Journal of Obesity and Overweight
Volume 8 | Issue 1
ISSN: 2455-7633

Research Article Open Access

Leptin Receptor Gene Variant Rs1137101 and Ghrelin Gene Variant Rs696217 are Associated with Body Mass Index in Brazilian Population: A Case-Control Study

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Citation: Ferreira-Júlio MA, Barato M, Molina GM, Noronha NY, Watanabe ML, et.al, (2022) Leptin Receptor Gene Variant Rs1137101 and Ghrelin Gene Variant Rs696217 are Associated with Body Max Index in Brazilian Population: A Case-Control Study. J Obes Overweig 8(1): 103

Abstract

Introduction: Obesity is a multifactorial condition influenced by environment and genetic factors. Controlling appetite and satiety involves complex interactions between the hypothalamus, which is responsible for homeostasis regulation energy, and hormones that regulate appetite including leptin and ghrelin. Leptin plays an important role in the regulation of food intake and energy expenditure, generating an increase in energy burning and decreasing food intake. And ghrelin is directly involved in the regulation of short-term energy balance.

Objectives: To verify frequency, biochemical profile and Body Mass Index (BMI) variations according to SNPs in *LEPR* and *GHRL* gene.

Subjects and Methods: 163 both genders subjects were classified into Study Group (SG): 103 subjects with obesity; Control Group (CG): 60 non-obese. Blood samples were collected to perform DNA extraction and biochemical profile analysis. Statistical significance was established at $p < 0.05$.

Results: The genotype and allele frequency were similar between groups for both polymorphisms. The *_/A* genotype of the *GHRL rs696217* polymorphism was associated to increased BMI in SG compared CG ($p = 0.003$) and increased triglycerides (TG) and very low density lipoprotein (VLDLc) values in CG ($p < 0, 05$). The *_/A* genotype was also associated with increased fasting glucose compared to *CC* genotype only in CG ($p = 0.031$). Considering the *LEPR rs1137101* polymorphism, *AA* genotype subjects presented higher BMI compared to *_/G* genotype subjects ($p = 0.024$). No difference between biochemical profile variables related to *LEPR rs1137101* polymorphism was found.

Conclusion: *AA* genotypes of the *LEPR rs1137101* polymorphism and *_/A* of the *GHRL rs696217* polymorphism suggest being risk factors for BMI and the latter is associated with fasting glucose, VLDLc and TG variation.

Keywords: Obesity, Biochemical Profile, Polymorphism, Leptin Receptor, Ghrelin

Introduction

Obesity is considered a worldwide epidemic and confers an increased risk for severe conditions including type 2 diabetes (T2D), dyslipidemia, insulin resistance, hypertension, kidney disorders, heart failure and proinflammatory state [1,2]. According to the World Health Organization (WHO), obesity is defined as a BMI of 30.0 kg/m² or greater [1].

Obesity is a complex and multifactorial condition related mainly to genetic and environmental aspects. Considering genetic factors, Single Nucleotide Polymorphisms (SNPs) seems to modify energy balance, food intake and satiety increasing the risk of obesity development [2].

Controlling appetite and satiety involves complex interactions between the hypothalamus, which is responsible for homeostasis regulation energy, and hormones that regulate appetite such as ghrelin and leptin [3,4]. In this scenario, hormones resistance promotes inconsistency between physiological signals and hunger/satiety feeling leading to adiposity and metabolic disorders [5].

Leptin is a aneroxigenic peptide hormone of 167-amino acid synthesized in white adipose tissue [6] which binds to specific leptin receptors (*LEPR*) found in the hypothalamus and others tissue as neuronal, hepatic, pancreatic, cardiac and intestinal [7]. This hormone is related to the fat body amount and it acts at central nervous system performing an important role in appetite regulation and energy homeostasis [6]. Despite higher circulating levels of leptin, obesity carriers can present resistant *LEPR* causing extra calorie intake [6].

Several SNPs have been described in the *LEPR* gene and associated with severe obesity and hyperphagia including the *rs1137101* (*Q223R, A>G*) polymorphism due to replacement of adenine by guanine at codon 223. This change results the replacement of glutamine amino acid by arginine leading to decreased expression of the receptor [4,8].

Ghrelin is a orexigenic peptide hormone of 28-amino acid encoded by the *GHRL* gene. It is produced mainly by gastric cells [3] and it participates of appetite regulation, fat storage, energy homeostasis, carbohydrate metabolism, inhibition of insulin secretion, growth hormone (GH) release stimulation, rewards system regulation, increase of gastric acid secretion and intestinal motility [9,10]. In contrast to leptin, the blood level of ghrelin increases during periods of fasting resulting in appetite stimulation [3].

Several of SNPs in the *GHRL* gene have been described including the *rs696217* polymorphism (*Leu72Met, C>A*) found in exon 2 of the *GHRL* gene due to replacement of a leucine amino acid by a methionine amino acid [11].

Genetic variability is a relevant factor that must be considered in obesity studies since variation in cellular and metabolic responses in population with this condition might be influenced by polymorphisms. Therefore, the objective of this study was to characterize the frequency of *rs1137101* (*LEPR* gene) and *rs696217* (*GHRL* gene) polymorphisms and possible association between this SNPs and other variables as BMI and biochemical profile in a Brazilian population comparing obesity and non-obesity carriers.

Subjects and Methods

Study Population

This case-control study included 163 subjects between 18 and 70 years old, regardless of gender and ethnicity, performed in two groups. The study group (SG) was composed of 103 obesity carriers (BMI ≥ 30kg/m²) and the control group (CG) was composed of 60 non-obese subjects (BMI between 18.5 and 24.9 kg/m²). Diabetes Mellitus carriers were excluded.

The subjects were selected at Hospital de Base/Medical School of São José do Rio Preto (HB/FAMERP). The study was approved by the Ethics Committee of São José do Rio Preto Medical School (CAAE: 19694913.8.0000.5415).

Anthropometric and Biochemical Assessment

Anthropometric data (height, weight and BMI) and samples of peripheral blood were collected. The DNA extraction for genotyping and biochemical profile measurement including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), very low-density lipoprotein (VLDLc), low-density lipoprotein cholesterol (LDL-c) and fasting glucose levels were performed. The LDL-c level was calculated through the Fridewald equation (LDL-c = CT - HDL-c - TG / 5) considering TG levels < 400mg/dL.

Genotyping

Genomic DNA was extracted from leukocytes using blood samples (5mL) collected via venipuncture with ethylenediamine tetra-acetic acid (EDTA). The extraction was performed according to the technique described by Salazar *et al*., 1998 [12]. Genotyping of *LEPR* gene *rs1137101* polymorphism and *GHRL* gene *rs696217* polymorphism were performed by polymerase chain reaction (PCR) technique. The PCR solution was composed of 0.5 µL deoxynucleotide (0.8 mM); 2.5µL of 10X PCR buffer; 2.5µL of 10% dimethyl sulfoxide; 2.0µL of each primer (2.5mM); 0.2µL of Taq polymerase (5U / µL); 10.8µL of Milli Q water; 2µL of diluted genomic DNA (0.2µg).

The *LEPR* gene primers were P1: 5 'ACC CTT TAA GCT GGG TGT CCC AAA TAG 3' and P2: 5 'AGC TAG CAA ATA TTT TTG TAA GCA ATT 3'. Thermocycler reactions included 1 cycle of 95ºC for 3 minutes; 40 cycles of 95ºC for 45 seconds, 58ºC for 30 seconds, 72ºC for 30 seconds; and final extension of 72ºC for 10 minutes. The amplification product was digested by restriction enzyme MspI and electrophoresis in 1.5% agarose gel was conducted to detect the following genotypes: *AA* (421bp); *AG* (421bp, 294bp and 127bp); *GG* (294bp and 127bp).

The primers for *GHRL* gene were P1: 5 'TGA CCT CAC TGT TTC TGG AAG 3' and P2: 5 'GGA CCC TGT TCA CTG CCA C 3'. Thermocycler reactions included 1 cycle of 95ºC for 3 minutes; 30 cycles of 95ºC for 30 seconds, 60ºC for 1 minute and 30 seconds, 72ºC for 2 minutes; and final extension of 72ºC for 10 minutes. The amplification product was digested by restriction enzyme BsrI and electrophoresis in 1.5% agarose gel was conducted to detect the following genotypes: *AA* (277bp); *AC* (277bp, 176bp and 101bp); *CC* (176bp and 101bp).

Statistical Analysis

Statistical analysis was performed by Graphpad Prism 4® program. Distribution of continuous variables was expressed as mean ± standard deviation (SD) and dichotomous variables were expressed as frequency and percentage. Difference between groups was analyzed by Student's t-test for parametric data and Mann-Whitney U test for non-parametric data. Data normality was tested with the Kolmogorov-Smirnov test. The genotype and allele frequency analysis were performed using Fisher's exact test or Chi-square test. Statistical significance was considered for $p < 0.05$.

Results

The mean age for the SG (N = 103) was 42.74 ± 10.52 years while for the CG (N = 60) the mean age was 47.85 ± 13, 42 years (p = 0.008). The female gender was prevalent in both groups and no statistical association was found between them (SG = 80.58%; CG = 70.00%; p = 0.130). SG had a higher BMI when compared to CG (EG = 41.27 ± 9.846; CG = 22.53 ± 2,397; $p < 0.001$).

The genotype and allele frequency of *rs1137101* (*LEPR* gene) and *rs696217* (*GHRL* gene) polymorphisms for both groups are presented in Table 1. The absolute frequency of the *A* allele of the *LEPR* gene was prevalent in both groups (SG = 0.64; CG = 0.66) with no statistical difference between them (p = 0.829) followed by the *AG* genotype (SG = 48.15%; GC = 64.71%; p = 0.09), *AA* (SG = 39.51%; GC = 33.33%; p = 0.59) and *GG* genotype (SG = 12.34%; GC = 1.96%, p = 0.050). The presence of at least *G* allele of *LEPR* did not differentiate SG and GC (SG = 60.5%; 66.67%; p = 0.6143).

Considering the *GHRL* gene, the absolute frequency of the *C* allele was higher in both groups and no statistically significant difference between them was detected (SG = 0.67; GC = 0.59; $p = 0.186$). No difference was detected in genotype frequency of the *CA* genotype (SG = 65.66%; GC = 75.00%; $p = 0.289$), *CC* genotype (SG = 34.34%; GC = 21.67%; $p = 0.129$) and *AA* genotype (EG = 0.00%; GC = 3.33%; $p = 0.141$). The presence of at least one *A* allele did not differentiate SG and CG (SG = 65.7%; CG = 78.3%; $p = 0.565$).

A total of 132 subjects was successfully genotyped for *LEPR* gene and 159 for the *GHRL* gene even the total number of participants was 163 because not all included subject's DNA samples had adequate results in genotyping for both genes (Table 1).

Genotype	**SG N=103**		**CG N=60**		***p*value (OR/CI)**
***LEPR* gene (*A>G*)**	**N**	**%**	**N**	**%**	
AA	32	39.51	17	33.33	0.5963
					1.31/(0.63-2.72)
AG	39	48.15	33	64.71	0.0928
					0.51/(0.45-1.04)
GG	10	12.34	1	1.96	0.050
					7.04/(0.87-56.78)
Total	81	100.00	51	100.00	0.6143
_G	49	60.49	34	66.67	0.77/(0.40-1.59)
Alleles	**N**	**Absol freq**	**N**	**Absolut freq**	
A	103	0.64	67	0.66	0.829
G	59	0.36	35	0.34	0.91/(0.54-1.53)
Total	162	1	102	1	
GHRL* gene *(C>A)	**N**	**%**	**N**	**%**	
CC	34	34.34	13	21.67	0.129
					1.89/(0.90-3.968)
CA	65	65.66	45	75.00	0.289
					0.64/(0.311-1.305)
AA	0	0.00	2	3.33	0.141
Total	99	100.00	60	100.00	0.565
_A	32	32.32	15	25.00	1.08/(0.53-2.17)
Alleles	**N**	**Abs freq**	**N**	**Abs freq**	
C	133	0.67	71	0.59	0.186
A	65	0.33	49	0.41	1.41/(0.88-2.26)
Total	198	1	120	1	

Fisher's Exact test or Chi-square test; N = total number of individuals; Abs. Freq. = Absolute frequency; OR/CI = Odds Ratio/Confidence interval; SG = Study group; CG = Control group; *p*-value = statistical significance

Table 1: Alleles and genotypes absolute and relative frequency distribution of the *rs1137101* (*LEPR* gene) and *rs696217* (*GHRL* gene) polymorphisms in obese (Study Group - SG) and non-obese subjects (Control Group - CG)

The relationship between three degrees of obesity and the presence of at least one *G* allele of SNP *rs1137101* (*LEPR*) and the presence of at least one *A* allele of SNP *rs696217* (*GHRL*) was analyzed (Table 2). SG subjects were classified into three groups according to the class obesity: class I (BMI: 30 - 34.9 kg/m^2), class II (BMI: 35 - 39.9 kg/m^2) and class III (BMI > 40 kg/m^2). The *A* allele of GHRL *rs696217* polymorphism was more frequent only in class I obesity ($p = 0.013$).

BMI	***LEPR*** *AA* x *_/G*		***p*-valor**	***GHRL*** *CC* x *_/A*		***p*-valor**
	N	**%**		**N**	**%**	
30 a 34.9 kg/m^2	41	39.8	0.66	40	38.8	0.013*
35 a 39.9 kg/m^2	17	16.5	0.47	17	16.5	0.19
>40.0 kg/m^2	23	22.3	0.10	42	40.8	0.94

Fisher's Exact test or Chi-square test; N = total number of individuals; BMI = Body Mass Index; *p*-value = statistical significance *$p < 0.05$

Table 2: Genotypes of LEPR and GHRL gene polymorphisms distributed between class I. II and III obesity A genotype combination of the two SNPs was performed to verify possible synergism between them. However. significant difference in frequency between groups was not verified and the data were not expressed

A genotype combination of the two SNPs was performed to verify possible synergism between them. However, significant difference in frequency between groups was not verified and the data were not expressed.

The variables related to the biochemical profile (CT, HDL-c, LDL-c, VLDLc, TG and fasting glucose) were measured and mean and SD are presented in Table 3. Both groups were within the reference values recommended by the Brazilian Society Cardiology guidelines, except SG HDL-c (HDL-c = 38.06 ± 21.24 mg/dL). Reference values for the variables are CT < 190 mg/dL; HDL-c > 40 mg/dL; TG < 150 mg/dL; VLDLc < 30 mg/dL; LDL-c < 130 mg/dL for patients classified as low cardiovascular risk. The cardiovascular risk assessment was not performed in this study. Considering serum fasting glucose, the Endocrinology Brazilian Society recommends values less than 100 mg/dL [13]. Difference between the groups regarding VLDLc level (SG = 23.88 ± 19.162 mg/dL; CG = 17.23 ± 15.19 mg/dL; $p = 0.016$) and TG level (SG = 119.06 ± 95,418 mg/dL; CG = 86.37 ± 75.92 mg/dL; $p = 0.017$) was found and they were higher in patients with obesity.

Biochemical profile	**SG (N=103)**	**CG (N=60)**	***p*-valor**
	M±SD	**M±SD**	
TC (mg/dL)	155.72±76.62	142.45±82.68	0.310
HDLc (mg/dL)	38.06±21.24	42.33±25.76	0.280
LDLc (mg/dL)	90.24±49.49	83.32±53.11	0.410
VLDLc (mg/dL)	23.88±19.16	17.23±15.19	0.016*
TG (mg/dL)	119.06±95.42	86.37±75.92	0.017*
Glucose (mg/dL)	84.72±48.09	78.17±52.18	0.428

T-test or Mann-Whitney U test; N = total number of individuals; TC = total cholesterol; HDLc = high density lipoproteins; LDLc = low density lipoprotein; VLDLc = very low density lipoprotein; TG = triglycerides; SG = Study group; CG = Control group; *p*-value = statistical significance *$p < 0.05$

Table 3: Biochemical profile mean values and standard deviation comparison between SG and CG

The BMI and biochemical profile mean values were compared between subjects with the *AA* genotype and the *_/G* genotype of the *LEPR rs1137101* polymorphism without group distinction. The BMI was higher in *AA* genotype subjects (BMI = 35.29 ± 12.63 kg/m^2) compared to *_/G* genotype (BMI = 30.25 ± 9.08 kg/m^2; $p = 0.024$). No difference was identified regarding these genotypes and biochemical profile mean values. The same analysis was performed for *GHRL rs696217* polymorphism (CC x _/A) and no difference was observed for BMI and biochemical profile means values (Table 4).

	LEPR			***GHRL***		
Biochemical	***AA***	***_/G***	***p-***	***CC***	***_/A***	***p-***
Profile; BMI	N=49	N=83	**value**	N=47	N=112	**value**
	M±SD	**M±SD**		**M±SD**	**M±SD**	
BMI (kg/m^2)	35.29±12.63	30.25±9.08	0.024*	32.91±9.35	34.21±13.26	0.976
TC (mg/dL)	182.83±48.58	179.90±20.34	0.695	184.30±44.82	181.45±41.97	0.740
HDLc (mg/dL)	48.94±13.07	51.34±14.6	0.403	48.97±14.23	49.24±13.97	0.923
LDLc (mg/dL)	107.28±31.55	108.59±33.04	0.843	108.31±31.20	107.13±32.57	0.857
VLDLc (mg/dL)	27.15±14.91	25.65±19.46	0.679	27.56±20.03	25.67±15.36	0.576
TG (mg/dL)	135.03±72.86	128.41±97.55	0.695	138.03±100.53	128.05±76.12	0.609
Glucose (mg/dL)	101.00±30.31	96.38±35.58	0.499	91.42±17.28	103.32±38.55	0.111

T-test or Mann-Whitney U test; N = total number of individuals; BMI = Body Mass Index; TC = total cholesterol; HDLc = high density lipoproteins; LDLc = low density lipoprotein; VLDLc = very low density lipoprotein; TG = triglycerides; *p*-value = statistical significance *p <0.05

Table 4: Biochemical profile and BMI mean values comparison according to the different genotypes of the *rs1137101* (*LEPR* gene) and the *rs696217* (*GHRL* gene) polymorphisms

Regarding SG subjects, the BMI and biochemical profile mean values were compared considering *AA* and *_/G* genotypes of the *rs1137101* polymorphism (*LEPR* gene). Higher BMI mean value was related to *AA* genotype (BMI = 41.81 ± 10.94 kg/m^2) when compared to *_/G* genotype (BMI = 36.08 ± 7.16kg / m^2; $p = 0.011$). The same comparison was performed in CG (Table 5) and no difference regarding BMI and the different genotypes was found. In addition, no statistical difference was detected when biochemical parameters mean values comparison was performed considering the different genotypes of *LEPR* gene (Table 5).

The same intragroup analysis was performed for the *GHRL rs696217* polymorphism (Table 5). The BMI and biochemical profile mean value of SG were compared considering *CC* and *_/A* genotypes. The same comparison was performed in CG. Only in SG was detected higher BMI mean value associated to *_/A* genotype (BMI = 43.13 ± 10.35 kg/m^2) when compared to the *CC* genotype (BMI = 36.91 ± 7.86 kg/m^2, $p = 0.003$). No statistical difference was detected considering biochemical parameters and the genotypes (*CC x _/A*) in the SG. In CG, fasting glucose value was higher in *_/A* genotype subjects (glucose = 99.23 ± 38.65 mg/dL) compared to the *CC* genotype (glucose = 80.33 ± 9.85 mg/dL; $p = 0.031$).

	SG			CG		
Biochemical	***AA***	***_/G***		***AA***	***_/G***	
Profile; BMI	N=32	N=49		N=18	N=35	
***LEPR* gene (*A*>*G*)**	**M±SD**	**M±SD**	***p*-valor**	**M±SD**	**M±SD**	***p*-valor**
BMI (kg/m^2)	41.81±10.94	36.08±7.16	0.011*	23.00±1.22	21.85±2.50	0.075
TC (mg/dL)	185.82±45.44	186.78±41.52	0.923	181.23±57.71	184.733±40.44	0.820
HDLc (mg/dL)	47.59±11.88	48.61±15.43	0.776	54.54±17.48	55.20±12.61	0.889
LDLc (mg/dL)	108.56±24.24	109.36±34.77	0.771	106.84±44.08	106.00±32.79	0.944
VLDLc (mg/dL)	29.62±16.46	28.35±21.11	0.794	21.84±8.58	21.71±16.02	0.425
TG (mg/dL)	146.92±80.24	141.94±106.05	0.838	109.69±42.97	108.58±79.98	0.361
Glucose (mg/dL)	104.59±34.79	92.81±20.97	0.289	93.38±13.99	99.53±46.21	0.861
	CC	***_/A***		***CC***	***_/A***	
	N=34	N=65		N=13	N=51	
***GHRL* gene (*C*>*A*)**	**M±SD**	**M±SD**	***p*-valor**	**M±SD**	**M±SD**	***p*-valor**
BMI (kg/m^2)	36.91±7.86	43.13±10.35	0.003*	22.46±1.33	21.87±2.62	0.830
TC (mg/dL)	187.78±45.75	182.84±38.19	0.601	164.80±37.21	180.06±48.17	0.497
HDLc	48.51±13.16	45.41±13.09	0.3146	40±20.86	55.00±14.00	0.605
LDLc (mg/dL)	110.93±29.57	108.51±29.62	0.725	94.00±39.41	105.06±36.75	0.527
VLDLc (mg/dL)	29.07±21.06	28.14±15.90	0.822	19.40±11.26	22.10±13.56	0.669
TG (mg/dL)	145.59±105.75	140.02±78.58	0.787	97.20±56.20	110.74±67.76	0.669
Glucose (mg/dL)	93.89±17.71	105.63±37.44	0.553	80.33±9.85	99.23±38.65	0.031*

T-test or Mann-Whitney U test; N = total number of individuals; BMI = Body Mass Index; TC = total cholesterol; HDLc = high density lipoproteins; LDLc = low density lipoprotein; VLDLc = very low density lipoprotein; TG = triglycerides; SG = Study group; CG = Control group; *p*-value = statistical significance *$p < 0.05$

Table 5: Biochemical profile and BMI mean values intragroup comparison according to the different genotypes of the rs1137101 (LEPR gene) and the rs696217 (GHRL gene) polymorphismsa

The comparison of BMI and biochemical profile mean values between groups considering the same genotype of *LEPR rs1137101* polymorphism is described in Table 6. No difference regarding these parameters was detected considering *AA* genotype ($p > 0.05$) and results were similar in *_/G* genotype analysis.

Considering the *_/A* genotype of *GHRL rs696217* polymorphism, SG subjects had higher levels of VLDLc (VLDLc = 28.14 ± 15.90 mg/dL) compared to the CG (VLDLc = 22.10 ± 13.56 mg/dL; $p = 0.044$). In addition, *_/A* genotype SG subjects also had a higher TG value (TG = 140.02 ± 78.58 mg/dL) compared to the CG (TG = 110.74 ± 67.76 mg/dL; $p = 0.048$). This analysis suggests that the presence of at least one *A* allele (*GHRL*) might be a risk factor for increased VLDLc and TG levels only in *_/A* genotype obesity carriers compared to non-obese. No difference between groups considering same genotype was expressed to the others biochemical parameters (Table 6).

	AA			*_/G*		
Biochemical	**SG**	**CG**	***p*-valor**	**SG**	**CG**	***p*-valor**
profile	N=32	N=18		N=49	N=37	
***LEPR* gene (*A*>*G*)**	**M±SD**	**M±SD**		**M±SD**	**M±SD**	
TC (mg/dL)	182.82+45.44	181.23+57.71	0.784	186.78+41.52	182.48+41.69	0.673
HDLc (mg/dL)	47.59+11.88	54.53+17.48	0.147	48.61+15.43	54.77+12.62	0.081
LDLc (mg/dL)	108.55+24.24	106.84+44.08	0.931	109.36+34.78	106.00+32.79	0.687
VLDLc (mg/dL)	29.62+16.46	21.84+8.58	0.084	28.35+21.11	21.70+16.02	0.155
TG (mg/dL)	146.93+80.25	109.69+42.98	0.083	141.95+106.05	108.56+79.98	0.154
Glucose (mg/dL)	104.59+34.79	93.38+13.99	0.828	92.81+20.97	99.53+46.02	0.618
	CC			*_/A*		
	SG	**CG**	***p*-valor**	**SG**	**CG**	***p*-valor**
	N=34	N=6		N=57	N=	
***GHRL* gene (*C*>*A*)**	**M±SD**	**M±SD**		**M±SD**	**M±SD**	
TC (mg/dL)	187.78±45.75	164.80±37.21	0.298	182.84±38.19	180.06±48.17	0.734
HDLc (mg/dL)	48.51±13.16	40.01±20.86	0.685	45.41±13.09	55.00±14.00	0.0005
LDLc (mg/dL)	110.93±29.57	94.00±39.41	0.271	108.51±29.62	105.06±36.75	0.598
VLDLc (mg/dL)	29.07±21.06	19.40±11.26	0.329	28.14±15.90	22.10±13.56	0.044*
TG (mg/dL)	145.59±105.75	97.20±56.20	0.331	140.02±78.58	110.74±67.76	0.048*
Glucose (mg/dL)	93.89±17.71	80.33±9.85	0.082	105.63±37.44	99.23±38.65	0.398

T-test or Mann-Whitney U test; N = total number of individuals; TC = total cholesterol; HDLc = high density lipoproteins; LDLc = low density lipoprotein; VLDLc = very low density lipoprotein; TG = triglycerides; SG = Study group; CG = Control group; *p*-value = statistical significance *$p < 0.05$

Table 6: Biochemical profile and BMI mean values comparison between SG and CG according to each genotype of the *rs1137101* (*LEPR* gene) and the *rs696217* (*GHRL* gene) polymorphisms

Discussion

GHRL Gene Rs696217 Polymorphism

The current literature is controversial about the association between the *GHRL rs696217* polymorphism and obesity [2]. Studies show this polymorphism is associated with obesity increased risk in Asian [14] and European [15] populations. The present study revealed prevalence of the *_/A* genotype in class I obesity subjects ($p = 0.013$) (Table 2) and increased BMI were found only in SG patients with at least one *A* allele ($p = 0.003$) (Table 5). However, in this polymorphism analysis no difference was found in the genotypes and alleles frequencies distribution between *GE* and *CG* ($p > 0.05$) (Table 1). This results corroborates with another Brazilian population study [16], which also found no association between the polymorphism and metabolic parameters including lipid profile and blood glucose. No correlation between the *GHRL* polymorphism and obesity has also been described in different populations [17,18].

Regarding the lipid profile, the present study found significant relationship between at least one polymorphic *A* allele and increased VLDLc ($p = 0.044$) and TG ($p = 0.048$) levels (Table 6) in SG compared to the GC. In a Czech study [19], the authors described a significant association between the *_/A* genotype of *rs696217* polymorphism and lower serum HDLc levels compared to *CC* genotype but no association was found in TG levels, BMI and waist-to-hip ratio. A Chinese study [18] found *AA* genotype subjects had decreased CT and LDLc plasma levels in non-obese female group and in obese male group compared to the *CC* genotype.

In the present study, no difference between polymorphism and higher fasting glucose was found when comparing SG and CG ($p > 0.05$) (Table 4). Similar results were reported in other studies associating the polymorphism with T2D and insulin resistance [14]. However, the present study found a significant relation between *_/A* genotype and increased fasting glucose levels in CG ($p = 0.031$) (Table 5), which was not observed in SG. Yan-yan Li *et al.* [20] found a significant correlation between the presence of *rs696217* polymorphism and T2D increased risk in the Chinese population.

Ghrelin is an orexigenic hormone responsible for food intake stimulation during fasting periods through hypothalamic stimulation triggering hunger and energy expenditure reduction [9]. Dosage of serum ghrelin is limited since it variates according to food intake but a study suggest postprandial ghrelin production do not reduce in obesity carriers [21]. In addition, ghrelin is considered a T2D risk factor since it participates in secondary physiological functions such as insulin secretion inhibition resulting in an increased serum glucose [10]. Moreover, polymorphisms found in the *GHRL* gene may be related to increased serum glucose levels as the present study has shown in CG with genotype *_/A* (Table 5).

LEPR Gene Rs1137101 Polymorphism

In the present study, no relation between obesity and the different genotypes and alleles frequencies of the *rs1137101* polymorphism (*LEPR*) was found. All genotypes had equally distribution among the groups, despite the *GG* genotype frequency being marginally significant in SG compares to CG ($p = 0.050$) (Table 1). Egyptian study found a higher frequency of the *GG* genotype in patients with obesity [22]. However, it is not possible to states that this genotype is a risk factor for obesity in the present study. No difference in genotype frequency was found in others studies comparing obese and non-obese subjects [23,24]. Farzam *et al.*, found prevalence of the *AA* genotype frequency in obesity carriers suggesting it is an obesity risk factor [25].

Despite the non-relationship between genotype frequency and obesity, higher BMI was related to *AA* genotype subjects compared to the *_/G* genotype ($p = 0.024$) suggesting that the *AA* genotype is a risk factor for increased BMI (Table 4). A similar study also suggested that at least one *G* allele presence was related to lower BMI values [26]. However, in other studies no relation was found between BMI and the different genotypes [4,22,27]. Another one found relation between higher BMI and the *GG* genotype [28]. This results demonstrate the polymorphism unconformity behavior in different populations according to the literature.

Considering the biochemical profile, no difference was found between the studied variables means when compared *AA* and *_/G* genotypes with no group distinguishing (Table 4). Other studies found similar results [4,22,23) but another study found higher mean values of TG and VLDLc in *AA* genotype subjects [26]. In a Tunisian study, lower HDLc and higher TC and glucose values were associated with the *GG* genotype [28].

Regarding the SG biochemical profile (Table 5), no difference was detected between means when compared *AA* and *_/G* genotype subjects. Similar results were found in *CG* analyses (Table 5). Similar study also found no difference between glucose, CT, HDLc, and VLDLc means for the different genotypes in non-obese subjects but it found lower LDLc mean value in *AG* genotype subjects [23]. A Sri Lankan population study found no difference in the biochemical profile for the different genotypes in obesity carriers [4]. Another study found *GG* genotype non-obese subjects have higher TC and LDLc values compared to *_/A*, while *GG* genotype have higher TG values compared to *_/A* only in obese group [27].

Leptin is produced in adipose tissue and it is an anorexigenic hormone responsible for hunger inhibition promoting satiety and energy expenditure. A study found that obese subjects have higher leptin levels [29] suggesting increased serum leptin may lead to hormone resistance by decreasing leptin receptors density at hypothalamus [7]. Therefore, the mechanism that promotes satiety in this group is unregulated. In the present study the presence of the *G* polymorphic allele (LEPR *rs1137101* polymorphism) seems to provide protection against increased BMI. However, different results in genotype analyses were demonstrated in different populations suggesting other events may interfere in obesity determination such as genes interaction, environmental factors, gender and age [30].

Biochemical Profile

Considering the biochemical profile, no difference was detected between SG and GC in TC, HDLc, LDLc and glucose mean values comparison. However, the SG presented higher VLDLc ($p = 0.016$) and TG ($p = 0.017$) mean when compared to the CG (Table 3). A similar study found an association between obesity and higher levels of CT, TG, fasting glucose and lower HDLc [24]. The present study found difference only in VLDLc and TG but, in general, the literature suggests entire biochemical profile alteration.

Study Limitations

The sample size was the main limitation of the present study although similar results were also observed in more robust studies [15,30]. The genotyping technique was also a limitation and real-time PCR would be recommended but the results may not be underestimated due to the strict laboratory practice criteria applied by specialists in molecular biology involved in this study.

Conclusion

The *AA* genotype of the *rs1137101* polymorphism (*LEPR*) in all subjects and *_/A* of the *rs696217* polymorphism (*GHRL*) in obese subjects appear to be risk factors for increased BMI. The presence of at least one *A* allele (*GHRL*) may be a risk factor for class I obesity. Furthermore, the *A* allele of *rs696217* polymorphism (*GHRL*) seems to be associated with biochemical profile alteration including increased fasting glucose, VLDLc and TG.

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