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Original Research

Contribution of polymorphisms in the *LEP*, *LEPR* and *RETN* genes on serum leptin and resistin levels in young adults from Mexico

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Abstract: Polymorphisms in the *LEP* (G–2548A and A19G), *LEPR* (A326G, A668G and G3057A) and *RETN* (C–420G and G+62A) genes were documented according to their association with alterations in biochemical parameters such as glucose, insulin and lipid profiles, along with serum leptin and resistin concentrations. The aim of the study was to establish any contribution of the G-2548A and A19G polymorphisms of the *LEP* gene, the A326G, A668G and G3057A polymorphisms of the *LEPR* gene, and the C-420G and G+62A polymorphisms of the *RETN* gene to serum leptin and resistin levels in Mexican young adults. Clinical and biochemical variables, serum leptin and resistin levels, and genotype profiles were analysed in 66 Mexican young adults. Seven polymorphisms in the *LEP*, *LEPR* and *RETN* genes were genotyped using polymerase chain reaction–restriction fragment length polymorphisms analysis. Individuals carrying allele 3057A of the G3057A polymorphism in the *LEPR* gene showed significantly higher leptin concentrations than those bearing the genotype G/G (43.78 ± 39.11 *vs* 28.20 ± 14.12 ng/mL; p = 0.021). There were no associations of serum leptin or resistin levels according to the genotype of the other six analysed polymorphisms. Our results suggest that the allele 3057A of the *LEPR* G3057A polymorphism contributes to increased serum leptin levels in Mexican young adults.

Key words: Resistin concentration; Leptin concentration; Adipokines; Genetic polymorphisms; Mexican young adults.

Introduction

Obesity is considered to be a chronic disease defined by an excessive expansion of adipose tissue. Initially, it is driven by an increase in the number of adipocytes (hyperplasia) and later by an increase in adipocyte size (hypertrophy), which together modify the expression of adipokines, resulting in the development of a low-grade chronic pro-inflammatory state (1, 2). This pro-inflammatory state is a health problem because it is associated with metabolic disturbances and pathologies such as insulin resistance, type 2 diabetes mellitus (T2DM), atherosclerosis, hypertension and cardiovascular disease (3, 4).

Obesity is a complex disease resulting from the interaction of genetic and environmental factors, leading to the malfunction of several signalling peptides involved in body energy balance and nutritional status (5, 6). Increases in the number of people with obesity in recent years have been associated with changes in lifestyle but are greatly influenced by genetic susceptibility. Reports on the heritability of obesity have identified nine loci in Mendelian forms of obesity, and 58 loci contribute in polygenic ways to the obese phenotype (7). Among these, the *LEP* and *LEPR* genes encoding leptin and leptin receptor, respectively, play major roles.

The relation between leptin levels and genetic polymorphisms in either the *LEP* or *LEPR* genes has been examined before. Thus, the influence of A19G and G–2548A polymorphisms in the *LEP* gene on leptin levels was evaluated in consanguineous Tunisian families (8), whereas in Korean pre- and postmenopausal women with breast cancer the influence of four polymorphisms in the *LEPR* gene including A326G, A668G and G3057A on leptin concentration was assessed (9). Studies considering genetic variants in both *LEP* and *LEPR* genes have been carried out in Romanians aged > 30 years, where the polymorphism G–2548A (*LEP*) was associated with higher leptin levels and the polymorphism A668G (*LEPR*) with increased triglycerides (TG) and glucose levels (10). In another study on men from the Pacific Island of Nauru with an average age of 31 years, the relation between a short tandem repeat in the *LEP* gene and the polymorphisms A668G and G3057A of the *LEPR* gene and obesity markers and glucose intolerance was also investigated (11), whereas in Malaysians aged 52 years, the relation of A19G and G–2548A variants of *LEP* and A326G and A668G variants of *LEPR* with leptin levels and obesity was assessed (12).

Information about the influence of the genetic polymorphisms in the *RETN* gene on the levels of resistin is scarcer compared to leptin. In a study performed in Fin-

nish subjects with hypertension aged 40–59 years, three polymorphisms in the *RETN* gene (C–420G, C+157T and G+299A) were studied with regard to resistin concentration, and to fasting insulin, glucose and lipid profiles (13). In Japanese subjects with T2DM aged 25–88 years, the relation between the genotype distribution of C–420G polymorphism, resistin levels and the presence of cerebrovascular disease was studied (14). The relation between the G+62A polymorphism and insulin resistance, together with inflammatory markers and lipid profile, has been studied in a Mexican population aged 20–69 years (15). None of these reports considered the age range of the young adult participants enrolled in the present study (18–25 years) or evaluated a combinatorial genetic approach (*LEP*, *LEPR* and *RETN* genes) with both leptin and resistin levels. Based on this knowledge, the aim of the present study was to describe the genotype and allele distributions of the G–2548A and A19G polymorphisms in *LEP*, A326G, A668G and G3057A in *LEPR*, and C–420G and G+62A in *RETN* genes. We also aimed to assess the associations of variants in the *LEP* and *LEPR* genes with serum leptin, and *RETN* variants with serum resistin levels, as well as with variables related to metabolic alterations in a group of young adults from Mexico.

Materials and Methods

Subjects

Sixty-six individuals (28 men and 38 women) were included in the study. The subjects were invited to participate in a cross-sectional study and were recruited at the Biochemistry Laboratory of Centro Universitario de Ciencias Exactas e Ingenierías of the Universidad de Guadalajara under the following inclusion criteria: active students, aged 18–25 years, male or female, born in Mexico (with a family history of Mexican ancestors at least back to the third generation) and being Mestizo (to have a Spanish-derived last name). Exclusion criteria were the following: pregnancy, use of contraceptives, alcohol consumption 72 hours before, being related to any other participant of the study. All participants signed a voluntary consent form for the use of their blood samples in this investigation. The study protocol was approved by the Research and Ethics Committee of the Centro de Investigación Biomedica de Occidente, Instituto Mexicano del Seguro Social (R-2011-1305-4).

Anthropometric measurements

Age and anthropometric measurements, including height, current weight, waist circumference (WC) and hip circumference (HC), as well as the waist-to-hip ratio (WC/HC), were obtained. Fat mass was obtained with the Tanita Body Composition Analyzer (TBF-300A; Tanita Corp., Tokyo, Japan), and body mass index (BMI) was calculated as weight/height squared (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured.

Biochemical analyses

A fasting venous blood sample was taken from all subjects. Glucose concentration and lipid profiles including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were measured using the Vitros DT II system (Ortho Clinical Diagnostics, Linden, NJ, USA). Serum insulin, leptin and resistin levels were measured using the Bio-Plex Pro Human Diabetes 10-Plex assay system (Bio-Rad Laboratories, Hercules, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol (16) and stored in Tris-EDTA buffer pH 8.0 at −20 °C until processed. The genotyping of polymorphisms A19G (rs2167270) from the *LEP* gene, A326G (rs1137100), A668G (rs1137101) and G3057A (rs1805096) from the *LEPR* gene and C–420G (rs1862513) and G+62A (rs3745368) from the *RETN* gene was carried out using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) using the primer sequences and PCR conditions as previously described (14, 17-20), except for the genomic region encompassing the G–2548A (rs7799039) polymorphism of the *LEP* gene, which was amplified using the following designed primers: forward 5′–TTT CCT GTA ATT TTC CCG TGA G–3′ and reverse 5′–AAA GCA AAG ACA GGC ATA AAA A–3′. The PCR products were generated in a 10 μL reaction volume containing 100 ng of genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 1 μM of each primer, and 0.25 U Taq DNA polymerase (Invitrogen Life Sciences, Carlsbad, CA, USA). Cycling conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 30 sec and a final extension step at 72 °C for 10 min. PCR products were digested with 5 U of *HhaI* restriction enzyme at 37 °C, according to the manufacturer's instructions (New England BioLabs, Ipswich, MA, USA); thus, in the presence of the G–2548 allele, the PCR product (242 bp) is cut into two fragments of 181 and 61 base pairs (bp) in length. Differences in bp length for all enzyme digestion products were visualized using electrophoresis in 6% polyacrylamide gels stained with silver nitrate.

Statistical analysis

Allele frequencies were determined by direct counting of observed genotype frequencies and it was verified if the genotype distributions are consistent with Hardy-Weinberg equilibrium. Mean values of anthropometric, clinical and biochemical parameters were compared using Student's *t* tests. Correlations between leptin and resistin serum concentrations with anthropometric clinical and biochemical variables were evaluated by calculating Pearson's correlation coefficients. Analysis of variance (ANOVA) followed by post hoc tests when necessary (Bonferroni's or Dunnet's T3-test) and Student's *t* tests or Mann–Whitney non-parametric *U* tests were used to compare quantitative variables in the genotype subgroups; we also assessed the dominant genetic model for each polymorphism comparison. Results were regarded as significant when $p < 0.05$. For data analysis, we used the statistical software package

Table 1. Anthropometric, clinical and biochemical variables of the study population.

Variables	Study group			p value
	Total population	Men	Women	
n	66	28	38	
Age (years)	21.6 ± 1.4	21.5 ± 1.5	21.6 ± 1.4	0.829
WC (cm)	95.2 ± 13.8	101.8 ± 14.2	90.7 ± 11.8	**0.001**
HC (cm)	108.7 ± 9.3	109.4 ± 11.6	108.3 ± 7.4	0.646
WC/HC ratio	0.87 ± 0.09	0.930 ± 0.07	0.836 ± 0.08	**< 0.001**
Weight (kg)	81.1 ± 16.2	91.0 ± 16.2	73.8 ± 11.8	**< 0.001**
Height (m)	1.69 ± 0.1	1.8 ± 0.05	1.6 ± 0.08	**< 0.001**
BMI (kg/m^2)	28.5 ± 5	28.9 ± 5.5	28.2 ± 4.6	0.585
Body fat mass (%)	30.7 ± 9.2	24.9 ± 9.8	34.9 ± 6	**< 0.001**
Body fat mass (kg)	24.8 ± 10.7	22.8 ± 13.2	26.2 ± 8.2	0.200
SBP (mm Hg)	120.3 ± 11.4	126.4 ± 11.2	115.8 ± 9.4	**< 0.001**
DBP (mm Hg)	82.5 ± 15.7	83.6 ± 11.7	81.7 ± 18.2	0.616
Glucose (mg/dL)	68.5 ± 9.2	68.9 ± 11.1	68.3 ± 7.7	0.787
Insulin (ng/mL)	2 ± 1.6	1.7 ± 0.7	2.2 ± 2	0.267
HOMA-IR	8 ± 6.8	7 ± 3.2	8.8 ± 8.5	0.310
TC (mg/dL)	160.7 ± 36.3	153.5 ± 39.4	166 ± 33.4	0.167
TGs (mg/dL)	104.1 ± 62.1	109.8 ± 69.9	99.9 ± 56.4	0.528
HDL (mg/dL)	47.5 ± 16.3	42.1 ± 14.5	51.4 ± 16.6	**0.020**
LDL (mg/dL)	90.5 ± 33.1	88.3 ± 35.1	92.2 ± 32	0.637
VLDL (mg/dL)	22.7 ± 15.6	23.1 ± 13.7	22.4 ± 17.1	0.849
Leptin (ng/mL)	40.2 ± 35.5	22.9 ± 16.2	53 ± 40.4	**< 0.001**
Resistin (ng/mL)	10.8 ± 4.9	10.6 ± 3.6	11 ± 5.7	0.818

Data are shown as the mean ± standard deviation. Data in bold type were found to be statistically significant using Student's *t* test.

IBM SPSS Statistics v. 21 (IBM Corp., Armonk, NY, USA).

Results

The anthropometric, clinical and biochemical characteristics of the study population are shown in Table 1. There were statistically significant differences in SBP ($p < 0.001$), WC ($p = 0.001$), WC/HC ratio ($p < 0.001$), weight ($p < 0.001$), height ($p < 0.001$), percentage of body fat mass ($p < 0.001$), HDL ($p = 0.020$) and leptin serum concentration ($p < 0.001$) between male and female subjects. The distribution of BMI between men and women by weight groups (normal weight 18.5-24.9 kg/m^2, overweight ≥25-29.9 kg/m^2 and obese ≥30 kg/m^2) was also assessed and there was not statistical difference ($p = 0.944$) (data not shown in tables).

There were significant positive correlations of leptin serum concentrations with HC, BMI and both percentage and kg of body fat mass, and a trend with insulin levels ($p = 0.050$). In addition, there was a negative correlation with height and SBP. On the other hand, resistin serum concentrations did not correlate significantly with any of the studied variables (Table 2).

The genotypic distribution of all the polymorphisms in the *LEP, LEPR* and *RETN* genes were consistent with Hardy-Weinberg equilibrium, with the exception of the A19G variant in the *LEP* gene (data not shown in tables).

Anthropometric, clinical and biochemical parameters were analysed according to the genotype distributions of polymorphisms of the *LEP* gene, as shown in Table 3. There were significant differences regarding TC ($p = 0.012$) according to the distribution of the G–2548A polymorphism, as the mean value for carriers of the G/A genotype was higher than that for A/A genotype carriers (168.2 ± 35.4 *vs* 133.3 ± 32.1 mg/dL). In addition, we found differences in LDL levels ($p = 0.005$); individuals bearing the G/G and G/A genotypes showed higher LDL levels than A/A genotype carriers (98.7 ± 32 and 95.6 ± 29.5 *vs* 63.1 ± 33.4 mg/dL, respectively). Concerning the A19G polymorphism, there were also differences in TC values ($p = 0.032$), as the A/G genotype carriers had higher concentrations than G/G genotype carriers (167.9 ± 33.5 *vs* 139.5 ± 36.5 md/dL, respectively), and LDL levels were significantly higher ($p = 0.015$) in individuals carrying the A/A and A/G genotypes than in carriers of the G/G genotype (102.4 ± 34.4 and 95.3 ± 29.7 *vs* 69.6 ± 34.5 mg/dL, respectively).

No significant differences were found regarding the mean values of the analysed variables according to the genotype distributions of the A326G, A668G and G3057A polymorphisms in the *LEPR* gene (Table 4).

Analysis of anthropometric, clinical and biochemical variables according to the distribution of genotypes of the C–420G and G+62A polymorphisms of the *RETN* gene revealed significant differences only when comparing the glucose levels ($p = 0.042$) for the distribution of the C–420G polymorphism, and VLDL levels ($p = 0.013$) for the G+62A polymorphism (Table 5). All the significant values in the ANOVA tests remained statistically significant after the Bonferroni´s post hoc correction ($p<0.05$).

Finally, serum leptin concentrations were not significantly different for the *LEP* and *LEPR* gene polymorphism distributions (Tables 3 and 4, respectively) or in the resistin levels for the *RETN* gene polymorphism distributions (Table 5). Nevertheless, when comparisons for all the polymorphisms were done assuming a dominant genetic model, leptin serum concentrations

Table 2. Correlations of leptin and resistin levels with anthropometric, clinical and biochemical variables.

	Leptin (ng/mL)		Resistin (ng/mL)	
Variable	***r***	**p value**	***r***	**p value**
WC (cm)	0.177	0.164	0.134	0.296
HC (cm)	0.435	**< 0.001**	0.027	0.833
WC/HC ratio	−0.124	0.332	0.173	0.176
Height (cm)	−0.381	**0.002**	0.050	0.690
Weight (kg)	0.093	0.460	0.063	0.615
BMI (kg/m^2)	0.399	**0.001**	0.051	0.683
Body Fat Mass (%)	0.574	**< 0.001**	0.190	0.126
Body Fat Mass (kg)	0.500	**< 0.001**	0.114	0.362
SBP (mm Hg)	−0.272	**0.027**	–0.157	0.207
DBP (mm Hg)	−0.028	0.820	–0.018	0.886
Glucose (mg/dL)	−0.055	0.660	–0.201	0.105
Insulin (ng/mL)	0.242	0.050	0.093	0.459
HOMA-IR	0.221	0.075	0.062	0.621
TC (mg/dL)	0.058	0.643	−0.145	0.245
TGs (mg/dL)	−0.107	0.393	−0.133	0.286
HDL (mg/dL)	0.200	0.107	0.184	0.139
LDL (mg/dL)	0.054	0.668	−0.217	0.080
VLDL (mg/dL)	−0.189	0.129	−0.70	0.577
Leptin (ng/mL)	–	–	0.149	0.232
Resistin (ng/mL)	0.149	0.232	–	–

Results show Pearson correlation coefficients (*r*) and their respective p values. Statistically significant data are shown in bold type ($p < 0.05$).

Table 3. Anthropometric, clinical and biochemical variables according to genotype for polymorphisms of the *LEP* gene.

	Polymorphism							
	G–2548A				**A19G**			
Variable	**G/G**	**G/A**	**A/A**	**p value**	**A/A**	**A/G**	**G/G**	**p value**
	18 (27%)	**36 (55%)**	**12 (18%)**		**10 (15%)**	**41 (62%)**	**15 (23%)**	
WC (cm)	92.4 ± 12.5	95.1 ± 14.8	99.9 ± 12.7	0.354	93.3 ± 13.8	95 ± 14.5	97.2 ± 12.8	0.783
HC (cm)	108.5 ± 6.4	108.6 ± 10	109.4 ± 11.2	0.967	107.7 ± 7.1	109.1 ± 9.5	108.5 ± 10.6	0.910
WC/HC ratio	0.850 ± 0.1	0.873 ± 0.1	0.915 ± 0.1	0.138	0.863 ± 0.1	0.869 ± 0.1	0.897 ± 0.1	0.525
BMI (kg/m^2)	27.2 ± 2.8	28.3 ± 5	30.9 ± 6.7	0.134	27.1 ± 3.5	28.3 ± 4.7	29.9 ± 6.3	0.376
Body fat mass (%)	29.2 ± 8.4	31.2 ± 8.8	31.3 ± 12	0.744	26.2 ± 6.9	32 ± 8.6	29.8 ± 11.5	0.193
Body fat mass (kg)	22.6 ± 7.1	25.3 ± 11.7	26.6 ± 12.3	0.561	20.8 ± 7	25.7 ± 11.1	25 ± 11.6	0.440
SBP (mm Hg)	120.1 ± 10.6	119.9 ± 12.3	122 ± 10.6	0.853	120.2 ± 12.5	119.7 ± 11.4	122.1 ± 11.2	0.792
DBP (mm Hg)	82.1 ± 11.2	84.1 ± 18.6	78.3 ± 11.5	0.533	85.5 ± 13.2	80.7 ± 9.8	85.3 ± 27.1	0.510
Glucose (mg/dL)	69 ± 7.3	68.7 ± 7.5	67.3 ± 15.4	0.881	70 ± 7.5	68.3 ± 7.4	68.3 ± 14.1	0.865
Insulin (ng/mL)	1.7 ± 0.8	1.9 ± 1.3	2.6 ± 2.8	0.266	1.8 ± 0.8	1.9 ± 1.2	2.3 ± 2.6	0.678
HOMA-IR	6.9 ± 2.8	7.6 ± 5.1	11 ± 12.9	0.236	7.4 ± 3.1	7.6 ± 4.8	9.6 ± 11.8	0.595
TC (mg/dL)	163.9 ± 33.8	168.2 ± 35.4	133.3 ± 32.1	**0.012***	163 ± 38.7	167.9 ± 33.5	139.5 ± 36.5	**0.032***
TGs (mg/dL)	106.1 ± 67.4	109.7 ± 56.9	84.5 ± 70.8	0.480	78.8 ± 41.4	114.4 ± 59.9	92.8 ± 75.5	0.196
HDL (mg/dL)	44.1 ± 10.3	50.7 ± 17.3	43 ± 19.8	0.213	44.9 ± 11.2	49.7 ± 16.8	43.1 ± 17.8	0.355
LDL (mg/dL)	98.7 ± 32	95.6 ± 29.5	63.1 ± 33.4	**0.005***	102.4 ± 34.4	95.3 ± 29.7	69.6 ± 34.5	**0.015***
VLDL (mg/dL)	21.2 ± 13.4	22 ± 11.4	27.3 ± 26.8	0.537	15.7 ± 8.2	22.9 ± 12	26.9 ± 25	0.216
Leptin (ng/mL)	39.5 ± 20.4	43 ± 38.6	33.2 ± 44.7	0.718	33 ± 16.4	45.2 ± 36.6	31.4 ± 40.9	0.348

Data are presented as means ± standard deviations compared using ANOVA. Statistically significant data are shown in bold type ($p < 0.05$).
*Bonferroni´s correction $p<0.05$

were significantly higher only in carriers of the 3057A allele (G/A+A/A genotypes) of the G3057A polymorphism in the *LEP* gene compared with individuals bearing the G/G genotype (43.78 ± 39.11 *vs* 28.20 ± 14.12 ng/mL, p = 0.021) (data not shown in tables). Considering this last finding, we also assessed the distribution of genotypes of G3057A polymorphism in the *LEPR* gene according to BMI groups (normal weight 18.5-24.9 kg/m^2, overweight ≥25-29.9 kg/m^2 and obese ≥30 kg/m^2), nevertheless, there was not statistical difference (p = 0.626) (data not shown in tables).

Discussion

To the best of our knowledge, this is the first report concerning the G–2548A and A19G polymorphisms in the *LEP* gene, the A326G, A668G and G3057A variants in the *LEPR* gene, and the C–420G and G+62A polymorphisms of the *RETN* gene, and assessing the contribution of *LEP* and *LEPR* genotypes to the serum levels of leptin and *RETN* genotypes to resistin levels.

It is remarkable that young adults in Mexico are an understudied group. These represent an immediate tran-

Table 4. Anthropometric, clinical and biochemical variables according to genotype for polymorphisms of the *LEPR* gene.

Variable	Polymorphism											
	A326G			p value	A668G			p value	G3057A			p value
	A/A 26 (39%)	A/G 29 (44%)	G/G 11 (17%)		A/A 16 (24%)	A/G 34 (52%)	G/G 16 (24%)		G/G 15 (23%)	G/A 35 (53%)	A/A 16 (24%)	
WC (cm)	94.4 ± 12.4	95.6 ± 15.9	96.5 ± 12	0.915	97 ± 13	94.6 ± 15	95.1 ± 12.7	0.858	94.93 ± 14.3	95.38 ± 14.3	95.40 ± 13.2	0.994
HC (cm)	108.7 ± 7.7	109.2 ± 10	107.7 ± 1	0.906	109 ± 9.1	109.5 ± 9.4	106.9 ± 9.7	0.671	106.07 ± 7.6	109.33 ± 10.3	110.11 ± 8.4	0.435
WC/HP ratio	0.869 ± 0.1	0.872 ± 0.1	0.898 ± 0.1	0.661	0.890 ± 0.1	0.861 ± 0.1	0.889 ± 0.1	0.466	0.893 ± 0.1	0.870 ± 0.1	0.865 ± 0.1	0.640
BMI (kg/m^2)	28 ± 4.5	28.9 ± 5.4	28.5 ± 5.4	0.796	28.4 ± 5.2	28.9 ± 5.1	27.8 ± 4.8	0.770	27.55 ± 4.1	28.57 ± 5.4	29.10 ± 5	0.684
Body fat mass (%)	29.1 ± 9.4	31.8 ± 8.2	31 ± 11.7	0.546	28.41 ± 9.4	31.31 ± 8.7	31.56 ± 10.5	0.539	29.54 ± 9.5	30.64 ± 10.1	31.79 ± 7.3	0.800
Body fat mass (kg)	23.4 ± 8.4	26.2 ± 12.2	24.3 ± 11.7	0.619	22.75 ± 8.4	26.10 ± 11.8	24.06 ± 10.4	0.565	22.08 ± 7.9	25.03 ± 12.3	26.81 ± 9.1	0.466
SBP (mm Hg)	120.9 ± 11.2	118.8 ± 12	122.7 ± 10.6	0.596	121.9 ± 11.7	117.9 ± 11.8	123.8 ± 9.4	0.195	120.93 ± 13.9	118.67 ± 10.5	123.25 ± 10.8	0.409
DBP (mm Hg)	79.5 ± 7.8	82.4 ± 12.4	89.7 ± 30.7	0.198	79.1 ± 7.6	84.2 ± 19.8	82.2 ± 11.5	0.567	81.27 ± 7.9	79.49 ± 9.7	90.25 ± 26.7	0.069
Glucose (mg/dL)	67.3 ± 8.4	69.4 ± 7.6	69 ± 14.6	0.686	66.2 ± 7	69.3 ± 7.8	69.3 ± 13.3	0.513	66.40 ± 11.9	67.43 ± 8.6	72.94 ± 6.6	0.083
Insulin (ng/mL)	1.7 ± 0.1	2.4 ± 0.4	1.5 ± 0.2	0.129	1.64 ± 0.7	2.29 ± 2	1.65 ± 0.6	0.241	1.72 ± 0.8	1.90 ± 1.3	2.40 ± 2.4	0.443
HOMA-IR	6.7 ± 3.0	9.9 ± 9.5	6.3 ± 3	0.151	6.39 ± 3.1	9.39 ± 8.9	6.82 ± 3	0.250	6.76 ± 3.4	7.50 ± 5.1	10.42 ± 11.1	0.262
TC (mg/dL)	158.1 ± 40.3	169 ± 33.4	144.6 ± 29.6	0.148	159.81 ± 33.5	163.09 ± 38.3	156.5 ± 36.5	0.835	160.53 ± 39.9	163.31 ± 37.1	155.13 ± 32.7	0.762
TGs (mg/dL)	100.9 ± 63.6	106.3 ± 58	105.7 ± 74.7	0.947	103.50 ± 67.7	98.68 ± 52.4	116.25 ± 76.8	0.654	106.93 ± 71.6	93.91 ± 59	123.75 ± 57.6	0.281
HDL (mg/dL)	47.7 ± 18.3	47.5 ± 13.9	46.7 ± 18.9	0.986	49.31 ± 21.8	46.18 ± 12.4	48.38 ± 18.3	0.797	48.87 ± 18.1	48.63 ± 17.8	43.62 ± 10.7	0.564
LDL (mg/dL)	90.1 ± 32.4	97.2 ± 32.3	73.7 ± 33.9	0.134	89.69 ± 27.3	94.47 ± 34.5	82.94 ± 36.1	0.521	88 ± 36.9	93.34 ± 33	86.69 ± 30.2	0.763
VLDL (mg/dL)	20.3 ± 12.8	24.3 ± 18.5	24.1 ± 13.9	0.608	20.81 ± 13.6	22.44 ± 17.2	25.19 ± 14.5	0.729	23.67 ± 13.6	21.34 ± 18	24.81 ± 11.7	0.741
Leptin (ng/mL)	43.7 ± 44.6	42.5 ± 30.7	26 ± 17.9	0.353	47.51 ± 54.7	42.15 ± 29.9	28.89 ± 17.6	0.305	28.20 ± 14.1	47.29 ± 45.2	36.09 ± 19.4	0.192

Data are presented as means ± standard deviations compared using ANOVA.

Table 5. Anthropometric, clinical and biochemical variables according to genotype for polymorphisms of the *LEPR* gene.

Variable	Polymorphism							
	C–420G			p value[a]	G+62A			p value[b]
	C/C 23 (35%)	C/G 35 (53%)	G/G 8 (12%)		G/G 63 (95%)	G/A 3 (5%)	A/A 0 (0%)	
WC (cm)	95.6 ± 12.3	95.7 ± 15.8	92.3 ± 8.1	0.837	32.1	30.1	–	0.874
HC (cm)	108 ± 7.9	109.3 ± 10.8	108.4 ± 5.7	0.888	31.8	35.7	–	0.744
WC/HC ratio	0.884 ± 0.1	0.873 ± 0.1	0.853 ± 0.1	0.723	32.4	24	–	0.464
BMI (kg/m^2)	28 ± 3.6	29.2 ± 6.1	26.4 ± 1.7	0.317	33.4	36	–	0.834
Body fat mass (%)	30.8 ± 10.5	30.9 ± 8.7	29.1 ± 8.6	0.880	33.2	39	–	0.637
Body fat mass (kg)	24.2 ± 9.6	25.9 ± 12.2	21.8 ± 5.3	0.600	33.3	37.5	–	0.732
SBP (mm Hg)	119 ± 12.3	120.3 ± 11.9	123.9 ± 5.5	0.525	33.8	26.3	–	0.562
DBP (mm Hg)	79.4 ± 13.2	85 ± 18.1	80.1 ± 8.6	0.376	34.4	14.8	–	0.089
Glucose (mg/dL)	64.7 ± 10.3	70.6 ± 7.7	70.6 ± 9.6	**0.042***	32.7	50.7	–	0.119
Insulin (ng/mL)	1.9 ± 1.1	2 ± 1.7	2 ± 2	0.962	33.6	31	–	0.837
HOMA-IR	7.5 ± 4.9	8.3 ± 8	8.2 ± 6.5	0.909	33.4	36	–	0.839
TC (mg/dL)	163.4 ± 34	154.1 ± 37.4	182 ± 32.7	0.132	33.7	29.5	–	0.732
TGs (mg/dL)	97.7 ± 67.8	103.4 ± 61.8	125.5 ± 47.5	0.557	33.1	42.5	–	0.428
HDL (mg/dL)	48.6 ± 19.6	45.1 ± 13.6	54.4 ± 16.9	0.325	33.3	37	–	0.767
LDL (mg/dL)	89.7 ± 41.3	88.3 ± 29.2	102.6 ± 22.7	0.545	34.5	12.7	–	0.053
VLDL (mg/dL)	25 ± 21	20.7 ± 12.4	25 ± 9.4	0.531	32.3	58.7	–	**0.013**
Resistin (ng/mL)	10.7 ± 4.6	11.2 ± 5.5	9.6 ± 2.7	0.717	33.9	26	–	0.516

[a]Mean values ± standard deviations were compared using ANOVA. Statistically significant data are shown in bold type ($p < 0.05$). [b]Mean ranges were compared using Mann–Whitney non-parametric *U* tests. *Bonferroni´s correction $p<0.05$.

sition to adults and present a realistic picture of what might happen in the future bearing in mind the baseline characteristics of this subgroup and also knowing the genetic background that might contribute to specific metabolically altered states.

It should be noted that the insulin levels and the HOMA-IR values are high in our studied individuals, although our own unpublished data show elevated values in another group of previously studied subjects within the same range of age 18-25 years. There are not reference reported values of HOMA-IR for the young adults (18-25 years) in Mexican population. The studies conducted in Mexicans have included children/adolescents or adults over 30 years. Particularly in a study carried out in Mexican children and adolescents a mean value of 2.0 for HOMA-IR in subjects of 13-18 years was established (21). In Chilean population, the established cutoff value of HOMA-IR was 2.53 in individuals from 19 to 40 years, with normal weight and normal glucose values (22). In Italian individuals, in ages from 2-17.8 years, the cutoff established value was 1.68 for normal weight individuals and 3.42 for obese subjects (23). In other study conducted in physical education male students (18-24 years) from Poland, the mean HOMA-IR was 1.54 (24). The high HOMA-IR values observed in the individuals of the present study are an unexpected finding, because these young adults are characterized by an insulin resistant state and therefore they are a risk group, for this reason, a follow up program must be established for an adequate management of these young individuals, as well as for preventing further metabolic alterations.

The young students who participated in this study are also characterized by an increased body fat mass and BMI, and these conditions could have an impact on other biochemical features like the increment of the insulin and leptin levels. It has been documented the interplay between insulin and leptin, where insulin stimulates leptin secretion and in turn, once leptin is secreted, it diminishes the expression of insulin gene, however, if there exists an insulin or leptin resistant state the feedback does not control this condition (25, 26). In our young adults, we cannot ensure if the sustained insulin resistant state appeared before, after or simultaneously as fat mass increased. Considering the correlation of leptin concentrations versus adiposity parameters, even when in the present study there is no significant difference in BMI values when comparing men and women or when comparing them by weight groups (normal weight, overweight and obese), we found that the percentage of fat mass is positively correlated with leptin levels. The correlation of leptin concentration with fat mass content has been previously described (26), whereas it has been suggested that BMI is not the best parameter to associate with adiposity and leptin levels because it underestimates the obesity prevalence particularly in women with leptin levels over 30 ng/mL (27).

Very low or high levels of leptin have been demonstrated in lipodystrophic or overweight/obese individuals, respectively (28). In overweight/obese individuals the increased leptin levels is the result of leptin resistance, likely due to a deficient leptin signalling more than an absence of leptin (29). In the present study, women displayed higher serum leptin concentrations than men, which might be caused by a leptin resistant state frequently observed in women given their hormone levels. Leptin levels difference between men and women has been documented, it is argued that several factors are involved in the regulation of these differences, such as fat mass content and body fat dis-

tribution, a suggested sexual dimorphism, birth weight, caloric restriction and physical activity (27, 29-31). In addition, it has been described that as higher the testosterone level, the lower the leptin concentration, since it has been shown that after a 3-month-caloric restriction diet in obese men, there is an increment in testosterone levels, which in turn diminishes the leptin levels and increases the insulin sensitivity assessed by the leptin/adiponectin ratio (30).

Another interesting finding in the present study was that leptin/fat mass ratio is approximately 1 for men and 2 for women. In two previous studies, one conducted by Peltz et al (32) and another carried out by Chow and Phoon (33), it also was found that women presented higher leptin levels with large standard deviation compared to men, however, in these studies the leptin/fat mass ratio, 1 for men and 2 for women, was not observed. Ratio leptin/fat mass is a parameter that can be inferred but not often reported, therefore it is difficult to suggest the fact that leptin levels double the fat mass content is a characteristic of the Mexican population.

In this study, we did not observe differences in serum leptin levels regarding the genotypes of the G–2548A and A19G polymorphisms of the *LEP* gene. This finding is consistent with that previously described for the G–2548A polymorphism (34, 35). However, these outcomes remain controversial, because in the populations from Romania (10), Brazil (36) and Tunisia (37), the leptin concentrations differed among the genotypes. Regarding the A19G polymorphism, the lack of association is consistent with studies conducted in Polish (35), British (34), Italian (38) and Malaysian (39) populations, where the leptin levels did not differ with respect to genotype. However, the 19G allele was associated with serum hyperleptinaemia in a study in Tunisians (8). Even though the G–2548A and A19G polymorphisms of the *LEP* gene were not associated with leptin levels, as an interesting result of the present study, the homozygous carriers for –2548A and 19G alleles had the lowest TC and LDL concentrations compared with other genotypes. These findings are in discordance with those reported in other studies (10, 12, 37). The associations of the –2548A and 19G alleles with low TC and LDL levels can be explained because leptin regulates lipid metabolism independent of food intake; thus, leptin administration inhibits *de novo* lipogenesis and stimulates lipolysis in adipose tissue and liver through the sympathetic nervous system. In addition, leptin stimulates fatty acid oxidation by up-regulation of peroxisome proliferator-activated receptor γ-coactivator-1α and decreases the storage of TG within white adipocytes and the liver (26, 39, 40). Sequence variants in the *LEP* gene might modify these processes and have an additive effect, although the functional consequence of these polymorphisms is not very clear.

The three polymorphisms analysed in the *LEPR* gene did not show an association with the anthropometric, clinical or biochemical variables considered here. Furthermore, we found that the serum leptin concentration was not significantly different among genotypes of the A326G and A668G polymorphisms. This lack of association for both polymorphisms was also reported in a study conducted in Japanese (41), which is discordant with the results described in Koreans where the carriers of the G/G genotype for both polymorphisms had significantly higher leptin levels (9). In studies assessing only the A326G or A668G polymorphisms, the results are controversial, because in a Malaysian population the 326G allele was associated with low plasma leptin levels (12), and in other populations, no association of the 668G allele with leptin concentrations was evident (9, 10, 12, 41-43). By contrast, in obese Tunisian patients bearing the 668G/G genotype, the leptin concentration was diminished (5). Regarding the G3057A polymorphism, we found that serum leptin concentrations were higher in carriers of the 3057A allele than in those individuals bearing the 3057G/G genotype (43.78 ± 39.11 ng/mL *vs* 28.20 ± 14.12). This finding is similar to that reported in a study carried out in individuals from Nauru, in which individuals homozygous for the 3057A allele showed higher serum leptin concentrations (11); while this seems to support our findings, other studies did not show significant results (9, 44). Even when the association of increased BMI with the presence of A allele in homozygous or heterozygous state of the *LEPR* G3057A polymorphism has been described (44), we only found a non-significant tendency of increasing BMI regarding the genotype (G/G<G/A<A/A). In addition, we assessed the genotype distribution according to specific weight classification (normal, overweight, obese), nevertheless, there was no association.

The relation between C–420G and G+62A polymorphisms in the *RETN* gene and resistin concentrations has been assessed in some studies (13-15, 45, 46). In this study, there was no association with either of the two polymorphisms. However, we found that the G allele of the C–420G polymorphism was associated with higher glucose levels, as described previously in adult Hindu women (47) and in Japanese patients with T2DM (45). This finding is in contrast with a study conducted in Finnish subjects with hypertension (13). Both the C and G alleles of the C–420G polymorphism show different affinities for stimulatory protein (Sp)1 and Sp3 transcription factors. Sp1 and Sp3 bind specifically to DNA with higher affinity in the presence of the –420G allele and increase the promoter activity of the *RETN* gene (45, 48). On the other hand, it has been reported that a hyperglycaemic state increases the resistin expression in rodents (48), and given that the –420G allele increases resistin expression, these two factors might have contributed to increased glucose levels in our study group. Therefore, we hypothesize that if the G allele carriers of C–420G polymorphism were exposed to a hyperglycaemic diet, this could mark the beginning of a positive feedback where the increase in glucose concentration might stimulate resistin production, and this, in turn, could promote sustained insulin resistance.

It has been reported that 3′ UTR polymorphisms could affect gene expression (49-51), and it has been proposed that the G+62A polymorphism might modify the *RETN* gene expression (46). However, few publications have evaluated the effect of this polymorphism on resistin levels. A study on a German population did not establish any association of the G+62A polymorphism with serum resistin concentrations (46). In a previous study in a population from western Mexico aged 20–69 years (15), carriers of the G/A genotype showed lower serum resistin, TG and VLDL levels. In our study popu-

lation, there was also an association with lower VLDL levels, but in the presence of the G/G genotype. It has been reported that resistin promotes intracellular lipid storage, as described by Ikeda et al. (52). Nevertheless, these results should be regarded with caution because of the low frequency of this polymorphism in most populations; besides, the sample size and the absence of the A/A genotype in both Mexican studies must be borne in mind, as this might influence the variability in results.

In conclusion, in this study, only the G3057A polymorphism in the *LEPR* gene was found to contribute significantly to variations in serum leptin levels. However, we should consider other factors that might have masked the effect of the other polymorphisms analysed, such as lifestyle, gender and pro-inflammatory markers. Nevertheless, our results represent an initial search for a characteristic genetic profile influencing leptin and resistin serum levels, which in turn could represent metabolically altered states in Mexican young adults. This is because when there is one alteration in such clinical or biochemical parameters, the subjects have an increased risk of developing two or more disturbances later on, leading to metabolic syndrome.

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Conflict of interest
The authors declare that they have no conflict of intrest.

Author's contribution
All co-authors have made substantial contributions taking due care to ensure the integrity of the work and have reviewed and accepted the final version of the manuscript. However, the specific contribution of every participant author is mentioned below.
Andres López-Quintero. Acquisition, analysis and interpretation of data, drafting of the article, and final approval of the version to be published.
Alejandra Guadalupe García-Zapién. Conception and design of the work, and final approval of the version to be published.
Silvia Esperanza Flores-Martínez. Critical revision for important intellectual content, drafting of the article, and final approval of the version to be published.
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References

1. Leal V de O, Mafra D. Adipokines in obesity. Clin Chim Acta 2013; 419:87–94.
2. Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. J Cardiol 2014; 63:250–259.
3. Jung U, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci 2014; 15:6184–6223.
4. Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014; 2014:943162.
5. Ben Ali S, Kallel A, Sediri Y, Ftouhi B, Feki M, Slimene H *et al*. *LEPR* p.Q223R Polymorphism influences plasma leptin levels and body mass index in Tunisian obese patients. Arch Med Res 2009; 40:186–190.
6. Wang Z, Nakayama T. Inflammation, a link between obesity and cardiovascular disease. Mediators Inflamm 2010; 2010:535918.
7. Choquet H, Meyre D. Molecular basis of obesity: current status and future prospects. Curr Genomics 2011; 12:154–168.
8. Fourati M, Mnif M, Kharrat N, Charfi N, Kammoun M, Fendri N *et al*. Association between leptin gene polymorphisms and plasma leptin level in three consanguineous families with obesity. Gene 2013; 527:75–81.
9. Woo HY, Park H, Ki CS, Park YL, Bae WG. Relationships among serum leptin, leptin receptor gene polymorphisms, and breast cancer in Korea. Cancer Lett 2006; 237:137–142.
10. Constantin A, Costache G, Sima AV, Glavce CS, Vladica M, Popov DL. Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects. Biochem Biophys Res Commun 2010; 391:282–286.
11. de Silva AM, Walder KR, Aitman TJ, Gotoda T, Goldstone AP, Hodge AM *et al*. Combination of polymorphisms in OB-R and the OB gene associated with insulin resistance in Nauruan males. Int J Obes Relat Metab Disord 1999; 23:816–822.
12. Fan SH, Say YH. Leptin and leptin receptor gene polymorphisms and their association with plasma leptin levels and obesity in a multi-ethnic Malaysian suburban population. J Physiol Anthropol 2014; 33:15.
13. Ukkola O, Kunnari A, Kesäniemi YA. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. Regul Pept 2008; 149:56–59.
14. Tsukahara T, Nakashima E, Watarai A, Hamada Y, Naruse K, Kamiya H *et al.* Polymorphism in resistin promoter region at −420 determines the serum resistin levels and may be a risk marker of stroke in Japanese type 2 diabetic patients. Diabetes Res Clin Pract 2009; 84:179–186.
15. Chavarria-Ávila E, Ruíz Quezada SL, Guzmán-Ornelas MO, Castro-Albarrán J, Aguila Aldrete ME, Vásquez-Del Mercado M, *et al*. [Association of resistin gene 3'UTR +62G>A polymorphism with insulin resistance, adiposity and the adiponectin-resistin index in Mexican population]. Nutr Hosp 2013; 28:1867–1876.
16. Gustincich S, Manfioletti G, Del Sal G, Schneider C, Carninci P. A fast method for high-quality genomic DNA extraction from whole human blood. Biotechniques 1991; 11:298–300.
17. Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD *et al*. Leptin receptor gene variation and obesity: lack of association in a white British male population. Hum Mol Genet 1997; 6:869–876.
18. Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T *et al*. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. Diabetologia 1997; 40:1204–1210.

19. Karvonen MK, Pesonen U, Heinonen P, Laakso M, Rissanen A, Naukkarinen H *et al*. Identification of new sequence variants in the leptin gene. J Clin Endocrinol Metab 1998; 83:3239–3242.
20. Krízová J, Dolinková M, Lacinová Z, Sulek S, Dolezalová R, Housová J *et al*. Adiponectin and resistin gene polymorphisms in patients with anorexia nervosa and obesity and its influence on metabolic phenotype. Physiol Res 2008; 57:539–546.
21. Aradillas-García C, Rodríguez-Morán M, Garay-Sevilla ME, Malacara JM, Rascon-Pacheco RA, Guerrero-Romero F. Distribution of the homeostasis model assessment of insulin resistance in Mexican children and adolescents. Eur J Endocrinol 2012; 166:301-306.
22. Acosta AM, Escalona M, Maiz A, Pollak F, Leighton F. [Determination of the insulin resistance index by the Homeostasis Model Assessment in a population of Metropolitan Region in Chile]. Rev Med Chil 2002; 130:1227-1231.
23. Shashaj B, Luciano R, Contoli B, Morino GS, Spreghini MR, Rustico C *et al*. Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. Acta Diabetol 2016; 53:251-260.
24. Keska A, Lutoslawska G, Czajkowska A, Tkaczyk J, Mazurek K. Variability in HOMA-IR, lipoprotein profile and selected hormones in young active men. ScientificWorldJournal 2013; 2013:412764.
25. Moon HS, Dalamaga M, Kim SY, Polyzos SA, Hamnvik OP, Magkos F *et al*. Leptin's role in lipodystrophic and nonlipodystrophic insulin-resistant and diabetic individuals. Endocr Rev 2013; 34:377-412.
26. Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism 2015; 64:24–34.
27. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. PLoS One 2012; 7:e33308.
28. Grundy SM. Adipose tissue and metabolic syndrome: too much, too little or neither. Eur J Clin Invest 2015; 45:1209-1217.
29. Ozcelik O, Dogan H, Celik H, Ayar A, Serhatlioglu S, Kelestimur H. Effects of different weight loss protocols on serum leptin levels in obese females. Physiol Res 2005; 54:271-277.
30. Schulte DM, Hahn M, Oberhäuser F, Malchau G, Schubert M, Heppner C *et al*. Caloric restriction increases serum testosterone concentrations in obese male subjects by two distinct mechanisms. Horm Metab Res 2014; 46:283-286.
31. Labayen I, Ruiz JR, Huybrechts I, Ortega FB, Rodríguez G, Dehenauw S *et al*. Sexual dimorphism in the early life programming of serum leptin levels in European adolescents: the HELENA study. J Clin Endocrinol Metab 2011; 96:E1330-1334.
32. Peltz G, Sanderson M, Pérez A, Sexton K, Ochoa Casares D, Fadden MK. Serum leptin concentration, adiposity, and body fat distribution in Mexican-Americans. Arch Med Res 2007; 38:563-570.
33. Chow VT, Phoon MC. Measurement of serum leptin concentrations in university undergraduates by competitive ELISA reveals correlations with body mass index and sex. Adv Physiol Educ 2003; 27:70-77.
34. Gaukrodger N, Mayosi BM, Imrie H, Avery P, Baker M, Connell JMC *et al*. A rare variant of the leptin gene has large effects on blood pressure and carotid intima-medial thickness: a study of 1428 individuals in 248 families. J Med Genet 2005; 42:474–478.
35. Cieslak J, Bartz M, Stachowiak M, Skowronska B, Majewska KA, Harasymczuk J *et al.* Effect of three common SNPs in 5′-flanking region of *LEP* and *ADIPOQ* genes on their expression in Polish obese children and adolescents. Mol Biol Rep 2012; 39:3951–3955.
36. Hinuy H, Hirata M, Forti N, Diament J, Sampaio M, Armaganijan D *et al*. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arq Bras Endocrinol Metab 2008; 52:611–616.
37. Ben Ali S, Kallel A, Ftouhi B, Sediri Y, Feki M, Slimane H *et al*. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. Clin Biochem 2009; 42:584–588.
38. Lucantoni R, Ponti E, Berselli ME, Savia G, Minocci A, Calò G *et al*. The A19G polymorphism in the 5′ untranslated region of the human obese gene does not affect leptin levels in severely obese patients. J Clin Endocrinol Metab 2000; 85:3589–3591.
39. Lee Y, Yu X, Gonzales F, Mangelsdorf DJ, Wang MY, Richardson C *et al*. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. Proc Natl Acad Sci U S A 2002; 99:11848–11853.
40. Minokoshi Y, Kim YB, Peroni OD, Fryer LGD, Müller C, Carling D *et al*. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002; 415:339–343.
41. Ogawa T, Hirose H, Yamamoto Y, Nishikai K, Miyashita K, Nakamura H *et al*. Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. Metabolism 2004; 53:879–885.
42. Becer E, Mehmetçik G, Bareke H, Serakinci N. Association of leptin receptor gene Q223R polymorphism on lipid profiles in comparison study between obese and non-obese subjects. Gene 2013; 529:16–20.
43. Répásy J, Bokor S, Erhardt É, Molnár D. Association of Gln223 Arg polymorphism of the leptin receptor gene with indicators of energy expenditure in obese children. Nutrition 2014; 30:837–840.
44. Ben Ali S, Sediri Y, Kallel A, Ftouhi B, Haj-Taib S, Omar S *et al*. The G3057A *LEPR* polymorphism is associated with obesity in Tunisian women. Nutr Metab Cardiovasc Dis 2011; 21:591–596.
45. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H *et al*. The G/G genotype of a resistin single-nucleotide polymorphism at −420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am J Hum Genet 2004; 75:678–686.
46. Gouni-Berthold I, Giannakidou E, Faust M, Kratzsch J, Berthold HK, Krone W. Resistin gene 3′-untranslated region +62G→A polymorphism is associated with hypertension but not diabetes mellitus type 2 in a German population. J Intern Med 2005; 258:518–526.
47. Kumar S, Gupta V, Srivastava N, Gupta V, Mishra S, Mishra S *et al*. Resistin 420C/G gene polymorphism on circulating resistin, metabolic risk factors and insulin resistance in adult women. Immunol Lett 2014; 162:287–291.
48. Chung SS, Choi HH, Kim KW, Cho YM, Lee HK, Park KS. Regulation of human resistin gene expression in cell systems: an important role of stimulatory protein 1 interaction with a common promoter polymorphic site. Diabetologia 2005; 48:1150–1158.
49. Jupe ER, Badgett AA, Neas BR, Craft MA, Mitchell DS, Resta R *et al*. Single nucleotide polymorphism in prohibitin 3' untranslated region and breast-cancer susceptibility. Lancet 2001; 357:1588–1589.
50. Pesole G, Mignone F, Gissi C, Grillo G, Licciulli F, Liuni S. Structural and functional features of eukaryotic mRNA untranslated regions. Gene 2001; 276:73–81.
51. Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M *et al*. A variation in 3′ UTR of *hPTP1B* increases specific gene expression and associates with insulin resistance. Am J Hum Genet 2002; 70:806–812.
52. Ikeda Y, Tsuchiya H, Hama S, Kajimoto K, Kogure K. Resistin affects lipid metabolism during adipocyte maturation of 3T3-L1 cells. FEBS J 2013; 280:5884–5895.