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Original research article

ADIPOQ-rs2241766 polymorphism is associated with changes in cholesterol levels of Mexican adolescents

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Abstract
Background: The *ADIPOQ* gene encodes a fat-derived protein hormone with a preponderant role in the homeostasis of glucose and fatty acids. However, previous association studies between *ADIPOQ* genetic variants and metabolic disorders have shown controversial results. In this study, we evaluated the effect of the *ADIPOQ*-rs2241766 polymorphism on diverse biochemical parameters (*i.e.*, insulin resistance, atherogenic index, overweight and obesity) in an adolescent population from Mexico.
Methods: A cross-sectional study with convenience sampling was carried out in 356 adolescents from Northern Mexico. They were classified by sex and BMI-z score. The biochemical parameters were measured from blood samples using conventional methods. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
Results: In low and normal weight groups, GG carriers had a significantly higher cholesterol level ($P \leq 0.05$) than TG and TT carriers. However, there was no association between *ADIPOQ*-rs2241766 polymorphism and atherogenic index, overweight, or obesity.
Conclusions: Our findings suggest that the cholesterol levels are under the influence of the *ADIPOQ*-rs2241766 polymorphism in Mexican adolescents and may explain how *ADIPOQ* variants increase the risk of developing metabolic disorders. Nevertheless, further studies are required to rule out the influence of other genetic and non-genetic factors.

Keywords: *ADIPOQ* gene; Biochemical parameters; Mexican population; rs2241766 gene polymorphism

Highlights:
- The evaluated biochemical parameters were significantly different according to the nutritional status.
- Cholesterol, HDL-C, and LDL-C levels were not different between males and females when stratified by nutritional status.
- Cholesterol levels are influenced by the *ADIPOQ*-rs2241766 polymorphism in Mexican adolescents.
- No association was found between *ADIPOQ*-rs2241766 polymorphism and nutritional status.

Introduction

Cardiovascular diseases (CVDs) are one of the most common health problems in the world and the leading cause of death worldwide (Roth et al., 2017). Several risk factors have been associated with CVDs, including dyslipidemia, smoking, hypertension, diabetes, and abdominal obesity (Stewart et al., 2017). Abnormal lipid metabolism is commonly observed in obese Mexican patients (Arjona-Villicaña et al., 2014; Costa-Urrutia et al., 2021). The latter has become a pandemic, since overweight and obesity affects >30% of the infant and adolescent populations (Hernández-Cordero et al., 2017). Overweight/obese persons also have high levels of free fatty acids, a condition that stimulates insulin resistance and promotes the development of Type 2 diabetes (T2D) and CVDs (Achari and Jain, 2017; Kasim et al., 2016).

The *ADIPOQ* gene is mainly expressed in adipose tissue and encodes adiponectin, a protein hormone involved in the homeostasis of glucose and fatty acids (Tureck et al., 2015). Adiponectin has insulin-sensitizing, anti-atherogenic, and anti-inflammatory effects and, in some cases, it can reduce body weight (Achari and Jain, 2017). Its effects are mediated through the activation of the AdipoR1 and AdipoR2 receptors, which promote the enhanced phosphorylation of AMPK (AMP activated protein kinase) and the suppression of energy-con-

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suming processes, such as lipogenesis and gluconeogenesis (Achari and Jain, 2017). Adiponectin also regulates the activity of PPARα (Peroxisome proliferator-activated receptor α ligand), which increases the metabolism of fatty acids (Wang and Scherer, 2016). Unlike other adipocytokines, adiponectin is a mitigating factor of cardio-metabolic alterations due to its reduced concentration in obese subjects. These characteristics have made adiponectin a biomarker for therapeutic intervention in cases of obesity, diabetes, and cardiovascular disorders (Kishida et al., 2014; Oh and Kang, 2014; Riestra et al., 2015).

Previous studies have reported that the expression pattern of adiponectin can be severely affected by single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene (Enns et al., 2011), such as –11391G>A (rs17300539), –11377C>G (rs266729), +45T>G (rs2241766), and +276G>T(rs1501299), all of which have been associated with high Body Mass Index (BMI), high blood glucose levels, increased T2D risk, obesity, and metabolic syndrome (Enns et al., 2011; Sanchez et al., 2019; Wu et al., 2014).

A study in a Mexican population suggested that the rs2241766 gene variant could have a protective role against ductal infiltrating breast cancer (Macías-Gómez et al., 2019). This polymorphism also has a direct effect on body fat distribution and obesity-related biochemical parameters, such as waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), and glucose levels (Macías-Gómez et al., 2019). It has also been implied that a rs2241766 TG genotype could have a direct impact on cholesterol levels (Macías-Gómez et al., 2019). On the other hand, the rs2241766 G allele (GG/GT genotype) has a protective effect against dyslipidemia, primarily in HIV/HCV-coinfected patients with steatosis (Pineda-Tenor et al., 2014).

The present study evaluated the effect of the *ADIPOQ*-rs2241766 polymorphism on diverse biochemical parameters (*i.e.*, BMI, insulin resistance, and atherogenic index) and its association with overweight/obesity in a population of Mexican adolescents.

Material and methods

Participants

A cross-sectional, observational, and descriptive study was conducted between December 2013 and August 2017. A group of 356 adolescents from Saltillo, Coahuila (Northern México), were invited to participate in the study. A total of 204 women and 152 men, between 11 and 19 years of age were included. Subjects with a previous diagnosis of Diabetes Mellitus type 1 or thyroid disease were excluded. The present study complied with the ethical guidelines of the Ethics and Research Committee of the University Hospital in Saltillo and was registered under record number INMED 01-1113. The participants, or their legal guardians, provided their informed consent prior to the start of the study.

Anthropometric parameters

Height was measured to the nearest 0.1 cm using a Seca stadiometer m0123 (Hamburg, Germany); weight was determined to the nearest 0.01 kg with a Tanita body composition analyzer mTBF-410 (Arlington Heights, IL). Nutritional status was determined according to BMI and z score (BMI-z), based on age and sex, and using the BMI percentile tables published by the WHO (2020). This nutritional status was classified as follows: Obese, >95th percentile; overweight, 85–94.9th percentile; normal weight, 5–84.9th percentile; and low weight, <5th percentile.

Biochemical profile

Peripheral blood samples (5 ml) were taken from the participants after a 12-hour fasting period. The following parameters were evaluated: glucose, total cholesterol, very low-density lipoprotein (VLDL-C), low density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), triglycerides, and insulin. A SLFIA immunoassay was used in this regard, performed in a InCCA model Diconex Clinical Chemistry Analyzer (Intelligent Clinical Chemistry Analyzer) (Diconex, USA), and a TOSOH AIA-600 analyzer (Tokyo, Japan) for insulin (Carranza-González et al., 2018). Glucose and insulin values were included in the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (Matthews et al., 1985). The atherogenic index (AI, or Castelli index I) was calculated by dividing the obtained cholesterol and HDL-C values (Millán et al., 2009).

DNA extraction and genotyping

A phenol-chloroform extraction (Barker et al., 1998) was used to isolate the genomic DNA from the peripheral blood samples, followed by precipitation in absolute ethanol. The DNA pellet was resuspended in Tris-EDTA (pH 7.8) at a final concentration of 0.1–1.0 μg/μl and stored at –20 °C until used for genetic analysis. The rs2241766 polymorphism was genotyped through PCR-RFLP using specific oligonucleotide primers and a MJ Mini PTC1148 thermocycler (Bio-Rad, Hercules, CA, USA) (Mackevics et al., 2006). The PCR included 200 ng of genomic DNA and 0.5 μm of each of primer (forward 5´-TGT GTG TGT GGG GTC TGT CT-3´and reverse 5´-TGT GAT GAA AGA GGC CAG AA-3´; IDT, Coralville, IA, USA), 0.2 mmol dNTPs, 1.5 mmol $MgCl_2$, and 1 unit of Green Taq DNA Polymerase (GenScript, Nueva Jersey, USA). The amplification program consisted of 35 cycles at 95 °C/45 s, 58 °C/45 s, and 72 °C/45 s. The amplicons, of 305 bp in length, were digested with the AvaI restriction enzyme (New England Biolabs, Ipswich, MA, USA), loaded in a 2% agarose gel prior to electrophoresis, stained with ethidium bromide, and visualized in a UVP m2UV High Performance Transilluminator (Upland, CA, USA). The expected results were two fragments (*i.e.*, 204 bp and 101 bp) for the mutant G allele and a single 305 bp fragment (*i.e.*, non-digested) for the wildtype T allele.

Statistical analysis

The statistical analysis was performed with the software SPSS v21.0 (IBM Corp., Armonk, NY, US). Assuming a variance of 986 for total cholesterol values (mg/dl), 95% confidence level, and α = 10%, we needed a minimum of 155 subjects to reach a statistical power of 80% ($P \leq 0.05$). Genotype distribution was assessed using Hardy–Weinberg equilibrium (HWE) with a goodness-of-fit test. The effect of the polymorphism on the biochemical parameters was determined with a Student's *t* or Mann–Whitney *U* test for comparisons between 2 groups. ANOVA or Kruskal–Wallis *H* tests were applied for comparisons between 3 groups, according to parametric or nonparametric distribution. Tukey, Bonferroni, Tamhane and Dunnet T3 *post-hoc* tests were used for pair-wise comparisons. To confirm the influence of the genetic variant, a linear regression analyses was performed. Possible associations between genotypes with the nutritional status were evaluated using χ^2 and Fisher's exact tests and validated through logistic regression analysis. All the included analyses used conventional genetic models (dominant and recessive) and were adjusted according to sex and nutritional status. The odds ratio (OR) was esti-

mated with a 95% CI. All P values were two-tailed. Corrected *P* values (Pc) were obtained using Bonferroni correction for exclusion of spurious associations. $P < 0.05$ was interpreted as statistically significant.

Results

Characteristics of the study subjects

The participants were non-related Mexican nationals, self-identified as Mestizo by descent and unspecified ethnic group. The participants were 11 to 19 years old at the time of the study ($\bar{x}$ = 14.28), 57.31% were female and 42.69% were male. They were classified according to BMI-z score: low weight (14.9%, *n* = 53), normal weight (24.4%, *n* = 87), overweight (24.4%, *n* = 87), and obese (36.3%, *n* = 129).

A statistical difference was observed between the low-, normal-, overweight and obese groups regarding the biochemical parameters evaluated, *i.e.*, glucose, insulin, triglycerides, and HOMA-IR ($P < 0.05$) – Table 1. This difference was also evident when considering BMI and biochemical parameters according to sex ($P < 0.05$) – Table 1. The overweight and obese subjects also showed a higher Atherogenic index and greater predisposition to insulin resistance according to the obtained HOMA-IR values and Plasma Atherogenic Index. There was no apparent difference between sex and nutritional status, mainly low- and normal weight, when evaluating the parameters of total cholesterol, HDL-C, LDL-C, and atherogenic index (Table 1).

Table 1. Biochemical parameters according to sex and nutritional status

Clinical/biochemical parameters	Nutritional status			
All subjects	Low weight (53)	Normal weight (87)	Overweight (87)	Obese (129)
BMI	17.63 ± 1.50 [a, b, c]	20.61 ± 2.13 [d, e]	24.74 ± 1.87 [f]	30.39 ± 3.58
Glucose (mg/dl)	84.72 ± 9.33 [c]	87.59 ± 9.54 [e]	85.77 ± 10.20 [f]	91.33 ± 12.15
Insulin (mU/ml)	7.94 ± 3.86 [b, c]	8.85 ± 4.73 [d, e]	14.44 ± 8.01 [f]	23.45 ± 13.14
HOMA-IR	1.67 ± 0.84 [b, c]	1.94 ± 1.20 [d, e]	3.08 ± 1.82 [f]	5.27 ± 3.24
Triglycerides (mg/dl)	72.34 ± 33.06 [b, c]	74.43 ± 41.71 [d, e]	98.39 ± 48.31 [f]	121.22 ± 61.53
Total cholesterol (mg/dl)	152.94 ± 27.70	152.44 ± 24.12 [e]	158.55 ± 31.21	161.74 ± 31.06
HDL-C (mg/dl)	63.43 ± 18.82 [b, c]	63.35 ± 16.03 [d, e]	53.38 ± 15.31	51.43 ± 12.04
LDL-C (mg/dl)	83.91 ± 20.97 [b, c]	86.39 ± 21.48 [d, e]	97.28 ± 26.69	100.46 ± 27.93
VLDL-C (mg/dl)	14.47 ± 6.61 [b, c]	14.89 ± 8.34 [d, e]	19.68 ± 9.66 [f]	24.25 ± 12.31
Atherogenic index	2.56 ± 0.73 [b, c]	2.50 ± 0.53 [d, e]	3.20 ± 1.25	3.29 ± 0.88
Females	Low weight (45)	Normal weight (37)	Overweight (51)	Obese (71)
BMI	17.86 ± 1.50 [a, b, c]	21.30 ± 2.40 [d, e]	25.28 ± 1.87 [f]	30.92 ± 3.39
Glucose(mg/dl) [*, #, &]	84.56 ± 9.68	85.24 ± 9.92	84.14 ± 10.65	89.93 ± 13.4 [k]
Insulin (mU/ml) *	7.56 ± 2.79 [a, b, c]	11.10 ± 5.43 [d, e]	14.95 ± 7.76 [f]	24.78 ± 12.24
HOMA-IR *	1.59± 0.64	2.43 ± 1.47	3.12 ± 1.72	5.53 ± 3.15
Triglycerides (mg/dl) [+, *]	75.60 ± 34.56 [c, g]	89.43 ± 53.31 [e]	96.86 ± 48.86 [f]	123.42 ± 57.08
Total cholesterol (mg/dl)	155.80 ± 27.97	157.62 ± 25.22	156.25 ± 29.76	164.69 ± 29.01
HDL-C (mg/dl)	64.26 ± 20.04 [b, c]	66.01 ± 15.88 [d, e]	54.18 ± 14.48	52.29 ± 12.62
LDL-C (mg/dl)	85.10 ± 21.33 [c]	91.58 ± 22.76	96.12 ± 25.26	102.05 ± 25.58
VLDL-C (mg/dl) [+, *]	15.12 ± 6.91 [c, h]	17.89 ± 10.66	19.37 ± 9.77 [f]	24.68 ± 11.42
Atherogenic index	2.59 ± 0.77 [b, c]	2.46 ± 0.52 [d, e]	3.06 ± 1.07 [k]	3.31 ± 0.92
Males	Low weight (8)	Normal weight (50)	Overweight (36)	Obese (58)
BMI	16.33 ± 0.57 [a, b, c]	20.11 ± 1.75 [d, e]	23.98 ± 1.59 [f]	29.76 ± 3.73
Glucose(mg/dl) [*, #, &]	85.63 ± 7.46 [c]	89.32 ± 8.95 [j]	88.08 ± 9.18 [f]	93.05 ± 10.21
Insulin (mU/ml) *	10.05 ± 7.47 [c]	7.19 ± 3.32 [d, e]	13.73 ± 8.41 [f]	21.83 ± 14.10
HOMA-IR *	2.13 ± 1.54 [c]	1.58 ± 0.79 [d, e]	3.02 ± 1.97 [f]	4.95 ± 3.35
Triglycerides (mg/dl) [+, *]	54.00 ± 12.65 [b, c]	63.34 ± 25.89 [d, e]	100.56 ± 48.13	118.53 ± 66.99
Total cholesterol (mg/dl)	136.88 ± 20.86	148.60 ± 22.76	161.81 ± 33.30	158.12 ± 33.29
HDL-C (mg/dl)	58.80 ± 9.21 [h]	61.39 ± 16.01 [d, e]	52.25 ± 16.55	50.39 ± 11.30
LDL-C (mg/dl)	71.58 ± 14.18 [b, c]	82.54 ± 19.83 [e, i]	98.93 ± 28.88	98.52 ± 30.68
VLDL-C (mg/dl) [+, *]	10.80 ± 2.53 [b, c]	11.20 ± 5.18 [d, e]	20.11 ± 9.63	23.71 ± 13.40
Atherogenic index	2.35 ± 0.44 [c]	2.53 ± 0.53 [e, i]	3.40 ± 1.45	3.24 ± 0.83

Data shown as mean ± SD; BMI, body mass index; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol. For [a] low versus normal $P \leq 0.01$, [b] low versus overweight $P \leq 0.01$, [c] low versus obese $P \leq 0.01$, [d] normal versus overweight $P \leq 0.01$, [e] normal versus obese $P \leq 0.01$, [f] overweight versus obese $P \leq 0.01$, [g] low versus overweight $P \leq 0.05$, [h] low versus obese $P \leq 0.05$, [i] normal versus obese $P \leq 0.05$, [j] overweight versus obese $P \leq 0.05$, [k] overweight versus obese $P \leq 0.05$; difference in nutritional status between men and women for + low weight, * normal weight, [#] overweight and [&] obese $P \leq 0.05$.

Association between ADIPOQ*-rs2241766 with clinical and biochemical parameters*

Therefore, we tested the effect that the gene polymorphism could have on these biochemical variables according to low-normal or overweight-obese phenotypes (Table 2).

The genotype analysis showed a frequency of 16.6% for the G allele among the included participants, of which only 9 (2.5%) had a GG genotype (Tables 2 and 3). These subjects also displayed high HDL-C and total cholesterol values ($P < 0.05$). In addition, there was a tendency toward higher total cholesterol levels in low- and normal- weight participants when grouped according to nutritional status (Table 2). No significant difference could be observed between the overweight and obese groups concerning total cholesterol, HDL-C, LDL-C, VLDL-C, and atherogenic index. It must be mentioned that genotype had no effect whatsoever on the atherogenic index (Castelli index I) of the participants ($P > 0.05$). Further, no statistical association was found between the nutritional status of the participants and their genotype ($P > 0.05$) – Table 3.

Table 2. *ADIPOQ*-rs2241766 genotype distribution in correlation with the evaluated biochemical parameters

Clinical and biochemical parameters	*ADIPOQ*-rs2241766		
	T/T	T/G	G/G
n (%)	247 (69.4%)	100 (28.1%)	9 (2.5%)
BMI	24.83 ± 5.29	24.56 ± 6.00	23.47 ± 5.96
Glucose (mg/dl)	88.23 ± 10.72	87.61 ± 11.87	89.00 ± 6.91
Insulin (mU/ml)	15.20 ± 11.46	16.08 ± 11.33	12.36 ± 6.15
HOMA-IR	3.36 ± 2.76	3.50 ± 2.65	2.69 ± 1.38
Triglycerides (mg/dl)	97.53 ± 56.83	95.82 ± 49.64	92.89 ± 26.17
Total cholesterol (mg/dl)	157.89 ± 30.00	154.45 ± 27.28	175.78 ± 21.80 [a, b]
HDL-C (mg/dl)	56.78 ± 15.46	55.04 ± 14.41	69.34 ± 33.44 [c, d]
LDL-C (mg/dl)	94.23 ± 26.83	92.55 ± 24.80	95.13 ± 16.80
VLDL-C (mg/dl)	19.51 ± 11.37	19.16 ± 9.93	18.58 ± 5.23
Atherogenic index	2.97 ± 1.02	2.95 ± 0.83	2.86 ± 0.67
Low and normal weight n *(%)*	94 (26.4%)	41 (11.5%)	5 (1.4%)
Glucose (mg/dl)	86.90 ± 9.30	85.17 ± 10.09	89.80 ± 9.50
Insulin (mU/ml)	8.44 ± 4.15	8.52 ± 5.05	9.68 ± 4.96
HOMA-IR	1.81 ± 0.99	1.86 ± 1.28	2.15 ± 1.18
Triglycerides (mg/dl)	75.28 ± 40.22	69.51 ± 37.05	76.80 ± 8.04
Total cholesterol (mg/dl)	153.45 ± 23.43	148.10 ± 28.58	174.40 ± 27.02 [e]
HDL-C (mg/dl)	62.94 ± 15.61	62.17 ± 14.93	81.62 ± 42.44
LDL-C (mg/dl)	85.98 ± 20.78	84.02 ± 22.92	87.15 ± 18.79
VLDL-C (mg/dl)	15.06 ± 8.04	13.90 ± 7.41	15.36 ± 1.61
Atherogenic index	2.55 ± 0.66	2.45 ± 0.49	2.51 ± 0.62
Overweight and obesity n *(%)*	153 (43%)	59 (16.6%)	4 (1.1%)
Glucose (mg/dl)	89.04 ± 11.46	89.31 ± 12.77	88.00 ± 2.16
Insulin (mU/ml)	19.35 ± 12.51	21.33 ± 11.53	15.70 ± 6.43
HOMA-IR	4.32 ± 3.05	4.64 ± 2.77	3.38 ± 1.46
Triglycerides (mg/dl)	111.20 ± 61.17	114.10 ± 49.30	113.00 ± 27.72
Total cholesterol (mg/dl)	160.62 ± 33.19	158.86 ± 25.66	177.50 ± 16.92
HDL-C (mg/dl)	52.99 ± 14.13	50.09 ± 11.82	53.98 ± 3.72
LDL-C (mg/dl)	92.30 ± 28.85	98.47 ± 24.50	105.10 ± 6.53
VLDL-C (mg/dl)	22.24 ± 12.23	22.82 ± 9.86	22.60 ± 5.54
Atherogenic index	3.23 ± 1.12	3.29 ± 0.85	3.30 ± 0.49

Data shown as mean ± SD; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment for insulin resistance; HDL-C, high density lipoproteins; LDL-C, low density lipoproteins. [a] G/G vs T/G $P = 0.033$; [b] G/G vs. T/T $P = 0.058$; [c] G/G vs T/G $P = 0.026$; [d] G/G vs. T/T $P = 0.05$; [e] GG vs TG genotype $P = 0.073$.

Table 3. *ADIPOQ*-rs2441766 genotype frequency in relation to nutritional status

Nutritional status	*ADIPOQ*-rs2241766			χ^2	OR	95% CI	*P* value
	T/T	T/G	G/G				
n (%)	247 (69.4%)	100 (28.1%)	9 (2.5%)				
Low and normal weight	97 (27.2%)	45 (12.6%)	5 (1.4%)	9.711			0.046
Overweight	40 (11.2%)	5 (1.4%)	0 (0%)				
Obese	110 (30.9%)	50 (14.0%)	4 (1.1%)				
	T/T + T/G		GG				
Low and normal weight	142 (39.9%)		5 (1.4%)	0.775	0.555	0.131–2.228	0.379
Overweight and obese	205 (57.6%)		4 (1.1%)				
	T/T	T/G + G/G					
Low and normal weight	97 (27.2%)	50 (14.0%)		1.359	0.764	0.484–1.207	0.244
Overweight and obese	150 (42.1%)	59 (16.6%)					

We observed a clear association between the *ADIPOQ*-rs2241766 polymorphisms and total cholesterol level under recessive conditions, *i.e.*, a higher total cholesterol level was observed in GG carriers in comparison with TT and TG carriers. The latter genotypes were prevalent in the participants with low- and normal-weight. Those observations were validated through a linear regression analysis ($P \leq 0.05$) – Table 4 .

Table 4. *ADIPOQ*-rs2241766 polymorphism and cholesterol levels

	Biochemical parameters by nutritional status	Dominant		*P*-value	Recessive		*P*-value
		TT	GG + TG		TG + TT	GG	
Cholesterol	All subjects	157.89 ± 30.00	156.21 ± 27.42	0.870	156.90 ± 29.25	175.78 ± 21.80	**0.046***
	Low and normal weight	153.45 ± 23.42	150.96 ± 29.32	0.588	151.82 ± 25.11	174.40 ± 27.02	**0.051***
	Overweight and obesity	160.62 ± 33.19	160.05 ± 25.51	0.884	160.13 ± 31.22	177.55 ± 16.92	0.269
HDL-C (mg/dl)	All subjects	56.78 ± 15.46	56.22 ± 16.99	0.409	56.28 ± 15.16	69.34 ± 33.44	0.341
	Low and normal weight	62.94 ± 15.61	64.29 ± 19.89	0.878	62.71 ± 15.35	81.62 ± 42.44	0.351
	Overweight and obesity	52.99 ± 14.13	50.34 ± 11.50	0.162	52.19 ± 13.57	53.98 ± 3.72	0.675

Data is shown as mean ± SD. * Validated by the linear regression analyses $P \leq 0.05$.

Discussion

Overweight and obesity have reached epidemic proportions in the adolescent population of Mexico, and thus become a serious health problem (Shamah-Levy et al., 2022). In the present study's population, almost 24% are overweight and 36% are obese, which represents a greater prevalence than in other regions of the world, such as Europe and Asia (Wang and Lim, 2012). With the exception of total cholesterol levels, a significant difference was observed in the values of the biochemical parameters analyzed when comparing the opposite ends of the nutritional status, which remained significant even after stratification according to sex. This same comparison showed that age (14.28 years) was a non-factor in the observed changes. For example, all groups had similar glucose levels; however, insulin levels differed >2-fold between low-weight and obese groups. The latter group had greater HOMA-IR values when compared to the low- and normal-weight groups, thus indicating the onset of insulin resistance from a very young age. The lipid profile, on the other hand, had lower variation when the comparison was made between nutritional status groups. Notably, total cholesterol levels were the exception in this case as well. The latter could be explained by the variable adiponectin levels in the participants, since it plays an important role in the regulation of glucose and lipid metabolism, whose alteration could be involved in some metabolic disorders (Frankenberg et al., 2017).

A previous study suggests that the *ADIPOQ* gene could be a susceptibility locus for metabolic syndrome, T2D, insulin resistance, and CVD (Enns et al., 2011). Previously identified as rs2241766, this polymorphism could not be associated with an increased risk for overweight and/or obesity in the tested population. Instead, it was associated with high cholesterol levels in GG genotype carriers. Although the population included in this study is rather small, the effects of this polymorphism could still be observed when it was stratified according to nutritional status, *i.e.*, low- and normal-weight. This observation was confirmed under the recessive genetic model and validated through linear regression analyses (Table 4). Interestingly, the effect of this genotype on HDL-C levels could not be observed in low- and normal-weight, in overweight and

obese participants, or under the dominant and recessive genetic models (Tables 2 and 4). Therefore, it may be possible that these findings are related to the small sample size.

A previous meta-analysis on *ADIPOQ* polymorphisms suggests that the SNPs rs17300539 and rs1501299 could be associated with a greater risk of obesity in Caucasians; on the other hand, the rs266729 SNP has been associated in this regard in Asians. So far, no association between the rs2241766 SNP and the risk of obesity has been established (Lu et al., 2014). In contrast, another meta-analysis including 2,818 obese subjects and 3,024 controls determined that the GG genotype of *ADIPOQ*-rs2241766 was associated with an increased risk of obesity. However, when performing the same analysis in different regions, it was discovered that the risk of obesity was only found in the Chinese population (Wu et al., 2014). Another study conducted in Mexican children evaluated the link between *ADIPOQ* (*i.e.*, rs182052, rs266729, rs2241766, and rs822393) and *ADIPOR2* polymorphisms (rs11061971) with obesity, reporting a significant overweight/obesity countereffect by the rs11061971 gene variant (Peralta Romero et al., 2015). A similar study evaluated the *ADIPOQ* polymorphisms rs182052, rs2241766, rs266729, rs822393, *ADIPOR1* rs10920533, and *ADIPOR2* rs11061971. However, this study could not establish a clear association between these genetic variants with adiponectin levels in serum or with other biochemical parameters (He et al., 2019). It must be mentioned that this previous study focused on the effect of these polymorphisms on the levels of adiponectin, foregoing any potential effect on other obesity-related biochemical parameters according to sex or nutritional status, which prevented the obtention of important additional data. In contrast, the present research did not find a clear association between a GG genotype and the development of overweight and obesity (Table 3).

The present study found that, when stratified according to nutritional status, the rs2241766 polymorphism has a significant effect on total cholesterol; however, this effect was independent of sex. It must be considered that the observations made in this, and another study conducted in Mexico, could be correlated to the nature of the population itself, which may present unforeseen epistatic effects due to its Mestizo background (Martínez-Cortés et al., 2012), in addition to the complex interactions between the environment, diet, and the genetic component of the populations (Matey-Hernandez et al., 2018). On the other hand, it should be noted that the rs2241766 polymorphism did not have an appreciable effect on the overweight or obese participants included in this study. In contrast, a previous study on the effect of the rs266729 polymorphism on weight loss, CVDs, and adiponectin under a low-calorie diet, showed a strong association between this polymorphism, increased adiponectin levels, and decreased LDL-C, glucose, insulin, and HOMA-IR after weight loss (de Luis et al., 2020). Therefore, the effect that diet and genetics could have in the variation of clinical and biochemical parameters should not be neglected.

Another study in a Mexican adult population (84 males and 158 females, aged 24 to 69 years), also self-identified as Mestizo, could not establish a clear association between the rs2241766 polymorphism with either lean, overweight, or obese phenotypes. This same study suggests that the *ADIPOQ* +45G allele could be associated with body fat distribution on the basis that TG genotype carriers have smaller biceps circumference and skin thickness than those with a TT genotype. According to an additive model in the same study, among other parameters a heterozygous TG + GG genotype results in lower body fat, hip circumference, skin thickness in biceps and triceps, than in TT carriers (Guzman-Ornelas et al., 2012). A study conducted on an Indian population diagnosed with T2D showed a positive correlation between the rs266729 polymorphism and high cholesterol/LDL levels, whereas its influence was null on HDL levels. The same study also suggests that this polymorphism may play a role in the development and progression of CVDs (Momin et al., 2017).

A case-control study correlated the rs2241766 polymorphism with high cholesterol levels in a Mexican TG carrier with ductal infiltrating breast cancer ($P = 0.145$) (Macías-Gómez et al., 2019). Interestingly, the previous report stated that the Mexican population has a low frequency of the GG genotype, an observation that was also made in the present study. The protective effect of rs2241766 polymorphism against dyslipidemia in HIV/HCV-coinfected patients with steatosis was also previously described, suggesting that GG/GT carriers have lower cholesterol and LDL levels in comparison with other genotypes ($p = 0.003$ and $p = 0.071$, respectively (Pineda-Tenor et al., 2014).

There is evidence that synonymous mutations can affect the secondary structure of the mRNA and thus convey an altered stability and/or translation (Presnyak et al., 2015). For example, the *ADIPOQ* allele +45T>G (rs2241766) is a synonymous mutation (GGT → GGG, Gly → Gly). However, this polymorphism still results in higher adiponectin levels in its carriers (Oliveira et al., 2011), which in turn leads to greater HDL level in plasma. This has an inverse correlation with very low-density lipoprotein (VLDL) and LDL levels (Izadi et al., 2013). Controversial results have been reported in a Mexican population, where *ADIPOQ* gene variants could not be associated with the concentration of adiponectin in serum (He et al., 2019), although these discrepancies have been accredited to the diverse genetic background of the included participants.

A recent meta-analysis of polymorphic *ADIPOQ* variants from 5,840 individuals suggested their association with obesity; in particular, carriers of the wild type genotype (GG) have an increased risk of obesity in comparison with other genotypes (Wu et al., 2014). Similarly, the present study also found a low frequency of G allele carriers (TT 69.38%, TG 28.09%, and GG 2.53%), which contradicts the previous report by Wu et al. (2014) (TT 60.67%, TG 32.67%, and GG 6.66%).

However, the available literature also reports a low frequency of the wild type genotype in all of the studied populations, ranging from 1.5% to 8.3% (National Center for Biotechnology Information, 2020). The published data in the 1,000 Genomes Project also support the results obtained in the present study (NCBI 1000 Genomes Project, 2020).

Previous research has been focused on the association between gene polymorphisms and obesity/BMI, trying to correlate the level of circulating adiponectin with diverse biochemical parameters, e.g., lipid profile. However, these reports were unable to establish a direct correlation between *ADIPOQ* gene variants and obesity-related biochemical parameters in a healthy population. Unlike these past studies, the present research describes the significant effect of the rs2241766 polymorphism on total cholesterol levels in low- and normal-weight healthy subjects. We also observed the potential effect of this polymorphism on HDL levels that may be contributing to the previous observation. Because total cholesterol and HDL levels are considered in the calculation of the atherogenic index, and they are usually within the same range across different genotypes, we could only associate the net differences in the Catelli index I (Atherogenic index) with nutritional status variations (Table 1).

Although the impact of rs2241766 polymorphism on total cholesterol levels is clear in the included population, there might be a potential effect on HDL levels that may or not have a cardioprotective effect. This study was not without its limitations, the first of which was the low frequency of the wild type genotype (GG) in the participants. Also, we do not have sufficient data to evaluate the impact of rs2241766 polymorphisms on the level of adiponectin, and thus are unable to establish a correlation between them. Finally, the effect that diet and the genetic background may have on the evaluated obesity-related biochemical parameters should also be explored in further detail across different nutritional status. Based on these limitations, additional studies are required to confirm or reject our findings.

Conclusions

The results obtained from this study suggest that cholesterol levels are under the influence of the *ADIPOQ*-rs2241766 polymorphism in Mexican adolescents. This observation could explain how *ADIPOQ* variants partially increase the risk of developing metabolic disorders. However, unlike previous reports including other populations, the present study could not prove a clear association between *ADIPOQ*-rs2241766 polymorphisms and obesity or insulin resistance. Therefore, additional studies should be performed in a population with a greater number of G allele carriers to confirm these results and to rule out the influence of other genetic and non-genetic factors.

Availability of data and material

The used and/or analyzed datasets in this study are available from the corresponding author upon request.

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Author's contributions

Study conception: MSS, RLC; Study design: RLC, MSS; Patient selection: MSS, DAT, PVO, RLC, Data analysis: MSS, RLC, DAT, PVO, VSV, CRI, CAN, JMC; Data interpretation: RLC, MSS, CRI; Drafting of the manuscript: RLC, CRI, MSS, DAT; Manuscript improvement: RLC, CRI, MSS; Critical reading of manuscript: RLC, MSS; Final approval: all.

Ethical approval and informed consent

The Ethics and Research Committee of the University Hospital in Saltillo approved and registered the present study under record number INMED 01-1113. Informed consent was obtained from the participants or their legal guardians.

Consent for publication

All the authors gave their signed consent and agree with the publication of the manuscript in the submitted form.

Conflict of interests

The authors have no conflict of interests to declare.

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