

Research Article

Associations of the rs12504538 and rs6824447 Polymorphisms of the Elovl6 Gene with Estimated Elongase and Desaturase Activity and Fatty Acid Concentrations in Mexican Women with Gestational Diabetes Mellitus

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Abstract

Background: This study aimed to investigate the possible associations of the rs12504538 and rs6824447 polymorphisms of the Elovl6 gene with estimated elongase and desaturase activity and saturated fatty acid concentrations in Mexican women with Gestational Diabetes Mellitus (GDM).

Methods: We recruited 172 women in the second and third trimesters of pregnancy who had undergone an oral glucose tolerance test, including 66 who had diagnosed with GDM according to the 2016 ADA criteria and 106 who had normal glucose tolerance test results, from the General Hospital and Health Centers of the Health Ministry of Guanajuato, México. Participants were matched by gestational week. Data on age, gestational week and anthropometric characteristics were collected. Blood samples were drawn after an overnight fast for the measurement of serum glucose, lipid, NEFA, serum fatty acid and insulin levels; SNP genotyping and quantification of fatty acids was performed and elongase and desaturase activity was estimated.

Findings: With the exception of HDL-cholesterol, all variables, including NEFA levels, were significantly greater in the GDM group than in the non-GDM group. The rs6824447 polymorphism of the Elovl6 gene, an age >25 years and HOMA-IR levels were associated with the development of GDM (OR=5.1, 95% CI 1.56-17.1, p=0.006; OR=4.89, 95% CI 1.65-14.4, p=0.003; OR=34.1 95% CI 8.7-133, p<0.000001, respectively). Palmitic acid (OR=1.08; 95% CI: 1.06-1.11; p<0.001) and oleic acid (OR=1.17; 95% CI: 1.14-1.2; p<0.001) concentrations were also associated with the development of GDM. No differences in the estimated elongase and desaturase activity among the non-GDM and GDM groups were found and only the activity of $\Delta 9D$ SCD18 desaturase was marginally high in GDM patients (p=0.050).

Conclusion: The rs6824447Elovl6 polymorphism is associated with the development of GDM, as are high serum palmitic acid, oleic acid and stearic acid concentrations and estimated $\Delta 9D$ SCD18 desaturase activity.

Keywords: Gestational Diabetes Mellitus; Rs6824447 Elovl6; Rs12504538 Elovl6; Fatty Acids; Elongase Activity; Desaturase Activity

Introduction

Gestational Diabetes Mellitus (GDM) is a pregnancy complication in which hyperglycemia spontaneously develops during pregnancy [1]. The rising obesity epidemic has driven an increase in the incidence of gestational diabetes mellitus, as well as in

pregnancy complications in terms of maternal and perinatal outcomes. These factors include known non-modifiable risk factors for a predisposition to GDM, such as advanced maternal age, the presence of overweight and obesity, ethnicity and a family history of Type 2 Diabetes Mellitus (T2DM) [2,3]. According to the most recent (2021) International Diabetes Federation (IDF) estimates, 21.1 million (16.7%) women who give birth to live-born infants have some form of hyperglycemia during pregnancy, 80.3% of whom have GDM [4].

The majority of women with GDM appear to have β -cell dysfunction with a background of chronic insulin resistance, to which the normal insulin resistance in pregnancy is partially additive [5]. The metabolism of the toxic intracellular metabolites Triacylglycerol (TAG) and Non-Esterified Fatty Acids (NEFAs) has been reported to cause severe insulin resistance [6-8]. Recently, a significant increase in NEFA and TAG levels in GDM patients compared with healthy pregnant women has been reported [9].

The enzymes required for the synthesis of Fatty Acids (FAs) are regulated by Sterol-Regulatory Element Binding Protein (SREBP-1) [10]. Overexpression of the SREBP-1a or SREBP-1c isoforms in the livers of mice results in increased rates of novo fatty acid synthesis and TAG accumulation [11,12]. Elov16 is an endoplasmic reticulum enzyme highly induced in the livers of SREBP transgenic mice [13] that is expressed in lipogenic tissues and regulated by SREBP-1 [14]. Elov16 catalyzes the chain elongation of C12 to C16 saturated and monounsaturated FAs to form C18, such as stearic (C18:0), oleic (C18:1n9) and vaccenic (C18:1n7) acids, the most important step for the de novo synthesis of endogenous long-chain FAs [15]. Elov16 gene disruption (E6KO) in mice decreases the proportion of stearic (C18:0) and oleic (C18:1n-9) acids and increases that of palmitic (C16:0) and palmitoleic (C16:1n-7) acids in the liver, confirming that Elov16 catalyzes the chain elongation of palmitic to stearic acids and the elongation of palmitoleic to vaccenic acids [15, 16]. In addition, E6KO mice are resistant to obesity-induced insulin resistance, despite being similar to wild-type mice in terms of hepatosteatosis and obesity, suggesting that Elov16 inhibition could be a potential therapeutic approach for T2DM treatment since Elov16 deficiency prevents T2DM progression by increasing the insulin secretory capacity of pancreatic β -cells in db/db mice and significantly improves hyperglycemia and elevates HbA1c levels [15-18]. It has been reported that the regulation of FA structure may participate in the modulatory effects of FAs on β -cells [19]. Subpopulations of MIN-6 and rat β -cells were shown to be resistant to palmitate-induced apoptosis and associated with increased expression of Stearoyl-CoA Desaturase (SCD1) and increased conversion of palmitate to Monounsaturated Fatty Acids (MUFAs) [20]. In addition, T2DM is associated with diminished islet expression of SCD1, which can disrupt the desaturation of saturated FAs to MUFAs, rendering β -cells more susceptible to saturated FA-induced ER stress and apoptosis [19].

Several studies have shown that genetic variations (SNPs) in the Elov16 gene are associated with the risk of T2DM and insulin resistance [21-23]. The rs6824447GG genotype was associated with lower insulin levels in a population from southern Spain [21]. Another study showed that the rs12504538 polymorphism is associated with an increase in insulin resistance and an increased risk of T2DM in the Han Chinese population [22]. In the Mexican population from Queretaro, the rs6824447 polymorphism was associated with lower total cholesterol and LDL cholesterol levels [23]. To date, there are few studies on polymorphisms of the Elov16 gene and their associations with insulin resistance and GDM. Therefore, the aim of this study was to investigate the possible associations of the rs12504538 and rs6824447 polymorphisms of the Elov16 gene with elongase and desaturase activity and fatty acid concentrations in Mexican women with gestational diabetes mellitus.

Methods

Patients

We conducted an observational transverse study of 172 women in the second and third trimesters of pregnancy, including 66 women who had been diagnosed with GDM according to the 2016 criteria of the American Diabetes Association (GDM group) and 106 women with a normal glucose tolerance test result, matched by gestational week, from the General Hospital (Approval # GTSSA002101) and health centers from Health Ministry number VII (Approval # JS7-04-230615) in Leon, Mexico. All participants provided informed consent. The study was approved by the Institutional Ethics Committee of the University of Guanajuato (Approval # CIBIUG-P-20-2015) and was performed following the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects.

The participants were recruited during the second and third trimesters of pregnancy after undergoing an Oral Glucose Tolerance Test (OGTT). According to the results of the OGTT, we formed two groups: the Non-Gestational Diabetes Mellitus (Non-GDM)

group and the Gestational Diabetes Mellitus (GDM) group. Data on age, obstetric history, gestational week, height, prepregnancy weight and actual weight were collected. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m²) (kg/m²). Blood samples were drawn after an overnight fast for the measurement of serum glucose, lipid, NEFA, fatty acid and insulin levels and DNA extraction. Serum glucose and lipid levels were measured using enzymatic methods with a semiautomatic chemical analyzer (SPINLAB SPINREACT). Serum insulin was measured by an immunoenzymatic assay (GeneWay Biotech Inc., San Diego, CA, USA) and NEFAs were measured by an enzymatic colorimetric method (Wako Life Sciences Inc., Mountain View, CA, USA). The intra-assay variation coefficients were 3.4 to 6.1% for insulin and 1.6% to 2.2% for NEFAs. The quantification of fatty acids was performed by gas chromatography.

Genotyping of Elovl6 Polymorphisms

DNA was extracted from peripheral blood leucocytes according to the TSNT protocol, quantified using a NanoDrop system (Roche) and stored at -20°C. Single nucleotide polymorphisms (SNPs) were detected by a combination of Polymerase Chain Reaction (PCR) and restriction fragment length polymorphism (RFLP) assays using appropriate restriction enzymes as described previously [22]. Polymerase Chain Reaction (PCR) was carried out in a total volume of 30 µL in a Thermal Cycler GeneAmp PCR System 2700 (Applied Biosystems). The PCR mixture contained 50 ng of DNA, 0.5 mM of dNTPs (Invitrogen), 10 pM of primers, 2.0 mM of MgCl₂, 0.6 µL of DMSO and 5 U of Taq polymerase (Invitrogen) for both polymorphisms. Amplification of the rs12504538 polymorphism was carried out using the following protocol: 95°C for 5 min; 20 cycles of 95°C for 30 s; 68°C for 45 s; 72°C for 45 s; and 72°C for 1 min. The annealing temperature decreased by 0.5 °C/cycle. This was followed by 20 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 40 s, with a final single extension step of 72°C for 6 min. rs6824447 polymorphism amplification was carried out using the following protocol: 95°C for 5 min; 35 cycles of 95°C for 30 s, 56°C for 45 s and 72°C for 1 min; and 72°C for 1 min. The PCR product for the rs12504538 polymorphism was a fragment of 295 bp that, after digestion with the restriction enzyme Pag I, an isoschizomer of Rca I (Thermo Scientific, Waltham, MA, USA), yielded 186 and 109 bp products for the C/C genotype and 295, 186 and 109 bp products for the C/T genotype, while the enzyme did not cut the normal T/T genotype sequence. For the rs6824447 polymorphism, PCR generated a product of 133-pb, which, after digestion with Ban II (New England BioLabs, Ipswich, MA, USA), yielded 85- and 48-bp fragments for the G/G genotype and 133, 85 and 48-pb fragments for the A/G genotype, while the PCR products with the normal A/A genotype were not digested. Both fragments were separated by electrophoresis on a 3% ethidium bromide-stained agarose gel [22].

Measurement of Serum Fatty Acids

Serum samples (500 µl) were dried under a gentle stream of nitrogen at room temperature and the residues were dissolved in 1 ml of NaOH and 0.5 M methanol. An internal standard consisting of 10 µl of nonadecanoic acid (C19:0, 5 mg/ml) was added. The temperature of the solution was held at 90°C for 1 h; each sample was then cooled to room temperature and 1 ml of boron trifluoride etherate in methanol (Sigma-Aldrich) was slowly added. The samples were reincubated at 90°C for 30 minutes. The solutions were cooled and transferred to a test tube, where 2 ml of deionized water and 4 ml of hexane were added, after which the organic phase was separated. Each solution was dried again under a stream of nitrogen at room temperature and dissolved in 400 µl of isooctane (Fisher Chemical). After that, an aliquot was injected into the chromatograph. Fatty acids were chromatographed on a 30 m fused-silica Zebron ZB-WAX column (0.25 mm i.d.). Analysis was performed with an Agilent Technologies 7890A gas chromatograph equipped with an Agilent Technologies 5975C flame ionization detector. The column temperature was held at 50°C for 3 min, subsequently increased in a stepwise fashion (10°C/min) to a plateau of 250°C and then held for 3 min. The injection temperature was 220°C. Helium was used as the carrier gas at 2 ml/min. The quantification was performed for 67 women without GDM and 64 women with GDM.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normality of the data. The anthropometric and metabolic data are expressed as the mean ± SD or median (25-75 quartiles). Differences between groups were examined using the independent t test, Mann-Whitney U test or one-way ANOVA and the Kruskal-Wallis test. Both groups were tested for Hardy-Weinberg equilibrium for the rs12504538 and rs6824447 Elovl6 polymorphisms. We compared allelic frequency between groups using Yates-corrected χ^2 tests. The association between the polymorphisms and the development of GDM was assessed with logistic regression analysis adjusted for age and IMC using the dominant model. Analyses were performed using a statistical package (Statistica, StatSoft Inc., Tulsa, OK). The level of statistical significance was defined as $P < 0.05$.

Results

We included a total of 172 pregnant women, including 66 with GDM and 106 with a normal glucose tolerance test result (non-GDM), with a median gestational week of 27 (25-30) weeks. The clinical and metabolic characteristics of both groups are shown in Table 1. The groups were similar in terms of gestational week, weight gain, total cholesterol levels, triglyceride levels and LDL cholesterol levels. With the exception of HDL cholesterol levels, all variables, including NEFA levels, were significantly greater in the GDM group. Table 2 summarizes the distributions of the rs6824447 and rs12504538 Elov6 genotypes and alleles between GDM patients and controls. The genotypic frequencies and allelic frequencies of rs12504538 Elov6 were similar between the groups ($\chi^2 = 0.35$, $p = 0.84$; $\chi^2 = 0.94$; $p = 0.75$, respectively). However, the allelic frequencies of the rs6824447 Elov6 polymorphism were significantly different between the groups ($\chi^2 = 4.87$, $p = 0.02$). The GG genotype frequency was significantly greater in the GDM group ($\chi^2 = 6.16$, $p = 0.04$) than in the non-GDM group. The rs12504538 SNP was in accordance with the Hardy-Weinberg equilibrium and the rs6824447 SNP showed slight deviation in the GDM group. In the comparison of the rs6824447 Elov6 genotype, we detected significantly lower levels of HDL cholesterol but significantly higher levels of triglycerides, palmitic acid (C18:0), stearic acid (C18:0), oleic acid (C18:1w9) and eicosenoic acid (C20:1w9) in GDM patients with one or two allelic variants (AG or GG) (Table 3). No differences in the comparison of other variables for genotypes were found. In the comparison of metabolic variables for the rs12504538 Elov6 genotype, there were no significant differences.

Estimation of Elongase and Desaturase Activity and Comparison Between Groups

In women with GDM, the palmitic acid, stearic acid, oleic acid and eicosenoic acid concentrations were significantly greater than those in women without GDM (Table 4). The estimated elongase activities of Elov13 (C20:1/C18:2), Elov6 (C18/C16) and Elov15 (C20:2/C18:2) and the estimated desaturase activities of D5D (C20:4/C20:3), Δ 6D (C18:3/C18:2), Δ 9D SCD16 (C16:1/C16:0) and Δ 9D SCD18 (C18:1/C18:0) were compared between the GDM and non-GDM groups. No differences between the non-GDM and GDM groups were found and only the activity of desaturase Δ 9D SCD18 was marginally elevated in the GDM group (Table 4).

Logistic Regression Analysis of Associations

According to the univariate logistic regression analysis, age, prepregnancy BMI ≥ 30 kg/m², NEFA levels, serum insulin level, HOMA-IR value, rs6824447 Elov6 polymorphism, palmitic acid, oleic acid and eicosenoic acid levels and the activity of desaturase Δ 9D SCD18 were significantly associated with gestational diabetes mellitus. Notably, the serum HDL cholesterol concentration had a strong protective effect (Table 5). Multiple logistic regression analysis adjusted for age, including previous variables, showed that age, HOMA-IR levels and the rs6824447 polymorphism remained significantly associated with GDM (Table 5).

	Non-GDM	GDM	t/z	p
	N = 106	N = 66		
Age (years old)	23 (20-29)	30 (25-34)	-5	0.000001
Gestational age (weeks)	27 (25-30)	28 (25-31)	-1.1	0.3
Height (cm)	155 (152-160)	155 (153-159)	-0.4	0.7
Pregestational BMI (kg/m ²)	26.5 $\pm$ 5.5	30.0 $\pm$ 6.0	-3.9	0.0001
BMI at collection (kg/m ²)	28.8 $\pm$ 5.3	32.5 $\pm$ 5.8	-4.2	0.00004
Weight gain (kg)	5 (3.5-9)	5 (2-9)	0.3	0.7
Glucose (mmol/L)	4.4 $\pm$ 0.4	5.3 $\pm$ 0.9	-7.9	0.000001
Triglycerides (mmol/L)	2.30 (1.83 - 2.8)	2.61 (2.03 - 3.41)	-2.2	0.02
Cholesterol (mmol/L)	5.56 (4.8 - 6.49)	5.74 (4.62 - 6.46)	0.1	0.9
HDL cholesterol (mmol/L)	1.83 (1.68 - 1.91)	1.57 (1.37 - 1.81)	5.8	0.000001
LDL cholesterol (mmol/L)	2.76 $\pm$ 1.08	2.84 $\pm$ 1.03	-0.4	0.7
NEFAs (mmol/L)	0.40 (0.34-0.45)	0.43 (0.35-0.52)	-2.2	0.03
Insulin (μ UI/mL)	19 (16-22)	23 (18-34)	-3.3	0.0009
HOMA-IR	3.6 (3-4)	5.3 (4-8)	5.7	0.000001
The values are expressed as mean $\pm$ SD or median and interquartile range; t = t Student; z = Mann and Whitney. GDM = Gestational diabetes mellitus; NGDM = Non-Gestational diabetes mellitus; BMI = Body mass index; LDL-cholesterol = Lipoprotein Density Low; HDL cholesterol = High Density Lipoprotein NEFAs = non-esterified fatty acid; HOMA-IR = Homeostasis model assessment.				

Table 1: Clinical and metabolic characteristics of the two groups of pregnant women.

SNP	n	Genotype frequency [n (%)]			Allele frequency [n (%)]	
rs12504538 (T/C)		TT	TC	CC	T	C
Non-GDM	106	57 (0.54)	46 (0.43)	3 (0.03)	-0.75	48 (0.25)
GDM	66	35 (0.53)	28 (0.42)	3 (0.05)	-0.74	31 (0.26)
		$\chi^2 = 0.35$ p = 0.84			$\chi^2 = 0.94$	p = 0.75
rs6824447 (A/G)		AA	AG	GG	A	G
Non-GDM	106	35 (0.33)	57 (0.54)	14 (0.13)	92 (0.60)	71 (0.40)
GDM	66	11 (0.17)	41 (0.62)	14 (0.21)	52 (0.48)	55 (0.52)
		$\chi^2 = 6.16$ p = 0.04			$\chi^2 = 4.87$ p = 0.02	
GDM = Gestational Diabetes Mellitus.						

Table 2: Genotype and allelic frequencies of rs12504538 and rs6824447.

Variable	NGDM AA N = 35	GDM AA N = 11	p	NGDM AG + GG N = 71	GDM AG + GG N = 55	*p	**p
Age (years)	25 (20 - 29)	30 (24 - 33)	0.07	23 (20 - 29)	31(25-34)	0.0006	0.00001
Pregestational Weight	64.4 ± 13.8	78 ± 17.1	0.01	64.5±13.9	72.3±15.5	0.003	0.001
Pregestational BMI (kg/m ²)**	25.1 ± 5.3	30.4 ± 6.7	0.01	25.1±5.6	28.5±5.9	0.004	0.002
Weight at collection	71.2 ± 14.8	84.4 ± 14.6	0.01	69.7±13.2	78.4±15.2	0.0009	0.0005
BMI (kg/m ²) at collection	27.8 ± 5.7	32.9 ± 5.1	0.01	27.2 ±5.1	30.6 ±5.7	0.006	0.0005
Weight gain (kg)	6 (4 - 9)	4 (1 - 12)	NS	5 (4 - 9)	6 (2 - 9)	NS	NS
Glucose (mmol/L)	4.38 (4.14 - 4.77)	5.38 (5.27 - 6.16)	0.00004	4.38 (4.0 - 4.6)	5.21 (4.6 - 5.99)	<0.00001	0.00001
Triglycerides (mmol/L)	2.41 (1.93 - 2.98)	2.15 (2.01 - 2.93)	NS	2.21 (1.81 - 2.73)	2.77 (2.14 - 3.5)	0.007	0.06
Total Cholesterol (mmol/L)	5.35 (5.0 - 4.37)	6.38 (4.37 - 6.82)	NS	5.55 (4.86 - 6.49)	5.58 (4.62 - 6.36)	NS	NS
HDL cholesterol (mmol/L)	1.83 (1.65 - 1.91)	1.65 (1.52 - 1.91)	NS	1.83 (1.73 - 1.91)	1.49 (1.31 - 1.78)	<0.00001	0.00001
LDL-cholesterol (mmol/L)	2.84 ± 1.21	2.76 ± 1.03	NS	2.74 ± 1.03	2.81 ± 1.0	NS	NS
NEFAS (mmol/L)	0.39 (0.36 - 0.47)	0.44 (0.38 - 0.49)	NS	0.40 (0.31 - 0.44)	0.43 (0.34 - 0.53)	0.04	NS
Insulin (μUI/mL)	19 (16 - 22)	24 (20 - 38)	0.05	19 (16 - 23)	23 (18 - 34)	0.007	0.01
HOMA-IR	3.7 (3.1 - 4.5)	5.5 (4.6 - 10.3)	0.006	3.5 (2.9 - 4.5)	5.3 (4.1 - 7.3)	0.000001	0.00001
*t student Test; *MWT = Man and Whitney Test; **ANOVA one way; KWT = Kruskal Wallis Test: The values are expressed as mean ± SD or median and interquartile range. GDM = Gestational diabetes mellitus; NGDM = Non-Gestational diabetes mellitus; BMI = Body mass index; LDL-cholesterol = Lipoprotein Density Low-cholesterol; HDL-cholesterol = High Density Lipoprotein-cholesterol; NEFAS = non-esterified fatty acid; HOMA-IR = Homeostasis model assessment.							

Table 3: Analysis by genotypes of polymorphism rs6824447 (dominant model) among groups.

	Non-GDM (n = 67)	GDM (n = 64)		
Variable	Mean $\pm$ SD	Mean	t student	p
Myristic acid (C14:0) $\mu\text{g/mL}$	15.1 $\pm$ 8.23	16.3 $\pm$ 9.3	0.768	NS
Palmitic acid (C16:0) $\mu\text{g/mL}$	857.4 $\pm$ 225	990 $\pm$ 321	2.75	0.006
Palmitoleic acid (C16:1) $\mu\text{g/mL}$	48.8 $\pm$ 21.3	54 $\pm$ 25	1.26	NS
Stearic acid (C18:0) $\mu\text{g/mL}$	122.3 $\pm$ 21.3	135.3 $\pm$ 32.6	2.70	0.007
Oleic acid (C18:1 w9) $\mu\text{g/mL}$	746.3 $\pm$ 194	888 $\pm$ 297	3.245	0.001
Elaidic acid (C18:1) $\mu\text{g/mL}$	58.6 $\pm$ 11.7	63.6 $\pm$ 18.2	1.85	0.06
Linoleic acid (C18:2 w6) $\mu\text{g/mL}$	1064.2 $\pm$ 271.3	1134 $\pm$ 295	1.40	NS
Alpha-Linolenic acid (C18:3 w3) $\mu\text{g/mL}$	21.7 $\pm$ 7.9	23.5 $\pm$ 8.8	1.23	NS
Eicosenoic acid (C20:1 w9) $\mu\text{g/mL}$	7.4 $\pm$ 4.2	8.7 $\pm$ 2.8	2.10	0.036
$\Delta 9\text{D SCD } 16$	0.057 $\pm$ 0.014	0.053 $\pm$ 0.012	1.087	NS
$\Delta 9\text{D SCD } 18$	6.1 $\pm$ 1.2	6.5 $\pm$ 1.4	-1.937	0.05
$\Delta 5\text{D}$	2.94 $\pm$ 1.0	3.04 $\pm$ 1.0	0.47	NS
$\Delta 6\text{D}$	0.02 $\pm$ 0.05	0.02 $\pm$ 0.05	0.256	NS
Elovl 3	0.007 $\pm$ 0.003	0.008 $\pm$ 0.001	1.22	NS
Elovl 5	0.007 $\pm$ 0.003	0.006 $\pm$ 0.0013	-1.33	NS
Elovl 6	0.15 $\pm$ 0.02	0.14 $\pm$ 0.02	-1.20	NS
Elovl = Elongase; $\Delta 5\text{D}$ = Delta 5Desaturase; $\Delta 6\text{D}$ = Delta 6Desaturase; SCD = stearyl-CoA desaturase GDM = Gestational diabetes mellitus; NGDM = Non-Gestational diabetes mellitus.				

Table 4: Comparison of fatty acids, elongases and desaturases activity among groups.

Univariate Model			
Variable	Odd Ratio	IC 95%	P
Age > 25 (years old)	3.75	1.92 - 7.34	0.00009
Pregestational BMI ≥ 30 (kg/m ²)	1.93	1.0 - 3.7	0.045
NEFAs (mMol/L)	23.9	1.72 - 333)	0.017
HDL-cholesterol (mg/dl)	0.86	0.81 - 0.90	0.000001
Insulin ($\mu\text{UI/mL}$)	1.06	1.02 - 1.10	0.0005
HOMA-IR	1.56	1.3 - 1.87	0.000001
rs6824447 (dominant model)	2.46	1.14 - 5.3	0.02
Palmitic acid (C16:0) $\mu\text{g/mL}$	1.08	1.06 - 1.11	<0.001
Oleic acid (C18:1w9) $\mu\text{g/mL}$	1.17	1.14 - 1.2	<0.001
Eicosenic acid (C20:1w6) $\mu\text{g/mL}$	4.56	2.15 - 9.63)	0.007
$\Delta 9\text{D SCD } 18$ activity	1.25	1.19 - 1.32	<0.001
Multivariate model (Adjusted for age)			
Age >25 years	4.89	1.65-14.4	0.0038
HOMA-IR	34.1	8.73-133.3	0.0000003
rs6824447	5.18	1.56 - 17.1	0.0067
HDL-cholesterol = High Density Lipoprotein-cholesterol; NEFAS = non-esterified fatty acid; HOMA-IR = Homeostasis model assessment; $\Delta 9\text{D SCD } 18$ = Stearyl-CoA desaturase (SCD)			

Table 5: Variables associated with the risk of development of gestational diabetes mellitus (Logistic regression analysis).

Discussion

In this study, we analyzed the metabolic phenotypes of women with GDM and healthy pregnant women and the associations of the rs12504538 and rs6824447 Elovl6 polymorphisms with GDM and insulin resistance. Additionally, the serum fatty acid concentration and estimated elongase and desaturase activities were evaluated in both groups. We found that the prepregnancy

BMI and triglyceride, NEFA, insulin and insulin resistance levels were significantly greater and that the HDL cholesterol concentration was lower in women with GDM. Similar results have been reported in other studies [9,24]. Compared with normoglycemic controls, women with GDM were at increased risk of developing T2DM, had abnormalities in insulin secretion and lipid metabolism and had elevated NEFA levels [25,26].

The rs12504538 Elov6 polymorphism has been associated with an increased risk of T2DM because of increased insulin resistance, but no association between the rs6824447 polymorphism and the development of T2DM has been reported in the Han Chinese population [22]. Instead, we found a significant association between the rs6824447 Elov6 polymorphism and the development of GDM but no association between the rs12504538 polymorphism and the development of T2DM. In nondiabetic subjects in the Spanish population, the rs6824447 polymorphism is associated with a low risk of having high HOMA-IR values [21]. In young subjects from Mexican populations with a normal BMI, rs6824447 has been shown to have a protective effect against high cholesterol and high LDL cholesterol levels. Interestingly, our results revealed that triglyceride levels were greater and that HDL cholesterol levels were lower in GDM patients who were carriers of the rs6824447AG and s6824447GG genotypes. There are very few reports on the association of Elov6 polymorphisms with T2DM and there are few reports on the association of Elov6 polymorphisms with GDM. To the best of our knowledge, this is the first report of the contribution of the rs6824447 polymorphism to the development of GDM. In addition, multiple logistic regression showed that age, insulin levels and the rs6824447 polymorphism are factors associated with the risk of GDM.

In our work, no differences in estimated Elov13, Elov15, or Elov6 activity were found between women without GDM and women with GDM. There are few studies concerning estimated Elov6 activity; in children aged 9-12 years, Elov6 activity is a significant predictor of Insulin Resistance (IR) [27]. High Elov6 activity was associated with high IR in Japanese subjects, low activity might be ideal for protection against atherosclerosis and in Nonalcoholic Steatohepatitis Hepatic (NASH) was proposed [28].

A previous study reported that the palmitic acid, myristic acid and oleic acid concentrations were significantly increased in GDM patients [29]. In our study, we found that serum palmitic acid, oleic acid and eicosenoic acid concentrations were significantly associated with the risk of GDM. Several reports have reported similar results [30-32]. An inverse association between C18:2n-6 and GDM has also been reported [32]. Another study showed that the activity of the Δ 5D and Δ 6D desaturases changed during pregnancy, the estimated Δ 6D desaturase activity was elevated and the Δ 5D desaturase activity was attenuated in the first trimester; however, changes were not detected during the second trimester. However, in the third trimester, Δ 5D and Δ 9D SCD-18 desaturase activities were elevated and Δ 6D desaturase activity was attenuated [33]. In our study, a similar tendency in Δ 9D SCD-18 activity (significantly marginal) was found in women with GDM in the third trimester of pregnancy. It has also been reported that increased activation of Δ 9D SCD-18, which converts stearic acid (C18:0) to oleic acid (C18:1), is associated with the development of GDM in the third trimester [33]. Controversial results have been reported, while Δ 5D desaturase activity was significantly lower and Δ 9D SCD-18 desaturase activity was significantly greater during pregnancy [34]. In another study, Δ 6D desaturase and Δ 9D SCD-18 desaturase activities were significantly lower, but there were no significant changes in Δ 5D desaturase activity in women with GDM at 32-35 weeks of gestation [35]. In our study, no differences in Δ 5D or Δ 6D desaturase activity were found; these results may be due to differences in sample size. Higher serum total n-6 Polyunsaturated Fatty Acid (PUFA), linoleic acid and arachidonic acid concentrations and estimated Δ 5D activity were associated with a lower risk of incident T2DM and participants with higher γ -linolenic acid and dihomo γ -linolenic acid concentrations and estimated Δ 6D activity had a greater risk of T2D [36]. In our study, we did not find significant differences in estimated elongase activity between the non-GDM and GDM groups. However, it has been reported that estimated Elov6 and Δ 5D activities might be useful markers of insulin resistance in Japanese subjects [28].

The elongation of C16 to C18 is mediated by Elov6 activity and is an important metabolic control point for the conversion of palmitic acid to stearic acid and for the action of Δ 9D SCD-18, it is converted to oleic acid. Both fatty acids influence the decrease in insulin secretion and β -cell function and eventually lead to diabetes development. Stearic acid (C18:0) also increased inflammation and the terminal UPR [18]. In our study, although the estimated Elov6 activity did not increase significantly in the GDM group, the conversion of palmitic acid to stearic acid suggested major Elov6 activity and the conversion of stearic acid to oleic acid also suggested major activity of Δ 9D SCD-18 in the second and third trimesters of pregnancy.

Conclusion

Our results are interesting but require additional confirmation given the sample size, particularly in the GDM group. In our work, Age, insulin levels and the rs6824447 Elov16 polymorphism are significantly associated with the development of GDM. Triglyceride levels were greater and HDL cholesterol levels were lower in GDM patients who were carriers of the rs6824447AG and rs6824447GG genotypes. Palmitic acid, oleic acid, eicosenoic acid and $\Delta 9$ D SCD-18 desaturase were significantly associated with the risk of GDM. Other estimated $\Delta 5$ D and $\Delta 6$ D desaturase and elongase activities were similar between the groups.

Conflict of Interest

The authors have no competing interest to declare.

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Statements and Declarations

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Authors Contribution

Manuel A. Muñoz-Nava, conceptualization, investigation and methods; Martha E. Fajardo-Araujo conceptualization, investigation, methods and project administration; Elva Perez-Luque, Data curation, formal analysis, writing original draft; Mónica I. Cardona Alvarado, Data curation, formal analysis; Enrique Ramírez-Chávez, methods; Jorge Molina-Torres, methods.

Data Availability

Not applicable

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