 lumbar vertebrae to determine abdominal VAT and SAT [104]. Basic anthropometry was completed before measurement of body fat percentage by DXA.

2.3.2. Blood sampling and analysis

Blood samples were drawn from the antecubital vein after an overnight (10-12 hours) fast for the determination of TAG, T-C, HDL-C, and LDL-C concentrations, and for DNA extraction. Serum TAG, T-C, and HDL-C concentrations were measured on the Roche Modular Auto Analyzer using enzymatic colorimetric assays. LDL-C was calculated using the Friedewald equation [218].

2.3.3. Dietary assessment

Dietary intake was estimated using a food frequency questionnaire from Steyn and Senekal that represents monthly intake [219] (Chapter 10, appendix), and has been validated in black SA women by De Villiers *et al.* [220]. The questionnaire comprises 100 food items with food photographs to determine portion size. The questionnaire was administered by registered dieticians. Nutrient intake was calculated by means of the software program FoodFinder, III™ (SA Medical Research Council, Cape Town, SA)[221]. Under-reporting and over-reporting were detected on the basis of the ratio of energy intake (kJ/day):estimated resting metabolic rate (kJ/day), using the Harris Benedict

equation [215]). Upper and lower cut-points were calculated assuming a sedentary lifestyle of 1.55 x basal metabolic rate as defined by the physical activity level values from the World Health Organisation (WHO) recommended energy requirements [222]. Participants were defined as adequate reporters if this ratio was between 1.05 and 2.28 [215]. They included 42 under-reporters, 268 adequate reporters, and 73 over-reporters. Only adequate reporters were included in the diet analyses. All subject characteristics, body composition, serum lipids and dietary intake of the reporters groups, both as a combined group (Table 2S I), and separately for black (Table 2S II) and white (Table 2S III) women, are described in Chapter 9 (Supplementary data).

The Goldberg cut-offs [215] were used to classify individuals into energy intake reporting groups as it removes bias in energy intake based on size. Resting energy expenditure varies considerably between individuals with a low and high body mass, markedly affecting their energy intake requirements. The effect of body size on energy intake reporting is of particular relevance in the SA population as it has recently been shown that in a study including 198 SA women, 45% of black women under-reported their energy intake, of which 83% of these black under-reporters were obese (Personal communication, Z. Mciza, Human Science Research Council, Nutrition Unit. Research done with the University of Cape Town, SA, Human Biology Department). As this study focused on obesity and the sample comprised normal-weight and obese women, it was important to consider body size when selecting the women to include in the analyses.

2.4. Genotyping

2.4.1. DNA extraction

Approximately 5 ml of venous blood was collected from the antecubital vein of the subjects into an ethylenediaminetertaacetic acid (EDTA) vacutainer tube. Samples were stored at 4°C for no longer than one week until DNA was extracted using a modification of the method of Lahiri and Nurnberger [223]. In brief, the blood samples were transferred into sterile 15 ml polypropylene tubes with 10 ml of TKM1 buffer (10 mM Tris-HCl, pH 7.6; 10 mM KCl; 10 mM $MgCl_2$; 2 mM EDTA) containing 2.5% Nonidet P-40 to lyse the red blood cells. After a 10 minute incubation period at room temperature, the white blood cells were centrifuged at 1200 X g for 10 minutes at room temperature. The supernatants were discarded and the pellets resuspended in 5 ml of TKM1 buffer without NP40, by vortexing for 2 minutes. The samples were then centrifuged at 1200 X g for 10 minutes at room temperature, the supernatants were discarded and the pellets resuspended in 800 µl of TKM2 buffer (10 mM Tris-HCl, pH 7.6; 10 mM KCl; 10 mM $MgCl_2$; 2 mM EDTA; NaCl 0.4 M) containing 0.6% sodium-dodecyl-sulphate (SDS) by vortexing for 4 minutes. The samples were then incubated at 55°C in a water bath for 1 hour or until the pellets had completely dissolved, after which 150 µl of 5 mM $NaClO_4$ and 500 µl of analytical grade chloroform were added to the dissolved pellets. After vortexing for 15 seconds, the solutions were transferred into sterile 1.5 ml microfuge tubes and centrifuged at 600 X g for 10 minutes at room temperature. The top aqueous phases (approximately 500 µl) were transferred to a new sterile microfuge tube, and 1 ml of absolute ethanol

was added to precipitate the DNA. The samples were then centrifuged at 600 X g for 10 minutes at room temperature, forming a pellet of DNA in the bottom of the tubes. The supernatants were removed and the DNA was left to air dry for up to 10 hours. The DNA was resuspended in 200 µl of Tris-EDTA buffer (10 mM Tris-HCl 10, pH 8.0; 1 mM EDTA). The samples were incubated for 15 minutes at 65°C and stored at 4°C until PCR analysis.

2.4.2. Genotype analysis

For all polymorphisms genotyped, several negative control samples (containing no DNA), and positive controls were included on each 96 well polymerase chain reaction (PCR) plate in order to detect PCR contamination. The positive controls included a known sample for each of the three genotypes and the negative controls included three sample with no DNA included in the reaction. In addition, several DNA samples were randomly selected and re-analysed to confirm genotype results. All the negative and positive control samples gave the same results during all the analyses.

2.4.2.1. TNFA -308 G>A

DNA samples were genotyped for the functional -308 G>A polymorphism (rs1800629) within the proximal promoter of the *TNFA* gene by restriction fragment length polymorphism (RFLP) analysis using a nested PCR based method as previously described [224]. The following four primers were used:

forward primers F1 (5'-CTG AAG CCC CTC CCA GTT CTA-3') and F2 (5'-AGG CAA TAG GTT TTG AGG GCC-3'), and reverse primers R1 (5'-GCT CAT CTG GAG GAA GCG GTA-3') and R2 (5'-TCC TCC CTG CTC CGA TTC CG-3') (Figure 2.1). The F2 primer contains a modified nucleotide (underlined C), which creates a *Nco*I restriction recognition site in the secondary wild type PCR product.

Figure 2.1. A 600 bp genomic sequence within the proximal promoter of the human *TNFA* gene is shown to indicate the position of the outer forward (F1) and reverse (R1) primers (solid arrows) designed to amplify the primary 326 bp fragment. The positions of the nested forward (F2) and reverse (R2) primers (dashed arrows) designed to amplify the secondary 107 bp PCR fragment, which contains the -308 G>A transition (rs1800629), is also indicated. In addition the primer sequences are also in bold. The F2 primer contains a modified nucleotide, which is in lower case (c) and introduces a *Nco*I restriction recognition site (5'-CCATGG-3') in the amplified wild type sequence. Polymorphism rs1800629 is indicated with an R and the *Nco*I site is underlined with a dashed line. The G to A substitution eliminates the *Nco*I restriction site, therefore the 107 bp fragment is only digested when the G allele is present (87 bp and 20 bp). The sequence was obtained from Ensembl Transcript TNF-001 ENST00000448781 (www.ensembl.org). Abbreviations: bp, base pair; PCR, polymerase chain reaction; *TNFA*, tumour necrosis alpha gene.

The primary PCR reaction included approximately 100 ng genomic DNA, 20 pmol of the F1 and R1 primers, 20 mM Tris-HCl (pH 8.4), 10 mM KCl, 2 Mm $MgCl_2$, 20 Mm $MgSO_4$, 125 µM each dATP, dCTP, dTTP, and dGTP, and 0.5 U of *Taq* polymerase (New England Biolabs, Ipswich, Massachusetts, USA) in a final volume of 50 µl. The nested PCR reaction was as described above, except; 10 µl of the primary 326 bp PCR product was used and the F2 and R2 primers, in a final volume of 50 µl.

Both the primary and secondary PCR amplifications were performed with an (i) initial denaturing step for two minute at 94°C; (ii) followed by 35 cycles of denaturing for 1 minute at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C; (iii) and a final extension at 72°C for 5 minutes (BIOER XP Thermal Cycler PCR machine, Bioer technology Co. Ltd. Tokyo, Japan).

The 107 bp secondary PCR products were digested with 5U of the restriction endonuclease *Nco*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 4 hours following the manufacturers instructions. The resultant fragments together with a 100 bp molecular weight marker (Promega Corporation, Madison, Wisconsin, USA), and *SYBER® Gold* nucleic acid stain (*Invitrogen Molecular probes™,* Oregon, USA) were separated on 8% non-denaturing polyacrylamide gels. The gels were photographed under UV light using the Uvitec photo documentation system (Uvitec Limited, Cambridge, UK) and the size of the DNA fragments determined. The A allele (restriction site absent) produces a 107 bp fragment while the G allele produces 87 bp and 20 bp fragments (Figure 2.2.).

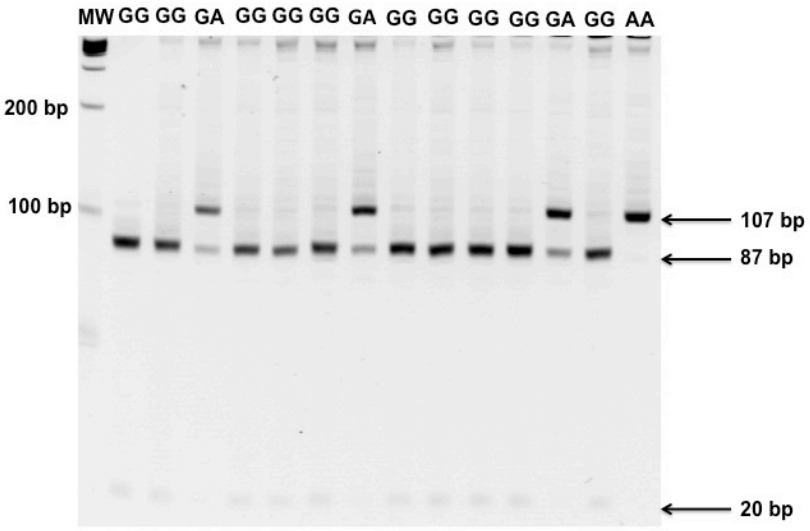

Figure 2.2. A typical 8% non-denaturing polyacrylamide gel showing the genotypes of the *TNFA* -308 G>A single nucleotide polymorphism. The 107 bp secondary PCR product was digested with *Nco*I to produce 87 bp and 20 bp fragments for the G allele. *Nco*I is unable to digest the 107 bp PCR product when the A allele is present. The genotype of the samples is indicated at the top of the lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs. Abbreviations: bp, base pair; PCR, polymerase chain reaction; *TNFA*, tumour necrosis alpha gene.

2.4.2.2. TNFA -238 G>A

DNA samples were genotyped for the -238 G>A polymorphism (rs361525) within the proximal promoter of the *TNFA* gene by RFLP analysis as previously described [225] using the following forward (5'-AAA CAG ACC ACA GAC CTG GTC-3') and reverse (5'-CTC ACA CTC CCC ATC CTC CCG GAT C-3') primers (Figure 2.3.). The reverse primer contains two modified nucleotides (underlined G and A), which creates a *BamHI* restriction site in the amplified wild type sequence. The PCR reactions included approximately 100 ng genomic DNA, 10 pmol of each primers, 5uL *Taq* polymerase buffer, 3 Mm $MgCl_2$, 125 µM each

dATP, dCTP, dTTP, and dGTP, and 0.5 U of *Taq* polymerase (New England Biolabs, Ipswich, Massachusetts, USA) in a final volume of 50 µl.

Figure 2.3. A 600 bp genomic sequence within the proximal promoter of the human *TNFA* gene. The positions of the forward (F1) and reverse (R1) primers are indicated with solid arrows. In addition the primer sequences are also in bold. The reverse primer contains two modified nucleotides, which are in lower case (t and c) and creates a *BamHI* (5'-GGATCC-3') restriction site in the amplified wild type sequence. The forward and reverse primers were used to amplify a 155 bp PCR fragment which contains the -238 G>A transition (rs361525). Polymorphism rs361525 is indicated with an R. The G to A substitution eliminates the *Bam*HI restriction site. Therefore only the 155 bp wild type PCR fragment is cleaved into 129 and 25 bp with *BamH*I. The sequence was obtained from Ensembl Transcript TNF-001 ENST00000448781 (www.ensembl.org). Abbreviations: bp, base pair; PCR, polymerase chain reaction; *TNFA*, tumour necrosis alpha gene.

The PCR amplifications were performed with an (i) initial denaturing step for two minute at 94°C; (ii) followed by 35 cycles of denaturing for 30 seconds at 94°C, annealing for 1 minute at 59°C, and extension for 2 minute at 72°C; (iii) and a final extension at 72°C for 5 minutes (BIOER XP Thermal Cycler PCR machine, Bioer technology Co. Ltd. Tokyo, Japan).

The 155 bp PCR products were digested with 5 U of the restriction endonuclease *Bam*HI (New England Biolabs, Ipswich, MA, USA) at 37°C for 4 hours following the manufacturers instructions. The resultant fragments together with a 100 bp molecular weight marker (Promega Corporation, Madison, Wisconsin, USA), and *SYBER® Gold* nucleic acid stain (*Invitrogen Molecular probes™,* Oregon, USA) were separated on 2% agarose gels. The gels were photographed under UV light using the Uvitec photo documentation system (Uvitec Limited, Cambridge, UK) and the size of the DNA fragments determined. The G to A substitution eliminates a *Bam*HI restriction site. The digested G allele produces 129 bp and 25 bp fragments, while the A allele remains uncut (155 bp) (Figure 2.4.).

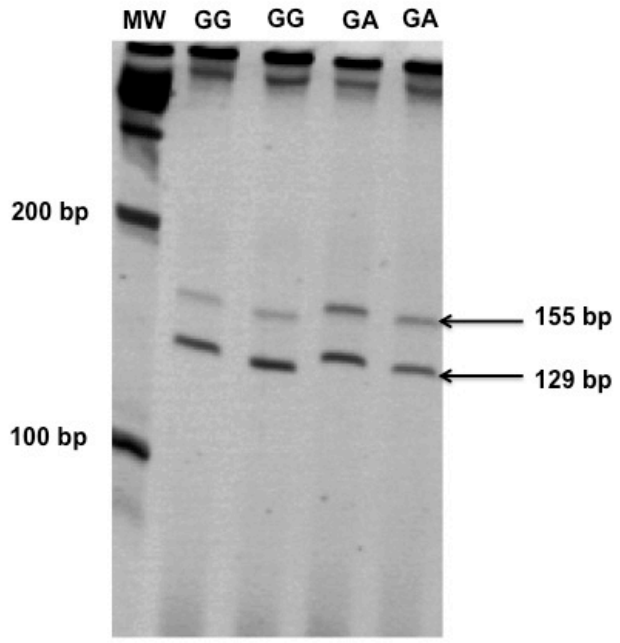

Figure 2.4. A typical 2% agarose gel showing the GG and GA genotypes of the *TNFA* -238 G>A polymorphism. The 155 PCR products were digested with *Bam*HI to produce 129 bp and 25 bp fragments with the G allele. *Bam*HI is unable to digest the 155 bp PCR product when the A allele is present. The genotype of samples is indicated at the top of each lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs (bp). Abbreviations: bp, base pair; PCR, polymerase chain reaction; *TNFA*, tumour necrosis alpha gene.

2.4.2.3. IL-6 polymorphism selection

The *IL-6* gene spans a physical region of 4.8 kb located on chromosome 7p21 and includes five exons and four introns. The information from the databases hosted by the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and Ensemble Genome Data Centre (www.ensembl.org) were used to construct a schematic representation of the exon and intron boundaries of *IL-6* and selected polymorphisms mapped to this region (Figure 2.5.).

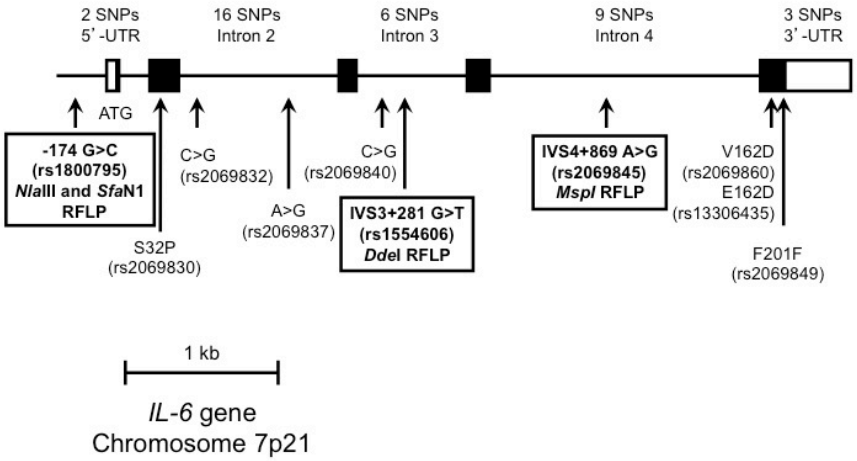

Figure 2.5. A schematic representation of the *IL-6* gene. The relative sizes and positions of the exons (boxes), introns (vertical lines) and proximal promoter (vertical line) are shown. The 5'- and 3'-UTRs are indicated as clear boxes, while the translated exons or part of the exons are indicated as black boxes. The number of SNPs identified within the UTRs and introns are indicated in Table 2S IV (Chapter 9) in Supplementary data. The accession numbers, amino acid substitutions and/or nucleotide changes of selected polymorphisms are annotated. Specifically the accession numbers as well as the RFLPs of the three *IL-6* polymorphisms used in this study (rs1800795, rs1554606, rs2069845) (boxed) are also annotated. All the information used to construct this figure was obtained from databases hosted by the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and Ensemble Genome Data Centre (www.ensembl.org). Abbreviations: *IL-6,* interleukin 6; RFLP, restriction fragment length polymorphism analysis; SNP, single nucleotide polymorphism; *TNFA*, tumour necrosis alpha gene; UTR, untranslated region.

Polymorphisms with the highest heterozygous frequencies (potentially informative polymorphisms) within both the Caucasian and black populations were identified (Table 2S IV, Chapter 9 (Supplementary data)) Preference was given to functional polymorphisms or nonsynonomous coding variants, i.e. polymorphisms that change the amino acid sequence. The most extensively studied polymorphism within the *IL6* gene is the G>C functional promoter polymorphism at position -174 has a low frequency (<5%) in the black population [133], and was therefore expected to be low in the SA black population. Only four polymorphisms were identified within the coding region of *IL-6* of which three were non-synonomous. However, the heterozygote frequencies of all four polymorphisms within the black population were either low (<10%) or not determined and were therefore not selected. These four polymorphisms were therefore considered non-informative in the black population and were not selected for this study. The large majority of the polymorphisms mapped to the non-coding regions of the *IL-6* gene; 19 polymorphisms were located within intron two, 11 polymorphisms within intron three, and eight polymorphisms within intron four. Two of these polymorphisms (rs1554606, rs2069845) had a high heterozygous frequency within both the black and Caucasian populations (Table 2S IV, Chapter 9 (Supplementary data)). In addition, these polymorphisms altered restriction enzyme recognition sequences, which facilitated RFLP analysis and they were therefore considered suitable for this genetic association study (Figure 2.5).

2.4.2.4. IL-6 -174 G>C

Genotyping of the -174 G>C polymorphism (rs1800795) within the promoter of the *IL-6* gene was performed by PCR amplification and digested with the *Sfa*N1 restriction enzyme as previously described [186] (Figure 2.6.). A 227 bp fragment corresponding to the -246 to -473 bp region of the *IL-6* gene promoter region, was PCR amplified using the forward primer 5'-TTT TCT CTT TGT AAA ACT TCG TGC ATC ACT T-3' (the modified nucleotide is underlined), in which an *Sfa*N1 site was created, and reverse primer 5'-TGG GGC TGA TTG GAA ACC TTA TTA AG-3' as previously described with slight modifications (Fishman *et al.* 1998). The PCR reactions were carried out in a final volume of 50 µl containing at least 100 ng genomic DNA, 20 pmol of the forward and reverse primers, 20 mM Tris-HCl (pH 8.4), 10 mM KCl, 2 mM $MgCl_2$, 20 mM $MgSO_4$, 125 µM each dATP, dCTP, dTTP, and dGTP, and 0.5 U of *Taq* DNA polymerase (New England Biolabs, Ipswich, Massachusetts, USA).

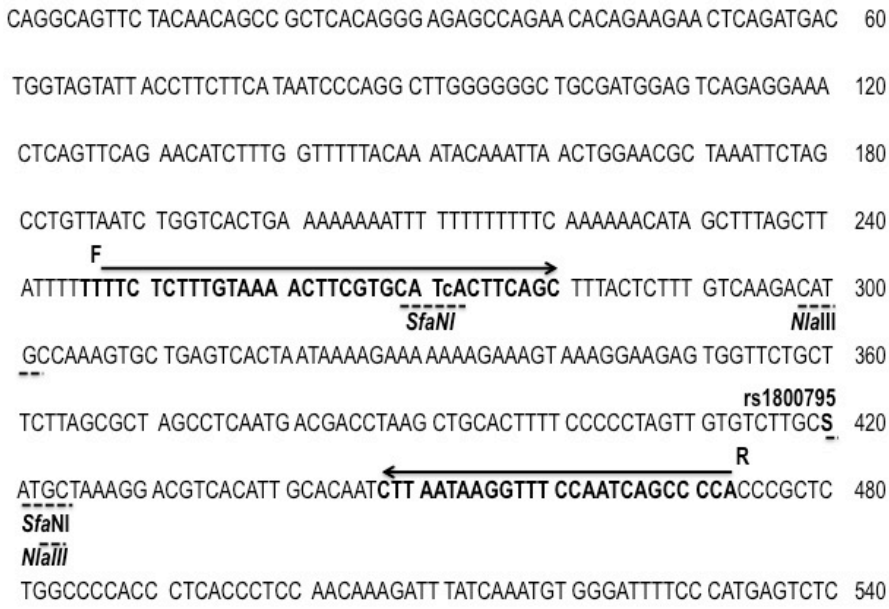

Figure 2.6. A 540 bp genomic sequence within the proximal promoter of the human *IL-6* gene. The positions of the forward (F) and reverse (R) primers are indicated with solid arrows. In addition the primer sequences are also in bold. The forward primer contains a modified nucleotide (lower case c) in which an internal *Sfa*N1 (5'-GCATG-3') site in the PCR fragment was created (positive digestion control). The naturally occurring positive digestion control *Nla*III (5'-CATG-3') site with the PCR fragment is also indicated. In addition the reverse complement sequences of the *Sfa*N1and *Nla*III restrictions sites, which includes polymorphism rs1800795 (C>G) are also indicated. polymorphism rs1800795 is indicated with an **S.** The 228 bp PCR product was digested with *Sfa*N1 to produce fragments of 196 bp and 32 bp for the C allele and 137 bp, 59 bp and 32 bp for the G allele. The *Nla*III enzyme produced 123 bp, 56 bp and 49 bp fragments for the C allele and 172 bp and 56 bp fragments for the G allele. The sequence was obtained from ensemble (www.ensembl.org) *IL-6* gene product ENSG00000136244. Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

The samples were amplified with a BIOER XP Thermal Cycler PCR machine (Bioer technology Co. Ltd. Tokyo, Japan), using the following conditions: (i) an initial denaturing step for 5 minutes at 94^{o}C; (ii) followed by 35 cycles of denaturing for 1 minute at 94^{o}C, annealing for 105 seconds at 60^{o}C, and extension for 1 minute at 72^{o}C; (iii) and a final extension step at 72^{o}C for 3 minutes.

The 228 bp PCR product was digested with *Sfa*N1 (New England Biolabs, Ipswich, MA, USA) at 37°C for 4 hours to produce fragments of 196 bp and 32 bp for the C allele and 137 bp, 59 bp and 32 bp for the G allele (Figure 2.7.). Genotypes were confirmed by also digesting the PCR products with *Nla*III (New England Biolabs, Ipswich, MA, USA) at 37°C for 4 hours to produce 123 bp, 56 bp and 49 bp fragments for the C allele and 172 bp and 56 bp fragments for the G allele (Figure 2.8.). The resultant fragments together with a 100 bp molecular weight marker (Promega Corporation, Madison, Wisconsin, USA), and *SYBER*® *Gold* nucleic acid stain (*Invitrogen Molecular probes*™*,* Oregon, USA) were separated on 8% non-denaturing polyacrylamide gels. The gels were photographed under UV light using the Uvitec photo documentation system (Uvitec Limited, Cambridge, UK) and the size of the DNA fragments determined.

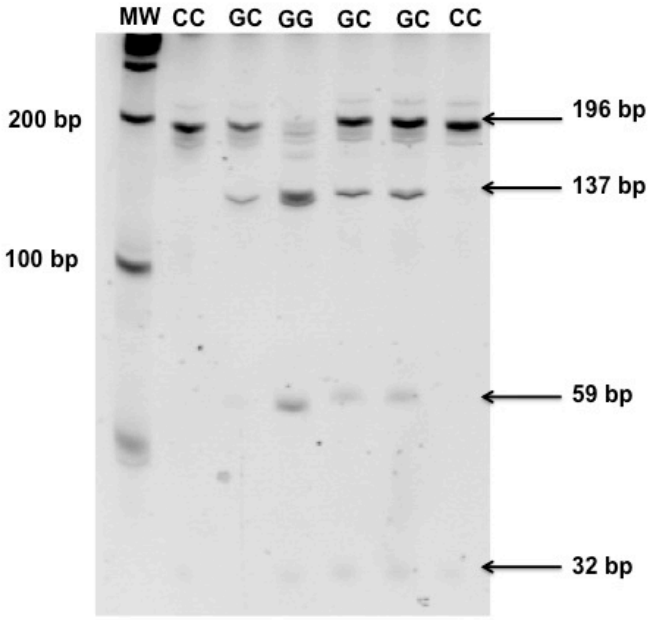

Figure 2.7. A typical 8% non-denaturing polyacrylamide gels showing the genotypes of the *IL-6* -174 G>C polymorphism. The 228 bp PCR product was digested with *Sfa*N1 to produce 196 bp and 32 bp fragments for the C allele and 137 bp, 59 bp and 32 bp for the G allele. The genotype of samples is indicated at the top of the lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs (bp). Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

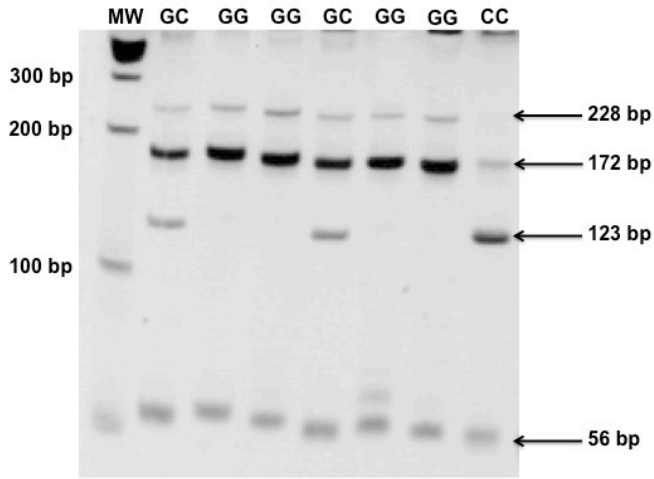

Figure 2.8. A typical 8% non-denaturing polyacrylamide gels showing the genotypes of the *IL-6* -174 G>C polymorphism. The 228 bp PCR product was digested with *Nla*III to produce 123 bp, 56 bp and 49 bp fragments for the C allele and 172 bp and 56 bp fragments for the G allele. The genotype of samples is indicated at the top of the lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs . Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

2.4.2.5. IL-6 IVS3+281 G>T

DNA samples were genotyped for the IVS3+281 G>T (rs1554606) polymorphism in intron 3 of the *IL-6* gene (Figure 2.9.). The 472 bp fragment of intron 3 of the human *IL-6* gene was PCR amplified using forward primer 5'-GTA CCA ACT TGT CGC ACT CA-3' and reverse primer 5'-GGA TCC TTC TCT GAT TGT CC-3' (Figure 2.11). The PCR reactions were performed in a final volume of 50 µl containing at least 100 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM each of dATP, dTTP, dCTP and dGTP, 0.5 U *Taq* polymerase, and 20pmol each of the forward and reverse primers.

Figure 2.9. A 540 bp genomic sequence, including nucleotides 1 to 540, of the 707 bp intron 3 of the human *IL-6* gene. The positions of the forward (F) and reverse (R) primers are indicated with solid arrows. In addition the primer sequences are also in bold. The 472 bp PCR fragment, contains the *IL-6* IVS3+281 (G>T) polymorphism (rs1554606), which is indicated with a K. The G to T substitution creates a *Dde*I restriction site (5'-CTCAG-3') in the amplified 472 bp fragment. The G allele eliminates the *Dde*I restriction site, therefore the 472 bp fragment is only digested when the T allele is present (191 bp, and 281 bp). The sequence was obtained from ensemble (www.ensembl.org) *IL-6* gene product ENSG00000136244. Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

Samples were amplified with a BIOER XP Thermal Cycler PCR machine (Bioer technology Co. Ltd. Tokyo, Japan), using the following conditions: (i) 5 minutes at 94˚C; (ii) 30 cycles of denaturing for 30 seconds at 94˚C, annealing for 30 seconds at 58˚C, and extension for 40 seconds at 72˚C; (iii) followed by a final extension step for 5 minutes at 72˚C. Twenty-five µl of PCR product was digested with 5U of the restriction endonuclease *Dde*I in a final volume of 30 µl overnight at 37˚C following the manufacturers instructions. The resultant fragments

together with a 100 bp molecular weight marker (Promega Corporation, Madison, Wisconsin, USA), and *SYBER® Gold* nucleic acid stain (*Invitrogen Molecular probes™,* Oregon, USA) were separated on 8% non-denaturing polyacrylamide gels. The gels were photographed under UV light using the Uvitec photo documentation system (Uvitec Limited, Cambridge, UK) and the size of the DNA fragments determined. The digested 472 bp PCR product produced fragments of 34 bp, and 438 bp for the G allele, and 34 bp, 191 bp, and 247 bp for the T allele (Figure 2.10.).

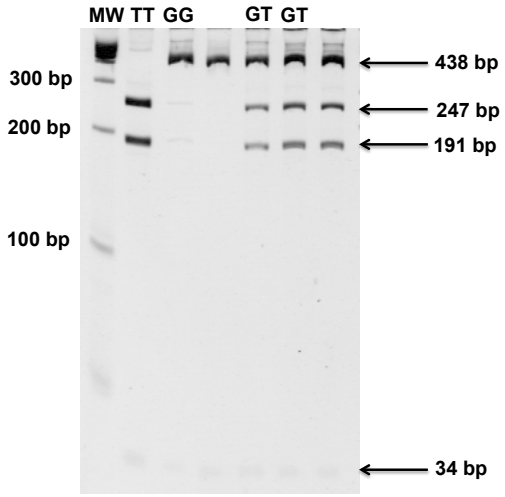

Figure 2.10. A typical 8% non-denaturing polyacrylamide gels showing the genotypes of the *IL-6* IVS3+281 G>T polymorphism. The 472 bp PCR products were digested with *Dde*I to produce 34 bp, and 438 bp for the G allele, and 34 bp, 191 bp, and 247 bp for the T allele. The genotype of samples is indicated at the top of the lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs. Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

2.4.2.6. IL-6 IVS4+869 A>G

DNA samples were genotyped for the IVS4+869 A>G (rs 2069845) polymorphism in intron 4 of the *IL-6* gene (Figure 2.11.). The 383 bp fragment corresponding to the 694 to 1076 region of intron 4 of the human *IL-6* gene was PCR amplified using the forward primer 5'-GAG TCT GAC TTA GCA AGC CTC CGG T-3' and the reverse primer 5'-CCA AGC CTG ACC AGC ATC ACT ATC-3' (Figure 2.11.). The forward primer contains a modified nucleotide (underlined C), which creates an *MspI* site in the amplified PCR product.

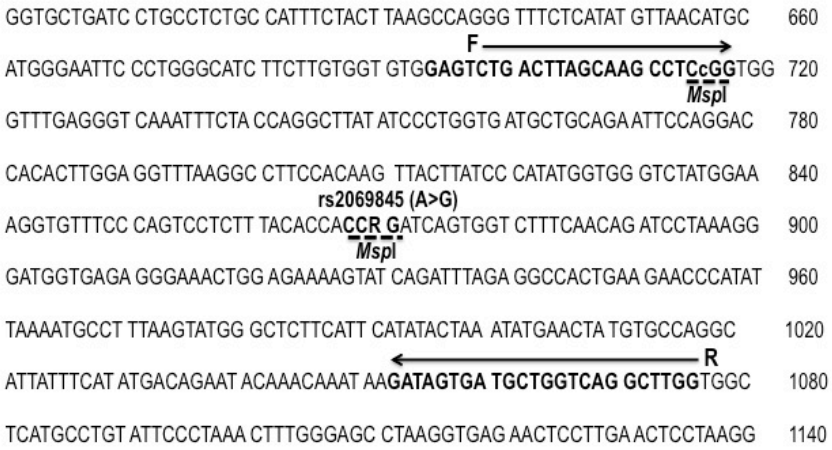

Figure 2.11. A 540 bp genomic sequence, including nucleotides 601 to 1140, of 1745 bp intron 4 of the human *IL-6* gene. The positions of the forward (F) and reverse (R) primers are indicated with solid arrows. In addition the primer sequences are also in bold. The forward primer contains a modified nucleotide, which is indicated by a lowercase c, to create an internal *MspI* (5'-CCGG-3') restriction site in the amplified PCR product. The forward and reverse primers were used to amplify a 383 bp PCR fragment, which contains the *IL-6* IVS4+869 (A>G) polymorphism (rs2069845). Polymorphism rs2069845 is indicated with an R. The A to G substitution creates a *MspI* restriction site in the amplified product. The digested 383 bp PCR product produced fragments of 21 bp, 155 bp, and 207 bp for the G allele, and 21 bp and 362 bp for the A allele. The sequence was obtained from ensemble (www.ensembl.org) *IL-6* gene product ENSG00000136244. Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

The PCR reactions were performed in a final volume of 50 µl containing at least 100 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM each of dATP, dTTP, dCTP and dGTP, 0.5 U *Taq* polymerase, and 20 pmol each of the forward and reverse primers. Samples were amplified with a BIOER XP Thermal Cycler PCR machine (Bioer technology Co. Ltd. Tokyo, Japan), using the following conditions: (i) 5 minutes at 94˚C; (ii) 30 cycles of denaturing for 30 seconds at 94˚C, annealing for 30 seconds at 58˚C, and extension for 40 seconds at 72˚C; (iii) followed by a final extension step for 5 minutes at 72˚C. Twenty five µl of PCR product was digested with 5U of the restriction endonuclease *Msp*I in a final volume of 30 µl overnight at 37˚C following the manufacturers instructions. The resultant fragments together with a 100 bp molecular weight marker (Promega Corporation, Madison, Wisconsin,

USA), and *SYBER*® *Gold* nucleic acid stain (*Invitrogen Molecular probes*™*,* Oregon, USA) were separated on 8% non-denaturing polyacrylamide gels. The gels were photographed under UV light using the Uvitec photo documentation system (Uvitec Limited, Cambridge, UK) and the size of the DNA fragments determined. The digested 383 bp PCR product produced fragments of 21 bp, 155 bp, and 207 bp for the G allele, and 21 bp and 362 bp for the A allele (Figure 2.12.).

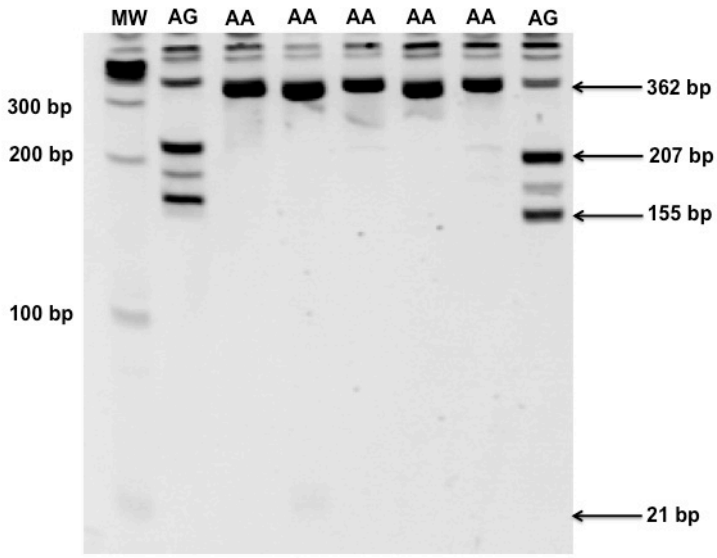

Figure 2.12. A typical 8% non-denaturing polyacrylamide gels showing the AA and AG genotypes of the *IL-6* IVS4+869 A>G polymorphism. The 383 bp PCR product was digested with *Msp*I to produce 21 bp, 155 bp, and 207 bp fragments for the G allele, and 21 bp and 362 bp fragments for the A allele. The genotype of the samples is indicated at the top of the lane. The left lane contains the 100 bp molecular weight marker (MW) and the appropriate fragment sizes are given in base pairs. Abbreviations: bp, base pair; *IL-6*, interleukin 6; PCR, polymerase chain reaction.

2.5. Statistical analysis

2.5.1. Study groups

In order to explore the metabolic effects of extreme ranges in BMI and hence most other measures of obesity and body fat distribution, while maintaining a unimodal distribution for the lipid profile, only normal-weight and obese women were recruited. Accordingly, 107 normal-weight and 120 obese black and 89 normal-weight and 62 obese white SA women were included in the analyses. However, for all diet and diet-gene analyses only adequate responders where included.

2.5.2. Summary statistics

Genotype and allelic frequency distributions are summarised as counts and percentage, according to BMI group, separately in black and white women (Tables 3.3., 4.1. and 5.1.). Exact tests of Hardy Weinberg Equilibrium (HWE), as well as linkage disequilibrium (LD) between polymorphisms were assessed for women of each BMI group and ethnic group.

Subject characteristics and body composition are summarised as mean ± SD. Serum lipid concentrations and dietary intake are summarised as median (interquartile range), because of skewed distributions.

2.5.3. Modelling

Logistic regression (a binomial model with logit link) was used to model the dichotomous outcomes, BMI group (belonging to the normal-weight or obese group) and ethnicity (belonging to the white or the black group). Numerical outcomes (subject characteristics, body composition, serum lipids and dietary intake), were either left untransformed, or transformed (adding a constant, then log transformed), when required to approximate normality for analysis. General linear models were used for numerical data. Modelling enabled adjustment for confounders, testing for associations with genotype, and also testing the interaction between genotype and dietary intake on outcomes by including them as terms in the models. Tests of the above associations and interactions on body composition variables were adjusted for age, whereas the tests of association and interaction on serum lipid variables were adjusted for age and fat mass. Associations and interactions were tested in white and black women separately and combined in one group, while covarying for ethnic group. In order to determine whether interaction effects differed between ethnic groups, three-way interactions between ethnic group and dietary intakes and polymorphisms were modeled in the combined group (of black and white women). In order to determine whether interaction effects were the same in the two ethnic groups, an indicator of ethnic group was included and tested in models of the two-way interaction between dietary intake and polymorphisms on outcome (body composition or serum lipids) in the combined group.

2.5.4. Polymorphisms

Genotype (2 degrees of freedom) models and additive allelic (number of minor alleles, 1 degree of freedom) models were tested, and based on only those estimates provided by significant models, other transition models, additive, dominant or recessive for the minor allele were investigated. The best model (smallest p-value) is reported in the results section.

2.5.5. Confounders

Different confounders were adjusted for in different parts of this study. As the obese women were older than the normal-weight, all analyses were adjusted for age. All joint (combined) analyses (including women from both ethnic groups) were adjusted for ethnic group. Tests for interaction between dietary intake and genotype on serum lipids were further adjusted for a measure of body fat, specifically BMI for the *TNFA* polymorphisms and fat mass for the *IL-6* polymorphisms. The models provided the effect estimates and p-values in the tables.

2.5.6. Interpreting interactions

Graphs are presented in this thesis to aid in the interpretation of significant interactions, because interaction effects, especially between transformed variables, are difficult to describe.

A significant interaction between dietary intake and polymorphisms on a numerical outcome suggests that there is a significant difference in rates of change in the outcome between the genotype groups. There are two possibilities: either only the difference is significant, but none of the individual rates of change are, or one or both of the 'slopes' change significantly. In this thesis only the significant genotype effects are described.

Boxplots are sometimes used to illustrate the interaction between genotype and BMI group on outcomes. Plots show predicted outcome, back-transformed if necessary, versus genotype, for a woman of average age.

The significant interaction between genotype and dietary fat intake as a percentage of total energy intake (%E) on risk of obesity (BMI group, a dichotomous outcome) were illustrated by graphing estimated OR of obesity with specific genotypes compared to other genotype, as a function of dietary fat intake (%E). The odds of being obese increases with age, but the OR (odds ratios) are the same for all ages. The term 'odds of being obese' was used to mean the odds of being obese relative to being of normal body weight. The study design (case-control study) did not enable the estimation of the odds of being obese, only the estimation of the obesity OR.

Significant interactions between genotype and dietary fatty acid intake (%E) on numerical outcomes are illustrated by plotting the observed raw values, as well as the modelled relationships (back transformed where necessary) for the specific genotypes, for a woman with average values of all the confounders we

adjusted for. Some outcomes were modelled with transformed values, with the result that the modelled relationships of the untransformed values (which we graph) are curves, not straight lines. The curves will move up or down with different values of the confounders, but the relative positions will remain the same. For example the curves will be slightly higher for older, but lower for younger (than average age) women.

2.5.7. Multiple testing

Results corresponding to p-values below 5% are described as significant. There was no adjustment for multiple testing because it has been suggested that corrections, such as Bonferroni, are too conservative when several associations are tested in the same group of individuals [226], and might not be appropriate in a situation such as this, where there is prior evidence that such effects exist [227]. Nyhold's correction is not appropriate for this thesis, where there are only 5 linked polymorphisms [226-228]). Furthermore, It is impossible to rule out a false positive result, whether one does a single or multiple tests. The Bonferroni, Benjamini-Yekutieli [4, 6] (and all other corrections for multiple testing) entails a decrease of the critical or cutoff p-value. Decreasing the critical p-value, decreases the probability of a type 1 error (false positives), while it increases the probability of missing a true association (false negatives).

Most 'correction for multiple testing' approaches are based on an assumption of statistically independent tests. Approaches such as the Bonferroni corrections are deemed to be inappropriate in a genetic study such as this, as the two *TNFA*

SNPs and the three *IL-6* SNP's are located close together on the same gene and in some cases are in linkage disequilibrium with each other, so it is expected to obtain similar associations with all outcomes. There is no consensus on the appropriate critical *p*-value for testing associations with multiple outcomes with SNPs in linkage disequilibrium [226, 228].

2.5.8. Software

R, a free language and environment for graphics and statistical computing, freely available from http://www.R-project.org [229] was used for statistical analysis. The R package genetics [229], available from the same site, was used for allele and genotype frequencies, HWE and LD testing. Power analysis was done with Quanto (http://hydra.usc.edu/gxe/).

An example demonstrating the statistical analysis described above is included in Chapter 9 (Supplementary data). This example, which is taken from Chapter 4, assesses the interaction between a diet variable and a genetic factor on a quantitative trait, followed by a discussion of the steps in general.

CHAPTER THREE

DIETARY FAT INTAKE AND THE RELATIONSHIP BETWEEN TUMOUR NECROSIS FACTOR-A GENE -308 G>A POLYMORPHISM AND SERUM LIPIDS, AND OBESITY RISK IN BLACK AND WHITE SOUTH AFRICAN WOMEN

The data and results presented in this chapter has been published in part in the following peer-reviewed articles:

Joffe YT, van der Merwe L, Carstens M, Collins M, Jennings C, Levitt NS, Lambert EV, Goedecke JH: Tumor necrosis factor-alpha gene -308 G/A polymorphism modulates the relationship between dietary fat intake, serum lipids, and obesity risk in black South African women. *The Journal of Nutrition* 2010, 140(5):901-907.

Joffe YT, van der Merwe L, Collins M, Carstens M, Evans J, Lambert EV, Goedecke JH: The -308 G/A polymorphism of the tumour necrosis factor-alpha gene modifies the association between saturated fat intake and serum total cholesterol levels in white South African women. *Genes & Nutrition* 2011, 6(4):353-359.

Statement of contribution to manuscripts and chapter:
Y.T.J, M. Collins, E.V.L, and J.H.G designed study; **Y.T.J**, M. Carstens, and C. Jennings conducted the research; **Y.T.J**, M. Collins, J.H.G and L.v.d.M analyzed the data; **Y.T.J** wrote the paper with editorial input from M. Collins, J.H.G and L.v.d.M; **Y.T.J** had final primary responsibility for final content.

CHAPTER THREE

3.1. INTRODUCTION

Excess adipose tissue associated with obesity has been linked with a low-grade, chronic inflammatory response, characterised by altered production of adipokines and raised inflammatory markers such as TNF ! [32, 230]. The proinflammatory cytokine TNF ! has also been shown to have important effects on whole-body lipid metabolism. Raised circulating levels of TNF ! have been associated with increased serum TAG, VLDL-C and low levels of HDL-C [231]. Moreover, circulating soluble TNF receptor levels, a surrogate marker of previous TNF ! effects, have been shown to circulate in proportion to LDL-C concentrations in apparently healthy subjects [232]. The link between obesity, inflammation and dyslipidaemia may be mediated through different pathways; one of which may include interactions between dietary fat intake and polymorphisms within the *TNFA* gene, potentially modulating the phenotype [170, 182, 183].

The A allele of the functional *TNFA* -308 G>A polymorphism has been shown to increase transcription and subsequently increase TNF ! production [40, 125, 187]. A number of studies have reported higher BMI and/or percent body fat [167-169, 233], lower HDL-C [179] and higher TAG [180], in carriers of the pro-inflammatory -308 A allele compared to those with the G allele (Table 1.3. in Chapter 1). In addition, the *TNFA* -308 A allele has been shown to modulate the relationship between dietary fat intake and obesity risk and dyslipidaemia in different populations. Nieters *et al.* found

that white German men and women with the *TNFA* -308 A allele, who were in the highest tertile for intake of the n-6 fatty acids LA and AA (%E), had increased risk of obesity [170]. In an ethno-racially diverse Canadian diabetic population,

Fontaine-Bisson *et al*. showed that dietary PUFA intake (%E) was inversely associated with HDL-C concentration in carriers of the *TNFA* -308 A allele, but not in those with the GG genotype [182].

The interactions between dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism may be of particular relevance in the SA context, given the high prevalence of obesity in black and white SA women [3], as well as the higher prevalence of dyslipidaemia and CVD in white women compared to black women [234]. Black SA women typically present with a more favourable lipid profile than white SA women, with lower TAG, T-C and LDL-C concentrations [22, 235]. However, in a cohort of black SA women of Xhosa ancestry (African tribe residing in southern and eastern SA), it was recently found that approximately 45% had low HDL-C levels [236]. The increased risk for obesity and low HDL-C in black SA women may be compounded by an increase in dietary fat intake associated with the nutrition transition in the country [135]. In addition, differences in the distribution of inflammatory gene polymorphisms and inflammatory gene expression between black and white populations may contribute to ethnic differences in phenotype [19, 118, 237]. Genetic studies have shown that African-American and sub-Saharan populations are more likely to carry allelic variants that up-regulate inflammation compared to white women [97]. Further, in a SA population, black women had a higher SAT inflammatory gene expression profile, including higher TNF ! mRNA levels, than white SA women [118].

Given these reported differences, it is important to investigate these diet-genotype interactions that have not as yet been examined in a black population. Therefore, the aims of this study were to i) characterise the body composition, serum lipid concentrations and dietary intake of black and white SA women; ii) explore the relationships between the *TNFA* -308 G>A polymorphism and obesity and serum lipid concentrations; and iii) to determine whether these relationships are modulated by dietary fatty acid intake; and iv) determine whether these interactions differ by ethnicity.

3.2. RESULTS

3.2.1. Subject characteristics

The study group included 107 normal-weight and 120 obese urban black women, and 89 normal-weight and 62 obese urban white SA women between the ages of 18 and 45 years. Basic subject characteristics of the black and white women are summarised by ethnicity and BMI group in Table 3.1. The white women were older than the black women, and the obese women older than the normal-weight women; as a result all analyses were adjusted for age ($P< 0.001$).

The white women were taller and heavier than the black women ($P<0.001$), but because of their shorter stature the black women had a higher BMI than the white women ($P=0.002$). The white women also had greater waist ($P=0.003$), waist hip

ratio (WHR), and VAT (P<0.001, respectively) than the black women, and the black women had higher body fat % (P=0.004), and SAT (P=0.028) than the white women. Irrespective of ethnicity, and by design of the study, all body composition variables were higher in the obese women compared to the normal-weight women

After adjusting for age and BMI group, TAG, T-C, HDL-C and LDL-C were higher in the white women compared to the black women (P<0.001). Only the total cholesterol : high-density lipoprotein cholesterol (T-C:HDL-C) ratio was not different (P=0.618). In addition, T-C (P=0.047), TAG, LDL-C and T-C:HDL-C ratio were all higher and HDL-C lower (P<0.001 for all variables), in the obese compared to the normal-weight women (Table 3.1).

Table 3.1. Subject characteristics, body composition and serum lipids of normal-weight and obese black and white women

	Black		White		P-values		
	Normal-weight	Obese	Normal-weight	Obese	BMI group	Ethnicity	Interaction
N	107	120	89	62			
Age, *years*	24 ± 5	30 ± 8	29 ± 7	34 ± 8	< 0.001	< 0.001	0.333
Body composition							
Height, *m*	1.61 ± 0.06	1.60 ± 0.06	1.67 ± 0.06	1.67 ± 0.07	0.005	< 0.001	0.911
Weight, *kg*	58 ± 6	93 ± 14	62 ± 6	98 ± 14	< 0.001	< 0.001	0.903
BMI, kg/m^2	22 ± 2	37 ± 5	22 ± 2	35 ± 4	< 0.001	0.002	0.201
Body fat, *%*	30 ± 5	45 ± 4	28 ± 5	45 ± 4	< 0.001	0.004	0.007
Fat Mass, *kg*	17 ± 4	42 ± 10	17 ± 4	44 ± 9	< 0.001	0.214	0.127
Waist, *cm*	74 ± 6	105 ± 12	77 ± 6	107 ± 11	< 0.001	0.003	0.278
WHR	0.75 ± 0.06	0.84 ± 0.08	0.79 ± 0.05	0.85 ± 0.06	< 0.001	< 0.001	0.122
VAT, cm^2	49 ± 20	98 ± 43	63 ± 21	156 ± 61	< 0.001	< 0.001	< 0.001
SAT, cm^2	193 ± 96	567 ± 153	166 ± 64	533 ± 119	< 0.001	0.028	0.812
Serum lipids							
TAG, *mmol/L*	0.60 (0.40 - 0.70)	0.70 (0.60 - 1.00)	0.80 (0.60 - 1.00)	1.10 (0.80 - 1.50)	< 0.001	< 0.001	0.940
T-C, *mmol/L*	3.9 (3.3 - 4.2)	4.0 (3.4 - 4.4)	4.5 (4.0 - 5.0)	5.0 (4.4 - 5.7)	0.047	< 0.001	0.059
HDL-C, *mmol/L*	1.5 (1.2 - 1.8)	1.2 (1.0 - 1.4)	1.7 (1.6 - 2.0)	1.5 (1.2 - 1.7)	< 0.001	< 0.001	0.995
LDL-C, *mmol/L*	2.0 (1.6 - 2.4)	2.4 (1.9 - 2.8)	2.3 (1.9 - 2.8)	2.8 (2.5 - 3.5)	< 0.001	< 0.001	0.052
TC;HDL-C ratio	2.6 (2.2 - 3.0)	3.3 (2.7 - 3.9)	2.6 (2.2 - 2.8)	3.6 (2.9 - 4.1)	< 0.001	0.618	0.132

Body composition: Summarised as mean ± SD. Serum lipids: Summarised as median (interquartile range). Abbreviations: BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; VAT, visceral adipose tissue; WHR, waist hip ratio

3.2.2. *TNFA* -308 G>A genotype and allele frequencies

The *TNFA* -308 G>A genotype (P=0.011) and allele frequencies (P=0.016) differed between the black and white women, after adjusting for BMI group (Table 3.2.). When the BMI groups were analysed, there were no significant differences in the *TNFA* -308 G>A genotype (P=0.287) or allele frequencies (P=0.115) between the normal-weight and obese women, after adjusting for ethnicity. Similarly, when the GA and rare AA genotypes were combined, the combined genotype differed between the black and white women (P=0.005) after adjusting for BMI group, whereas there was no significant difference on average between the normal-weight and obese women (P=0.139) after adjusting for ethnicity. Subsequently, because of the low frequency of the -308 AA genotype, the GA and AA genotypes were combined for all further analyses.

Similar genotype distributions were obtained for the black women when the normal-weight and obese women of only Xhosa ancestry were compared (P=0.914), indicating that there was reduced likelihood of population stratification in the black group. The *TNFA* genotype distribution of the normal-weight and obese black women were in Hardy-Weinberg equilibrium (HWE) (P=0.718), as were the white women (normal-weight, P=0.138; and obese, P=0.264).

Table 3.2. The *TNFA* -308 G>A genotype and allele distribution of normal-weight and obese black and white women

	Black		White		P-values	
	Normal-weight	Obese	Normal-weight	Obese	Ethnicity	BMI group
N	105	118	88	60		
AA genotype	3.8 (4)	2.5 (3)	2 (2)	2 (1)		
GA genotype	31.4 (33)	23.7(28)	43 (38)	40 (24)		
GG genotype	64.8 (68)	73.7 (87)	55 (48)	58 (35)	0.011	0.287
GA+AA genotype	35.2 (37)	26.3 (31)	45 (40)	42 (25)	0.005	0.139
G allele	80.5 (169)	85.6 (202)	76 (134)	78 (94)		
A allele	19.5 (41)	14.4 (34)	24 (42)	22 (26)	0.016	0.115
HWE p-value	0.718	0.718	0.138	0.264		

n is the number of subjects. Values are summarised as percentage (count). *TNFA* -308 G>A genotype frequency distribution, minor allele frequency and p-values for tests of association with ethnicity and BMI group, respectively, each adjusted for the other. Abbreviations: BMI, body mass index; Hardy-Weinberg equilibrium (HWE); *TNFA*, tumour necrosis factor ! gene

3.2.3. *TNFA* -308 G>A polymorphism, body composition and serum lipids

The normal-weight and obese subjects were divided into two groups based on their *TNFA* -308 G>A genotype in order to investigate associations between genotype and phenotype. Those who were homozygous wild type (GG) were included in one group, while those who were heterozygous and homozygous for the variant A allele were combined in the second group. The physical characteristics, body composition and serum lipid concentrations, according to *TNFA* -308 G>A genotype for black and white women are presented in Tables 3.3. and 3.4. In the black and white women there were no independent genotype effects for any measures of body composition, nor serum lipid concentrations. There was however an interaction between BMI groups and *TNFA* -308 G>A genotypes on BMI. BMI was greater in the obese white women with the GA+AA genotypes compared to those with the AA genotype (P=0.020) .

Table 3.3. Physical characteristics and body composition of normal-weight and obese black and white women according to *TNFA* -308 G>A genotype

Black women	Normal-weight, n=105		Obese, n=118		P-values		
	GG	GA+AA	GG	GA+AA	BMI group	*TNFA* -308	Interaction
N	68	37	87	31			
Age, years	23 ± 5	25 ± 7	29 ± 8	34 ± 8	< 0.001	0.316	0.186
Height, cm	161 ± 5	160 ± 7	161 ± 6	158 ± 5	0.659	0.603	0.340
Weight, kg	57.4 ± 6.3	57.5 ± 6.3	93.7 ± 15.1	89.1 ± 13.0	< 0.001	0.960	0.166
BMI, kg/m^2	22.4 ± 2.5	22.5 ± 2.5	36.6 ± 5.9	34.8 ± 5.1	< 0.001	0.722	0.391
Body fat, %	29.9 ± 4.2	30.5 ± 4.9	44.9 ± 4.5	43.9 ± 3.5	< 0.001	0.531	0.244
Waist, cm	73.1 ± 5.9	72.4 ± 6.8	103.7 ± 12.3	102.4 ± 13.2	< 0.001	0.740	0.822
WHR	0.75 ± 0.06	0.74 ± 0.07	0.83 ± 0.08	0.84 ± 0.09	< 0.001	0.821	0.430
VAT, cm^2	48 ± 23	46 ± 14	94 ± 42	104 ± 44	< 0.001	0.769	0.294
SAT, cm^2	195 ± 95	194 ± 108	564 ± 153	527 ± 157	< 0.001	0.989	0.420
White women	**Normal-weight, n=88**		**Obese, n=60**		**P-values**		
	GG	**GA+AA**	**GG**	**GA+AA**	**BMI group**	***TNFA* -308**	**Interaction**
N	48	40	35	25			
Age, years	29.8 ± 7.5	29 ± 7.1	33.4 ± 8.7	36.1 ± 7.8	0.034	0.652	0.191
Height, cm	1.67 ± 0.07	1.68 ± 0.07	1.67 ± 0.06	1.66 ± 0.07	0.739	0.403	0.458
Weight, kg	61.5 ± 7.0	62.2 ± 5.8	94.8 ± 12.3	101.3 ± 16.4	< 0.001	0.732	0.130
BMI, kg/m^2	21.6 ± 1.8	21.6 ± 1.9	33.9 ± 3.8	36.4 ± 4.6	< 0.001	0.931	0.020
Body fat, %	27.7 ± 5.1	27.5 ± 5.3	46.2 ± 3.2	44.6 ± 4.3	< 0.001	0.896	0.346
Waist, cm	77.6 ± 6.4	77.2 ± 6.0	106.1 ± 10.6	108.6 ± 11.1	< 0.001	0.857	0.355
WHR	0.79 ± 0.05	0.78 ± 0.05	0.86 ± 0.07	0.85 ± 0.05	< 0.001	0.569	0.855
VAT, cm^2	64.1 ± 23.7	61.5 ± 17.9	152.7 ± 67.6	161.7 ± 55.9	< 0.001	0.826	0.960
SAT, cm^2	163 ± 67	169 ± 63	567 ± 140	516 ± 104	< 0.0001	0.8330	0.131

Values are summarised as mean ± SD (standard deviation). *P*-values are from a linear model testing the interaction between BMI group and *TNFA* -308 G>A genotype, adjusted for age. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; ratio, VAT, visceral adipose tissue; WHR, waist hip ratio

Table 3.4. Serum lipids of normal-weight and obese black and white women according to *TNFA* -308 G>A genotype

Black women	Normal-weight, *n*=108		Obese, *n*=115		P-values		
	GG	GA+AA	GG	GA+AA	BMI group	*TNFA* -308	Interaction
N	72	36	85	30			
TAG, mmol/L	0.60 (0.40-0.70)	0.60 (0.48-0.73)	0.70 (0.60-1.00)	0.80 (0.60-1.10)	0.001	0.893	0.637
T-C, *mmol/L*	3.80 (3.35-4.20)	3.90 (3.28-4.60)	4.10 (3.50-4.50)	3.95 (3.35-4.38)	0.775	0.757	0.726
HDL-C, *mmol/L*	1.50 (1.20-1.80)	1.60 (1.18-1.90)	1.10 (1.00-1.40)	1.30 (1.00-1.58)	< 0.001	0.929	0.449
LDL-C, *mmol/L*	2.00 (1.53-2.40)	1.95 (1.60-2.70)	2.50 (1.90-2.90)	2.25 (1.90-2.58)	0.202	0.607	0.623
TC:HDL-C ratio	2.62 (2.17-3.00)	2.57 (2.18-2.96)	3.44 (2.80-4.17)	3.33 (2.53-3.81)	< 0.001	0.972	0.384
White women	**Normal-weight, *n*=88**		**Obese, *n*=60**		**P-values**		
	GG	**GA+AA**	**GG**	**GA+AA**	**BMI group**	***TNFA* -308**	**Interaction**
N	48	40	35	25			
TAG, mmol/L	0.80 (0.60-1.05)	0.75 (0.60-1.03)	1.20 (0.65-1.60)	1.00 (0.90-1.50)	0.003	0.974	0.965
T-C, *mmol/L*	4.30 (3.85-4.95)	4.50 (4.08-5.10)	4.90 (4.40-5.80)	5.00 (4.40-5.70)	0.023	0.827	0.956
HDL-C, *mmol/L*	1.80 (1.60-2.00)	1.70 (1.60-2.00)	1.50 (1.35-1.85)	1.40 (1.10-1.60)	0.014	0.659	0.096
LDL-C, *mmol/L*	2.30 (1.85-2.70)	2.30 (1.85-2.80)	2.70 (2.25-3.50)	3.20 (2.60-3.50)	0.006	0.992	0.348
TC:HDL-C ratio	2.53 (2.17-2.90)	2.67 (2.20-2.82)	3.29 (2.58-4.06)	3.82 (3.15-4.82)	< 0.001	0.799	0.122

Values are summarised as median (interquartile range). P-values are from a linear model testing the interaction between BMI group and *TNFA* -308 G>A genotype, adjusted for age. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

3.2.4. Dietary intake of adequate reporters

A further aim of this chapter was to assess whether dietary fatty acids modified the relationship between *TNFA* -308 genotypes, obesity and serum lipid concentrations. When examining dietary intake, only those who were classified as adequate reporters according to the Goldberg cut-offs, were included in the analysis. Accordingly, 73 normal-weight and 74 obese black, and 73 normal-weight and 48 obese white women were studied and their dietary intake is summarised in Table 3.5. The black women consumed more energy (kJ), CHO (%E)(P< 0.001) and total fat (%E)(P=0.002) than the white women. When individual dietary fatty acid intake was analysed, the black women consumed more total PUFAs (%E) and n-6 PUFAs (%E), including greater amounts of LA and AA (%E)(P=0.001), and the n-3 PUFAS; EPA and DHA (%E)(P<0.001), than the white women. The polyunsaturated fat:saturated ratio (PUFA:SFA ratio) and n-6:n-3 PUFA ratio were also higher in the black women compared to the white women (P<0.001). In contrast, the white women consumed more protein, SFA and ALA (P<0.001) compared with the black women. MUFA (%E)(P=0.844) and total n-3 PUFA (%E)(P=0.997) intake were not different between the ethnic groups. All results presented in this paragraph were adjusted for age and BMI group.

When comparing dietary intake of the BMI groups, the obese group consumed more energy (kJ; P<0.001), total fat (%E)(P=0.023), SFA (%E)(P=0.041) and PUFA (%E)(P=0.007) compared to the normal-weight group. When individual dietary fatty acids were analysed, the obese group also had a greater intake of total n-6 PUFAs (%E)(P=0.010), the n-6 PUFA's LA (%E)(P=0.011) and AA (%E)(P<0.001), and the

n-3 PUFA's EPA (P=0.037) and DHA (P=0.043), compared to the normal-weight group. Intake of protein (%E), CHO (%E), MUFA (%E), n-3 PUFA (%E), ALA (%E), and the PUFA:SFA and n-6:n-3 PUFA ratios did not differ between the obese and normal-weight groups. All results presented in this paragraph were adjusted for age and ethnic group.

Table 3.5. Dietary intakes of normal-weight and obese black and white adequate reporter women

	Black		White		P-value		
	Normal-weight	Obese	Normal-weight	Obese	BMI group	Ethnic group	Interaction
n	73	74	73	48			
Energy intake (kJ/d)	11573 (9723-14340)	13257 (9727-17264)	8899 (7098-9987)	8627 (7310-10850)	< 0.001	< 0.001	0.098
Protein (%E)	12 (10.8-13.6)	12.4 (11.1-14.0)	15 (12.3-16.5)	15.8 (14.2-18.1)	0.210	< 0.001	0.034
CHO (%E)	54.6 (49.5-59.6)	52.6 (47.9-57.3)	51.3 (47.8-56.0)	48.8 (46.0-54.1)	0.163	< 0.001	0.598
Fat (%E)	33.7 (28.3-37.2)	34.7 (31.1-38.5)	29.6 (25.7-32.5)	30.6 (26.3-33.9)	0.023	0.002	0.581
SFA (%E)	9.2 (7.9-10.7)	9.5 (8.3-10.9)	9.7 (8.3-12.0)	11.2 (9.1-12.3)	0.041	< 0.001	0.131
MUFA (%E)	10.3 (8.8-11.8)	11.4 (9.8-12.9)	10.2 (8.7-11.6)	10.4 (8.4-12.1)	0.050	0.844	0.578
PUFA (%E)	8.4 (6.8-10.2)	9.5 (7.7-11.0)	6.1 (4.9-7.9)	6.1 (4.8-7.6)	0.007	< 0.001	0.407
P:S ratio	0.91 (0.73-1.17)	1 (0.81-1.21)	0.62 (0.46-0.78)	0.56 (0.48-0.72)	0.205	< 0.001	0.097
n-3 PUFA (%E)	0.28 (0.22-0.34)	0.36 (0.27-0.46)	0.33 (0.26-0.41)	0.32 (0.27-0.39)	0.296	0.997	0.184
n-6 PUFA (%E)	7.9 (6.6-10.3)	9.1 (7.3-10.7)	5.5 (4.2-7.5)	5.5 (4.4-7.2)	0.010	< 0.001	0.507
n-6:n-3 PUFA ratio	27 (20.0-39.9)	25.7 (18.3-36.5)	15.9 (12.3-22.5)	16.4 (13.0-24.9)	0.203	< 0.001	0.770
ALA (%E)	0.21 (0.18-0.25)	0.22 (0.18-0.27)	0.26 (0.21-0.30)	0.25 (0.21-0.29)	0.372	< 0.001	0.381
LA (%E)	8.1 (6.70-10.6)	9.3 (7.50-10.9)	5.5 (4.20-7.40)	5.5 (4.40-7.20)	0.011	< 0.001	0.496
AA (%E)	0.04 (0.030-0.060)	0.06 (0.040-0.070)	0.03 (0.020-0.040)	0.04 (0.030-0.050)	< 0.001	< 0.001	0.174
EPA (%E)	0.017 (0.009-0.035)	0.037 (0.016-0.065)	0.014 (0.009-0.023)	0.018 (0.009-0.025)	0.037	< 0.001	0.195
DHA (%E)	0.037 (0.023-0.076)	0.079 (0.038-0.128)	0.047 (0.029-0.063)	0.045 (0.028-0.066)	0.043	< 0.001	0.468

Dietary intake includes adequate reporters only. Summarised as median (interquartile range). Outcomes were log-transformed when required for modelling. P-values are from age-adjusted linear models of outcomes, testing the difference between BMI and ethnic groups each adjusted for the other.

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; CHO, carbohydrate; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid.

3.2.5. Diet-genotype interactions

3.2.5.1. Diet-genotype interactions on body composition

In black women, there was a significant interaction observed between total dietary fat intake (%E) and *TNFA* -308 G>A genotypes on obesity risk, (P=0.036) (Figure 3.1.). Figure 3.1. shows the obesity OR for genotype GA+AA versus genotype GG, at each fat intake value (%E). At low relative dietary fat intakes (%E), the odds of being obese for the GA+AA genotype were lower than for the GG genotype. However, the increase was greater for the GA+AA genotype so that when dietary fat intake was = 40.6 (%E) the estimated odds of obesity for the GA+AA genotype was equal to the odds of obesity with the GG genotype. For those with the GG genotype the OR for obesity was 1.12 and 1.26 for fat = 35 and 40 (%E) compared to 30 %E. For those with the GA+AA genotype, the obesity OR was 3.02 and 9.12 for fat = 35 and 40 (%E) compared to 30 %E.

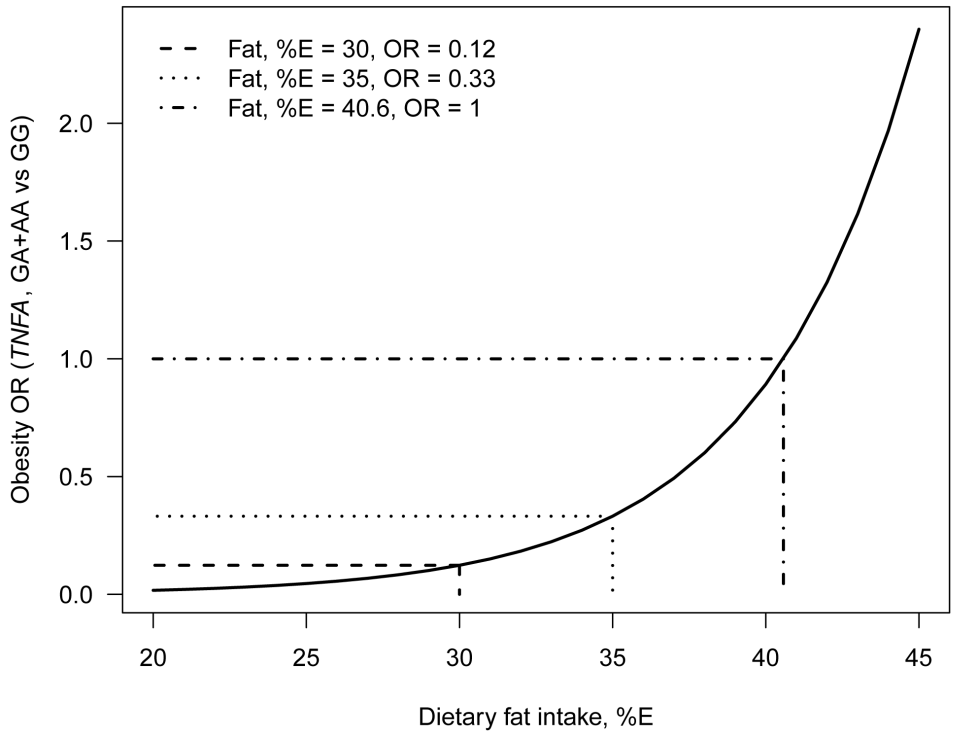

Figure 3.1. The modelled relationship between the odds of being obese (odds of being obese versus being normal weight), *TNFA* -308 genotype and dietary fat intake (%E) for black women. The graph gives the modelled obesity OR for genotype GA+AA versus genotype GG, at each fat intake (%E). Lines shows the dietary fat intake (%E) of equal odds (OR=1, for the genotype groups), namely 40.6 (%E), the OR for fat = 30 (%E) namely 0.12 and the OR for fat intake = 35 (%E), namely 0.33. Abbreviations: OR, odds ratio; TNFA, tumour necrosis factor-! gene.

In the white women there was a significant interaction between the intake of the n-3 PUFA, EPA (%E) and *TNFA* -308 G>A genotypes on WHR (P=0.043) (Table 3S III). With increasing EPA intake (%E), WHR decreased in the white women with the -308 GA+AA genotype, whereas there was no effect in those with the GG genotype.

The ethnic-specific nature of these diet-genotype interactions was investigated by examining three-way interactions between the ethnic group, dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism on obesity (Table 3S V), as well as by including both black and white women in the same model and adjusting for ethnicity (Tables 3S IV and 3S VIII).

When the black and white women were combined and adjusted for age and ethnicity, interactions between ALA (%E) intake and *TNFA* -308 genotypes on SAT, and EPA (%E) intake and *TNFA* -308 genotypes on most measures of body composition were identified (weight, BMI, body fat %, fat mass, waist and SAT). In addition, the n-3 PUFA, DHA (%E) interacted with the *TNFA* -308 genotypes on weight, BMI, fat mass and waist (Table 3S IV). Of these, only the interaction between EPA (%E) intake and *TNFA* -308 genotypes on WHR was significant in the white women (P=0.043), but not in the black women. This suggests that the interactions identified in the combined group were not different for the black and white women, and were observed only because there was more power in this larger group to detect effects. No diet-gene interactions were identified in the 3-way interactions on obesity (Table 3S V).

3.2.5.2. Diet-genotype interactions on serum lipid concentrations

In the black women, the interaction between the n-3 PUFA, ALA (%E) and *TNFA* -308 G>A genotypes on the T-C:HDL-C ratio was significant *(*P=0.028) (Table 3S VI) (Figure 3.2.). With increasing ALA intake (%E), T-C:HDL-C ratio decreased in those with the -308 GA+AA genotype, and increased in the GG genotype.

In the white women, a significant interaction between dietary SFA intake (%E) and *TNFA* -308 G>A genotypes on serum T-C concentrations (P=0.047) was observed (Table 3S VII) (Figure 3.3.). Serum T-C levels decreased for the GG genotype and increased for the GA+AA genotype with increasing SFA intake (%E).

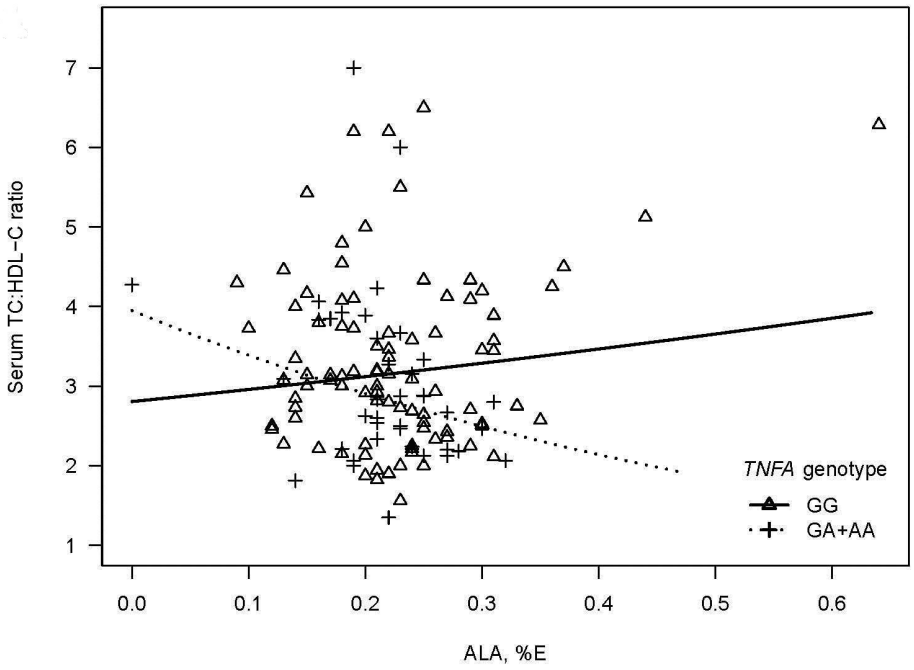

Figure 3.2. Interaction between *TNFA* -308 G>A genotype and dietary ALA intake (%E) on T-C:HDL-C ratio in normal weight and obese black women. Symbols represent, for each woman, observed values. The regression curves are modelled relationships for a woman of average age (27.5 years). Abbreviations: ALA, !-linolenic acid; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene.

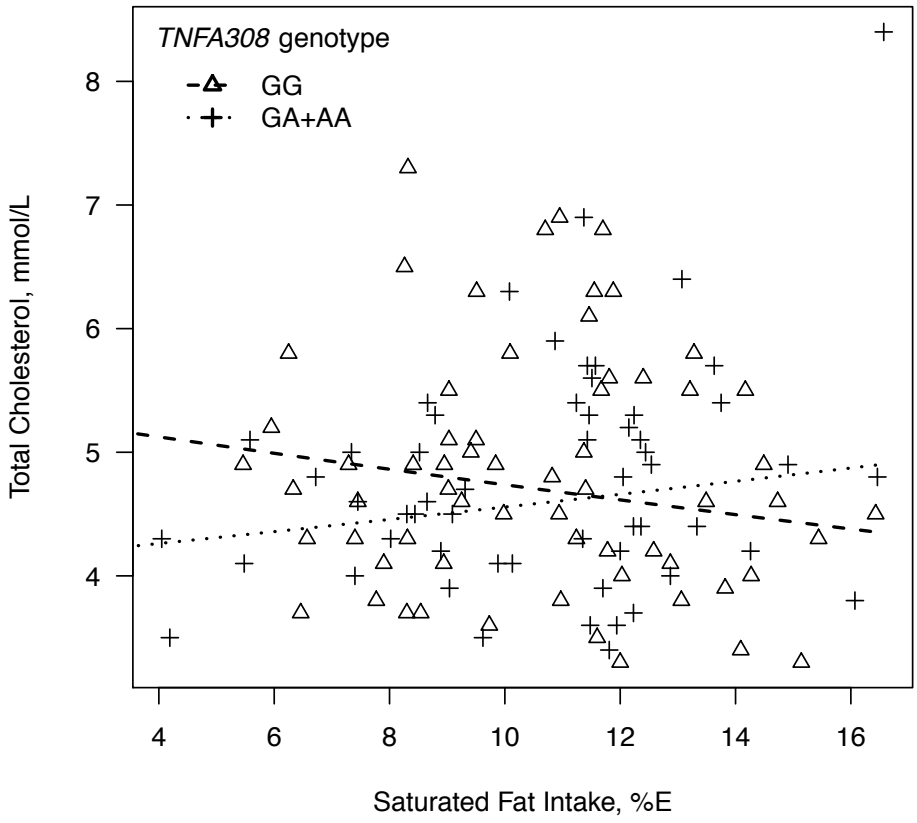

Figure 3.3. The relationship between dietary SFA, serum T-C and *TNFA* -308 G>A genotype in normal-weight and obese white women. The modelled relationship is for white women adequate reporters with mean age (30.6 years) and BMI (26.7 kg/m^2). With increasing dietary SFA (%E), T-C concentration decreases in those with the GG genotype and increases in those with the GA+AA genotype. The difference in rates of change is statistically significant (P=0.047). Curves (because of the logs being modelled) will shift up or down according to age and BMI. Abbreviations: BMI, body mass index; SFA, saturated fat; T-C, total cholesterol; *TNFA*, tumour necrosis factor-! gene.

Table 3S IX, which lists p-values for three-way interactions between ethnic group, dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism on serum lipid concentrations, shows a significant interactions between ALA (%E) intake and the *TNFA* -308 genotypes on LDL-C (P=0.026) and T-C:HDL-C ratio (P=0.044). These interactions were therefore different for the black and white women. However, only the interaction between ALA (%E) intake and *TNFA* -308 genotypes on T-C:HDL-C ratio was significant in the black women, the interaction on LDL-C was not (P=0.120) (Table 3S VI). Neither interaction was significant in the white women (Table 3S VII). When the black and white women were combined and adjusted for age and ethnicity, only the interaction between EPA (%E) intake and *TNFA* -308 genotypes on T-C was significant (P=0.046) (Table 3S VIII). This interaction was not significant in the black or white women when analysed separately, suggesting that the interaction identified was not different for the black and white women, and was observed only because there was more power in the larger combined group to detect the effect.

3.3. DISCUSSION

This study has reported ethnic differences in body composition, serum lipid concentrations, dietary intake and *TNFA* -308 G>A genotype frequencies between black and white SA women. In addition, although no independent genotype associations between the *TNFA* -308 G>A polymorphism and obesity and serum lipid concentrations were observed, for the first time, interactions between dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism on obesity risk, adiposity and serum lipid concentrations in a black and white SA population were identified. These

results highlight the complexity of understanding the role these many factors play in the development and prevalence of obesity and associated co-morbidities in black and white SA populations. It is therefore important to understand these ethnic differences in the development of effective interventions.

CAD and dyslipidaemia are important co-morbidities associated with obesity, but their prevalence differs by ethnicity, with white women being at greater risk for CAD than black women [8, 13]. In this study, serum lipid concentrations were higher in the white compared to the black women. Black SA women, similar to African Americans, have previously been shown to have a less atherogenic lipid profile than white women [21-23], potentially attributable to relatively low levels of VAT [21], also reported in this study. Studies in SA women have generally reported no ethnic difference in HDL-C levels [22], however, in this study, similar to that reported by Goedecke *et al.* [19, 24], HDL-C was lower in black women compared to white women. The reasons for this are not clear, but could relate to their lower total cholesterol levels.

Ethnic differences in the obese phenotype and serum lipid profiles may also be due, in part, to differences in the genotype or allele distribution of *TNFA* polymorphisms [97, 98]. Specifically, several studies have reported associations between polymorphisms in the *TNFA* gene and obesity and dyslipidaemia [40, 187]. The pro-inflammatory A allele of the *TNFA* -308 G>A polymorphism has been associated with a higher BMI and/or percent body fat [167-169, 233], lower HDL-C [179] and higher TAG [180], than those with the G allele. These studies are summarised in Table 1.3. in Chapter 1. Although the white women in this study had higher *TNFA* -

308 GA+AA genotype and A allele frequencies than the black women, this study found no independent genotype effects of the *TNFA* -308 G>A polymorphism on obesity risk, adiposity or serum lipid concentrations in the black or white women, nor in the combined group (Table 3S I). This is unlikely to be due to differences in genotype frequency, as the frequency of the A allele in this study was between 14% and 24% (Table 3.3.), not different from those reported in Caucasian, African American, and other African populations, summarised in Table 1.1. in Chapter 1 [170, 182, 233, 238-240].

TNFA polymorphisms have been shown to interact with dietary fatty acids [40, 187]. When dietary fat intake was included in the study analyses, interactions between dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism on obesity risk, adiposity and serum lipid concentrations in both black and white women were observed. In the black women, the obesity odd ratio (OR) of those with the -308 A allele compared to the GG genotype, was higher with increasing fat intake (%E). Those with the A allele appeared to be more responsive to an increase in dietary fat intake (%E) in their risk of being obese versus normal-weight.

In the white women in this study there was a significant interaction between the intake of the n-3 PUFA, EPA (%E) and *TNFA* -308 G>A genotypes on WHR, a measure of central adiposity (P=0.043) (Table 3S III). With increasing EPA intake (%E), WHR decreased in those with the -308 GA+AA genotype, there was no effect in the GG genotype. The identification of this diet-gene interaction on WHR and not waist circumference or VAT suggests that it may be the ratio of central to peripheral adiposity that is important, with peripheral fat being more protective. Whereas this

result showed an interaction between higher n-3 PUFA intake and reduced central adiposity, in German Caucasian men and women Nieters *et al.* identified an increased OR for the risk of obesity (based on BMI) with a higher intake of n-6 PUFAs (LA and AA (%E)) [170], both these interactions were only observed in those with the -308 A allele. These findings are of relevance in the white SA women, who present with greater levels of central adiposity compared to the black women [241].

In addition to the effects on body composition, diet-gene interactions have also been shown to impact serum lipid concentrations [96]. In the black women in this study; with increasing ALA intake (%E), the T-C:HDL-C ratio decreased, and with increasing PUFA (%E) intake, LDL-C concentrations increased, but only in subjects with the *TNFA* -308 A allele. In the white women, with increasing SFA intake (%E), T-C concentrations increased for the –308 GA + AA genotype and decreased for the GG genotype. These interactions between PUFA intake (%E) and LDL-C in the black women, and SFA intake (%E) and T-C in the white women are notable, in that the dietary analysis reported that black women consumed greater amounts of PUFAs compared to the white women, and the white women consumed greater amounts of SFA compared to the black women (Table 3.3).

Only the studies by Fontaine-Bisson *et al*. have previously investigated interactions between dietary fat intake and the -308 G>A polymorphism on serum lipids [182, 183]. In an ethno-racially diverse Canadian population they found that PUFA intake (%E) was inversely associated with HDL-C concentrations in *TNFA* -308 A allele carriers, but not in those with the GG genotype [182]. However, in a subsequent study using a diabetes-free population, with participants being mostly lean and

younger, they found that the association between PUFA (%E) intake and HDL-C amongst each genotype were in the opposite direction [183]. While this study did not show an interaction between the *TNFA* -308 G>A polymorphism, PUFA intake (%E) and HDL-C, the results of this study, and those of Nieters *et al.* [170] and Fontaine-Bisson *et al.* [182, 183] suggest that individuals with the pro-inflammatory *TNFA* -308 A allele may be more responsive to changes in dietary fat intake (%E) than those with the GG genotype (33), and that different dietary fatty acids impact the phenotype in different ways.

Grimble *et al.* has suggested that the inherent underlying inflammatory status of a population may alter the impact of different dietary fats on inflammation [165, 183, 242]. An individual's inherent inflammatory status may be due to an individual's genotype and / or dietary intake or a pre-existing condition such as obesity [41]. The -308 A allele has been shown to increase transcription and subsequently increase TNF ! production [125]. In this study, the -308 A allele frequency was greater in the white women compared to the black women. However, dietary fat intake was also different between the black and white women. It is therefore possible that these differences in genotype frequency and dietary fat intake may contribute to the ethnic variability in diet-gene interactions observed in this study, and the differences in lipid profiles between these two groups.

Interpreting the diet-genotype interactions reported in this study would be enhanced by knowing the impact of dietary fatty acids on the release of different cytokines [40, 41]. Unfortunately, circulating levels of TNF ! or TNF-R, were not measured in this study. However, previous studies have reported that SFAs have been shown to have

a pro-inflammatory action, increasing TNF ! concentration [160], whereas cell culture and human studies have demonstrated that n-3 PUFAs act in an anti-inflammatory manner, inhibiting the production of a number of cytokines, including TNF ! [40, 41]. Discussed in detail in Chapter 1, section 1.8.3.

These differences in the inflammatory impact of different dietary fatty acids are relevant to the results in this study as most dietary fat intake variables investigated in this study differed between the white and black SA women (Table 3.3). The black women consumed more total PUFAs (%E) and n-6 PUFAs (%E), and showed a greater n-6:n-3 PUFA ratio compared to the white women. In contrast, the white women consumed more SFA (%E) compared with the black women. Total n-3 PUFA (%E) intake was not different between the ethnic groups. In contrast to the study of Nieters *et al.* in which the relative total dietary fat intake was higher [170] than this study, their n-6:n-3 PUFA ratio was almost 3 times lower than that reported in the white SA women and 4 times lower than that reported in the black SA women in this study. Further, their reported n-3 (% E) intake was two times greater than in the white and black SA women in this study [170]. Dietary fat intake in the black SA women in this study are a cause for concern, highlighted by the very high n-6:n-3 PUFA ratio (28.2:1 and 29.9:1 in the obese and normal weight groups, respectively), far exceeding the WHO recommendation of a 5–10:1 ratio [243].

It is generally believed that multiple genes, each with modest effects, may underlie the obesity phenotype and associated risk factors [96, 244, 245]. This study was limited to the examination of only one polymorphism from a single gene [170] [182]. It has also been shown that there is constancy in TNF ! production in post-menopausal women and men but not pre-menopausal women, suggesting that

TNF ! production may be regulated by sex hormones [246]. This study included only pre-menopausal women, which may also affect the study results. Furthermore, the study group was small and therefore may not have been able to detect differences that may exist in the black and white population. For example, only when the black and white women were combined, an interaction between EPA (%E) intake and *TNFA* -308 genotypes on T-C concentrations was identified. An interaction that was not significant in the black or white women when analysed separately, suggesting that the interaction may only have been observed because there was more power in the larger combined group to detect the effect.

In order to improve the validity of the dietary data in this study, a validated food frequency questionnaire was used, developed specifically for the SA population, and included only adequate reporters in the analysis [215]. The results, like the results of all genetic association studies, should be treated with caution until independently replicated.

In conclusion, although the *TNFA* -308 G>A polymorphism was not independently associated with obesity risk or serum lipid concentrations in this sample of black and white SA women, dietary fat intake modified the relationship between the *TNFA* -308 G>A polymorphism, obesity risk and serum lipid concentrations. In light of these findings, the results of this and other similar studies should be confirmed in larger cohorts. Additional polymorphisms within the *TNFA* gene such as *TNFA* -238 G>A, as well as polymorphisms in other genes involved in inflammation such as *IL-6* -174 G>C should be investigated. Results from diet-gene studies such as these will contribute towards an understanding of how ethnic populations respond differentially

CHAPTER FOUR

DIETRAY FAT INTAKE AND THE RELATIONSHIP BETWEEN TUMOUR NECROSIS FACTOR-ǃ GENE -238 G>A POLYMORPHISM, OBESITY RISK AND SERUM LIPID CONCENTRATIONS IN BLACK AND WHITE SOUTH AFRICAN WOMEN.

The data and results presented in this chapter have been published in the following peer-reviewed article:

Joffe YT, van der Merwe L, Evans J, Collins M, Lambert EV, September A, Goedecke JH: The tumor necrosis factor-alpha gene -238 G>A polymorphism, dietary fat intake, obesity risk and serum lipid concentrations in black and white South African women. *Eur J Clin Nutr* 2012, 66(12):1295-1302.

Statement of contribution to manuscript and chapter:
Y.T.J, M. Collins, E.V.L, and J.H.G designed the study; **Y.T.J**, M. Carstens, and J. Evans. conducted the research; **Y.T.J**, M.Collins, J.H.G and L.v.d.M analyzed data; **Y.T.J** wrote the paper and chapter with editorial input from M. Collins, A. September, J.H.G and L.v.d.M; **Y.T.J** had final primary responsibility for final content.

CHAPTER FOUR

4.1. INTRODUCTION

The previous chapter has shown that dietary fat intake modifies the relationship between the *TNFA* -308 G>A polymorphism (rs1800629), obesity, adiposity, and serum lipid concentrations. Common to the results of Chapter 3 and those of other studies [170, 182] was that the pro-inflammatory A allele of the functional *TNFA* -308 G>A polymorphism was associated with increased risk for obesity and dyslipidaemia, and appeared to be more responsive to dietary fat intake. Notably, these associations differed between black and white SA women. Differences in these relationships may be explained, in part, by ethnic differences in obesity risk, dietary fatty acid intake, and serum lipid concentrations [19], as well as the distribution of inflammatory gene polymorphisms and inflammatory gene expression between black and white SA women [102, 236].

The functional -308 G>A polymorphism within the promoter region of the *TNFA* gene is in close proximity to the downstream *TNFA* -238 G>A polymorphism (rs361525). While a number of studies have investigated the *TNFA* -308 G>A polymorphism, only a few have reported on the *TNFA* –238 G>A polymorphism and obesity [177, 181], and only two studies have investigated the interaction between dietary fat intake and this polymorphism on serum lipid concentrations [182, 183]. However, there are no studies that have explored these associations in healthy populations of different ethnicity.

Therefore, the aims of this study were to explore whether the *TNFA* -238 G>A polymorphism is independently associated with adiposity and serum lipid concentrations, whether these associations differ depending on dietary fat intake in

apparently healthy premenopausal black and white urban SA women, and if these associations and interactions differ by ethnicity.

4.2. RESULTS

4.2.1. Subjects

The study group included 107 normal-weight and 120 obese urban black women, and 89 normal-weight and 62 obese urban white SA women between the ages of 18 and 45 years. Only 72 normal-weight and 70 obese black, and 74 normal-weight and 47 obese white women, who were classified as adequate-reporters according to the Goldberg cutoffs were included in the diet-gene analysis. Subject characteristics and dietary intake for normal-weight and obese, black and white women in this study are the same as those described in the previous chapter, sections 3.2.1 and 3.2.4, respectively, and can be found in Tables 3.1. and 3.6.

4.2.2. *TNFA* -238 G>A genotype and allele frequencies

The genotype and minor allele frequency distribution for normal-weight and obese, black and white women are presented in Table 4.1. There were no significant differences in the *TNFA* -238 G>A genotype (P=0.285) or allele frequencies (P=0.352) between the normal-weight and obese women, after adjusting for ethnicity. However, the differences in genotype distribution and allele frequency between black and white women were highly significant after adjusting for BMI group (P<0.001); the A allele frequency was higher in black than white women.

The normal-weight and obese white and the normal-weight black groups were in HWE (P=0.600, P=1.00 and P=0.113 respectively), but the black obese group was not (P<0.001). As reported in Table 4.1., the *TNFA* -238 G>A polymorphism was in tight LD with *TNFA* -308 G>A only in the normal-weight white women (D'=0.99).

There was only one black normal-weight woman with the *TNFA* -238 AA homozygous genotype. For all analyses, she was grouped with those with a GA genotype, thus assuming a dominant effect of the A allele for this polymorphism, as done previously by other authors such as Fontaine-Bisson *et al.* [182, 247].

Table 4.1. *TNFA* -238 G>A genotype and allele frequency in black and white women

	Black		**White**		**P-values**	
	Normal-weight	**Obese**	**Normal-weight**	**Obese**	**Ethnicity**	**BMI group**
n	107	120	89	62		
GG	63%	52%	76%	81%	<0.001	0.285
GA	36%	48%	24%	19%		
AA	1%	-	-	-		
A allele	19%	24%	12%	10%	<0.001	0.352
HWE p-value	0.113	< 0.001	0.600	1.000		
LD between *TNFA* -238 G>A and *TNFA* -308 G>A						
n	107	111	88	59		
D'	0.05	0.10	0.99	0.03		
r	0.05	0.07	-0.20	-0.01		
LD p-value	0.475	0.289	0.007	0.954		

Values are expressed as frequency (%). *TNFA* -238 G>A genotype frequency distribution, minor allele frequency and p-values for tests of association with ethnicity and BMI group, respectively, each adjusted for the other. P-values for exact tests of HWE. Summary statistics for LD with *TNFA* -308 G>A. Abbreviations: BMI, body mass index; HWE, Hardy Weinberg equilibrium; LD, linkage disequilibrium; *TNFA*, tumour necrosis factor-! gene.

4.2.3. *TNFA* -238 G>A polymorphism, body composition and serum lipids

Summaries of body composition and serum lipid concentrations, according to *TNFA* -238 G>A genotypes and BMI group are presented in Tables 4.2. and 4.3., respectively, with age-adjusted p-values comparing the outcomes between BMI groups and the *TNFA* -238 G>A genotypes.

In the black women, those with the *TNFA* -238 GA genotype had a greater body fat % than the GG genotype group (P<0.001), independent of age and BMI group (Table 4.2.). In the black and white women there were no other independent genotype effects for all measures of body composition or serum lipid concentrations (Tables 4.2 .and 4.3.).

Table 4.2. Physical characteristics and body composition of normal-weight and obese black and white women according to *TNFA* -238 G>A genotype.

Black women	**Normal-weight, n=107**		**Obese, n=120**		
	GG	**GA**	**GG**	**GA**	**Genotype P-values**
n	67	40	63	57	
Age (years)	23 ±5	24 ± 6	31 ± 8	30 ±8	0.802
Height (m)	1.61 ± 0.05	1.60 ± 0.06	1.60 ± 0.06	1.59 ± 0.06	0.349
Weight (kg)	58 ± 6	57 ± 7	93 ± 14	94 ± 14	0.826
BMI (kg/m^2)	22 ± 2	22 ± 2	36 ± 5	37 ± 5	0.441
Body fat (%)	29 ± 5	32 ± 5	44 ± 4	46 ± 4	< 0.001
Fat mass (kg)	17 ± 4	18 ± 4	41 ± 10	43 ± 10	0.193
Waist (cm)	73 ± 6	74 ± 7	104 ± 12	107 ± 12	0.183
White women	**Normal-weight, n=89**		**Obese, n=62**		**Genotype P-values**
	GG	GA	GG	GA	
n	68	21	50	12	
Age (years)	29.8 ± 7.5	28.1 8 ± 6.2	33.9 ± 8.6	36.5 ± 7.9	0.963
Height (m)	1.68 ± 0.06	1.67 ± 0.07	1.67 ± 0.07	1.67 ± 0.06	0.754
Weight (kg)	62 ± 7	62 ± 6	97 ± 15	98 ± 10	0.738
BMI (kg/m^2)	22 ± 2	22 ± 2	35 ± 5	35 ± 3	0.561
Body fat (%)	27 ± 5	29 ± 6	46 ± 4	44 ± 3	0.397
Fat mass (kg)	17 ± 4	18 ± 4	44 ± 10	43 ± 4	0.844
Waist (cm)	77 ± 6	78 ± 6	106 ± 11	110 ± 6	0.220

Values are summarised as mean ± SD (standard deviation). *P*-values are from a linear model testing the interaction between BMI group and *TNFA* -238 G>A genotype, adjusted for age. Abbreviations: BMI, body mass index.

Table 4.3. Serum lipids of normal-weight and obese black and white women according to *TNFA* -238 G>A genotype

Black women	**Normal-weight, n=107**		**Obese, n=120**		
	GG	**GA**	**GG**	**GA**	**Genotype P-values**
n	67	40	63	57	
TAG (mmol/L)	0.60 (0.50-0.78)	0.60 (0.40-0.70)	0.70 (0.50-1.10)	0.80 (0.60-1.20)	0.602
T-C (mmol/L)	3.85 (3.30-4.28)	3.90 (3.35-4.45)	4.20 (3.60-4.43)	3.80 (3.30-4.40)	0.873
HDL-C (mmol/L)	1.50 (1.20-1.80)	1.50 (1.18-1.73)	1.25 (1.00-1.50)	1.20 (1.00-1.40)	0.475
LDL-C (mmol/L)	1.90 (1.50-2.40)	2.10 (1.68-2.80)	2.45 (2.08-2.90)	2.30 (1.70-2.70)	0.881
TC:HDL-C ratio	2.53 (2.18-3.00)	2.75 (2.26-3.10)	3.27 (2.70-4.02)	3.33 (2.71-3.92)	0.615
White women	**Normal-weight, n=89**		**Obese, n=62**		**Genotype P-values**
	GG	GA	GG	GA	
n	68	21	50	12	
TAG (mmol/L)	0.70 (0.60-0.95)	0.90 (0.60-1.30)	1.05 (0.80-1.70)	1.20 (0.60-1.35)	0.357
T-C (mmol/L)	4.40 (4.00-4.95)	4.80 (3.70-5.30)	4.95 (4.33-5.70)	4.95 (4.40-5.93)	0.578
HDL-C (mmol/L)	1.70 (1.60-2.00)	1.90 (1.60-2.00)	1.50 (1.20-1.68)	1.45 (1.00-1.83)	0.665
LDL-C (mmol/L)	2.30 (1.90-2.80)	2.50 (1.60-2.80)	2.80 (2.50-3.50)	3.10 (2.58-3.70)	0.987
TC:HDL-C ratio	2.56 (2.25-2.94)	2.57 (2.10-2.79)	3.62 (2.91-4.13)	3.22 (2.97-5.03)	0.999

Values are summarised as median (interquartile range). P-values are from a linear model testing the interaction between BMI group and *TNFA* -238 G>A genotype, adjusted for age. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

4.2.4. Diet-genotype interactions

4.2.4.1. Diet-genotype interactions on body composition

In black women, a number of interactions between dietary fat intake and the *TNFA* -238 G>A polymorphism on measures of adiposity were identified (Table 4S II). With increasing total fat and SFA intake (%E), weight (P=0.034 and P=0.017), waist (P=0.036 and P=0.012), and fat mass (P=0.047 for SFA only) increased for those with the GA genotype, but not the GG genotype. With increasing MUFA intake (%E), weight (P=0.044) increased for the GA genotype, but not the GG genotype, and with increasing ALA intake (%), the rates of change in waist (P=0.007), BMI (P=0.012), body fat % (P=0.006), waist (P=0.044), and fat mass (P=0.005) also differed, but neither individual rate was significant. As an example, the interaction between SFA intake, the *TNFA* -238 G>A polymorphism and waist in black women is illustrated in Figure 4.1.

No diet-genotype interactions with obesity or body composition were identified in the white women (Table 4S III).

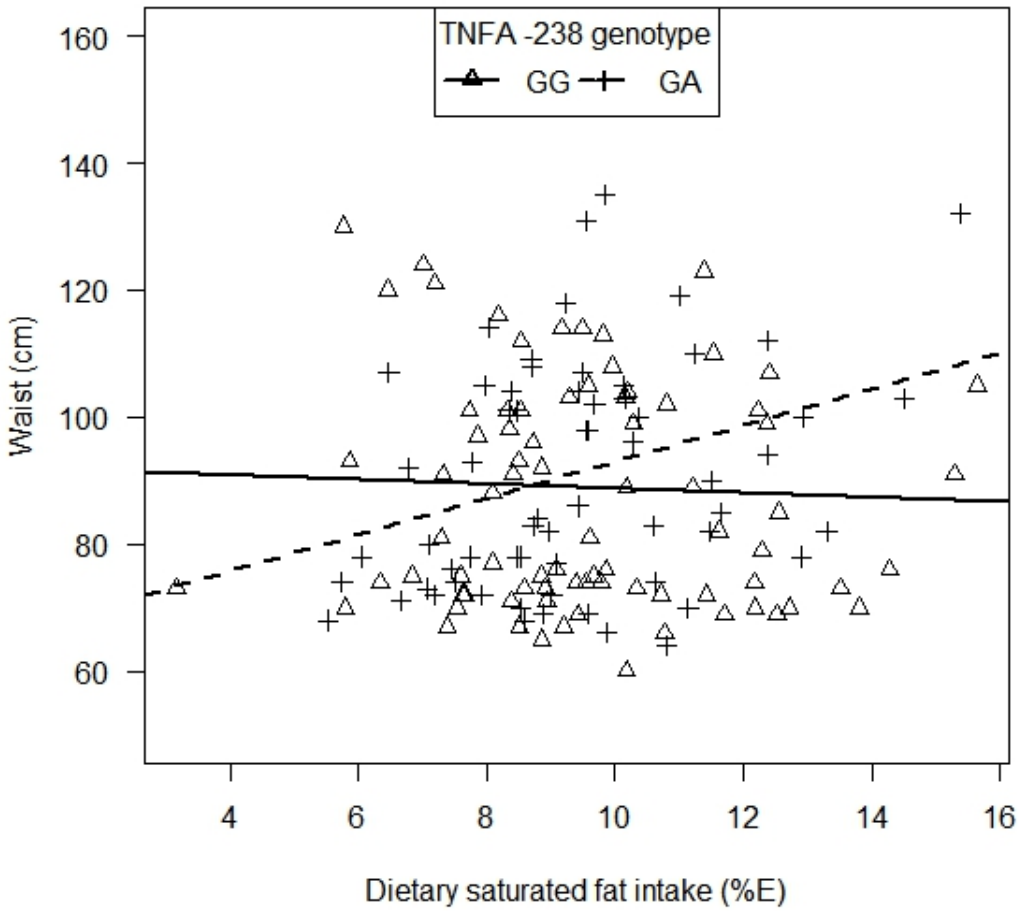

Figure 4.1. The relationship between adiposity, *TNFA* -238 G>A genotype, and dietary saturated fat intake in normal-weight and obese black adequate reporter women. Symbols represent, for each woman, observed values. The lines are modelled relationships for a woman of average age (27.3 y). With increasing saturated fat intake (%E), adiposity increased for the A allele, while the GG genotype did not.

The ethnic-specific nature of these diet-genotype interactions was identified by examining three-way interactions between ethnic group, dietary fat intake (%E) and the *TNFA* -238 G>A polymorphism on obesity (Table 4S V), as well as by including both black and white women in the same model and adjusting for ethnicity (Tables 4S IV and 4S VIII).

The association showing a greater body fat % in those with the *TNFA* -238 GA genotype compared to subjects with the GG genotype was observed in black women, as well as in the combined black and white group, independent of ethnicity (Table 4S I). This suggests that this association was not different for the black and white women, and was observed only because there was more power in this larger group, and the white group may have been too small to detect the effect. No diet-gene interactions on obesity were identified in the combined group (Table 4S IV) nor in the 3-way interactions on obesity (Table 4S V).

4.2.4.2. Diet-genotype interactions on serum lipid concentrations

In black women, interactions between dietary fat intake and the *TNFA* -238 G>A polymorphism on serum lipid concentrations were identified (Table 4S VI). With increasing P:S ratio, HDL-C concentrations decreased in those with the GA (P=0.013) but not the GG genotype, and as expected T-C:HDL-C ratio increased in those with the GA genotype but not the GG genotype (P=0.032). With increasing n-6:n-3 PUFA ratio, the rates of change in HDL-C concentration (P=0.032) and T-C:HDL-C ratio (P=0.004) differed between GG and GA, but did not change significantly with either of the genotypes. In addition, with increasing n-3 PUFA (P=0.012) intake (%E), T-C:HDL-C ratio decreased in those with the GA, but not in those with GG genotype (Table 4S VI). As an example, interactions between n-6:n-3 PUFA ratio, the *TNFA* -238 G>A polymorphism, HDL-C and T-C:HDL-C ratio are illustrated in Figure 4.2.

In white women, with increasing EPA (P=0.020)(%E) intake, LDL-C decreased in those with the GG genotype but not the GA genotype (Table 4S VII). Similarly, with increasing EPA (%E) (P=0.041) and DHA (%E) intakes (P=0.040), T-C decreased in those with the GG genotype and not the GA genotype, but the individual rates were not significant (Table 4S VII).

When the black and white women were combined and adjusted for age and ethnicity (Table 4S VIII), the interactions between MUFA (%E), n-3 PUFA (%E) and ALA (%E) intake and *TNFA* -238 genotypes on T-C:HDL-C ratio were significant (P=0.038, P=0.019, and P=0.015, respectively). Of these, only the interaction between n-3 PUFA (%E) intake and *TNFA* -238 genotypes on T-C:HDL-C ratio was significant in the black women (P=0.012), but not in the white women. This suggests that the interactions identified in the combined group were not different for the black and white women, and were observed only because there was more power in this larger group to detect effects.

Table 4S IX, which reports the p-values for three-way interactions between ethnic group, dietary fat intake (%E) and the *TNFA* -238 G>A polymorphism on serum lipid concentrations, identified significant interactions between the n-6:n-3 PUFA ratio and the *TNFA* -238 G>A genotypes on T-C:HDL-C ratio (P=0.015), and between EPA (%E) and DHA (%E) and the *TNFA* -238 genotypes on T-C (P=0.019 and P=0.004, respectively) and LDL-C (P=0.014 and P=0.006, respectively). This suggests that these interactions are different between the black and white women.

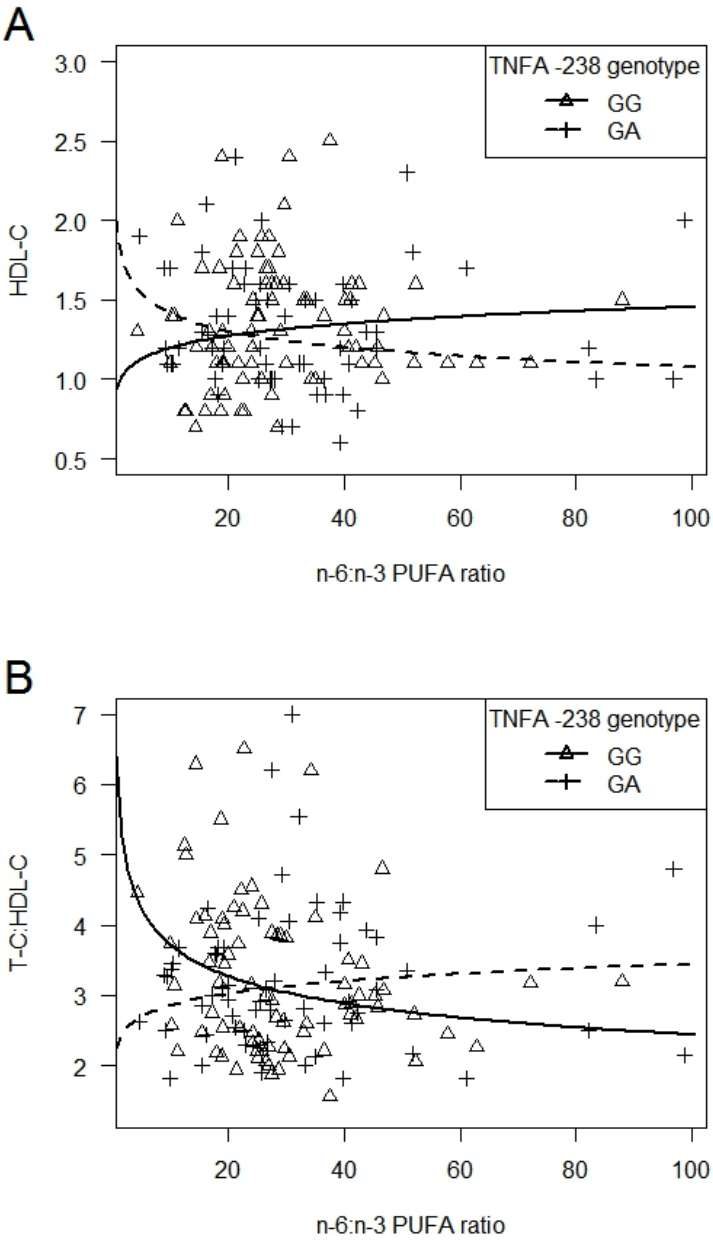

Figure 4.2. The relationship between serum lipids, *TNFA* -238 G>A genotype, and n-6:n-3 PUFA ratio in normal-weight and obese black adequate reporter women. Symbols represent, for each woman, observed values. The curves on each graph are from general linear models of age, fat mass, and the interaction between *TNFA* -238 G>A genotype and specific dietary fat intake for a woman aged 27.3 y with fat mass 29.4 kg.

(A) With increasing n-6:n-3 PUFA ratio, HDL-C concentrations increased in those with the GG genotype and decreased in those with the GA genotype.

(B) With increasing n-6:n-3 PUFA ratio, T-C:HDL-C ratio increased in those with the GA genotype and decreased in those with the GG genotype.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

4.3. DISCUSSION

The main findings of this study were that black women with the *TNFA* -238 GA genotype had a greater body fat % than those with the GG genotype, and that interactions between the -238 G>A polymorphism and serum lipid concentrations differed depending on dietary fat intake; moreover, these interactions differed between the black and white women.

Previous studies have found no association between the *TNFA* -238 G>A polymorphism and BMI [177, 181]. This is the first study to report that the -238 GA genotype is associated with greater body fat % than the GG genotype. This effect was demonstrated in black women, as well as in the combined black and white group, independent of ethnicity. In Chapter 3, the proinflammatory A allele of the *TNFA* -308 G>A polymorphism was discussed as being independently associated with obesity risk [167, 169], but this has not been reported in all studies [175, 176]. Obesity is associated with chronic sub-clinical systemic inflammation, characterized by increased production of pro-inflammatory cytokines, such as TNF!, released by adipose tissue [248]. Unlike with the *TNFA* -308 G>A polymorphism [125], there is controversy regarding the functional significance of the *TNFA* -238 G>A polymorphism. Studies have reported that the -238 A allele either increases [249], decreases [250, 251] or has no effect on *TNFA* transcription [252, 253], depending on the conditions of the experiment. The inflammatory impact of this polymorphism needs to be confirmed. Measures of circulating TNF! or TNF! receptor levels may be informative in this respect, but were unfortunately not measured in this study.

It is notable that the A allele frequency in this study, particularly in the black women is higher than that reported in the Ensemble database in Caucasian, African American, and other African populations, summarised in Table 1.1. in Chapter 1. There are however no studies that have reported this genotype in Southern African populations (as opposed to Sub-Saharan Africans, which refers to West Africans).

Human studies have also shown that TNF ! expression is regulated by dietary fatty acids [163-165]. Results presented in Chapter 3, as well as previous studies on the *TNFA* -308 G>A polymorphism, have shown that the relationship between this polymorphism and obesity risk differed depending on dietary fat intake [170]. In agreement with this, this study is the first to show that with increasing dietary fat intake, irrespective of the type and quality, select measures (weight, BMI, body fat %, fat mass, and waist) of body composition increased in black women with the *TNFA* -238 GA genotype and decreased or did not change in those with the GG genotype (Figure 4.1.). These findings suggest that the A allele of the -238 polymorphism may be responsive to dietary fatty acid intake, as has been shown for the -308 A allele [167-170].

This chapter also showed that the relationship between the *TNFA* -238 G>A polymorphism and serum HDL-C concentration differed depending on the intake of specific PUFAs in black, but not white women (Figure 4.2.). Similarly,
Fontaine-Bisson *et al.* reported that PUFA (%E) intake was positively associated with serum HDL-C concentrations in carriers of the -238 A allele within a mixed ethnicity, diabetic Canadian population [182]. The greatest contribution to PUFA intake is from the n-6 PUFA's, specifically LA. However the effects of n-6 PUFAs on inflammation

are contradictory and inconclusive [254, 255]. Nonetheless, n-6 PUFAs have been shown to reduce LDL-C concentrations and T-C:HDL-C ratio, although some studies have also reported a decrease in HDL-C [254, 256]. A high n-6:n-3 PUFA ratio is more consistently regarded as pro-inflammatory and has been associated with cardiovascular, inflammatory and autoimmune diseases [203, 255, 257, 258]. Notably, the intake of n-6 PUFAs (%E) and the n-6:n-3 PUFA ratio in the black women were higher than that of the white women in this study (8.3% vs. 5.6%, and 26.4:1 vs. 16.1:1, for black and white women, respectively), and that reported in other populations (4.0 – 6.0% for n-6 PUFA (%E) [254], and !15:1 for n-6:n-3 PUFA ratio [259]). In this study the interaction between the *TNFA* -238 G>A polymorphism and n-6:n-3 PUFA ratio on T-C:HDL-C ratio was only observed in the black women. These findings are relevant in this context as the black women in this study had lower HDL-C concentrations than the white women. These findings need to be replicated in other African populations as it has been shown that other African populations, as well as African Americans have higher HDL-C concentrations than their white counterparts [13, 21, 22].

Apart from the higher HDL-C concentrations, white women in this study had a more atherogenic lipid profile than black women, characterised by higher TAG, T-C, and LDL-C concentrations in the white women compared to the black women [21, 234]. This study reported that the relationship between the *TNFA* -238 G>A polymorphism and serum LDL-C and T-C concentrations differed depending on the intake of the n-3 PUFA's EPA and DHA, in white, but not black women. The intake of n-3 PUFA (%E) in this study was the same for the black and white women, but is approximately half of that previously reported in the Fontaine-Bisson *et al.* study which included a

non-diabetic Canadian population [183]. These differences are relevant and worth exploring further as n-3 PUFAs have been shown to attenuate inflammation. In particular, EPA and DHA regulate inflammatory processes and have been shown to improve serum lipid concentrations and vascular endothelial function [62, 260]. As evidenced by the findings of this study and others, dietary fatty acids affect inflammation and serum lipids in different ways, for this reason the inclusion of individual FAs in diet-genotype studies is important. The differences in dietary fat intake, as well as differences between serum lipid profiles [93] between black and white SA women, may partly explain the ethnic differences in these diet-gene interaction findings.

This study is novel and adds to the literature as it is the first to highlight differences in the associations between the *TNFA* -238 G>A polymorphism, body composition and serum lipid concentrations, and their interaction with dietary fat intake in different ethnic groups. There are however limitations to this study that should be considered. Accuracy of diet reporting is always difficult. As mentioned in Chapter 3, the validity of the dietary intake data was improved by using a validated FFQ developed specifically for the SA population, and by including only adequate reporters in the analyses. Other lifestyle factors, such as physical activity and socioeconomic factors may also interact with these variables, but were not included in this study. The sample size was small, included no men and had fewer white than black women. Therefore, small associations that were detected in the black women may not be detected in the white women, as demonstrated by the independent genotype association between the -238 G>A polymorphism and body fat % that was shown in the black women and the combined group, but not the white women.

This study also investigated the -238 G>A and -308 G>A polymorphisms as a haplotype. Although these polymorphisms are only 69 bp apart (http://www.ensembl.org), they were only in LD in the white normal-weight women and not in the obese white women, nor the black groups. For this reason the haplotypes were not informative, and were not reported here. It is not surprising that the two variants were not in LD in the black populations, as rapid decay in LD has been shown to be more associated with populations of African descent compared to populations of non-African descent [261]. It is also not surprising to note the differences in the frequency distribution of the genotypes and alleles for these two variants within the various populations. This study therefore highlights the importance of exploring this particular genetic interval defined by these two variants in populations of African descent to unravel the functional significance of *TNFA* in modulating an individuals' response to dietary fat intake.

In conclusion, a novel association between the *TNFA* -238 G>A polymorphism and body fat % was observed, as well as responsiveness of the -238 A allele to increasing dietary fat intake for adiposity in black SA women. This thesis has also shown that the relationship between the *TNFA* -238 G>A polymorphism and serum lipid concentrations differed depending on PUFA intake, in both black and white SA women, but that these interactions were ethnic-specific. While the results of this study provides new insight into the *TNFA* -238 G>A polymorphism, they also highlight the need for further research to study the functional significance of this polymorphism in both men and women of different ethnic origin.

CHAPTER FIVE

GENETIC POLYMORPHISMS IN THE INTERLEUKIN-6 GENE AND THEIR ASSOCIATION WITH OBESITY AND DYSLIPIDAEMIA IN BLACK AND WHITE SOUTH AFRICAN WOMEN.

The data presented in this chapter has been submitted for publication, but has not as yet been accepted.

Statement of contribution to this chapter:

Y. Joffe, M. Collins and J. Goedecke designed the study; **Y. Joffe** conducted the research; **Y. Joffe**, M. Collins, J. Goedecke and L. van der Merwe analyzed the data; **Y. Joffe** wrote the chapter with editorial input from M. Collins, J. Goedecke and L. van der Merwe; **Y. Joffe** had final primary responsibility for final content.

CHAPTER FIVE

5.1. INTRODUCTION

In previous studies and in Chapters 3 and 4 of this thesis, polymorphisms in the cytokine gene *TNFA* have been associated with obesity, measures of adiposity and serum lipid concentrations. Furthermore, it has been shown that total dietary fat intake, as well as individual dietary fatty acids, in particular SFAs and the n-3 and n-6 PUFAs, interact with *TNFA* polymorphisms to modulate these relationships. In addition to the *TNFA* gene, *IL-6* was also selected for investigation as a candidate gene for association with obesity and dyslipidaemia, based on the biological function of the encoded IL-6 protein, and the role it plays in both systemic and adipose tissue inflammation.

The cytokine IL-6 is known to regulate inflammation [189]. Higher circulating concentrations of IL-6 have also been associated with obesity and VAT deposition [190-192], lipid metabolism [189] and increased risk for CVD [209]. In addition, there is a growing body of evidence linking polymorphisms within the *IL-6* gene to increased risk of obesity and dyslipidaemia [187, 189, 206-208].

Within the *IL-6* gene, the most frequently studied polymorphism is *IL-6* -174 G>C (rs1800795). This polymorphism has been shown to be functional, with most studies showing the C allele to be associated with raised concentrations of IL-6 and the acute phase protein, CRP [126, 209-212]. However, association studies between this *IL-6* gene polymorphism, obesity and dyslipidaemia have yielded conflicting results. A recent large meta-analysis by Yu *et al.* found the -174 G>C polymorphism to be associated with obesity [126] but this finding was not repeated in two other

meta-analyses [210, 213]. Although Qi *et al.* found no association between the -174 G>C polymorphism and obesity, they identified an *IL-6* haplotype to be associated with adiposity in healthy American men and women [210].

Conversely, several studies have found an association between the -174 G>C polymorphism and serum lipid concentrations, independent of obesity, with most showing the *IL-6* -174 G allele to be associated with higher T-C, LDL-C and TAG concentrations [186, 206, 208], and lower HDL-C concentrations [208, 214].

Taken together, these findings suggest that this polymorphism is a strong candidate for obesity and dyslipidaemia. However, the -174 G>C polymorphism has been shown to be rare in persons of African descent [262]. Therefore, the aim of this study was to identify informative (more common) polymorphisms in both black and white SA populations, and to investigate associations between these *IL-6* polymorphisms (*IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G) and obesity and dyslipidaemia in black and white SA women.

5.2. RESULTS

5.2.1. Subjects

The study group included 107 normal-weight and 120 obese urban black women, and 89 normal-weight and 62 obese urban white SA women between the ages of 18 and 45 years. Subject characteristics for normal-weight and obese, black and white women in this study are the same as those described in Chapter 3, sections 3.2.1

and summarised in Table 3.1. The white women were older than the black women, and the obese women older than the normal-weight women; as a result all analyses were adjusted for age (P< 0.001).

5.2.2. *IL-6* genotype and allelic frequency distributions

The genotype and minor allele frequency distribution of the *IL-6* polymorphisms investigated in this study for normal-weight and obese, black and white women are presented in Table 5.1. There were no significant differences between the normal-weight and obese women, after adjusting for ethnicity, for the *IL-6* -174 G>C genotype (P=0.104) or allele frequencies (P=0.515), *IL-6* IVS3+281 G>T genotype (P=0.914) or allele frequencies (P=0.950), and *IL-6* IVS4+869 A>G genotype (P=0.293) or allele frequencies (P=0.474).

However, the difference in the *IL-6* -174 G>C and IVS3+281 G>T genotype distribution and allele frequency between black and white women was highly significant after adjusting for BMI group (P<0.001). Although the IVS4+869 A>G genotype distribution between black and white women was also significant after adjusting for BMI group (P<0.001), the allele frequency was not different (P=0.688). The frequency of the minor allele of the -174 G>C and IVS3+281 G>T polymorphisms were higher in the white compared to the black women. All genotype frequencies reported in this study were similar to European and African populations reported in the Ensembl database (Chapter 1, Table 1.1), however none of the African populations reported in the Ensembl database were representative of populations from Southern Africa.

Deviations from HWE were detected for the -174 G>C polymorphism in the black obese group and for the IVS4+869 A>G polymorphism in both the normal-weight and obese white women (Table 5.1.). Only one individual was homozygous for the IVS4+869 G allele, despite the frequency of the minor G allele being over 30% in the white SA women (Table 5.1.). In the white women, all the polymorphisms were in high LD in both the normal-weight and obese groups. In the black women, of the three pairs of polymorphisms genotyped, only IVS4+869 A>G and IVS3+281 G>T were in LD, in both the normal-weight and obese groups (Figure 5.1.).

Table 5.1. Genotype and minor allele frequencies of normal-weight and obese black and white women

	Normal-weight		Obese		P-values	
	Black	**White**	**Black**	**White**	**Ethnicity**	**BMI group**
***IL-6* 174 G>C**						
n	106	89	123	63		
GG	97	30	95	32		
GC	3	58	3	46		
CC	0	12	2	22	< 0.001	0.104
C	1	40	3	45	< 0.001	0.515
HWE p-value	1.000	0.077	0.003	0.613		
***IL-6* IVS3+281 G>T**						
n	100	89	115	63		
GG	54	35	5	30		
GT	38	48	37	51		
TT	8	17	9	19	< 0.001	0.914
T	27	41	27	44	< 0.001	0.950
HWE p-value	0.799	1.000	0.477	1.000		
***IL-6* IVS4+869 A>G**						
n	99	88	112	63		
AA	51	42	54	37		
GA	39	57	40	3		
GG	10	1	6	1	< 0.001	0.293
G	30	30	26	32	0.688	0.474
HWE p-value	0.630	0.001	0.811	< 0.001		

Values are expressed as percentage (%). *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G genotype frequency distribution, minor allele frequency and p-values for tests of association with ethnicity and BMI group, respectively, each adjusted for the other. P-values for exact tests of HWE. Abbreviations: BMI, body mass index; HWE, Hardy Weinberg equilibrium; *IL-6*, Interleukin 6.

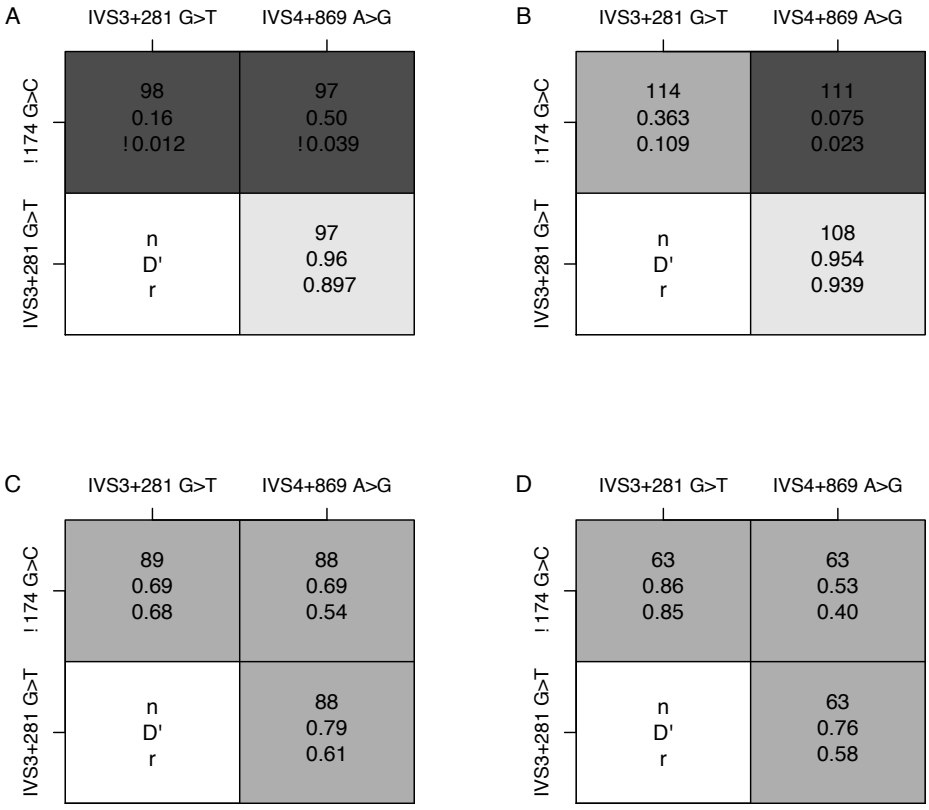

Figure 5.1. LD structure of *IL-6* polymorphisms (n, number of women; D', Lewontin's D; r, coefficient of correlation) for *IL-6* polymorphisms of normal-weight and obese black and white women. LD plots showing A: Normal Black; B Obese Black; C Normal White; D Obese White. Shading determined by value of D' – light indicates high value.

5.2.3. Body composition and genotype

Physical characteristics and body composition of normal-weight and obese black and white women according to *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G genotypes are described in Tables 5.2., 5.3. and 5.4., respectively.

Table 5.2. Physical characteristics and body composition of normal-weight and obese black and white women according to *IL-6* -174 G>C genotype.

Black women									
	Normal-weight			Obese			P-values		
	GG	GC	CC	GG	GC	CC	BMI group	*IL-6 -174*	Interaction
n	103	3	0	117	4	2			
Age, years	24 ± 5	25 ± 2	-	30 ± 8	31 ± 8	32 ± 1	< 0.001	0.885	0.968
Height, cm	1.61 ± 0.06	1.63 ± 0.04	-	1.60 ± 0.06	1.61 ± 0.07	1.51 ± 0.05	0.467	0.062	0.823
Weight, kg	58 ± 6	59 ± 4	-	93 ± 14	94 ± 4	80 ± 14	< 0.001	0.228	0.908
BMI, kg/m^2	22 ± 2	22 ± 2	-	37 ± 5	36 ± 4	36 ± 9	< 0.001	0.922	0.962
Body fat, %	30 ± 5	29 ± 2	-	45 ± 4	48 ± 2	47 ± 4	< 0.001	0.730	0.327
Fat mass, kg	17 ± 4	17 ± 3	-	42 ± 10	44 ± 5	38 ± 10	< 0.001	0.674	0.710
Waist, cm	74 ± 6	74 ± 2	-	105 ± 12	104 ± 5	100 ± 15	< 0.001	0.664	0.744
WHR	0.75 ± 0.06	0.76 ± 0.06	-	0.84 ± 0.08	0.84 ± 0.05	0.82 ± 0.07	< 0.001	0.893	0.878
VAT, cm^2	49 ± 20	31	-	99 ± 43	89 ± 36	No data	< 0.001	0.442	0.898
SAT, cm^2	194 ± 96	158	-	567 ± 156	580 ± 28	No data	< 0.001	0.971	0.773

Values are summarised as mean ± SD. SD not presented when only one subject. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* -174G>C genotype, adjusted for age. Each p-value is adjusted for the ones before it. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 5.2 continued.

White women									
	Normal-weight			Obese			P-values		
	GG	GC	CC	GG	GC	CC	BMI group	*IL-6 -174*	Interaction
n	27	52	10	20	29	14			
Age, years	28 ± 7	30 ± 8	29 ± 6	33 ± 9	35 ± 8	36 ± 7	< 0.001	0.369	0.745
Height, cm	1.67 ± 0.07	1.68 ± 0.06	1.68 ± 0.08	1.67 ± 0.05	1.67 ± 0.08	1.66 ± 0.06	0.456	0.987	0.899
Weight, kg	62 ± 6	62 ± 7	64 ± 4	99 ± 14	97 ± 15	96 ± 13	< 0.001	0.805	0.565
BMI, kg/m^2	22 ± 2	21 ± 2	23 ± 2	35 ± 5	35 ± 4	35 ± 4	< 0.001	0.749	0.539
Body fat, %	27 ± 5	28 ± 5	28 ± 6	47 ± 4	45 ± 4	45 ± 4	< 0.001	0.914	0.374
Fat mass, kg	17 ± 4	17 ± 4	18 ± 5	47 ± 9	43 ± 9	43 ± 8	< 0.001	0.517	0.164
Waist, cm	78 ± 6	77 ± 6	79 ± 6	108 ± 12	106 ± 10	106 ± 12	< 0.001	0.725	0.604
WHR	0.79 ± 0.06	0.78 ± 0.05	0.81 ± 0.06	0.86 ± 0.05	0.85 ± 0.06	0.85 ± 0.07	< 0.001	0.771	0.467
VAT, cm^2	61 ± 18	63 ± 22	74 ± 27	149 ± 65	163 ± 61	152 ± 61	< 0.001	0.917	0.302
SAT, cm^2	174 ± 68	158 ± 60	186 ± 68	537 ± 121	548 ± 120	504 ± 119	< 0.001	0.720	0.294

Values are summarised as mean ± SD. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* -174G>C genotype, adjusted for age. Each p-value is adjusted for the ones before it. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 5.3. Physical characteristics and body composition of normal-weight and obese black and white women according to *IL-6* IVS3+281 G>T genotype.

Black women									
	Normal-weight			Obese			P-values		
	GG	GT	TT	GG	GT	TT	BMI group	IVS3+*281*	Interaction
n	54	38	8	63	42	10			
Age, years	24 ± 6	24 ± 5	23 ± 4	31 ± 8	29 ± 8	34 ± 10	< 0.001	0.424	0.407
Height, cm	1.60 ± 0.06	1.62 ± 0.06	1.58 ± 0.03	1.59 ± 0.06	1.59 ± 0.06	1.61 ± 0.04	0.485	0.481	0.154
Weight, kg	58 ± 7	59 ± 6	56 ± 6	93 ± 14	94 ± 13	98 ± 18	< 0.001	0.569	0.446
BMI, kg/m^2	22 ± 2	22 ± 2	22 ± 2	36 ± 5	37 ± 5	38 ± 8	< 0.001	0.604	0.540
Body fat, %	31 ± 5	30 ± 4	31 ± 5	45 ± 5	46 ± 4	45 ± 5	< 0.001	0.968	0.514
Fat mass, kg	17 ± 5	17 ± 4	17 ± 3	41 ± 10	43 ± 8	46 ± 14	< 0.001	0.472	0.371
Waist, cm	74 ± 6	74 ± 6	72 ± 6	103 ± 12	108 ± 12	108 ± 11	< 0.001	0.152	0.215
WHR	0.75 ± 0.06	0.75 ± 0.05	0.75 ± 0.04	0.83 ± 0.09	0.86 ± 0.07	0.84 ± 0.10	< 0.001	0.192	0.408
VAT, cm^2	50 ± 25	48 ± 12	51 ± 7	92 ± 39	102 ± 39	115 ± 52	< 0.001	0.371	0.331
SAT, cm^2	194 ± 101	182 ± 82	240 ± 52	553 ± 150	594 ± 156	612 ± 164	< 0.001	0.422	0.422

Values are summarised as mean ± SD. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* -174G>C genotype, adjusted for age. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 5.3 continued

White women

	Normal-weight			Obese			P-values		
	GG	GT	TT	GG	GT	TT	BMI group	IVS3+*281*	Interaction
n	31	43	15	19	32	12			
Age, years	29 ± 7	30 ± 8	29 ± 7	35 ± 8	33 ± 9	38 ± 6	< 0.001	0.543	0.126
Height, cm	1.66 ± 0.07	1.68 ± 0.06	1.68 ± 0.08	1.67 ± 0.05	1.66 ± 0.07	1.67 ± 0.07	0.453	0.726	0.614
Weight, kg	60 ± 6	63 ± 7	63 ± 5	98 ± 11	96 ± 15	100 ± 18	< 0.001	0.781	0.652
BMI, kg/m^2	21 ± 2	22 ± 2	22 ± 2	35 ± 5	35 ± 4	35 ± 5	< 0.001	0.813	0.805
Body fat, %	27 ± 6	28 ± 5	27 ± 4	46 ± 4	45 ± 4	44 ± 4	< 0.001	0.696	0.512
Fat mass, kg	17 ± 4	17 ± 4	17 ± 3	46 ± 8	43 ± 9	44 ± 11	< 0.001	0.882	0.327
Waist, cm	77 ± 6	78 ± 7	77 ± 4	108 ± 11	106 ± 10	108 ± 13	< 0.001	0.995	0.609
WHR	0.78 ± 0.06	0.79 ± 0.06	0.79 ± 0.04	0.86 ± 0.05	0.85 ± 0.06	0.85 ± 0.07	< 0.001	0.963	0.685
VAT, cm^2	59 ± 18	66 ± 25	66 ± 17	163 ± 62	153 ± 64	153 ± 61	< 0.001	0.897	0.537
SAT, cm^2	176 ± 72	153 ± 57	179 ± 58	533 ± 109	540 ± 116	519 ± 147	< 0.001	0.864	0.427

Values are summarised as mean ± SD. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* -174G>C genotype, adjusted for age. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 5.4. Physical characteristics and body composition of normal-weight and obese black and white women according to *IL-6* IVS4+869 A>G genotype.

Black women									
	Normal-weight			Obese			P-values		
	AA	AG	GG	AA	AG	GG	BMI group	*IVS4+869*	Interaction
n	50	39	10	60	45	7			
Age, years	24 ± 5	24 ± 5	24 ± 4	31 ± 8	29 ± 8	34 ± 9	< 0.001	0.571	0.481
Height, cm	1.60 ± 0.06	1.62 ± 0.06	1.58 ± 0.03	1.60 ± 0.06	1.60 ± 0.06	1.61 ± 0.05	0.625	0.678	0.229
Weight, kg	58 ± 7	59 ± 6	55 ± 7	92 ± 13	96 ± 13	101 ± 19	< 0.001	0.133	0.103
BMI, kg/m^2	22 ± 2	23 ± 1	22 ± 2	36 ± 4	38 ± 5	39 ± 9	< 0.001	0.061	0.057
Body fat, %	30 ± 5	31 ± 4	30 ± 4	45 ± 4	46 ± 4	46 ± 4	< 0.001	0.218	0.715
Fat mass, kg	17 ± 5	18 ± 4	16 ± 3	40 ± 9	44 ± 10	49 ± 14	< 0.001	0.050	0.034
Waist, cm	74 ± 6	74 ± 6	72 ± 6	103 ± 13	109 ± 11	109 ± 11	< 0.001	0.026	0.077
WHR	0.75 ± 0.06	0.75 ± 0.06	0.76 ± 0.04	0.83 ± 0.09	0.85 ± 0.07	0.83 ± 0.06	< 0.001	0.370	0.247
VAT, cm^2	48 ± 21	49 ± 13	52 ± 6	93 ± 41	104 ± 38	106 ± 59	< 0.001	0.401	0.504
SAT, cm^2	171 ± 71	201 ± 90	205 ± 91	557 ± 130	610 ± 174	585 ± 128	< 0.001	0.100	0.786

Values are summarised as mean ± SD. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* +869 genotype, adjusted for age.
Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 5.4 continued.

White women									
	Normal-weight			Obese			P-values		
	AA	AG	GG	AA	AG	GG	BMI group	*IVS4+869*	Interaction
n	37	50	1	23	40	0			
Age, years	28 ± 7	30 ± 8	40	34 ± 8	35 ± 9	-	< 0.001	0.220	0.871
Height, cm	1.68 ± 0.06	1.67 ± 0.07	1.73	1.67 ± 0.05	1.67 ± 0.07	-	0.510	0.672	0.785
Weight, kg	62 ± 6	61 ± 7	59	100 ± 14	96 ± 14	-	< 0.001	0.484	0.495
BMI, kg/m^2	22 ± 2	22 ± 2	19	36 ± 4	34 ± 4	-	< 0.001	0.312	0.307
Body fat, %	28 ± 5	28 ± 5	22	46 ± 4	45 ± 4	-	< 0.001	0.344	0.488
Fat mass, kg	17 ± 4	17 ± 4	13	45 ± 9	43 ± 9	-	< 0.001	0.605	0.586
Waist, cm	78 ± 6	77 ± 6	71	110 ± 11	105 ± 10	-	< 0.001	0.247	0.183
WHR	0.78 ± 0.06	0.79 ± 0.05	0.76	0.86 ± 0.05	0.85 ± 0.06	-	< 0.001	0.843	0.322
VAT, cm^2	61 ± 22	66 ± 20	No data	155 ± 66	156 ± 60	-	< 0.001	0.944	0.955
SAT, cm^2	174 ± 70	161 ± 58	No data	551 ± 120	523 ± 119	-	< 0.001	0.232	0.675

Values are summarised as mean ± SD. SD not presented when only one subject. *P*-values are from a linear model testing the interaction between BMI group and *IL-6* +869 genotype, adjusted for age. Abbreviations: BMI, body mass index, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

The IVS4+869 A>G was the only polymorphism to be associated with body composition. In the black women, waist circumference was 3.38 cm (95%CI: 0.79-5.98) higher in those with the AG and GG genotypes compared to the AA genotype (P=0.011, dominant model). An interaction between this polymorphism and BMI group on fat mass in black women (P=0.034) is illustrated in Figure 5.2. The differences in fat mass between genotypes in the normal-weight group were not significant, but in the obese group, each G allele added, on average, 4.28 kg (95%CI: 1.90-6.65) to fat mass (P=0.010, additive allelic model).

The ethnic-specific nature of these genotype-phenotype associations were examined by including both black and white women in the same model and adjusting for ethnicity (Table 6S I). The associations between the IVS4+869 A>G polymorphism and waist and fat mass described above were significant in the black women, but not in the white group, nor in the combined group (Table 6S I), suggesting that these associations were different for the black and white women.

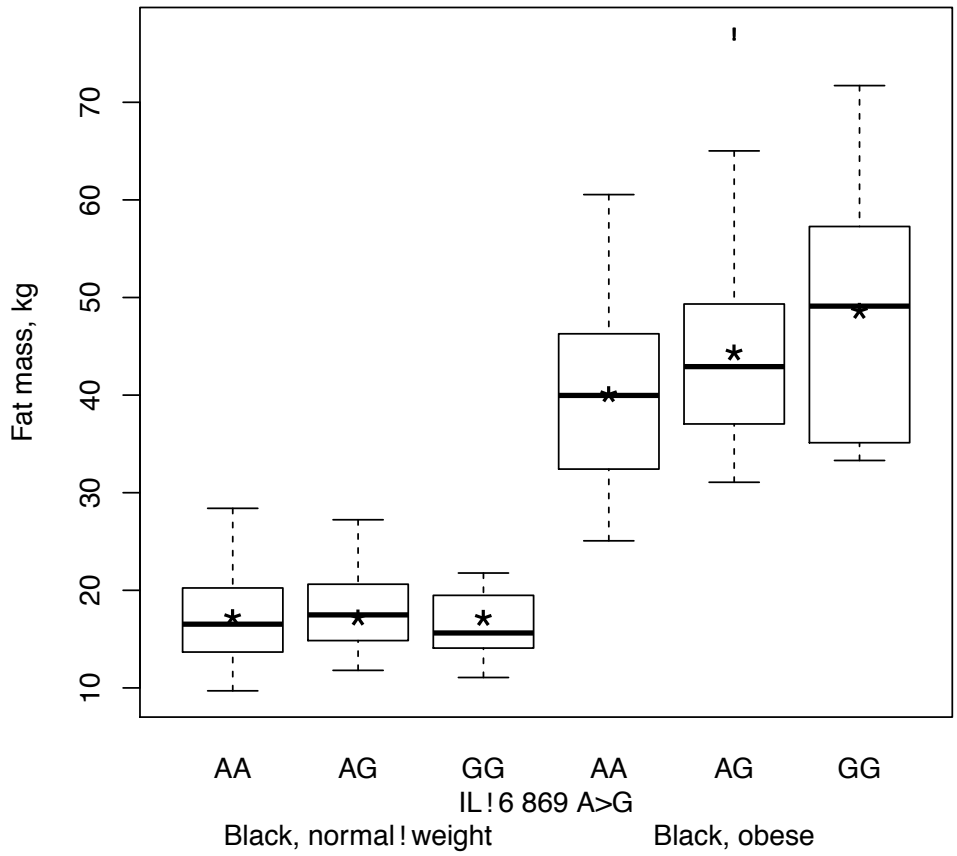

Figure 5.2. Interaction between *IL-6* IVS4+869 A>G polymorphism and BMI group on fat mass in black women. Figure contains boxplots of fat mass in black women, separated by BMI group and genotype. Boxplots indicate the median, the quartiles and the minimum and maximum values for each group. Predicted fat mass (*) from the additive allelic interaction model, for a woman of average age, is also indicated for each genotype by BMI group combination. Abbreviations: BMI, body mass index; *IL-6*, Interleukin-6.

5.2.4. Serum lipid concentrations and genotype

Serum lipid concentrations of normal-weight and obese black and white women according to *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G genotypes are described in Tables 5.5, 5.6 and 5.7, respectively.

Table 5.5. Serum lipid concentrations of normal-weight and obese black and white women according to *IL-6* -174 G>C genotype

Black women									
	Normal-weight			Obese			P-values		
	GG	GC	CC	GG	GC	CC	BMI group	*IL-6 -174*	Interaction
n	103	3	0	117	4	2			
TAG, mmol/L	0.60 (0.43-0.70)	0.40 (0.40-0.55)	-	0.70 (0.60-1.10)	1.15 (0.88-1.30)	0.55 (0.53-0.58)	< 0.001	0.438	0.214
T-C, *mmol/L*	3.9 (3.3-4.3)	3.5 (3.5-4.6)	-	4.0 (3.4-4.4)	4.0 (3.6-4.5)	4.1 (3.8-4.4)	0.579	0.916	0.757
HDL-C, *mmol/L*	1.5 (1.2-1.8)	1.8 (1.8-1.9)	-	1.1 (1.0-1.4)	1.6 (1.3-1.7)	1.5 (1.4-1.5)	< 0.001	0.072	0.968
LDL-C, *mmol/L*	2.0 (1.6-2.5)	1.5 (1.5-2.5)	-	2.4 (1.9-2.8)	2.3 (1.9-2.6)	2.4 (2.1-2.6)	0.113	0.808	0.655
TC:HDL-C ratio	2.6 (2.2-3.0)	1.9 (1.9-2.4)	-	3.4 (2.8-4.1)	3.1 (2.6-3.3)	2.8 (2.7-3.0)	< 0.001	0.127	0.848
White women									
	Normal-weight			Obese			P-values		
	GG	GC	CC	GG	GC	CC	BMI group	*IL-6 -174*	Interaction
n	27	52	10	20	29	14			
TAG, mmol/L	0.90 (0.65-1.10)	0.70 (0.60-1.00)	0.70 (0.60-0.90)	1.20 (0.98-1.73)	0.90 (0.60-1.50)	1.20 (0.83-1.70)	< 0.001	0.266	0.178
T-C, *mmol/L*	4.3 (4.0-4.9)	4.6 (3.9-5.1)	4.6 (4.1-4.9)	5.2 (4.5-5.8)	4.8 (4.1-5.4)	5.6 (4.9-6.2)	0.002	0.490	0.114
HDL-C, *mmol/L*	1.8 (1.6-2.0)	1.7 (1.6-2.0)	2.0 (1.7-2.1)	1.5 (1.3-1.8)	1.5 (1.2-1.7)	1.4 (1.1-1.5)	< 0.001	0.392	0.212
LDL-C, *mmol/L*	2.2 (1.8-2.8)	2.3 (1.9-2.9)	2.5 (1.8-2.6)	2.8 (2.5-3.6)	2.6 (2.2-3.3)	3.5 (2.9-4.1)	< 0.001	0.549	0.067
TC:HDL-C ratio	2.5 (2.1-2.9)	2.6 (2.4-2.9)	2.5 (1.9-2.8)	3.4 (2.7-4.1)	3.3 (2.5-4.0)	4.2 (3.7-4.8)	< 0.001	0.394	0.056

Values are median (interquartile range). *P*-values are from a linear model testing the interaction between BMI group and *IL-6* -174 G>C genotype, adjusted for age. Each p-value is adjusted for the ones before it. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 5.6. Serum lipid concentrations of normal-weight and obese black and white women according to *IL-6* IVS3+281 G>T genotype.

Black women									
	Normal-weight			Obese			P-values		
	GG	GT	TT	GG	GT	TT	BMI group	IVS3+*281*	Interaction
n	54	38	8	63	42	10			
TAG, mmol/L	0.60 (0.43-0.80)	0.60 (0.40-0.70)	0.50 (0.45-0.63)	0.75 (0.50-1.20)	0.70 (0.60-1.00)	0.70 (0.50-1.00)	0.001	0.496	0.959
T-C, *mmol/L*	3.9 (3.3-4.3)	3.7 (3.3-4.2)	4.0 (3.9-4.7)	4.0 (3.1-4.4)	4.1 (3.6-4.7)	4.3 (4.1-4.6)	0.813	0.198	0.254
HDL-C, *mmol/L*	1.5 (1.2-1.7)	1.5 (1.2-1.8)	1.5 (1.1-1.7)	1.1 (0.9-1.3)	1.2 (1.1-1.6)	1.3 (1.2-1.6)	< 0.001	0.146	0.133
LDL-C, *mmol/L*	2.0 (1.5-2.5)	1.8 (1.6-2.3)	2.5 (2.2-3.0)	2.4 (1.7-2.9)	2.4 (2.2-2.8)	2.3 (2.2-2.8)	0.088	0.101	0.288
TC:HDL-C ratio	2.5 (2.1-3.0)	2.7 (2.2-2.9)	3.2 (2.4-3.8)	3.5 (2.8-4.2)	3.2 (2.8-3.7)	3.2 (2.9-3.6)	< 0.001	0.636	0.253
White women									
	Normal-weight			Obese			P-values		
	GG	GT	TT	GG	GT	TT	BMI group	IVS3+*281*	Interaction
n	31	43	15	19	32	12			
TAG, mmol/L	0.90 (0.63-1.20)	0.70 (0.60-0.95)	0.70 (0.60-0.85)	1.40 (0.95-1.75)	0.90 (0.60-1.35)	1.20 (0.88-1.58)	< 0.001	0.024	0.217
T-C, *mmol/L*	4.6 (4.0-5.1)	4.4 (4.0-5.1)	4.3 (3.8-4.8)	5.2 (4.5-6.0)	4.9 (4.1-5.5)	5.0 (4.6-5.9)	0.002	0.215	0.472
HDL-C, *mmol/L*	1.8 (1.6-2.0)	1.7 (1.6-2.0)	1.8 (1.6-2.1)	1.5 (1.3-1.7)	1.5 (1.2-1.8)	1.4 (1.1-1.5)	< 0.001	0.334	0.172
LDL-C, *mmol/L*	2.4 (1.8-2.8)	2.3 (1.9-2.8)	2.2 (1.9-2.5)	3.2 (2.5-3.7)	2.7 (2.2-3.4)	3.3 (2.7-3.8)	< 0.001	0.834	0.265
TC:HDL-C ratio	2.6 (2.2-2.8)	2.6 (2.3-2.9)	2.4 (2.1-2.6)	3.6 (3.1-4.4)	3.3 (2.5-4.0)	4.1 (3.8-4.5)	< 0.001	0.675	0.079

Values are median (interquartile range). *P*-values are from a linear model testing the interaction between BMI group and *IL-6* IVS3+281 G>T genotype, adjusted for age. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 5.7. Serum lipid concentrations of normal-weight and obese black and white women according to *IL-6* IVS4+869 A>G genotype

Black women									
	Normal-weight			Obese			P-values		
	AA	AG	GG	AA	AG	GG	BMI group	*IVS4+869*	Interaction
n	50	39	10	60	45	7			
TAG, mmol/L	0.60 (0.50-0.80)	0.60 (0.40-0.70)	0.50 (0.43-0.60)	0.70 (0.50-1.10)	0.70 (0.60-1.10)	0.85 (0.63-1.00)	0.002	0.634	0.562
T-C, *mmol/L*	4.0 (3.3-4.3)	3.6 (3.2-4.1)	4.0 (3.9-4.6)	4.0 (3.1-4.4)	4.1 (3.5-4.6)	4.5 (4.2-4.8)	0.951	0.323	0.325
HDL-C, *mmol/L*	1.4 (1.2-1.7)	1.6 (1.2-1.8)	1.5 (1.2-1.7)	1.1 (0.9-1.4)	1.2 (1.0-1.6)	1.4 (1.2-1.6)	< 0.001	0.072	0.567
LDL-C, *mmol/L*	2.1 (1.6-2.5)	1.8 (1.5-2.1)	2.3 (2.1-2.9)	2.5 (1.8-2.9)	2.4 (2.0-2.7)	2.8 (2.4-3.0)	0.102	0.065	0.415
TC:HDL-C ratio	2.6 (2.2-3.1)	2.6 (2.2-2.8)	2.9 (2.5-3.3)	3.5 (2.8-4.2)	3.2 (2.8-3.7)	3.2 (2.9-3.9)	< 0.001	0.161	0.551
White women									
	Normal-weight			Obese			P-values		
	AA	AG	GG	AA	AG	GG	BMI group	*IVS4+869*	Interaction
n	37	50	1	23	40	0			
TAG, mmol/L	0.90 (0.60-1.23)	0.70 (0.60-0.90)	0.70 (0.70-0.70)	1.20 (0.80-1.70)	1.05 (0.68-1.50)	-	< 0.001	0.136	0.876
T-C, *mmol/L*	4.7 (4.0-5.1)	4.3 (3.9-5.1)	4.9 (4.9-4.9)	5.2 (4.5-5.9)	4.9 (4.2-5.6)	-	0.002	0.353	0.505
HDL-C, *mmol/L*	1.8 (1.6-2.0)	1.7 (1.6-1.9)	2.0 (2.0-2.0)	1.4 (1.3-1.6)	1.5 (1.2-1.7)	-	< 0.001	0.656	0.598
LDL-C, *mmol/L*	2.5 (1.9-2.8)	2.2 (1.8-2.8)	2.6 (2.6-2.6)	3.2 (2.6-3.7)	2.8 (2.3-3.4)	-	< 0.001	0.737	0.334
TC:HDL-C ratio	2.6 (2.3-2.8)	2.6 (2.2-2.9)	2.5 (2.5-2.5)	3.7 (3.1-4.2)	3.4 (2.6-4.3)	-	< 0.001	0.854	0.345

Values are median (interquartile range). *P*-values are from a linear model testing the interaction between BMI group and *IL-6*+869 A>G genotype, adjusted for age. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

The only association identified between the *IL-6* polymorphisms and serum lipid concentrations, was in the white women, between the IVS3+281 G>T polymorphism and serum TAG concentrations (P=0.024) (Figure 5.3). White women with a IVS3+281 T allele (GT and TT genotype), had lower TAG concentrations than those with a G allele; the estimated effect of any T allele was 82% of the GG genotype (P=0.008, dominant model). The association was also significant in the combined group (Table 6S I), suggesting that this association was not different for the black and white women.

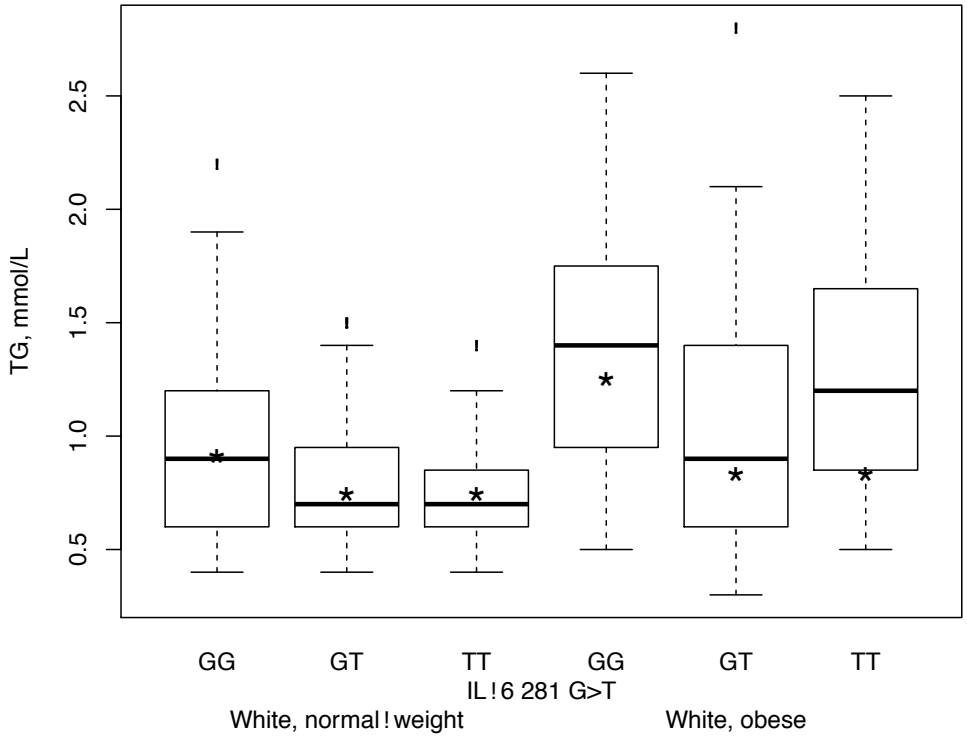

Figure 5.3. Association of *IL-6* IVS3+281 G>T polymorphisms with triglyceride concentration in white women. The figure contains boxplots of triglyceride concentrations in white women, separated by BMI group and genotype, as well as predicted triglyceride concentration (*) for a white woman of average age per *IL-6* IVS3+281 G>T genotype and BMI group, from the dominant model. Abbreviations: BMI, body mass index; *IL-6*, interleukin 6; TAG, triglycerides.

5.3. DISCUSSION

This study identified associations between the G allele of the *IL-6* IVS4+869 A>G polymorphism and increasing adiposity in the black women, and the T allele of the *IL-6* IVS3+281 G>T polymorphism and lower TAG concentrations in the white women. Although the functional nature of the IVS4+869 A>G and IVS3+281 G>T polymorphisms have not been determined, these polymorphisms are in strong LD in both black and white SA women, identifying a region of the *IL-6* gene that may contribute to obesity and dyslipidaemia.

Previous studies in varied populations have shown the C allele of the *IL-6* -174 G>C polymorphism to be associated with increased adiposity [126], and the T allele of the IVS3+281 G>T polymorphism to be associated with adiposity when part of a haplotype [210]. However, in agreement with previous studies in black populations, the black SA women had a very low -174 C allele frequency [97, 262, 263], which may explain why no association was found with this allele. It is notable that black SA women have a higher prevalence of obesity [3], greater SAT [102], and a higher SAT inflammatory gene expression profile compared to white SA women [118]. The findings of the present study are supported by the previous studies in Chapters 3 and 4 examining polymorphisms within the pro-inflammatory cytokine gene *TNFA*. In these chapters it was reported that for both the *TNFA* -308 G>A and -238 G>A polymorphisms, the -308 A allele and the -238 A allele were associated with obesity risk and adiposity in black, but not white SA women.

Conversely, the association between the IVS3+281 G>T polymorphism and lower TAG concentrations was found only in white women. White SA women have a more

atherogenic lipid profile than black women [19], which may be related to their higher levels of VAT [21]. Limited data is available concerning the role of *IL-6* polymorphisms in lipid metabolism [214, 264] in Caucasian and African American populations [264-266]. Most studies, but not all [214], found -174 G allele carriers to have higher T-C, LDL-C and TAG concentrations, [186, 206, 208] and lower HDL-C concentrations [208, 214]. Henningson *et al.* reported the -174 CC genotype was associated with lower TAG, T-C and LDL-C concentrations in Swedish women, however in men, C allele carriers displayed elevated TAG concentrations [206]. In contrast, Riikola *et al.* reported that T-C and LDL-C concentrations were higher in Finnish men with the -174 GG genotype compared to the CG and CC genotypes, but not in women. These conflicting results highlight the complexity of these associations [187, 208].

Unfortunately, little is known about the biological function and therefore the inflammatory nature of the IVS4+869 A>G and IVS3+281 G>T polymorphisms analysed in the present study, which limits the interpretation of these results. Circulating levels of IL-6 were not measured in this study, but in all studies these should be cautiously interpreted due to the contribution of both adipocytes and myocytes to IL-6 concentrations [194]. Studies on the -174 G>C polymorphism have yielded conflicting results. Specifically, the majority of studies show the -174 C allele results in greater gene expression and higher IL-6 concentrations [209, 212, 265, 267], while others have reported a positive association between the G allele and IL-6 concentrations [186, 266].

A limitation of the study is the small subjects numbers. In addition, we focused solely on the *IL-6* gene and the three polymorphisms analysed. However, these were included because of their high reported heterozygosity frequency in both white and black SA populations, and their established LD in both the black and white SA populations. Studies need to be undertaken in larger, more representative samples and need to explore the functional nature and biological relevance of these polymorphisms. Furthermore, the *IL-6* gene contains a number of polymorphisms that may interact with each other, and other inflammatory genes should also be considered. It is also important to consider that genotype-phenotype relationships may be impacted by environmental factors such as dietary intake. Previous studies have shown that dietary fatty acid intake impacts IL-6 production and *IL-6* gene expression, thereby influencing inflammatory status [40]. A few studies have also identified interactions between *IL-6* polymorphisms and dietary fatty acid intake on obesity and serum lipid concentrations [184, 185]. Therefore, studies that include dietary intake data, investigating diet, *IL-6* genotype and phenotype interactions should be undertaken.

In conclusion*, IL-6* genotype findings in this study were associated with obesity in black SA women and serum lipids in the white women. These findings are novel in that, no other studies have reported on the association between the IVS4+869 A>G polymorphism and adiposity. This study is also the first to report on ethnic-specific associations between *IL-6* genotypes, obesity and associated risk in different SA populations, which may provide insight into mechanisms underlying these pathologies.

CHAPTER SIX

INTERACTIONS BETWEEN DIETARY FAT INTAKE AND INTERLEUKIN-6 GENE POLYMORPHISMS ON OBESITY AND SERUM LIPID CONCENTRATIONS IN BLACK AND WHITE SOUTH AFRICAN WOMEN.

The data presented in this chapter has been submitted for publication, but has not as yet been accepted.

Statement of contribution to this chapter:

Y. Joffe, M. Collins, and J. Goedecke designed the study; **Y. Joffe** conducted the research; M Carstens assisted with dietary assessment; **Y. Joffe**, M. Collins, J. Goedecke and L. van der Merwe analyzed the data; **Y. Joffe** wrote the chapter with editorial input from M. Collins, J. Goedecke and L. van der Merwe; **Y. Joffe** had final primary responsibility for final content.

CHAPTER SIX

6.1. INTRODUCTION

DNA sequence variants within the *IL-6* gene, in particular the functional -174 G>C polymorphism, have been extensively studied, but inconclusively associated with obesity and dyslipidaemia [187, 189, 206-208]. In the previous chapter the *IL-6* IVS4+869 A>G polymorphism was associated with adiposity in black SA women and the IVS3+281 G>T polymorphism with serum lipids in the white women. Differences in study findings may relate to differences in environmental factors, such as dietary intake. Chapters 3 and 4 in this thesis have described how dietary fatty acid intake, in particular, total fat, SFA and the n-3 and n-6 PUFAs modulate the relationship between polymorphisms in the *TNFA* gene and obesity and dyslipidaemia.

To our knowledge, only two studies have reported on the relationship between *IL-6* polymorphisms and dietary intake, and both studies investigated only the -174 G>C polymorphism. Corpeleijn *et al.* reported that the ability to increase fat oxidation after a high fat load was increased in obese European Caucasians with the *IL-6* -174 C allele [184]. In Spanish men and women with a high CVD risk, the -174 CC genotype was associated with higher levels of adiposity at baseline, however after three years of nutritional intervention, those with the -174 CC genotype following a Mediterranean-style diet, had the greatest reduction in body weight [185].

In Chapter 5, the *IL-6* -174 G>C polymorphism was shown not to be informative in a black SA population as the minor C allele is rare [133, 262]. For this reason, the two *IL-6* intronic polymorphisms, IVS3+281 G>T and IVS4+869 A>G were identified, which were common in both black and white women. The G allele of the *IL-6* polymorphism IVS4+869 A>G (rs2069845) was associated with waist and fat mass

in black SA women, whereas the T allele of IVS3+281 G>T (rs1554606) was associated with lower TAG in white women (Chapter 5). However, it is not known whether these relationships are impacted by dietary fat intake, which has been shown to alter an individuals' inflammatory profile [41]. The aim of this study was to investigate the impact of dietary fat intake on the relationship between the *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms and obesity and serum lipids in black and white SA women, and whether these interactions are ethnic specific.

6.2. RESULTS

6.2.1. Subjects

The study group included 107 normal-weight and 120 obese urban black women, and 89 normal-weight and 62 obese urban white SA women between the ages of 18 and 45 years. Only 73 normal-weight and 74 obese black, and 73 normal-weight and 48 obese white women classified as adequate-reporters according to the Goldberg cut-offs were included in the analysis. Subject characteristics and dietary intake for normal-weight and obese, black and white women in this study are the same as those described in Chapter 3, sections 3.2.1 and 3.2.4 and summarised in Tables 3.1. and 3.5. The white women were older than the black women, and the obese women older than the normal-weight women; as a result all analyses were adjusted for age.

In Chapter 5, physical characteristics and body composition of normal-weight and obese black and white women according to *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G genotypes are described in Tables 5.2., 5.3. and 5.4., respectively. Serum lipid concentrations of normal-weight and obese black and white women according to *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G genotypes are described in Chapter 5 in Tables 5.5., 5.6. and 5.7., respectively. Genotype frequency distributions and allele frequencies are discussed in Chapter 5 (section 5.2.2) and summarised in Table 5.1.

6.2.2. Diet-genotype interactions on BMI and body composition

Diet-genotype interactions on body composition are summarised in Table 6.1, and then described in greater detail below.

Table 6.1. Summary of *IL-6* diet-gene interactions on body composition

		Black				White				
	SNP	Weight, kg	BMI, kg/m^2	Fat mass, kg	Waist, cm	BMI, kg/m^2	Fat mass, kg	Body fat %	Waist, cm	WHR
Total fat	IVS3+281 G>T	↑GG or GT ↓TT	↑GG or GT ↓TT	↑GG or GT ↓TT	↑GG or GT ↓TT					
	IVS4+869 A>G	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA ↓GG[a]					
SFA	IVS3+281 G>T		↑GG or G[b] ↓TT[b]	↑GG or GT ↓TT						
	IVS4+869 A>G	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]					
MUFA	IVS3+281 G>T		↓TT	↑GG or GT ↓TT						
	IVS4+869 A>G	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]	↑AG or AA[a] ↓GG[a]					
PUFA	IVS3+281 G>T	↑GG or GT ↓TT	↑GG or GT[b] ↓TT[b]	↑GG or GT ↓TT	↑GG or GT ↓TT					
n-6 PUFA	IVS3+281 G>T	↑GG or GT ↓TT	↑GG or G[b] ↓TT[b]	↑GG or GT ↓TT						
LA	IVS3+281 G>T	↑GG or GT ↓TT	↑GG or G[b] ↓TT[b]	↑GG or GT ↓TT						
AA	IVS3+281 G>T	↑GG or GT ↓TT	↑GG or GT[b] ↓TT[b]	↑GG or GT ↓TT						
	IVS4+869 A>G	↑AG or AA	↑AG or AA	↑AG or AA						
n-3 PUFA	-174 G>C					↓C allele				
	IVS4+869 A>G						↓AG or GG			
EPA	-174 G>C					↓C allele				
DHA	-174 G>C					↓C allele				
ALA	IVS3+281 G>T					↓T allele			↓T allele	↓T allele
	IVS4+869 A>G						↓AG or GG[b] ↑AA[ab]			
n-6:n-3 ratio	-174 G>C					↑C allele				
	IVS3+281 G>T					↑T allele[b]		↑T allele	↑T allele[b]	
	IVS4+869 A>G						↑AG or GG[c]			

The interactions are described as follows: With increasing dietary fat variable, the body composition outcome either ↑ (increases) or ↓ (decreases) for a particular genotype or allele. All interactions are diet-genotype unless stated as being allelic. Dietary fat intake is calculated as a percentage of total energy intake. [a] These interactions were significant but the individual rates of change were not significant; [b] These interactions were ethnic specific; [c] This interaction was not different for black and white women. Abbreviations: WHR, waist:hip ratio; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6 : omega-3 polyunsaturated fatty acid ratio; ALA, !-linolenic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

6.2.2.1. IL-6 -174 G>C

In white women, interactions between the *IL-6* -174 G>C polymorphism and the intake of different dietary fatty acids (%E) on BMI were found (Table 6S II A & 6S IV A, and summarised in Table 6.1.). With increasing total n-3 PUFA (P=0.027) (Figure 6.1A), EPA (P=0.040) and DHA (P=0.043) intake (%E), a decrease in BMI was observed in individuals with a -174 C allele (CC or GC genotypes, dominant). In addition, as the n-6:n-3 PUFA ratio increased, BMI increased equally with each additional -174 C allele (additive, P=0.028) (Figure. 6.1B). Diet-genotype interactions, as well as the impact of ethnicity for the -174 G>C polymorphism on body composition were not analysed due to the rare frequency of the C allele in the black women.

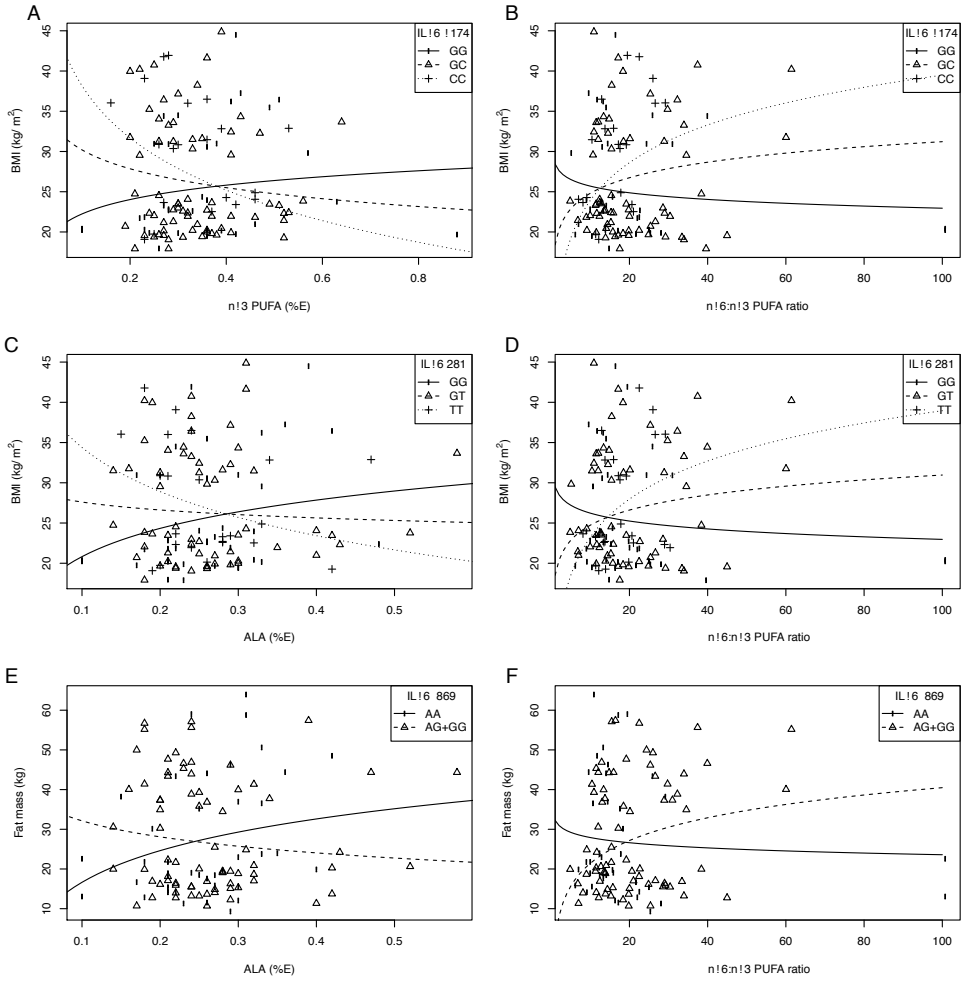

Figure 6.1. The relationship between BMI and fat mass, *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms and dietary fat intake in white women. Symbols represent, for each woman, observed values. The lines are modelled relationships for a woman of average age (27.3 y).

A. With increasing n-3 PUFA intake (%E), BMI decreased in those with the -174 CC or GC genotypes.
B. With increasing n-6:n-3 PUFA ratio, BMI increased equally with each additional -174 C allele.
C. With increasing ALA intake (%E), BMI decreased with each additional IVS3+281 T allele.
D. With increasing n-6:n-3 PUFA ratio, BMI increased with each additional IVS3+281 T allele.
E. With increasing ALA intake (%E), fat mass decreased in those with the IVS4+869 AG or GG genotype.
F. With increasing n-6:n-3 PUFA ratio, fat mass increased in those with the IVS4+869 AG or GG genotype; compared to those with the AA genotype.

Abbreviations: ALA, !-linolenic acid; BMI, body mass index; *IL-6*, Interleukin-6 gene; n-6:n-3 PUFA, omega-6:omega-3 polyunsaturated fatty acids ratio.

6.2.2.2. IVS3+281 G>T

Interactions were also observed in white women between IVS3+281 G>T and ALA intake (%E) on BMI (P=0.032), waist (P=0.038) and WHR (P=0.037), as well as between IVS3+281 G>T and n-6:n-3 PUFA ratio on BMI (P=0.030), waist (P=0.048), and body fat % (P=0.044) (Table 6SII B & 6SIV B, and summarised in Table 6.1.). More specifically, with increasing ALA intake (%E), BMI decreased with each T allele (Figure. 6.1C); while with increasing n-6:n-3 PUFA ratio, BMI increased with each additional IVS3+281 T allele (additive models) (Figure. 6.1D). In all cases, the rate of change (increase and decrease) was significant for the minor homozygotes, TT. Similar to the -174 G>C interactions described above, slopes were in the opposite direction depending on the fatty acids consumed.

In black women, we also identified interactions between IVS3+281 G>T and dietary fat intake (P=0.006), PUFA (P=0.004), SFA (P=0.010), MUFA (P=0.046), n-6 PUFA (P=0.004), LA (P=0.006), and AA (P=0.005), intake (%E) on BMI (Table 6SII B & 6SIV B, and summarised in Table 6.1). With increasing dietary fat intake, BMI decreased in those with the IVS3+281 TT genotype and increased in those with the GG or GT genotype (recessive). With increasing MUFA intake (P=0.046), BMI also decreased in those with the IVS3+281 TT genotype, but the increase in those with the GG or GT genotype was not significant. Similar effects were detected for weight (total dietary fat (P=0.012), PUFA (P=0.007), n-6 PUFA (P=0.009), LA (P=0.009) and AA (P=0.008)), waist (for total dietary fat (P=0.019) and PUFA (P=0.013)), and for fat mass (total fat (P=0.005), SFA (P=0.010), MUFA (P=0.013), PUFA (P=0.004), n-6 PUFA (P=0.005), LA (P=0.006) and AA (P=0.004)). For all these interactions, with

increasing dietary fat intake, adiposity decreased in those with the IVS3+281 TT genotype and increased in those with the GG or GT genotype.

The ethnic-specific nature of these diet-genotype interactions was identified by examining three-way interactions between ethnic group, dietary fat intake (%E) and the *IL-6* polymorphisms on body composition (Table 6S X A (genotype) and 6S XII A (allelic), as well as by including both black and white women in the same model and adjusting for ethnicity (Tables 6S VI A and 6S VII A (genotype) and Tables 6S VIII A and 6S IX A (allelic)).

Tables 6S X A (genotype) and XII A (allelic), which report the p-values for three-way interactions between ethnic group, dietary fat intake (%E) and the IVS3+281 G>T polymorphism on body composition, identified significant interactions between ethnicity and SFA (P=0.045), PUFA (P=0.024), n-6 PUFA (P=0.027), LA (P=0.029), AA (P=0.005), and n-6:n-3 PUFA ratio (P=0.023) and the IVS3+281 G>T genotypes on BMI. Interactions between ethnicity, n-6:n-3 PUFA ratio and IVS3+281 G>T genotypes (P=0.015) and alleles (P=0.049) on waist were also observed. This suggests that these interactions, reported above, were different between the black and white women. Significant diet-gene interactions identified for the IVS3+281 G>T polymorphism, but not found to be significant in the three-way interactions, were also not significant in the combined group (Tables 6S VI A and 6S VII A (genotype) and Tables 6S VIII A and 6S IX A (allelic)).

6.2.2.3. IVS4+869 A>G

In white women, we also detected interactions between IVS4+869 A>G polymorphism and ALA (%E) (P=0.048, Figure. 6.1E), n-3 PUFA (%E) (P=0.034) and n-6:n-3 PUFA ratio (P=0.034, Figure. 6.1F) on fat mass (Table 6SII C & 6SIV C, and summarised in Table 6.1.). With increasing n-3 PUFA (%E) intake, fat mass decreased in those with AG or GG genotype, while with increasing n-6:n-3 PUFA ratio, fat mass increased in those with an AG+GG genotype; compared to those with the AA genotype (Figure. 6.1F). With increasing ALA (%E) intake, fat mass increased in those with the AA genotype and decreased in those with the AG+GG genotype, however the individual rates were not significant (Figure.. 6.1E). These interactions are similar to -174 G>C and IVS3+281 G>T in the white women where opposite slopes were observed for n-3 PUFA and n-6:n-3 PUFA ratio.

In black women, we also observed a number of diet-gene interactions with the IVS4+869 A>G polymorphism on adiposity (Table 6S II C & S6 IV C, and summarised in Table 6.1), which where similar to interactions shown for the IVS3+281 G>T polymorphism. With increasing total dietary fat (P=0.009), MUFA (P=0.010), and SFA (P=0.007) intake (%E), BMI, (as well as weight, waist and fat mass) increased in those with the IVS4+869 AA+AG genotype and decreased in those with the IVS4+869 GG genotype. However, the individual rates of change were not significant. With increasing AA intake (%E), BMI (P=0.010), weight (P=0.016) and fat mass (P=0.014) increased for the AA+AG genotypes (recessive effects), but were not significant for the GG genotype.

Tables 6S X B (genotype) and XII B (allelic), which reports the p-values for three-way interactions between ethnic group, dietary fat intake (%E) and the IVS4+869 A>G polymorphism on body composition, identified significant interactions between ethnicity, ALA intake and the IVS4+869 A>G genotypes (P=0.010) and alleles (P=0.036) on fat mass. This suggests that these interactions, reported above, were different between the black and white women.

When the black and white women were combined and adjusted for age and ethnicity (Tables 6S VI B and VIII B), the interactions between total fat (%E) and SFA (%E) and the IVS4+869 A>G polymorphism on weight, BMI, waist and fat mass were significant (P=0.009, P=0.029, P=0.011 and P=0.018, for total fat intake respectively, and P=0.014, P=0.009, P=0.015 and P=0.049, for SFA intake respectively). These interactions were only significant in the black women. In the combined group an interaction between n-6:n-3 PUFA ratio and IVS4+869 A>G polymorphism on fat mass was significant (P=0.035), but this was only observed in the white women. This suggests that these interactions identified in the combined group were not different for the black and white women.

6.2.3. Diet-genotype interactions on serum lipid concentrations

Diet-genotype interactions on body composition are summarised in Table 6.2

Table 6.2. Summary of *IL-6* diet-gene interactions on serum lipids.

		Black			White		
	SNP	**TAG, *mmol/L***	**LDL-C, *mmol/L***	**T-C:HDL-C ratio**	**TAG, *mmol/L***	**HDL-C, *mmol/L***	**T-C:HDL-C ratio**
Total fat	**IVS3+281 G>T**		↓ GT	↓ GT			
	IVS4+869 A>G	↕ GG ↓ AA		↕ GG [a] ↓ AA [a]			
MUFA	**-174 G>C**				↓ C allele		
	IVS3+281 G>T		↓ GT	↓ GT			
PUFA	**IVS3+281 G>T**		↓ GT [b]	↓ GT			
n-6 PUFA	**IVS3+281 G>T**		↓ GT [b]	↓ GT			
LA	**IVS3+281 G>T**		↓ GT [b]	↓ GT			
n-3 PUFA	**-174 G>C**					↑ C allele	
EPA	**-174 G>C**				↓ C allele		↓ CC
	IVS3+281 G>T						↓ TT
	IVS4+869 A>G					↓ G allele [a]	
DHA	**-174 G>C**					↑ C allele	↓ CC
	IVS4+869 A>G					↓ G allele [a]	
ALA	**-174 G>C**					↑ C allele	
	IVS3+281 G>T					↑ T allele	↓ TT [b]
n-6:n-3 ratio	**IVS4+869 A>G**	↕ GG ↓ AA		↕ GG ↓ AA			

The interactions are described as follows: With increasing dietary fat variable, the serum lipid outcome either ↑ (increases) or ↓ (decreases) for a particular genotype or allele. All interactions are diet-genotype unless stated as being allelic. Dietary fat intake is calculated as a percentage of total energy intake. [a] These interactions were ethnic specific; [b] These interactions were not different for black and white women. Abbreviations: TAG, triglycerides; T-C, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; T-C:HDL-C ratio, total cholesterol: high density lipoprotein cholesterol ratio, SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P:S ratio, polyunsaturated fatty acid : saturated fatty acid ratio; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6 : omega-3 polyunsaturated fatty acid ratio; ALA, !-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

6.2.3.1. IL-6 -174 G>C

In white women, we detected interactions between -174 G>C polymorphism and the intake of different dietary fatty acids (%E) on T-C, TAG, HDL-C, and T-C:HDL-C ratio (Table 6S III A & 6S V A, and summarized in Table 6.2.). With increasing MUFA (P=0.015), and EPA (P=0.044) intakes, TAG decreased with each -174 C allele, and with increasing n-3 PUFA (P=0.047) and ALA (P=0.022) intakes (%E), HDL-C increased with each -174 C allele (additive effects). With increasing EPA and DHA intakes (%E) (P=0.021 and P=0.014, respectively), the T-C:HDL-C ratio decreased in those with the -174 CC compared to CG+GG genotype (recessive effect).
Diet-genotype interactions, as well as the impact of ethnicity for the -174 G>C polymorphism on serum lipids were not analysed due to the rare frequency of the C allele in the black women.

6.2.3.2. IVS3+281 G>T

In white women, with increasing n-3 PUFA (P=0.015), and ALA (P=0.029) intakes (%E), T-C:HDL-C ratio decreased only in those with the IVS3+281 TT genotype compared to TG+GG genotype (recessive), and with increasing ALA intake (%E), HDL-C increased significantly with each additional IVS3+281 T allele (P=0.018) (Table 6S III B & 6S V B, and summarized in Table 6.2.).

In black women, the interactions between IVS3+281 G>T and the intake of dietary fatty acids on serum lipids were unusual, in that with increasing dietary fat intake (%E) (Fat, MUFA, PUFA, n-6 PUFA and LA), the serum lipid levels (LDL-C and

T-C:HDL-C ratio) decreased in those with a GT genotype, but there was no effect (increasing, but no statistical significance was observed) in those who were homozygous for either allele.

The ethnic-specific nature of these diet-genotype interactions was investigated by examining three-way interactions between ethnic group, dietary fat intake (%E) and the *IL-6* polymorphisms on serum lipids (Table 6S X A (genotype) and 6S XII A (allelic)), as well as by including both black and white women in the same model and adjusting for ethnicity (Tables 6S VI A and 6S VII A (genotype) and Tables 6S VIII A and 6S IX A (allelic)).

No diet-gene interactions were identified in the 3-way interactions on serum lipids (Tables 6S XI A and XII A). When the black and white women were combined and adjusted for age and ethnicity (Tables 6S VII A and IX A), the interactions between PUFA (%E), n-6 PUFA (%E) and LA (%E) intake and IVS3+281 G>T genotypes on LDL-C were significant (P=0.021, P=0.026, and P=0.026, respectively), and between ALA intake and IVS3+281 G>T genotypes on T-C:HDL-C ratio (P=0.027). Of these, the interactions on LDL-C were only significant in the black women and the interaction on T-C:HDL-C ratio was significant in the white women. This suggests that these interactions described above, and identified in the combined group were not different for the black and white women.

6.2.3.3. IVS4+869 A>G

In white women, with increasing EPA and DHA (%E) intakes (P=0.047 and P=0.023, respectively), HDL-C decreased with each IVS4+869 G allele (additive); in those the minor homozygote GG, the decrease in HDL-C was significant (Table 6S III C & 6S V C, and summarized in Table 6.2.).

In black women, an interaction was observed between increasing total dietary fat intake (%E) and n-6:n-3 PUFA ratio, and TAG (P=0.049), and T-C:HDL-C ratio (P=0.029) respectively, with each IVS4+869 G allele. With increasing dietary fat intake, a decrease in serum lipids was noted in individuals who were homozygous AA, compared to increases in serum lipids in individuals who were homozygous GG.

Table 6S XIII B (allelic), which reports the p-values for three-way interactions between ethnic group, dietary fat intake (%E) and the IVS3+281 G>T polymorphism on serum lipids, identified significant interactions between EPA (P=0.026) and DHA (P=0.013), and the IVS4+869 A>G alleles on HDL-C reported above, suggesting that these interactions are different between the black and white women.

When the black and white women were combined and adjusted for age and ethnicity (Tables 6S VII B and IX B), only the interaction between total fat (%E) and IVS4+869 A>G polymorphism on T-C:HDL-C ratio was significant (P=0.013)(Table 6S VII B), and only in the black women, suggesting that it is different between the black and white women.

6.3. DISCUSSION

For the first time dietary fatty acids have been shown to modulate the relationship between *IL-6* polymorphisms and measures of obesity and serum lipids in both black and white SA women. In this study, the -174 G>C, IVS4+869 A>G and IVS3+281 G>T polymorphisms showed similar diet-gene interactions for their minor alleles, some of these relationships differed between the black and white women.

As previously discussed, the overall prevalence of overweight and obesity in SA women is high, with black women being more affected than white women (58.5% vs. 52.9%) [3]. Obesity in black women is associated with a higher prevalence of insulin resistance and type 2 diabetes, whereas obese white women traditionally have a higher prevalence of dyslipidaemia and associated CVD [8]. As ethnicity encompasses genetic, environmental, lifestyle (including diet) and cultural differences, and given that diet and environment contribute significantly to the pathogenesis and development of these chronic diseases, it is unlikely that genetic differences alone would underlie these ethnic differences [268]. SA has undergone a rapid diet transition from a rural diet, high in complex carbohydrates, to a more westernised urban intake, high in fat [135], with subsequent increases in the prevalence of diseases of lifestyle [8]. In contrast, polymorphism frequencies are unlikely to change in this time span. It is therefore hypothesized that individuals may take different routes in their development of obesity and comorbidities, and that the quantity and quality of dietary fat intake, (as well as other environmental and lifestyle factors), and the relative frequency of genetic variants may impact the obese phenotype.

CHAPTER SIX

In Chapter 5 it was reported that the IVS4+869 AG and GG genotypes were associated with greater waist and fat mass in black SA women and the IVS3+281 T allele was associated with lower TAG levels in white SA women. However, here it is shown for the first time that both the quality and quantity of dietary fat intake modulates this relationship. When dietary fat intake was included in the analyses, it was observed in the white women that with increasing intake of the anti-inflammatory n-3 PUFAs, measures of adiposity decreased, and with increasing n-6:n-3 PUFA ratio, adiposity increased in those with the -174 C, IVS3+281 T and IVS4+869 G minor alleles. These findings are supported by Razquin *et al.* who showed that with increasing n-3 PUFA intake, the -174 CC genotype was associated with lower measures of adiposity [185]. Further, in white women in this study, an increasing intake of n-3 PUFAs was associated with a decrease in TAG (only for -174 G>C) and T-C:HDL-C ratio, and an increase in HDL-C in those with the -174 C and IVS3+281 T alleles. These findings suggest that anti-inflammatory n-3 PUFAs have a favourable effect on adiposity and serum lipids, more so in individuals with the -174 C, IVS3+281 T and IVS4+869 G minor alleles.

In contrast, in the black women it appears that an increasing intake of dietary fat, rather than the quality of individual fatty acids, was associated with increasing measures of adiposity in those with the IVS3+281 GT+GG and IVS4+869 AG+AA genotypes, and decreasing measures of adiposity in those with the IVS3+281 TT and IVS4+869 GG genotypes. Further, with increasing total fat intake and n-6:n-3 PUFA ratio, TAG and T-C:HDL-C ratio increased with each IVS4+869 G allele. Similarly, in Chapters 3 and 4 of this thesis, it was observed that in black women,

total dietary fat intake was associated with greater measures of obesity for the *TNFA* -308 and -238 A alleles irrespective of the quality of the dietary fat.

Studies have shown that dietary fatty acids modulate IL-6 production, thereby influencing inflammatory status [40]. A number of studies have investigated the effect of different dietary fatty acids on IL-6 levels in different models, with human studies showing similar results to cell culture and rodent models [40]. He *et al.* showed that increased n-3 PUFA intake and fish consumption were associated with decreased plasma concentrations of IL-6 and other inflammatory markers [202]. Further, Ferrucci *et al.* reported that plasma levels of PUFAs, especially n-3 PUFAs, were independently associated with lower levels of pro-inflammatory markers and higher levels of anti-inflammatory markers [203]. Rats fed a diet high in n-6 PUFA-rich sunflower oil showed a moderate IL-6 release from adipocytes, and lower plasma IL-6 than when fed a SFA-rich diet, but greater than when fed a MUFA-rich diet [200]. Only one controlled feeding trial investigated the effect of a SFA and MUFA–rich diet on serum lipid concentrations and whole-genome microarray gene expression profiles of adipose tissue [204]. The eight week consumption of a SFA-enriched diet resulted in increased expression of genes involved in inflammatory processes in adipose tissue, including IL-6 and NF-!B signalling, whereas the MUFA enriched diet led to a more anti-inflammatory gene expression profile, accompanied by a decrease in serum LDL-C concentration [204].

These findings support this study hypothesis that dietary fatty acids may modulate the relationship between *IL-6* polymorphisms, obesity and dyslipidaemia. For the *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms reported here it

appears that the minor alleles of these polymorphisms may be more responsive to the quantity and quality of the dietary fat being consumed, compared to the major allele. Similar results were observed for the *TNFA* polymorphisms in Chapters 3 and 4, where the minor A allele was more responsive to changes in dietary fat intake. Notably, in this study the individual diet-gene interactions differed between the two ethnic groups. The reasons for this are not known, but may relate to differences in the frequency of the polymorphisms and/or differences in dietary intake, specifically dietary fatty acid intake, between black and white women included in this study.

The minor allele frequencies of the -174 G>C and IVS3+281 G>T polymorphisms were higher in the white compared to the black women, but all frequencies were similar to those from European and other African populations. In this study the diet-gene interactions in the white women, for both body composition and serum lipids, were observed only with regards the n-3 PUFA's and the n-6:n-3 PUFA ratio, whereas in the black women, the diet-gene interactions were observed for total fat intake, SFA, MUFA and PUFA, and for all the n-6 fatty acids. The intake of n-6 PUFAs (%E) and the n-6:n-3 PUFA ratio in the black women is alarmingly high, and higher than that of the white women (8.3% vs. 5.6%, and 26.4:1 vs. 16.1:1, for black and white women respectively), and that reported in other populations, 4.0 – 6.0% for n-6 PUFA (%E) [254], and !15:1 for the n-6:n-3 PUFA ratio [259].

While the functional nature of the -174 G>C polymorphism has been investigated, little is known of the functional nature of the two additional intronic polymorphisms included in this study, especially their impact on markers of inflammation. Unfortunately, circulating IL-6 levels were not measured in this study. Furthermore

other polymorphisms such as those found on the fatty acid desaturase 1 (*FADS1*) and *FADS2* genes may alter PUFA metabolism and these gene-gene interactions may need to be considered [141]. Further limitations to this study should be considered, as described in the previous chapters. In brief, our sample size was small and included fewer white than black women. It is always difficult to report accurate dietary intake. To improve the validity of the dietary intake data we used a validated FFQ developed specifically for the SA population, and included only adequate reporters in the analyses. In addition, no lifestyle factors such as physical activity and socioeconomic factors were included, that may also impact the obese phenotype.

In conclusion, this novel study showed that dietary fat intake, and the quality of the dietary fatty acids consumed, modulated the relationship between the *IL-6* -174 G>C, IVS3+281 G>T and IVS4+869 A>G polymorphisms, on measures of obesity and serum lipids, with different effects in black and white women. Importantly, two polymorphisms that are informative as markers of gene-diet interactions in both black and white SA women were identified. This final chapter further highlights the importance of understanding the inflammatory impact of different dietary fatty acids and how they may differentially interact with genes in different ethnic populations.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

CHAPTER SEVEN

The first SADHS published in 2002, highlighted the high overall prevalence of overweight and obesity in SA, with more than 29% of men and 56% of women classified as overweight or obese [3]. Black SA women had the highest prevalence of overweight and obesity (58.5%), higher than white SA women (52.9%)[3]. In addition, urban women were found to have a higher BMI than their rural counterparts. Although the prevalence of obesity is high in both black and white SA women, the prevalence of co-morbidities associated with obesity in these populations differ [8]. Insulin resistance and diabetes are more prevalent in black SA women, while the prevalence of dyslipidaemia and CAD is greater in white SA women [8].

On account of the high prevalence of overweight and obesity in women compared to men in SA, this thesis focused only on women, [3, 7]. Of the SA populations, only black and white women were compared, due to the disparities in obesity risk, serum lipid profiles and CAD prevalence. In line with previous studies [7], the white women in the study group had greater weight and central adiposity (waist, WHR, and VAT) than the black women, while the black women had higher BMI, body fat %, and SAT than the white women. The study sampling procedures also resulted in the white women being older than the black women; as a result all analyses were adjusted for age. Attempts to recruit older normal-weight black women were unsuccessful; likely reflective of the demographic of this population. Based on the SADHS, the mean BMI of SA women in the 15 to 24 year and 25 to 34 year old groups were 23.4 and 26.8 kg/m^2, respectively, compared to the 35-44 year old women who had a mean BMI of 28.9 kg/m^2 [3].

CAD is an important co-morbidity associated with obesity, and one that has shown differences in prevalence by ethnicity. In 2000, of the total age-standardised death rates in South Africans, CAD contributed 23% for black men and women compared to 41% for white men and women [8, 13], and the cholesterol-attributable mortality estimates were significantly lower in black than white SA women [20]. Black SA women, similar to African Americans have been shown to have a less atherogenic lipid profile than white women [21-23], potentially attributable to relatively low levels of VAT [21]. This was also observed in this thesis sample group. All serum lipid concentrations, except the T-C:HDL-C ratio were higher in the white compared to the black women. Studies in SA women have generally reported no ethnic difference in HDL-C levels [22], whereas African Americans have been shown to have higher HDL-C levels than their white counterparts [23]. In this study, similar to that reported by Goedecke *et al.,* HDL-C was lower in black women compared to white women [19, 24]. In addressing the healthcare burden of chronic diseases of lifestyle in SA, It is therefore important to identify and understand factors contributing to these differences, enabling the development of effective interventions.

There is growing scientific evidence establishing obesity as a condition of chronic low-grade inflammation [191]. Adipocyte hypertrophy results in increased chemokine secretion, and a subsequent increase in macrophage infiltration, which in turn secretes cytokines such as IL-6 and TNF ! [33]. Low-grade chronic inflammation also mediates all stages of atherosclerosis. LDL-C, VLDL-C and intermediate-density molecules in the intima undergo oxidative modification, inducing expression of pro-inflammatory cytokines, chemokines and other mediators of inflammation

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Ethnic differences in the inflammatory phenotype may be due, in part, to differences in the distribution of DNA sequence variants (genotype frequencies) and gene expression in genes such as *TNFA* and *IL-6* [97, 98]. Polymorphisms in these inflammatory genes have been shown to impact molecular processes of the inflammatory pathways, serum lipids and the obese phenotype. Specifically, several studies have reported associations between polymorphisms in the cytokine genes *TNFA* and *IL-6,* and obesity and dyslipidaemia [40, 187]. However, there are no studies that have examined these associations in Southern African populations.

Accordingly, the aim of this thesis was to examine associations between *TNFA* and *IL-6* polymorphisms (*TNFA* -308 G>A and -238 G>A & *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G) and measures of adiposity and serum lipid concentrations in urbanised black and white SA women, to examine the effects of dietary fatty acid intake on these relationships, and to determine if these relationships were ethnic specific. Summaries of the main findings of this thesis are presented in Tables 7.1. and 7.2.

Association studies between the *TNFA* -308 G>A and -238 G>A polymorphisms with obesity and serum lipid concentrations are described in Chapters 3 and 4, respectively. No independent associations between the *TNFA* -308 G>A polymorphism and obesity and serum lipid concentrations were observed in either the black or white women. However, this is the first study to show that black SA women with the *TNFA* -238 GA genotype had a greater body fat percentage than those with the GG genotype.

Within the *IL-6* gene, the most frequently studied polymorphism is *IL-6* -174 G>C (rs1800795). However, it has been shown to be rare in persons of African descent, and as expected the -174 C allele was rare in the black women in this study, representing only 1% of the normal-weight and 3% of the obese black women. Two *IL-6* polymorphisms, IVS3+281 G>T (rs1554606) in intron 3, and IVS4+869 A>G (rs2069845) in intron 4 (Chapter 2) were identified. Both polymorphisms showed an adequate heterozygosity in both the white and black SA populations in this study. The IVS3+281 T allele was present in 27% of the black women (both normal-weight and obese), and approximately 42% for the normal-weight and obese white women. For the IVS4+869 A>G polymorphism, the G allele was present in approximately 30% of the normal-weight and obese, black and white women.

Chapter 5 describes the genotype-phenotype associations investigated between *IL-6* 174 G>C, IVS3+281 G>T, and IVS4+869 A>G polymorphisms and obesity and serum lipid concentrations (Table 7.1. and 7.2.). It was observed that the IVS4+869 G allele was associated with higher adiposity in the black women This association was different for the black and white women. In the white women, those with the T allele of the IVS3+281 G>T polymorphism had lower TAG concentrations than those with the IVS3+281 GG genotype. This association was not different for the black and white women. These findings are novel in that no other studies have reported on associations between the IVS4+869 A>G and IVS3+281 G>A polymorphisms and obesity and serum lipids.

Overall, this thesis reported genotype associations with obesity in the black women, and associations with serum lipids in the white women. One of the difficulties in

interpreting these associations and understanding the impact of these polymorphisms on inflammation, the obese phenotype and serum lipids is the necessity of understanding the impact of these polymorphisms on *TNFA* and *IL-6* gene transcription, and subsequent TNF ! and IL-6 plasma levels. There is controversy regarding the functional significance of the *TNFA* -238 polymorphism, and an absence of functional studies on the IVS4+869 A>G and IVS3+281 G>A polymorphisms. Studies have reported that the -238 A allele either increases [249], decreases [250, 251] or has no effect on *TNFA* transcription [252, 253], depending on the conditions of the experiment. Although the functional nature of the two intronic polymorphisms has not yet been determined, they are in strong LD in both black and white SA women, identifying a region of the *IL-6* gene that may contribute to obesity and dyslipidaemia, which requires further investigation. Unfortunately, in this thesis, circulating levels of IL-6, TNF ! or TNF-R, were not measured, which could have provided insight into the inflammatory phenotype of the black and white women.

Dietary fatty acids also regulate cytokine gene expression and secretion, thereby impacting inflammation [69, 160, 162, 200, 205]. *TNFA* and *IL-6* polymorphisms have been shown to interact with dietary fatty acids, modulating these relationships (Chapter 1) [40, 187]. Little dietary intake data of SA populations has been published since the early 1990's. Data from the early BRISK (black urban) and VIGHOR (white urban) SA dietary studies [139, 140] found that black and white urban populations consumed similar amounts of PUFA, however total fat and saturated fat intake was higher in white urban compared to black urban diets [139, 140]. In contrast to these studies, it was reported in this thesis that black women consumed more total fat and PUFA, but less SFA than white women (Chapter 3). The continued impact of the

nutrition transition may have altered these intakes over the past 20 years [135, 136]. Unfortunately, no previous studies have described the intake of individual dietary fatty acids in SA populations, making a comparison with these dietary intake results difficult. Nonetheless, these results reports that black SA women consumed more n-6 PUFA, EPA and DHA (%E), as well as had a higher n-6:n-3 PUFA ratio than the white women (Chapter 3). Notably, the n-6:n-3 PUFA ratio in the black women was alarmingly high, higher than that of the white women, and that reported in other populations [254, 259], and far exceeding the WHO recommendation of a n-6:n-3 PUFA ratio of 5–10:1 [243].

In addition to the established associations between dietary fat, obesity, serum lipids and CVD [72, 79, 254, 255, 269, 270], dietary fatty acids interact with genetic variants to regulate the development and progression of obesity and serum lipids. These complex nutrigenetic interactions may partly explain differences observed in the obese phenotype and serum lipid concentrations that vary both within and across populations [40, 96, 187].

When dietary intake was included in the study analyses, interactions between dietary fat intake (%E) and the *TNFA* -308 G>A polymorphism on adiposity and serum lipid concentrations in both black and white women were observed (Chapter 3). In the black women, the obesity OR of those with the -308 A allele compared to the GG genotype, was higher with increasing fat intake (%E). Furthermore, with increasing dietary fat intake, irrespective of the type of fat, weight and adiposity was higher in black women with the -238 GA genotype and decreased or did not change in those with the GG genotype (Chapter 4).

With regards diet - *IL-6* gene interactions, only two studies have previously reported on the relationship between the *IL-6* -174 G>C polymorphism and dietary intake [184, 185], and none including the IVS3+281 G>T and IVS4+869 A>G polymorphisms. As described in Chapter 6 and detailed in Tables 7.1. and 7.2., for the first time, it is shown that both the amount and type of dietary fatty acid intake modulated the relationship between *IL-6* -174 G>C, IVS3+281 G>T, and IVS4+869 A>G polymorphisms and obesity and serum lipid profiles.

In the white women, with increasing intake of n-3 PUFAs, measures of adiposity decreased, and with increasing n-6:n-3 PUFA ratio, adiposity increased in those with the -174 C, IVS3+281 T and IVS4+869 G minor alleles (Chapter 6). In contrast, in the black women it appeared that an increasing intake of total dietary fat, rather than individual dietary fatty acids, was associated with increasing measures of adiposity in those with the IVS3+281 GT+GG and IVS4+869 AG+AA genotypes, and decreasing measures of adiposity in those with the IVS3+281 TT and IVS4+869 GG genotypes.

When analysing diet-genotype interactions on serum lipid concentrations; with increasing ALA intake (%E), the T-C:HDL-C ratio decreased in black women with the *TNFA* -308 A allele. In contrast, increasing PUFA intake, comprising mostly the n-6 PUFA LA, was associated with increasing LDL-C concentrations only in black women with the –308 A allele. In the white women, with increasing SFA intake (%E), T-C concentrations increased for the –308 GA + AA genotype and decreased for the GG genotype. These interactions are described in detail in Table 7.2.

Despite many studies examining the -308 G>A polymorphism, only the studies by Fontaine-Bisson *et al*. investigated interactions between dietary fat intake and the -308 G>A and -238 G>A polymorphisms on serum lipids in an ethno-racially diverse Canadian population (Chapter 1) [182, 183]. Interactions between the *TNFA* -238 G>A polymorphism and dietary fatty acids on obesity and serum lipid concentrations are described in Chapter 4. The relationship between the -238 G>A polymorphism and serum lipid concentrations differed depending on the individual PUFAs consumed. As expected and similar to that observed for the *TNFA* -308 G>A polymorphism, the n-3 PUFAs had a favourable effect, and the n-6:n-3 PUFA ratio an unfavourable effect on serum lipid concentrations, but different diet-gene interactions were observed in the black and white women (Table 7.2.).

Similar to the findings for the *TNFA* gene polymorphism, increasing intake of n-3 PUFAs was associated with a decrease in TAG (only for *IL-6* -174 G>C) and T-C:HDL-C ratio, and an increase in HDL-C in those with the *IL-6* -174 C and IVS3+281 T alleles. Further, in black women, with increasing total fat intake and n-6:n-3 PUFA ratio, TAG and T-C:HDL-C ratio increased with each *IL-6* IVS4+869 G allele.

Only some of the diet-gene interactions described above for *TNFA* and *IL-6* were different for the black and white women. However, a number of factors were common to all these interactions. In brief, in both black and white women, dietary fatty acids interacted in different ways; a high total fat, SFA and n-6:n-3 PUFA ratio produced an unfavourable effect, and the n-3 PUFAs produced a favourable effect on obesity measures and serum lipids concentrations. Furthermore, for almost all the

interactions the minor allele of all polymorphisms showed the greatest responsiveness. Specifically, these thesis results reported that increasing intake of total fat, SFA and the n-6:n-3 PUFA ratio were associated with higher BMI, adiposity, TAG, T-C, LDL-C and T-C:HDL ratio, and lower HDL-C, in those with the *TNFA* and *IL-6* polymorphism minor alleles. In contrast, an increase in total n-3 PUFAs, as well as the individual n-3 PUFAs; EPA, DHA and ALA, were associated with a decrease in BMI, adiposity and TAG, T-C, LDL-C and T-C:HDL ratio in those with the *IL-6* polymorphisms minor allele. This is not completely unexpected as a higher intake of n-3 PUFAs has been associated with a reduction in inflammation and improved obesity and serum lipid outcomes [41, 81]. In contrast, a higher intake of total fat and a greater n-6:n-3 PUFA ratio has been associated with increased inflammation and increased measures of obesity and serum lipid concentrations [41, 81, 84, 260, 271].

The nutrigenomics literature suggests that either the wild-type or variant allele will exhibit a greater responsiveness to changes in dietary intake. The findings in this thesis suggest that both the *TNFA* -308 and -238 minor A alleles produce a greater response to changes in dietary fat intake than the G allele. Similarly, the minor alleles of all three *IL*-6 polymorphisms (-174 G>C, IVS3+281 G>T, and IVS4+869 A>G), appear to show consistently, greater responsiveness to changes in dietary fatty acid intake than the wild-type alleles. While, the responsive allele was the same for black and white women, some of the associations and interactions observed in this thesis were different between the two populations.

Table 7.1. Summary of the results of this thesis, investigating associations between *TNFA* polymorphisms and obesity and serum lipids, and diet-gene interactions between dietary fat intake and *TNFA* polymorphisms on obesity and serum lipids.

TNFA	Main findings	Chapter
Obesity		
TNFA -308 G>A	• Increasing dietary fat intake (%E) was associated with an increase in obesity risk in -308 GA+AA genotype compared with the GG genotype.	Chapter 3
TNFA -238 G>A	• Black women with the -238 A allele had a greater body fat % than those with the GG genotype. • In black women with increasing total fat and SFA intake (%E), adiposity increased for the -238 GA genotype, but not the GG genotype, and • In black women, increasing MUFA (%E), weight increased for the -238 GA genotype, but not the GG genotype.	Chapter 4
	Serum lipids	
TNFA -308 G>A	• With increasing ALA intake (%E) the T-C:HDL-C ratio increased for the -308 GG genotype and decreased for the GA+AA genotype. • With increasing SFA intake (%E), serum T-C decreased for the -308 GG genotype and increased for the GA+AA genotypes.	Chapter 3
TNFA -238 G>A	• In black women, with increasing P:S ratio and n-6:n-3 PUFA ratio, HDL-C decreased, and T-C:HDL-C ratio increased in those with the -238 GA genotype but not the GG genotype. • In black women, with increasing n-3 PUFA intake T-C:HDL-C ratio decreased in those with the -238 GA genotype, but not in those with the GG genotype. • In white SA women, with increasing EPA (%E) intake, LDL-C decreased in the -238 GG genotype but not the GA genotype.	Chapter 4

Abbreviations: ALA, !-linolenic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; *TNFA*, tumour necrosis factor alpha; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 7.2. Summary of the results of this thesis, investigating associations between *IL-6* polymorphisms and obesity and serum lipids, and diet-gene interactions between dietary fat intake and *IL-6* polymorphisms on obesity and serum lipids.

IL-6	**Main findings**	**Chapter**
Obesity		
IL-6 -174 G>C	• In white women, with increasing n-3 PUFA, EPA and DHA intake (%E), BMI decreased in those with the -174 CC+GC genotypes. • In white women with increasing n-6:n-3 PUFA ratio, BMI increased equally with each additional -174 C allele.	Chapter 6
IL-6 IVS3 +281 G>T	• In white women, with increasing ALA intake (%E), BMI decreased with each T allele; while with increasing n-6:n-3 PUFA ratio, BMI increased with each additional IVS3+281 T allele. • In black women, with increasing dietary fat intake, BMI decreased in those with the IVS3+281 TT genotype and increased in those with the GG+GT genotype. • In black women with increasing MUFA, BMI also decreased in those with the IVS3+281 TT genotype, but the increase in those with the GG+GT genotype was not significant.	Chapter 6
IL-6 IVS4 +869 G>A	• The IVS4+869 G allele was also associated with greater waist and fat mass in black women. • In white women, with increasing ALA (%E) intake, fat mass increased in those with the IVS4+869 AA genotype and decreased in those with the AG+GG genotype. • Also in white women, with increasing n-3 PUFA (%E) intake, fat mass decreased in those with IVS4+869 AG+GG genotype, while with increasing n-6:n-3 PUFA ratio, fat mass increased in those with the AG+GG genotype; compared to those with the AA genotype. • In black women, with increasing total fat, MUFA, and SFA, intake (%E), BMI, (as well as weight, waist and fat mass) increased in those with the IVS4+869 AA+AG genotype and decreased in those with the GG genotype.	Chapter 5 Chapter 6

Abbreviations: ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; MUFA, mono-unsaturated fat; percentage energy; %E; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 7.2. continued

IL-6	Main findings	Chapter
Serum lipids		
IL-6 -174 G>C	• In white women, with increasing MUFA, and EPA intakes, TAG decreased with each -174 C allele, and with increasing n-3 PUFA intake (%E), HDL-C increased with each -174 C allele. With increasing EPA and DHA intake (%E), the T-C:HDL-C ratio decreased in those with the -174 CC compared to CG+GG genotype.	Chapter 6
IL-6 IVS3 +281 G>T	• The IVS3+281 T allele had lower TAG concentrations than the GG genotype in white women. • In white women, with increasing n-3 PUFA intake (%E), T-C:HDL-C ratio decreased only in those with the IVS3+281 TT genotype compared to TG+GG genotype, and with increasing ALA intake (%E), HDL-C increased significantly with each additional IVS3+281 T allele.	Chapter 5 Chapter 6
IL-6 IVS4 +869 G>A	• In white women, with increasing EPA and DHA intake (%E) HDL-C decreased with each IVS4+869 G allele; in those the minor homozygote GG, the decrease in HDL-C was significant. • In black women, with increasing total fat intake (%E) and n-6:n-3 PUFA ratio, TAG (P=0.049) increased with each IVS4+869 G allele, this was also seen for T-C:HDL-C ratio (P=0.029). For both scenarios, with increasing dietary fat intake, the serum lipid decreases in the major homozygotes, AA, and increases in minor homozygotes, GG.	Chapter 6

Abbreviations: ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; MUFA, mono-unsaturated fat; percentage energy; %E; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

CHAPTER SEVEN

There is little doubt that ethnicity is a significant confounder when investigating obesity and serum lipids. As discussed in Chapter 1, ethnicity encompasses genetic, environmental, diet and lifestyle, and cultural differences, highlighting the importance of including these variables in all studies. This thesis showed differences between the black and white women for body composition, serum lipid profiles, dietary fat intake, genotype frequency, and certain genotype-phenotype associations and diet-genotype interactions. These differences emphasise the role these factors play in the development of obesity and dyslipidaemia, and potentially in the differences observed in the prevalence of obesity-associated co-morbidities in black and white SA populations.

This thesis also draws attention to the importance of including individual dietary fatty acid data in diet and diet-gene studies. By analysing only total fat intake or intake of the dietary fat sub-groups such as SFA, MUFA and PUFA; diet-gene interactions contributing to the development of obesity and dyslipidaemia may not have been identified. The thesis findings reiterate how different dietary fatty acids differentially impact phenotype. The diet-gene interactions identified here show that dietary fatty acids, especially PUFAs, differentially impact obesity and serum lipid profiles, but that this is not true for all individuals. Rather, individuals carrying certain genotypes will show a greater or lesser responsiveness to the beneficial or harmful effects of different dietary fatty acids, potentially impacting dietary requirements and recommendations.

It is important to note that there are limitations to the studies in this thesis. The

subjects were not randomly sampled, but were recruited through local newspapers, church groups and universities. Therefore the data may not be representative of the black and white SA population. The sample size was small, and included no men. As discussed, only women were included because of their high prevalence of overweight and obesity [3, 7], and only black and white women were compared, due to the disparities in their obesity risk, serum lipid profiles and prevalence of CAD [8]. There were also fewer white than black women, which means that associations in the white women may not have been detected, which were detected in the black women.

Accuracy of diet reporting is always difficult. In order to improve the validity of the dietary data in this study, a validated food frequency questionnaire, developed specifically for the SA population was used, and only adequate reporters were included in the analysis [215]. For future studies, the measurement of serum or red cell fatty acid composition will be objective and informative. Other lifestyle factors, such as physical activity and socioeconomic factors may also impact obesity and serum lipids, but were not included in this study.

Multiple genes, each with modest effects, may underlie the obese phenotype and serum lipid profiles [96, 244, 245]. This thesis included only two of the inflammatory cytokine genes and only five polymorphisms, however many other adipokines, chemokines and cytokines have been associated with obesity and serum lipids (e.g. adiponectin, IL-1, IL-10, and MCP-1), and are sensitive to dietary fatty acid intake and should be studied further [187]. These results

should also be confirmed in larger cohorts, including additional polymorphisms within the *TNFA* and *IL-6* gene, as well as polymorphisms in other genes involved in inflammation. Furthermore other polymorphisms such as those found on the *FADS1* and *FADS2* genes may alter PUFA metabolism and these interactions may need to be considered [141].

In addition to the genes and polymorphisms chosen, it is also important to consider the study designs selected for this thesis. The first results from a GWAS were reported in 2005 [272] and 2006 [273], however the 2007 Wellcome Trust Case Control Consortium (WTCCC) paper published in Nature is regarded as a starting point and the first successful GWAS [272, 274]. Since then, GWAS have become the method of choice to identify common polymorphisms with small effects that are associated with complex phenotypes such as obesity, and diseases such as CVD. The advantage of this technique is that it simultaneously analyses the association of a few 100,000 to several millions polymorphisms with a well-defined trait without a previous hypotheses about potential mechanisms. The GWAS approach has yielded many successful discoveries [274], identifying at least 50 loci associated with BMI, WHR, body fat percentage and extreme obesity [272], as well as genetic variants associated with variation in serum lipid concentrations [275]. However, a major limitation of GWAS in the context of this thesis is that they have been conducted primarily in white European populations, with only one study including a population from the African continent and none from Southern Africa. A study of 1,931 African Nigerians and African Americans with a follow-up sample of 3,700 did not identify any new significant genome-wide associations

with anthropometric measures [276]. However, they did show variants near *DLEU7* (deleted in lymphocytic leukemia) for height and *MC4R* (Melanocortin 4 Receptor) for BMI, previously confirmed in European populations. The authors suggest that sample sizes comparable to European GWAS are required to identify replicable associations, with additional studies in African-ancestry populations to improve power to detect novel associations [276, 277].

Notably, GWAS have also not to date been used to address gene-diet and gene-environment interactions, which are essential to our understanding of how diet and environment may modulate genotype-phenotype associations identified. Many important polymorphisms that interact with diet could potentially be missed using the GWAS approach since the role of diet and other factors in the development of obesity and dyslipidaemia are not taken into account in GWAS.

There is therefore still a place for the use of the more traditional candidate gene association studies in nutrigenetic research and in clarifying the association of gene variants identified using GWAS. In this approach, candidate genes and variants are selected if there is good evidence that; i) the candidate gene is biologically relevant to the phenotype, ii) the genetic variant influences the function of the gene, and iii) that the polymorphisms of the selected genes are frequent enough in the population to allow meaningful statistical analysis [278]. These studies have however been implemented with small subject numbers and are susceptible to false-positives, therefore the replication of these studies is of the utmost importance.

In this thesis, although the *TNFA* and *IL-6* genes have not been identified in GWAS [275, 279], they were selected for this thesis as they fulfilled the criteria for a hypothesis-driven candidate gene study. Their selection was based on established associations between *TNFA* and *IL-6*, inflammation, obesity and serum lipid concentrations, and the impact of different dietary fatty acids on *TNFA* and *IL-6* expression and serum levels. In addition, two of the five polymorphisms studies have been shown to influence the function of the gene, and except for the *IL-6* -174 G>C polymorphism in the black women, the other polymorphisms had adequate genotype and allele frequencies in the black and white SA populations included in this thesis, ethnic populations that have not previously been studied.

Limitations of the study also include the fact that there was no adjustment for multiple testing. Nevertheless, it has been suggested that the Bonferroni correction is too conservative when several associations are tested in the same group of individuals [226], and might not be appropriate in a situation such as this, where there is prior evidence that such effects exist [227]. Based on prior evidence the studies in this thesis tested specific hypotheses. These results, like the results of all genetic association studies, should be treated with caution until independently replicated.

Based on the findings in this thesis, it is recommended that future research investigating obesity and CAD should include detailed diet and genotype data, different ethnic populations, and both male and female subjects. Particular consideration should be given to the inclusion of polymorphisms and genes,

considering SNP-SNP and gene-gene interactions. In addition to diet-polymorphism interactions, it is also important to understand the combined effect of a number of polymorphisms on the same or different genes interacting with the environment. For this reason, the identification and analysis of haplotype-diet interactions may provide additional insights in future research.

It is also important that future research unravel the molecular mechanisms that govern the endogenous metabolism of serum lipids, which has also been shown to differ between black and white women, and the impact of dietary fatty acids on inflammation [19]. These mechanisms remain unclear, and appear to act via multiple pathways. It is known that different groups of dietary fatty acids (SFA, MUFA, and n-3, n-6 & n-9 PUFA) differ in their effect on inflammatory gene expression and plasma levels. The nutrigenetic interactions described in this thesis are complex and it is difficult to assess the magnitude of their impact in managing an individual or a populations' diet. Rather than the cross-sectional study design employed in this thesis, randomised controlled trials with dietary interventions are required to measure whether nutrigenetic-driven dietary recommendations alter phenotypic outcomes. Furthermore, the inter-ethnic variability reported here should caution us with regards generalised dietary recommendations, as it cannot be assumed that dietary fatty acids and other nutrient metabolism is uniform for all populations.

The future study of nutrigenomics offers the opportunity to clarify the underlying molecular mechanisms governing the interactions between dietary fatty acids and the inflammatory and obese phenotype, potentially elucidating the observed

CHAPTER EIGHT

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CHAPTER NINE

SUPPLEMENTARY DATA

CHAPTER NINE

9.1. Characteristics and dietary intake of under-, adequate and over-reporters.

Table 2S I. describes subject characteristics, body composition, serum lipid concentrations and dietary intake of under, adequate and over-reporters. These variables are described separately for black (Table 2S III) and white (Table 2S III) women.

Under-reporters were older than adequate reporters, and over-reporters younger than adequate reporters. Weight, BMI, waist, WHR and fat mass were higher in the under-reporter group compared to the adequate and over-reporter groups. There was a significant group*ethnicity interaction for body fat percentage, waist and fat mass, suggesting that the body fat measures of the dietary reporting groups were different between the black and white women. The example of body fat % is shown in the boxplots below, illustrating the differences (Figure 2S 1.). After adjusting for age; in white women; body fat percentage increased from over to under-reporters (Figure 2S 1.). In black women, adequate reporters had lower adiposity than under-reporters, with over- reporters in-between (Figure 2S 1.). It was also found that T-C and LDL-C concentrations were higher in the under-reporters and adequate reporters compared to the over-reporters.

All measures of dietary fat intake differed between reporter groups, except for total n-3 PUFA, and ALA and DHA intake (%E). Over-reporters consumed more dietary fat than under- and adequate reporters (Table 2S I.).

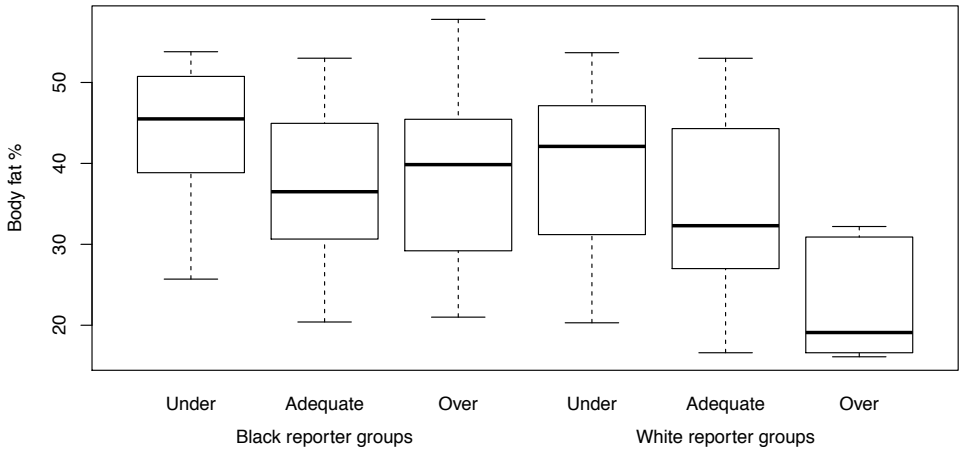

Figure 2S 1. Group*ethnicity interaction; in white women; over-reporters have lower body fat % than under-reporters, with adequate reporters in the middle. In black women, adequate reporters have lower body fat % than under-reporters, with over- reporters in-between.

Table 2S I. Subject characteristics, body composition, serum lipids and dietary intake of under, adequate and over reporters.

	Under-reporters	Adequate reporters	Over-reporters	P values		
				Reporter group	Ethnicity	Group* Ethnicity
N	42	268	73			
Age, *years*	35 ± 8	29 ± 8	26 ± 7	< 0.001	< 0.001	0.069
Body composition						
Height, *m*	1.65 ± 0.08	1.63 ± 0.07	1.61 ± 0.06	0.020	< 0.001	0.930
Weight, *kg*	89 ± 21	75 ± 20	76 ± 22	0.011	0.062	0.050
BMI, kg/m^2	33 ± 8	28 ± 8	29 ± 8	0.017	< 0.001	0.054
Body fat, *%*	40 ± 10	36 ± 9	37 ± 10	0.090	< 0.001	0.002
Fat Mass, *kg*	37 ± 15	28 ± 14	29 ± 15	0.021	0.005	0.013
Waist, *cm*	101 ± 17	89 ± 17	90 ± 19	0.010	0.019	0.041
WHR	0.84 ± 0.07	0.80 ± 0.08	0.80 ± 0.09	0.066	0.467	0.312
VAT, cm^2	114 ± 59	86 ± 54	73 ± 35	0.488	0.008	0.296
SAT, cm^2	447 ± 223	353 ± 215	377 ± 236	0.117	< 0.001	0.057
Serum lipids						
TAG, *mmol/L*	0.80 (0.50-1.10)	0.70 (0.60-1.10)	0.60 (0.50-0.85)	0.321	< 0.001	0.134
T-C, *mmol/L*	4.20 (3.90-5.10)	4.30 (3.70-4.90)	3.90 (3.35-4.30)	0.005	< 0.001	0.475
HDL-C, *mmol/L*	1.50 (1.20-1.80)	1.50 (1.10-1.70)	1.40 (1.15-1.80)	0.854	< 0.001	0.849
LDL-C, *mmol/L*	2.45 (2.10-2.88)	2.40 (1.80-2.90)	2.00 (1.60-2.50)	0.016	0.007	0.781
TC;HDL-C ratio	3.00 (2.46-3.79)	2.91 (2.44-3.73)	2.69 (2.28-3.21)	0.129	0.012	0.678

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 2S I. continued

Dietary intake	Under-reporters	Adequate reporters	Over-reporters	P values Reporter group	Ethnicity	Group* Ethnicity
Fat (%E)	28 (25-32)	32 (28-36)	36 (32-39)	< 0.001	< 0.001	0.657
SFA (%E)	9.0 (7.5-10.4)	9.6 (8.4-11.7)	9.7 (8.6-11.0)	0.020	< 0.001	0.506
MUFA (%E)	9.4 (8.2-11.5)	10.6 (8.9-12.3)	11.6 (10.2-13.4)	< 0.001	0.519	0.463
PUFA (%E)	6.2 (4.6-7.5)	7.7 (5.9-9.5)	9.6 (7.7-11.1)	< 0.001	< 0.001	0.823
P:S ratio	0.69 (0.51-1.05)	0.78 (0.55-1.03)	1.00 (0.80-1.19)	< 0.001	< 0.001	0.810
(n-3) PUFA (%E)	0.32 (0.27-0.41)	0.31 (0.26-0.41)	0.32 (0.23-0.43)	0.942	0.963	0.415
(n-6) PUFA (%E)	5.8 (4.2-7.1)	7.2 (5.4-9.2)	9.3 (7.4-11.0)	< 0.001	< 0.001	0.914
(n-6):(n-3) PUFA ratio	17.2 (12.2-26.0)	22.0 (14.7-30.6)	26.3 (18.8-38.8)	0.002	< 0.001	0.553
ALA (%E)	0.25 (0.19-0.28)	0.23 (0.20-0.27)	0.21 (0.18-0.27)	0.221	< 0.001	0.452
LA (%E)	5.8 (4.2-7.1)	7.3 (5.4-9.2)	9.4 (7.6-11.3)	< 0.001	< 0.001	0.913
AA (%E)	0.04 (0.03-0.05)	0.04 (0.02-0.06)	0.05 (0.03-0.07)	0.024	< 0.001	0.055
EPA (%E)	0.02 (0.01-0.03)	0.02 (0.01-0.04)	0.03 (0.01-0.05)	0.030	< 0.001	0.148
DHA (%E)	0.05 (0.04-0.07)	0.05 (0.03-0.09)	0.05 (0.03-0.10)	0.324	0.001	0.096

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 2S II. Subject characteristics, body composition, serum lipids and dietary intake of black under, adequate and over-reporter women.

	Under-reporters	Adequate reporters	Over-reporters	P value
N	17	146	68	
Age, *years*	34 ± 10	27 ± 7	25 ± 6)	< 0.001
Body composition				
Height, *m*	1.60 ± 0.07	1.60 ± 0.06	1.61 ± 0.05)	0.417
Weight, *kg*	93 ± 22	75 ± 19	77 ± 22)	0.033
BMI, *kg/m²*	36 ± 8	29 ± 8	30 ± 8)	0.072
Body fat, *%*	43 ± 9	37 ± 8	38 ± 9)	0.207
Fat Mass, *kg*	41 ± 15	29 ± 14	30 ± 15)	0.036
Waist, *cm*	105 ± 18	89 ± 18	91 ± 19)	0.030
WHR	0.85 ± 0.07	0.79 ± 0.08	0.80 ± 0.09)	0.061
VAT, *cm²*	109 ± 50	73 ± 42	72 ± 35)	0.192
SAT, *cm²*	561 ± 215	384 ± 219	393 ± 238)	0.188
Serum lipids				
TAG, *mmol/L*	1.00 (0.50-1.10)	0.60 (0.50-0.90)	0.60 (0.50-0.80)	0.659
T-C, *mmol/L*	4.05 (3.65-4.23)	3.90 (3.30-4.40)	3.85 (3.23-4.30)	0.367
HDL-C, *mmol/L*	1.40 (1.05-1.50)	1.30 (1.10-1.60)	1.40 (1.10-1.80)	0.216
LDL-C, *mmol/L*	2.30 (2.15-2.50)	2.30 (1.70-2.83)	2.05 (1.60-2.50)	0.204
TC;HDL-C ratio	3.00 (2.69-3.78)	3.00 (2.47-3.84)	2.70 (2.25-3.25)	0.065

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose

Table 2S II. continued

Dietary intake	Under-reporters	Adequate reporters	Over-reporters	P value
Fat (%E)	30 (26-33)	34 (29-37)	36 (33-39)	0.024
SFA (%E)	7.6 (7.0-9.5)	9.2 (8.1-10.7)	9.7 (8.8-10.9)	0.013
MUFA (%E)	9.3 (8.0-11.5)	10.8 (9.2-12.3)	11.6 (10.2-13.4)	0.001
PUFA (%E)	7.2 (6.1-10.9)	8.7 (7.2-10.3)	9.8 (7.8-11.2)	0.028
P:S ratio	1.10 (0.80-1.37)	0.95 (0.77-1.16)	1.01 (0.82-1.19)	0.602
(n-3) PUFA (%E)	0.31 (0.23-0.37)	0.31 (0.25-0.41)	0.32 (0.23-0.44)	0.884
(n-6) PUFA (%E)	7.0 (5.8-10.6)	8.3 (6.8-10.2)	9.6 (7.5-11.3)	0.243
(n-6):(n-3) PUFA ratio	28.2 (15.6-36.5)	26.4 (19.1-37.3)	27.1 (18.8-39.4)	0.654
ALA (%E)	0.21 (0.18-0.28)	0.22 (0.18-0.25)	0.21 (0.18-0.27)	0.400
LA (%E)	7.1 (5.9-10.8)	8.5 (6.9-10.4)	9.6 (7.6-11.6)	0.253
AA (%E)	0.04 (0.03-0.06)	0.05 (0.03-0.07)	0.05 (0.04-0.07)	0.349
EPA (%E)	0.02 (0.01-0.03)	0.03 (0.01-0.06)	0.03 (0.01-0.06)	0.203
DHA (%E)	0.04 (0.04-0.06)	0.05 (0.03-0.11)	0.06 (0.03-0.11)	0.259

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 2S III. Subject characteristics, body composition, serum lipids and dietary intake of white under, adequate and over-reporter women.

	Under-reporters	Adequate reporters	Over-reporters	P value
N	25	122	5	
Age, *years*	35 ± 8	31 ± 8	35 ± 9	0.024
Body composition				
Height, *m*	1.68 ± 0.06	1.67 ± 0.07	1.67 ± 0.06	0.558
Weight, *kg*	86 ± 21	75 ± 20	62 ± 6	0.023
BMI, kg/m^2	30 ± 7	27 ± 7	22 ± 2	0.033
Body fat, *%*	39 ± 10	35 ± 10	23 ± 8	0.002
Fat Mass, *kg*	35 ± 15	28 ± 15	14 ± 6	0.011
Waist, *cm*	98 ± 17	89 ± 17	77 ± 7	0.022
WHR	0.84 ± 0.06	0.81 ± 0.07	0.80 ± 0.04	0.205
VAT, cm^2	117 ± 66	100 ± 63	86 ± 35	0.472
SAT, cm^2	367 ± 196	318 ± 207	175 ± 70	0.152
Serum lipids				
TAG, *mmol/L*	0.80 (0.60-1.40)	0.90 (0.60-1.20)	0.80 (0.60-0.90)	0.426
T-C, *mmol/L*	4.70 (4.00-5.20)	4.70 (4.10-5.30)	3.90 (3.80-5.10)	0.276
HDL-C, *mmol/L*	1.60 (1.30-1.90)	1.60 (1.40-1.90)	1.50 (1.40-2.10)	0.789
LDL-C, *mmol/L*	2.50 (2.10-3.40)	2.60 (2.10-3.20)	2.00 (2.00-2.70)	0.388
TC;HDL-C ratio	3.00 (2.32-3.75)	2.81 (2.40-3.56)	2.52 (2.50-2.60)	0.361

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose t

Table 2S III. continued

	Under-reporters	Adequate reporters	Over-reporters	P value
Dietary intake				
Fat (%E)	27 (25-30)	31 (26-34)	29 (26-32)	0.053
SFA (%E)	9.6 (8.8-10.6)	11.2 (8.7-12.2)	7.9 (7.8-13.0)	0.137
MUFA (%E)	9.5 (8.3-11.3)	10.4 (8.7-12.2)	10.2 (9.3-10.5)	0.155
PUFA (%E)	6.0 (4.5-6.9)	6.1 (4.9-8.0)	5.3 (5.2-8.2)	0.208
P:S ratio	0.59 (0.48-0.70)	0.57 (0.46-0.78)	0.67 (0.63-0.70)	0.785
(n-3) PUFA (%E)	0.34 (0.28-0.42)	0.32 (0.27-0.39)	0.26 (0.25-0.35)	0.529
(n-6) PUFA (%E)	4.8 (4.1-6.3)	5.6 (4.4-7.6)	5.0 (4.5-7.8)	0.130
(n-6):(n-3) PUFA ratio	14.9 (11.9-20.2)	16.1 (12.4-25.0)	20.2 (18.9-20.9)	0.205
ALA (%E)	0.26 (0.22-0.28)	0.25 (0.21-0.30)	0.20 (0.19-0.24)	0.402
LA (%E)	4.7 (4.1-6.2)	5.6 (4.4-7.6)	5.0 (4.5-7.8)	0.125
AA (%E)	0.04 (0.03-0.05)	0.03 (0.02-0.04)	0.02 (0.02-0.03)	0.301
EPA (%E)	0.01 (0.01-0.03)	0.01 (0.01-0.02)	0.02 (0.01-0.03)	0.864
DHA (%E)	0.05 (0.04-0.10)	0.05 (0.03-0.07)	0.05 (0.05-0.05)	0.683

Body composition: Summarised as mean ± SD. Serum lipids and dietary intake: Summarised as median (interquartile range). Other than age, all p-values are from age-adjusted linear models of outcomes. A significant group*ethnicity interaction for a characteristic means that the groups means are different between the black and white women. Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; (n-6) PUFA, (n-6) polyunsaturated fatty acids; (n-3) PUFA, (n-3) polyunsaturated fatty acid; (n-6):(n-3) PUFA ratio, (n-6):(n-3) polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

9.2. Polymorphisms of the human *IL-6* gene

Table 2S IV. Single nucleotide polymorphisms (SNPs) within the 5'- and 3'-untranslated region (UTR), exon and introns of the human *IL-6* gene tabulating the rare allele and the reported minor allele frequencies (MAF) within the black and white populations (pop). The presence (restriction fragment length polymorphisms, RFLPs) or absence (none) of restriction endonuclease sites that encompass the SNP of potentially informative markers is also indicated. SNPs selected for this thesis are indicated in bold.

IL-6 Gene	No	SNP Accession No	SNP	Rare Allele	Black Pop MAF	White Pop MAF	RFLP
5'-UTR	1	rs2069857	A>C	A	0.0	2.2	
5'-UTR	2	rs3087226	C>T	G			
Exon 2	1	rs2069830	C>T	T	9.2	0.0	
Intron 2	1	rs2069831	C>T	T	2.1	0.0	
Intron 2	2	rs2069858	A>G	A	0.0	4.8	
Intron 2	3	rs2069832	A>G	A	2.1	52.5	
Intron 2	4	rs2069833	C>T	C	2.2	50.0	
Intron 2	5	rs2069834	C>T	T	3.3	0.0	
Intron 2	6	rs2069835	C>T	C	10.8	10.0	
Intron 2	7	rs3087230	A>G	G			
Intron 2	8	rs1474348	C>G	G	2.1	54.3	
Intron 2	9	rs2069836	G>T	T	2.3	0.0	
Intron 2	10	rs2069837	A>G	G	13.4	6.7	
Intron 2	11	rs1474347	G>T	G	8.7	50.0	
Intron 2	12	rs3087231	C>T	T			
Intron 2	13	rs3087232	C>T	T			
Intron 2	14	rs1524107	C>T	T	8.3	4.2	
Intron 2	15	rs3087233	G>T	T			
Intron 2	16	rs2066992	G>T	T	8.7	4.2	
Intron 3	1	rs2069838	C>T				
Intron 3	2	rs2069839	A>G				
Intron 3	3	rs2069840	C>G	G	17.4	37.5	None
Intron 3	**4**	**rs1554606**	**G>T**	**T**	**41.3**	**56.5**	***DdeI***
Intron 3	5	rs2069841	A>G				
Intron 3	6	rs13306433	A>G				
Intron 4	1	rs2069842	A>G				
Intron 4	2	rs1548216	C>G				
Intron 4	3	rs2069843	A>G				
Intron 4	4	rs2069844	A>C				
Intron 4	**5**	**rs2069845**	**A>G**	**G**	**38.6**	**54.8**	***MspI***
Intron 4	6	rs2069859	C>T				
Intron 4	7	rs2069846	A>G				
Intron 4	8	rs2069847	A>G				
Intron 4	9	rs2069848	A>T				
Exon 5	1	rs2069860	T>A				
Exon 5	2	rs13306435	A>T				
Exon 5	3	rs2069849	C>T	T	20.8	6.5	
3'-UTR	1	rs2069850	C>T				
3'-UTR	2	rs13306436	A>G				
3'-UTR	3	rs2069851	A>G				

9.3. Statistical analysis: an example

The following provides an example from Chapter 4; assessing the interaction between a diet variable and a genetic factor on a quantitative trait, followed by a discussion of the steps in general.

In nutrition, as in many other disciplines, it is becoming clear that many factors do not affect outcome in a linear way, but rather modify the effect of each other. Testing for interactions between dietary intake and a genetic factor, such as genotypes on an outcome such as body composition (e.g. BMI, body fat percentage) or serum lipids (e.g. T-C, HDL-C) is often necessary.

In this example, we assess the interaction between a genotype (*TNFA* -238 G>A) and dietary intake (n-3 PUFA) on the T-C:HDL-C ratio in a convenience sample of 129 black SA women. The group was selected to include both normal-weight and obese women. The T-C:HDL-C ratio is significantly associated with both age (years) and fat mass (kilograms), and is therefore adjusted for these in our model.

Preparing the data for analysis

The n-3 PUFA values were converted to a percentage of total energy intake (% E), to make the effect sizes comparable. Because there were positive outliers, the n-3 PUFA intakes were log-transformed towards symmetry, to avoid spurious association, indicated with log(n-3 PUFA).

The distribution of the outcome, T-C:HDL-C ratio is skewed to the right, so it was also log-transformed towards symmetry. There was a single minor *TNFA* -238 homozygote (AA), which was grouped with the heterozygotes (GA), hence assuming a dominant model. The *TNFA* -238 G>A genotype is therefore a factor with only two levels, the wild type homozygote, GG, and women bearing at least one A allele, AA+AG. This SNP has previously been analysed as dominant [182, 183, 247].

The analysis

We use a general linear model to write log(T-C:HDL-C ratio) as a linear function of age and fat mass and the interaction between the *TNFA* -238 G>A SNP and log(n-3 PUFA). The interaction term means that T-C:HDL-C ratio is written as a different function of n-3 PUFA for the *TNFA -238* wild type (GG) as for other *TNFA* -238 A allele carriers. It also means that the effect of *TNFA* -238 G>A SNP on T-C:HDL-C ratio depends on n-3 PUFAs.

Table 2S V. The analysis of variance for the linear model for *TNFA* -238 G>A

Response: log(T-C:HDL-C ratio)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age	1	0.80	0.80	10.6	0.001
Fat mass	1	0.89	0.89	11.8	0.001
TNFA -238 G>A	1	0.00	0.00	0.0	0.859
log(n-3 PUFA)	1	0.24	0.24	3.2	0.077
TNFA -238 G>A**:**log(n-3 PUFA)	1	0.49	0.49	6.4	0.012
Residuals	123	9.27	0.08		

The last term in the table, is the interaction term, with the colon indicating statistical interaction (*TNFA* -238 G>A**:**log(n-3 PUFA)). Main effects are, and should be, automatically included in the model. The sums of squares were calculated

successively, the sum of squares for fat mass, after removal of age, the sum of squares for *TNFA* -238 G>A, after removal of the sums of squares for both age and fat mass. That means that each F-test is covaried for those preceding it (those higher up in the table). For the interaction term, the blend of effect size and precision, the p-value, is below 5%, a signal to explore further. This means that there is a statistical interaction between the *TNFA* -238 G>A SNP and n-3 PUFA, over and above the main effects, and that the regression lines of T-C:HDL-C ratio on n-3 PUFA, have different slopes for the different genotypes. A different but equivalent interpretation is that the genotype effect on T-C:HDL-C ratio depends on n-3 PUFA intake.

Table 2S VI. Summary table for interaction between TNFA -238 G>A SNP and n-3 PUFAs on T-C:HDL-C ratio

	Effect size	SE	t value	P-value
(Intercept)	1.098	0.150	7.3	< 0.001
Age	0.007	0.004	1.8	0.075
Fat mass	0.006	0.002	3.1	0.003
TNFA -238 AG+AA	-0.373	0.150	-2.5	0.014
log(n-3 PUFA)	0.285	0.093	3.1	0.003
TNFA -238 AG+AA:log(n-3 PUFA)	-0.315	0.124	-2.5	0.012

Residual standard error: 0.2746 on 123 degrees of freedom

Multiple R-squared: 0.2067, Adjusted R-squared: 0.1745

F-statistic: 6.411 on 5 and 123 DF, p-value: 0.0001

Multiple R-squared means that over 20% of the variation in T-C:HDL-C ratio can be explained by the factors in the linear model. All the statistics for factors listed in this table are adjusted for all the other factors in the model. Those that are statistically

significant are said to be independently significant, meaning that they remain significant after adjusting for confounders. Note that only the p-values in the last line of the ANOVA and summary (coefficient) tables are the same. The others are not, because of the way they are calculated.

The standard errors of the estimates, in the second column, are the indication of the precision of the estimate. The smaller the standard error, the more precise is the estimate. The t-value is a statistic that simultaneously tests whether the estimate is significantly large and significantly precise. The t-values and their corresponding p-values blend those two tests.

The effect estimates in the table

If you want to estimate the outcome (log (T-C:HDL-C ratio)) for a specific woman, start with the intercept 1.098 then add 0.007 for every year of age, in other words multiply her age with 0.007 and add that to the intercept. The effect of one year of age is to add 0.007 to the outcome. Similarly, each kilogram of fat mass adds 0.006 to the outcome. Next is genotype, with only two possible values: GG (wild type) and GA+AA and women with GA and AA subtract 0.373 from their outcome. Each unit of log(n-3 PUFA) adds 0.285 to her estimated outcome (in other words multiply log(n-PUFA) with 0.285). If there was no interaction between the last two factors, that would be the end of the linear model. But for women with GA+AA genotype, we subtract 0.315 for each unit of log(n-3 PUFA) from her estimated outcome.

For a specific example: woman aged 30 years, fat mass 40 kg, TNFA -238 genotype GG. Her outcome will be:

1.098 + 30 x 0.007 + 40 x 0.006 + 0.285 x log(n-3 PUFA),

which is 1.548 + 0.285 x log(n-3 PUFA), because the terms with *TNFA* -238 GA and AA genotype do not apply to her.

As n-3 PUFA is her dietary consumption of n-3 PUFA, we can calculate an outcome for each possible value of log(n-3 PUFA). This can be plotted on a graph (figure 2.1). In our data, n-3 PUFA ranged from 0.13 to 0.81. This means log(n-3 PUFA) ranged from -2.04 to -0.12. And our corresponding values of outcomes from 1.548 – 0.285 * 2.04 to 1.548 – 0.285 * 0.12. This is represented by the solid line on the plot.

If her *TNFA* -238 genotype was not GG, but GA or AA, then the model explaining her outcome has to change in two ways. Subtract 0.373 (main effect) from the intercept and subtract -0.315 multiplied with) log(n-3 PUFA). The GA model then becomes: 1.548 – 0.373 + (0.285-0.315) x log(n-3 PUFA) which is 1.175 – 0.030 x log (n-3 PUFA). These relationships are illustrated in Figure 2S 2A.

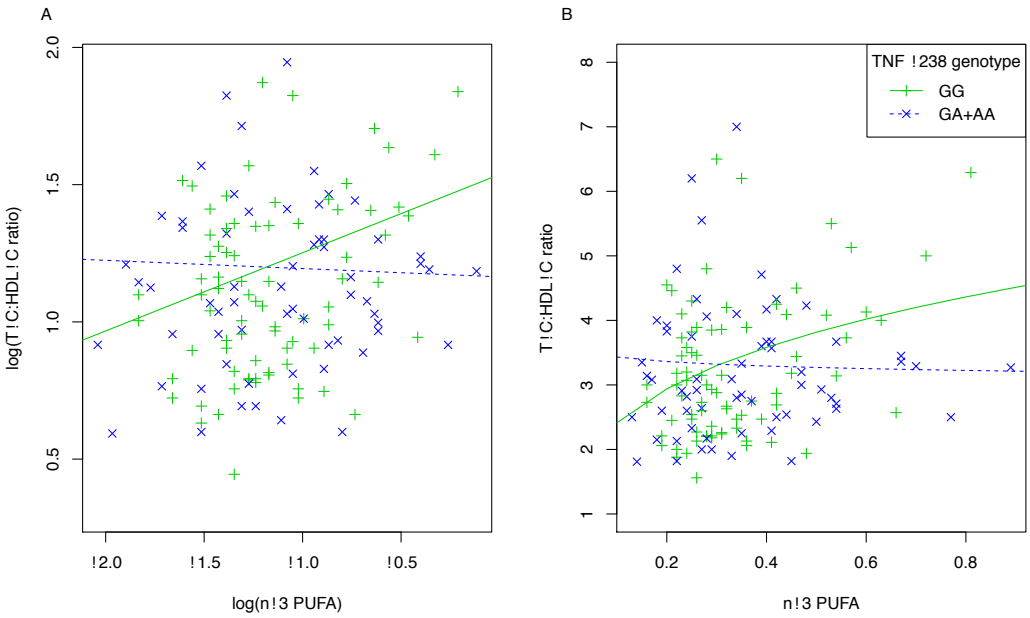

Figure 2S 2. Modelled relationship between *TNFA* -238 G>A and log(n-3 PUFA) on log(T-C:HDL-C ratio). Points are observed values (logs in A, original in B). Lines are A: linear model in log(T-C:HDL-C ratio) and log(n-3 PUFA), and B: back transformed to original scale. Abbreviations: n-3 PUFA, omega-3 polyunsaturated fatty acid; *TNFA,* tumour necrosis factor alpha gene; T-C:HDL-C ratio, total cholesterol: high-density lipoprotein cholesterol ratio.

The graph for older women will simply move 0.007 up for every year that she is older than 30 or down for younger. But the modelled relationship between *TNFA* -238 G>A and n-3 PUFA and T-C:HDL-C ratio relative to one another remains the same. If you have the wild type genotype (GG), your log(T-C:HDL-C ratio) increases significantly with increasing log(n-3 PUFA), while if you have the GG+AA genotype, your n-3 PUFA intake does not make much difference. The significant interaction is the significant difference between the GG genotype slope and the AG+AA genotype slope. Generally, the significant interaction does not necessarily mean that either of the individual slopes are significantly different from zero.

It is preferable to report effect sizes in terms of the original measurements, where possible transforming our model back to the original units, by taking the antilogs

(exp). Figure 2S 2B. illustrates that if a woman with the *TNFA* -238 GG genotype consumes a larger percentage of n-3 PUFA, her T-C:HDL-C ratio will increase significantly, but the increase is logarithmically, becoming smaller as intake increases. For the women with the GA+AA genotype, we see a small, not-significant decrease in T-C:HDL-C ratio with increasing n-3 PUFA intake. Figure 2S 2. includes the modelled values, as well as the observed data superimposed on it.

9.4. Chapter three supplementary tables

Supplementary Table 3S I. P-values for black and white women combined, testing association with BMI group, ethnic group and *TNFA* -308 G>A genotype on body composition and serum lipids, each adjusted for age and the factors listed before it.

	BMI group	**Ethnic group**	***TNFA* -308 G>A genotype**
Body composition			
Height (m)	< 0.001	< 0.001	0.815
Weight (kg)	< 0.001	0.002	0.710
BMI (kg/m^2)	< 0.001	0.001	0.605
Body fat (%)	< 0.001	0.016	0.271
Fat Mass (kg)	< 0.001	0.205	0.850
Waist (cm)	< 0.001	0.036	0.889
WHR	< 0.001	0.012	0.853
VAT	< 0.001	< 0.001	0.914
SAT	< 0.001	0.011	0.864
Serum lipids			
TAG (mmol/L)	< 0.001	< 0.001	0.841
T-C (mmol/L)	0.917	< 0.001	0.749
HDL-C (mmol/L)	< 0.001	< 0.001	0.848
LDL-C (mmol/L)	0.003	< 0.001	0.575
TC:HDL-C ratio	< 0.001	0.516	0.616

Abbreviations: BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-！gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 3S II. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on body composition in 143 black adequate reporter women, adjusted for age.

Dietary intake	Height, *m*	Weight, *kg*	BMI, kg/m^2	Body fat, *%*	Fat Mass, *kg*	Waist, *cm*	WHR	VAT, cm^2	SAT, cm^2
Fat (%E)	0.032	0.155	0.439	0.447	0.280	0.360	0.772	0.966	0.217
SFA (%E)	0.318	0.860	0.881	0.416	0.722	0.788	0.937	0.948	0.646
MUFA (%E)	0.156	0.407	0.648	0.812	0.709	0.569	0.942	0.931	0.267
PUFA (%E)	0.022	0.632	0.795	0.965	0.752	0.928	0.551	0.742	0.962
P:S ratio	0.090	0.792	0.785	0.692	0.675	0.868	0.615	0.778	0.780
n-3 PUFA (%E)	0.172	0.950	0.658	0.777	0.757	0.390	0.435	0.738	0.886
n-6 PUFA (%E)	0.016	0.691	0.713	0.971	0.838	0.930	0.688	0.647	0.933
n-6:n-3 PUFA ratio	0.411	0.562	0.771	0.661	0.481	0.430	0.225	0.787	0.941
ALA (%E)	0.372	0.235	0.313	0.465	0.360	0.605	0.371	0.768	0.174
LA (%E)	0.016	0.688	0.718	0.983	0.833	0.942	0.675	0.643	0.936
AA (%E)	0.732	0.615	0.513	0.869	0.857	0.945	0.608	0.866	0.744
EPA (%E)	0.107	0.530	0.255	0.186	0.291	0.222	0.984	0.737	0.446
DHA (%E)	0.112	0.543	0.264	0.250	0.320	0.245	0.998	0.941	0.507

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 3S III. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on body composition in 122 white adequate reporter women, adjusted for age.

Dietary intake	Height, *m*	Weight, *kg*	BMI, kg/m^2	Body fat, *%*	Fat Mass, *kg*	Waist, *cm*	WHR	VAT, cm^2	SAT, cm^2
Fat (%E)	0.079	0.932	0.795	0.630	0.762	0.841	0.217	0.152	0.853
SFA (%E)	0.294	0.347	0.246	0.248	0.385	0.253	0.164	0.070	0.232
MUFA (%E)	0.017	0.595	0.905	0.914	0.863	0.544	0.949	0.449	0.726
PUFA (%E)	0.649	0.663	0.749	0.595	0.710	0.913	0.383	0.606	0.773
P:S ratio	0.528	0.405	0.341	0.849	0.953	0.541	0.917	0.422	0.320
n-3 PUFA (%E)	0.653	0.527	0.339	0.795	0.518	0.371	0.109	0.603	0.659
n-6 PUFA (%E)	0.622	0.503	0.575	0.670	0.899	0.709	0.534	0.834	0.612
n-6:n-3 PUFA ratio	0.960	0.427	0.356	0.768	0.893	0.481	0.645	0.896	0.552
ALA (%E)	0.131	0.742	0.896	0.826	0.922	0.871	0.500	0.905	0.963
LA (%E)	0.617	0.502	0.576	0.670	0.900	0.707	0.540	0.837	0.616
AA (%E)	0.685	0.871	0.952	0.989	0.809	0.908	0.373	0.576	0.457
EPA (%E)	0.681	0.115	0.074	0.112	0.086	0.051	0.043	0.360	0.238
DHA (%E)	0.585	0.276	0.210	0.312	0.210	0.178	0.198	0.582	0.701

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 3S IV. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on body composition in 263 white and black adequate reporter women, adjusted for age and ethnicity.

Dietary intake	Height, *m*	Weight, *kg*	BMI, kg/m^2	Body fat, *%*	Fat Mass, *kg*	Waist, *cm*	WHR	VAT, cm^2	SAT, cm^2
Fat (%E)	0.005	0.542	0.961	0.958	0.772	0.941	0.312	0.236	0.741
SFA (%E)	0.062	0.605	0.965	0.358	0.920	0.919	0.280	0.484	0.793
MUFA (%E)	0.002	0.443	0.883	0.889	0.860	0.630	0.585	0.603	0.427
PUFA (%E)	0.142	0.472	0.234	0.393	0.400	0.312	0.518	0.121	0.258
P:S ratio	0.954	0.220	0.184	0.587	0.285	0.182	0.827	0.316	0.203
n-3 PUFA (%E)	0.124	0.596	0.248	0.521	0.321	0.158	0.027	0.487	0.872
n-6 PUFA (%E)	0.124	0.523	0.262	0.413	0.428	0.321	0.553	0.156	0.279
n-6:n-3 PUFA ratio	0.850	0.877	0.908	0.755	0.987	0.917	0.345	0.591	0.517
ALA (%E)	0.163	0.057	0.122	0.214	0.162	0.206	0.359	0.533	0.039
LA (%E)	0.133	0.483	0.242	0.407	0.404	0.302	0.554	0.145	0.254
AA (%E)	0.952	0.339	0.310	0.387	0.374	0.108	0.025	0.937	0.611
EPA (%E)	0.315	0.015	0.003	0.009	0.005	0.002	0.037	0.074	0.025
DHA (%E)	0.343	0.070	0.016	0.053	0.025	0.013	0.128	0.283	0.158

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 3S V. P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *TNFA* -308 G>A genotype on body composition in 268 white and black adequate reporter women, adjusted for age.

Dietary intake	Height, *m*	Weight, *kg*	BMI, kg/m^2	Body fat, *%*	Fat Mass, *kg*	Waist, *cm*	WHR	VAT, cm^2	SAT, cm^2
Fat (%E)	0.820	0.326	0.423	0.383	0.326	0.384	0.292	0.394	0.251
SFA (%E)	0.848	0.499	0.628	0.941	0.821	0.397	0.449	0.384	0.252
MUFA (%E)	0.626	0.737	0.716	0.921	0.839	0.875	0.845	0.859	0.461
PUFA (%E)	0.215	0.997	0.676	0.660	0.634	0.980	0.321	0.897	0.846
P:S ratio	0.096	0.699	0.407	0.896	0.809	0.589	0.735	0.473	0.361
n-3 PUFA (%E)	0.654	0.575	0.602	0.976	0.688	0.770	0.514	0.807	0.554
n-6 PUFA (%E)	0.226	0.778	0.488	0.749	0.848	0.701	0.516	0.918	0.673
n-6:n-3 PUFA ratio	0.521	0.791	0.591	0.622	0.773	0.918	0.631	0.751	0.636
ALA (%E)	0.697	0.479	0.349	0.717	0.536	0.709	0.777	0.758	0.263
LA (%E)	0.229	0.778	0.491	0.743	0.846	0.707	0.513	0.914	0.677
AA (%E)	0.546	0.707	0.542	0.929	0.938	0.966	0.809	0.646	0.890
EPA (%E)	0.203	0.366	0.530	0.653	0.438	0.415	0.138	0.864	0.613
DHA (%E)	0.177	0.580	0.786	0.922	0.652	0.673	0.298	0.972	0.995

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:p,omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; *TNFA*, tumour necrosis factor-! gene; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 3S VI. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on serum lipids in 142 black adequate reporter women, adjusted for fat mass and age.

Dietary intake	TAG *(mmol/L)*	T-C *(mmol/L)*	HDL-C *(mmol/L)*	LDL-C *(mmol/L)*	T-C:HDL-C ratio
Fat (%E)	0.504	0.815	0.544	0.778	0.441
SFA (%E)	0.858	0.949	0.710	0.913	0.748
MUFA (%E)	0.915	0.577	0.966	0.769	0.709
PUFA (%E)	0.888	0.213	0.705	0.114	0.193
P:S ratio	1.000	0.265	0.602	0.158	0.173
n-3 PUFA (%E)	0.824	0.777	0.810	0.506	0.983
n-6 PUFA (%E)	0.908	0.289	0.854	0.190	0.330
n-6:n-3 PUFA ratio	0.922	0.622	0.518	0.602	0.310
ALA (%E)	0.672	0.190	0.230	0.120	0.028
LA (%E)	0.919	0.299	0.860	0.201	0.342
AA (%E)	0.173	0.492	0.916	0.397	0.673
EPA (%E)	0.471	0.129	0.851	0.058	0.172
DHA (%E)	0.399	0.162	0.734	0.061	0.156

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 3S VII. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on serum lipids in 122 white adequate reporter women, adjusted for fat mass and age.

Dietary intake	TAG *(mmol/L)*	T-C *(mmol/L)*	HDL-C *(mmol/L)*	LDL-C *(mmol/L)*	T-C:HDL-C ratio
Fat (%E)	0.832	0.086	0.534	0.111	0.529
SFA (%E)	0.672	0.047	0.496	0.101	0.473
MUFA (%E)	0.784	0.111	0.310	0.102	0.872
PUFA (%E)	0.996	0.718	0.701	0.521	0.537
P:S ratio	0.994	0.356	0.272	0.749	0.703
n-3 PUFA (%E)	0.946	0.184	0.180	0.175	0.755
n-6 PUFA (%E)	0.983	0.683	0.545	0.414	0.389
n-6:n-3 PUFA ratio	0.738	0.660	0.167	0.893	0.326
ALA (%E)	0.988	0.330	0.650	0.137	0.797
LA (%E)	0.980	0.691	0.540	0.418	0.389
AA (%E)	0.635	0.156	0.378	0.236	0.861
EPA (%E)	0.712	0.093	0.076	0.225	0.633
DHA (%E)	0.935	0.187	0.216	0.204	0.819

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 3S VIII. P-values for interaction between dietary fat intake (%E) and *TNFA* -308 G>A genotype on serum lipids in 263 white and black adequate reporter women, adjusted for age, fat mass and ethnicity.

Dietary intake	TAG *(mmol/L)*	T-C *(mmol/L)*	HDL-C *(mmol/L)*	LDL-C *(mmol/L)*	T-C:HDL-C ratio
Fat (%E)	0.493	0.274	0.111	0.561	0.459
SFA (%E)	0.812	0.082	0.416	0.141	0.631
MUFA (%E)	0.502	0.270	0.278	0.248	0.811
PUFA (%E)	0.676	0.223	0.672	0.215	0.633
P:S ratio	0.948	0.855	0.909	0.700	0.805
n-3 PUFA (%E)	0.899	0.279	0.126	0.299	0.497
n-6 PUFA (%E)	0.867	0.274	0.753	0.238	0.622
n-6:n-3 PUFA ratio	0.844	0.825	0.466	0.790	0.385
ALA (%E)	0.623	0.963	0.418	0.764	0.455
LA (%E)	0.866	0.290	0.741	0.258	0.651
AA (%E)	0.517	0.149	0.248	0.240	0.959
EPA (%E)	0.912	0.046	0.089	0.119	0.863
DHA (%E)	0.661	0.072	0.312	0.065	0.729

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 3S IX. P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *TNFA* -308 G>A genotype on serum lipid levels in 263 white and black adequate reporter women, adjusted for age and ethnic group and fat mass.

Dietary intake	TAG *(mmol/L)*	T-C *(mmol/L)*	HDL-C *(mmol/L)*	LDL-C *(mmol/L)*	T-C:HDL-C ratio
Fat (%E)	0.549	0.195	0.909	0.210	0.291
SFA (%E)	0.689	0.402	0.966	0.535	0.567
MUFA (%E)	0.870	0.164	0.566	0.229	0.646
PUFA (%E)	0.902	0.473	0.960	0.416	0.568
P:S ratio	0.999	0.154	0.815	0.189	0.411
n-3 PUFA (%E)	0.740	0.580	0.560	0.741	0.882
n-6 PUFA (%E)	0.965	0.629	0.792	0.682	0.921
n-6:n-3 PUFA ratio	0.761	0.550	0.751	0.651	0.894
ALA (%E)	0.795	0.086	0.438	0.026	0.044
LA (%E)	0.971	0.637	0.784	0.697	0.936
AA (%E)	0.441	0.806	0.708	0.999	0.858
EPA (%E)	0.411	0.866	0.198	0.458	0.173
DHA (%E)	0.479	0.758	0.301	0.495	0.221

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

9.5. Chapter four supplementary tables

Supplementary Table 4S I. P-values for black and white women combined, testing association with ethnic group, *TNFA* -238 G>A genotype and BMI group, on body composition and serum lipids, each adjusted for age and the factors listed before it.

	Ethnic group	BMI group	*TNFA* -238 G>A genotype
Body composition			
Height (m)	< 0.001	0.367	0.365
Weight (kg)	< 0.001	< 0.001	0.706
BMI (kg/m^2)	< 0.001	< 0.001	0.283
Body fat (%)	< 0.001	< 0.001	0.004
Fat Mass (kg)	< 0.001	< 0.001	0.263
Waist (cm)	< 0.001	< 0.001	0.061
Serum lipids			
TAG (mmol/L)	< 0.001	< 0.001	0.295
T-C (mmol/L)	< 0.001	0.085	0.888
HDL-C (mmol/L)	< 0.001	< 0.001	0.990
LDL-C (mmol/L)	0.002	< 0.001	0.947
T-C:HDL-C ratio	0.037	< 0.001	0.591

Abbreviations: BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; WHR, waist hip ratio.

Supplementary Table 4S II. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on body composition in 142 black adequate reporter women, adjusted for age.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat mass, kg	Waist, cm
Fat, % E	0.034	0.092	0.217	0.096	0.036
SFA, % E	0.017	0.055	0.143	0.048	0.013
MUFA, % E	0.044	0.090	0.170	0.090	0.087
PUFA, % E	0.952	0.785	0.608	0.657	0.918
P:S ratio	0.087	0.093	0.098	0.059	0.092
n-3 PUFA. % E	0.978	0.952	0.536	0.838	0.451
n-6 PUFA, % E	0.996	0.870	0.604	0.704	0.917
n-6:n-3 PUFA	0.579	0.417	0.099	0.270	0.919
ALA, % E	0.007	0.013	0.006	0.006	0.045
LA, % E	0.998	0.861	0.607	0.700	0.917
AA, % E	0.204	0.323	0.088	0.106	0.775
EPA, %E	0.245	0.336	0.956	0.525	0.047
DHA, %E	0.301	0.374	0.998	0.507	0.086

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; *TNFA*, tumour necrosis factor-! gene.

Supplementary Table 4S III. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on body composition in 121 white adequate reporter women, adjusted for age.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat mass, kg	Waist, cm
Fat, *% E*	0.820	0.868	0.933	0.949	0.992
SFA, % E	0.630	0.484	0.754	0.594	0.574
MUFA, *% E*	0.535	0.609	0.900	0.792	0.925
PUFA, *% E*	0.714	0.573	0.815	0.607	0.607
P:S ratio	0.471	0.252	0.705	0.446	0.390
n-3 PUFA. *% E*	0.742	0.721	0.454	0.751	0.834
n-6 PUFA, *% E*	0.717	0.574	0.812	0.609	0.572
n-6:n-3 PUFA	0.985	0.928	0.735	0.937	0.884
ALA, *% E*	0.690	0.614	0.692	0.830	0.969
LA, *% E*	0.711	0.569	0.810	0.605	0.569
AA, *% E*	0.580	0.686	0.458	0.953	0.737
EPA, %E	0.850	0.636	0.848	0.900	0.820
DHA, %E	0.710	0.873	0.392	0.602	0.589

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; *TNFA*, tumour necrosis factor-! gene.

Supplementary Table 4S IV. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on body composition in 263 white and black adequate reporter women, adjusted for age and ethnicity.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat mass, kg	Waist, cm
Fat, *% E*	0.091	0.145	0.249	0.193	0.102
SFA, % E	0.108	0.124	0.423	0.177	0.064
MUFA, *% E*	0.056	0.088	0.207	0.150	0.150
PUFA, *% E*	0.847	0.700	0.985	0.672	0.862
P:S ratio	0.294	0.220	0.631	0.265	0.227
n-3 PUFA. *% E*	0.856	0.747	0.743	0.668	0.812
n-6 PUFA, *% E*	0.897	0.777	0.991	0.733	0.872
n-6:n-3 PUFA	0.573	0.405	0.494	0.328	0.681
ALA, *% E*	0.115	0.143	0.243	0.111	0.155
LA, *% E*	0.899	0.775	0.969	0.744	0.876
AA, *% E*	0.187	0.251	0.300	0.178	0.494
EPA, %E	0.711	0.955	0.657	0.860	0.381
DHA, %E	0.540	0.818	0.955	0.800	0.276

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; *TNFA*, tumour necrosis factor-! gene

Supplementary Table 4S V. P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *TNFA* -238 G>A genotype on body composition in 263 white and black adequate reporter women, adjusted for age.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat mass, kg	Waist, cm
Fat, *% E*	0.820	0.868	0.933	0.949	0.992
SFA, % E	0.630	0.484	0.754	0.594	0.574
MUFA, *% E*	0.535	0.609	0.900	0.792	0.925
PUFA, *% E*	0.714	0.573	0.815	0.607	0.607
P:S ratio	0.471	0.252	0.705	0.446	0.390
n-3 PUFA. *% E*	0.742	0.721	0.454	0.751	0.834
n-6 PUFA, *% E*	0.717	0.574	0.812	0.609	0.572
n-6:n-3 PUFA	0.985	0.928	0.735	0.937	0.884
ALA, *% E*	0.690	0.614	0.692	0.830	0.969
LA, *% E*	0.711	0.569	0.810	0.605	0.569
AA, *% E*	0.580	0.686	0.458	0.953	0.737
EPA, %E	0.850	0.636	0.848	0.900	0.820
DHA, %E	0.710	0.873	0.392	0.602	0.589

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; *TNFA*, tumour necrosis factor-! gene.

Supplementary Table 4S VI. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on serum lipids in 142 black adequate reporter women, adjusted for age.

	TAG (mmol/L)	T-C (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	T-C:HDL-C ratio
Fat (%E)	0.978	0.736	0.827	0.745	0.974
SFA (%E)	0.506	0.847	0.191	0.773	0.239
MUFA (%E)	0.758	0.936	0.351	0.664	0.320
PUFA (%E)	0.822	0.670	0.051	0.801	0.103
P:S ratio	0.622	0.655	0.013	0.714	0.032
n-3 PUFA (%E)	0.911	0.304	0.100	0.112	0.012
n-6 PUFA (%E)	0.970	0.723	0.060	0.808	0.107
n-6:n-3 PUFA ratio	0.777	0.374	0.032	0.122	0.004
ALA (%E)	0.748	0.837	0.054	0.751	0.072
LA (%E)	0.981	0.734	0.058	0.792	0.101
AA (%E)	0.580	0.359	0.757	0.415	0.306
EPA (%E)	0.750	0.288	0.396	0.166	0.087
DHA (%E)	0.567	0.207	0.358	0.093	0.053

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 4S VII. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on serum lipids in 121 white adequate reporter women, adjusted for age.

	TAG (mmol/L)	T-C (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	T-C:HDL-C ratio
Fat (%E)	0.225	0.063	0.613	0.072	0.068
SFA (%E)	0.107	0.067	0.741	0.090	0.103
MUFA (%E)	0.519	0.119	0.493	0.062	0.077
PUFA (%E)	0.698	0.571	0.589	0.554	0.357
P:S ratio	0.620	0.666	0.819	0.693	0.928
n-3 PUFA (%E)	0.125	0.763	0.287	0.918	0.430
n-6 PUFA (%E)	0.824	0.486	0.612	0.464	0.327
n-6:n-3 PUFA ratio	0.440	0.455	0.728	0.540	0.847
ALA (%E)	0.167	0.209	0.318	0.070	0.067
LA (%E)	0.824	0.480	0.614	0.457	0.325
AA (%E)	0.287	0.811	0.576	0.874	0.712
EPA (%E)	0.317	0.041	0.417	0.020	0.500
DHA (%E)	0.226	0.040	0.145	0.078	0.946

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 4S VIII. P-values for interaction between dietary fat intake (%E) and *TNFA* -238 G>A genotype on serum lipids in 263 white and black adequate reporter women, adjusted for age, fat mass and ethnicity.

	TAG (mmol/L)	T-C (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	T-C:HDL-C ratio
Fat (%E)	0.316	0.082	0.810	0.110	0.161
SFA (%E)	0.159	0.172	0.360	0.098	0.072
MUFA (%E)	0.519	0.182	0.378	0.078	0.038
PUFA (%E)	0.572	0.437	0.135	0.996	0.618
P:S ratio	0.750	0.967	0.066	0.433	0.196
n-3 PUFA (%E)	0.381	0.347	0.181	0.139	0.019
n-6 PUFA (%E)	0.653	0.410	0.151	0.917	0.694
n-6:n-3 PUFA ratio	0.548	0.838	0.058	0.305	0.065
ALA (%E)	0.355	0.405	0.053	0.105	0.015
LA (%E)	0.639	0.410	0.148	0.922	0.697
AA (%E)	0.272	0.251	0.843	0.254	0.199
EPA (%E)	0.611	0.869	0.604	0.937	0.320
DHA (%E)	0.602	0.960	0.208	0.688	0.162

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 4S IX. P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *TNFA* -238 G>A genotype on serum lipid levels in 263 white and black adequate reporter women, adjusted for age and fat mass.

	TAG (mmol/L)	T-C (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	T-C:HDL-C ratio
Fat (%E)	0.196	0.176	0.914	0.220	0.210
SFA (%E)	0.247	0.165	0.395	0.377	0.955
MUFA (%E)	0.490	0.237	0.797	0.307	0.604
PUFA (%E)	0.759	0.699	0.268	0.404	0.065
P:S ratio	0.689	0.666	0.203	0.873	0.136
n-3 PUFA (%E)	0.251	0.355	0.918	0.176	0.185
n-6 PUFA (%E)	0.770	0.589	0.307	0.337	0.055
n-6:n-3 PUFA ratio	0.834	0.165	0.438	0.052	0.015
ALA (%E)	0.315	0.393	0.697	0.543	0.764
LA (%E)	0.767	0.579	0.302	0.326	0.052
AA (%E)	0.681	0.371	0.748	0.351	0.287
EPA (%E)	0.561	0.019	0.907	0.004	0.054
DHA (%E)	0.397	0.014	0.592	0.006	0.088

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; *TNFA*, tumour necrosis factor-! gene; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

9.6. Chapter six supplementary tables

Supplementary Table 6S I. P-values for black and white adequate reporter women combined, testing association with ethnic group, *IL-6* polymorphisms *(allelic)* and BMI group, on body composition and serum lipids, each adjusted for age and the factors listed before it.

	***IL-6* -174 G>C**			***IL-6* IVS3+281 G>T**			***IL-6* IVS4+869 A>G**		
Outcome	Ethnic group	BMI group	-174	Ethnic group	BMI group	IVS3+281	Ethnic group	BMI group	IVS4+869
Body composition									
Weight, kg	0.065	< 0.001	0.319	0.016	< 0.001	0.037	0.039	< 0.001	0.377
BMI, kg/m^2	< 0.001	< 0.001	0.380	< 0.001	< 0.001	0.147	< 0.001	< 0.001	0.376
Body fat, %	< 0.001	< 0.001	0.369	< 0.001	< 0.001	0.827	< 0.001	< 0.001	0.848
Fat Mass	< 0.001	< 0.001	0.471	< 0.001	< 0.001	0.160	< 0.001	< 0.001	0.079
Waist, cm	0.012	< 0.001	0.473	0.005	< 0.001	0.113	0.012	< 0.001	0.278
WHR	0.168	< 0.001	0.804	0.174	< 0.001	0.460	0.151	< 0.001	0.339
VAT, cm^2	< 0.001	< 0.001	0.173	< 0.001	< 0.001	0.276	< 0.001	< 0.001	0.167
SAT, cm^2	< 0.001	< 0.001	0.706	< 0.001	< 0.001	0.953	< 0.001	< 0.001	0.905
Serum Lipids									
TAG, mmol/L	< 0.001	< 0.001	0.765	< 0.001	< 0.001	0.011	< 0.001	< 0.001	0.019
T-C, mmol/L	< 0.001	0.133	0.766	< 0.001	0.084	0.626	< 0.001	0.058	0.722
HDL-C, mmol/L	< 0.001	< 0.001	0.195	< 0.001	< 0.001	0.700	< 0.001	< 0.001	0.276
LDL-C, mmol/L	0.056	< 0.001	0.726	0.049	< 0.001	0.932	0.062	< 0.001	0.736
T-C:HDL-C ratio	0.008	< 0.001	0.309	0.005	< 0.001	0.974	0.004	< 0.001	0.212

Abbreviations: BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LDL-C, low-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Tables 6S II (A,B,C). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms *(*genotype) on body composition in black and white adequate reporter women, adjusted for age.

Table 6S II A.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* -174 G>C n=144**								***IL-6* -174 G>C n=122**							
Fat, % E	0.271	0.169	0.184	0.186	0.412	0.467	0.265	0.738	0.687	0.634	0.419	0.562	0.796	0.495	0.483	0.390
SFA, % E	0.199	0.097	0.149	0.160	0.481	0.915	0.237	0.515	0.852	0.803	0.888	0.816	0.853	0.708	0.500	0.282
MUFA, % E	0.199	0.140	0.124	0.135	0.376	0.616	0.255	0.417	0.810	0.659	0.636	0.829	0.749	0.329	0.460	0.444
PUFA, % E	0.198	0.147	0.087	0.148	0.387	0.581	0.296	0.099	0.469	0.546	0.408	0.685	0.654	0.637	0.958	0.913
P:S ratio	0.086	0.038	0.031	0.051	0.224	0.926	0.147	0.148	0.208	0.203	0.548	0.426	0.237	0.896	0.344	0.191
n-3 PUFA, % E	0.218	0.260	0.164	0.186	0.278	0.461	0.390	0.149	0.141	0.068	0.619	0.330	0.169	0.599	0.133	0.059
n-6 PUFA, % E	0.142	0.099	0.054	0.097	0.312	0.686	0.284	0.111	0.559	0.603	0.530	0.769	0.701	0.599	0.959	0.898
n-6:n-3 PUFA ratio	0.062	0.059	0.021	0.037	0.109	0.450	0.294	0.120	0.100	0.041	0.434	0.204	0.068	0.904	0.188	0.139
ALA, % E	0.071	0.031	0.025	0.035	0.157	0.667	0.208	0.159	0.259	0.143	0.895	0.466	0.264	0.283	0.127	0.139
LA, % E	0.130	0.087	0.047	0.087	0.295	0.720	0.268	0.113	0.557	0.600	0.533	0.770	0.698	0.597	0.959	0.895
AA, % E	0.385	0.515	0.323	0.341	0.416	0.378	0.644	0.417	0.371	0.237	0.151	0.210	0.530	0.708	0.915	0.752
EPA, %E	0.499	0.685	0.507	0.530	0.507	0.418	0.433	0.649	0.168	0.097	0.137	0.187	0.150	0.729	0.480	0.207
DHA, %E	0.589	0.852	0.620	0.640	0.547	0.415	0.273	0.881	0.118	0.088	0.152	0.161	0.109	0.595	0.347	0.174

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S II B.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* IVS3+281 G>T n=130**								***IL-6* IVS3+281 G>T n=121**							
Fat, % E	0.011	0.009	0.137	0.005	0.015	0.563	0.033	0.037	0.231	0.510	0.602	0.320	0.310	0.540	0.265	0.760
SFA, % E	0.017	0.007	0.167	0.006	0.058	0.617	0.081	0.242	0.341	0.569	0.528	0.360	0.234	0.611	0.204	0.668
MUFA, % E	0.010	0.010	0.357	0.008	0.040	0.846	0.009	0.043	0.446	0.979	0.921	0.569	0.824	0.090	0.985	0.598
PUFA, % E	0.112	0.104	0.626	0.097	0.112	0.561	0.201	0.098	0.406	0.352	0.429	0.450	0.590	0.229	0.397	0.458
P:S ratio	0.410	0.607	0.787	0.590	0.411	0.838	0.572	0.407	0.808	0.489	0.697	0.820	0.899	0.185	0.814	0.488
n-3 PUFA, % E	0.816	0.749	0.607	0.654	1.000	0.399	0.019	0.467	0.163	0.138	0.092	0.034	0.186	0.192	0.043	0.051
n-6 PUFA, % E	0.147	0.156	0.654	0.140	0.156	0.457	0.226	0.240	0.508	0.417	0.409	0.426	0.730	0.147	0.549	0.511
n-6:n-3 PUFA ratio	0.291	0.400	0.381	0.323	0.233	0.376	0.061	0.127	0.199	0.166	0.090	0.054	0.324	0.709	0.102	0.084
ALA, % E	0.303	0.300	0.363	0.210	0.706	0.456	0.102	0.492	0.096	0.092	0.141	0.048	0.142	0.172	0.006	0.007
LA, % E	0.157	0.166	0.670	0.151	0.165	0.470	0.235	0.254	0.505	0.414	0.406	0.423	0.727	0.145	0.548	0.506
AA, % E	0.016	0.010	0.433	0.014	0.052	0.646	0.254	0.909	0.581	0.585	0.468	0.503	0.576	0.885	0.746	0.930
EPA, %E	0.535	0.446	0.664	0.318	0.915	0.415	0.057	0.599	0.448	0.273	0.090	0.074	0.484	0.852	0.488	0.514
DHA, %E	0.470	0.405	0.767	0.304	0.888	0.444	0.052	0.682	0.455	0.289	0.240	0.135	0.507	0.974	0.646	0.501

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S II C.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* IVS4+869 A>G n=130**								***IL-6* IVS4+869 A>G n=121**							
Fat, % E	0.020	0.012	0.129	0.006	0.032	0.597	0.054	0.112	0.914	0.859	0.988	0.853	0.801	0.437	0.433	0.502
SFA, % E	0.060	0.031	0.249	0.024	0.148	0.592	0.253	0.715	0.936	0.911	0.832	0.930	0.983	0.683	0.739	0.632
MUFA, % E	0.052	0.046	0.629	0.038	0.158	0.538	0.025	0.257	0.958	0.816	0.981	0.893	0.790	0.317	0.466	0.553
PUFA, % E	0.020	0.014	0.616	0.013	0.041	0.575	0.110	0.051	0.956	0.848	0.686	0.962	0.983	0.477	0.881	0.860
P:S ratio	0.205	0.255	0.768	0.267	0.264	0.841	0.529	0.189	0.811	0.674	0.698	0.805	0.900	0.622	0.640	0.639
n-3 PUFA, % E	0.961	0.942	0.652	0.924	0.868	0.510	0.008	0.233	0.409	0.209	0.427	0.369	0.237	0.182	0.426	0.270
n-6 PUFA, % E	0.025	0.019	0.674	0.019	0.051	0.491	0.116	0.126	0.909	0.729	0.527	0.879	0.955	0.461	0.815	0.734
n-6:n-3 PUFA ratio	0.075	0.095	0.762	0.122	0.085	0.517	0.024	0.064	0.254	0.078	0.128	0.198	0.101	0.537	0.265	0.245
ALA, % E	0.746	0.805	0.636	0.613	0.844	0.521	0.028	0.248	0.270	0.098	0.456	0.259	0.099	0.070	0.101	0.110
LA, % E	0.027	0.021	0.679	0.021	0.055	0.501	0.124	0.134	0.905	0.724	0.520	0.872	0.953	0.461	0.812	0.731
AA, % E	0.012	0.007	0.214	0.008	0.067	0.794	0.359	0.882	0.265	0.235	0.205	0.305	0.495	0.816	0.781	0.703
EPA, %E	0.708	0.620	0.739	0.506	0.966	0.575	0.035	0.425	0.462	0.453	0.103	0.281	0.416	0.814	0.827	0.730
DHA, %E	0.621	0.549	0.947	0.449	0.937	0.636	0.035	0.548	0.455	0.401	0.237	0.334	0.353	0.698	0.556	0.627

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 6S III (A,B,C). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms *(*genotypes) on serum lipids in 144 black and 122 white adequate reporter women, adjusted for age and fat mass.

Table 6S III A.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	IL-6 174 G>C, n=144					**IL-6 174 G>C, n=122**				
Fat, % E	0.217	0.291	0.540	0.723	0.828	0.214	0.123	0.562	0.186	0.560
SFA, % E	0.129	0.056	0.416	0.261	0.851	0.346	0.064	0.405	0.115	0.574
MUFA, % E	0.054	0.469	0.714	0.564	0.362	0.105	0.107	0.681	0.142	0.421
PUFA, % E	0.423	0.680	0.359	0.706	0.295	0.425	0.701	0.834	0.607	0.621
P:S ratio	0.639	0.730	0.408	0.584	0.237	0.358	0.254	0.889	0.241	0.468
n-3 PUFA, % E	0.467	0.903	0.059	0.563	0.088	0.101	0.321	0.970	0.317	0.465
n-6 PUFA, % E	0.409	0.644	0.517	0.713	0.387	0.306	0.538	0.866	0.462	0.529
n-6:n-3 PUFA ratio	0.544	0.442	0.143	0.853	0.615	0.066	0.318	0.791	0.248	0.307
ALA, % E	0.846	0.957	0.071	0.856	0.157	0.141	0.117	0.799	0.123	0.351
LA, % E	0.406	0.644	0.523	0.715	0.390	0.288	0.497	0.892	0.432	0.520
AA, % E	0.919	0.879	0.404	0.346	0.334	0.153	0.571	0.668	0.442	0.388
EPA, %E	0.083	0.468	0.124	0.123	0.021	0.178	0.595	0.994	0.598	0.671
DHA, %E	0.122	0.547	0.060	0.159	0.014	0.244	0.823	0.794	0.752	0.660

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S III B.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	IVS3+281 G>T n=135					**IVS3+281 G>T n=122**				
Fat, % E	0.777	0.667	0.514	0.593	0.754	0.090	0.107	0.966	0.051	0.187
SFA, % E	0.339	0.624	0.590	0.536	0.389	0.135	0.519	0.902	0.346	0.713
MUFA, % E	0.456	0.907	0.278	0.978	0.346	0.148	0.327	0.934	0.155	0.336
PUFA, % E	0.165	0.216	0.561	0.356	0.746	0.171	0.114	0.789	0.060	0.091
P:S ratio	0.144	0.181	0.825	0.257	0.465	0.758	0.192	0.740	0.184	0.192
n-3 PUFA, % E	0.874	0.647	0.554	0.393	0.380	0.988	0.831	0.308	0.898	0.532
n-6 PUFA, % E	0.112	0.176	0.424	0.401	0.845	0.211	0.148	0.821	0.095	0.126
n-6:n-3 PUFA ratio	0.577	0.601	0.216	0.816	0.425	0.419	0.190	0.372	0.147	0.186
ALA, % E	0.835	0.501	0.631	0.741	0.981	0.607	0.979	0.455	0.968	0.571
LA, % E	0.114	0.173	0.418	0.396	0.846	0.211	0.146	0.833	0.095	0.130
AA, % E	0.405	0.619	0.303	0.742	0.541	0.833	0.940	0.226	0.959	0.365
EPA, %E	0.561	0.233	0.065	0.752	0.379	0.825	0.824	0.506	0.862	0.681
DHA, %E	0.470	0.143	0.029	0.510	0.318	0.673	0.707	0.537	0.798	0.857

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S III C.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	***IL-6* IVS4+869 A>G n=130**					***IL-6* IVS4+869 A>G n=121**				
Fat, % E	0.496	0.525	0.771	0.821	0.929	0.401	0.057	0.759	0.010	0.056
SFA, % E	0.480	0.188	0.284	0.468	0.821	0.080	0.702	0.980	0.520	0.784
MUFA, % E	0.464	0.355	0.341	0.444	0.360	0.665	0.192	0.597	0.040	0.082
PUFA, % E	0.261	0.757	0.974	0.917	0.952	0.513	0.109	0.522	0.023	0.034
P:S ratio	0.659	0.674	0.436	0.788	0.727	0.821	0.172	0.567	0.069	0.060
n-3 PUFA. % E	0.223	0.397	0.114	0.184	0.015	0.791	0.853	0.323	0.948	0.552
n-6 PUFA, % E	0.287	0.757	0.920	0.959	0.990	0.592	0.119	0.544	0.033	0.041
n-6:n-3 PUFA ratio	0.800	0.901	0.140	0.464	0.111	0.672	0.202	0.738	0.136	0.383
ALA, % E	0.239	0.764	0.059	0.274	0.029	0.888	0.813	0.616	0.674	0.375
LA, % E	0.284	0.756	0.919	0.958	0.991	0.578	0.118	0.550	0.033	0.043
AA, % E	0.913	0.325	0.780	0.230	0.776	0.991	0.918	0.333	0.970	0.488
EPA, %E	0.304	0.216	0.432	0.150	0.084	0.636	0.723	0.405	0.871	0.736
DHA, %E	0.169	0.122	0.301	0.075	0.055	0.493	0.566	0.371	0.762	0.826

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 6S IV (A,B,C). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on body composition in 144 black and 122 white adequate reporter women, adjusted for age.

Table 6S IV A.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* -174 G>C n=144**								***IL-6* -174 G>C n=122**							
Fat, % E	0.271	0.169	0.184	0.186	0.412	0.467	0.265	0.738	0.450	0.376	0.593	0.343	0.468	0.299	0.386	0.183
SFA, % E	0.199	0.097	0.149	0.160	0.481	0.915	0.237	0.515	0.691	0.576	0.890	0.578	0.903	0.865	0.607	0.320
MUFA, % E	0.199	0.140	0.124	0.135	0.376	0.616	0.255	0.417	0.641	0.390	0.915	0.613	0.452	0.144	0.360	0.230
PUFA, % E	0.198	0.147	0.087	0.148	0.387	0.581	0.296	0.099	0.988	0.688	0.902	0.928	0.912	0.304	0.892	0.845
P:S ratio	0.086	0.038	0.031	0.051	0.224	0.926	0.147	0.148	0.649	0.365	0.889	0.590	0.624	0.589	0.448	0.324
n-3 PUFA. % E	0.218	0.260	0.164	0.186	0.278	0.461	0.390	0.149	0.053	0.027	0.421	0.151	0.061	0.322	0.120	0.060
n-6 PUFA, % E	0.142	0.099	0.054	0.097	0.312	0.686	0.284	0.111	0.926	0.627	0.950	0.960	0.887	0.263	0.927	0.751
n-6:n-3 PUFA ratio	0.062	0.059	0.021	0.037	0.109	0.450	0.294	0.120	0.085	0.028	0.379	0.150	0.054	0.759	0.101	0.070
ALA, % E	0.071	0.031	0.025	0.035	0.157	0.667	0.208	0.159	0.107	0.055	0.687	0.253	0.130	0.436	0.058	0.093
LA, % E	0.130	0.087	0.047	0.087	0.295	0.720	0.268	0.113	0.921	0.623	0.961	0.970	0.883	0.261	0.924	0.745
AA, % E	0.385	0.515	0.323	0.341	0.416	0.378	0.644	0.417	0.681	0.714	0.506	0.487	0.607	0.486	0.923	0.716
EPA, %E	0.499	0.685	0.507	0.530	0.507	0.418	0.433	0.649	0.069	0.040	0.118	0.102	0.101	0.586	0.434	0.109
DHA, %E	0.589	0.852	0.620	0.640	0.547	0.415	0.273	0.881	0.054	0.043	0.106	0.089	0.073	0.467	0.572	0.131

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio

Table 6S IV B.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* IVS3+281 G>T n=135**								***IL-6* IVS3+281 G>T n=122**							
Fat, % E	0.350	0.218	0.445	0.308	0.381	0.388	0.474	0.912	0.703	0.630	0.875	0.578	0.727	0.442	0.460	0.309
SFA, % E	0.485	0.506	0.118	0.662	0.871	0.718	0.652	0.934	0.761	0.745	0.816	0.852	0.906	0.899	0.504	0.352
MUFA, % E	0.418	0.303	0.476	0.408	0.494	0.349	0.526	0.733	0.831	0.558	0.860	0.661	0.543	0.157	0.373	0.289
PUFA, % E	0.288	0.162	0.705	0.154	0.177	0.151	0.384	0.815	0.756	0.572	0.447	0.881	0.840	0.237	0.840	0.794
P:S ratio	0.685	0.513	0.479	0.478	0.448	0.309	0.538	0.760	0.507	0.365	0.497	0.569	0.696	0.346	0.328	0.329
n-3 PUFA. % E	0.879	0.721	0.235	0.848	0.918	0.502	0.340	0.715	0.200	0.081	0.206	0.170	0.096	0.074	0.198	0.133
n-6 PUFA, % E	0.307	0.169	0.770	0.185	0.164	0.122	0.260	0.909	0.659	0.460	0.331	0.739	0.767	0.221	0.814	0.670
n-6:n-3 PUFA ratio	0.772	0.790	0.910	0.839	0.468	0.211	0.235	0.996	0.111	0.030	0.044	0.076	0.048	0.396	0.103	0.097
ALA, % E	0.362	0.482	0.180	0.317	0.510	0.557	0.209	0.413	0.107	0.032	0.241	0.110	0.038	0.037	0.029	0.037
LA, % E	0.303	0.168	0.770	0.185	0.163	0.127	0.260	0.926	0.653	0.455	0.326	0.730	0.762	0.221	0.811	0.665
AA, % E	0.314	0.270	0.250	0.188	0.829	0.466	0.180	0.826	0.386	0.417	0.360	0.342	0.423	0.580	0.659	0.521
EPA, %E	0.492	0.416	0.379	0.410	0.906	0.726	0.675	0.809	0.467	0.338	0.159	0.305	0.430	0.521	0.881	0.562
DHA, %E	0.434	0.375	0.616	0.298	0.839	0.648	0.510	0.777	0.537	0.404	0.292	0.417	0.453	0.400	0.837	0.721

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S IV C.

	Black								White							
	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
	***IL-6* IVS4+869 A>G n=130**								***IL-6* IVS4+869 A>G n=121**							
Fat, % E	0.725	0.426	0.390	0.518	0.698	0.652	0.729	0.604	0.159	0.290	0.309	0.200	0.176	0.914	0.265	0.760
SFA, % E	0.535	0.543	0.162	0.715	0.966	0.599	0.756	0.702	0.260	0.414	0.360	0.256	0.154	0.453	0.204	0.668
MUFA, % E	0.693	0.542	0.413	0.623	0.916	0.917	0.938	0.776	0.286	0.607	0.517	0.343	0.508	0.252	0.985	0.598
PUFA, % E	0.770	0.428	0.916	0.440	0.422	0.221	0.331	0.776	0.248	0.166	0.194	0.265	0.329	0.535	0.397	0.458
P:S ratio	0.745	0.963	0.971	0.962	0.824	0.433	0.310	0.876	0.553	0.265	0.394	0.528	0.755	0.375	0.814	0.488
n-3 PUFA. % E	0.984	0.919	0.510	0.910	0.757	0.371	0.208	0.790	0.319	0.328	0.258	0.112	0.417	0.423	0.043	0.051
n-6 PUFA, % E	0.835	0.454	0.817	0.522	0.404	0.168	0.222	0.827	0.324	0.207	0.185	0.252	0.434	0.383	0.549	0.511
n-6:n-3 PUFA ratio	0.833	0.981	0.525	0.820	0.664	0.229	0.157	0.866	0.148	0.111	0.052	0.034	0.231	0.869	0.102	0.084
ALA, % E	0.234	0.268	0.141	0.186	0.279	0.239	0.127	0.203	0.179	0.199	0.294	0.112	0.285	0.323	0.006	0.007
LA, % E	0.822	0.446	0.832	0.514	0.401	0.175	0.220	0.850	0.321	0.205	0.182	0.249	0.432	0.378	0.548	0.506
AA, % E	0.518	0.488	0.320	0.295	0.928	0.340	0.116	0.856	0.840	0.927	0.840	0.803	0.957	0.502	0.746	0.930
EPA, %E	0.496	0.454	0.783	0.390	0.964	0.607	0.537	0.994	0.632	0.474	0.209	0.163	0.742	0.864	0.488	0.514
DHA, %E	0.459	0.427	0.932	0.283	0.884	0.587	0.391	0.959	0.553	0.393	0.344	0.197	0.645	0.876	0.646	0.501

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Tables 6S V (A,B,C). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on serum lipids in 144 black and 122 white adequate reporter women, adjusted for age and fat mass.

Table 6S V A.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	IL-6 174 G>C, n=144					**IL-6 174 G>C, n=122**				
Fat, % E	0.214	0.123	0.562	0.186	0.560	0.098	0.119	0.313	0.552	0.867
SFA, % E	0.346	0.064	0.405	0.115	0.574	0.131	0.017	0.198	0.094	0.623
MUFA, % E	0.105	0.107	0.681	0.142	0.421	0.015	0.239	0.535	0.321	0.154
PUFA, % E	0.425	0.701	0.834	0.607	0.621	0.520	0.564	0.385	0.983	0.687
P:S ratio	0.358	0.254	0.889	0.241	0.468	0.766	0.574	0.853	0.524	0.825
n-3 PUFA. % E	0.101	0.321	0.970	0.317	0.465	0.237	0.775	0.047	0.991	0.100
n-6 PUFA, % E	0.306	0.538	0.866	0.462	0.529	0.560	0.576	0.486	0.895	0.798
n-6:n-3 PUFA ratio	0.066	0.318	0.791	0.248	0.307	0.680	0.318	0.084	0.636	0.365
ALA, % E	0.141	0.117	0.799	0.123	0.351	0.628	0.768	0.022	0.568	0.056
LA, % E	0.288	0.497	0.892	0.432	0.520	0.562	0.580	0.488	0.899	0.797
AA, % E	0.153	0.571	0.668	0.442	0.388	0.866	0.694	0.850	0.600	0.910
EPA, %E	0.178	0.595	0.994	0.598	0.671	0.044	0.480	0.707	0.948	0.386
DHA, %E	0.244	0.823	0.794	0.752	0.660	0.068	0.374	0.465	0.432	0.184

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S V B.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	IVS3+281 G>T n=135					**IVS3+281 G>T n=122**				
Fat, % E	0.478	0.173	0.849	0.725	0.516	0.300	0.714	0.419	0.841	0.650
SFA, % E	0.714	0.070	0.469	0.847	0.266	0.194	0.671	0.129	0.421	0.234
MUFA, % E	0.539	0.760	0.288	0.755	0.126	0.501	0.284	0.426	0.348	0.284
PUFA, % E	0.610	0.209	0.853	0.902	0.944	0.976	0.620	0.804	0.986	0.738
P:S ratio	0.771	0.755	0.872	0.838	0.837	0.377	0.962	0.391	0.446	0.505
n-3 PUFA. % E	0.124	0.604	0.875	0.174	0.515	0.416	0.191	0.407	0.091	0.246
n-6 PUFA, % E	0.699	0.274	0.947	0.834	0.873	0.948	0.544	0.741	0.890	0.806
n-6:n-3 PUFA ratio	0.232	0.282	0.644	0.304	0.423	0.419	0.556	0.655	0.303	0.501
ALA, % E	0.689	0.935	0.468	0.279	0.250	0.829	0.453	0.565	**0.018**	0.104
LA, % E	0.690	0.267	0.939	0.815	0.873	0.941	0.544	0.733	0.883	0.800
AA, % E	0.517	0.943	0.600	0.311	0.836	0.717	0.768	0.232	0.456	0.270
EPA, %E	0.278	0.473	0.976	0.357	0.716	0.110	0.134	0.157	0.959	0.351
DHA, %E	0.211	0.405	0.864	0.378	0.871	0.107	0.072	0.050	0.934	0.084

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S V C.

	Black					White				
	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
	***IL-6* IVS4+869 A>G n=130**					***IL-6* IVS4+869 A>G n=121**				
Fat, % E	0.049	0.788	0.989	0.993	0.848	0.862	0.703	0.817	0.529	0.957
SFA, % E	0.073	0.481	0.526	0.205	0.262	0.335	0.601	0.705	0.555	0.464
MUFA, % E	0.301	0.658	0.630	0.343	0.436	0.509	0.936	0.444	0.989	0.505
PUFA, % E	0.059	0.504	0.593	0.459	0.321	0.152	0.252	0.872	0.323	0.508
P:S ratio	0.433	0.614	0.288	0.338	0.170	0.157	0.206	0.971	0.265	0.392
n-3 PUFA. % E	0.828	0.969	0.104	0.495	0.111	0.930	0.740	0.408	0.415	0.312
n-6 PUFA, % E	0.074	0.555	0.638	0.481	0.381	0.106	0.207	0.706	0.364	0.592
n-6:n-3 PUFA ratio	0.166	0.459	0.089	0.149	0.029	0.585	0.618	0.260	0.825	0.477
ALA, % E	0.481	0.922	0.114	0.435	0.115	0.796	0.576	0.794	0.764	0.881
LA, % E	0.073	0.558	0.640	0.483	0.384	0.107	0.203	0.699	0.360	0.595
AA, % E	0.627	0.642	0.216	0.921	0.406	0.492	0.539	0.230	0.704	0.488
EPA, %E	0.616	0.850	0.326	0.552	0.275	0.594	0.230	0.047	0.785	0.315
DHA, %E	0.513	0.991	0.398	0.746	0.419	0.491	0.136	0.023	0.512	0.290

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 6S VI (A,B). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (genotype) on body composition in 266 white and black adequate reporter women, adjusted for age and ethnicity.

Table 6S VI A.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS3+281 G>T n=257**				
Fat, % E	0.150	0.085	0.383	0.093	0.078	0.169	0.124	0.124
SFA, % E	0.736	0.578	0.515	0.630	0.622	0.554	0.409	0.699
MUFA, % E	0.448	0.188	0.889	0.308	0.300	0.146	0.065	0.270
PUFA, % E	0.416	0.359	0.557	0.354	0.289	0.130	0.780	0.268
P:S ratio	0.770	0.809	0.604	0.718	0.672	0.383	0.976	0.586
n-3 PUFA. % E	0.433	0.222	0.692	0.299	0.384	0.595	0.212	0.420
n-6 PUFA, % E	0.459	0.444	0.559	0.411	0.349	0.120	0.773	0.336
n-6:n-3 PUFA ratio	0.378	0.376	0.421	0.276	0.434	0.649	0.457	0.154
ALA, % E	0.645	0.465	0.707	0.392	0.567	0.659	0.244	0.827
LA, % E	0.475	0.463	0.563	0.430	0.361	0.126	0.781	0.351
AA, % E	0.385	0.352	0.896	0.366	0.551	0.922	0.735	0.942
EPA, %E	0.304	0.182	0.377	0.219	0.374	0.614	0.144	0.480
DHA, %E	0.340	0.211	0.451	0.218	0.375	0.641	0.094	0.476

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S VI B.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS4+869 A>G n=251**				
Fat, % E	0.009	0.029	0.179	0.011	0.018	0.861	0.069	0.042
SFA, % E	0.014	0.009	0.378	0.015	0.049	0.759	0.050	0.255
MUFA, % E	0.057	0.113	0.747	0.096	0.260	0.510	0.121	0.131
PUFA, % E	0.157	0.272	0.310	0.187	0.275	0.559	0.380	0.135
P:S ratio	0.461	0.523	0.559	0.514	0.747	0.613	0.899	0.450
n-3 PUFA. % E	0.645	0.678	0.606	0.393	0.620	0.106	0.010	0.139
n-6 PUFA, % E	0.201	0.350	0.288	0.203	0.376	0.423	0.420	0.194
n-6:n-3 PUFA ratio	0.096	0.167	0.053	0.035	0.171	0.444	0.041	0.030
ALA, % E	0.774	0.776	0.845	0.722	0.894	0.296	0.049	0.420
LA, % E	0.209	0.364	0.289	0.211	0.385	0.444	0.431	0.201
AA, % E	0.208	0.184	0.688	0.306	0.472	0.415	0.795	0.708
EPA, %E	0.807	0.688	0.694	0.483	0.893	0.137	0.073	0.502
DHA, %E	0.656	0.561	0.707	0.374	0.941	0.198	0.133	0.578

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 6S VII (A,B). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (genotype) on serum lipids in 266 white and black adequate reporter women, adjusted for age, fat mass, and ethnicity.

Table 6S VII A.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS3+281 G>T n=257**		
Fat, % E	0.565	0.522	0.276	0.057	0.038
SFA, % E	0.526	0.190	0.322	0.263	0.583
MUFA, % E	0.268	0.596	0.328	0.327	0.100
PUFA, % E	0.271	0.158	0.286	0.021	0.133
P:S ratio	0.738	0.059	0.435	0.041	0.475
n-3 PUFA. % E	0.589	0.230	0.332	0.134	0.014
n-6 PUFA, % E	0.306	0.162	0.250	0.026	0.103
n-6:n-3 PUFA ratio	0.496	0.056	0.704	0.006	0.055
ALA, % E	0.518	0.386	0.278	0.111	0.027
LA, % E	0.296	0.155	0.244	0.026	0.102
AA, % E	0.906	0.855	0.285	0.988	0.735
EPA, %E	0.492	0.309	0.718	0.336	0.110
DHA, %E	0.329	0.251	0.902	0.202	0.129

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S VII B.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS4+869 A>G n=251**		
Fat, % E	0.527	0.212	0.242	0.037	0.013
SFA, % E	0.669	0.644	0.987	0.596	0.596
MUFA, % E	0.989	0.444	0.407	0.139	0.041
PUFA, % E	0.097	0.195	0.133	0.029	0.017
P:S ratio	0.219	0.360	0.278	0.132	0.089
n-3 PUFA. % E	0.953	0.706	0.733	0.881	0.722
n-6 PUFA, % E	0.078	0.214	0.119	0.039	0.017
n-6:n-3 PUFA ratio	0.311	0.278	0.473	0.076	0.148
ALA, % E	0.963	0.746	0.620	0.936	0.790
LA, % E	0.077	0.214	0.114	0.039	0.017
AA, % E	0.477	0.998	0.284	0.733	0.238
EPA, %E	0.837	0.883	0.763	0.726	0.474
DHA, %E	0.865	0.865	0.742	0.883	0.814

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 6S VIII (A,B). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on body composition in 266 white and black adequate reporter women, adjusted for age and ethnicity.

Table 6S VIII A.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS3+281 G>T n=257**				
Fat, % E	0.487	0.266	0.670	0.454	0.466	0.209	0.315	0.523
SFA, % E	0.763	0.692	0.279	0.746	0.854	0.933	0.520	0.640
MUFA, % E	0.477	0.222	0.851	0.350	0.313	0.064	0.198	0.265
PUFA, % E	0.579	0.428	0.958	0.576	0.368	0.070	0.620	0.825
P:S ratio	0.958	0.864	0.762	0.939	0.635	0.208	0.923	0.763
n-3 PUFA. % E	0.229	0.098	0.549	0.147	0.211	0.382	0.217	0.350
n-6 PUFA, % E	0.678	0.530	0.886	0.731	0.403	0.061	0.598	0.893
n-6:n-3 PUFA ratio	0.480	0.355	0.312	0.265	0.616	0.548	0.633	0.507
ALA, % E	0.637	0.463	0.974	0.578	0.430	0.396	0.103	0.516
LA, % E	0.678	0.535	0.890	0.746	0.410	0.067	0.595	0.880
AA, % E	0.263	0.234	0.781	0.247	0.502	0.996	0.940	0.798
EPA, %E	0.191	0.084	0.248	0.108	0.278	0.481	0.473	0.531
DHA, %E	0.210	0.098	0.315	0.111	0.284	0.497	0.923	0.724

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio

Table 6S VIII B.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS4+869 A>G n=251**				
Fat, % E	0.443	0.896	0.204	0.571	0.503	0.634	0.734	0.459
SFA, % E	0.715	0.935	0.123	0.632	0.362	0.380	0.185	0.537
MUFA, % E	0.685	0.860	0.394	0.766	0.729	0.419	0.890	0.969
PUFA, % E	0.428	0.660	0.378	0.534	0.773	0.173	0.899	0.491
P:S ratio	0.373	0.417	0.607	0.422	0.760	0.217	0.629	0.557
n-3 PUFA. % E	0.548	0.560	0.735	0.312	0.907	0.551	0.287	0.437
n-6 PUFA, % E	0.432	0.663	0.331	0.467	0.846	0.114	0.700	0.535
n-6:n-3 PUFA ratio	0.241	0.336	0.133	0.123	0.659	0.172	0.804	0.322
ALA, % E	0.863	0.992	0.829	0.792	0.922	0.566	0.164	0.568
LA, % E	0.432	0.659	0.338	0.461	0.837	0.120	0.697	0.544
AA, % E	0.917	0.916	0.507	0.805	0.629	0.305	0.602	0.657
EPA, %E	0.540	0.403	0.504	0.242	0.958	0.663	0.488	0.850
DHA, %E	0.440	0.329	0.485	0.178	0.831	0.574	0.728	0.833

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 6S IX (A,B). P-values for interaction between dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on serum lipids in 266 white and black adequate reporter women, adjusted for age, fat mass and ethnicity.

Table 6S IX A.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS3+281 G>T n=257**		
Fat, % E	0.998	0.939	0.444	0.698	0.245
SFA, % E	0.973	0.086	0.490	0.108	0.584
MUFA, % E	0.229	0.326	0.370	0.208	0.087
PUFA, % E	0.474	0.203	0.291	0.391	0.622
P:S ratio	0.763	0.095	0.175	0.215	0.700
n-3 PUFA. % E	0.290	0.491	0.106	0.157	0.004
n-6 PUFA, % E	0.456	0.208	0.228	0.451	0.505
n-6:n-3 PUFA ratio	0.357	0.169	0.901	0.129	0.155
ALA, % E	0.572	0.225	0.130	0.037	0.008
LA, % E	0.454	0.195	0.212	0.439	0.489
AA, % E	0.965	0.915	0.544	0.912	0.856
EPA, %E	0.175	0.582	0.406	0.437	0.037
DHA, %E	0.154	0.438	0.607	0.385	0.068

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S IX B.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS4+869 A>G n=251**		
Fat, % E	0.290	0.464	0.624	0.736	0.647
SFA, % E	0.593	0.548	0.840	0.457	0.280
MUFA, % E	0.825	0.962	0.377	0.615	0.222
PUFA, % E	0.023	0.211	0.614	0.500	0.799
P:S ratio	0.053	0.230	0.738	0.415	0.497
n-3 PUFA. % E	0.812	0.775	0.569	0.982	0.341
n-6 PUFA, % E	0.018	0.203	0.500	0.550	0.966
n-6:n-3 PUFA ratio	0.082	0.364	0.848	0.536	0.459
ALA, % E	0.608	0.736	0.300	0.903	0.293
LA, % E	0.018	0.204	0.477	0.565	0.998
AA, % E	0.239	0.981	0.423	0.906	0.497
EPA, %E	0.652	0.598	0.855	0.491	0.444
DHA, %E	0.683	0.552	0.517	0.578	0.759

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 6S X (A,B). P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *IL-6* polymorphisms (genotype) on body composition in 266 white and black adequate reporter women, adjusted for age.

Table 6S X A.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS3+281 G>T n=257**				
Fat, % E	0.171	0.114	0.512	0.118	0.259	0.957	0.962	0.429
SFA, % E	0.112	0.045	0.424	0.054	0.241	0.856	0.605	0.872
MUFA, % E	0.144	0.162	0.815	0.154	0.418	0.980	0.536	0.504
PUFA, % E	0.057	0.024	0.725	0.075	0.077	0.975	0.566	0.228
P:S ratio	0.267	0.231	0.758	0.400	0.249	0.990	0.570	0.348
n-3 PUFA. % E	0.692	0.622	0.348	0.588	0.455	0.156	0.063	0.134
n-6 PUFA, % E	0.065	0.027	0.680	0.082	0.085	0.961	0.512	0.376
n-6:n-3 PUFA ratio	0.055	0.023	0.500	0.081	0.015	0.224	0.077	0.151
ALA, % E	0.384	0.313	0.604	0.410	0.306	0.141	0.022	0.052
LA, % E	0.069	0.029	0.679	0.086	0.089	0.963	0.518	0.387
AA, % E	0.010	0.005	0.045	0.016	0.054	0.692	0.668	0.576
EPA, %E	0.643	0.645	0.087	0.339	0.639	0.515	0.445	0.484
DHA, %E	0.554	0.507	0.282	0.400	0.570	0.510	0.619	0.617

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S X B.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS4+869 A>G n=251**				
Fat, % E	0.761	0.518	0.476	0.718	0.591	0.358	0.683	0.137
SFA, % E	0.834	0.479	0.352	0.588	0.725	0.583	0.946	0.374
MUFA, % E	0.706	0.359	0.488	0.665	0.404	0.157	0.534	0.105
PUFA, % E	0.915	0.865	0.889	0.923	0.887	0.605	0.523	0.389
P:S ratio	0.477	0.884	0.886	0.671	0.409	0.497	0.639	0.614
n-3 PUFA. % E	0.231	0.204	0.099	0.062	0.275	0.368	0.179	0.231
n-6 PUFA, % E	0.833	0.867	0.869	0.880	0.872	0.582	0.529	0.590
n-6:n-3 PUFA ratio	0.974	0.827	0.635	0.588	0.972	0.807	0.280	0.992
ALA, % E	0.027	0.027	0.046	0.010	0.093	0.223	0.007	0.011
LA, % E	0.850	0.847	0.856	0.863	0.885	0.571	0.520	0.609
AA, % E	0.134	0.101	0.167	0.183	0.080	0.494	0.963	0.601
EPA, %E	0.626	0.452	0.127	0.175	0.522	0.916	0.905	0.865
DHA, %E	0.635	0.472	0.414	0.356	0.568	0.909	0.862	0.819

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 6S XI (A,B). P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *IL-6* polymorphisms (genotype) on serum lipids in 266 white and black adequate reporter women, adjusted for age and fat mass.

Table 6S XI A.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS3+281 G>T n=257**		
Fat, % E	0.512	0.259	0.957	0.962	0.429
SFA, % E	0.424	0.241	0.856	0.605	0.872
MUFA, % E	0.815	0.418	0.980	0.536	0.504
PUFA, % E	0.725	0.077	0.975	0.566	0.228
P:S ratio	0.758	0.249	0.990	0.570	0.348
n-3 PUFA. % E	0.348	0.455	0.156	0.063	0.134
n-6 PUFA, % E	0.680	0.085	0.961	0.512	0.376
n-6:n-3 PUFA ratio	0.500	0.015	0.224	0.077	0.151
ALA, % E	0.604	0.306	0.141	0.022	0.052
LA, % E	0.679	0.089	0.963	0.518	0.387
AA, % E	0.045	0.054	0.692	0.668	0.576
EPA, %E	0.087	0.639	0.515	0.445	0.484
DHA, %E	0.282	0.570	0.510	0.619	0.617

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S XI B.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS4+869 A>G n=251**		
Fat, % E	0.476	0.591	0.358	0.683	0.137
SFA, % E	0.352	0.725	0.583	0.946	0.374
MUFA, % E	0.488	0.404	0.157	0.534	0.105
PUFA, % E	0.889	0.887	0.605	0.523	0.389
P:S ratio	0.886	0.409	0.497	0.639	0.614
n-3 PUFA. % E	0.099	0.275	0.368	0.179	0.231
n-6 PUFA, % E	0.869	0.872	0.582	0.529	0.590
n-6:n-3 PUFA ratio	0.635	0.972	0.807	0.280	0.992
ALA, % E	0.046	0.093	0.223	0.007	0.011
LA, % E	0.856	0.885	0.571	0.520	0.609
AA, % E	0.167	0.080	0.494	0.963	0.601
EPA, %E	0.127	0.522	0.916	0.905	0.865
DHA, %E	0.414	0.568	0.909	0.862	0.819

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Supplementary Table 6S XII (A,B). P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on body composition in 266 white and black adequate reporter women, adjusted for age.

Table 6S XII A.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS3+281 G>T n=257**				
Fat, % E	0.749	0.609	0.710	0.833	0.735	0.909	0.769	0.434
SFA, % E	0.717	0.716	0.283	0.835	0.862	0.777	0.912	0.639
MUFA, % E	0.667	0.688	0.718	0.810	0.923	0.910	0.744	0.696
PUFA, % E	0.336	0.157	0.402	0.293	0.264	0.718	0.577	0.958
P:S ratio	0.430	0.259	0.326	0.351	0.393	0.818	0.292	0.608
n-3 PUFA. % E	0.384	0.327	0.086	0.347	0.196	0.091	0.196	0.179
n-6 PUFA, % E	0.301	0.128	0.344	0.256	0.223	0.671	0.473	0.794
n-6:n-3 PUFA	0.151	0.078	0.097	0.124	0.049	0.135	0.056	0.205
ALA, % E	0.076	0.055	0.080	0.066	0.062	0.086	0.024	0.051
LA, % E	0.295	0.126	0.340	0.251	0.221	0.677	0.469	0.780
AA, % E	0.999	0.851	0.146	0.920	0.642	0.346	0.379	0.472
EPA, %E	0.924	0.916	0.111	0.800	0.604	0.470	0.946	0.509
DHA, %E	0.950	0.966	0.280	0.945	0.665	0.346	0.933	0.579

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Table 6S XII B.

	Weight, kg	BMI, kg/m^2	Body fat, %	Fat Mass	Waist, cm	WHR	VAT, cm^2	SAT, cm^2
				***IL-6* IVS4+869 A>G n=251**				
Fat, % E	0.168	0.206	0.636	0.147	0.201	0.922	0.164	0.924
SFA, % E	0.227	0.338	0.757	0.289	0.344	0.999	0.319	0.935
MUFA, % E	0.276	0.463	0.895	0.287	0.594	0.383	0.856	0.498
PUFA, % E	0.251	0.115	0.271	0.163	0.206	0.778	0.207	0.654
P:S ratio	0.752	0.362	0.448	0.566	0.692	0.890	0.528	0.627
n-3 PUFA. % E	0.391	0.446	0.188	0.176	0.361	0.210	0.027	0.071
n-6 PUFA, % E	0.339	0.145	0.297	0.176	0.256	0.827	0.274	0.670
n-6:n-3 PUFA ratio	0.268	0.199	0.188	0.091	0.216	0.518	0.032	0.183
ALA, % E	0.069	0.088	0.081	0.036	0.123	0.123	0.002	0.004
LA, % E	0.332	0.142	0.290	0.173	0.254	0.840	0.273	0.655
AA, % E	0.874	0.777	0.431	0.742	0.847	0.810	0.911	0.703
EPA, %E	0.982	0.896	0.245	0.464	0.770	0.772	0.489	0.553
DHA, %E	0.925	0.821	0.485	0.598	0.718	0.720	0.570	0.551

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, IL-6, Interleukin-6 gene; LA, linoleic acid; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist hip ratio.

Supplementary Table 6S XIII (A,B) P-values for 3-way interaction between ethnic group, dietary fat intake (%E) and *IL-6* polymorphisms (allelic) on serum lipids in 266 white and black adequate reporter women, adjusted for age and fat mass.

Table 6S XIII A.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS3+281 G>T n=257**		
Fat, % E	0.298	0.496	0.816	0.767	0.967
SFA, % E	0.213	0.641	0.550	0.873	0.472
MUFA, % E	0.264	0.957	0.628	0.831	0.901
PUFA, % E	0.891	0.934	0.846	0.918	0.894
P:S ratio	0.961	0.533	0.395	0.782	0.555
n-3 PUFA. % E	0.598	0.502	0.961	0.586	0.690
n-6 PUFA, % E	0.969	0.835	0.767	0.883	0.965
n-6:n-3 PUFA ratio	0.938	0.773	0.845	0.805	0.982
ALA, % E	0.795	0.892	0.583	0.812	0.986
LA, % E	0.979	0.833	0.770	0.877	0.954
AA, % E	0.969	0.531	0.952	0.449	0.536
EPA, %E	0.419	0.283	0.360	0.534	0.771
DHA, %E	0.432	0.131	0.371	0.261	0.583

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

Table 6S XIII B.

	TAG, mmol/L	T-C, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	T-C:HDL-C ratio
			***IL-6* IVS4+869 A>G n=251**		
Fat, % E	0.211	0.800	0.831	0.653	0.974
SFA, % E	0.072	0.848	0.773	0.430	0.499
MUFA, % E	0.171	0.762	0.728	0.612	0.976
PUFA, % E	0.675	0.486	0.452	0.774	0.915
P:S ratio	0.316	0.444	0.358	0.877	0.780
n-3 PUFA. % E	0.802	0.763	0.110	0.299	0.080
n-6 PUFA, % E	0.526	0.417	0.336	0.788	0.940
n-6:n-3 PUFA ratio	0.980	0.857	0.053	0.441	0.080
ALA, % E	0.674	0.623	0.509	0.301	0.106
LA, % E	0.535	0.414	0.330	0.784	0.942
AA, % E	0.760	0.957	0.691	0.999	0.947
EPA, %E	0.641	0.334	0.026	0.817	0.252
DHA, %E	0.408	0.196	0.013	0.556	0.283

Abbreviations: AA, arachidonic acid; ALA, !-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid, HDL-C, high-density lipoprotein cholesterol; IL-6, Interleukin-6 gene; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MUFA, mono-unsaturated fat; percentage energy; %E; PUFA, polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6:n-3 PUFA ratio, omega-6:omega-3 polyunsaturated fatty acids ratio; P:S ratio, polyunsaturated:saturated fat ratio; SFA, saturated fatty acid; TAG, triacylglycerol; T-C, total cholesterol; T-C:HDL-C ratio, total cholesterol:HDL-cholesterol ratio.

CHAPTER TEN

APPENDIX

Reagents for the isolation of DNA from Whole Blood

TKM1 Buffer (pH 7.6)

	Final Conc.	MW	For 500ml	For 1000ml
Tris-HCl	10mM	121.00	0.6056	1.2112
KCl	10mM	74.56	0.3728	0.7456
$MgCl_2.6H_2O$	10mM	203.20	1.016	2.032
EDTA	2mM	372.24	0.372	0.744
dH_2O			to 500ml	

- Set the pH with HCl
- Autoclave
- Make up 1 volume which includes 2.5% NP40 and 1 volume without NP40

TKM2 Buffer (pH 7.6)

	Final Conc.	MW	For 200ml
Tris-HCl	10mM	121.00	0.242
KCl	10mM	74.56	0.149
$MgCl_2.6H_2O$	10mM	203.20	0.406
EDTA	2mM	372.24	0.1488
NaCl			4.675
dH_2O			to 200 ml

- Set the pH with HCl
- Autoclave

10% SDS

	Final Conc.	MW	For 200ml
SDS	10%		20
dH_2O			to 200 ml

- Autoclave

1X TE buffer (pH 8.0)

	Final Conc.	MW	For 100ml
Tris-HCl	10mM	121.00	0.121
EDTA	1mM	372.24	0.037
dH_2O			to 100 ml

- Set the pH with HCl
- Autoclave
-

5M $NaClO_4$

	Final Conc.	MW	For 100ml
$NaClO_4$	5M	122.4	61.2
dH_2O			to 100 ml

- Autoclave

Other Chemicals and Reagents

- Chloroform
- NP40
- Absolute ethanol

DNA Extraction from Whole Blood

- Draw 5mls of blood into an EDTA vacutainer tube (Purple top).
- Blood can be stored at 4ºC up to 1 week before the DNA is extracted.
- Transfer the blood to a sterile 15ml polyporpolene tube.
- Add 2 volumes (10ml) of TKM1 buffer containing 2.5% NP40.
- Mix by inverting several times and incubate at room temperature for 10 minutes in order to enhance the haemolysis of red blood cells.
- Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
- Decant off the supernatant containing leaving the white pellet at the bottom of the tube.
- Add 1 volume (5ml) of TKM1 buffer (without NP40)
- Invert and vortex the solution.
- Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
- Decant the supernatant leaving the white pellet in the bottom of the tube.
- Repeat steps 7-10 until the pellet in the bottom of the tube is clean and white.
- Add 800ul of TKM2 buffer and 50ul of the 10% SDS solution.
- Vortex and then mix using a blue pipette tip in order to assist in the lyses of the white blood cells.
- Incubate for 10 minutes at 55ºC in a water bath (make sure the element of the water bath is totally covered with dH_20).
- Add 150ul of 5M $NaClO_4$.
- Add 500ul of molecular biology grade chloroform.

- Vortex the solution.
- Transfer the solution to sterile 1.5ml microfuge tubes.
- Centrifuge at 1300rpm at room temperature for 5 minutes.
- Carefully transfer 500ul of the top aqueous phase to a new sterile microfuge tube.
- Add 1ml of absolute ethanol.
- Invert until DNA precipitates.
- Centrifuge at 1300rpm at room temperature for 5 minutes.
- Carefully tip off supernatant leaving the pellet in the bottom of the tube.
- Allow pellet to air dry completely.
- Add 100ul of 1XTE buffer.
- Incubate the tubes at 65ºC for 15 minutes in a heating block.
- Store DNA at 4ºC.

Reference: Lahiri et al.(1991) Nucleic acids research 19:54444

UCT/SSI STUDY 2010

NAME _____ SUBJECT NUMBER_____
DATE OF COMPLETION_____
COMPLETED BY _____

A. Food items (with FPM numbers)	B. Description of food item	C. Item Code	D. Amount usually eaten (g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten at least once a month
				T / day	D/week	T / month
DAIRY – BLUE						
1. Tea						
1. Coffee		**4037**				
1. Sugar in tea coffee						
2. Milk in tea/coffee						
2. Milk with porridge						
3. Buttermilk/maas						
4. Milk drinks						

A. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
5. Yoghurt						
6. Cottage cheese						
7. Hard cheese						
8. Processed cheese						
9. Ice cream & ice-lollies						
Other						
STARCH – BROWN						
1. Brown bread/rolls						
1. White bread/rolls						
2. Traditional bread/roti						
2. Fat cakes						
3. Breakfast cereals						
4. Maize porridge soft						

B. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
4. Maize porridge stiff						
4. Mabele/maltabella-soft						
4. Mabele /stiff						
4. Oats		**3239**				
5. Pasta without sauce						
6. Pasta dishes						
7. Rice						
7. Samp/mealie rice						
7. Wheat rice						
8. Pizza & savoury tart						
Other						
FATS – TAN						
1. Brick margarine		3484				

C. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
1. Tub margarine						
1. White margarine						
1. Butter						
2. Animal fat, i.e. lard						
3. Cream & substitutes						
4. Oils						
5.Salad dressing						
5. Mayonnaise						
Other						
SPREADS – PINK						
Cheese spread						
Fish paste		3109				
Honey/syrup		3988				

D. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
Jam						
Marmite		4030				
Meat spread i.e. Bovril		4029				
Peanut butter		3485				
Sandwich spread		3522				
Other						
EGGS – YELLOW						
Boiled		2867				
Fried						

Omelette						
Scrambled						
Other						
FRUIT – ORANGE						
1. Apples						
2. Bananas		3540				
3. Berries						
4. Figs/ prickly pears						
5. Fruit salad						
6. Grapes		3550				
7. Guavas						
8. Mango/paw paw						
9. Melons						
10. Naartjies						

E. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
11. Oranges						
12. Peaches						
13. Pears						
14. Pineapple						
15. Plums						
16. Dry fruit						
17. Fruit juice						
Other						
SOUP, LEGUMES & NUTS						
1. Soups						
2. Beans & lentils						
3. Nuts & seeds						
Other						

F. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
FISH & SEAFOOD – BEIGE						
1. Fried fish						
2. Grilled/smoked/dried fish						
3. Pilchard & sardines						
3. Tuna						
Other						
MEAT – RED						
1. Beef & ostrich						
2. Patties & mince						
3. Burgers & take-aways						
4. Chicken& turkey						
5. Cold meat						
6. Meat fillings						
7. Meat pies						

G. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
8. Mutton						
9. Pork						
10. Sausage & viennas						
11. Traditional & organ meats						
12. Vegetarian products						
13. Dry sausage & biltong						
Other						
VEGETABLES – GREEN						
1. Asparagus						
2. Avocado						
3. Baby marrows						
4. Beetroot						
5. Butternut & pumpkin						

H. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
6. Broccoli/cauliflower						
7. Cabbage						
8. Carrots						
9. Gem squash						
10. Green beans						
11. Mealies						
12. Mixed vegetables						
13. Mushrooms						
14. Peas						
15. Potatoes						
16. Potato chips						
17. Salad vegetables						
18. Spinach/marog						

19. Sweet potatoes						
20. Tomatoes						
Other						
BISCUITS, CAKE & PUDDING						
1. Biscuits/cookies						
2. Biscuits/savoury						
3. Buns/muffins/scones						
4. Cakes & Tarts						
5. Doughnuts/eclairs						
6. Pancakes/waffles						
7. Puddings & custard						
8. Rusks						
9. Special breads						
Other						

I. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
SNACKS, SWEETS & COLD DRINKS,– PINK						
1. Carbonated cold drinks						
1. Diet cold drinks						
2. Energy drinks						
2. Squashes						
3. Crisps & popcorn						
4. Sweets/chocolates						
Other						
SAUCES & CONDIMENTS – GREY						
1. Cheese & white sauces						
2. Chakalaka/Atjar						
2. Tomato sauce & other						
3. Salt, spices & seasoning						
Other						

J. Food items	B. Description	C. Code	D. Amount usually eaten (g)	E. Every day	F. Every week	G. ! once a month
ALCOHOLIC DRINKS – GREY						
1. Beer & cider & coolers						
2. Wine						
3. Spirits						
4. Liqueurs & fortified wine						
Other:						

RELATIVE CONTRIBUTION TO DIFFERENT ASPECTS OF THIS THESIS

Concepts and ideas

- The concepts for this thesis and study design were developed in collaboration with Dr Julia Goedecke and Professor Malcolm Collins. Professor Vicki Lambert assisted in the early stages of the thesis.

Operational

- Development of the research protocols and recruitment and testing of the black women was done with Dr Courtney Jennings.
- Recruitment and testing of the white women was done with Dr Juliet Evans.
- Selection of the *IL-6* polymorphisms was with the assistance of Robyn Johnson, an honours student.
- Statistical analysis was done with the assistance of the biostatistician Dr Lize van der Merwe.
- DNA isolation was done with the assistance of Dr Courtney Jennings and Dr Juliet Evans.
- Genotyping was completed with the support and assistance of Neezaam Kariem.
- Administration of the dietary intake questionnaire and dietary data analysis was performed with the assistance of the dietitian Madelaine Carstens.

- Data analysis was completed under the supervisions of Dr Julia Goedecke and Professor Malcolm Collins.

Writing and compilations of the thesis

All writing of the thesis and its compilation was performed by myself.