Clinical and Applied Thrombosis/Hemostasis
Volume 26: 1-9

DOI: 10.1177/1076029620915286
journals.sagepub.com/home/cat
SAGE

Association Between Genetic Variants in FADS1-FADS2 and ELOVL2 and Obesity, Lipid Traits, and Fatty Acids in Tunisian Population

Wided Khamlaoui, PhD[1], Sounira Mehri, PhD[1], Sonia Hammami, PR[1,2], Souha Hammouda, PhD[1], Imed Chraeif, PhD[1], Roberto Elosua, PR[3], and Mohamed Hammami, PR[1]

Abstract

The aim of this study was to determine whether genetic variants in FADS1/FADS2 and ELOVL2 are associated with overweight–obesity and body mass index (BMI) and to assess the association between these genetic variants and lipid profile and fatty acid levels. A total of 259 overweight–obese patients were compared to 369 healthy controls. *FADS1*, *FADS2*, and *ELOVL2* genes were associated with BMI and overweight–obesity ($P \leq .001$). In an additive model, the C allele in each of these variants was associated with a lower BMI: −1.18, −0.90, and −1.23 units, respectively. Higher amounts of total cholesterol, low-density lipoprotein cholesterol, total saturated fatty acids (lauric [12:0], myristic [C14:0], palmitic [C16:0], stearic [C18:0], arachidic [20:0], lignoceric [24:0]), monounsaturated fatty acids (myristoleic [C14:1], erucic [C22:1 n-9]), and polyunsaturated fatty acids (α-linolenic [ALA, 18:3 n-3], docosahexaenoic [DHA, C22:6 n-3], eicosapentaenoic acid [EPA, C20:5n-3], arachidonic acid [AA, 20:4n-6], and conjugated linolenic acids [CLA1 and CLA2]) were shown in patients. A significant increase in D6D activities presented by 20:4n-6/18:2n-6 and 18:3n-6/18:2n-6, Δ9 desaturase (D9D) activity, estimated by the ratio 18:1n-9/18:0 and elongase activities (AE), and estimated by the ratio of docosatetraenoic/AA and DPA/EPA in patients. The C minor allele of FADS1 had significantly lower DHA. A significant decrease in stearic acid, EPA, and AE activity (docosatetraenoic/AA) was revealed in patients with the minor allele carriers of FADS2. The C minor allele of ELOVL2 had significantly lower ALA, EPA, DPA, and D6D activity (C20:4 n-6/C18:2n-6). These data suggest that variations in FADS1, FADS2, and ELOVL2 affect the risk of overweight–obesity and the level of circulating fatty acids and could point to a key molecular pathway of metabolic syndrome and its related comorbidities.

Keywords

overweight–obesity, *FADS1/FADS2*, *ELOVL2*, genotypes, lipid profile, fatty acid, Tunisia

Date received: 30 October 2019; revised: 19 January 2020; accepted: 25 February 2020.

Introduction

Obesity is a chronic metabolic disease defined as abnormal fat accumulation.[1] It has been considered the 21st-century epidemic in both developed and developing countries.[2,3] Its prevalence is rapidly increasing to alarming rates. The worldwide prevalence of overweight–obesity had nearly tripled between 1975 and 2014.[4] By 2030, 38% of the world's adult population could be overweight and 20% obese.[5] As a serious and complex condition, obesity has multifactorial origin stemming from environmental factors and genetic background.[6] The fundamental cause of overweight and obesity is an energy imbalance between energy intake (an increase in energy intake that is high in fat) and energy expended because of a lack of regular physical activity due to an inappropriate lifestyle characterized by changing modes of transportation, increasing urbanization, and

[1] Biochemistry Laboratory, LR12ES05 "Nutrition-Functional Foods and Vascular Health," Faculty of Medicine, University of Monastir, Tunisia
[2] Department of Internal Medicine, CHU F. Bourguiba, Monastir, Tunisia
[3] Cardiovascular Epidemiology and Genetics, Institute Hospital del Mar d'Investigacions Mediques, Barcelona, Spain

Corresponding Author:
Sounira Mehri, Faculty of Medicine, University of Monastir, Avicenne St, 5019 Monastir, Tunisia.
Email: mehrisounira@yahoo.fr

a rapidly changing modern sedentary environment (nature of many forms of work).[6] Moreover, genetic factors also play a relevant role in obesity which heritability has been estimated to be around 40% to 70%.[6,7] To date, the characterization of genes that increase the susceptibility to develop overweight and obesity has become a priority question for health, allowing the identification of persons who are at risk and may lead to new strategies and approaches for prevention and/or treatment to curb rising obesity rates. Recent genome-wide association studies have discovered several loci associated with obesity-related traits.[8] Most of these studies have been performed in European, Asian, or African American populations.[8,9] However, information about the genetic background of obesity in North-African populations is scanty.

It is widely recognized that polyunsaturated fatty acids (PUFAs) have a major impact on human health.[10] Epidemiological studies have demonstrated that PUFAs are associated with numerous clinical outcomes, including obesity,[11,12] cardiovascular complications, and metabolic syndrome.[13-15] Candidate gene studies have focused on the contribution of genetic variants in fatty acid desaturases 1 (FADS1) and FADS2 to circulating and cellular levels of PUFAs.[16-18] The *FADS1* and *FADS2* genes, localized on the human chromosome 11 (11q12-q13.1),[19] encoded delata-5-desaturase (D5D or Δ5) and delta-6-desaturase (D6D or Δ6), respectively, serving as key enzymes in PUFA metabolism.[10] In fact, ELOVL2 is located on the human chromosome 6 (6q24.2) and known as another essential enzyme in the homeostasis of long-chain fatty acids.[20] The *FADS1*, *FADS2*, and *ELOVL2* genes are essential in the homeostasis of PUFA[21] and have been associated with lipid profile.[10] Our main aim was to determine the association between genetic variants in FADS1, FADS2, and ELOVL2 and obesity and body mass index (BMI) in a Tunisian population. We also assessed the association between these genetic variants and lipid profile and fatty acid profile in this population.

Patients and Methods

Design and Patients

An age- and sex-matched case–control study was designed, with 259 obese patients and 369 nonobese patients. Cases were individuals with overweight or obesity recruited from the Department of Endocrinology from Fattouma Bourguiba University Hospital (Monastir, Tunisia). All these patients were prospectively invited to participate in this study. Controls were normoweight individuals and were also prospectively selected and invited to participate among those attending a routine checkup as part of annual physical examination. Controls were living in the same geographic area as cases. Inclusion criteria were male and female patients, being Tunisian national, above 18 years old, with a BMI $\geq$30 kg/m^2 for obese patients and <25 kg/m^2 for control individuals, able to give consent, no use of any medication affecting body weight, lipid/fatty acid profiles, and energy expenditure, and patients who had essential hypertension and were treated with selective antihypertensive medication (diuretics, beta blockers, and calcium channel blockers) for about 3 years or more than that in monotherapy or combination therapy, with fasting plasma glucose $\geq$7.0 mmol/L and no treatment used for diabetes (diet and/or oral antidiabetic drugs [biguanidine and/or sulfamide or metformin] and/or insulin to achieve glycemic control) in patients and controls. The exclusion criteria were pregnancy, patients undergoing dialysis or with hematological, hepatic, or thyroid diseases, malignancy, or other liver diseases, including chronic viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, and drug-induced liver disease.

The study was approved by the local ethics committee, and all the participants gave informed consent before their enrollment. An interview was conducted with each participant to collect information about age, family history of previous cardiovascular diseases, cigarette smoking, and medications. Moreover, a trained nurse measured blood pressure following a standardized protocol. The presence of cardiovascular risk factors was defined based on standardized criteria. Diabetes was diagnosed according to the World Health Organization criteria. Hypertension was defined as a systolic blood pressure $\geq$140 mm Hg or diastolic $\geq$90 mm Hg or use of antihypertensive drugs. Dyslipidemia was defined according to the new European Guidelines by low-density lipoprotein (LDL) level >3.1 mmol/L.[22]

Genotyping

Genomic DNA was extracted from blood samples using a salting out procedure.[23] Allelic discrimination between variants was performed by standard polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis. rs174556 at FADS1, rs174617 at FADS2, and rs3756963 at ELOVL2 were selected as genetic markers. They were all intronic with a C/T base change. One hundred nanograms of DNA were used for polymorphism analysis by PCR. The reverse primer was used in combination with forward primer (http://www.ncbi.nlm.nih.gov/SNP/): at rs174556 of F5′AAGCAGGGACCTCAAGAC3′; R5′AGCCCACCAAGAATGTAA3′; at rs174617 of F5′GAACTGTCAGAGGCAACG3′; R5′CTGGGCAATAAAGCAAGA3′; and that at rs3756963 at F5′CCTTTGTGCGAGAACCT3′; R5′ATCCCAAGCGACAGACCC3′.

Reaction conditions were as follows: predenaturation at 94°C for 5 minutes, 35 cycles of 95°C for 40 seconds, 57°C to 60°C for 1 minute, and an elongation step at 72°C for 1 minute. A final step of extension at 72°C for 10 minutes happened. PCR product of 5 μL was analyzed by 2% agarose gel electrophoresis. Then, an endonuclease digestion took place. Restriction enzymes MboI, MspI, and HhaI were used for digestion. The different genotypes (TT, CT, and CC) were determined after electrophoresis on 3% agarose gel.

Anthropometric Variables

Weight (kg), height (cm), and waist circumference (WC, cm) were determined according to a standard protocol in the

Nutrition Center by trained nurses. Body mass index was calculated: weight (kg)/height (m^2). According to BMI, 3 groups were defined: normoweight (BMI = 18.5-24.9 kg/m^2), overweight (BMI = 25.0-29.9 kg/m^2), and obesity (BMI $\geq$ 30 kg/m^2; National Heart, Lung, and Blood Institute in cooperation with the National Institute of Diabetes and Digestive and Kidney Diseases, 1998).

Biochemical Measurements

Venous blood samples were drawn into tubes containing EDTA, from each patient after at least 12 hours (overnight) fasting, for genomic DNA extraction and biochemical analysis. The tubes were immediately placed on ice until they arrived at the laboratory. Then plasma and red blood cells were separated by centrifugation. All samples were stored at −80°C until analysis.

Biochemical measurements were carried out according to validated methods. Plasma glucose concentration was evaluated using an enzymatic assay (glucose oxidase; Randox, Antrim, United Kingdom), total cholesterol, and triglycerides by enzymatic methods using Randox reagents, and LDL and high-density lipoprotein cholesterol (HDL-C) were determined as described by Smaoui et al.[24]

Fatty Acid Extraction and Determination

Fatty acids were analyzed using gas chromatography (GC; Hewlett Packard, Palo Alto, California) equipped with a flame ionization detector, on a 30 m × 0.25 mm internal diameter and 0.25 μm polar fused silica capillary column [HP-INNOWax, is a polyethylene glycol (PEG) stationary phase that features high polarity and high upper temperature limits]. Total fat from plasma was extracted, according to the method of Folch et al.[25]

Briefly, lipid extraction was performed with chloroform–methanol (2:1, vol/vol) mixture, containing 0.01% butylated hydroxytoluene (as the antioxidant). The fatty acid C17:0 was added to the samples as an internal standard. The chloroform phase containing lipids was collected and subjected to methylation (total lipids were converted into methyl esters) using 14% methanol–boron trifluoride (BF3) at 50°C for 30 minutes. One microliter of each sample was injected into the GC system. The oven temperature was programmed to increase from 180°C to 250°C at a rate of 10°C/min, and the injector and detector temperatures were 220°C and 280°C, respectively. Fatty acid methyl esters were identified by comparison with fatty acid standard, and area and its percentage for each resolved peak were analyzed using an HP Chemstation integrator.

Desaturase and elongase activities (AEs) were calculated using relevant fatty acid product–precursor ratios as described previously.[10] The Δ6 desaturase (D6D) activities were estimated by dividing the percentage composition of C18:3n-6/C18:2n-6 and C20:4n-6/C18:2n-6, and the D5D activity was estimated by dividing the percentage composition of C20:4n-6/C20:3 n-6. The D9D activity was estimated by dividing the percentage composition of C18:1 n-9/C18:0. The ratios C22:4n-6/ C20:4n-6 and C22:5n-3/ C20:5n-3 for elongase were used.

Table 1. Demographic and Biochemical Characteristics of the Participants in the Study Stratified by the Presence of Overweight–Obesity.

	Cases (n = 259)	Controls (n = 369)	*P* Value
Age, years	48.85 ± 14.70	48.08 ± 14.11	.506
Sex, M/F	154/105	202/167	.240
Smoking, n (%)	174 (67.2)	231 (62.6)	.238
Hypertension, n (%)	139 (53.7)	152 (41.2)	.002
Dyslipidemia, n (%)	155 (59.8)	49 (13.3)	<.001
Diabetes, n (%)	108 (41.7)	132 (35.8)	.132
Fasting glucose, mmol/L	6.01 ± 1.31	6.37 ± 2.62	.043
Triglycerides, mmol/L	1.49 ± 0.83	1.40 ± 0.67	.154
Total cholesterol, mmol/L	5.43 ± 1.77	4.66 ± 1.28	<.001
HDL-C, mmol/ L	1.23 ± 0.54	1.40 ± 0.53	<.001
LDL-C, mmol/ L	3.04 ± 1.38	2.65 ± 0.91	<.001
BMI, kg/m^2	30.72 ± 3.78	21.71 ± 2.22	<.001
Waist circumference, cm	117.83 ± 12.95	98.22 ± 11.04	<.001
Normal weight, n (%)	-	369	
Overweight, n (%)	103 (39.77)	-	
Obesity, n (%)	156 (60.23)	-	

Abbreviations: BMI, body mass index; F, female; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M, male.

Statistical Analysis

Continuous variables are presented as mean (standard deviation) and categorical as frequency (percentage). We used analysis of variance and chi-square to compare distribution of continuous and categorical variables between groups, respectively. Those variables associated with the genetic variants of interest and with BMI or overweight–obesity were considered as potential confounders. We used multivariate logistic regression analysis and multivariate linear regression analysis to evaluate the association between genetic variants and overweight–obesity and BMI, respectively. These multivariate models were adjusted for all the confounder variables. A multilocus genetic risk score (GRS) was computed for each individual as the sum of the number of risk alleles across the variants that were associated with overweight–obesity or BMI. The statistical analyses were carried out using SPSS 22.0 (SPSS, Chicago, Illinois).

Results

The characteristics of the participants in this study are shown in Table 1. There was no significant difference in either the age or the gender distribution between the 2 groups included. Hypertension and dyslipidemia were significantly higher in overweight–obese patients compared to controls. Smoking, diabetes, and triglycerides level were similar in the 2 groups. Higher BMI, WC, total cholesterol, LDL-C, and fasting glucose were also shown in overweight–obese patients group. A significantly high level of HDL-C was found in the control group than patients.

Genotype frequencies of FADS1, FADS2, and ELOVL2 polymorphisms are shown in Table 2. The genotype distributions of FADS1, FADS2, and ELOVL2 polymorphisms were in

Table 2. The Distribution of the FADS1, FADS2, and ELOVL2 Polymorphisms in Cases and Controls.

	Cases (n = 259)	Controls (n = 369)	*P* Value
FADS1 polymorphism			
TT, n (%)	101 (38.99)	89 (24.12)	<.001
CT, n (%)	110 (42.47)	172 46.61)	
CC, n (%)	48 (18.53)	108 (29.27)	
FADS2 polymorphism			.014
TT, n (%)	78 (30.11)	86 (23.31)	
CT, n (%)	125 (48.26)	164 (44.44)	
CC, n (%)	56 (21.62)	119 (32.25)	
ELOVL2 polymorphism			
TT, n (%)	81 (31.27)	58 (15.72)	<.001
CT, n (%)	117 (45.17)	158 (42.82)	
CC, n (%)	61 (23.55)	153 (41.46)	

Table 3. Univariate and Multivariate Linear Regression Analysis Between the Analyzed Genotypes and BMI and Between the Summary Genetic Risk Score (GRS) and BMI.[a]

		β	SE	*P* Value
FADS1 (C allele)	Univariate	−1.24	0.28	<.001
	Multivariate	−1.18	0.28	<.001
FADS2 (C allele)	Univariate	−1.06	0.29	<.001
	Multivariate	−0.90	0.28	.001
ELOVL2 (C allele)	Univariate	−1.40	0.28	<.001
	Multivariate	−1.23	0.28	<.001
GRS (C allele)	Univariate	−1.34	0.17	<.001
	Multivariate	−1.19	0.16	<.001

Abbreviations: BMI, body mass index; SE, Error standard.
[a]Multivariate analysis adjusted for age, sex, fasting glucose, diabetes, hypertension, dyslipidemia, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol.

agreement with Hardy-Weinberg equilibrium. The frequencies of the FADS1, FADS2, and ELOVL2 genotypes were significantly different between the 259 patients and the 369 controls included in the analyses, respectively (FADS1, $P = .001$; FADS2, $P = .014$; and ELOVL2, $P < .001$; Table 2).

The univariate and multivariate effect size on BMI per each C allele is carried by an individual across genetic variants and in the multilocus GRS. The results of the univariate and multivariate logistic regression analyses are shown in Table 3. The presence of the C allele was also associated with lower odds of being overweight–obese. In an additive model, the C allele in each of these variants was associated with a lower BMI: −1.18, −0.90, and −1.23 units, respectively.

The characteristics of the participants with regard to the FADS1, FADS2, and ELOVL2 genotypes are summarized in Table 4. The results showed that the triglycerides and total cholesterol were significantly lower in overweight–obese patients with the minor allele of FADS1. Lower triglycerides, total cholesterol, and LDL-C were related to C minor allele of FADS2 in overweight–obese patients. Smoking, hypertension, dyslipidemia, and diabetes were significantly different between

Table 4. Demographic and Biochemical Characteristics of Patients Across the 3 Genetic Variants Analyzed and Corresponding Genotypes.

	FADS1				FADS2				ELOVL2			
	TT (n = 101)	CT (n = 110)	CC (n = 48)	*P*	TT (n = 78)	CT (n = 125)	CC (n = 56)	*P*	TT (n = 81)	CT (n = 117)	CC (n = 61)	*P*
Age, years	46.91 ± 14.30	50.06 ± 14.53	50.12 ± 15.61	.237	48.41 ± 14.08	48.94 ± 15.05	49.25 ± 14.84	.944	48.43 ± 14.43	48.48 ± 14.83	50.10 ± 14.83	.747
Sex, M/F	61/40	66/44	27/21	.925	51/27	74/51	30/26	.326	46/35	67/50	41/20	.320
Smoking, n (%)	69 (68.3)	75 (68.2)	31 (63.3)	.799	48 (61.5)	85 (68.0)	42 (73.7)	.323	63 (77.8)	72 (61.5)	40 (64.5)	.049
Hypertension, n (%)	55 (54.5)	58 (52.7)	27 (55.1)	.950	43 (55.1)	71 (56.8)	26 (45.6)	.360	53 (65.4)	56 (47.9)	31 (50.8)	.040
Dyslipidemia, n (%)	60 (59.4)	70 (63.6)	25 (51.0)	.326	48 (61.5)	74 (59.2)	33 (57.9)	.905	58 (71.6)	65 (55.6)	32 (51.6)	.026
Diabetes, n (%)	37 (36.6)	53 (48.2)	19 (38.8)	.209	22 (28.2)	65 (52.0)	22 (38.6)	.003	48 (59.3)	45 (38.5)	16 (25.8)	<.001
Fasting glucose, mmol/L	5.89 ± 1.25	6.12 ± 1.30	6.02 ± 1.47	.454	5.78 ± 1.26	6.16 ± 1.30	6.02 ± 1.40	.139	6.56 ± 1.34	5.80 ± 1.22	5.70 ± 1.24	<.001
Triglycerides, mmol/L	1.69 ± 0.98	1.47 ± 0.75	1.13 ± 0.50	<.001	1.84 ± 1.04	1.48 ± 0.70	1.28 ± 0.66	<.001	1.58 ± 0.95	1.68 ± 0.90	1.33 ± 0.67	.015
Total cholesterol, mmol/L	5.79 ± 1.70	5.46 ± 1.88	4.59 ± 1.36	<.001	6.00 ± 1.69	5.65 ± 2.16	4.96 ± 1.49	<.001	5.17 ± 1.86	5.31 ± 1.72	5.96 ± 1.66	.018
HDL-C, mmol/L	1.42 ± 0.54	1.4 ± 0.50	1.29 ± 0.57	.285	1.48 ± 0.47	1.40 ± 0.54	1.30 ± 0.58	.170	1.33 ± 0.50	1.47 ± 0.56)	1.37 ± 0.50	.158
LDL-C, mmol/L	3.22 ± 1.51	2.99 ± 1.40	2.76 ± 0.98	.147	3.36 ± 1.44	3.17 ± 1.51	2.77 ± 1.24	.008	3.04 ± 1.42	3.43 ± 1.34	2.82 ± 1.34	.020
BMI, kg/m^2	30.82 ± 3.96	30.73 ± 3.51	30.52 ± 4.02	.902	30.99 ± 3.99	30.49 ± 3.47	30.87 ± 4.13	.623	31.8 ± 4.18	30.5 ± 3.66	30.10 ± 1.22	.005
Waist circumference, cm	118.1 ± 13.16	118.5 ± 12.90	115.8 ± 12.56	.473	116.6 ± 13.21	117.1 ± 11.36	121.1 ± 15.27	.094	120.3 ± 14.94	116.2 ± 12.01	117.6 ± 11.36	.087

Abbreviations: BMI, body mass index; F, female; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M, male.

Table 5. Fatty Acids Profile in Total Population.

Fatty Acids	Cases (n = 259)	Controls (n = 369)	*P* Value
Lauric C12:0	6.70 ± 11.50	3.26 ± 3.15	<.001
Myristic C14:0	7.88 ± 7.94	0.95 ± 2.60	<.001
Palmitic C16:0	9.10 ± 3.54	2.58 ± 4.64	<.001
Stearic C18:0	6.04 ± 2.80	1.72 ± 0.92	<.001
Arachidic C20:0	1.10 ± 1.18	0.55 ± 0.78	<.001
Behenic C22:0	1.29 ± 0.40	1.36 ± 0.44	.059
Lignoceric C24:0	3.76 ± 1.99	1.47 ± 0.48	<.001
Myristoleic C14:1	8.18 ± 3.99	4.39 ± 0.44	<.001
Heptadecenoic C17:1	3.93 ± 2.03	3.69 ± 1.95	.132
Oleic C18:1 n-9	5.79 ± 2.18	5.82 ± 2.19	.898
Cis-vaccenic C18:1 n-7	87.85 ± 27.22	88.36 ± 29.72	.838
Paulinic C20:1 n-9	6.24 ± 2.42	6.11 ± 2.34	.494
Erucic C22:1 n-9	5.90 ± 2.34	2.10 ± 2.01	<.001
Elaidic C18:1 trans 9	8.35 ± 4.21	8.32 ± 4.30	.944
Vaccenic C18:1 trans 11	7.23 ± 2.63	7.28 ± 2.71	.805
Linoleic C18:2 n-6	298.06 ± 203.55	263.74 ± 243.45	.069
Eicosadienoic C20:2 n-6	5.40 ± 3.85	2.37 ± 2.09	<.001
Docosadienoic C22:2 n-6	3.18 ± 1.65	3.44 ± 1.84	.079
CLA1 C18:2 cis 9, trans 11	2.76 ± 0.98	2.58 ± 0.83	.002
CLA2 C18:2 trans 10, cis 12	5.32 ± 2.10	5.02 ± 1.90	.007
α-linolenic C18:3 n-3	2.37 ± 2.85	1.55 ± 1.51	<.001
Eicosatrienoic C20:3 n-3	3.21 ± 3.04	2.81 ± 2.55	.089
EPA C20:5 n-3	3.54 ± 3.90	2.29 ± 2.93	<.001
DPA C22:5 n-3	4.08 ± 3.20	4.38 ± 5.01	.405
DHA C22:6 n-3	4.74 ± 3.68	3.66 ± 3.20	<.001
Glinoleic C18:3 n-6	3.64 ± 3.20	4.03 ± 6.04	.348
DGLA C20:3 n-6	2.57 ± 2.49	2.30 ± 2.26	.169
AA C20:4 n-6	7.51 ± 7.89	6.16 ± 4.61	.007
Docosatetraenoic C22:4 n-6	5.33 ± 5.96	4.55 ± 5.33	.092
C20:4 n-6/C20:3 n-6 (D5D)	3.39 ± 3.59	3.77 ± 3.15	.278
C18:3 n-6/C18:2 n-6 (D6D)	6.25 ± 4.12	5.10 ± 3.37	.027
C20:4 n-6/C18:2 n-6 (D6D)	5.66 ± 2.17	1.82 ± 0.91	<.001
C18:1 n-9/C18:0 (D9D)	4.12 ± 2.52	1.23 ± 0.89	<.001
Docosatetraenoic/AA (AE)	3.71 ± 2.81	1.19 ± 1.71	<.001
DPA/EPA (AE)	6.45 ± 4.77	2.15 ± 2.05	<.001

Abbreviations: AA, arachidonic acid; AE, elongase activity; ALA, α-linolenic Acid; CLA, conjugated linolenic acid; D5D, Δ5desaturase activity; D6D, Δ6 desaturase activity; D9D, Δ9desaturase activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

ELOVL2 genotypes in overweight–obese patients. The C minor allele of ELOVL2 was related to lower BMI, fasting glucose, triglycerides, total cholesterol, and LDL-C in overweight–obese patients.

To establish a fatty acid profile, analyses by gas chromatography were conducted on the plasma of overweight–obese patients and controls. The fatty acid profile is shown in Table 5.

The overweight–obese patients showed higher amounts of total saturated fatty acids (SFAs), lauric acid (12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (20:0), lignoceric (24:0) monounsaturated fatty acids (MUFAs), myristoleic (C14:1), and erucic (C22:1 n-9) than the controls group ($P < .001$).

For the n-3 polyunsaturated fatty acids (n-3 PUFAs), a significant increase in ALA (18:3 n-3), docosahexaenoic acid (DHA, C22:6 n-3), and eicosapentaenoic acid (EPA, C20:5 n-3) was observed in patients compared to controls.

For the n-6 PUFAs, a significant increase in the levels of arachidonic acid (AA; 20:4 n-6) and conjugated linolenic acids (CLA1; C18:2 cis 9, trans 11 and CLA 2, C18:2 trans 10, cis 12) was found in patients compared to the controls.

As a consequence, a significant increase in D6D activities, presented by 20:4n-6/ 18:2n-6 and 18:3n-6/18:2n-6, was observed in the patients compared to controls ($P = .027$ and $P < .001$). The D9D activity, estimated by the ratio 18:1n-9/ 18:0, was significantly higher in the group of patients than controls ($P < .001$). Significant increase in AE, estimated by the ratio docosatetraenoic/AA and DPA/EPA, was found in patients compared to the controls group ($P < .001$).

Fatty acid profile of the patients' group with regard to the FADS1, FADS2, and ELOVL2 genotypes is summarized in Table 6. The overweight–obese patients with the minor allele carriers of rs174556 FADS1 had significantly lower DHA. A significant decrease in stearic acid, EPA, and AE activity (docosatetraenoic/AA) was revealed in patients with the minor allele carriers of rs2236212 FADS2. Patients with minor allele carriers of rs3756963 ELOVL2 had significantly lower ALA, EPA, DPA, and D6D activity (C20:4 n-6/C18:2n-6).

Discussion

We report an association between rs174556, rs174617, and rs3756963 of the FADS1, FADS2, and ELOVL2 genes and obesity. The presence of the C allele was also associated with lower odds of being overweight–obese. Despite a lack of studies analyzing the association between obesity risk and FADS1, FADS2, and ELOVL2 polymorphisms, some studies have found that minor alleles of FADS confer a higher risk of obesity related to increased triglyceride levels and decreased HDL-C concentrations.[26,27] Our results showed that the minor allele of FADS1 related to decreased triglycerides and total cholesterol. Lower triglycerides, total cholesterol, and LDL-C levels were associated with the C minor allele of FADS2. Lower BMI, fasting glucose, triglycerides, total cholesterol, and LDL-C were related to the minor allele of ELOVL2. Several genome-wide association studies have reported a consistent association between FADS1 and FADS2 loci and serum lipid traits in European,[28] East Asian,[9] Afro-American,[8] and Hispanic populations.[29]

We analyzed the fatty acids involved in enzymatic reactions encoded by the *FADS* and *ELOVL* genes (substrates, products, or indexes [product/substrates]). In our study, the amount of total SFAs in plasma was significantly higher in the overweight–obese patients; these SFAs include lauric acid (12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (20:0), and lignoceric (24:0), which are related to the incidence of obesity.[30] Additionally, total SFAs may increase cardiovascular disease risk by increasing levels of LDL-C and total cholesterol.[30] Indeed, the overweight–obese group of our study showed higher total cholesterol and LDL-C. Similarly, the level of total MUFAs, myristoleic (C14:1) and erucic (C22:1 n-9) acids, increased in the overweight–obese patients. Our study showed only slight differences in the levels

Table 6. Fatty Acids Profile in Patients Across the 3 Genetic Variants Analyzed and Corresponding Genotypes.

	FADS1				FADS2				ELOVL2			
Fatty Acids	TT (n = 101)	CT (n = 110)	CC (n = 48)	*P*	TT (n = 78)	CT (n = 125)	CC (n = 56)	*P*	TT (n = 81)	CT (n = 117)	CC (n = 61)	*P*
Lauric C12:0	7.33 ± 1.61	6.22 ± 2.57	6.34 ± 2.42	.763	7.41 ± 4.75	7.18 ± 4.28	4.58 ± 2.89	.294	7.66 ± 3.57	6.24 ± 2.57	6.22 ± 2.54	.650
Myristic C14:0	7.74 ± 1.94	8.05 ± 2.68	7.62 ± 1.16	.937	7.09 ± 3.11	8.62 ± 4.84	7.19 ± 3.23	.322	8.20 ± 4.88	8.27 ± 3.62	6.62 ± 4.87	.374
Palmitic C16:0	9.41 ± 4.65	8.97 ± 2.48	8.58 ± 3.04	.380	9.45 ± 4.64	8.94 ± 3.20	8.82 ± 2.53	.520	9.58 ± 5.21	8.73 ± 2.29	9.04 ± 2.82	.262
Stearic C18:0	1.86 ± 1.18	1.80 ± 0.98	1.59 ± 0.72	.162	1.86 ± 0.96	1.84 ± 1.04	1.46 ± 0.65	.008	1.70 ± 0.73	1.85 ± 1.11	1.54 ± 0.81	.096
Arachidic C20:0	0.48 ± 0.41	0.65 ± 0.52	0.45 ± 0.37	.208	0.50 ± 0.46	0.53 ± 0.13	0.64 ± 0.23	.558	0.46 ± 0.51	0.61 ± 0.39	0.55 ± 0.38	.440
Behenic C22:0	1.27 ± 0.39	1.28 ± 0.41	1.37 ± 0.43	.347	1.32 ± 0.42	1.27 ± 0.38	1.31 ± 0.43	.629	1.32 ± 0.42	1.27 ± 0.38	1.29 ± 0.43	.716
Lignoceric C24:0	1.50 ± 0.51	1.46 ± 0.47	1.47 ± 0.49	.828	1.43 ± 0.46	1.49 ± 0.51	1.52 ± 0.48	.568	1.47 ± 0.50	1.47 ± 0.48	1.50 ± 0.50	.920
Myristoleic C14:1	0.43 ± 0.29	0.37 ± 0.16	0.49 ± 0.30	.530	0.37 ± 0.22	0.47 ± 0.26	0.35 ± 0.13	.372	0.44 ± 0.38	0.38 ± 0.35	0.45 ± 0.30	.666
Heptadecenoic C17:1	3.87 ± 1.99	3.88 ± 1.97	4.16 ± 2.25	.671	3.70 ± 1.98	3.95 ± 2.12	4.20 ± 1.89	.360	3.99 ± 2.13	4.01 ± 2.02	3.69 ± 1.92	.575
Oleic C18:1 n-9	5.75 ± 2.23	5.86 ± 1.95	5.73 ± 2.55	.905	5.57 ± 1.86	6.06 ± 2.49	5.51 ± 1.75	.171	5.54 ± 1.78	6.07 ± 2.55	5.61 ± 1.82	.176
Cis-vaccenic C18:1 n-7	90.54 ± 36.59	85.85 ± 18.14	87.26 ± 20.85	.451	88.69 ± 15.27	89.46 ± 35.20	83.58 ± 18.28	.385	88.83 ± 17.32	88.22 ± 36.26	86.26 ± 15.66	.847
Paulinic C20:1 n-9	6.31 ± 2.36	6.25 ± 2.49	6.03 ± 2.42	.795	6.19 ± 2.14	6.23 ± 2.42	6.29 ± 2.80	.972	6.42 ± 2.59	6.22 ± 2.40	5.99 ± 2.24	.585
Erucic C22:1 n-9	5.75 ± 2.29	5.91 ± 2.31	6.08 ± 2.60	.725	5.57 ± 2.20	5.90 ± 2.52	6.25 ± 2.12	.250	5.93 ± 2.46	6.03 ± 2.37	5.53 ± 2.16	.382
α-linolenic C18:3 n-3	262.27 ± 131.42	264.43 ± 164.51	267.27 ± 168.76	.993	268.20 ± 167.15	269.41 ± 172.06	246.97 ± 148.20	.834	246.11 ± 162.35	247.91 ± 163.60	318.27 ± 201.83	.132
Eicosadienoic C20:2 n-6	5.65 ± 4.37	5.38 ± 3.49	4.92 ± 3.35	.550	5.01 ± 3.80	5.59 ± 4.16	5.51 ± 3.15	.570	4.81 ± 3.82	5.60 ± 3.93	5.79 ± 3.68	.237
Glinoleic C18:3 n-6	3.48 ± 2.06	3.71 ± 2.22	4.99 ± 2.89	.177	3.38 ± 3.02	4.29 ± 3.11	3.61 ± 3.18	.381	3.68 ± 3.21	3.33 ± 3.09	5.12 ± 3.96	.059
Linoleic C18:2 n-6	2.10 ± 2.01	2.78 ± 2.22	2.04 ± 1.34	.149	2.25 ± 2.15	2.33 ± 2.04	2.65 ± 1.16	.708	2.43 ± 2.07	1.88 ± 1.29	3.26 ± 2.25	.008
DGLA C20:3 n-6	2.39 ± 2.24	2.73 ± 2.22	2.56 ± 2.39	.615	2.49 ± 2.32	2.59 ± 2.09	2.62 ± 2.01	.946	2.59 ± 2.29	2.40 ± 2.39	2.85 ± 2.03	.509
Eicosatrienoic C20:3 n-3	3.04 ± 2.83	3.32 ± 3.04	3.23 ± 2.44	.805	2.62 ± 2.34	3.56 ± 3.34	3.19 ± 2.29	.100	3.24 ± 2.76	3.21 ± 2.70	3.11 ± 2.67	.965
AA C20:4 n-6	7.61 ± 4.30	7.47 ± 3.56	7.25 ± 3.86	.967	7.34 ± 6.66	6.99 ± 5.77	8.75 ± 6.07	.373	6.53 ± 4.54	7.88 ± 4.99	7.97 ± 5.01	.423
EPA C20:5 n-3	4.23 ± 2.55	3.22 ± 2.59	2.85 ± 2.32	.065	4.55 ± 3.75	3.07 ± 2.17	3.21 ± 2.85	.023	4.29 ± 3.26	2.55 ± 2.17	4.44 ± 2.58	.001
Docosadienoic C22:2 n-6	3.12 ± 1.75	3.27 ± 1.63	3.09 ± 1.51	.749	3.12 ± 1.86	3.15 ± 1.59	3.32 ± 1.49	.761	3.25 ± 1.52	3.07 ± 1.62	3.28 ± 1.87	.656
Docosatetraenoic C22:4 n-6	5.28 ± 3.88	5.28 ± 3.41	5.45 ± 3.24	.749	4.80 ± 2.17	5.02 ± 2.64	6.66 ± 3.37	.149	4.21 ± 2.18	5.70 ± 2.67	6.02 ± 2.30	.124
DPA C22:5 n-3	3.93 ± 2.29	4.03 ± 3.16	4.45 ± 3.13	.636	4.71 ± 3.77	3.80 ± 2.85	3.80 ± 3.02	.111	4.46 ± 3.49	3.50 ± 2.79	4.65 ± 3.40	.030
DHA C22:6 n-3	5.30 ± 2.22	4.00 ± 2.92	5.14 ± 3.84	.025	5.16 ± 4.07	4.47 ± 3.41	4.67 ± 3.72	.434	4.60 ± 3.61	4.39 ± 3.52	5.50 ± 4.03	.152
C20:4 n-6/C20:3 n-6 (D5D)	3.22 ± 1.67	3.45 ± 1.55	3.62 ± 1.61	.870	3.66 ± 2.57	3.10 ± 2.30	3.67 ± 2.70	.615	3.33 ± 2.74	3.22 ± 2.14	3.81 ± 2.22	.706
C18:3 n-6/C18:2 n-6 (D6D)	6.72 ± 2.60	6.46 ± 1.24	4.80 ± 1.31	.460	7.06 ± 4.35	6.69 ± 4.40	4.20 ± 3.45	.151	7.62 ± 4.31	5.97 ± 4.32	5.00 ± 3.67	.213
C20:4 n-6/C18:2 n-6 (D6D)	0.73 ± 0.72	1.09 ± 0.24	0.43 ± 0.21	.105	0.91 ± 0.13	0.62 ± 0.37	1.17 ± 0.53	.173	1.15 ± 0.23	0.47 ± 0.14	1.07 ± 0.46	.024
C18:1 n-9/C18:0 (D9D)	4.32 ± 2.48	3.92 ± 2.15	4.17 ± 3.29	.504	4.54 ± 2.55	4.09 ± 2.64	3.63 ± 2.12	.112	3.90 ± 2.34	4.09 ± 2.68	4.49 ± 2.42	.374
Docosatetraenoic/AA (AE)	3.37 ± 2.85	3.52 ± 2.11	4.82 ± 2.49	.327	2.42 ± 2.80	4.63 ± 2.17	3.44 ± 2.22	.028	2.94 ± 2.51	4.54 ± 2.95	3.15 ± 2.62	.112
DPA/EPA (AE)	1.88 ± 1.08	2.38 ± 2.03	2.21 ± 1.75	.208	2.36 ± 2.25	2.07 ± 2.04	2.06 ± 1.80	.569	2.40 ± 1.96	2.01 ± 1.93	2.11 ± 1.38	.421

Abbreviations: AA, arachidonic acid; AE, elongase activity; ALA, α-linolenic Acid; CLA, conjugated linolenic acid; D5D, Δ5desaturase activity; D6D, Δ6 desaturase activity; D9D, Δ9desaturase activity; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

of n-3 PUFAs, specifically ALA (18:3 n-3), DHA, and EPA, which were higher in the overweight–obese patients. The levels of total n-6 PUFAs, CLAs (CLA1 and 2), and AA (C20:4, n-6) were higher in the overweight–obese patients.

The n-6 PUFAs are thought to promote adipogenesis and increase the expression of lipogenic genes.[31,32] We also found that there were significant differences in desaturases and AE between the patients and control groups. Then, we found a significant increase in Δ6 and Δ9desaturases activities in the overweight–obese patients. These findings are in agreement with some previous studies.[33,34] The differences observed in desaturases activities may be responsible for the disturbance of long-chain PUFA metabolism in overweight/obesity.[11] The overweight–obese patients had increased AE, estimated by the ratio docosatetraenoic/AA and DPA/EPA in our population. Regarding the overweight–obese group, minor allele carriers of rs174556 (*FADS1*) were associated with a lower DHA. Several studies have observed that the DHA status, or that of the n-3 series, is less influenced by genetic variants in the *FADS* genes.[10,35,36] FADS1 was reported to be associated with Δ9desaturase activity (AA/LA) in Caucasians and Asians,[33] but the findings were inconsistent in Chinese population.[17,34]

The minor allele carriers of rs2236212 (*FADS2*) was associated with a lower stearic acid and EPA. Additionally, among the minor allele carriers of FADS2, overweight–obese patients showed significantly lower AE activity (docosatetraenoic/AA). Several studies have also found an association between FADS2 and lower levels of AA and D6D activity (AA/LA).[10,35] We also observed that ELOVL2 genetic variant affected fatty acid concentrations. Overweight–obese patients with minor allele carriers of ELOVL2 had significantly lower levels of ALA, EPA, and DPA. Furthermore, among minor homozygotes, overweight–obese patients also showed lower D6D activity (C20:4n-6/C18:2n-6). Several genome-wide association studies have also reported a consistent association between FADS1, FADS2, and ELOVL2 loci and plasmatic fatty acids in European,[16] East Asian,[17] Afro-American, and Hispanic populations.[18] Our results suggest that the FADS1/FADS2 locus could be an important metabolic pathway regulating fatty acid metabolism and the clustering of some metabolic disorders, such as obesity and dyslipidemia, which are important components of metabolic syndrome and could increase the risk of cardiovascular diseases. These results support the role of desaturase activities in the regulation of the metabolism of long-chain PUFAs that seem to be altered in obesity[11] or may contribute to obesity risk.[36] High Δ9-desaturase activity has been associated with obesity, hypertriglyceridemia, metabolic syndrome, and increased risk of insulin resistance.[37] Additionally, hepatic lipid composition changes caused by obesity are related to desaturase expression.[38] Obesity is in turn associated with several metabolic disorders, such as insulin resistance and dyslipidemia. On the other hand, some studies have shown that changes in the composition of dietary fatty acids could be efficacious to improve lipid profile.[39] Adipose tissue dysfunction, which is involved in atherosclerotic vascular diseases and type 2 diabetes development, is a state of hypersecretion of proatherogenic, pro-inflammatory, and prodiabetic adipocytokines.[40] Therefore, overweight and/or obese status, fatty acid composition, and desaturase activity are related to each other.

A major strength of these preliminary results indicates that our study is the first to demonstrate the impact of this FA-related single-nucleotide polymorphism (SNP) on obesity in a Tunisian population. The present study has several limitations. Among the limitations, we have to mention the limited number of SNPs analyzed in this study. However, they could be considered as tag-SNPs of the loci previously reported to be associated with the phenotypes of interest and therefore are good candidates to capture most of the common genetic variability existing in those loci. On the other hand, the sample size of our study population is modest hampering our statistical power. However, we did observe and replicate some associations previously reported in other ethnic groups. Body structure and habitual diets can differ substantially between races and countries. Diet and regular physical activity as behavior habits are associated with strong beliefs that influence one's own health, too. Diet is of paramount importance in the management of weight and obesity-dependent complications. It is accepted that a composition of dietary changes (diet with less calories and fat) and increased regular physical activities is the proficiency method to promote weight loss and to maintain the weight loss. Numerous studies[41,42] were undertaken to analyze the association between fatty acid intake and obesity, which warrants further investigation. Our results require personalized nutrition to show if metabolism is affected by nutritional status or genes.

Conclusion

In conclusion, this case–control study preliminary indicates that variations in FADS1, FADS2, and ELOVL2 affect the risk of obesity and the level of circulating fatty acids and could indicate a key molecular pathway of metabolic syndrome. Further biological investigation including measurement of FADS1, FADS2, and ELOVL2 levels and activity would be necessary to understand the influences of these genes on the development of this cluster of events.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by The Ministry of Higher Education, Scientific Research and Information and Communication Technologies, Tunisia.

ORCID iD

Sounira Mehri https://orcid.org/0000-0002-2221-7193

References

1. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic*. Geneva, Switzerland: World Health Organization; 2000.
2. Hruby A, Hu FB. The epidemiology of obesity: a big picture. *Pharmacoeconomics*. 2015;33(7):673-689.
3. Roberto CA, Swinburn B, Hawkes C, et al. Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking. *Lancet*. 2015;385(9985):2400-2409.
4. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19· 2 million participants. *Lancet*. 2016;387(10026):1377-1396.
5. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int j obes*. 2008;32(9): 1431.
6. Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015; 518(7538):197.
7. Popkin BM, Hawkes C. Sweetening of the global diet, particularly beverages: patterns, trends, and policy responses. *Lancet Diabetes Endocrinol*. 2016;4(2):174-186.
8. Hoffmann TJ, Theusch E, Haldar T, et al. A large electronic-health-record-based genome-wide study of serum lipids. *Nat Genet*. 2018;50(3):401.
9. Spracklen CN, Chen P, Kim YJ, et al. Association analyses of East Asian individuals and trans-ancestry analyses with European individuals reveal new loci associated with cholesterol and triglyceride levels. *Hum Mol Genet*. 2017;26(9):1770-1784.
10. Yeates AJ, Love TM, Engstrom K, et al. Genetic variation in FADS genes is associated with maternal long-chain PUFA status but not with cognitive development of infants in a high fish-eating observational study. *Prostaglandins Leukot Essent Fatty Acids*. 2015;102-103:13-20.
11. Fekete K, Gyorei E, Lohner S, Verduci E, Agostoni C, Decsi T. Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. *Obes Rev*. 2015;16(6): 488-497.
12. Wang L, Manson JE, Rautiainen S, et al. A prospective study of erythrocyte polyunsaturated fatty acid, weight gain, and risk of becoming overweight or obese in middle-aged and older women. *Eur J Nutr*. 2016;55(2):687-697.
13. Aslibekyan S, Jensen MK, Campos H, et al. Fatty acid desaturase gene variants, cardiovascular risk factors, and myocardial infarction in the Costa Rica study. *Front Genet*. 2012;3:72.
14. Wu JH, Lemaitre RN, King IB, et al. Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study. *Circulation*. 2014;130(15): 1245-1253.
15. Praagman J, de Jonge EA, Kiefte de Jong JC, et al. Dietary saturated fatty acids and coronary heart disease risk in a Dutch middle-aged and elderly population. *Arterioscler Thromb Vasc Biol*. 2016;36(9):2011-2018.
16. Guan W, Steffen BT, Lemaitre RN, et al. Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genetics*. 2014;7(3):321-331.
17. Dorajoo R, Sun Y, Han Y, et al. A genome-wide association study of n-3 and n-6 plasma fatty acids in a Singaporean Chinese population. *Genes Nutr*. 2015;10(6):53.
18. Mozaffarian D, Kabagambe EK, Johnson CO, et al. Genetic loci associated with circulating phospholipid trans fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *Am J Clin Nutr*. 2014; 101(2):398-406.
19. Marquardt A, Stohr H, White K, Weber BH. cDNA cloning, genomic structure, and chromosomal localization of three members of the human fatty acid desaturase family. *Genomics*. 2000; 66(2):175-183.
20. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lip Res*. 2006;45(3):237-249.
21. Lankinen M, Uusitupa M, Schwab U. Genes and dietary fatty acids in regulation of fatty acid composition of plasma and erythrocyte membranes. *Nutrients*. 2018;10(11):1785.
22. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J*. 2016;37(39):2999-3058.
23. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
24. Smaoui M, Hammami S, Chaaba R, et al. Lipids and lipoprotein(a) concentrations in Tunisian type 2 diabetic patients relationship to glycemic control and coronary heart disease. *J Diabetes Complications*. 2004;18(5):258-263.
25. Folch J, Lees M, Stanley GS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957;226(1):497-509.
26. Nakayama K, Bayasgalan T, Tazoe F, et al. A single nucleotide polymorphism in the FADS1/FADS2 gene is associated with plasma lipid profiles in two genetically similar Asian ethnic groups with distinctive differences in lifestyle. *Hum Genet*. 2010;127(6):685-690.
27. Liu F, Li Z, Lv X, Ma J. Dietary n-3 polyunsaturated fatty acid intakes modify the effect of genetic variation in fatty acid desaturase 1 on coronary artery disease. *PLoS One*. 2015;10(4): e0121255.
28. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11): 1274.
29. Klarin D, Damrauer SM, Cho K, et al. Genetics of blood lipids among ~ 300,000 multi-ethnic participants of the million veteran program. *Nat Genet*. 2018;50(11):1514-1523.
30. Flock MR, Kris Etherton PM. Diverse physiological effects of long-chain saturated fatty acids: implications for cardiovascular disease. *Curr Opin Clin Nutr Metab Care*. 2013;16(2): 133-140.
31. Cebrian SL, Costa AG, Carretero SN, Zabala M, Martínez JA, Moreno Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. *J Physiol Biochem*. 2013;69(3):633-651.

32. Muhlhausler BS, Ailhaud GP. Omega-6 polyunsaturated fatty acids and the early origins of obesity. *Curr Opin Endocrinol, Diabetes Obes*. 2013;20(1):56-61.
33. Abdelmagid SA, Clarke SE, Roke K, et al. Ethnicity, sex, FADS genetic variation, and hormonal contraceptive use influence delta-5-and delta-6-desaturase indices and plasma docosahexaenoic acid concentration in young Canadian adults: a cross-sectional study. *Nutrit Metab*. 2015;12(1):14.
34. Hu Y, Li H, Lu L, et al. Genome-wide meta-analyses identify novel loci associated with n-3 and n-6 polyunsaturated fatty acid levels in Chinese and European-ancestry populations. *Hum Mol Genet*. 2016;25(6):1215-1224.
35. Nagahara Y, Motoyama S, Sarai M, et al. The ratio of serum eicosapentaenoic acid to arachidonic acid as a predictor of high risk plaque characteristics on coronary computed tomography angiography. *J Am Coll Cardiol*. 2015;65(10 suppl):A1255.
36. Yang Q, Yin RX, Cao XL, Wu DF, Chen WX, Zhou YJ. Association of two polymorphisms in the FADS1/FADS2 gene cluster and the risk of coronary artery disease and ischemic stroke. *Int J Clin Exp Pathol*. 2015;8(6):7318.
37. Vessby B, Gustafsson IB, Tengblad S, Berglund L. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. *Br J Nutr*. 2013; 110(5):871-879.
38. Wang Y, Botolin D, Xu J, et al. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. *J Lipid Res*. 2006;47(9):2028-2041.
39. Figueiredo PS, Inada AC, Marcelino G, et al. Fatty acids consumption: the role metabolic aspects involved in obesity and its associated disorders. *Nutrients*. 2017;9(10):1158.
40. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J*. 2008; 29(24):2959-2971.
41. Raatz S, Conrad Z, Johnson L, Picklo MJ, Jahns L. Relationship of the reported intakes of fat and fatty acids to body weight in US adults. *Nutrients*. 2017;9(5):E438.
42. Zhuang P, Zhang Y, He W, et al. Dietary fats in relation to total and cause-specific mortality in a prospective cohort of 521 120 individuals with 16 years of follow-up. *Circ Res*. 2019;124(5): 757-768.