

Relationship between variants of the leptin gene and obesity and metabolic biomarkers in Brazilian individuals

Associação entre variantes do gene de leptina e obesidade e biomarcadores metabólicos em indivíduos brasileiros

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ABSTRACT

Objective: The relationship between variants of the leptin gene (*LEP*) and obesity and metabolic biomarkers was investigated in Brazilian individuals. **Subjects and methods:** One-hundred-ten obese (BMI > 30 kg/m²) and 100 non-obese individuals (145 women and 65 men, aged 49 ± 14 years) were randomly selected. Plasma leptin, glycemia, serum lipid measurements and *LEP* -2548G>A and 3'HVR polymorphisms were analyzed. **Results:** The *LEP* -2548GG genotype was associated with a 2.2% and 2.0% increase in BMI (p = 0.009) and plasma leptin (p = 0.031), respectively. 3'HVR I/II (classes I/I+I/II) genotypes contributed with 1.8% of BMI values (p = 0.046). *LEP* I/G combined genotypes (I/IGG, I/IGA and I/IIGG) were associated with obesity, and increased BMI, waist circumference, leptin and triglycerides (p < 0.05). These relationships were found in women (p < 0.05) but not in men. *LEP* I/G combined genotypes were not associated with hypertension, hyperglycemia, dyslipidemia and coronary artery disease. **Conclusions:** *LEP* I/G combined genotypes are associated with obesity-related metabolic biomarkers and phenotype in a gender-dependent manner. Arq Bras Endocrinol Metab. 2010;54(3):282-8

Keywords

Leptin; gene polymorphism; obesity; metabolic biomarkers; plasma leptin

RESUMO

Objetivo: A relação entre as variantes do gene da leptina (*LEP*) e obesidade e biomarcadores metabólicos foi investigada em indivíduos brasileiros. **Sujeitos e métodos:** Cento e dez indivíduos obesos (IMC > 30 kg/m²) e 100 não obesos (145 mulheres e 65 homens, idade 49 ± 14 anos) foram selecionados aleatoriamente. Leptina plasmática, glicemia, lípidos séricos e polimorfismos *LEP* -2548G>A e 3'HVR foram analisados. **Resultados:** O genótipo -2548GG foi associado com aumento de 2,2% e 2,0% no IMC (p = 0,009) e leptina plasmática (p = 0,031), respectivamente, enquanto os genótipos 3'HVR I/II (classes I/I+I/II) contribuíram com 1,8% dos valores de IMC (p = 0,046). Os genótipos combinados *LEP* I/G (I/IGG, I/IGA e I/IIGG) foram associados com obesidade e IMC aumentado, circunferência abdominal, leptina e triglicérides aumentados (p < 0,05). Essas relações foram encontradas em mulheres (p < 0,05), mas não em homens. Os genótipos *LEP* I/G combinados não foram associados com hipertensão, hiperglicemia, dislipidemia e doença arterial coronariana. **Conclusões:** Genótipos combinados *LEP* I/G são associados com biomarcadores metabólicos e fenótipo de obesidade de forma gênero-dependente. Arq Bras Endocrinol Metab. 2010;54(3):282-8

Descritores

Leptina; polimorfismo genético; obesidade; biomarcadores metabólicos; leptina plasmática

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INTRODUCTION

Leptin is a metabolic and neuroendocrine hormone produced and released mainly by adipocytes (1). Several systemic effects are attributed to leptin such as body mass control, lipid and glucose metabolism, thermogenesis, angiogenesis, immunity, reproductive, endocrine and cardiovascular functions, among other (2). Increased leptin is associated with adiposity and it is expected to reduce food intake and increase energy expenditure by binding to leptin receptors that further activate the hypothalamic melanocortin pathway and control body fat stores (3).

Several studies have suggested that variants in the leptin gene (*LEP*) may be important in the pathophysiology of human obesity (4-6). A common single nucleotide polymorphism (SNP) within the 5' promoter region (-2548G>A) of the *LEP* has been associated with variations in plasma leptin and body mass index (BMI) in obese individuals (6-9).

Microsatellite markers such as the highly variable tetranucleotide repeat (TTTC)_n located in the 3'-flanking region of the *LEP* (3'HVR) has also been associated with obesity-related traits and leptin plasma levels (6,10,11). However, the relation of this variant with other polymorphisms in the *LEP* gene on metabolic and obesity-related characteristics remains to be investigated.

In this study, the relationship between *LEP* 3'HVR and -2548G>A variants and obesity-related phenotype and metabolic biomarkers was investigated in a sample of the Brazilian population.

SUBJECTS AND METHODS

Study population

Two-hundred-ten unrelated individuals were randomly selected from Instituto Dante Pazzanese de Cardiologia (IDPC), Sao Paulo, Brazil. These individuals were declared to have European ancestry (non-African) by physical examination, however lack of relationship between color and genomic ancestry have been found in Brazilian samples (12). Sample size was estimated as 267 individuals considering 50% the frequency of *LEP* alleles related with obesity (95% confidence level, 5% confidence interval, and over 20,000 population size). Individuals with thyroid, liver or kidney diseases, evaluated by clinical and laboratory analyses, and pregnant women were not included in this sample.

Anthropometric measurements, such as BMI, waist circumference (WC) and waist-to-hip ratio (WHR) were taken from each participant. Individuals with BMI ≥ 30 kg/m² were classified as obese and those with systolic/diastolic blood pressure over 140/90 mmHg or under lowering-pressure therapy were considered hypertensive. Individuals with fasting glycemia > 100 mg/dL were considered hyperglycemic. Cigarette smoking was considered when individuals smoked 3 or more cigarettes/day. Sedentary life style was considered when individuals practiced less than a 30 min walk 3 times a week. The presence of coronary artery disease (CAD) was investigated by coronary angiography. The study protocol was approved by the Local Ethics Committees (Institute of Cardiology Dante Pazzanese and School of Pharmaceutical Sciences, University of Sao Paulo).

Biomarker analysis

Blood samples were collected from each participant after a 12-hour fasting for serum chemistry tests. Glucose, triglycerides, and total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by enzymatic-colorimetric assays using a Roche-Hitachi 912 automated analyzer (Hitachi, Nakakojo, Japan). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol were calculated. Apolipoproteins AI (apoAI) and B (apoB) were determined in serum by nephelometry. Plasma leptin was determined by an ELISA method (Alexis Biochemical/Vendor BioAgency, Sao Paulo, Brazil). The atherogenic index of plasma (AIP) calculated as log (triglycerides/HDL cholesterol), and ApoB/ApoAI ratio were used for evaluation of the cardiovascular risk.

DNA genotyping

Genomic DNA was extracted from 1 mL EDTA-anti-coagulated whole blood by a salting-out method (13). *LEP* -2548G>A and 3'HVR polymorphisms were genotyped by polymerase chain reaction (PCR) and fragment analysis as previously described (8,11). The accuracy of the genotyping was evaluated by performing duplicate analysis of 20% samples randomly selected. Moreover, heterozygous *LEP* -2458G>A and 3'HVR samples were included as genotype controls in each run.

Statistical analysis

LEP 3'HVR alleles were grouped in class I (short) and class II (long) as previously described (11). *LEP* com-

bined genotypes were formed by grouping 3'HVR and -2548G>A genotypes. The agreement of genotype frequencies with Hardy-Weinberg equilibrium (HWE) expectations was tested by the chi-square test. The linkage disequilibrium between LEP variants was estimated using the SNPAnalyzer software (D' index) (14). Relationships between the genotypes and categorical variables were evaluated by the chi-square test or Exact Fisher test.

Continuous variables without normal distribution (BMI, WC, leptin, glucose and lipid profile) were log transformed prior to the statistical analysis. Anthropometric and blood chemistry parameters were compared between LEP variants by One-way ANOVA and *t*-test. Pearson's correlation coefficients were used to estimate the association between continuous variables.

Logistic and linear regression analyses were used to verify the relationships between obesity, LEP variants and other variables such as BMI, WC, WHR and leptin. The models were adjusted by the covariates of age, gender, hypertension, hyperglycemia, CAD, cigarette smoking and sedentary life style. Statistical tests were performed by SAS System for Windows software version 8.02 (SAS Institute Inc, 1999-2001, Cary, NC, USA). The level of significance was considered $p < 0.05$.

RESULTS

Anthropometric, demographic and biochemical data of the studied group are shown in table 1. Four women refused to inform their ages. One woman and one man did not have their waist and hip measured. Obese individuals (BMI > 30 kg/m²) had higher means of age and anthropometric data (BMI, WC and WHR) as well as higher frequencies of hypertension, hyperglycemia, cigarette smoking, sedentary life style and CAD when compared with the non-obese group ($p < 0.05$). Plasma leptin was also higher in obese than in the non-obese group ($p < 0.05$).

LEP 3'HVR class I/I and I/II genotypes were more frequent in obese (I/I: 24%, I/II: 56%) than in non-obese individuals (I/I: 21%, I/II: 44%, $p = 0.043$) (Table 1). For -2548G>A polymorphism, the frequency of the GG genotype in obese (41%) was similar to that found in non-obese (28%) ($p = 0.090$) individuals. Both LEP 3'HVR and -2548G>A variants were under HWE in this sample (Table 1). A linkage disequilibrium was found between these polymorphisms (D' index = 0.7113). We found nine LEP combined genotypes

that were grouped as: I/G (I/IGG + I/IGA + I/IIGG); II/A (I/IIAA + II/IIAA + II/IIGA); and others (I/IAA + I/IIGA + II/IIGG). Obese individuals had higher frequencies of I/G combined genotypes (45%) than non-obese subjects (27%, $p = 0.018$).

Results from univariate logistic regression analysis are shown in table 2. Variables were adjusted by the covariates of age, hypertension, hyperglycemia, cigarette smoking, sedentary life style and CAD due to their association with obesity previously demonstrated (13). Individuals carrying 3'HVR class I/II genotype had 3 times higher risk for obesity (OR: 3.12, 95% CI: 1.21 - 8.08) compared to those with class II/II genotype ($p = 0.019$). The carriers of -2548GA and GG genotypes had, respectively, 4 times (OR: 4.75, 95% CI: 1.30 - 17.36) and 8 times (OR: 8.695, 95% CI: 2.22 - 34.05)

Table 1. Anthropometric and laboratory data of the study group

	Non-obese	Obese	p-value
Number of individuals	110	100	
Age, years	45 ± 15	52 ± 13	< 0.001
Gender, women	59%	41%	0.378
Hypertension	9%	72%	< 0.001
Hyperglycemia	1%	25%	< 0.001
Coronary artery disease	8%	14%	< 0.001
Cigarette smoking	30%	43%	0.020
Sedentary life style	59%	75%	0.015
Body mass index, kg/m ² *	24.2 ± 3.1	35.6 ± 4.7	< 0.001
Waist circumference, cm	87.3 ± 10.8	109.6 ± 11.4	< 0.001
Waist-hip ratio	0.87 ± 0.07	0.92 ± 0.06	< 0.001
Plasma leptin, ng/mL*	12.0 ± 12.0	35.1 ± 21.3	< 0.001
LEP 3'HVR			
Class I/I genotype	21% (23)	24% (24)	0.043
Class I/II genotype	44% (48)	56% (56)	
Class II/II genotype	35% (39)	20% (20)	
HWE (p-value)	0.256	0.223	
LEP -2548G > A			
GG genotype	28% (31)	41% (41)	0.090
GA genotype	52% (57)	47% (47)	
AA genotype	20% (22)	12% (12)	
HWE (p-value)	0.649	0.793	
LEP combined genotypes			
I/G (I/IGG + I/IGA + I/IIGG)	27% (30)	45% (45)	0.018
II/A (I/IIAA + II/IIAA + II/IIGA)	31% (34)	19% (19)	
Others (I/IAA + I/IIGA + II/IIGG)	42% (46)	36% (36)	

Continuous variables are presented as mean ± SD and compared by *t*-test. Categorical variables were compared by chi-square test. HWE, Hardy-Weinberg equilibrium. Four women refused to inform their ages. One man and a woman did not have their waist and hip measured. Obesity: body mass index ≥ 30 kg/m². Hypertension: blood pressure > 140/90 mmHg or use of lowering-pressure therapy. Hyperglycemia: blood glucose > 100 mg/dL. Cigarette smoking: 3 cigarettes/day. Sedentary life style: to practice less than a 30 min walk 3 times a week. (*) Values were log transformed.

Table 2. Univariate logistic regression analysis of the *LEP* variants associated with obesity

Variables	Categories	Odds ratio	95% CI	p-value
3'HVR genotypes	Class II/II	1.00	-	-
	Class I/II	3.12	1.21 - 8.08	0.019
	Class I/I	1.89	0.60 - 5.98	0.278
-2548G > A genotypes	AA	1.00	-	-
	GA	4.75	1.30 - 17.36	0.019
	GG	8.69	2.22 - 34.05	0.002
<i>LEP</i> combined genotypes	II/A	1.00	-	-
	I/G	3.97	1.30 - 5.55	0.012
	Other	2.62	0.69 - 2.85	0.068

Variables were adjusted for covariates: age, gender, hypertension, hyperglycemia, coronary artery disease, cigarette smoking and sedentary life style. CI, confidence interval. I/G genotypes, I/IGG + I/IGA + I/IGG; II/A genotypes, I/IIAA+II/IIAA+II/GA; Other genotypes, I/IIA + I/IIA + II/II + GG.

more risk for obesity than the -2548AA genotype carriers ($p < 0.05$). In addition, the risk for obesity was 4 times higher in I/G carriers (OR: 3.97, 95% CI: 1.30 - 5.55) than in those carrying II/A ($p = 0.012$).

Multivariate logistic regression analysis using stepwise criteria for selection of the genetic variables related with obesity was also tested. After adjustment for covariates (age, hypertension, hyperglycemia, cigarette smoking, sedentary life style and CAD), only the *LEP* -2548GG genotype was associated with an increased risk for obesity (OR: 9.43, 95% CI: 1.84 - 48.31; $p = 0.007$) (data not shown).

The relationship between *LEP* variants and anthropometric and blood chemistry variables were also investigated in this study (Table 3). Individuals carrying I/G genotypes had higher serum triglycerides when compared to the II/A carriers ($p < 0.05$). AIP values were also higher in I/G carriers than in those carrying other genotypes ($p < 0.05$). We investigated whether the relationship between *LEP* variants and these variables were influenced by gender, considering that leptin and obesity-related traits (BMI, WC, WHR) were higher in women than in men ($p < 0.05$) (data not shown). I/G genotypes were associated with higher WC, BMI and leptin values in women but not in men (Figure 1).

Univariate linear regression analysis showed that the variations in log-transformed values of the BMI were partially explained by *LEP* 3'HVR class I/II genotype (1.80%, $p = 0.046$) and -2548GG genotype (2.20%, $p = 0.009$), as indicated by the determinant coefficient (R^2) (Table 4). Moreover, only 2% of variation in log-transformed plasma leptin is explained by *LEP* -2548GG genotype ($p = 0.031$). Regression coefficients (estimates) indicate that the carriers of the

Table 3. Anthropometric and blood chemistry data in individuals carrying the *LEP* genotypes

Variables	I/G	II/A	Others	p-value
Number of individuals	75	53	82	
Body mass index, kg/m ² *	30.9 ± 6.9	28.0 ± 6.4	29.6 ± 7.1	0.070
Waist circumference, cm	101 ± 16	95 ± 15	96 ± 15	0.056
Waist-hip ratio, %	91 ± 7	89 ± 7	88 ± 7	0.082
Leptin, ng/mL*	26.1 ± 20.6	17.8 ± 15.6	23.4 ± 14.7	0.228
Glucose, mg/dL*	104 ± 27	101 ± 96	103 ± 30	0.850
Total cholesterol, mg/dL*	215 ± 42	214 ± 47	204 ± 42	0.203
HDL-c, mg/dL*	53 ± 13	56 ± 14	55 ± 15	0.461
LDL-c, mg/dL*	132 ± 34	129 ± 41	122 ± 34	0.222
VLDL-c, mg/dL*	30 ± 11	28 ± 15	27 ± 13	0.101
Triglycerides, mg/dL*	153 ± 60 ^a	147 ± 83 ^b	132 ± 65 ^{a,b}	0.039
ApoA1, mg/dL*	136 ± 26	137 ± 26	136 ± 26	0.995
ApoB, mg/dL*	112 ± 27	108 ± 28	102 ± 24	0.099
ApoB/ApoA1 ratio*	0.84 ± 0.25	0.82 ± 0.28	0.77 ± 0.21	0.202
AIP [log (triglycerides/ HDL-c)]*	0.44 ± 0.23 ^a	0.37 ± 0.29 ^{a,b}	0.34 ± 0.27 ^b	0.048

Results are presented as mean ± SD and compared by one-way ANOVA. Values in a row with different superscript letters are significantly different, $p < 0.05$ (Tukey's test). AIP, atherogenic index of plasma; ApoA1, apolipoprotein I; ApoB, apolipoprotein B; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very low-density lipoprotein cholesterol. (*) log-transformed data.

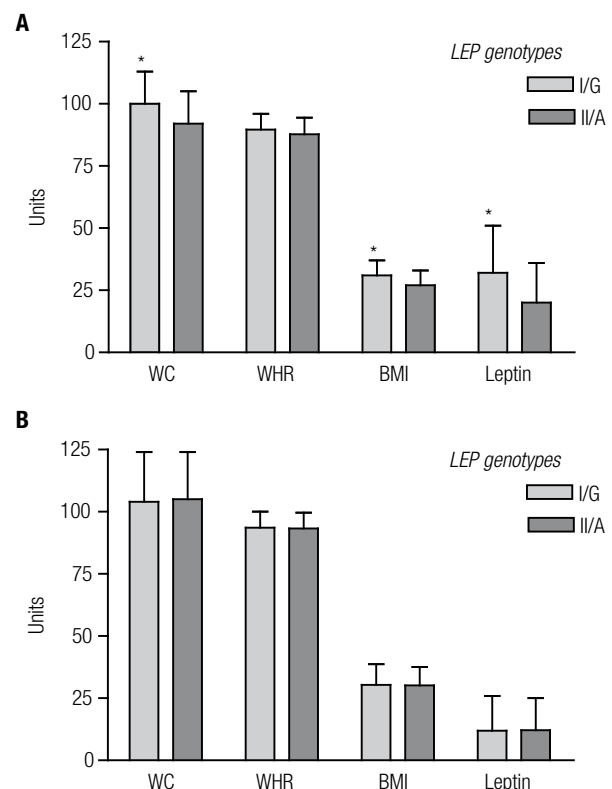


Figure 1. Anthropometric and plasma leptin values in women (A) and men (B) carrying *LEP* I/G and II/A combined genotypes. Waist circumference (WC, cm), waist-hip ratio (WHR, %), body mass index (BMI, kg/m²), plasma leptin (ng/mL). Values presented as mean ± SD are compared by t-test (* $p < 0.05$).

Table 4. Univariate linear regression analysis of the variables associated with *LEP* variants

Dependent variables	<i>LEP</i> variants	R ²	Estimate (SE)	p-value
Waist circumference	3'HVR class	0.0121	3.74 (2.07)	0.073
	I/II genotype			
	-2548GG genotype	0.0077	4.15 (2.67)	0.122
	I/G genotypes	0.0081	3.70 (2.30)	0.109
Waist-hip ratio	3'HVR class	0.0037	0.01 (0.01)	0.884
	I/II genotype			
	-2548GG genotype	0.0011	0.01 (0.01)	0.565
	I/G genotypes	0.0061	0.01 (0.01)	0.590
Body mass index*	3'HVR class	0.0180	0.03 (0.01)	0.046
	I/II genotype			
	-2548GG genotype	0.0220	0.04 (0.02)	0.009
	I/G genotypes	0.0131	0.02 (0.01)	0.077
Leptin*	3'HVR class	0.0099	0.11 (0.06)	0.060
	I/II genotype			
	-2548GG genotype	0.0200	0.20 (0.07)	0.031
	I/G genotypes	0.0177	0.12 (0.06)	0.054

Variables were adjusted to the covariates: age, gender, hypertension, hyperglycemia, CAD, cigarette smoking and sedentary file stile. SE, standard error. (*) log-transformed data.

3'HVR class I/II genotype had log-transformed BMI 0.03 more than the non-carriers. Moreover, log plasma leptin was 0.11 higher in 3'HVR class I/II genotype and 0.20 higher in -2548GG genotype in comparison with those carrying other genotypes (Table 4).

We also investigated whether the *LEP* variants were associated with hypertension, hyperglycemia, dyslipidemia and CAD but no relationship was found between these clinical conditions and *LEP* variants in this sample (data not shown).

DISCUSSION

The *LEP* variants evaluated in this sample were associated with obesity. Individuals carrying the -2548GG genotype have higher risk for increased BMI than carriers of the -2548GA>AA genotypes. Moreover, the -2548GG genotype carriers have 8 times more risk for obesity than the non-carriers suggesting that *LEP* -2458G>A polymorphism maybe a good predictor for obesity.

The association between *LEP* -2548G>A polymorphism and increased BMI was also found in overweight Europeans and Taiwanese Aborigines with extreme obesity (15,16). We have also found a relationship between the -2548G>A variant and increased plasma leptin and BMI in Brazilian women (8). It is important to mention that the relationship between -2548GG genotype and obesity was found in recessive and co-dominant models, but not in the dominant model (16).

In addition, common variants located in the 5' region of the *LEP*, including -2548G>A, were associated with increased BMI in men (17). The presence of the GG genotype was also associated with increase in BMI and weight gain in persons treated with olanzapine (18-20). However, no association was found between this polymorphism and obesity in a population-based case control study in Spain (21).

In this study, *LEP* -2458GG genotype was also shown to be a predictor for increased plasma leptin. This result confirms the findings from other investigations that showed an association between -2458AA (-/-) genotype and lower leptin levels in Caucasian obese girls and diabetic individuals from China (22,23). Conversely, as commented previously, -2458AA genotype was associated with increased plasma leptin in obese individuals (15). Moreover, carriers of this genotype had lesser decrease in circulating leptin after gastric banding surgery (24). The results from these studies are suggestive that the relationship between *LEP* -2548G>A and obesity-related phenotypes may be influenced by sample characteristics such as gender, sample size, population and other.

The variant -2548G>A has been implicated in the regulation of *LEP* mRNA expression and consequently leptin plasma levels (25). Gong and cols. (26) have described two repetitive sequences MER11 and Alu located at the *LEP* promoter (-2514 to 1545) that may regulate *LEP* expression (26). In addition, the 3-kb 5'-flanking region contains several putative binding sites for known transcription factors, including CREB, GRE, Sp1, LP1, C/EBP and TATA motifs (26,27). A Sp1 functional placental enhancer was found within the MER11 repetitive element suggesting that expression of leptin is the result of insertion of this element (28). Recently, Moreno-Aliaga and cols. (29) reported that the Sp1 binding site has a key role in the transcriptional activation of the *LEP* promoter by insulin-mediated glucose metabolism (29). Whether the -2548G>A variant located close to a Sp1 binding site (-2539) also modulates the transcription of the *LEP* remains to be investigated.

The role of leptin in body fat control is mediated by leptin receptors (LEPR) in specific hypothalamic centers that stimulate the production of anorectic peptides and inhibit the orexigenic molecules (30). LEPR gene (*LEPR*) polymorphisms have also been associated with increased BMI and other obesity-related characteristics in several populations (4,31-33). The combination of *LEP* -2548G>A and *LEPR* Q223R variants was related

to a 58% increase in obesity risk in another sample of our population (34). Therefore, the relationship between *LEP* -2548G>A and increased BMI found in this study may be due to its linkage to Q223R or other *LEPR* polymorphisms.

The *LEP* 3'HVR is a (TTTC)_n tetranucleotide repeat located at the 3' end of the *LEP* that has been associated with increased risk for obesity and high plasma leptin levels as well (10-11). These effects may be due to the linkage of the 3'HVR with -2548G>A variant.

The presence of the *LEPI*/G genotypes was associated with an increased risk for obesity-related phenotype (BMI and WC) and the biomarker of plasma leptin. The influence of I/G variant was further demonstrated to be related to gender, even though this variable did not affect the distribution of the *LEP* I/G genotypes. Accordingly, it has been shown that the association between a *LEP* variant (A19G) and risk for obesity was restricted to females from the Atherosclerosis Risk in Communities (ARIC) Study (35).

Differences in plasma glucose were not related to *LEPI*/G genotypes, confirming the lack of association between these variants and hyperglycemia. This result suggests that *LEP* variants may not be implicated in glucose homeostasis. Accordingly, insulin responses to glucose and insulin sensitivity were similar among Japanese carriers of *LEP* 3'HVR genotypes (36). Serum triglycerides and AIP values were found to be higher in *LEPI*/G carriers suggesting a possible role of *LEP* combined genotypes in lipid metabolism and cardiovascular risk. However, association analyses did not show relationship of the *LEP* variants with dyslipidemia and CAD evaluated by coronary angiography. Therefore, further investigations are necessary to evaluate these data in larger populations.

The lack of association between hypertension or cardiovascular disease and *LEP* 3'HVR has been also shown in other populations (37-39). Therefore, the association between obesity and hypertension and other risk factors for CAD previously found in our sample (8) seems to be independent of the *LEPI*/G genotypes.

It is noteworthy that the results from this study may be influenced by the limited sample size, which reduced the power of the statistical tests. Therefore studies with larger samples from the Brazilian population are necessary to confirm our findings.

In conclusion, -2548G>A and 3'HVR variants of the *LEP* gene are in linkage disequilibrium, and I/G combined genotypes are associated with obesity-related

biomarkers and phenotype and it is likely that they contribute to the regulation of circulating leptin and triglycerides in a gender-dependent manner.

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