IMPACT OF FATTY ACID DESATURASE (FADS) GENOTYPES ON THE RELATIONSHIP BETWEEN SERUM LIPIDS AND DIETARY FAT INTAKE IN YOUNG MEXICAN COLLEGE STUDENTS

BY

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DISSERTATION

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ABSTRACT

Background: The Mexican population has been found to exhibit an environmental and genetic predisposition to develop blood lipid disorders [1, 2]. The fatty acid desaturase (FADS) gene cluster encodes two key enzymes which are involved in the synthesis of long-chain polyunsaturated fatty acids (PUFA). Delta-5 desaturase (D5D) and Delta-6 desaturase (D6D) are encoded by FADS1 and FADS2, respectively. FADS gene cluster polymorphisms have recently been determined to be associated with high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels in Europeans [3]. Moreover, the dietary intake of PUFAs affects FADS-blood lipids interactions [4]. Gene-nutrient interactions and individual genetic variations of the FADS gene cluster have not been described in young Mexicans to date.

Objectives: The first aim was to report genetic associations between FADS1-rs174546 and FADS2-rs1535 polymorphisms and blood lipid profiles in Mexican college-age students. The second aim was to determine whether dietary intake of omega-3 and omega-6 PUFAs modified genetic associations between the FADS gene cluster and lipid profiles.

Methods: Body mass indexes (BMI), blood lipid profiles, dietary intakes of omega-3 and omega-6 fatty acids, and genotypes in the FADS gene cluster were evaluated in 462 individuals from the "UP AMIGOS" cohort, aged 18-25 yrs. FADS-SNPs were in Hardy-Weinberg equilibrium with a minor allele frequency of 0.29 and 0.28 for rs174546 and rs1535, respectively. All of the data was analyzed using SAS 9.3 (SAS Institute Inc., NC, USA), assuming a significance level of $p<0.05$ for multiple comparisons. Non-normal distributed variables were log transformed prior to analysis. The primary analysis was conducted using general linear regression models to examine associations between FADS SNPs and the blood

lipid profiles adjusted for sex, age and BMI. A secondary analysis tested the impact of dietary intake of omega-3 (g/d) and omega-6 (g/d) on the association between FADS SNPs and the lipid profiles adjusted for sex, age, BMI and total calorie intake.

Results: Carriers of the minor C-allele of FADS1-rs174546 and A-allele of FADS2-rs1535 had significantly higher LDL-C, TC and non-HDL-C concentrations compared with homozygotes for the major allele (T and G alleles, respectively). No significant associations were observed between the FADS genotypes and HDL-C, TG and VLDL concentrations. Moreover, BMI status has a strong impact on the FADS genotype and the lipid profile. Second, a significant association was observed between FADS1/FADS2 and dietary intake of omega-3, but this was not the case for omega-6 with respect to LDL-C levels. In addition, as a categorical variable, carriers of the C-allele were associated with lower LDL-C among those with lower intakes of omega-3.

Conclusion: Carriers of the minor allele of FADS1/FADS2 exhibited higher levels of atherogenic lipoproteins such as higher LDL-C. Moreover, FADS polymorphisms interact with dietary omega-3 to effect plasma cholesterol concentrations, which should be investigated further in future studies.

Future directions: There has been a documented rapid increase in the prevalence of Mexican college-age students who are overweight and obese coupled with a high prevalence of dyslipidemias [5-9]. We propose a new approach for identifying individuals who are at risk for dyslipidemias. This approach investigates potential gene-nutrient interactions that may provide insight into potential future therapies and dietary intervention strategies for preventing or delaying cardiovascular disease.

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LIST OF ABBREVIATIONS

CVD	Cardiovascular disease
FFQ	Food frequency questionnaire
GWA	Genome-wide association
SNP	Single nucleotide polymorphism
SE	Standard error
FADS	Fatty acid desaturase
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
LA	Linoleic acid
ALA	Alpha-linoleic acid
GLA	γ-linoleic acid
DGLA	Dihomo- γ-linoleic acid
AA	Arachidonic acid
ETA	Eicosatetraenoic acid
EPA	Eicopentaenoic acid
DPA	Docosapentaenoic acid
DHA	Docosahexaenoic acid
D5D	Delta-5 desaturase
D6D	Delta-6 desaturase
BMI	Body mass index

CM Chylomicrons

TC Total cholesterol

TG Triglycerides

HDL-C High-density lipoprotein cholesterol

LDL-C Low-density lipoprotein cholesterol

VLDL Very-low density lipoprotein

LCAT Lecithin-cholesterol acyltranferase

CETP Cholesteryl ester transfer protein

NPC1L1 Niemann-PickC1 like

FFE Free fatty acids

MAG 2-monoacylglycerols

OW Overweight

OB Obese

LP lipoprotein lipase

CHAPTER 1
INTRODUCTION

In 2012, Mexico was found to have the highest levels of obesity in North America. 72% of Mexican adults were overweight (~40%) or obese (32.8%) [10-12]. In addition, 16.7% of preschool children, 26.2% of school children and 30.9% of adolescents in Mexico were found to be overweight or obese [10]. More particularly, among college-aged students the prevalence of being overweight and obese increased from 13.2% to 37.3% from 1994 to 2008 [13].

Mexico is currently facing an increase in non-communicable diseases such as diabetes, cardiovascular disease (CVD) and chronic kidney disease, and this has impacted the national health-care budget [14]. Recent evidence concerning the economic burden of obesity in the U.S. reported significantly higher health care expenses and expenditures for obese children compared with their normal-weight peers [15]. The annual expenses associated with childhood obesity in the U.S. were ~$14.1 billion [15]. In Mexico, the total costs of obesity-related diseases was estimated to be $806 million in 2010, and this is projected to increase to $1.2 billion and $1.7 billion in 2030 and 2050, respectively [16].

Over half of all deaths from CVD in Mexico can be attributed to dyslipidemia [17]. The most common lipid disorders among Mexican adults aged 20 to 69 years were: low high-density lipoprotein cholesterol (HDL-C) (60.5%), high low-density lipoprotein cholesterol (LDL-C) (46%), high total cholesterol (TC) (43.6%), and high triglycerides (TG) (31.5%) [18]. Similar trends have been reported in the younger age groups, where 13.5% of children and adolescents, and 51.3% of young adults had low HDL-C [19-22]. It is well-known that the management of

lipids is essential for significantly reducing and preventing cardiovascular events, and consuming a balanced diet is a key factor in prevention [23-25].

The American Heart Association (AHA) provides dietary recommendations for promoting an overall healthy lifestyle with the goal of reducing the risk of CVD. These recommendations primarily involve nutritional adequacy and energy balance [26]. Dietary recommendations include consuming a diet rich in vegetables and fruits, choosing whole-grains, limiting the intake of dietary fat to 20-35% of energy intake, limiting the consumption of saturated fat (SFA) to 7% of total energy intake, increasing the consumption of n-3 polyunsaturated fatty acids (PUFA) and limiting intake of *trans* fats [27]. However, the recommended nutritional goals that can help prevent chronic diseases are not being met in children and adolescents. In a study across thirty countries including Mexico, less than half of the children and adolescents met the SFA and PUFA intake goals established by AHA [28].

The current Mexican diet is characterized by high levels of consumption of soft drinks, refined grains, corn tortillas, pastries and low levels of consumption of dairy products, seafood and whole grains [29]. In addition, the excessive intake of SFA and PUFA have been reported [30]. This dietary pattern has been associated with the high prevalence of dyslipidemia and central obesity among both younger Mexicans and adults [31, 32]. In contrast, the traditional (prudent) diet which involves high levels of consumption of fruits, vegetables, and legumes, and low levels of consumption of pastries and cereals has been associated with lower body mass index (BMI) and low TG [12].

Diet is one of a number of factors which affect blood lipids, and genetics also plays a key role [33]. Several studies have found that the Mexican population has a genetic predisposition for

dyslipidemias, particularly low HDL-C and high TG levels [1, 34-38]. However, research on identifying polymorphisms associated with LDL-C levels is lacking for the young Mexican population.

Genome-wide analysis (GWA) studies conducted in Europeans have found that blood lipid profiles are highly heritable, including ~40-60% for HDL-C, ~40-50% for LDL-C and ~35-48% for triglycerides [39, 40]. Currently, 95 genetic loci have been determined to be associated with plasma blood lipids which are also associated with coronary heart disease [41]. However, genetic variations in the lipid loci explain only ~12% of the overall variation of plasma lipids [41].

Blood lipid levels have been reported to have a strong genetic component, with heritability as high as 80% in childhood [42]. Childhood serum lipid levels have been shown to predict lipid measurements in adulthood [43-46]. A longitudinal study of Finnish children and adolescents estimated the effect of individual single-nucleotide polymorphism (SNPs) on lipids measurements. This study found that 26.7% of the total variance of HDL-C in males, and 21.9% in females, was explained by SNPs in children (age 3 to 6 years old), and 10% in adults over 20 years old [47].

The Mexican population has a genetic predisposition toward low HDL-C and high serum triglycerides levels [1, 34, 35, 37, 48], and 65% of the adult population has at least one type of dyslipidemia [18, 49]. Taking this into consideration leads to the notion that studying the effect of genetic variations which influence lipid levels in a young population could provide useful information for the early detection of lipid disorders and the increased risk of coronary heart disease.

Epidemiological evidence supports the contention that there are health benefits to be derived from including omega-3 [alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid, (DHA)] in the diet [50]. Human trials and dietary interventions demonstrate that increasing the amount of omega-3 in the diet significantly reduces blood lipid profiles, insulin sensitivity and the risk of CVD [51-55]. These benefits have also been described in the Mexican population [56-58]. In particular, Mexican obese children respond better to a dietary intervention involving omega-3 (1.8g/d omega-3, EPA + DHA) than treatment with metformin [56]. However, more than 50% of the Mexican population consumes an insufficient intake of PUFAs and an excessive intake of SFA [30].

In addition to dietary sources, PUFAs can also be derived endogenously from precursor essential fatty acids, linoleic acid (LA; C18:2n-6) and alpha-linoleic acid (ALA; C18:3n-3), by two enzymes: delta-5 (D5D) and delta-6 (D6D) desaturases. These enzymes are encoded by FADS1 and FADS2 genes, respectively in the fatty acid desaturase (FADS) gene cluster [59]. The FADS gene cluster is thus a good candidate for genetic association studies which focus on determining gene-nutrient interactions which may affect individual variations of blood lipid levels. Gene-nutrient interaction studies of the young Mexican population have been lacking so far.

Several studies have identified the genetic variations in the FADS gene cluster that not only influence fatty acid metabolism [60], but also influence glucose metabolism and other blood lipids such as TC, low LDL-C and high TG [61-63]. Several studies have identified that carriers of the minor alleles of single-nucleotide polymorphisms (SNPs) in the FADS gene cluster are associated with higher TC and TG, and lower HDL-C levels [61-63]. In addition, PUFAs have been shown to have genetic variants which interact with genetic variants FADS to affect blood

lipids [64-67]. Moreover, the intake of dietary fatty acids modifies the effect of FADS genotype categories on blood lipids [4, 68-71].

Gene-nutrient studies have shown that dietary fatty acids interact with a wide variety of SNPs in order to modulate TC and LDL-C levels [52, 72-74]. More particularly, SNPs in the FADS gene cluster have been shown to affect the activities of the enzymes which metabolize essential fatty acids in order to modulate the genetic risk in CVD [68, 75-78]. Therefore, it is of interest to identify and characterize the SNPs in the FADS gene cluster which may be mediated by dietary fat intake.

Study goals

The Mexican population has a high prevalence of dyslipidemias due to their genetic background of the population [1, 34, 37].

In particular, the prevalence of dyslipidemias among college students pointed us to our first goal: identifying genetic variations in the FADS gene cluster associated with blood lipid levels. Moreover, population-based research regarding the association between the FADS genotype, dietary fatty acids intake (e.g. omega-3 and omega-6), and serum lipid relationships in young Mexican students is incomplete. Therefore, our second goal was to examine whether or not dietary fat intake modifies the association between SNPs in the FADS gene cluster and blood lipid concentrations.

CHAPTER 2
LITERATURE REVIEW

2.1 Lipoprotein metabolism

Lipoproteins are large macromolecular complexes which are composed of a core of cholesterol esters and triglycerides (TG) surrounded by phospholipids and free cholesterol [79].

The plasma lipoproteins are divided into five major categories based on their relative densities: chylomicrons (CM), very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The size and density of a lipoprotein depends on the content of lipids and apolipoproteins per particle. HDL is the smallest and densest lipoprotein (1.063-1.210 g/mL). CM (triglyceride-rich lipoprotein) and VLDL are the largest and least dense lipoprotein particles, and have densities that are less than 1.006 g/mL [79].

Other particles associated with lipoproteins are known as apolipoproteins. There are four types of apolipoproteins: ApoA-1, ApoB, ApoC and ApoE. These proteins have four major functions: (1) assembly and secretion (ApoB100 and apoB48); (2) structure (ApoB, ApoE, ApoAI, ApoAII); (3) coactivators or inhibitors of enzymes (Apo AI, CI, CII, CIII); and (4) ligands for cell surface receptors (Apo-AI, B100, E) [79].

ApoA-I is synthesized in the liver and intestine and is found in all HDL particles. Two-thirds of all HDL particles are of the Apo-AII type, the second most abundant type of HDL particle [79]. ApoB is the major structural protein of the chylomicrons VLDL, IDL, and LDL. There are two types of ApoB: ApoB-48, which is present in CMs, and ApoB-100 which is present in VLDL, IDL or LDL. ApoE is present in CM, VLDL and IDL, and plays a critical role in the

metabolism and clearance of triglycerides-rich particles. There are three types of ApoC: ApoC-I, ApoC-II and ApoC-III, all of which participate in the metabolism of triglycerides-rich lipoproteins [79].

The main function of lipoprotein particles is the transport of TG, cholesterol and fat-soluble vitamins from the liver to the peripheral tissues and the transport of cholesterol from the peripheral tissues to the liver [79]. In addition, lipoproteins play an essential role in the absorption of cholesterol, fatty acids and fat-soluble vitamins from the diet [79]. Most plasma triglycerides are transported in CM or VLDL, and most of the plasma cholesterol is carried in the form of cholesterol esters in LDL and HDL [79].

The transport of fatty acids (FA) in the body is thought to involve two pathways. The first pathway is an exogenous pathway involved in the absorption and transport of dietary lipids from the intestine to peripheral tissues and then back to the liver. The second pathway is an endogenous pathway involving the hepatic secretion of ApoB-containing lipoproteins and their metabolism.

Exogenous pathway

After a meal, most lipids, like other macronutrients (carbohydrates and proteins), are broken down into smaller compounds for absorption in the gastrointestinal tract. In the intestinal lumen, free fatty acids (FFA) and 2-monoacylglycerols (MAG) are taken up by the enterocytes via passive diffusion and specific transporters such as FABPpm, FATP4 and FAT/CD36, while cholesterol is transported by an active transporter Niemann-Pick C1 like 1 protein (NPC1L1) [80].

Once inside the enterocyte, FFA and MAG are reassembled into triglycerides (TGs), and cholesterol is transformed into cholesterol-esters [80]. New TGs are packaged with phospholipids and Apo B48 to form CM, and are secreted from the intestinal cells into the lymphatic system where they enter the bloodstream via the thoracic duct. HDL donates ApoC-II and ApoE to the nascent CM, and HDL delivers FFA to cells in the heart, skeletal muscle and adipose tissue [80, 81]. Once it enters the blood, the enzyme lipoprotein lipase (LPL) that is attached to the inside wall of most cells is activated by ApoC-II in order to transfer TGs from the CM to the cells for energy or storage [82]. Then, smaller CMs called CM remnant travel back to the liver to be excreted out the body.

Endogenous pathway

The endogenous pathway transports fat from the liver to the peripheral tissues, and then returns to the liver. In the liver, TG-rich lipoproteins are packed into molecules known as VLDL which enter the circulatory system [82]. VLDL is a triglyceride-rich lipoprotein that is smaller than CMs. Both are similar in protein composition, but contain ApoB-100 instead of ApoB-48 and have a high ratio of cholesterol to TG [82].

In the peripheral tissues, lipoprotein lipase (LPL) hooks up with VLDL and TGs are released, and become intermediate-density lipoproteins (IDL). Hepatic lipase (HL) and LPL then remove additional TGs from IDLs in order to generate low-density lipoproteins (LDLs) [82]. LDL-C and CM remnants are removed from the blood by the liver [82].

Another important pathway in the metabolism of lipoproteins is the uptake of cholesterol from peripheral tissues. This is known as reverse cholesterol transport, and it is accomplished by HDL particles [82]. The intestine and liver synthesize ApoA-I in order to assemble nascent HDL.

When nascent HDL is in circulation, it collects free cholesterol from the cells and becomes mature HDL, and transports free cholesterol from the peripheral tissues back to the liver. The cholesterol that is found inside HDL lipoproteins is esterified into cholesterol-esters by HDL associated lecithin-cholesterol acyltransferase (LCAT) [82]. When in circulation, cholesterol ester transfer protein (CETP) exchanges TG from VLDL for cholesterol esters of HDL. While in the liver, HL hydrolyses TGs from HDL, which induces the formation of smaller particles of HDL which restarts the reverse cholesterol transport pathway.

2.2 Lipoprotein and atherosclerosis

Cardiovascular disease (CVD) refers to any disease that affects the cardiovascular system. CVD had diverse causes, and atherosclerosis and hypertension are the most common causes [83]. Atherosclerosis is the accumulation of fatty materials in the artery walls, and the accumulation of lipids which is converted from fibromuscular cap to form what is known as fibrous atherosclerotic plaque [84-86]. When LDL is not taken up by the body, it is oxidized by free radicals, and the scavenger cells (macrophages and white cells) remove oxidized LDL from circulation [82]. Over time, high concentrations of LDL in the circulatory system lead to the accumulation of cholesterol in the scavenger cells and accumulations in the inner lining of the arteries. This can produce conditions such as a heart attack, stroke and other cardiovascular disease [83, 87].

Since the 1980s, atherosclerosis plaque and CVD have been determined to be associated with high cholesterol levels. The Framingham Study's researchers analyzed the association between mortality and serum cholesterol values for 4,374 participants who were followed for 30 years [88]. This study found that for every 10 mg/dL increase in blood cholesterol levels, CVD deaths

increased by 9% [88]. Furthermore, the Multiple Risk Factor Intervention Trial (MRFIT) confirmed that 49% of all CVD deaths were associated with serum cholesterol levels of 180 mg/dL or above [89]. More recent cohort studies such as the Seven Country Study [90] and a meta-analysis [91] concluded that an increase of 1 mmol/l (38.67 mg/dL) in blood cholesterol was strongly associated with a 20-35% increase in CVD risk [90], and with a 12% increase in CVD mortality risk [91]. In addition, cohort and case-control studies highlight that low HDL-C is involved in CVD and mortality [92-95].

CVD does not usually occur until the fifth or sixth decade of life [96]. However, the atherosclerosis process is known to begin early in childhood [95, 97, 98]. The Bogalusa Heart Study has demonstrated that the occurrence of elevated cholesterol levels during childhood puts them at risk for having high adult cholesterol levels [99, 100].

Atherogenic dyslipidemia, which is characterized by high TG, low HDL-C and high LDL-C, is commonly found in obese children and adolescents [84, 101]. The early stage and progression of atherosclerosis in youths is associated with dyslipidemia and obesity [43, 95].

Hypertriglyceridemia in obesity is a consequence of increased fasting and postprandial TG which leads to elevated levels of free fatty acids (FFA). The liver processes those FFA, and increases the production of VLDL, and CETP later increases the exchange of cholesterol esters and TG between VLDL and HDL, and LDL. Furthermore, HDL concentrations decrease and small dense LDL increases [9]. As mentioned above, small dense LDL become trapped on the arterial walls and enhances its atherogenicity [84, 93].

2.3 Diet and CVD risk factors

Diet is an important environmental factor in the prevention of cardiovascular disease (CVD) [102-105]. Evidence from several epidemiological studies reflect that omega-3 fatty acids such as EPA and DHA (2-4g/day) reduce TG by 25-30%, with greater decreases found among subjects with baseline hypertriglyceridemia [106, 107]. On the other hand, high SFA intake has been confirmed to correlate with an increased incidence of CVD [25, 102, 103, 108].

Data from clinical trial show that restriction of total fat intake (~7%) can reduce total TC by 20 mg/dl, TG by 40 mg/dL, and increase HDL-C by 5 mg/dL [109]. Moreover, the evidence suggests that reduced SFA intake reduces the risk of CVD events by 14% [105]. Current guidelines recommend that 20% to 35% of daily caloric energy come from dietary fat, and specifically recommend limiting the consumption of saturated fats to no more than 7-10% of total caloric energy, and less than 300 mg/day of cholesterol [110]. Western diets containing excessive SFA and very little n-3 (polyunsaturated fatty acid PUFAs) are associated with increases in TC and LDL-C concentrations [102, 104, 105].

The Lyon Diet Heart Study showed that the Mediterranean diet reduces the rate of recurrence of myocardial infarction and reduces mortality [111]. Moreover, the Diet and Reinfarction Trial (DART) supported the contention that a diet that is rich in eicopentaecoic acid (EPA; C20:5n-3) was associated with 29% reduction in all-cause mortality, and reduced the incidence of ischemic complications due to atherosclerosis [112]. In addition, a multi-ethnic cohort of 2,837 U.S adults showed that high levels of circulatory EPA and docosapentaenoic acid (DHA; C22:6n-3) were inversely correlated with a lower incidence of CVD [55]. Current dietary recommendations

advise reducing the intake of SFA and trans-fatty acids (TFA) and increasing the intake of PUFAs in order to reduce CVD risk [113].

As mentioned above, a diet that is high in SFA is associated with increased risk of dyslipidemia and obesity [114, 115]. A study which assessed the intake of fats by children and adolescents in thirty countries around the world show that less than half of the children and adolescents met the minimum dietary recommendations for SFA and PUFAs intake [28]. Table 1 shows dietary intakes in adolescents (aged 11-18 years) around the world.

A recent study performed by the Nutrition and Chronic Diseases Expert Group found that, in Central Latin America, the intake of saturated fat increased by 0.5% E (0.1 to 1.0% E/day) and the intake of omega-6 increased by 1.3% E (0.6 to 2.1% E/day) between 1990 and 2010 [116]. On the other hand, the consumption of omega-3, particularly in in Mexico, is less than 50mg/day [116]. Notably, human intakes of *trans* fats are higher in Mexico, Canada and Egypt (>2% E) compared with other nations [116].

The Mexican National Health and Nutrition survey of 2006 reported that 61% of human energy intake comes from carbohydrates, and 25% of energy intake comes from total fat, and this constitutes a large part of the typical Mexican adult diet [117]. More particularly, high saturated fat consumption (SFA) among children and adolescents (0.5g/d or 0.04% E) is a major public concern regarding the Mexican population [30].

A cross-sectional study of Mexican school-aged children (8-10years) in Mexico City found that high intake of SFA (~12% E) was significantly associated with higher diastolic hypertension [118]. Therefore, future research is needed to explore the association between the intake of fatty acids and the widespread prevalence of dyslipidemias among Mexican college students.

LA is the primary PUFA found in significant quantities in many vegetable oils (e.g., corn, safflower, soybean and sunflower oil) and in products made from such oils (e.g., margarines). The PUFA-rich oils are divided into those oils which have small amounts of ALA and high levels of LA, and those which have high levels of ALA. Flaxseed and canola are rich in ALA, whereas soy and walnut oil provide sufficient ALA as well as LA [143]. On the other hand, olive oil and avocado oils are oils rich in monosaturated mono-unsaturated fatty acids.

In Mexico, the main sources of LA and ALA are chicken, eggs, corn tortillas, vegetable oils (e.g., corn, soy, sunflower, safflower or olive oil), and sweet breads [144]. Dietary fatty acids (n-3) intake includes DHA, ALA and EPA, all of which are found in chicken, eggs, salmon, fresh fish. However, their consumption in Mexico is lower than foods with n-6 fatty acids [144].

A cross-sectional study of Mexican 20-70 years of age found that dietary patterns high in refined cereals, pastries, corn tortillas and sugared sodas were associated with significant increases in BMI [31] and dyslipidemia (low HDL-C and high TG) [32].

Dietary fat mirrors fatty acid composition in serum lipids, erythrocyte membranes and adipose tissue [145]. The activity of the delta-5 (D5D), delta-6 (D6D) and enlogases play an important role in several metabolic risk factors [146, 147].

Table 1. Dietary fat intake in adolescents 12-18 years old in 8 countries†

Country	Age (years)	Subjects (n)	TFA (% E)	SFA (% E)	MUFA (% E)	PUFA (% E)
Australia†	12-18	1086	32.9 ± n.a	14.0 ± n.a	11.7 ± n.a	4.5 ± n.a
Costa Rica†	12-18	275	31.7 ± 6.5	11.4 ± 3.3	3.6 ± 3.0	5.7 ± 2.9
India†	13-18	797	34.0 ± 6.0	14.7 ± 2.5	11.6 ± 1.8	4.9 ± 1.3
Israel†	12-18	5760	33.3 ± 4.7	11.3 ± 2.4	10.3 ± 2.2	6.9 ± 1.7
Spain†	14-24	1576	39.9 ± 4.0	13.0 ± 2.0	16.4 ± 2.0	5.2 ± 1.0
U.K†	11-18	864	35.7 ± 4.7	13.8 ± 2.2	11.8 ± 2.0	6.3 ± 1.6
USA†	12-19	2115	33.2 ± 15.1	11.5 ± 4.3	12.1 ± 7.8	6.6 ± 3.2
Mexico*	12-19	7731	26.6 ± 0.17	10.7 ± 0.08	6.5 ± 0.05	9.4 ± 0.06

Data is presented in mean and standard deviation as percentage of energy (% E); n/a, not available. †Table was adapted from R. K. Harika et al. "Fatty acid intakes of children and adolescents are not in line with the dietary intake recommendations for future cardiovascular health: a systematic review of dietary intake data from thirty countries," *Br J Nutr*, vol. 106, pp. 307-16 [28].*Dietary fat in Mexican adolescents in percentage of energy and standard error of the mean (Ramirez-Silva, Villalpando et al. 2011).

2.4 Impact of genotype on the blood lipid profiles

Among the environmental factors discussed in the previous section, blood lipid levels are influenced by multiple genetic factors. Other factors such as age, gender, diet and obesity modulate the association between genes and lipid traits.

Since the introduction of genomics techniques, our understanding of the genetic basis of CVD has increased. Genome-wide association studies (GWAS) enable the scanning of the entire genome in order to identify the genes and/or loci which are significantly associated with CVD. The GWAS has identified genes and/or loci associated with atherosclerosis [119], inflammation [120], and lipid traits [40, 121, 122].

GWAS has been used to determine that blood lipid profiles are highly heritable, including ~40-60% for HDL-C, ~40-50% for LDL-C and ~35-48% for triglycerides [39, 40]. However, genetic variation only explains ~12% of the overall variation of plasma lipids [41, 123].

There are several example of GWAS having been used to identify genes that have been implicated for CVD, including blood pressure [124] and ischaemic stroke [125]. Moreover, GWA studies have identified candidate genes suspected of influencing plasma lipid concentrations that may contribute to the pathogenesis of atherosclerosis [33, 40, 122, 126].

Today, 52 genes for HDL-C, 42 genes for LDL-C, 59 genes for TC, and 39 genes for TG have been identified [127]. More GWAS have replicated these results in non-Hispanic Whites, non-Hispanic Blacks, Mexican-Americans, Hispanics and people of European descent [123, 128, 129]. Furthermore, a GWAS determined that there are many loci which are associated with CVD risk in children. The Bogalusa Heart Study (BHS) is a longitudinal study of participants of European ancestry which identified seven associations that increased the risk of CVD [130].

GWAS has been a particularly useful approach for identifying loci that influence the development of CVD, and for identifying genetic risk factors in children. However, GWAS requires replication and validation of the findings in additional population samples, so the GWAS approach might have limited applicability.

Although the genetic variations of genes may be related to the development of CVD later in life, the combination of genes, environmental, and behavioral factors is the key to preventing or delaying the onset of CVD.

In addition to candidate gene studies, some GWAS tests have identified genes that are associated with energy and macronutrient intakes. A meta-analysis of GWAS associated with the intake of macronutrients from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) found that genetic variants in the FTO gene were associated with protein intake and SNPs on chromosome 19 were associated with the intake of carbohydrates and fat [131]. Similarly, the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) which was performed using Black and White subjects who had linked regions in the 1p21 and 20q and dietary energy and nutrient intakes [132]. As regards Hispanic children, a GWAS identified chromosome 18q as being related to physical activity and carbohydrate intake [133].

Although recent GWAS have identified numerous genetic risk factors for complex diseases, including plasma lipids, some technical issues are relevant and need to be described. GWAS detected small effects associated with common variants, and used a large sample size of individuals. It requires replications of significant findings, and has the potential for producing false negative results [134]. Thus, the genotyping of selected SNPs is a better approach for genetic studies.

Single-nucleotide polymorphism (SNP) is a variation of a single base pair which represents the most frequent form of polymorphism in the human genome. The term minor allele is reserved for variants where the frequency exceeds 1% [134]. The data that is available from the International HapMap Project shows that many SNPs exhibit a high correlation between SNP genotypes and selected markers due to their shared evolutionary history [135]. This concept for finding efficient sets of SNPs is known as tagging SNPs (TagSNPs). The following section will discuss the most relevant findings concerning using the TagSNPs approach in gene-nutrient studies.

Current research focuses on finding interactions between diets and genetic variants affecting CVD risk, and obesity is widely recognized as contributor to CVD risk.

A large cohort from three independent populations include the Framingham offspring Study, the Genetics of lipid lowering drugs and diet network (GOLDN) study, and the Boston-Puerto Rican CPHHD study. All of these studies found that of C-allele of the APOA2 SNP consuming high intakes of SFA, were strongly associated with body mass index (BMI) [136]. Moreover, this study found that C-allele carriers have higher consumption of total fat, SFA, protein, carbohydrates and fructose than T-allele carriers [136]. However, one limitation of this gene-diet interaction is that it only applies to a small percentage of the population. In this case, the C-allele of the APOA2 SNP was found in only 10-15% of the participants.

Another variant that shows significant gene-nutrient interactions is found in the FTO gene. A cross-sectional study examined 4839 subjects in the population-based Malmö Diet and Cancer study. It found that there was a strong association between the genetic variant FTO (rs9939609) and BMI only among those who reported high fat intake [137]. This association was replicated in

two US populations using the GOLD study and the Multi-Ethnic study of atherosclerosis (MESA), which highlighted that SFA intake modulates interactions between genetic variants and obesity [138]. Furthermore, numerous reports have shown that individual genetic variations interact with PUFA intake in modulating lipid metabolism.

The Framingham Heart study found a gene-diet interaction exists between the APOA5-1131T>C polymorphism, PUFA intake and blood lipids. This study identified that carriers of the 1131C allele had higher TG levels when their consumption exceeded 6% of total energy intake [52]. In addition, it was previously shown that carriers of A-allele in the promoter region of the APOA1 gene (-75G/A SNP) exhibited higher HDL-C levels when they consumed high intakes of PUFA (approximately 40% of total energy intake) [139].

Another classic example of how gene-nutrient interactions affect blood lipid profiles was seen in the Canadian trial of dietary carbohydrate in Diabetes study. This study suggests that higher PUFA intake (8% of total energy intake) was positively associated with higher HDL levels [140]. The EPIC Norfolk cohort, which included data from ~23,000 individuals, similarly demonstrated that plasma LDL-C was positively related to SFA intake, with a particular degree of risk associated with the E4 carriers of ApoE [141].

In addition to the above studies, intervention studies showed that modulating the PUFA/SFA ratio in the diet contributes to the genetic effects on blood lipids. An intervention trial found that carriers of the ApoE genotype exhibited a 10% increase in LDL-C after a DHA intervention (3.7g DHA/day) [142].

The preceding studies evaluated dietary fat interactions with specific genes. Genes in the fatty acid desaturase (FADS) cluster have been studied extensively because they contribute to

PUFA concentrations in plasma. A GWAS of PUFA produced evidence of the association in the region of chromosome 11 that encodes FADS1, FADS2 and FADS3 [75].

2.5 Fatty acid desaturase (FADS) gene cluster

The human fatty acid desaturase (FADS) gene cluster (which includes FADS1, FADS2 and FADS3) comprises 91.9 kb on chromosome 11q12-13. It has a head-to-head orientation of FADS1 and FADS2 and a tail-to-tail orientation of FADS2 and FADS3 [59]. Both genes are separated by an 11kb region, which suggests the possibility of a coordinated transcription of FADS1 and FADS2 [59].

FADS1 and FADS2 gene encode two desaturases: delta-5 desaturase (D5D) and delta-6 desaturase (D6D), which are membrane-bound proteins [59]. The function of the protein product of the FADS3 gene is currently unknown.

D5D and D6D are rate-limiting enzymes which are responsible for double bond formation in long-chain polyunsaturated fatty acids (PUFAs) [59]. D6D introduces a double bond between the pre-existing double bond and the carboxyl end of the fatty acid [59].

D6D catalyzes the conversion of linoleic acid (LA; C18:2n-6, ω-6) and α-linolenic acid (ALA; C18:3n-3) into γ-linolenic acid (GLA, C18:3n-6) and stearidonic acid (C18:4n-3), respectively. This is followed by an elongation step, after which D5D introduces a double bond at the Δ5 position in a 20-carbon fatty acid chain. D5D catalyzes the conversion of dihomo- γ-linolenic acid (DGLA; C20:3n-6) and eicosatetraenoic acid (ETA; C20:4n-3) into arachidonic acid (AA, C20:4n-6) and eicosapentaenoic acid (EPA, C20:5n-3), respectively (Fig 1). The major downstream product of the omega-3 family is docosahexaenoic acid (DHA; C22:6n-3)

[59]. More particularly, EPA and DHA have numerous important functions in several biological processes [76].

LA is the primary PUFA found in significant quantities in many vegetable oils (e.g, corn, safflower, soybean and sunflower oil) and in products made from such oils (e.g, margarines). The PUFA-rich oils are split into those with small amounts of ALA but high levels of LA, and those with high amounts of ALA. Flaxseed and canola are rich in ALA, whereas soy and walnut oil provide sufficient ALA as well as LA [143]. On the other hand, olive oil and avocado oils are oils rich in monosaturated fatty acids.

In Mexico, the main sources of LA and ALA are chicken, eggs, corn tortillas, vegetable oils (e.i. corn, soy, sunflower, safflower or olive oil), and sweet breads in a high frequency of consumption per day [144]. Intakes of n-3 such as DHA, ALA and EPA are consumed from chicken, eggs, salmon, fresh fish, however their consumption is lower [144].

In a cross-sectional study in Mexicans aged 20-70years old was found that dietary patterns high in refined cereals, pastries, corn tortillas and sodas were associated with a significant increase in BMI [31] and dyslipidemia (low HDL-C and high TG) [32].

It is well known that dietary fat is a mirror of the fatty acid composition in serum lipids, erythrocyte membranes and adipose tissue [145]. The activity of the delta-5 (D5D), delta-6 (D6D) and elongase play an important role in several metabolic risk factors [146, 147].

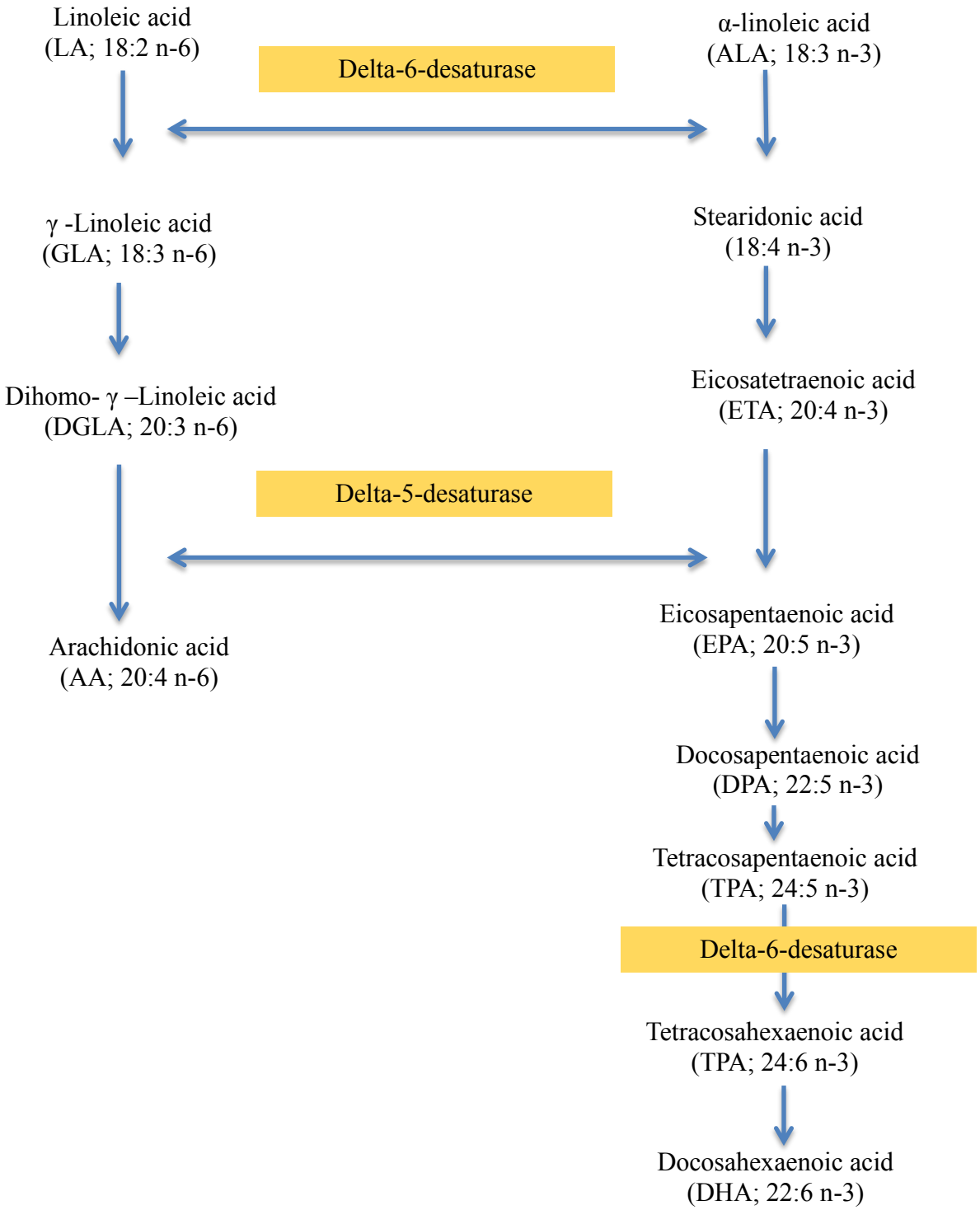


Fig 1. Fatty acid desaturases in PUFA biosynthesis.

Studies of substrate specificity and enzyme kinetics have identified that D5D has only two substrates, DGLA and ETA, while D6D has at least five substrates, such as LA, ALA, tetracosahexaenoic acid (C24:4n-6), tetracosapentaenoic acid (C24:5n-3), and palmitic acid (C16:0) [76]. The amount of substrates (LA and ALA) derived from the diet is equivalent to the fatty acid composition of the body [148, 149]. Moreover, changes in the type of dietary fat affects D5D and D6D activity in serum and skeletal muscles in human subjects [150].

Thus, the activities of D5D and D6D are modulated by the diet [59, 70]. In this manner, high estimates of D6D activity and low estimates of D5D lead to greater production of AA, which is the precursor of chemical mediators of inflammation which play an important role in atherosclerosis and CVD [151].

Previous studies suggest that higher D6D activity is related to higher CVD mortality [152], insulin resistance [151] and obesity [147]. In contrast, higher D5D activity has been determined to be associated with decreased CVD mortality [152], and lower metabolic risk factors [146]. However, dietary intake influences the activities of desaturase enzymes, and genetic variants within FADS1 and FADS2 impact plasma PUFA concentrations [76, 153-155]. At present, 13 SNPs have been identified as being associated with the AA/LA ratio [149, 156].

Martinelli, et al., showed in a haplotype analysis that the carriers of an elevated number of risk alleles in the FADS gene cluster metabolized greater amounts of AA from dietary LA [149]. High plasma concentrations of AA may promote a proinflammatory response and is associated with a greater risk of myocardial infarction [156].

Individual genetic variation in the FADS gene cluster has recently been extensively studied. FADS1-rs174546 in particular belongs to a highly preserved LD block. There are reports that

FADS1-rs174546 tags fifteen other SNPs in the Caucasian population [67]. Moreover, several studies have linked FADS1-rs174546 to PUFA concentration in plasma erythrocytes [67, 157, 158]. In addition, carriers of the minor allele of FADS1-rs174546 have experienced significantly lower D5D activity. Therefore, subjects with the minor allele experienced lower AA concentrations independent of the diet [67].

Haplotype analysis has linked FADS polymorphism, particularly FADS-rs174546, with changes in serum cholesterol and triglycerides [33, 121, 159, 160]. Several studies of Europeans have shown that carriers of the minor alleles had lower levels of HDL-C, and higher levels of TG and LDL-C [33, 62, 63, 126]. Similar trends have been found in Caucasian, Chinese and Japanese adults (Appendix A).

Similar trends in FADS SNPs and lipid profiles have been found among children in two independent studies which have shown that carriers of the minor allele of FADS1-rs174546 were associated with higher TG concentrations [161], and lower TC and HDL-C [61]. As regards HDL-C, the association between the minor allele of the FADS1-rs174546 and HDL-C become significant only among those with high intakes of n-3 PUFAS or ALA [4, 69]. However, gene-nutrient interactions and individual genetic variations in the FADS gene cluster have not been described for young Mexicans. FADS1 SNPs are perfect candidates for studying CVD risk factors such as abnormal lipid profiles (lower HDL-C and higher LDL-C) that might increase the incidence of CVD later in life. Appendix B summarizes the main findings from gene-nutrient interaction studies conducted using European and Caucasian populations.

2.6 Environmental and genetic factors associated with dyslipidemia in Mexico

Mexico has the world's highest rate of obesity (32.8%), followed by the United States (31.8%) [162]. Obesity, age, and high TG are risk factors associated with early-onset diabetes and CVD in the Mexican population [10]. More particularly, there are reports that of an increase in the number of overweight and obese college students [13]. A cross-sectional study of a large representative sample of Mexican college students found that the prevalence of overweight and obese students increased from 12.1% to 26.7%, and from 13.2% to 37.3%, respectively, between 1994 and 2008 [13].

One major contributor to the increased rate of overweight and obese people in Mexico may be changes in dietary patterns. Since 1980, Mexicans have shifted their traditional diet towards more calorie-dense foods due to the commercial dominance of transnational food companies in the Mexican market [163, 164]. Flores, et al., state that the Mexican adult population exhibits three major dietary patterns; 1) a Western diet pattern that includes pastries, refined cereals, corn tortillas, and soft drinks; 2) 40% of the population consumes the traditional Mexican diet, which was 47.1% maize-based foods and refined foods, and 3) 20% of the population consumed a diverse diet (low consumption of maize and the high consumption of whole-fat dairy products, rice, pasta, meat, poultry, eggs, saturated fats, fruits and vegetables). Those who were classified as diverse consumed refined foods and sweets, habits associated with a higher BMI [165].

Diverse and refined diets are characterized by the high consumption of cholesterol, fats, and the low consumption of fiber [165]. Furthermore, this dietary pattern is associated with higher BMI, higher prevalence of metabolic syndrome, high serum TG, and low HDL-C [31, 32].

The most common dyslipidemia in the Mexican population is low HDL-C, followed by hypercholesterolemia [18]. Among the adult population 20 to 69 years of age, 60.5% had low HDL-C and 43.6% had hypercholesterolemia [18]. In addition, a large percentage of young Mexicans older than 20 years have also been affected by these lipid disorders; 61.9% had low HDL-C and 22.3% had hypertriglyceridemia [18].

These metabolic abnormalities are associated with the Native American genetic heritage of the Mexican population [35].

Polymorphisms in cholesterol transfer to and from HDL particles, and are associated with metabolic abnormalities in the Mexican population [35]. For instance, ATP-binding cassette transporter sub-family A member 1 (ABC-A1) is a protein responsible for transferring cholesterol and phospholipids across the cell membranes of peripheral tissues so they can be incorporated into HDL via ApoA1. It has been learned that 1 in 10 Mexican mestizos have a variant of ABCA1 (the R230C variant), which is associated with lower HDL levels. More specifically, this variant is common among Mayans, Purepechas, Yaquis, and Teenek, but is absent in European, African, South Asian, or Chinese populations [35].

ApoA1 is the major lipoprotein for HDL, and is responsible for accepting peripheral cholesterol that is passed along to the HDL particle from tissues and VLDL. The minor allele of the rs964184 APOA1/C3/A4/A5 gene cluster region is significantly more common among Mexicans (27%) than whites (12%), and this allele is strongly associated with triglycerides [37].

In addition to this private mutation of the ABCA1 gene, a mutation in the LDL receptor and APOB gene has been found to exist exclusively among Mexicans [166, 167]. Thus, familial

combined hyperlipidemia (FCHL) is the most frequent primary dyslipidemia among Mexicans [2].

Apolipoprotein B (ApoB) is found in chylomicrons and LDL, and high levels of ApoB are related to heart disease [168]. In the Mexican population, two polymorphisms were found in a case-control study to be associated with ApoB. The first polymorphism was localized near the ApoB messenger RNA editing enzyme (APOBEC1-rs1349411). This enzyme is important for the production of ApoB, which exists in two forms: ApoB-48, which is produced in the small intestine from ApoB-100, and is necessary for the assembly of chylomicrons, and ApoB-100 which is synthesized in the liver and is found in VLDL, LDL-C and IDL particles. The second polymorphism was located in a highly consecutive noncoding region (rs142032) that was predicted to function as a regulatory element for serum ApoB [38].

The FADS gene cluster is one of the loci that has recently been associated with plasma TG, HDL-C and LDL-C concentrations in populations that have European ancestry [33, 121]. The FADS gene is a promising candidate for studying gene-environment interactions because two of the key enzymes involved in the metabolism of PUFAs are encoded by the FADS gene. Moreover, SNPs for blood lipid levels in the FADS cluster gene have been less extensively studied in the Mexican population.

CHAPTER 3

MATERIAL AND METHODS

3.1 Study design

San Luis Potosí is a centrally located state in Mexico and is the state capital. The overwhelming majority of applicants were from this city and surrounding areas. The Autonomous University of San Luis Potosi in Mexico (UASLP) routinely screens college applicants as part of their admission process. This pre-admission screening takes place from January to June. They screen about 10,000 students each year.

In 2009, the UASLP and the University of Illinois began a multidisciplinary investigation on genetics, obesity and social-environment (UP AMIGOS project) during the pre-admission process of college age individuals. The UP AMIGOS project is a cross-sectional study that seeks to evaluate genetics, obesity, and social-environmental factors among young Mexican college students, ages 18-25 years who hail from the city of San Luis Potosí in Mexico. The protocol was reviewed and approved by the Institutional Review Board at UASLP and University of Illinois Champaign-Urbana. Informed consent was obtained from every participant.

In brief, the UP AMIGOS project used a 309-item questionnaire [UP AMIGOS survey (Detección del estilo de vida, fenotipo y genotipo de riesgo para prevenir enfermedades crónico-metabólicas: Diabetes Mellitus y Arteriosclerosis en adultos jóvenes de San Luis Potosí)] adapted for the Mexican population. This survey provided information about socio-economic status, family medical history, a food frequency questionnaire, physical activity and participant self-esteem. The UP AMIGOS survey was administered by trained interviewers in Spanish (Appendix C). The English translation can be also found here (Appendix D).

After the volunteers completed the UP AMIGOS survey, they were given health screenings at the university clinic following overnight fasts. The health screening consisted of: (1) anthropometric measurements, including height, weight, and blood pressure; (2) physician-conducted medical interviews and physical exams, and (3) blood samples were draws to check blood biomarkers including fasting glucose and lipid profiles.

The initial sample (n=854), a subset of individuals was selected for this project with a total of 445 subjects were selected from the UP AMIGOS cohort for this nutrition-genetic study. More details about which participants were included and excluded participants can be found in Appendix E.

3.2 Anthropometric and blood lipids measures

Participants were barefoot and wore light clothing while their measurements were taken. Height was measured using a stadiometer and recorded to the nearest centimeter. Weight was measured using a calibrated scale and recorded to the nearest 0.01 kg. Body mass index (BMI) was calculated (BMI=kg/m^2) and classified using the adult International Classification (WHO) as follows: underweight (≤18.49 kg/m^2), normal weight (18.50-24.99 kg/m^2), overweight (25.00-29.99 kg/m^2), and obese (≥30.00 kg/m^2).

After an overnight fast, blood was drawn from the participants into EDTA-containing tubes by trained nurses at the Health Center at the UASLP. The blood samples were kept at 4°C and were separated within one hour of collection according to the SOP (standard operation procedure) previously established by the personal at UASLP. Aliquots were held at -80°C until laboratory testing.

Plasma lipid concentrations were determined in the Clinical Research Laboratory of the Chemistry School at UASLP on campus. TC was determined using enzymatic hydrolysis and oxidation. HDL-C was measured using a direct method, and serum TGs were determined according to the glycerol phosphate-oxidase peroxidase method, based on a colorimetric enzymatic reaction. Further details of these techniques can be found in the Appendix F. LDL-C, VLDL and non-HDL-C was calculated indirectly using a formula [169].

3.3 Measurement of dietary intake

The 116-item food frequency questionnaire (FFQ) used in our study was developed by the National Institute of Public Health (INSP) in Mexico [170, 171], and has been widely used to collect dietary information from pre-school (< 5years of age) and school-age children (5-11 years of age), adolescents (12-19 years of age), adults (20-59 years of age) and older adults (60 – 99 years of age) for both sexes [172].

The National Institute of Public Health validated the FFQ by comparing it with a 24H recall [170]. The reproducibility was assessed twice during a one-year interval, and the validity was compared by the FFQ with samples obtained during a four-day 24H recall at three-month intervals [170].

The correlation coefficients between the questionnaires and the mean values from the 24H recalls ranged from 0.4 or higher for total calories, carbohydrates, protein, total fat, and saturated, monosaturated, and polyunsaturated fatty acids intakes [170]. Parra, et al., validated this FFQ in order to evaluate dietary fatty acid intake by comparing the FFQ with the relative amounts of fatty acids in erythrocytes [173]. Estimates of fatty acid intakes obtained using this FFQ were moderately correlated using Pearson correlation coefficients among α-linolenic acids

(ALA: 0.32), docosahexaenoic acid (DHA: 0.35) and eicopentaenoic acid (EPA: 0.36). Each was significant at the $p<0.05$ level of significance for the composition of fatty acids from erythrocytes [173].

The FFQ appears in the Procedure Handbook for Nutrition Projects [174] published by the National Institute of Public Health in Mexico [162]. The FFQ included 116 food items for adults and adolescents, and is organized into 12 "ad-hoc" selected food groups. The FFQ already has a section on the dietary sources of long chain n-3 fatty acids; fresh fish, sardines, tuna, seafood (an average of ½ cup containing shrimp, crab, shellfish and octopus), cod oil, fresh and canned salmon, and a small dry fish known as *charales*. In addition, the questionnaire had a section on oils so as to account for the n-6 fatty acids (including safflower oil, olive oil, soya oil and canola oil). It also had a section on other meat sources that contain arachidonic acid (AA), such as beef and pork, and a section on vegetable sources such green leaf products [174] (Appendix C and D).

3.3.1 Calculation of nutrient intake

Calculations were performed using SNUT 3.0 software (System of Nutritional Habits and Nutrient Intake), which was developed at the National Institute of Public Health (INSP), Mexico, and patented by the Ministry of Education, copyright Division in 1999 [173]. SNUT estimates the total amount of energy and nutrients found in each particular food and in the total diet consumed by each individual using a nutritional-composition database compiled by the INSP. It combines the USDA Food composition tables with other tables from Mexico and Latin America [175-177]. These tables include analytical information for over 300 foods consumed by the Mexican population [176-178].

Daily dietary nutrient intakes for study participants were estimated for each food. A nutrient score was calculated using the food nutrient content information in the food table, adjusted for the specific portion size in the questionnaire. This score was multiplied by the weight which corresponds to the frequency of use. The weights used were: 6 for reported frequencies of 6 or more times per day; 4.5 for 4-5 per day; 2.5 for 2-3 per day; 1 for 1 per day; 0.8 for 5-5 per week; 0.43 for 2-4 per week, 0.08 for 2-3 per month and 0.016 for 1 per month or less [170].

Only one answer per food item was allowed, and the survey accounted for seasonal availability [121].We calculated the number of grams per day of specific dietary fatty acids using the previously-mentioned weights.

Total energy intake, total fat (TFA), saturated fat (SFA), monounsaturated fat (MUFA) and polyunsaturated fat (PUFA) were estimated using SNUT. This was reported as intake in terms of number of grams per day (g/day), and as percentage contribution to the total energy intake (%E). Dietary intake (g/day) of long-chain omega-3 PUFAs was calculated on the basis of the sum of 20:5n3 (EPA), 22:6n3 (DHA) and 22:5n3 (DPA). Total omega-3 was calculated on the basis of the sum of 18:3n-3 (ALA) and long-chain omega-3. Total omega-6 was calculated on the basis of the sum of 20:4n6 (AA), 18:3n-6 (GLA) and 18:2n6 (LA) [172, 179]. The methodology for the analysis of dietary data from the Mexican National Health and Nutrition survey 2006 indicates that dietary intakes which are more than 5 standard deviations away from the general distribution of energy, and less than 500 kcal, were excluded from this analysis [172].

3.4 DNA extraction from whole blood

Blood to be used for DNA extraction was collected in EDTA tubes, and DNA was extracted using a commercial DNA extraction procedure (Gentra Puregene Blood Kits, Quiagen, CA,

USA). Three volumes of RBC lysis buffer were added to 5ml of blood. The samples were centrifuged at 20,000g for 25 min. Most of the aqueous upper layer was discarded, leaving behind 1 ml which was used to prevent the loss of cells, and a brown pellet. The pellet was resuspended by adding 5 ml of cell lysis solution. Once the pellet was dissolved in the cell lysis, 2 ml of protein precipitation solution was added to continue the extraction of DNA. The samples were then centrifuged at max speed (50,000g) for 20 min. After centrifugation, the aqueous layer was transferred into 50ml tubes containing 6 ml of isopropanol. All of the tubes were gently inverted several times, followed by centrifugation at 10,000g for 20 min. The supernatant was discarded and 10 ml of 70 % ethanol was added to wash the pellet. Once the pellet was resuspended, all of the tubes were centrifuged at 10,000g for 10 min. All of the tubes were air-dried in a laminar air flow during 30-35 min. The dried pellet was rehydrated by adding 300-500 µl DNA hydration solution buffer to a stock concentration of 50ng/ul (for small pellets ~250 µl, and big pellet ~450 µl) and were labeled and stored at −80°C.

3.5 Single-nucleotide polymorphisms (SNPs) selection and genotyping

HapMap-Mex database (Project SNP database used the NCBI B36 assembly-HapMap Genome Browser data release 28, phase II+III build 16, August 10) to select FADS SNPs. The tagger procedure in Haploview V4.2 was used to determine tag SNPs using a minor allele frequency ≥15% and pairwise tagging (r^2 >0.8) in the FADS gene cluster in the Mexican population. Two SNPs, rs174546 (FADS1, position chr11:61569830) and rs1535 (FADS2, position chr11:61597972) were selected. FADS1-rs174546 and FADS2-rs1535 were in high linkage disequilibrium (LD) (r^2 = 0.958; P < 0.001) with each other. Pairwise tagging revealed that rs1535 tags 5 (r^2 = 0.88-0.95 for rs174556, rs174568, rs174549 and rs174555) in this LD block. In addition, the FADS1-rs174546 polymorphism has been described as interacting with

dietary PUFA intake and affecting plasma cholesterol concentrations in European adults and adolescents [4, 69]. Therefore, we decided to genotype both FADS SNPs in order to compare our associations with other populations. Detailed genotype determination methods were previously presented in Teran, *et al.* [174]. Appendix G is a global overview of the genotype frequencies and allele frequencies for different populations.

The sequences of the primers and PCR conditions are shown in Appendix H. Genotyping was performed using fluoropolarization technology for selected SNPs in the FADS gene (those with minor allele frequencies –MAF- greater than 10%) (Appendix I). Fluorescent signals were read using the VICTOR5 Multi-label Plate Reader (Perkin Elmer Life Sciences, Massachusetts, USA).

3.6 Statistical methods

All of the analyses were performed using Statistical Analysis System software package version 9.3 (SAS Institute, Cary, NC, USA). Covariates were assessed for normality using the Shapiro Wilk's test. Distributions of continuous variables were expressed in terms of adjusted means ± standard error (SE). Triglycerides (TG), and very-low-density liporprotein (VLDL) intakes of long-omega-3 and omega-6 were log-transformed for statistical testing.

General linear models (GLM) assume that additive, recessive and dominant models of inheritance were used to investigate the association of the FADS1/FADS2 genotype with the lipid profile. Model 1 was adjusted for age and sex (basic analysis), and model 2 was adjusted thereafter for age, sex, and BMI, followed by *Bonferroni* correction in both cases.

In order to study how gene-diet interactions determine blood lipid concentrations, we used multivariate linear regression models which included the main effects and interaction terms in a

model with dietary variables which were expressed in terms of grams per day adjusted for total calorie intake, as previously described by others [175]. However, we also tested our model using percentage of energy (%E) as a continuous or categorical variable. Dietary variables were also considered as categorical (tertiles based on the population distribution) and as continuous. Dietary intakes of omega-3 were classified in tertiles according to the omega-3-specific median %E (low: <0.06%E, medium: >0.06 and <0.16%E, and high: >0.16%E). The results are expressed as means ± standard error (S.E). All reported p-values for t-test were 2-tailed, and statistical significance was defined at the $\alpha = 0.05$.

CHAPTER 4

Genetic variations in the FADS gene cluster associated with blood lipid profile in young Mexican college students.

ABSTRACT

Introduction*:* Recent genome-wide association studies (GWA) of the Mexican population has identified several genetic loci associated with blood lipid levels in adults. The fatty acid desaturase (FADS) gene cluster in young Mexican college students has not been studied before. ***Objective***: Our aim was to report on the SNP in the FADS gene cluster associated with lipid profiles in young Mexicans from the college-age UP AMIGOS cohort. ***Methods***: Anthropometrics, cardiovascular disease history, and serum lipid profile were determined (n=445, aged 18-25 yrs.). Genotyping was conducted using the fluorescent polarization method. Genotype-phenotype associations were assessed using multiple linear regression models adjusted for sex, age and BMI. ***Results****:* Significant associations were found between carriers of the minor allele of FADS1/FADS2 genotypes and TC, LDL-C and non-HDL-C concentrations. In contrast, the association between the FADS1/FADS2 genotype and HDL-C, TG and VLDL were not significant. ***Conclusion*****:** Our results suggest that FADS1/FADS2 genotypes were associated with TC, LDL-C and non-HDL-C concentrations starting at a young age. Moreover, these results replicate previous findings concerning European and Asian adult populations. Other factors such as diet should also be taken into consideration in order to better understand the onset of dyslipidemia among young Mexican college students.

4.1. INTRODUCTION

Elevated low-density lipoprotein (LDL-C) cholesterol and low levels of high-density lipoprotein (HDL-C) cholesterol play an important role in the development of atherosclerosis and coronary heart disease [95, 168].

In Mexico, 65% of the adult population has low HDL-C, and 43.6% has hypercholesterolemia [18]. Similar trends have been found among young populations, where 62% have low HDL-C and 22.3% have hypertriglyceridemia [18].

A recent genome-wide association study in adults identified the genes which are associated with HDL-C and TG, and which contribute to the increasing predisposition toward dyslipidemia among Mexicans [1, 34, 37]. Furthermore, plasma lipid levels in childhood are known to be correlated with plasma levels in adulthood [182, 183].

Several genome-wide association (GWAS) studies have consistently reported that the fatty acid desaturase (FADS) gene cluster is associated with lower total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and higher triglycerides (TG) [33, 40, 126, 159, 184, 185]. Several studies have identified that the minor alleles of SNPs in the FADS gene cluster are associated with higher total cholesterol and TG and lower HDL-C levels. Moreover, other studies have shown that the minor alleles affect the activity of the desaturases (D5D and D6D) changing the ratios between the desaturase substrates and their products [3, 143]. Thus, the present study investigated whether individual genetic variations of the FADS gene cluster were associated to blood lipid profiles in young Mexican adults.

4.2 RESULTS

The clinical characteristics of the participants are exhibited in Table 2. No extreme values in the blood lipid profiles were found. Participant blood levels did not differ between males and females, except for weight, height and HDL-C concentrations.

Table 2. Anthropometric measurements and blood lipid profiles for young Mexicans college students.

Variables	**Males**	**Females**	**[1]p-value**
N	**219**	**208**	
Age, *yrs.*	18.7 ± 1.2	18.8 ± 1.2	0.80
Weight, *kg*	73.0 ± 15.4	61.0 ±12.6	<0.0001
Height, *m*	1.7 ± 0.1	1.6 ± 0.1	<0.0001
BMI, kg/m^2	24.5 ± 4.5	23.7 ± 4.4	0.04
TC, *mg/dL*	169.3 ± 32.6	173.7 ± 34.6	0.09
HDL-C, *mg/dL*	46.8 ± 11.5	50.4 ± 11.8	0.0001
TG, *mg/dL*[§]	104.7 ± 1.5	102.3 ± 1.5	0.31
LDL-C, *mg/dL*	99.3 ± 26.7	99.6 ± 27.2	0.90
VLDL, *mg/dL*[§]	20.9 ± 1.5	20.4 ± 1.5	0.31
Non-HDL-C, *mg/dL*	122.5 ± 31.8	122.1 ± 31.2	0.85

Values are presented as means ± standard deviation.

[§]Log-transformed values. [1]p-values are adjusted by age and sex.

Abbreviations: BMI: body mass index; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL: Very-low-density lipoprotein.

4.2.1 Genetic distribution of selected SNPs

Among the pool of study participants, 49% were homozygotic carriers for the major (MM) T-allele (TT), 44% were heterozygotic, and 7% were homozygotic carriers for the minor (mm) C-allele (CC) for FADS1-rs174546. The genotype and allele frequencies of the two SNPs included in the analysis are exhibited in Table 3. The FADS1-rs174546 was in the Hardy-Weinberg equilibrium (HWE) with a minor allele frequency (MAF) of 0.29, and the FADS2-rs1535 of 0.29.

Table 3. Characteristics of the SNPs in the FADS gene cluster

Genotype			**HapMap**[1]		**UP AMIGOS MEX**				
SNP	**Gene**	**Alleles**	**CEU**	**MEX-AM**	**MM**	**Mm**	**mm**	**MAF**	**HWE**
		M/m	**MAF**	**MAF**	**n (%)**	**n (%)**	**n (%)**		
rs174546	FADS1	T/C	0.23	0.45	220 (49%)	193 (44%)	32 (7%)	0.29	0.23
rs1535	FADS2	G/A	0.34	0.34	224 (51%)	190 (43%)	28 (6%)	0.29	0.14

[1]HapMap

CEU: Utah residents with Northern and Western European ancestry from the CEPH collection (n=113)

MEX-AM: Mexican ancestry in Los Angeles, California (n=58).

UP AMIGOS: Mexicans in San Luis Potosí, México.

4.2.2 Test for Hardy-Weinberg equilibrium

The results of the LD analysis of rs174546 and rs1535 are illustrated in Fig 2. Analysis of the linkage disequilibrium (LD) block structure for the Mexican HapMap population revealed two LD blocks in the region of interest, with the most highly significant SNPs primary appearing in the first block (Fig 2).

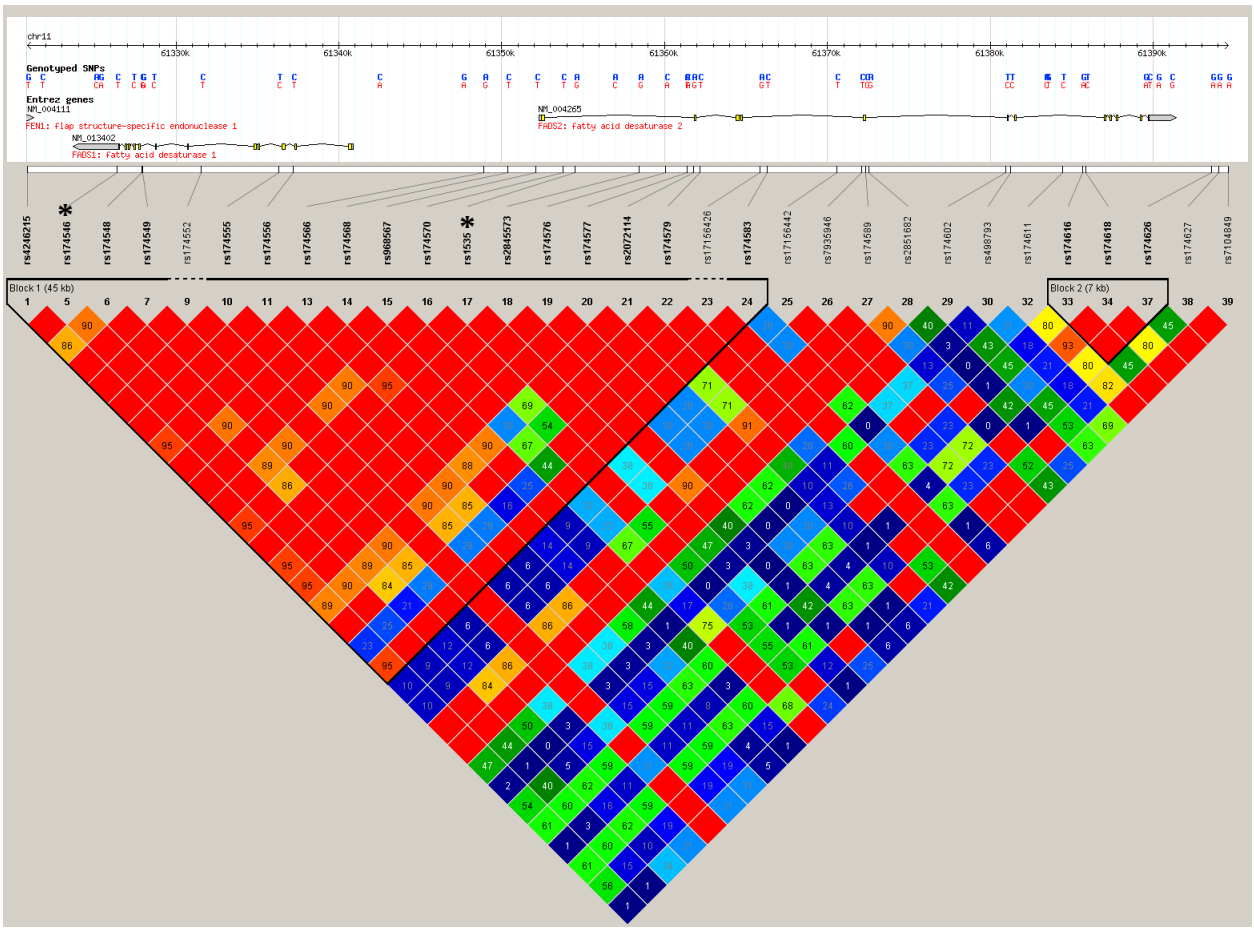


Fig 2. Linkage disequilibrium (LD) structure of SNPs near the FADS1/FADS2 locus in Mexicans. The HapMap Mex LD plot for the Mexican population is shown with the darkness of the shaded cells representing the degree of correlation between pairs of SNPs. *Marked SNPs were selected. The Scheme of the representation of LD follows the default setting of the Haploview Software.

4.2.3 Associations between the FADS1/FADS2 genotypes and blood lipids

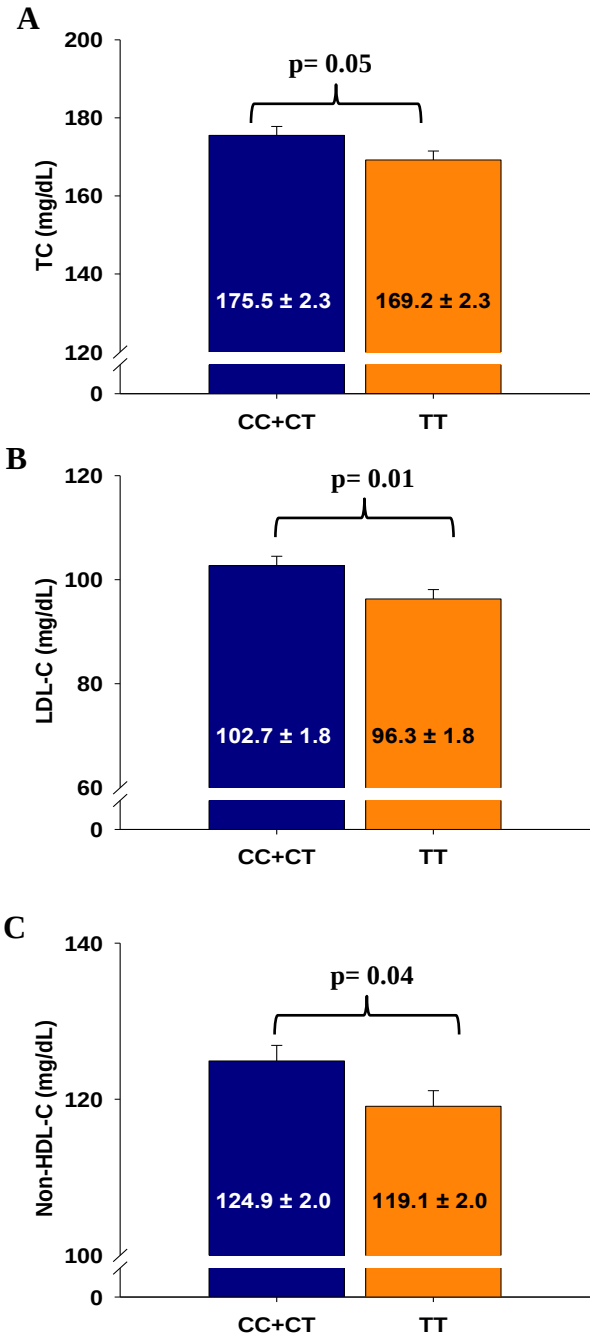
The results of the FADS SNPs associations' analysis of blood lipid profiles are summarized for TC, LDL-C and non-HDL-C using graphical representations in Fig 3 and Fig 4. Additional information can be found in appendix J.

A strong association was observed between FADS1/FADS2 SNPs and TC, LDL-C and non-HDL-C levels (p-value <0.05). However, no associations were found between any FADS SNPs and HDL-C, TG and VLDL concentrations (Appendix J).

In the dominant model, carriers of the C-allele of FADS1-rs174546 had significantly higher LDL-C and non-HDL-C ($p<0.05$) levels compared with those who are homozygous for the T allele (Fig 3 A, B, and C, respectively). A similar trend was found for carriers of the A-allele of FADS2-rs1535 compared with those homozygous of the G allele (Fig 4 A, B, and C, respectively). Thus, the C-allele of FADS1-rs174546 and the A-allele of FADS2-rs1535 might be considered to be risk alleles for higher levels of TC, LDL-C and non-HDL-C in the Mexican population.

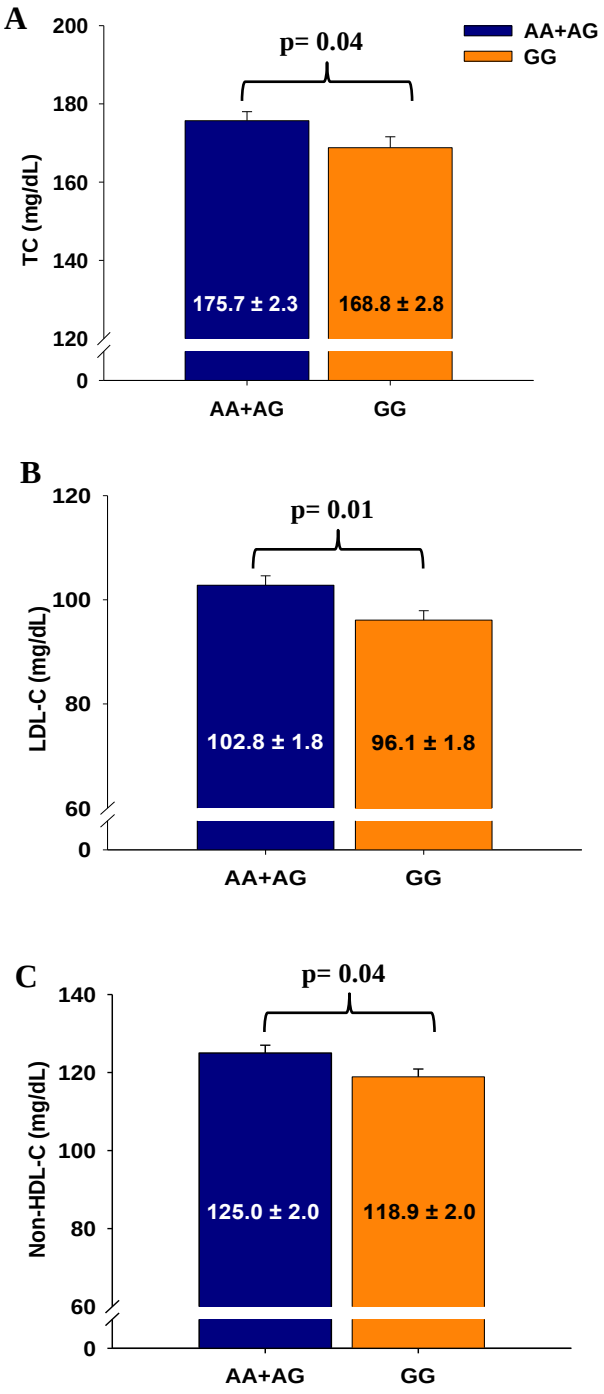
Furthermore, we observed a significant contribution of BMI on FADS1-rs174546 genotypes and the blood lipid profile. Carriers of the C-allele who were overweight or obese (OW/OB) had high blood concentrations comparable to carriers of the T-allele who were of normal weight (NW) (Fig 5, and Fig 6 for FADS1 and FADS2 genotypes, respectively). Thus, normal weight C-allele carriers had lower blood lipid levels than overweight/obese C-allele carriers. A similar trend was found among NW vs. OW/OB T-allele carriers. This highlights that being OW/OB might affect the protective effect of the T-allele for low levels of LDL-C. Additional information can be found in Appendix K

Fig 3. Association between FADS1-rs174546 genotypes and TC, LDL-C and non-HDL-C in Mexicans[1]



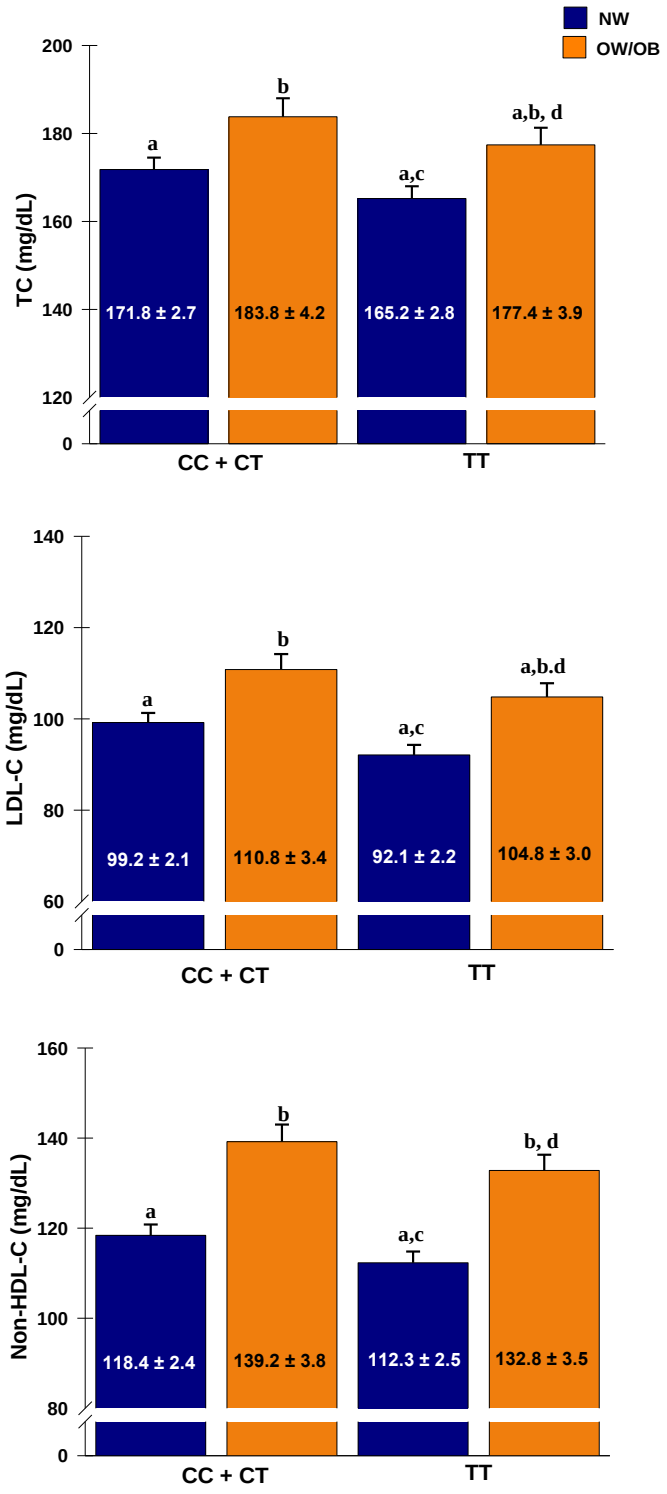
[1]Values are means ± SE adjusted for sex, age and BMI, P-value (P<0.05) under the dominant model. Sample size CC + CT (M/F: 104/116) vs. TT (M/F: 115/101).

Fig 4. Association between FADS2-rs1535 genotypes and TC, LDL-C and non-HDL-C in Mexicans[1]



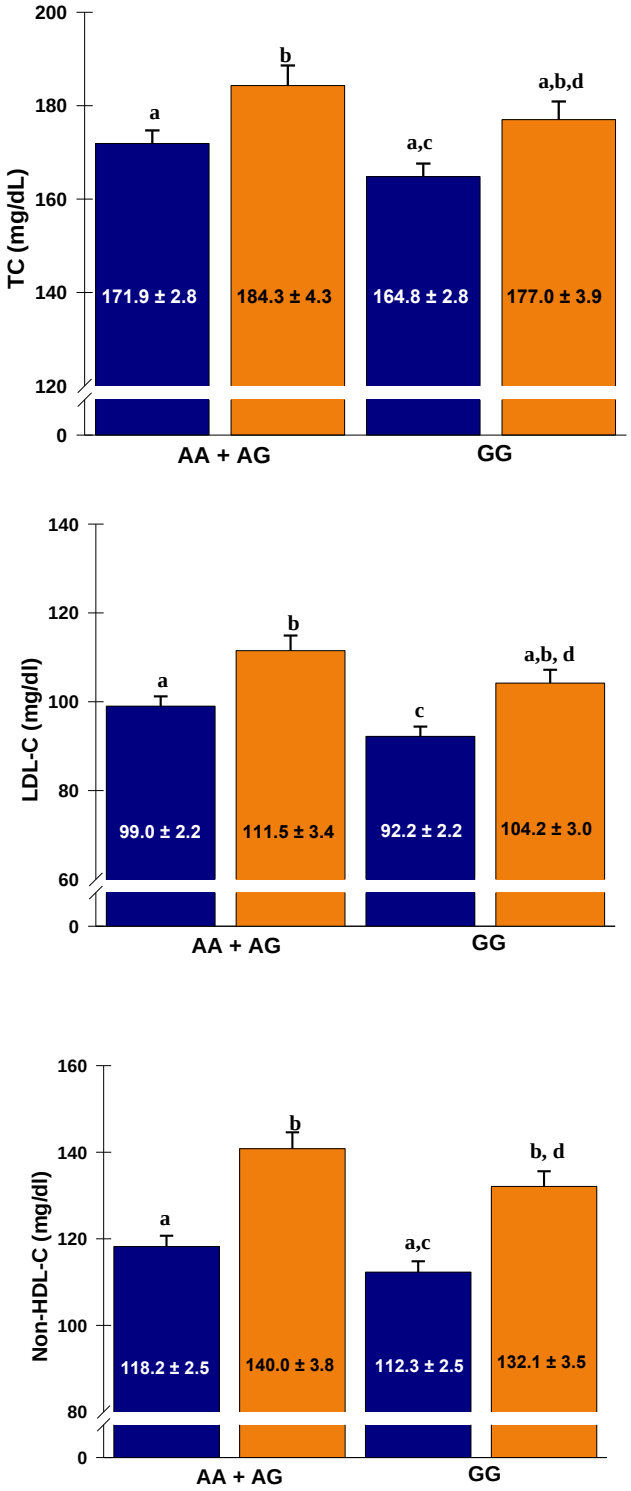
[1]Values are means ± SE adjusted by sex, age and BMI, P-value (P<0.05) under the dominant model. Sample size AA + AG (M/F: 122/99) vs. GG (M/F: 106/116).

Fig 5. Association between FADS2-rs1535 genotypes and TC, LDL-C and non-HDL-C in Mexicans[1]



[1]Values are means SE adjusted by sex, age and BMI, P-value ($P<0.05$) under the dominant model. NW: CC + CT (M/F: 66/83) vs. TT (M/F: 81/81); OW/OB: CC + CT (M/F: 40/35) vs. TT (M/F: 45/23). Values are significantly different between genotypes if they do not share the same letter (a, b, c or d; $p<0.05$).

Fig 6. Association between FADS2-rs1535 genotypes and TC, LDL-C and non-HDL-C in Mexicans[1]



[1]Values are means SE adjusted by sex, age and BMI, P-value (P<0.05) under the dominant model. NW: AA + AG (M/F: 67/86) vs. GG (M/F: 80/75); OW/OB: AA + AG (M/F: 41/34) vs. GG (M/F: 44/24). Values are significantly different between genotypes if they do not share the same letter (a, b, c or d; p<0.05).

4.3 DISCUSSION

Our study is the first to investigate the association between genetic variants of the FADS SNPs and blood lipid profiles in young Mexicans. Our results indicate that FADS1/FADS2 genotypes tend to be associated with TC, LDL-C and non-HDL-C concentrations. Our results replicate previously published research on European populations that reported an association between the minor allele and blood cholesterol [63, 121].

Minor alleles of FADS1/FADS2 SNPs were significantly associated with higher TC, LDL-C and non-HDL-C concentrations compared with carriers of the major alleles. What is remarkable is that FADS1 genotypes explained up to 8% of the variance in TC (R^2=0.08, p=0.05), 10% of the variance in LDL-C (R^2=0.10, p=0.01), and 15% of the variance in non-HDL-C (R^2=0.15, p=0.03) in young Mexicans. Along these lines, Molto-Puigmarti, et al., found that FADS genotypes in children explained 2.4 – 2.9% of the variance in TC, and around 2% of the variance for HDL-C [61]. In addition, Standl, et al., found that, for 10-year old children, the FADS SNP explains 1.2% of the variance in LDL-C concentrations [186].

A recent genome-wide association (GWA) study identified novel common variants associated with HDL-C, LDL-C and TG levels in children [187]. This study did not find any association between FADS SNPs and lipid profiles of youths. This study did highlight that different genes are expressed in childhood and adolescence compared with adulthood [187]. Other studies identified an association between FADS1-rs174546 and TG in adults [188, 189]. However, we were unable to replicate this association.

In a larger hypertriglyceridemia cohort, there is a strong association between the FADS genotype and TG [188]. It is clear that in young Mexican adults the effects of FADS

polymorphisms on TC, LDL-C and non-HDL concentrations are strongest between 18-25 years old.

Several limitations of our study should be addressed. First, no data was available regarding medications, smoking habits or alcohol consumption for our population. Second, we did not use random samples, which might have produced some form of bias. Further investigations which include controls for dyslipidemia are necessary in order to identify factors that directly influence blood lipid profiles. Moreover, longitudinal studies are needed to elucidate the genetic mechanism which underlies high LDL-C concentrations and their future consequences for this population.

4.4 CONCLUSION

In summary, the present study showed that FADS1-rs174546 and FADS2-rs1535 are associated with the plasma concentrations of TC, LDL-C and non-HDL-C in Mexican population. Most notably, we found that carriers of the minor allele of FADS1/FADS2 exhibited higher levels of atherogenic lipoproteins such as LDL-C.

According to the NCEP (National Cholesterol Program), lipid management is essential for significantly reducing and preventing cardiovascular events [23, 24, 190, 191]. However, it is not presently known whether or not the FADS genotype has an impact on the development of CVD later in life. Nevertheless, these results indicate that young Mexican population need to engage in appropriate management of their serum blood lipids starting early in life, particular LDL-C levels, depending on their genetic predispositions in their FADS1/FADS2 genotypes. Furthermore, longitudinal studies are needed to identify the best strategy for future interventions.

CHAPTER 5

Gene-nutrient variation in the FADS gene cluster associated with dietary fat intake profile on blood lipid profiles in young Mexican college students.

ABSTRACT

Introduction: Genetic variation in the FADS gene plays an important role modulating the association between dietary n-3 on plasma cholesterol concentrations in European population ***Objective:*** Our aim was to examine whether genetic variations in the FADS gene cluster interact with dietary intake of omega-3 and omega-6 to affect the lipid profiles. ***Methods:*** Anthropometrics, dietary intake, and serum lipid profile were determined (n=445, aged 18-25 yrs.). Genotyping was conducted using the fluorescent polarization method. Genotype-phenotype associations were assessed by multiple linear regression models adjusted by sex, age, BMI, and calorie intake. ***Results***: Significant associations between FADS1/FADS2 polymorphism and TC, LDL-C and non-HDL-cholesterol concentrations were observed. The minor C-allele of FADS1-rs174546 and minor A-allele of FADS2-rs1535 were associated with higher TC and LDL-C. We observed significant association between FADS2-rs1535 and long-chain omega-3 intakes on TC (p=0.04), LDL-C (p=0.01) and non-HDL-cholesterol (p=0.03) concentrations. On the other hand, FADS1-rs17454 and long-omege-3 was only associated on LDL concentrations. In addition, significant interaction was observed between rs1535 and long-omega-3 on HDL-C (p-interaction=0.03). ***Conclusion:*** Genetic variation in the FADS gene cluster potentially interacts with dietary intake of long-omega-3 to affect TC, LDL-C and non-HDL-C concentrations in the young Mexican population. Gene-nutrient interaction influencing blood lipid concentrations should be investigated further in other studies.

5.1 INTRODUCTION

High rates of obesity, dyslipidemias and metabolic syndrome have been described in the Mexican population [10, 18, 192]. Recent studies indicate that a Western dietary pattern is the most common type of diet among young Mexicans. This diet has been linked to the widespread prevalence of obesity, metabolic syndrome and dyslipidemias recently reported in Mexico [31, 32, 165, 193].

Dietary intakes of ~250mg/d of docosahexanoic acid (DHA; C22:6 n-3) and eicosapentanoic acid (EPA; C20:5 n-3) have been reported to be associated with a reduced prevalence of metabolic syndrome [50], cardiovascular disease [111], and decrease serum TG and increased HDL-C [51]. These daily requirements might be the result of the consumption of two servings of seafood per week (4 oz. per serving) [194].

The FADS1 and FADS2 genes code for the delta-5 (D5D) and delta-6 desaturases (D6D). Both D5D and D6D are crucial enzymes responsible for the formation of long chain polyunsaturated fatty acids (PUFAs) [59]. Carriers of functional risk alleles in the FADS locus can form more AA from dietary LA, and may have greater susceptibility to atherosclerotic vascular damage [68, 77, 78, 195]. Moreover, dietary intake of omega-3 and omega-6 PUFAs modulates the association between the FADS gene variants and lipid levels in European children, adolescents and adults [64, 196, 197]. Given that Mexican adolescents obtain ~10% of their daily energy from PUFAs [30], the overall objective of the second aim is to test whether the dietary intake of omega-3 and omega-6 PUFAs modifies genetic associations in the FADS1-rs174546 cluster gene and lipid profiles described above.

5.2 RESULTS

The dietary fat intakes did not differ significantly between FADS1/FADS2 polymorphisms (Appendix L**).**

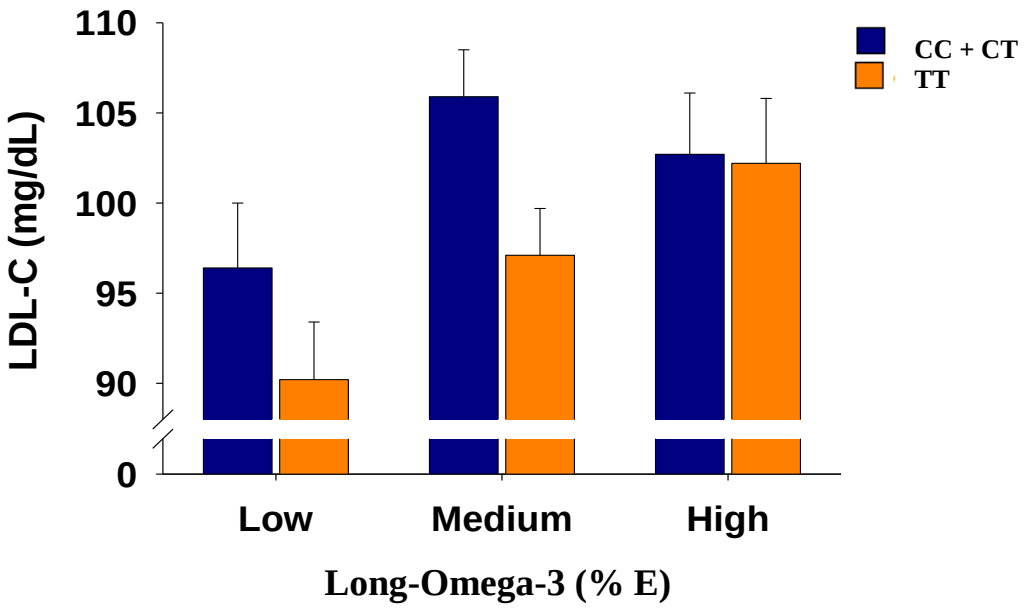
Whatever the genetic model tested, there was no significant association between dietary fatty acid intake and the FADS1/FADS2 polymorphism in the lipid profiles, except for dietary intake of DHA, DPA, long-omega-3 and total-omega-3 (Appendix M).

We found a significant association between the FADS1-rs174546 and FADS2-rs1535 polymorphism and intake of long-omega-3 on LDL-C (p=0.005), as a continuous variables (Appendix M). On the other hand, we found borderline significant associations on TC (p=0.06) and non-HDL-C (p=0.05) between the FADS1/FADS2 genotypes and intake of long-omega-3 (g/d) (Appendix M). In spite of this further adjustment, the intake of long-omega-3 did not substantially change the results observed between FADS1/FADS2 polymorphisms alone.

There was a significant interaction between the FADS1-rs174546 polymorphism and long-omega-3 (in g/d) on HDL-C concentrations (p-interaction=0.045) under the dominant model despite the lack of significant association between FADS1-rs174546 polymorphism and HDL-C concentrations in any model. No significant interactions were found between FADS2-rs1535 polymorphism and the blood lipid profiles.

After stratification by long-omega-3 intake in tertiles, there was still a significant association between FADS1-rs174546 and long-omega-3 tertiles (g/d) on LDL-C concentrations (p=0.03) (Fig. 7), but no interactions were found. Similar trend was found for FADS2-rs1535 (data not shown). No significant associations were found between the FADS1/FADS2 polymorphisms and intakes of omega-6 on blood cholesterol concentrations.

Fig 7. Association between FADS1-rs174546 and LDL-C concentrations in strata of long-omega-3 intake in percent of energy (%E) in the UP AMIGOS cohort.



Multiple linear regression model adjusted for sex, age, and BMI. P-value for trend across genotypes (p=0.03), long-omega-3 intake (p=0.01), and p-value of the interaction omega-3*FADS1 (p=0.35). Dietary long-omega-3 intakes (%E) were classified in tertiles according to the study population distribution: Low <0.06%E, medium >0.06 to <0.16%E, and high >0.16%E.

5.3 DISCUSSION

This study reports a significant association between the dietary intake of total omega-3 and the FADS1/FADS2 polymorphism on blood lipid concentrations in the Mexican population. Low intakes of omega-3 were associated with lower LDL-C concentrations in carriers of the C-allele. It is necessary to point out that in European populations for FADS1-rs174546, the minor T allele was associated with decreased LDL-C. By contrast, in the Mexican population, the C-allele was the minor allele and was associated with higher levels of LDL-C. This switch in the minor allele frequency has been described before as being a consequence of an adaptive evolution of the FADS gene cluster [198, 199]. Hence, our results replicate, the previously reported association between FADS1-rs174546 and LDL-C in European populations [4, 69], where carriers of the C-allele with high intakes of n-3 PUFA intake were associated with higher LDL-C. However, the associations between omega-3 and FADS1-rs174546 on TC, HDL and non-HDL-C were not replicated. Thus, our data suggests that dietary intake of omega-3 modulates the impact of FADS1 genetic variants on LDL-C concentrations at a young age.

Our results showed that higher omega-3 intakes were associated with higher LDL-C concentrations. This association has been described before by several intervention studies, where fish oil supplementation increased LDL-C and decreased TG concentrations [200]. However, the mechanism remains unclear.

Previous studies have found that genetic variations in the FADs gene cluster influence the activity of the enzyme delta-5 desaturase (D5D) [67, 184, 201-203]. It appears from these studies that carriers of the minor allele exhibited significantly lower D5D activity than was the case for

subjects with the major allele [158, 201]. Furthermore, in haplotypes carrying the FADS1-rs174546 minor allele has the largest effect affecting D5D activity [201].

D5D and deta-6 desaturase (D6D) are key enzymes in endogenous PUFA metabolism [59]. These enzymes are encoded by the FADS1 and FADS2 genes. Several studies have revealed that a defect in the activity of D5D and D6D might be a risk factor for the development of atherosclerosis [204], obesity [147, 205], diabetes [206] and dyslipidemia [63].

Several studies reported associations between FADS SNPs and serum and erythrocyte fatty acids. These studies have shown that carriers of the minor allele of the FADS gene cluster had enhanced levels of LA, EDA, DGLA (n-6) and ALA (n-3), and decreased levels of GLA, AA, EPA and DHA [67, 155]. It is well known that arachidonic acid-derived eicosanoids produce primarily pro-inflammatory effects, whereas EPA-derived eicosanoids are less inflammatory [143]. Likewise, different studies suggest that dietary intakes of different PUFAs interact with FADS polymorphisms to affect blood lipid levels [4, 62]. In addition, the activity of D5D and D6D might be affected by diet [205].

A cross-sectional study of two genetically similar Asian ethnic groups found that differences in lifestyle modifies the effect of FADS polymorphism on lipid profiles [62]. This study showed that in individuals with higher intakes of fish product, the risk allele of FADS1-rs174547 was associated with TG and HDL-C concentrations, whereas among individuals with higher intakes of livestock products and higher n-6/n-3 PUFAs ratios, the risk allele of FADS1-rs174547 was associated with decreased LDL-C but not with TG or HDL-C [62]. FADS1-rs174547 was in tight LD with FADS1-rs174546 in an Asian population. This study thus brings more information about the impact of lifestyle on FADS-lipid profiles, and highlights that individuals with risk

alleles in the FADS gene cluster may experience different responses to the dietary intake of PUFAs.

Our study has several limitations. First, we investigated only one SNP in the FADS1-rs174546 in high LD with FADS2-rs1535 in the FADS gene cluster. However, other genetic variants may be involved. Second, the nutrition survey used in this project was established by the Mexican National Institute of Public Health and published in the health and nutrition reports in 2012 [172, 179]. Even when the nutritional survey shows that there is a high correlation between the questionnaire and serum and erythrocytes fatty acid levels in blood [144, 170, 173, 179]. There is no evidence (experimental) to indicate that the diet reflects the fatty acid composition in the body or the activity of the desaturases. Still, this is the first study to analyze dietary fatty acids in a genetic study, and future studies which include fatty acid composition in serum or erythrocytes may support our hypothesis. Third, we did not investigate the effect of other fatty acids, such as lauric acid (C12:0), myristic (C14:0) and palmitic acid (C16:0), all of which are quite prevalent in Western diets. This is important because a recent review showed that these fatty acids increase LDL-C concentrations [207].

5.4 CONCLUSION

In conclusion, genetic variations in the FADS1/FADS2 gene cluster were associated with the dietary intake of omega-3 on blood lipid concentrations in a young Mexican population. Further investigations are needed to explore the potential mechanism of the genetic variations in the FADS gene cluster in a dietary intervention, and to investigate how this association can be modified by physical activity, smoking or drinking habits in this age group.

CHAPTER 6

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

The Mexican population has a genetic predisposition towards dyslipidemias [1, 34, 37]. Our results show that FADS1-rs174546 and FADS2-rs1535 are associated with TC, LDL-C and non-HDL-C levels in young Mexicans. These results replicate previous findings for European adult populations where FADS1-rs174546 was associated with TC and LDL-C [208]. On the other hand, we were unable to replicate the association between FADS SNP and HDL- and TG results reported to exist in children and adults [61, 189, 208].

The most significant piece of evidence found in this research is the strong association between FADS SNPs and LDL-C concentrations in young Mexican populations. In addition, our study showed that FADS SNPs explain the high variability in blood lipid profiles in young Mexican adults. This is the most significant piece of evidence in this research because the age of our target population might be the key to identifying genes associated with cardiovascular risk profiles.

Evidence from 2-year-old infants shows that FADS SNPs are associated with plasma TC and HDL-C concentrations [61]. This study shows that FADS polymorphisms explained 2.4 – 2.9% of the variance in TC, and around 2% of the variance for HDL-C [61]. In European children (~10yrs old), six FADS SNPs explain 1.2% of the variance for TC, HDL-C, LDL-C and TG concentrations [186]. In adolescents (~14yrs old), FADS SNPs have been associated exclusively with TC and non-HDL-C concentrations [69]. Finally, among people 60 years of age and older, FADS SNPs have been associated with higher risk of coronary artery disease [63, 78, 154, 209]. Thus, our study pointed out that ages 18 through 25 years may be the crucial period in life where

the development of atherosclerosis and consequently CVD is most heavily influenced by genetics.

A longitudinal study supports our evidence drawn from a young adult population. Tikkanen, et al., found that lipid levels are strongly explained by the known lipid loci at young ages to a greater degree than in adults. This study showed that between 3 and 6 years of age, and again from 15 to 18 years of age, ~20% of the variance in HDL-C and TC can be explained by genetics, while among individuals between 33- to 45 years of age, the variance in blood lipids can be described only by ~10% [47]. Thus, among adults, intensive lifestyle interventions early in life might be the key to reducing the widespread prevalence of cardiometabolic risk factors.

Cardiovascular disease (CVD) is the most common cause of death among Mexican adults [210]. LDL-C in particular is a strong predictor of CVD [211]. According to the ENSANUT 2006, thirty percent of Mexican adults require lipid-lowering treatments [212].

Plasma accumulations of LDL-C are the result of defective LDL-C clearance [213]. This lipid metabolism disturbance is a genetic disorder described in familial hypercholesterolemia (FH) [213]. Along these lines, four mutations in the gene of the LDL receptor have been described in the Mexican population [167, 214]. Thus, screening for dyslipidemia among young Mexican population could be the best strategy for future interventions. Moreover, young Mexican adults have high SFA and TFA content in their diets [215], and this population requires interventions designed to improve their lipid profiles by reducing TFA in their diets. Other studies have shown that changes in the dietary fat intake, particularly TFA intake, were associated with a significant decrease in TC and LDL-C [216]. In addition, one strong factor

affecting the lipid profiles is obesity, and several studies have shown that in changes in the diets of obese individuals are less effective than is the case for lean individuals [216, 217].

Several studies have identified the different genes that are significantly associated with status BMI that influences serum lipid profiles [218-223]. In the indigenous Mexican population, 8 SNPs in 6 genes (ADIPOQ, FTO, TMEM18, INSIGN2, FAIM2/BCDIN3 and BDNF) were associated with increased BMI in children and adults [224].

In the UP AMIGOS cohort the association of FTO-rs805704 with obesity and obesity-related phenotype was replicated [225]. Still, additional evidence is necessary in order to better understand the connection between the widespread prevalence of obesity and dyslipidemia in the Mexican population.

This study found that BMI status has a strong impact on the FADS1/FADS2-lipid profile interaction. Moreover, our results highlight that dietary fat, particularly omega-3 intake, contributed to LDL-C concentrations. Thus, the benefit of the dietary intake of omega-3 on the blood lipid profile might depend on particular FADS genotypes.

It is important to replicate these results using a well-powered cohort with good-quality dietary data and fatty acid composition in order to determine which factors (genetic or environmental) might contribute to the atherogenic phenotype. In addition, we learned from randomized trial studies that lifestyle interventions combined with physical activity result in significant weight losses and changes in cardiometabolic risk factors [226, 227]. Additional studies are clearly needed to determine the long-term effects of the interaction between genetics and diet on the blood lipid profiles in young adults later in life.

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Appendix A

Gene-phenotype studies with significant associations between FADS polymorphism and lipid concentrations.

	SNPs	Population	Gene-phenotype interaction studies
Aulchenko et al. 2009	rs174570	17,797 – 22,562 European population cohort aged 18-104 years old.	Carriers of the major G allele had higher levels of TC and LDL-C than carriers of the minor A allele.
Kathiresan et al. 2009	rs174547	19,840 Caucasian adults	Carriers of the minor C allele had lower levels of HDL-C and higher levels of TG.
Sabatti et al. 2009	rs174537, rs102275, rs174546, rs174556 and rs1535	4763 individuals from the Northern Finland Birth Cohort	Carriers of the minor alleles had lower LDL-C levels.
Nakayama et al. 2010	rs174547	21,004 Japanese adults and 1,203 Mongolian adults.	In Japanese population carriers of C allele was associated with increased TG levels and decreased of HDL-C. In Mongolian population carriers of the C allele had decreased LDL-C.
Liu et al. 2012	rs174546	30,500 Chinese adults	Carriers of the minor C allele had higher TG, low HDL-C levels and increased coronary heart disease (CHD) risk.

Qin et al. 2011	rs174556	391 Chinese adults	Carriers of the minor T allele were associated with higher prevalence of CHD.
Merino et al. 2011	rs174547	78 Caucasian adults and 69 Asian aged 20 -29 years	Carriers of the C allele had lower desaturase activity than carriers of the T allele in both Caucasian and Asian.
Martinelli et al. 2008	rs174545, r174570, rs174583, rs1000778	876 adults from European community.	Carriers of the risk allele had higher desaturase activity associated with a higher pro-inflammatory response.
Bokor et al. 2010	rs174546, rs968567, rs174572, rs174611, rs174616, rs174602	1,144 European adolescents	Minor alleles of nine SNPs were associated with higher LA, AA and lower D5D activity.
Tanaka et al. 2009	rs174537	1075 European adults	Carriers of the minor T allele had lower concentration of AA, decreased LDL-C and TC compared to the carriers of the major allele.
Sergeant et al. 2012	rs174537, rs102275, rs174546, rs174556, rs1535, rs174576, rs174579	166 European American (EA) and 63 African American (AfA) adults.	In EA seven SNPs in the FADS locus were strongly associated with AA, EPA and DGLA. Carriers of the T allele had lower AA levels and higher DGLA levels than carriers of the major allele. In AfA carriers of the G allele had higher AA levels.

Schaeffer et al. 2006	rs174544, rs174553, rs174556, rs174561, rs174568, rs968567, rs99780, rs174570, rs2072114, rs174583, rs174589	727 Caucasian adults from Germany	SNPs in the FADS cluster were strongly associated with changes in phospholipids fatty acid composition. Carriers of the minor allele had increases levels of ALA and LA and decreased levels of AA.
Malerba et al. 2008	rs174545, rs174556, rs174561, rs3834458, rs174570, rs2224299, rs174583, rs174589, rs174611, rs174627	658 Italian adults	Minor alleles were associated to higher levels of LA, ALA, EPA and lower levels of AA in serum phospholipids and in erythrocyte cell membranes.

Appendix B

Gene-phenotype-nutrient interaction studies with significant associations between FADS polymorphism on lipid concentrations

Author	**SNPs**	**Population**	**Gene-phenotype interaction studies**	**Gene-nutrient-phenotype interaction studies**
Hellstrand et al. 2012	rs174547	4,635 Swedish adult population	Carrier of the C allele had lower LDL-C levels compared to those with the major allele.	Carriers of the C-allele had lower LDL-C among individuals within the lowest tertile of long-chain ω-3 PUFAS intake.
Lu et al. 2010	rs174546, rs482548, rs174570	3575 subjects of the Doetichem Cohort Study (Netherlands).	Carriers of the C allele had higher levels of TC, non-HDL-C and HDL-C levels.	In individuals consuming high intakes of ω-6 PUFAs had higher levels of TC.
Mathias et al. 2011	rs174537, rs174545, rs174546, rs174553, rs174556, rs174561, rs174568, rs99780	224 individuals with European descent.		Minor allele across all SNPs was consistently associated with decreased ω-6 PUFAs, and increased levels of DGLA.
Al-Hilal et al. 2013	rs174537	310 Caucasian adults	Minor allele was associated with decreased activities of	Increasing EPA and DHA intake significantly decreased D5D and

			D5D and D6D.	decreased D6D activity.
Dumont et al. 2011	rs174546	573 European adolescents	Carriers of the minor allele had decreased TC.	Carriers of the minor T allele had decreased TC and non-HDL-C levels in adolescents reporting a high dietary ALA.
Standl et al. 2012	rs174545, rs174546, rs174556, rs174561, rs174575, rs3834458	2006 European children aged 10 years old.	Minor alleles were significantly associated with higher levels of TG and lower levels of TC, HDL-C and LDL-C levels.	A higher ω-3 PUFA intake was associated with lower concentrations of TC, LDL-C, HDL-C and higher TG levels, independently of the genotype.
Cormier et al. 2012	rs174546	254 Canadians aged 18-50 years old.	Carriers of the minor allele had higher TG than those with the major allele.	Carriers of the C allele had higher TG levels, independently of ω-3 PUFAS intake.

Appendix C

UP AMIGOS survey- Spanish version

CUESTIONARIO DE ALIMENTACION (FRECUENCIA DE CONSUMO).

Adaptado del Instituto Nacional de Salud Pública. Centro de Investigación en Salud Pública.

Por favor indique con una cruz en la columna que corresponda a la opción más cercana a su realidad y en la columna de la derecha registre el número correspondiente a la frecuencia de consumo reportada.

FRECUENCIA DE CONSUMO												
ALIMENTO **Productos lácteos**	NUNCA	MENOS DE UNA VEZ AL MES	VECES AL MES	VECES A LA SEMANA			VECES Al DIA				**CLAVE**
	(01)	(02)	1-3 (03)	1 (04)	2-4 (05)	5-6 (06)	1 (07)	2-3 (08)	4-5 (09)	6 (10)	
1 Un vaso de leche entera	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
2 Una rebanada de queso fresco o ½ taza de queso cottage	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
3 Una rebanada de queso Oaxaca	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
4 Una rebanada de queso Manchego o Chihuahua	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
5 Una cucharada de queso crema	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
6 Una taza de yogurt o búlgaros	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
7 Un barquillo helado de leche	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]

Durante el año previo a este día ¿Con que frecuencia consumió usted FRUTAS? Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad. Incluya las frutas que estuvieron disponibles solo en temporada.

FRECUENCIA DE CONSUMO											
ALIMENTO **Frutas**	NUNCA	MENOS DE UNA VEZ AL MES	VECES AL MES	VECES A LA SEMANA			VECES Al DIA				**CLAVE**
	(01)	(02)	1-3 (03)	1 (04)	2-4 (05)	5-6 (06)	1 (07)	2-3 (08)	4-5 (09)	6 (10)	
8 Un plátano	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
9 Una naranja	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
10 Un vaso con jugo de naranja o toronja	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
11 Una rebanada de melón	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
12 Una manzana fresca	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
13 Una rebanada de sandia	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
14 Una rebanada de piña	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
15 Una rebanada de papaya	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
16 Una pera	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
17 Un mango	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
18 Una mandarina	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
19 Una porción de fresas (~10)	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
20 Un durazno, chabacano o nectarina	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
21 Una porción de uvas (de 10 a 15)	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
22 Una tuna	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
23 Una porción de ciruelas (~6)	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
24 Una rebanada de mamey	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
25 Un zapote	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]

Durante el año previo a este día ¿Con que frecuencia consumió usted CARNES, HUEVO Y EMBUTIDOS? Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad.

FRECUENCIA DE CONSUMO

ALIMENTO **Carnes, huevo y embutidos**	NUNCA (01)	MENOS DE UNA VEZ AL MES (02)	VECES AL MES 1-3 (03)	VECES A LA SEMANA 1 (04)	2-4 (05)	5-6 (06)	VECES AI DIA 1 (07)	2-3 (08)	4-5 (09)	6 (10)	**CLAVE**
26 Un huevo de gallina	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
27 Una pieza de pollo	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
28 Una rebanada de jamón	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
29 Un plato de carne de res	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
30 Un plato de carne de cerdo	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
31 Una porción de atún	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
32 Un pedazo de chicharrón	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
33 Una salchicha	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
34 Una rebanada de tocino	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
35 Un bistec de hígado o hígados de pollo	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
36 Un trozo de chorizo o longaniza	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
37 Un plato de carne de pescado fresco (i.e. mojarra)	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
38 Un plato de sardinas en tomate	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
39 Media taza de mariscos	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
40 Un plato de carnitas	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
41 Un plato de barbacoa	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]

Durante el año previo a este día ¿Con que frecuencia consumió usted VERDURAS? Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad.

FRECUENCIA DE CONSUMO

ALIMENTO **Verduras**	NUNCA (01)	MENOS DE UNA VEZ AL MES (02)	VECES AL MES 1-3 (03)	VECES A LA SEMANA 1 (04)	2-4 (05)	5-6 (06)	VECES AI DIA 1 (07)	2-3 (08)	4-5 (09)	6 (10)	**CLAVE**
42 Un jitomate en salsa o guisado	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
43 Un jitomate crudo o en ensalada	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
44 Una papa o camote	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
45 Media taza de zanahoria	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
46 Una hoja de lechuga	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
47 Media taza de espinacas u otra verdura de hoja verde (acelgas)	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
48 Media taza de calabacitas o chayotes	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
49 Media taza de nopalitos	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
50 Una rebanada de tocino	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
51 Un plato de sopa crema de verduras	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
52 Medio aguacate	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
53 Media taza de flor de calabaza	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
54 Media taza de coliflor	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
55 Media taza de ejotes	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
56 Una cucharadita de salsa picante o chiles con sus alimentos	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
57 Un platillo con chile seco	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]
58 Un elote	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___]	[___][___]

Durante el año previo a este día ¿Con que frecuencia consumió usted LEGUMINOSAS o CEREALES? Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad.

FRECUENCIA DE CONSUMO											
ALIMENTO **Leguminosas**	NUNCA (01)	MENOS DE UNA VEZ AL MES (02)	VECES AL MES 1-3 (03)	VECES A LA SEMANA 1 (04)	2-4 (05)	5-6 (06)	VECES AI DIA 1 (07)	2-3 (08)	4-5 (09)	6 (10)	**CLAVE**
59 Un plato de frijoles	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
60 Media taza de chicharos	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
61 Un plato de habas verdes	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
62 Un plato de habas secas	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
63 Un plato de lentejas o garbanzos	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]

FRECUENCIA DE CONSUMO											
ALIMENTO **Cereales**	NUNCA (01)	MENOS DE UNA VEZ AL MES (02)	VECES AL MES 1-3 (03)	VECES A LA SEMANA 1 (04)	2-4 (05)	5-6 (06)	VECES AI DIA 1 (07)	2-3 (08)	4-5 (09)	6 (10)	**CLAVE**
64 Una tortilla de maíz	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
65 Una tortilla de trigo (tortilla de harina)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
66 Una rebanada de pan de caja (tipo Bimbo)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
67 Una rebanada de pan de caja integral	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
68 Un bolillo o telera	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
69 Una pieza de pan de dulce	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
70 Un plato de arroz	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
71 Un plato de sopa de pasta	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
72 Un plato de avena	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
73 Un tazón de cereal de caja (tipo hojuelas de maíz) ¿Qué tipo?_____	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
74 Cereal alto en fibra ¿Cuál? _____	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]

Durante el año previo a este día ¿Con que frecuencia consumió usted GOLOSINAS o POSTRES? Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad.

FRECUENCIA DE CONSUMO											
ALIMENTO **Golosinas o postres**	NUNCA (01)	MENOS DE UNA VEZ AL MES (02)	VECES AL MES 1-3 (03)	VECES A LA SEMANA 1 (04)	2-4 (05)	5-6 (06)	VECES AI DIA 1 (07)	2-3 (08)	4-5 (09)	6 (10)	**CLAVE**
75 Una rebanada de pastel	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
76 Una cucharadita de ate, miel, mermelada, cajeta o leche condensada	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
77 Una cucharadita de chocolate en polvo	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
78 Una tablilla/barra de chocolate	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]
79 Una bolsa de frituras	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__][__]

Por favor, indique cualquier otro alimento que usted consumió al menos una vez por semana y que no encontró en los alimentos anteriores, además de la siguiente lista, al año previo a este día. Por favor indique con una cruz, en la columna de frecuencias, la opción que considere más cercana a su realidad.

FRECUENCIA DE CONSUMO

ALIMENTO **Otros**	VECES A LA SEMANA			VECES AI DIA				CLAVE
	1 (04)	2-4 (05)	5-6 (06)	1 (07)	2-3 (08)	4-5 (09)	6 (10)	
Charales secos	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
Aceite de hígado de bacalao	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
Salmon fresco	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
Salmon enlatado	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
Sardina en aceite	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[___][___]

OTRAS PREGUNTAS

		CLAVE
¿Cuántas cucharaditas de azúcar le agrega usted a su alimentos al o largo del día? Tome en cuenta lo que le pone al café, licuado, otras bebidas, etc.	Cucharaditas	[___][___]
¿Le agrega usted sal a sus alimentos antes de probarlos?	a) Si b) No	[___]
¿Se come usted el pellejo del pollo?	a) Si b) No	[___]
¿Se come usted el "gordito" de la carne?	a) Si b) No	[___]
¿Cuántos meses del año pasado consumió usted vitaminas?	0 (a) 1-2 (b) 3-4 (c) 5-6 (d) 7-8 (e) 9-10 (f) 11-12 (g)	[___]
¿Cuál o cuáles? _____		
¿Cuántos meses del año pasado consumió usted suplemento de calcio?	0 (a) 1-2 (b) 3-4 (c) 5-6 (d) 7-8 (e) 9-10 (f) 11-12 (g)	[___]
¿Cuál o cuáles? _____		
¿Considera usted que su alimentación ha cambiado durante el ultimo año?	a) Si b) No	[___]
Si ha cambiado ¿Por qué? _____ _____ _____		
Observaciones _____ _____ _____		

Appendix D

UP AMIGOS survey- English version

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Dairy products	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
1. A glass of whole milk (%Fat____)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
2. A slice of cheese or ½ cup cottage cheese(%Fat____)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
3. A slice of Oaxaca cheese	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
4. A slice of Manchego cheese or Chihuahua cheese	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
A slice of fresh cheese	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
5. A spoonful of cream cheese	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
6. A cup of Yogurt or Bulgarians(%Fat____)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
7. A milk ice cream cone	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Fruits	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
8. A banana	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
9. An Orange	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
10. A glass of orange juice or grapefruits	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
11. A slice of melon	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
12. A fresh apple	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
13. A slice of Watermelon	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
14. A slice of pineapple	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
15. A slice of papaya	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
16. A pear	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
17. A mango	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
18. A tangerine	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
19. One serving of strawberries (~10)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
20. A peach, apricot or nectarine	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
21. One serving of grapes (10 to 15)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
22. A prickly pear	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
23. A plum portion (~ 6)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
24. A slice of mamey sapote	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
25. A Zapote	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Meats, egg and sausage	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
26. A chicken egg	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
27. A piece of chicken	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
28. A slice of ham	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
29. A plate of beef meat	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
30.A plate of pork meat (carnitas)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
31. A portion of tuna fish	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
32. A piece of pork skin (dry)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
33. A sausage or frank	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
34. A slice of bacon	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
35. A liver steak or chicken livers	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
36. A piece of sausage or chorizo	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
37. A plate of fresh fish (i.e. perch)	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
38. A plate of sardines in tomato	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
39. Half cup of seafood	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
40. Plate of shredded pork meat	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
41. A plate of Shredded beet meat-BBQ	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
A plate of Cooked Lamb	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

FREQUENCY OF CONSUMPTION															
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?	METHOD OF COOKING			
													COOKED		
Vegetables	(1)	(2)	1-3 (3)	1 (4)	2-4 (5)	5-6 (6)	1 (7)	2-3 (8)	4-5 (9)	6 (10)		RAW (A)	IN WATER (B)	IN OIL (C)	BAKED (D)
42. A tomato in sauce or stew	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
43. A raw tomato or in a salad	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
44. A potato	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
A Sweet Potato	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
45. Half a cup of carrot	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
46. A cup of lettuce	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
47. Half cup of spinach or other leafy green vegetables	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
48. Half cup of squash or chayote squash	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
49. Half cup of cactus	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
50. Canned hot green chili peppers	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
Dry hot chili pepper dish	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
51. A plate of cream vegetable soup	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
52. Half of a avocado	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
53. Half a cup of pumpkin flower/zucchini blossoms	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
54. Half a cup of cauliflower	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
55. Half a cup of green beans	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
56. A teaspoon of hot sauce or peppers with your food	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	___	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋

FREQUENCY OF CONSUMPTION										
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY			
Legumes	(1)	(2)	1-3 (3)	1 (4)	2-4 (5)	5-6 (6)	1 (7)	2-3 (8)	4-5 (9)	6 (10)
59. A plate of beans	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
60. Half cup of peas	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
61. A plate of fava beans	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
62. A plate of dried fava beans	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
63. A dish of lentils or chickpeas	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋
A plate of lima beans	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋	⌊__⌋

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Cereals	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
64. A corn tortilla	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
65. A flour tortilla	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
66. A slice of boxed white bread	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
67. A slice of boxed whole bread	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
68. A dinner or Kaiser roll	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
69. A piece of sweet bread	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
70. A plate of rice	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
71. A plate of pasta soup	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
72. A plate of oat	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
73. A bowl of cereal box (type of corn flakes). What kind?_____	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
74. High-fiber cereal. Which?_____	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Junk food or dessert	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
75. A piece of cake	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
A piece of Pastry (pie, donut, etc.)	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
76. A teaspoon of honey, jam, sweet-condensed milk or caramel?	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
77. A teaspoon of chocolate powder	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
78. A chocolate tablet/bar	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____
79. A bag of chips	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	⎕	____

FREQUENCY OF CONSUMPTION										
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY			
			1-3	1	2-4	5-6	1	2-3	4-5	6
Beverages	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
80. A medium size (can) cola drink	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
81. A flavored drink that has gas	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
82. A diet drink	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
83. A glass of sugared water	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
84. A cup of coffee without sugar	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
A cup of Instant coffee	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
85. A cup of "atole" without milk	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
86. A cup of "atole" with milk	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
87. A beer	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
88. A cup of table wine	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]
89. A drink with rum, brandy or tequila	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]

FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY/ MUCH EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Oils and fats	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
90. Corn oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
91. Soy oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
92. Sunflower oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
93. Safflower oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
94. Olive oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
95. A teaspoon of margarine	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
96. A teaspoon of butter	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
97. A teaspoon of sour cream	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
98. A teaspoon of mayonnaise	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
99. A teaspoon of vegetable shortening	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
100. A teaspoon of animal fat	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

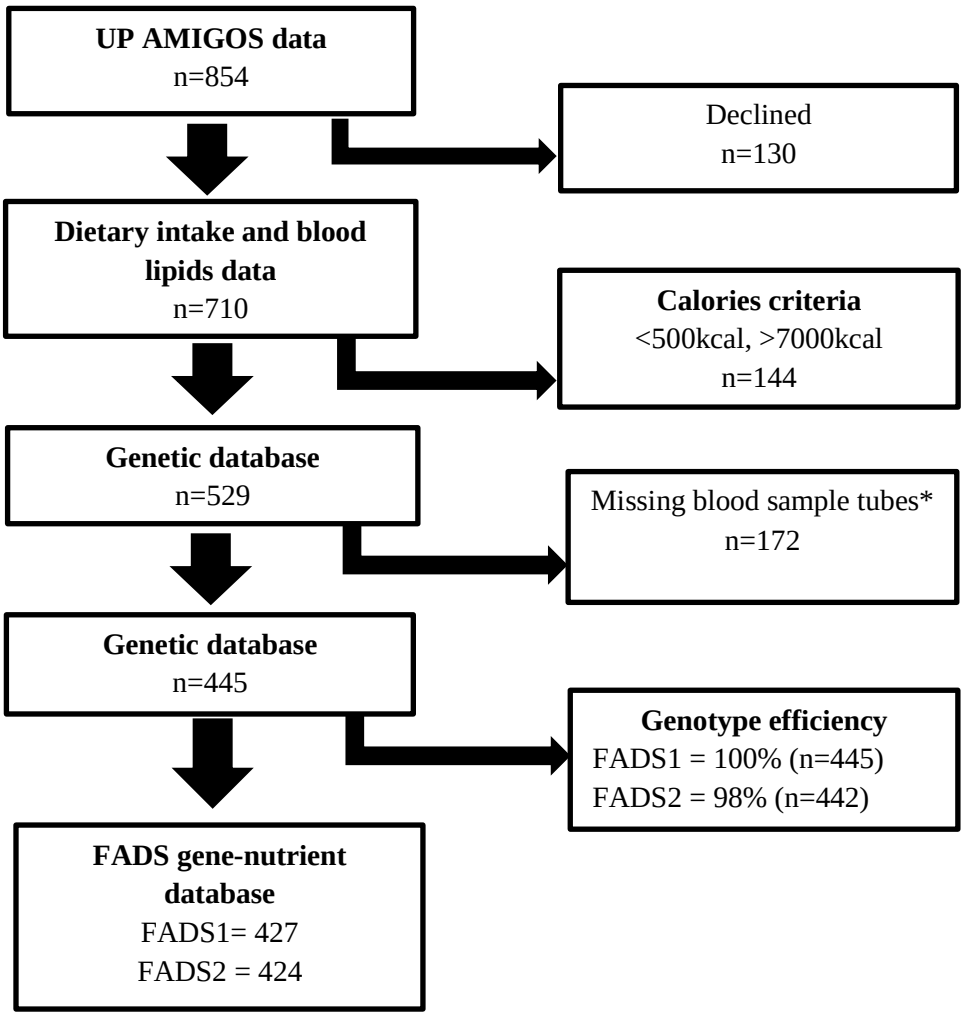
FREQUENCY OF CONSUMPTION											
FOOD	NEVER	LESS THAN ONCE A MONTH	TIMES A MONTH	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
			1-3	1	2-4	5-6	1	2-3	4-5	6	
Cravings	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
101. A taco of pork	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
102. A sope or quesadilla	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
103. A plate of pozole	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
104. A tamal	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

FREQUENCY OF CONSUMPTION								
FOOD	TIMES A WEEK			TIMES A DAY				HOW MANY EACH TIME?
	1	2-4	5-6	1	2-3	4-5	6	
Others	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
Dry Fish, charales	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
Cod liver oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
Fresh salmon	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
Canned salmon	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
Sardines in Oil	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____
	[__]	[__]	[__]	[__]	[__]	[__]	[__]	____

OTHER QUESTIONS								
								KEY
How many teaspoons of sugar you add to your food, or during the day? Consider what you put on coffee, milk shakes, other drinks, etc.					Teaspoon			[__] [__]
Do you add salt to your food before testing it?	a) Yes				b) No			[__]
Do you eat the skin of the chicken?	a) Yes				b) No			[__]
Do you eat the "fat" of the meat?	a) Yes				b) No			[__]
How many months last year did you consume vitamins? Which?_____	0	1-2	3-4	5-6	7-8	9-10	11-12	[__]
	a	b	c	d	e	f	g	
How many months last year did you consume a calcium supplement? Which?_____	0	1-2	3-4	5-6	7-8	9-10	11-12	[__]
	a	b	c	d	e	f	g	
Do you consider that you have changed your eating habits during last year?	a) Yes				b) No			[__]

APPENDIX E

Sample size and study population



*__NOTE:__ Individuals with TG (>500mg/dL), HDL (>100mg/dl) and BMI > 40kg/m^2 were excluded for this study.

APPENDIX F

Principles for quantification of glucose and lipid profiles

Chemistry of the Glucose measurement (GOD-PAP method)

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD) based on the following chemical reaction:

Enzyme glucose oxidase (GOD)

Glucose + oxygen + water ----------> gluconolactona + H_2O_2

Enzyme peroxidase

$2H_2O_2$ + 4-aminofenazona + fenol ----------> colored complex + $4H_2O_2$

The intensity of the colored complex is directly proportional to the glucose concentration in the sample, and conversion factor of mg/dL x 0.0555 = mmol/L was used.

Chemistry of the CHOD-PAP method of cholesterol measurement.

The cholesterol is determined after enzymatic hydrolysis and oxidation based on the following chemical reaction:

Cholesterol esterase

Cholesterol ester + H_2O ----------> Cholesterol + Fatty acids

Cholesterol oxidase

Cholesterol + O_2 ----------> Cholestene-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + phenol + 4-Aminoantipyrine ----------> quinoneimine (colored complex) + $4H_2O$

The indicator quinoneimine (colored complex) is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The intensity of the colored complex

is directly proportional to the total cholesterol concentration in the sample, and a conversion factor of mg/dL x 0.0259 = mmol/L was used.

Chemistry of the GOP-PAP method of triglycerides measurement

Triglycerides are generally determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of glycerol released based on the following chemical reaction:

$$\text{Triglycerides} + 3H_20 \xrightarrow{\text{Lipoprotein Lipase}} \text{glycerol} + 3\text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Enzyme glycerol kinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol Phosphate oxidase (GPO)}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine (4-AAP)} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Colored complex} + 4\ H_2O_2$$

The intensity of the colored complex is directly proportional to the triglycerides concentration in the sample, and a conversion factor of mg/dL x 0.0113 = mmol/L was used.

Chemistry of the HDL measurement

The method used in the present procedure is based on the following reactions.

Step 1

Cholesterol esterase (CE)

CM + VLDL + LDL-C esters ----------> Cholesterol + fatty acids

Cholesterol Oxidase (CO)

Cholesterol esters + O_2 ----------> Δ4-Cholesterone + H_2O_2

Catalase

2 H_2O_2 ----------> 2 H_2O_2+ O_2

Step 2

Cholesterol esterase (CE)

HDL-Cholesterol esters + O_2 ----------> Cholesterol + fatty acids

Cholesterol Oxidase (CO)

Cholesterol esters + O_2 ----------> Δ4-Cholesterone + H_2O_2

Peroxidase

2 H_2O_2 + 4-aminoantipyrine + HDAOS+ H+ + H_2O ----------> Colored complex + 5H_2O

HDAOS= (N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

The intensity of the colored complex is directly proportional to HDL-cholesterol concentration in the sample, and a conversion factor of mg/dL x 0.0259 = mmol/L was used.

Appendix G

FADS genotype and frequency distribution among population

Table G.1. FADS1 genotype and frequency distribution among population

Population	FADS1-rs174546 Genotype frequencies									
	genotype	freq	count	genotype	freq	Count	genotype	freq	count	Total
ASW (A)	C/C	0.84	48	C/T	0.16	9	T/T	0	0	57
CEU (C)	C/C	0.44	50	C/T	0.43	49	T/T	0.12	14	113
CHB (H)	C/C	0.40	55	C/T	0.45	61	T/T	0.15	21	137
CHD (D)	C/C	0.28	31	C/T	0.42	46	T/T	0.30	32	109
GIH (G)	C/C	0.82	83	C/T	0.18	18	T/T	0	0	101
JPT (J)	C/C	0.47	53	C/T	0.43	48	T/T	0.11	12	113
LWK (L)	C/C	0.96	106	C/T	0.04	4	T/T	0	0	110
MEX (M)	C/C	0.14	8	C/T	0.43	25	T/T	0.43	25	58
MKK (K)	C/C	0.87	135	C/T	0.13	20	T/T	0.01	1	156
TSI (T)	C/C	0.59	60	C/T	0.33	34	T/T	0.08	8	102
YRI (Y)	C/C	0.98	144	C/T	0.02	3	T/T	0	0	147

Population	Allele frequencies						
	Ref-allele			Other-allele			
	allele	freq	count	allele	freq	Count	Total
ASW (A)	C	0.92	105	T	0.08	9	114
CEU (C)	C	0.66	149	T	0.34	77	226
CHB (H)	C	0.62	171	T	0.38	103	274
CHD (D)	C	0.50	108	T	0.50	110	218
GIH (G)	C	0.91	184	T	0.09	18	202
JPT (J)	C	0.68	154	T	0.32	72	226
LWK (L)	C	0.98	216	T	0.02	4	220
MEX (M)	C	0.35	41	T	0.65	75	116
MKK (K)	C	0.93	290	T	0.07	22	312
TSI (T)	C	0.76	154	T	0.25	50	204
YRI (Y)	C	0.99	291	T	0.01	3	294
Note: the 'reference' allele is the base observed in the reference genome sequence at this location							

.

Table G.2 FADS2 genotype and frequency distribution among population

Population	FADS2 -rs1535			Genotype frequencies						
	genotype	freq	count	genotype	freq	count	genotype	freq	count	Total
ASW (A)	A/A	0.77	44	A/G	0.21	12	G/G	0.020	1	57
CEU (C)	A/A	0.44	50	A/G	0.43	49	G/G	0.12	14	113
CHB (H)	A/A	0.40	55	A/G	0.45	61	G/G	0.15	21	137
CHD (D)	A/A	0.29	31	A/G	0.44	47	G/G	0.28	30	108
GIH (G)	A/A	0.82	83	A/G	0.18	18	G/G	0	0	101
JPT (J)	A/A	0.46	52	A/G	0.43	49	G/G	0.11	12	113
LWK (L)	A/A	0.85	93	A/G	0.15	16	G/G	0.01	1	110
MEX (M)	A/A	0.14	8	A/G	0.41	24	G/G	0.45	26	58
MKK (K)	A/A	0.69	107	A/G	0.30	46	G/G	0.02	3	156
TSI (T)	A/A	0.57	58	A/G	0.34	35	G/G	0.09	9	102
YRI (Y)	A/A	0.78	115	A/G	0.21	31	G/G	0.01	1	147

Population	Allele frequencies						
	Ref-allele			Other-allele			
	allele	freq	count	allele	freq	count	Total
ASW (A)	A	0.88	100	G	0.12	14	114
CEU (C)	A	0.66	149	G	0.34	77	226
CHB (H)	A	0.62	171	G	0.38	103	274
CHD (D)	A	0.50	109	G	0.50	107	216
GIH (G)	A	0.91	184	G	0.09	18	202
JPT (J)	A	0.68	153	G	0.32	73	226
LWK (L)	A	0.92	202	G	0.08	18	220
MEX (M)	A	0.35	40	G	0.66	76	116
MKK (K)	A	0.83	260	G	0.17	52	312
TSI (T)	A	0.74	151	G	0.26	53	204
YRI (Y)	A	0.89	261	G	0.11	33	294
Note: the 'reference' allele is the base observed in the reference genome sequence at this location							

Appendix G (cont.)

Population descriptors:

ASW (A): African ancestry in Southwest USA

CEU (C): Utah residents with Northern and Western European ancestry from the CEPH

CHB (H): Han Chinese in Beijing, China.

CHD (D): Chinese in Metropolitan Denver, Colorado

GIH (G): Guajarati Indians in Houston, Texas.

JPT (J): Japanese in Tokyo, Japan.

LWK (L): Luhya in Webeye, Kenya.

MEX (M): Mexican ancestry in Los Angeles, California.

MKK (K): Maasai in Kinyawa, Kenya

TSI (T): Tuscan in Italy

YRI (Y): Yoruban in Ibadan, Nigeria

Appendix H

PCR conditions

Primers design

SNPs		Sequence
FADS1		
rs174546	**F**	ACCCCCTCTGAGTATTAAACTAT
	R	AGATGGAAGAAGGCCTTAAC
FADS2		
rs1535	**F**	GACCTTTCTGGTTAATTACCCAT
	R	ACCAGATTTATAGTCCATGAAAGT

Polymerase Chain Reaction (PCR) Gradient

1. PCR Master Mix

QIAGEN Mix (3.5mM MgCl2)	Final Conc.	Per reaction (µl)
Qiagen PCR buffer (10X, 15mM MgCl2)	1X	1
MgCl2(25mM)	3.5mM total	0.8
dNTPs (5mM each)	100µM each	0.4
F-primer (10µM)	120nM	0.12
R-primer (10µM)	120nM	0.12
Taq (5U/µl)		0.04
dH2O µl		2.52
Total µl		5

QIAGEN Mix (1.5mM MgCl2)	Final Conc.	Per reaction (µl)
Qiagen PCR buffer (10X, 15mM MgCl2)	1X	1
MgCl2(25mM)	3.5mM total	0.0
dNTPs (5mM each)	100µM each	0.4
F-primer (10µM)	120nM	0.12
R-primer (10µM)	120nM	0.12
Taq (5U/µl)		0.04
dH2O µl		3.32
Total µl		5

2. Add 5µl of each mix and 5µl of genomic DNA @1ng/ µl (5ng DNA/rxn).

3. PCR (Start program & wait until block is ~80˚C before adding tubes to machine)

	95ºC	2 min
35 cycles	95ºC	30 sec
	61.5ºC	30 sec
	72ºC	1.5 min
	Repeat 34 cycles	
	72ºC	5 min
	Hold 4ºC	

Agarose gel (1.5%) preparation

1. Set up the gel tray with comb.

2. Mix 0.75g agarose with 50ml of 1x TAE buffer.

3. Microwave this mixture for 30 second intervals until the agarose is dissolved..

4. Allow the mixture to cool until it can be handled.

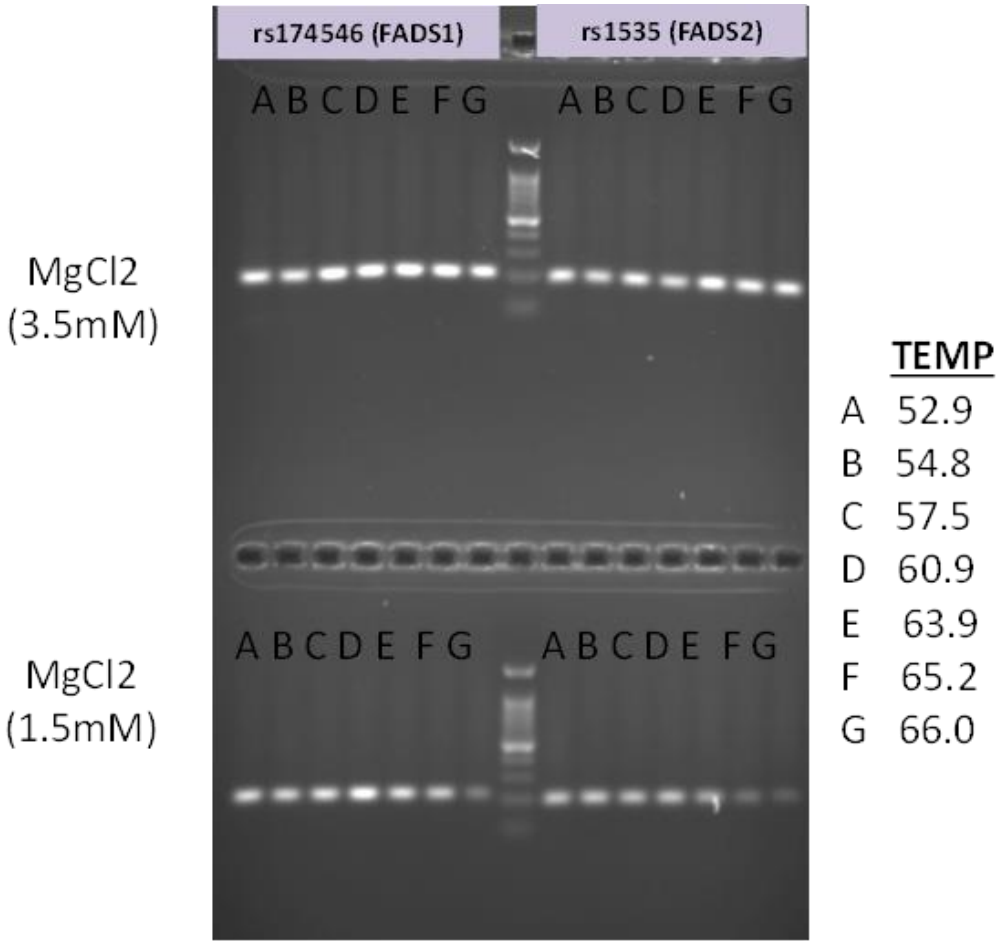
5. Add 3 µl of Ethidium Bromide to the mixture.

5. Pour the solution into the gel tray and allow it to solidify (approximately 20-30min).

6. Prepare and load the DNA, for every 5 µl of a DNA sample add 5 µl of the master mix described before.

7. Run the gel for 35 min at a constant of 100 volts

8. Take a picture and save your data



PCR Conditions

SNPs		MgCl2	Temperature
FADS1	**rs174546**	3.5mM	62ºC
FADS2	**rs1535**	3.5mM	60ºC

Appendix I

Fluoropolarization Method (FP)

Prepare a 396 Black plates samples with 5 µl of dry DNA at 1ng/µl.

1. Master Mix

QIAGEN Mix (3.5mM $MgCl_2$)	**Per reaction (µl)**	**410 reactions(µl)**
Qiagen PCR buffer (10X, 15mM $MgCl_2$)	0.6	264
$MgCl_2$(25mM)	0.48	196.8
dNTPs (2.5mM each)	0.24	98.4
F-primer (10µM)	0.072	29.5
R-primer (10µM)	0.072	29.5
Taq (5U/µl)	0.024	9.84
dH_2O µl	4.512	1850
Total µl	6	2460

Select the PCR conditions according to the SNPs

2. SAP.

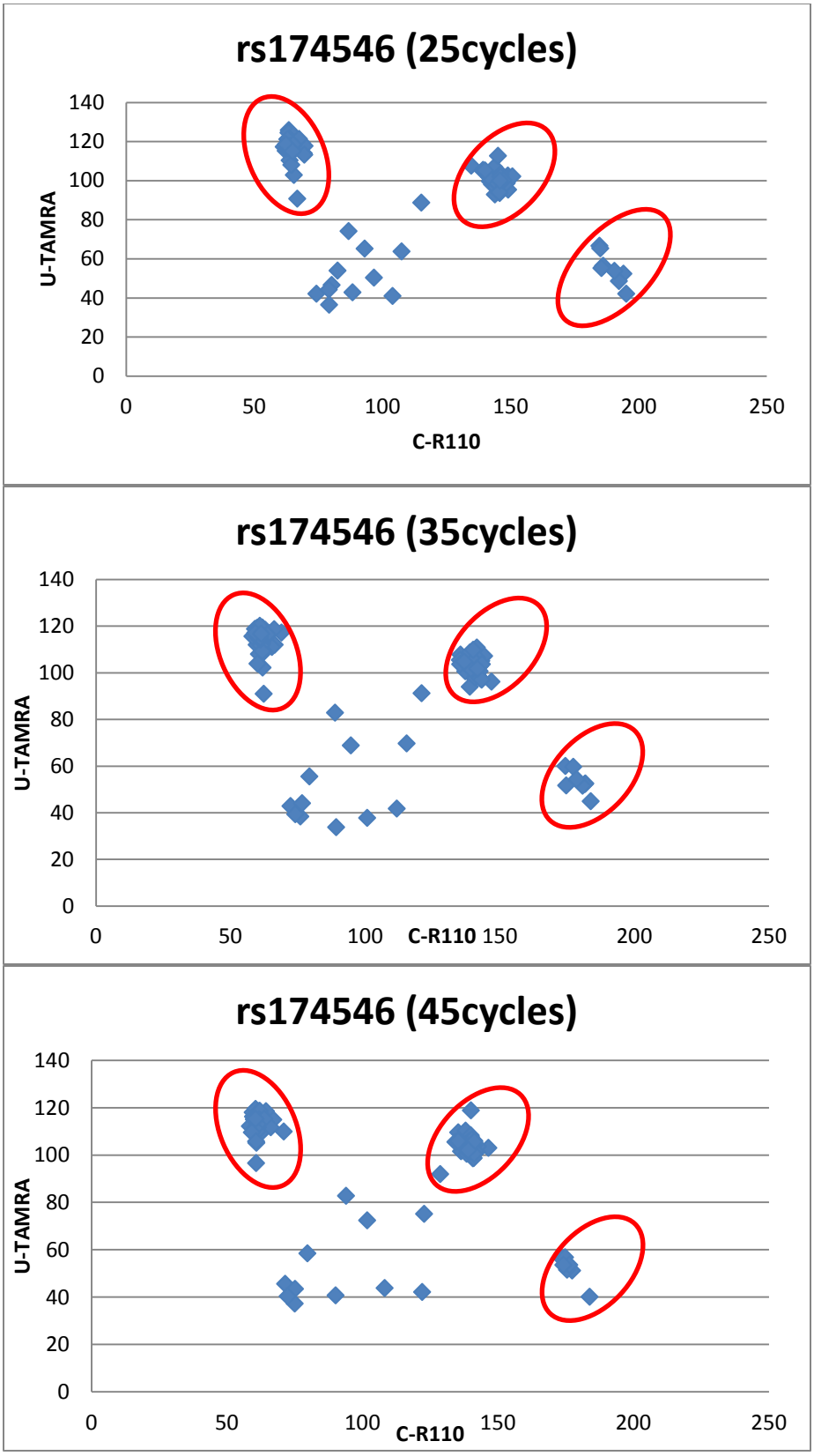
	Per reaction (µl)	**410 reactions (µl)**
ExoSAPit µl	0.65	266.5
dH_2O µl	4.35	1783.5
Total µl	5	2050

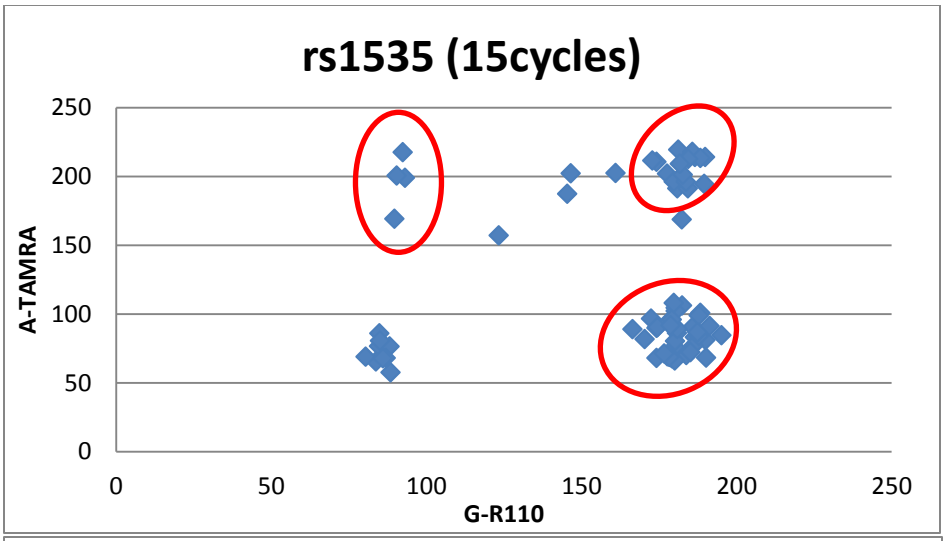
Select FPSAP

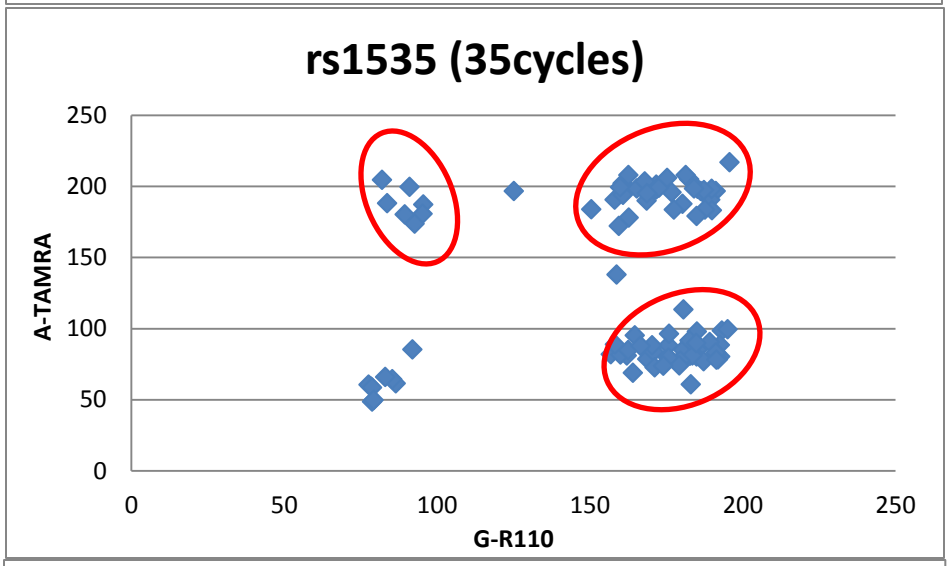
3. Single Base Pair Extension for primers

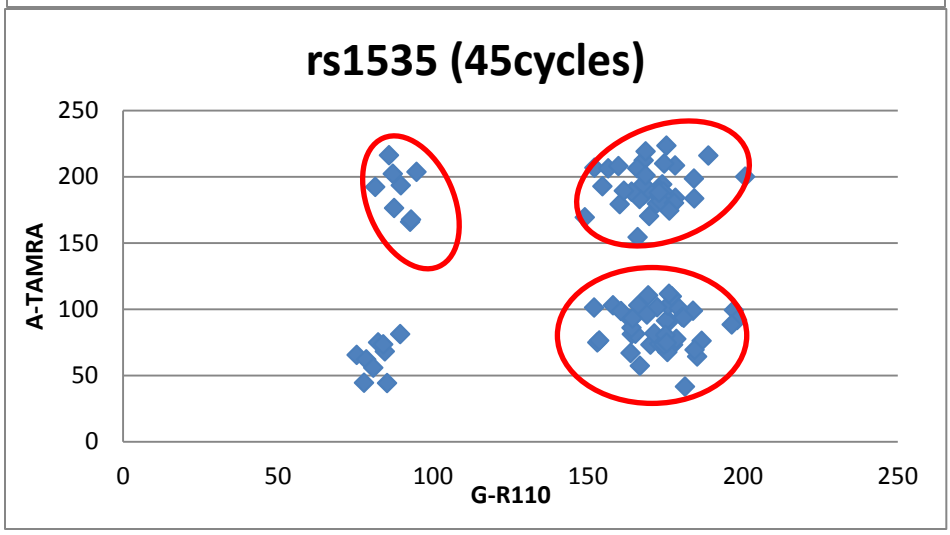
	Per reaction (µl)	**410 reactions(µl)**
Single Base Extension buffer	2	820
Primer (P3 or P4)	0.5	205
Terminator mix	0.5	205
Thermosequenase	0.025	10.3
dH_2O µl	6.975	2860
Total µl	10	4100

Appendix I


rs174546 (25cycles)
U-TAMRA
C-R110
0
20
40
60
80
100
120
140
0
50
100
150
200
250
rs174546 (35cycles)
U-TAMRA
C-R110
rs174546 (45cycles)
U-TAMRA
C-R110


rs1535 (15cycles)
A-TAMRA
G-R110
0
50
100
150
200
250


rs1535 (35cycles)
A-TAMRA
G-R110
0
50
100
150
200
250


rs1535 (45cycles)
A-TAMRA
G-R110
0
50
100
150
200
250

APPENDIX J

Supplementary tables for chapter 4

Table J.1 FADS1-rs174546 and blood lipid profiles.

	FADS1 (rs174546)						
Variable	**CC (n=28)**	**CT (n=187)**	**TT (n=212)**	**CC + CT (n=215)**	[1]p	[2]p	[3]p
TC	166.3±6.3	177.0±2.4	169.2±2.3	175.5±2.3	0.04	0.29	0.05
HDL-C	49.6±2.2	49.4±0.9	49.6±0.8	49.4±0.8	0.98	0.85	0.88
TG [§]	92.9±6.9	101.2±2.9	104.2±47.8	100.0±2.5	0.32	0.23	0.27
LDL-C	96.4±4.9	103.7±1.9	96.3±1.8	102.7±1.8	0.01	0.48	0.01
VLDL[§]	18.6±1.4	20.2±0.6	20.8±0.5	20.0±0.5	0.32	0.23	0.28
Non-HDL-C	116.5±5.5	126.1±2.2	119.1±2.0	124.9±2.0	0.04	0.32	0.04

All variables are adjusted means (mg/dL) ± standard error. [§]Log-transformed values

[1]Additive model (CC vs CT vs TT), [2]Recessive model (CC vs CT+TT) and [3]Dominant (CC+CT vs TT) were adjusted by sex, age, age^2, age^3 and BMI.

APPENDIX J (cont.)

Supplementary tables for chapter 4

Table J.2 FADS2-rs1535 and blood lipid profiles.

	FADS2 (rs1535)						
Variable	**AA (n=24)**	**GA (n=186)**	**GG (n=214)**	**AA + AG (n=210)**	[1]p	[2]p	[3]p
TC	166.3±6.8	176.9±2.4	168.8±2.8	175.7±2.3	0.04	0.34	0.04
HDL-C	48.8 ±2.4	49.5±0.9	49.4±0.8	49.4±0.8	0.96	0.63	0.98
TG[§]	90.8 ±7.4	101.9±2.8	103.5±2.6	100.5±2.5	0.31	0.18	0.44
LDL-C	97.8 ±5.3	103.5±1.9	96.1±1.8	102.8±1.8	0.02	0.70	0.01
VLDL[§]	18.2 ±1.5	20.4±0.6	20.7±0.5	20.1±0.5	0.31	0.18	0.44
Non-HDL-C	117.2 ±6.0	126.0±2.2	118.9±2.0	125.0±2.0	0.04	0.42	0.04

All variables are adjusted means (mg/dL) ± standard error. [§] Log-transformed values.

[1]Additive model (AA vs GA vs GG), [2]Recessive model (AA vs AG+GG), and [3]Dominant model (AA+AG vs GG) were adjusted by sex, age, age^2, age^3 and BMI.

APPENDIX K

Supplementary tables for chapter 4

Table K.1 FADS1-rs174546 and the lipid profiles in the UP AMIGOS cohort by BMI categories[1].

	FADS1 (rs174546)				
	CC + CT		TT		
	NW	**OW/OB**	**NW**	**OW/OB**	[1]p-
N (M/F)	**(66,83)**	**(40,35)**	**(81,81)**	**(45,23)**	value
TC, *mg/dL*	171.8 ± 2.7	183.8 ± 4.2	165.2 ± 2.8	177.4 ± 3.9	0.06
HDL-C, *mg/dL*	51.2 ± 0.9	45.5 ± 1.5	52.1 ± 1.0	44.7 ± 1.4	0.99
TG, *mg/dL*[§]	90.8 ± 2.9	124.5 ± 6.0	94.0 ± 3.2	128.8 ± 5.9	0.40
LDL-C, *mg/dL*	99.2 ± 2.1	110.8 ± 3.4	92.1 ± 2.2	104.8 ± 3.0	0.01
VLDL, *mg/dL*[§]	18.2 ± 0.5	24.9 ± 1.2	18.8 ± 0.6	25.8 ± 1.2	0.40
Non-HDL-C, *mg/dL*	118.4 ± 2.4	139.2 ± 3.8	112.3 ± 2.5	132.8 ± 3.5	0.04

All variables are adjusted means ± standard error. [§] Log-transformed values.

[1]Dominant model (CC+CT vs TT) was adjusted by sex, age, and BMI.

Abbreviations: M: males; F; females; NW; normal weight; OW: overweight; and OB: obese.

APPENDIX K (cont.)

Supplementary tables for chapter 4

Table K.2. FADS2-rs1535 and the lipid profiles in the UP AMIGOS cohort by BMI categories[1].

	FADS2 (rs1535)				
	AA + AG		GG		
	NW	OW/OB	NW	OW/OB	[1]p-value
N (M/F)	(67,86)	(41,34)	(80,75)	(44,24)	
TC, *mg/dL*	171.9 ± 2.8	184.3 ± 4.3	164.8 ± 2.8	177.0 ± 3.9	0.04
HDL-C, *mg/dL*	51.3 ± 1.0	45.3 ± 1.5	51.7 ± 1.0	44.9 ± 1.4	0.99
TG, *mg/dL*[§]	91.0 ± 2.9	125.3 ± 6.1	93.1± 3.0	104.0 ± 4.8	0.60
LDL-C, *mg/dL*	99.0 ± 2.2	111.5 ± 3.4	92.2± 2.2	104.2 ± 3.0	0.01
VLDL, *mg/dL*[§]	18.2 ± 0.6	25.1 ± 1.2	18.6 ± 0.6	25.6 ± 1.2	0.60
Non-HDL-C, *mg/dL*	118.2 ± 2.5	140.0 ± 3.8	112.3 ± 2.5	132.1 ± 3.5	0.03

All variables are adjusted means ± standard error. [§] Log-transformed values.

[1]Dominant model (AA + AG vs GG) was adjusted by sex, age, and BMI.

Abbreviations: M: males; F; females; NW; normal weight; OW: overweight; and OB: obese.

APPENDIX L

Supplementary tables for chapter 5

Table L.1 Dietary intake by genotypes of rs174546 in *FADS* gene cluster region.

	CC (n=32)	CT (n=193)	TT (n=220)	CC + CT (n=225)	Additive model p-value	Dominant model p-value
TFA, g/d	92.1 ± 3.7	89.5 ± 1.5	88.1 ± 1.4	89.9 ± 1.4	0.53[1]	0.35[1]
SFA, g/d	30.4 ± 1.4	27.5 ± 0.6	28.0 ± 0.5	27.9 ± 0.5	0.14[1]	0.91[1]
MUFA, g/d	36.3 ± 1.6	33.9 ± 0.7	34.0 ± 0.6	34.2 ± 0.6	0.38[1]	0.77[1]
Omega 3, g/d§	0.31 ± 0.1	0.22 ± 0.0	0.21 ± 0.0	0.20 ± 0.0	0.08[1]	0.23[1]
Omega 6, g/d§	8.8 ± 0.6	8.5 ± 0.2	8.4 ± 0.3	8.5 ± 0.2	0.79[1]	0.68[1]
Omega 6/3r	12.1 ± 0.7	11.8 ± 0.3	12.4 ± 0.3	11.8 ± 0.3	0.38[2]	0.18[2]
TFA, %E	37.1 ± 1.4	36.8 ± 0.6	35.5 ± 0.5	36.9 ± 0.5	0.16[2]	0.06[2]
SFA, %E	12.2 ± 0.5	11.4 ± 0.2	11.2 ± 0.2	11.5 ± 0.3	0.18[2]	0.21[2]
MUFA, %E	14.7 ± 0.6	14.0 ± 0.3	13.6 ± 0.2	14.1 ± 0.2	0.19[2]	0.12[2]
Omega 3, %E§	0.13 ± 0.0	0.10 ± 0.0	0.9 ± 0.0	0.10 ± 0.0	0.10[2]	0.23[2]
Omega 6, %E§	3.9 ± 0.3	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.0	0.72[2]	0.53[2]

Data are expressed as means ± standard error. §Log-transformed values. [1]Model adjusted by sex, age, age^2, age^3, body mass index (BMI) and total energy intake. [2]Model adjusted by sex, age, age^2, age^3 and BMI. Abbreviations: TFA, SFA, MUFA, Omega 3: EPA, DPA and DHA, Omega 6: LA, GLA, and AA. Omega-6/Omega-3 ratio.

Table L.2 Dietary intake by genotypes of rs1535 in *FADS* gene cluster region.

	AA (n=28)	**GA (n=190)**	**GG (n=224)**	**AA + AG (n=218)**	**Additive model p-value**	**Dominant model p-value**
TFA, g/d	93.0 ± 3.9	89.3 ± 1.5	88.5 ± 1.4	89.8 ± 1.4	0.54[1]	0.51[1]
SFA, g/d	30.2 ± 1.5	27.6 ± 0.6	28.1 ± 0.5	27.9 ± 0.5	0.22[1]	0.80[1]
MUFA, g/d	36.2 ± 1.8	33.8 ± 0.7	34.1 ± 0.6	34.1 ± 0.6	0.46[1]	0.96[1]
Omega 3, g/d§	0.30 ± 0.1	0.20 ± 0.0	0.20 ± 0.0	0.20 ± 0.1	0.05[1]	0.26[1]
Omega 6, g/d§	9.2 ± 0.7	8.4 ± 0.3	8.4 ± 0.2	8.5 ± 0.1	0.53[1]	0.73[1]
Omega 6/3 r	12.1 ± 0.8	11.8 ± 0.3	12.4 ± 0.3	11.8 ± 0.3	0.30[1]	0.13[1]
TFA, %E	37.4 ± 1.4	36.7 ± 0.6	36.5 ± 0.5	36.8 ± 0.5	0.19[2]	0.08[2]
SFA, %E	12.0 ± 0.5	11.5 ± 0.2	11.2 ± 0.2	11.5 ± 0.2	0.34[2]	0.26[2]
MUFA, %E	14.5 ± 0.7	14.0 ± 0.3	13.6 ± 0.2	14.0 ± 0.2	0.29[2]	0.16[2]
Omega 3, %E§	0.14 ± 0.0	0.10 ± 0.0	0.09 ± 0.0	0.1 ± 0.0	0.07[2]	0.23[2]
Omega 6, %E§	4.1 ± 0.3	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.0	0.44[2]	0.48[2]

Data are expressed as means ± standard error. §Log-transformed values. [1]Model adjusted by sex, age, age^2, age^3, body mass index (BMI) and total energy intake. [2]Model adjusted by sex, age, age^2, age^3 and BMI. Abbreviations: TFA, SFA, MUFA, Omega 3: EPA, DPA and DHA, Omega 6: LA, GLA, and AA. Omega-6/Omega-3 ratio.

APPENDIX M

Supplementary tables for chapter 5

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value TFA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.046	0.95	0.01	167.6± 6.4	176.5 ± 2.5	169.4 ± 2.3
HDL-C, *mg/dL*	0.029	0.92	0.98	49.2 ± 2.3	49.6 ± 0.9	49.4 ± 0.8
TG, *mg/dL*[§]	0.037	0.01	0.42	2.0 ± 0.034	2.0 ± 0.013	2.0 ± 0.01
LDL-C, *mg/dL*	0.051	0.66	0.045	97.6 ± 5.0	103.2 ± 2.0	96.5 ± 1.8
VLDL, *mg/dL*[§]	0.037	0.01	0.42	1.28 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.046	0.56	0.14	118.5 ± 5.9	125.4 ± 2.3	119 ± 2.1

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value TFA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.048	0.88	0.06	167.3 ± 7.0	176.6 ± 2.5	169.0 ± 2.3
HDL-C, *mg/dL*	0.027	0.88	0.92	48.7 ± 2.5	49.6 ± 0.9	49.3 ± 0.8
TG, *mg/dL*[§]	0.038	0.007	0.47	1.97 ± 0.04	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.050	0.74	0.04	98.7 ± 5.5	103.1 ± 2.0	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.038	0.007	0.47	1.27 ± 0.038	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.048	0.48	0.11	118.6 ± 6.4	125.5 ± 2.3	119.1 ± 2.1

All variables are adjusted means ± standard error. [§]Log-transformed values.[1]Models were adjusted for sex, age, age^2, age^3, total energy intake and total fat intake (TFA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value TFA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.043	0.91	0.07	175.3 ± 2.3	169.4 ± 2.3
HDL-C, *mg/dL*	0.029	0.93	0.93	49.5 ± 0.8	49.4 ± 0.8
TG, *mg/dL*[§]	0.037	0.009	0.22	1.99 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.048	0.69	0.02	102.4 ± 1.8	96.5 ± 1.8
VLDL, *mg/dL*[§]	0.037	0.009	0.22	1.29 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.044	0.53	0.09	124.5 ± 2.1	119 ± 2.1

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value TFA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.044	0.84	0.046	175.6 ± 2.3	169.0 ± 2.3
HDL-C, *mg/dL*	0.027	0.89	0.86	49.5 ± 0.84	49.3 ± 0.8
TG, *mg/dL*[§]	0.037	0.006	0.39	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.050	0.77	0.02	102.6 ± 1.87	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.037	0.006	0.39	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.046	0.45	0.06	124.7 ± 2.2	119.1 ± 2.1

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, total energy intake and total fat intake (TFA).

[2]P-values for differences in lipid concentrations with assumption of a dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value TFA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.08	0.83	0.04	166.5 ± 6.33	176.9 ± 2.4	169.1 ± 2.3
HDL-C, *mg/dL*	0.10	0.73	0.98	49.7 ± 2.2	49.4 ± 0.85	49.6 ± 0.8
TG, *mg/dL*[§]	0.17	0.002	0.38	1.98 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.84	0.02	96.5 ± 5.0	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.17	0.002	0.38	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.36	0.04	116.8± 5.5	126.± 2.8	119.0 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value TFA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.085	0.76	0.04	166.6 ± 6.9	176.9 ± 2.5	169.0 ± 2.3
HDL-C, *mg/dL*	0.097	0.69	0.98	49.0 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.178	0.001	0.37	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.93	0.02	98.1 ± 5.4	103.4 ± 2.0	96.1± 1.8
VLDL, *mg/dL*[§]	0.177	0.001	0.37	1.26 ± 0.034	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.153	0.29	0.04	117.6 ± 6.0	126.0 ± 2.2	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and total fat intake (TFA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value TFA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.08	0.79	0.048	175.6 ± 2.28	169.1 ± 2.29
HDL-C, *mg/dL*	0.099	0.73	0.89	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.17	0.002	0.32	2.0 ± 0.01	2.01 ± 0.01
LDL-C, *mg/dL*	0.099	0.87	0.011	102.7 ± 1.8	96.3 ± 1.78
VLDL, *mg/dL*[§]	0.17	0.002	0.32	1.3 ± 0.012	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.33	0.04	124.9 ± 2.02	119.0 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value TFA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.08	0.72	0.033	175.77 ± 2.3	168.8 ± 2.3
HDL-C, *mg/dL*	0.097	0.69	0.97	49.44 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.17	0.001	0.48	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.96	0.009	102.8 ± 1.8	96.1 ± 1.77
VLDL, *mg/dL*[§]	0.17	0.001	0.48	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.15	0.27	0.032	125.0 ± 2.05	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and total fat intake (TFA). [2]P-values for differences in lipid concentrations with assumption of a dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	**p-value SFA**	**[2]p-value SNP**	**CC**	**CT**	**TT**
TC, *mg/dL*	0.08	0.32	0.05	166.9 ± 6.34	176.8 ± 2.4	169.2 ± 2.3
HDL-C, *mg/dL*	0.099	0.99	0.98	49.7 ± 2.2	49.4 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.18	0.0004	0.38	1.98 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.97	0.02	96.5 ± 5.0	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.18	0.0004	0.38	1.28 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.18	0.04	117.2± 5.6	126.0.± 2.2	119.1 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	**p-value SFA**	**[2]p-value SNP**	**AA**	**GA**	**GG**
TC, *mg/dL*	0.087	0.30	0.04	166.9 ± 6.9	176.8 ± 2.5	169.0 ± 2.3
HDL-C, *mg/dL*	0.097	0.98	0.98	49.0 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.182	0.0005	0.38	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.91	0.02	98.1 ± 5.3	103.4 ± 2.0	96.1± 1.8
VLDL, *mg/dL*[§]	0.18	0.0005	0.38	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.155	0.17	0.05	117.9 ± 6.0	126.0 ± 2.2	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and saturated fat intake (SFA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value SFA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.08	0.26	0.05	175.5 ± 2.28	169.2 ± 2.29
HDL-C, *mg/dL*	0.099	0.99	0.90	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.18	0.0003	0.24	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.099	0.87	0.011	102.7 ± 1.8	96.3 ± 1.78
VLDL, *mg/dL*[§]	0.18	0.0003	0.24	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.14	0.04	124.8 ± 2.02	119.1 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value SFA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.08	0.26	0.036	175.7 ± 2.3	168.8 ± 2.3
HDL-C, *mg/dL*	0.097	0.99	0.96	49.45 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.18	0.0003	0.39	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.86	0.009	102.8 ± 1.8	96.1 ± 1.77
VLDL, *mg/dL*[§]	0.18	0.0003	0.39	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.15	0.14	0.036	125.0 ± 2.05	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and saturated fat intake (SFA). [2]P-values for differences in lipid concentrations with assumption of a dominant genetic model

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value MUFA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.09	0.29	0.05	166.8 ± 6.3	176.9 ± 2.4	169.1 ± 2.3
HDL-C, *mg/dL*	0.10	0.26	0.98	49.6 ± 2.2	49.4 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.18	0.0005	0.38	1.98 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.72	0.01	96.7 ± 5.0	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.18	0.0005	0.38	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.14	0.04	117.1± 5.5	126.1.± 2.2	119.0 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value MUFA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.088	0.22	0.04	166.9 ± 6.9	176.9 ± 2.4	169.0 ± 2.3
HDL-C, *mg/dL*	0.099	0.23	0.98	49.0 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.18	0.0002	0.36	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.58	0.02	98.2 ± 5.3	103.4 ± 2.0	96.1± 1.8
VLDL, *mg/dL*[§]	0.18	0.0002	0.37	1.26 ± 0.03	1.30 ± 0.0	1.32 ± 0.0
Non-HDL-C, *mg/dL*	0.157	0.91	0.05	117.8 ± 6.0	126.0 ± 2.2	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and monounsaturated fat intake (MUFA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	**p-value MUFA**	**[2]p-value SNP**	**CC + CT**	**TT**
TC, *mg/dL*	0.08	0.25	0.048	175.6 ± 2.28	169.1 ± 2.29
HDL-C, *mg/dL*	0.102	0.26	0.89	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.18	0.0004	0.27	2.0 ± 0.01	2.01 ± 0.01
LDL-C, *mg/dL*	0.10	0.66	0.011	102.8 ± 1.8	96.2 ± 1.78
VLDL, *mg/dL*[§]	0.18	0.0004	0.27	1.3 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.12	0.04	124.9 ± 2.02	119.0 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	**p-value MUFA**	**[2]p-value SNP**	**AA+AG**	**GG**
TC, *mg/dL*	0.08	0.19	0.034	175.74 ± 2.3	168.8 ± 2.3
HDL-C, *mg/dL*	0.099	0.23	0.97	49.44 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.18	0.0002	0.42	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.55	0.009	102.8 ± 1.8	96.1 ± 1.77
VLDL, *mg/dL*[§]	0.18	0.0002	0.42	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.15	0.08	0.033	125.0 ± 2.05	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and monounsaturated fat intake (MUFA). [2]P-values for differences in lipid concentrations with assumption of a dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value PUFA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.08	0.99	0.04	166.4 ± 6.3	176.9 ± 2.4	169.2 ± 2.3
HDL-C, *mg/dL*	0.10	0.57	0.98	49.7 ± 2.2	49.3 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.17	0.018	0.31	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.73	0.01	96.5 ± 54.9	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.17	0.02	0.31	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.33	0.03	116.5± 5.5	126.2 ± 2.2	119.0 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value PUFA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.09	0.93	0.04	166.5 ± 6.9	176.9 ± 2.5	169.0 ± 2.3
HDL-C, *mg/dL*	0.10	0.57	0.98	49.1 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.17	0.01	0.29	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.64	0.02	98.1 ± 5.3	103.5 ± 1.9	96.1± 1.8
VLDL, *mg/dL*[§]	0.17	0.01	0.29	1.26 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.27	0.04	117.3 ± 6.0	126.0 ± 2.2	118.8 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and polyunsaturated fat intake (PUFA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value PUFA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.08	0.95	0.05	175.6 ± 2.28	169.2 ± 2.29
HDL-C, *mg/dL*	0.10	0.58	0.88	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.16	0.02	0.32	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.099	0.75	0.01	102.8 ± 1.8	96.3 ± 1.78
VLDL, *mg/dL*[§]	0.16	0.02	0.32	1.3 ± 0.01	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.35	0.04	124.9 ± 2.02	119.0 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value PUFA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.08	0.94	0.034	175.7 ± 2.3	168.8 ± 2.3
HDL-C, *mg/dL*	0.097	0.57	0.98	49.43 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.17	0.01	0.48	2.0 ± 0.012	2.01 ± 0.01
LDL-C, *mg/dL*	0.10	0.65	0.008	102.8 ± 1.8	96.1 ± 1.8
VLDL, *mg/dL*[§]	0.17	0.01	0.48	1.30 ± 0.012	1.32± 0.01
Non-HDL-C, *mg/dL*	0.15	0.27	0.03	125.0 ± 2.05	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, BMI, total energy intake and polyunsaturated fat acid intake (PUFA). [2]P-values for differences in lipid concentrations with assumption of a dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value ALA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.09	0.23	0.05	166.4 ± 6.3	176.8 ± 2.4	169.3 ± 2.3
HDL-C, *mg/dL*	0.099	0.81	0.98	49.7 ± 2.2	49.4 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.69	0.31	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.66	0.02	96.6 ± 4.9	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.69	0.31	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.58	0.04	116.6 ± 5.5	126.0.± 2.2	119.1 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value ALA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.088	0.23	0.05	166.3 ± 6.9	176.8 ± 2.5	169.0 ± 2.3
HDL-C, *mg/dL*	0.097	0.79	0.98	49.0 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.71	0.29	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.68	0.02	98.1 ± 5.3	103.4 ± 2.0	96.1± 1.8
VLDL, *mg/dL*[§]	0.16	0.71	0.29	1.26 ± 0.03	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.60	0.05	117.2 ± 6.0	126.0 ± 2.2	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and alpha-linoleic acid (ALA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				**Means ± SE**	
	R^2	**p-value ALA**	[2]**p-value SNP**	**CC + CT**	**TT**
TC, *mg/dL*	0.08	0.22	0.06	175.4 ± 2.28	169.3 ± 2.29
HDL-C, *mg/dL*	0.099	0.81	0.89	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.15	0.67	0.26	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.64	0.01	102.7 ± 1.8	96.3 ± 1.78
VLDL, *mg/dL*[§]	0.15	0.67	0.26	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.56	0.05	124.8 ± 2.03	119.1 ± 2.02

FADS2-rs1535[1]				**Means ± SE**	
	R^2	**p-value ALA**	[2]**p-value SNP**	**AA+AG**	**GG**
TC, *mg/dL*	0.08	0.23	0.04	175.6 ± 2.3	168.9 ± 2.3
HDL-C, *mg/dL*	0.097	0.79	0.98	49.43 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.15	0.71	0.42	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.68	0.009	102.8 ± 1.8	96.1 ± 1.77
VLDL, *mg/dL*[§]	0.15	0.71	0.42	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.15	0.60	0.038	125.0 ± 2.06	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and alpha-linoleic acid (ALA). [2]P-values for differences in lipid concentrations with assumption of dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value LA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.09	0.13	0.04	166.2 ± 6.3	176.9 ± 2.4	169.2 ± 2.3
HDL-C, *mg/dL*	0.099	0.97	0.98	49.7 ± 2.2	49.4 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.81	0.31	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.13	0.01	96.4 ± 5.0	103.7 ± 1.9	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.81	0.31	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.19	0.03	116.4 ± 5.5	126.1 ± 2.2	119.1 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value LA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.089	0.15	0.03	166.1 ± 6.9	177.0 ± 2.4	168.8 ± 2.3
HDL-C, *mg/dL*	0.097	0.94	0.98	49.0 ± 2.4	49.5 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.74	0.30	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.15	0.02	97.8 ± 5.3	103.5 ± 1.9	96.1 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.74	0.30	1.26 ± 0.03	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.155	0.22	0.04	117.0 ± 6.0	126.0 ± 2.2	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and linoleic acid (LA).

[2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value LA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.08	0.14	0.05	175.5 ± 2.3	169.2 ± 2.3
HDL-C, *mg/dL*	0.099	0.97	0.90	49.4 ± 0.8	49.56 ± 0.8
TG, *mg/dL*[§]	0.15	0.79	0.28	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.13	0.011	102.7 ± 1.8	96.3 ± 1.8
VLDL, *mg/dL*[§]	0.15	0.79	0.28	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.19	0.04	124.8 ± 2.0	119.1 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value LA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.08	0.16	0.035	175.7 ± 2.3	168.8 ± 2.3
HDL-C, *mg/dL*	0.097	0.94	0.96	49.45 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.15	0.69	0.44	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.16	0.009	102.8 ± 1.8	96.1 ± 1.77
VLDL, *mg/dL*[§]	0.15	0.69	0.44	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.15	0.25	0.035	125.0 ± 2.05	118.9 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, BMI, total energy intake and linolenic acid (LA).

[2]P-values for differences in lipid concentrations with assumption of dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value ARA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.08	0.85	0.05	166.5 ± 6.3	177.0 ± 2.5	169.6 ± 2.3
HDL-C, *mg/dL*	0.098	0.85	0.88	49.6 ± 2.2	49.1 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.15	0.36	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.47	0.02	96.7 ± 4.9	103.9 ± 1.9	96.6 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.15	0.36	1.27 ± 0.03	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.95	0.04	116.8 ± 5.6	126.5.± 2.2	119.4 ± 2.0

FADS2-rs1535[1]				Means ± SE		
	R^2	p-value ARA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.084	0.81	0.04	166.6 ± 6.9	177.2 ± 2.5	169.3 ± 2.3
HDL-C, *mg/dL*	0.096	0.83	0.94	49.0 ± 2.4	49.2 ± 0.86	49.5 ± 0.8
TG, *mg/dL*[§]	0.16	0.16	0.34	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.45	0.02	98.2 ± 5.3	103.9±1.95	96.5 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.16	0.34	1.26 ± 0.03	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.15	0.99	0.04	117.5 ± 6.0	126.5 ± 2.2	119.2± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and arachidonic acid (ARA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value ARA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.08	0.84	0.06	175.6 ± 2.3	169.6 ± 2.32
HDL-C, *mg/dL*	0.098	0.84	0.66	49.1 ± 0.8	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.15	0.37	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.10	0.46	0.014	102.9 ± 1.8	96.6 ± 1.79
VLDL, *mg/dL*[§]	0.16	0.15	0.37	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.96	0.04	125.2 ± 2.05	119.4 ± 2.02

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value ARA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.08	0.84	0.04	176.0 ± 2.3	169.3 ± 2.3
HDL-C, *mg/dL*	0.096	0.83	0.74	49.2 ± 0.81	49.5 ± 0.8
TG, *mg/dL*[§]	0.16	0.15	0.59	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.10	0.46	0.009	103.2 ± 1.8	96.5 ± 1.78
VLDL, *mg/dL*[§]	0.16	0.15	0.59	1.30 ± 0.012	1.31 ± 0.012
Non-HDL-C, *mg/dL*	0.15	0.97	0.03	125.5 ± 2.07	119.2± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and arachidonic acid (ARA). [2]P-values for differences in lipid concentrations with assumption of dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	p-value EPA	[2]p-value SNP	CC	CT	TT
TC, *mg/dL*	0.11	0.68	0.16	170.2 ± 6.8	178.1 ± 2.6	171.5 ± 2.5
HDL-C, *mg/dL*	0.10	0.52	0.98	50.3 ± 2.3	49.8 ± 0.91	49.9 ± 0.9
TG, *mg/dL*[§]	0.16	0.55	0.40	1.99 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	0.22	0.07	98.5 ± 5.3	104.3 ± 2.1	97.7 ± 1.9
VLDL, *mg/dL*[§]	0.16	0.55	0.40	1.30 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.17	0.40	0.19	119.7 ± 6.0	126.6 ± 2.3	121.0 ± 2.2

FADS2-rs1535[1]				Means ± SE		
	R2	p-value EPA	[2]p-value SNP	AA	GA	GG
TC, *mg/dL*	0.11	0.70	0.16	170.3 ± 7.3	178.1 ± 2.6	171.3 ± 2.5
HDL-C, *mg/dL*	0.09	0.51	0.99	49.6 ± 2.5	49.8 ± 0.92	49.8 ± 0.8
TG, *mg/dL*[§]	0.16	0.59	0.45	1.98 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	0.25	0.08	100.4 ± 5.7	104.1 ± 2.1	97.7 ± 1.9
VLDL, *mg/dL*[§]	0.16	0.59	0.45	1.28 ± 0.04	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.18	0.42	0.19	120.6 ± 6.4	126.6 ± 2.3	120.9 ± 2.2

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, BMI, total energy intake and eicosapentaenoic acid (EPA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	p-value EPA	[2]p-value SNP	CC + CT	TT
TC, *mg/dL*	0.106	0.73	0.12	177.0 ± 2.5	171.5 ± 2.5
HDL-C, *mg/dL*	0.096	0.52	0.99	49.9 ± 0.8	49.9 ± 0.9
TG, *mg/dL*[§]	0.16	0.55	0.18	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.13	0.25	0.04	103.5 ± 1.9	97.7 ± 1.95
VLDL, *mg/dL*[§]	0.16	0.55	0.18	1.3 ± 0.01	1.33 ± 0.013
Non-HDL-C, *mg/dL*	0.17	0.44	0.13	125.7 ± 2.2	121.0 ± 2.2

FADS2-rs1535[1]				Means ± SE	
	R^2	p-value EPA	[2]p-value SNP	AA+AG	GG
TC, *mg/dL*	0.11	0.75	0.09	177.2 ± 2.5	171.3 ± 2.5
HDL-C, *mg/dL*	0.093	0.50	0.99	49.82 ± 0.86	49.82 ± 0.86
TG, *mg/dL*[§]	0.16	0.56	0.27	2.0 ± 0.012	2.02 ± 0.012
LDL-C, *mg/dL*	0.13	0.26	0.03	103.7 ± 2.0	97.7 ± 1.95
VLDL, *mg/dL*[§]	0.16	0.56	0.27	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.17	0.45	0.11	125.9 ± 2.2	120.9 ± 2.2

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and eicosapentaenoic acid (EPA). [2]P-values for differences in lipid concentrations with assumption of dominant genetic model.

FADS1-rs174546[1]				**Means ± SE**		
	R^2	**p-value DPA**	[2]**p-value SNP**	**CC**	**CT**	**TT**
TC, *mg/dL*	0.10	*0.01*	*0.055*	166.7 ± 6.4	176.8 ± 2.4	169.4 ± 2.3
HDL-C, *mg/dL*	0.10	0.26	0.97	49.6 ± 2.2	49.3 ± 0.86	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.56	0.42	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	0.005	0.02	96.7 ± 5.0	103.6 ± 1.9	96.4 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.56	0.42	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.17	*0.02*	*0.05*	117.1 ± 5.6	126.0.± 2.2	119.2 ± 2.0

FADS2-rs1535[1]				**Means ± SE**		
	R^2	**p-value DPA**	[2]**p-value SNP**	**AA**	**GA**	**GG**
TC, *mg/dL*	0.103	**0.01**	**0.04**	167.0 ± 7.0	177.0 ± 2.4	169.0 ± 2.3
HDL-C, *mg/dL*	0.097	0.26	0.98	48.9 ± 2.5	49.4 ± 0.86	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.57	0.42	1.96 ± 0.03	2.00 ± 0.01	2.01 ± 0.01
LDL-C, *mg/dL*	0.12	**0.005**	**0.02**	98.4 ± 5.4	103.5 ± 1.9	96.2 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.57	0.42	1.27 ± 0.03	1.31 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.17	*0.02*	*0.05*	118.2 ± 6.2	126.0 ± 2.2	119.0 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and docosapentaenoic acid (DPA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				**Means ± SE**	
	R^2	**p-value DPA**	[2]**p-value SNP**	**CC + CT**	**TT**
TC, *mg/dL*	0.096	*0.01*	*0.06*	175.5 ± 2.3	169.3 ± 2.3
HDL-C, *mg/dL*	0.100	0.25	0.84	49.4 ± 0.8	49.6 ± 0.8
TG, *mg/dL*[§]	0.16	0.53	0.32	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	**0.01**	**0.01**	102.7 ± 1.8	96.4 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.53	0.32	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.16	*0.03*	*0.05*	124.9 ± 2.03	119.2 ± 2.02

FADS2-rs1535[1]				**Means ± SE**	
	R^2	**p-value DPA**	[2]**p-value SNP**	**AA+AG**	**GG**
TC, *mg/dL*	0.099	**0.01**	**0.03**	175.9 ± 2.3	169.0 ± 2.3
HDL-C, *mg/dL*	0.097	0.26	0.97	49.4 ± 0.81	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.52	0.53	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.12	**0.006**	**0.009**	102.9 ± 1.8	96.2 ± 1.77
VLDL, *mg/dL*[§]	0.16	0.52	0.53	1.30 ± 0.012	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.16	**0.03**	**0.03**	125.2 ± 2.05	119.0 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age[2], age[3], BMI, total energy intake and docosapentaenoic acid (DPA). [2]P-values for differences in lipid concentrations with assumption of dominant genetic model.

FADS1-rs174546[1]				Means ± SE		
	R^2	**p-value DHA**	**[2]p-value SNP**	**CC**	**CT**	**TT**
TC, *mg/dL*	0.10	*0.02*	*0.05*	167.3 ± 6.4	177.3 ± 2.5	169.5 ± 2.3
HDL-C, *mg/dL*	0.10	0.19	0.98	49.7 ± 2.2	49.6 ± 0.87	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.87	0.35	1.97 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	**0.02**	**0.03**	97.1 ± 5.0	103.8 ± 2.0	96.7 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.87	0.35	1.27 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.16	0.05	0.06	117.5 ± 5.6	126.2 ± 2.2	119.5 ± 2.1

FADS2-rs1535[1]				Means ± SE		
	R^2	**p-value DHA**	**[2]p-value SNP**	**AA**	**GA**	**GG**
TC, *mg/dL*	0.10	**0.02**	**0.04**	167.6 ± 7.0	177.4 ± 2.5	169.2 ± 2.3
HDL-C, *mg/dL*	0.102	0.19	0.91	49.0 ± 2.4	49.7 ± 0.87	49.2 ± 0.8
TG, *mg/dL*[§]	0.16	0.88	0.38	1.96 ± 0.03	2.00 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	**0.02**	**0.03**	98.9 ± 5.5	103.7±1.96	96.5 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.88	0.38	1.26 ± 0.03	1.30 ± 0.01	1.32 ± 0.01
Non-HDL-C, *mg/dL*	0.165	0.05	0.07	118.6 ± 6.2	126.2 ± 2.2	119.4 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, BMI, total energy intake and docosahexaenoic acid (DHA). [2]P-values for differences in lipid concentrations with assumption of an additive genetic model.

FADS1-rs174546[1]				Means ± SE	
	R^2	**p-value DHA**	**[2]p-value SNP**	**CC + CT**	**TT**
TC, *mg/dL*	0.10	*0.03*	*0.051*	175.9 ± 2.3	169.5 ± 2.34
HDL-C, *mg/dL*	0.10	0.18	0.86	49.6 ± 0.8	49.4 ± 0.8
TG, *mg/dL*[§]	0.16	0.85	0.25	2.0 ± 0.01	2.02 ± 0.01
LDL-C, *mg/dL*	0.12	0.02	0.02	102.9 ± 1.8	96.7 ± 1.82
VLDL, *mg/dL*[§]	0.16	0.85	0.25	1.3 ± 0.011	1.32 ± 0.012
Non-HDL-C, *mg/dL*	0.16	*0.05*	*0.06*	125.0 ± 2.07	119.5 ± 2.06

FADS2-rs1535[1]				Means ± SE	
	R^2	**p-value DHA**	**[2]p-value SNP**	**AA+AG**	**GG**
TC, *mg/dL*	0.01	0.03	0.03	176.3 ± 2.4	169.2 ± 2.3
HDL-C, *mg/dL*	0.10	0.19	0.74	49.6 ± 0.82	49.2 ± 0.8
TG, *mg/dL*[§]	0.16	0.85	0.44	2.0 ± 0.012	2.01 ± 0.012
LDL-C, *mg/dL*	0.12	0.02	0.01	103.1 ± 1.8	96.5 ± 1.8
VLDL, *mg/dL*[§]	0.16	0.85	0.44	1.30 ± 0.012	1.32± 0.012
Non-HDL-C, *mg/dL*	0.16	*0.06*	*0.04*	125.3 ± 2.09	119.3 ± 2.0

All variables are adjusted means ± standard error. [§]Log-transformed values

[1]Models were adjusted for sex, age, age^2, age^3, BMI, total energy intake and docosahexaenoic acid (DHA). [2]P-values for differences in lipid concentrations with assumption of dominant genetic model.