The Arg64 Allele of the β_3-Adrenoceptor Gene But Not the −3826G Allele of the Uncoupling Protein 1 Gene Is Associated With Increased Leptin Levels in the Spanish Population

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To determine whether there are variations in leptin levels according to the β_3-adrenoceptor (β_3-AR) Trp64Arg and uncoupling protein 1 (UCP1) −3826A→G polymorphisms, given the regulatory role of catecholamines through the β_3-AR in leptin production and the previously reported association of the UCP1 −3826A→G variant with obesity. A total of 160 men and 172 women randomly chosen from a nationwide population-based obesity cross-sectional survey in Spain were studied. Body mass index (BMI), waist-to-hip ratio (WHR), leptin, insulin, fasting and 2-hour post-glucose load glycemia, high-density lipoprotein (HDL)-, low-density lipoprotein (LDL)-, and total cholesterol, and triglyceride plasma levels were measured. β_3-AR Trp64Arg and UCP1 −3826A→G genotypes were determined by restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR). UCP1 −3826G allele frequency was higher in men than in women (0.31 *v* 0.22, *P* = .015) and in obese women than in non-obese women (0.31 *v* 0.17, *P* = .008). Women carriers of the Arg64 or the alleles also showed higher leptin levels than noncarriers. Multiple linear regression analysis showed that the Arg64 allele is associated with higher leptin levels after the adjustment for gender, age, WHR, and the degree of glucose tolerance. In conclusion, the β_3-AR Trp64Arg polymorphism might have an impact on the mechanisms involved in leptin release from adipose tissue. Furthermore, our results agree with the previously reported association between UCP1 −3826G allele and obesity and point to a gender-related effect.

THE β_3-adrenoceptor (β_3-AR) is known to stimulate uncoupling protein 1 (UCP1)-mediated thermogenesis in brown adipose tissue[1] and lipolysis in white adipose tissue in rodents,[2] thus influencing energy expenditure. In addition, human lipolysis has also been shown to be, in part, regulated by this receptor.[3] The Trp64Arg polymorphism in the β_3-AR gene[4] has been shown to be associated with impairment of its lipolytic function,[5] a lower resting metabolic rate,[4] and a tendency to gain weight in obese individuals.[6] On the other hand, the role of UCP1-mediated thermogenesis in human obesity remains unclear,[7] but the presence of the −3826A→G variant in the promoter region of the UCP1 gene[8] has been associated with a greater weight gain,[9] lower weight loss under a low-calorie diet,[10] and some other metabolic features of the obese phenotype.[11] Moreover, an additive effect of both genetic variants on the development of obesity has also been reported,[9,12] although other investigators failed to find such an effect on obese phenotype.[13,14]

Since there is evidence of a regulatory role for catecholamines through the β_3-AR in leptin production in humans[15] and given the previously reported association of UCP1 −3826A→G variant with obesity, the aim of the current study was to determine the effects of the β_3-AR Trp64Arg and UCP1 −3826A→G polymorphisms on circulating leptin levels in obese and non-obese subjects. We also studied other parameters that are markers of the metabolic syndrome in relation to these polymorphisms.

MATERIALS AND METHODS

Population

We have studied 332 nonrelated Caucasian men (n = 160, 48.2%) and women (n = 172, 51.8%) aged 35 to 65 years, randomly chosen from a nationwide population-based survey on obesity and related conditions including insulin resistance and cardiovascular risk factors in Spain.[16] Subjects with previous diagnosis of type 1 diabetes mellitus were excluded from the study. All study subjects were unrelated and gave their written consent to participate in the study after being informed of its nature. The study protocol was approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, Spain.

Phenotype Measurements

Anthropometric measurements included body mass index (BMI, kg/m^2) and waist-to-hip ratio (WHR). For laboratory studies, 20 mL of blood was obtained from an antecubital vein without compression after a >10-hour overnight fasting period. Blood samples were collected in EDTA-coated tubes and immediately centrifuged at room temperature. Plasma glucose was determined in duplicate by a glucose-oxidase method adapted to an autoanalyzer (Hitachi 704, Boehringer Mannheim, Mannheim, Germany). Total cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol were determined by enzymatic methods using commercial kits (Boehringer Mannheim). Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald formula. Plasma insulin and leptin concentrations were determined by radioimmunoassay (RIA; Human Insulin Specific RIA kit and Human Leptin RIA Kit, respectively, Linco Research, St Louis, MO).

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Table 1. Genotype and Allele Frequencies of Trp64Arg β_3-AR and −3826A→G Polymorphisms According to Gender and Obesity (BMI ≥ 30)

	All Subjects			All Subjects			Men			Women		
Genotype	Men (n = 160)	Women (n = 172)	P*	Obese (n = 93)	Non-obese (n = 239)	P†	Obese (n = 38)	Non-obese (n = 122)	P†	Obese (n = 55)	Non-obese (n = 117)	P†
β_3-AR Trp64Arg												
Trp64Trp64	139 (86.8%)	155 (90.1%)		81 (87.1%)	213 (89.1%)		33 (86.8%)	106 (86.9%)		48 (87.3%)	107 (91.5%)	
Trp64Arg64	20 (12.6%)	17 (9.9%)		12 (12.9%)	25 (10.5%)		5 (13.2%)	15 (12.3%)		7 (12.7%)	10 (8.5%)	
Arg64Arg64	1 (0.6%)	0 (0%)	NS	0 (0%)	1 (0.4%)	NS	0 (0%)	1 (0.8%)	NS	0 (0%)	0 (0%)	NS
UCP1 −3826A→G												
AA	55 (48.7%)	85 (61.2%)		41 (50%)	99 (58.2%)		20 (51.3%)	35 (47.3%)		21 (48.8%)	64 (66.7%)	
AG	46 (40.7%)	48 (34.5%)		33 (40.2%)	61 (35.9%)		16 (41%)	30 (40.5%)		17 (39.6%)	31 (32.3%)	
GG	12 (10.6%)	6 (4.3%)	.049	8 (9.8%)	10 (5.9%)	NS	3 (7.7%)	9 (12.2%)	NS	5 (11.6%)	1 (1%)	.002
Allele frequencies												
Trp64	0.93	0.95		0.94	0.94		0.94	0.94		0.94	0.96	
Arg64	0.07	0.05	NS	0.06	0.06	NS	0.06	0.06	NS	0.06	0.04	NS
A	0.69	0.78		0.70	0.76		0.72	0.68		0.69	0.83	
G	0.31	0.22	.015	0.30	0.24	NS	0.28	0.32	NS	0.31	0.17	.008

NOTE. Values were compared by chi-square analysis.
Abbreviation: NS, not significant.
*Men *v* women.
†Obese subjects *v* non-obese subjects.

Table 2. Anthropometric Parameters According to Trp64Arg β_3-AR and −3826A→G UCP1 Genotypes

	Trp64Arg β_3-AR Genotype			−3826A→G UCP1 Genotype		
	Trp64Trp64	Arg64/	P	−3826A-3826A	−3826G/	P
Age (yr)						
Men	47.6 ± 8.6 (138)	48.1 ± 9.0 (21)	NS	51.3 ± 9.4 (55)	48.8 ± 9.2 (58)	NS
Women	47.2 ± 8.6 (155)	50.1 ± 9.1 (17)	NS	48.2 ± 8.7 (85)	48.8 ± 9.4 (53)	NS
BMI (kg/m^2)						
Men	27.2 ± 3.7 (139)	27.4 ± 3.1 (21)	NS	28.5 ± 4.2 (55)	27.6 ± 4.0 (58)	NS
Women	28.0 ± 5.1 (155)	28.6 ± 5.7 (17)	NS	27.7 ± 4.7 (85)	29.2 ± 4.5 (54)	.058
WHR						
Men	0.99 ± 0.05 (139)	1.01 ± 0.18 (21)	NS	0.99 ± 0.06 (55)	0.98 ± 0.04 (58)	NS
Women	0.93 ± 0.06 (155)	0.94 ± 0.12 (17)	NS	0.92 ± 0.07 (85)	0.95 ± 0.09 (54)	NS

NOTE. Values are mean ± SD (n).

Table 3. Analytical Parameters According to Trp64Arg β_3-AR Genotype

	Whole Population			Obese			Non-obese		
	Trp64Trp64	Arg64/	P	Trp64Trp64	Arg64/	P	Trp64Trp64	Arg64/	P
Triglycerides (mmol/L)									
Men	1.50 ± 0.89 (129)	1.28 ± 0.91 (18)	NS	1.67 ± 0.59 (30)	1.31 ± 0.47 (5)	NS	1.45 ± 0.96 (99)	1.26 ± 1.05 (13)	NS
Women	1.07 ± 0.53 (146)	1.11 ± 0.53 (17)	NS	1.15 ± 0.53 (43)	1.25 ± 0.39 (7)	NS	1.04 ± 0.53 (103)	1.01 ± 0.62 (10)	NS
Total cholesterol (mmol/L)									
Men	5.80 ± 0.98 (129)	5.56 ± 1.24 (18)	NS	6.11 ± 0.83 (30)	6.52 ± 1.51 (5)	NS	5.70 ± 1.00 (99)	5.19 ± 0.94 (13)	NS
Women	5.51 ± 1.01 (146)	5.62 ± 0.76 (17)	NS	5.28 ± 1.05 (43)	5.89 ± 0.71 (7)	NS	5.61 ± 0.98 (103)	5.42 ± 0.77 (10)	NS
HDL-cholesterol (mmol/L)									
Men	1.26 ± 0.32 (129)	1.27 ± 0.32 (18)	NS	1.17 ± 0.27 (30)	1.14 ± 0.28 (5)	NS	1.29 ± 0.33 (99)	1.32 ± 0.32 (13)	NS
Women	1.48 ± 0.35 (146)	1.52 ± 0.38 (17)	NS	1.32 ± 0.27 (43)	1.43 ± 0.27 (7)	NS	1.54 ± 0.35 (103)	1.58 ± 0.45 (10)	NS
LDL-cholesterol (mmol/L)									
Men	3.85 ± 0.87 (129)	3.71 ± 1.12 (18)	NS	4.18 ± 0.74 (30)	4.78 ± 1.50 (5)	NS	3.75 ± 0.89 (99)	3.30 ± 0.60 (13)	NS
Women	3.55 ± 0.86 (146)	3.59 ± 0.69 (17)	NS	3.44 ± 0.91 (43)	3.89 ± 0.71 (7)	NS	3.59 ± 0.83 (103)	3.38 ± 0.62 (10)	NS
Leptin (μg/L)									
Men	4.76 ± 3.57 (90)	5.73 ± 3.56 (18)	NS	8.31 ± 5.11 (16)	7.62 ± 3.66 (4)	NS	3.99 ± 2.60 (74)	5.19 ± 3.47 (14)	NS
Women	16.4 ± 10.6 (99)	25.9 ± 21.3 (8)	NS	26.5 ± 11.4 (26)	43.7 ± 13.3 (4)	.010	12.9 ± 7.6 (73)	8.03 ± 5.72 (4)	NS
Glucose (mmol/L)									
Men	5.51 ± 1.16 (130)	5.39 ± 0.80 (18)	NS	5.42 ± 0.89 (30)	5.98 ± 1.03 (5)	NS	5.54 ± 1.24 (100)	5.17 ± 0.60 (13)	NS
Women	5.14 ± 0.94 (148)	4.88 ± 0.66 (17)	NS	5.24 ± 1.05 (44)	5.13 ± 0.69 (7)	NS	5.10 ± 0.89 (104)	4.69 ± 0.60 (10)	NS
2h Glucose (mmol/L)									
Men	5.81 ± 2.08 (102)	5.94 ± 2.01 (11)	NS	6.15 ± 0.38 (24)	5.96 ± 2.79 (4)	NS	5.71 ± 1.99 (78)	5.94 ± 1.69 (7)	NS
Women	5.54 ± 1.62 (112)	4.70 ± 0.84 (13)	NS	5.74 ± 1.91 (38)	4.73 ± 0.92 (6)	NS	5.43 ± 1.45 (74)	4.67 ± 0.83 (7)	NS
Insulin (μU/mL)									
Men	12.4 ± 6.5 (138)	15.6 ± 16.7 (21)	NS	13.8 ± 8.1 (33)	15.0 ± 9.1 (5)	NS	11.9 ± 5.8 (105)	15.8 ± 18.7 (16)	NS
Women	12.6 ± 6.0 (154)	12.8 ± 6.9 (17)	NS	13.4 ± 6.3 (47)	14.6 ± 8.5 (7)	NS	12.2 ± 5.9 (105)	11.5 ± 3.0 (10)	NS

NOTE. Values are mean ± SD (n).

Table 4. Analytical Parameters According to −3826A→G UCP1 Genotype

	Whole Population			Obese			Non-obese		
	−3826A-3826A	−3826G/	*P*	−3826A-3826A	−3826G/	*P*	−3826A-3826A	−3826G/	*P*
Triglycerides (mmol/L)									
Men	1.72 ± 1.67 (55)	1.59 ± 0.85 (58)	NS	1.70 ± 0.74 (20)	1.53 ± 0.89 (19)	NS	1.73 ± 2.04 (35)	1.62 ± 0.83 (39)	NS
Women	1.13 ± 0.74 (85)	1.09 ± 0.53 (54)	NS	1.26 ± 1.06 (21)	1.25 ± 0.57 (22)	NS	1.09 ± 0.60 (64)	0.98 ± 0.48 (32)	NS
Total cholesterol (mmol/L)									
Men	5.83 ± 0.99 (55)	5.83 ± 0.94 (58)	NS	6.17 ± 1.05 (20)	5.92 ± 0.94 (19)	NS	5.63 ± 0.92 (35)	5.79 ± 0.95 (39)	NS
Women	5.79 ± 1.04 (85)	5.58 ± 1.14 (54)	NS	5.85 ± 1.01 (21)	5.39 ± 1.24 (22)	NS	5.78 ± 1.05 (64)	5.71 ± 1.06 (32)	NS
HDL-cholesterol (mmol/L)									
Men	1.19 ± 0.29 (55)	1.21 ± 0.33 (58)	NS	1.14 ± 0.28 (20)	1.19 ± 0.29 (19)	NS	1.22 ± 0.30 (35)	1.22 ± 0.35 (39)	NS
Women	1.50 ± 0.35 (85)	1.43 ± 0.29 (54)	NS	1.45 ± 0.26 (21)	1.30 ± 0.24 (22)	.052	1.52 ± 0.37 (64)	1.52 ± 0.30 (32)	NS
LDL-cholesterol (mmol/L)									
Men	3.85 ± 0.85 (55)	3.89 ± 0.81 (58)	NS	4.26 ± 0.88 (20)	4.03 ± 0.74 (19)	NS	3.62 ± 0.75 (35)	3.83 ± 0.85 (39)	NS
Women	3.77 ± 0.89 (85)	3.65 ± 0.99 (54)	NS	3.82 ± 0.88 (21)	3.52 ± 1.03 (22)	NS	3.76 ± 0.90 (64)	3.75 ± 0.96 (32)	NS
Leptin (μg/L)									
Men	8.16 ± 1.39 (30)	5.45 ± 0.75 (39)	NS	9.13 ± 1.73 (11)	9.60 ± 1.80 (12)	NS	7.60 ± 1.98 (19)	3.60 ± 0.40 (27)	NS
Women	15.7 ± 1.5 (53)	21.4 ± 1.9 (35)	.019	27.6 ± 3.8 (12)	30.1 ± 3.4 (13)	NS	12.2 ± 1.1 (41)	16.3 ± 1.6 (22)	.033
Glucose (mmol/L)									
Men	5.69 ± 1.23 (55)	5.36 ± 1.14 (59)	NS	5.68 ± 1.25 (20)	5.49 ± 1.56 (19)	NS	5.69 ± 1.23 (35)	5.30 ± 0.89 (39)	NS
Women	5.17 ± 1.05 (85)	5.09 ± 1.05 (54)	NS	5.28 ± 0.80 (21)	5.20 ± 1.51 (22)	NS	5.14 ± 1.13 (64)	5.01 ± 0.56 (32)	NS
2h Glucose (mmol/L)									
Men	6.38 ± 3.10 (44)	6.05 ± 2.71 (49)	NS	7.58 ± 3.81 (17)	6.60 ± 2.21 (14)	NS	5.62 ± 2.33 (27)	5.83 ± 2.86 (35)	NS
Women	5.48 ± 1.74 (72)	5.62 ± 2.18 (47)	NS	5.86 ± 1.51 (18)	5.60 ± 2.95 (21)	NS	5.35 ± 1.80 (54)	5.63 ± 1.33 (26)	NS
Insulin (μU/mL)									
Men	14.1 ± 14.5 (55)	11.3 ± 5.9 (58)	NS	18.6 ± 22.6 (20)	14.1 ± 7.5 (19)	NS	11.5 ± 5.4 (35)	10.0 ± 4.6 (39)	NS
Women	11.2 ± 5.5 (85)	12.1 ± 5.0 (54)	NS	11.5 ± 5.1 (21)	11.9 ± 4.2 (22)	NS	11.1 ± 5.7 (64)	12.3 ± 5.5 (32)	NS

NOTE. Values are mean ± SD (n).

An oral glucose tolerance test (OGTT) using 75 g of glucose according to the World Health Organization recommendations was performed. One and 2 hours after glucose administration, blood samples were obtained for determination of glucose and insulin plasma levels. Insulin resistance was estimated according to the homeostasis model assessment (HOMA-IR) method from fasting glucose and insulin concentrations, according to the formula: Insulin (μU/mL) × Glucose (mmol/L)/22.5.[17]

According to the Expert Committee criteria on the diagnosis and classification of diabetes mellitus,[18] subjects were classified as having normoglycaemia (NG), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or type 2 diabetes (DM).

Genotype Measurements

Genomic DNA was extracted from leucocytes by digestion with proteinase K followed by phenol/chloroform extraction.[19] The polymerase chain reaction (PCR) amplification of the DNA segments containing codon 64 of the β_3-AR gene was performed as previously described.[4] The forward primer was 5′-CCAGTGGGCTGCCAGGGG-3′ and the reverse primer was 5′-GCCAGTGGCGCCCAACGG-3′. The amplified product (248 bp) was digested with *Bst*NI, resulting in fragments of 97, 64, and 61 bp in Trp64 homozygous and 158 and 64 bp in Arg64 homozygous.

For the PCR amplification of the promoter region containing the UCP1 −3826A→G variant, forward and reverse primers were 5′-CTTGGGTAGTGACAAAGTAT-3′ and 5′-CCAAAGGGTCAGATTTCTAC-3′,[20] respectively. The 470-bp amplified product was digested with *Bcl*I, resulting in fragments of 250 and 220 bp for −3826A homozygous and remaining uncut for −3826G homozygous.

Statistical Analysis

Allele frequencies for each polymorphic site were estimated by the gene-counting method. Genotype and allele frequency distributions were compared using the chi-square test. The distribution of single diallelic restriction fragment length polymorphisms (RFLPs) was tested for Hardy-Weinberg equilibrium with a chi-square test. Student's *t* test and analysis of variance (ANOVA) were used to compare continuous variables—expressed as the mean ± SD—while categorical variables were compared using the chi-square test. Multiple linear regression was performed to investigate the effect of these polymorphisms, both individually and combined, on fasting leptin levels (log-transformed). Adjusted odds ratios (adjOR) and their 95% confidence intervals (95% CI) were calculated and the existence of interactions was evaluated. The null hypothesis was rejected in each statistical test when $P < .05$. Analyses were performed using the Windows SPSS version 11.0 software (SPSS, Inc, Chicago, IL).

RESULTS

Subjects with BMI ≥ 30 kg/m^2 were classified as obese (n = 93, 38 men and 55 women) and according to the degree of glucose tolerance[18] as having normoglycemia (n = 217), IFG (n = 0), IGT (n = 17), and type 2 DM (n = 19).

Genotype distributions and allele frequencies for the β_3-AR Trp64Arg and the UCP1 −3826A→G polymorphisms are shown in Table 1. Allele frequencies were in Hardy-Weinberg equilibrium in every study group. Differences in the UCP1 −3826A→G polymorphism genotype distribution when comparing men to women ($P = .049$) and obese to non-obese women ($P = .002$) were found. Accordingly, the −3826G allele frequency was higher in men than in women (0.31 *v* 0.22, $P = .015$) and in obese women than in non-obese women (0.31 *v* 0.17, $P = .008$). As regards to the β_3-AR Trp64Arg genotype,

Table 5. Multivariate Linear Regression Analysis for Leptin Levels

	adjOR	95% CI	*P*
Gender (women/men)	1.93	1.76-2.12	.000
Age	1.01	0.99-1.01	.597
WHR	6.93	3.15-15.24	.000
Arg64 carriers *v* Trp64Trp64	1.16	1.02-1.33	.026

NOTE. Data were adjusted for the degree of glucose tolerance.

Table 6. Analytical Parameters According to Combined Trp64Arg β_3-AR and −3826A→G UCP1 Genotypes

	Whole Population				
	Trp64Trp & −3826AA	Trp64Trp & −3826G/X	Arg64 & −3826AA	Arg64 & −3826G/X	*P*
BMI (kg/m^2)					
Men	28.7 ± 3.9 (22)	37.5 ± 4.0 (33)	27.7 ± 3.5 (2)	27.3 ± 0.0 (1)	NS
Women	27.8 ± 4.7 (40)	28.8 ± 4.5 (26)	29.7 ± 4.7 (6)	30.6 ± 8.0 (4)	NS
WHR					
Men	0.970 ± 0.217 (22)	0.980 ± 0.053 (33)	0.860 ± 0.113 (2)	1.00 ± 0.00 (1)	NS
Women	0.852 ± 0.064 (41)	0.816 ± 0.167 (26)	0.850 ± 0.094 (6)	0.813 ± 0.060 (4)	NS
Age (yr)					
Men	47.0 ± 8.5 (22)	46.8 ± 9.2 (33)	51.0 ± 4.2 (2)	38.0 ± 0.0 (1)	NS
Women	49.5 ± 9.3 (41)	46.0 ± 8.3 (26)	49.0 ± 8.2 (6)	51.3 ± 10.0 (4)	NS
Triglycerides (mmol/L)					
Men	1.79 ± 1.37 (22)	1.55 ± 0.67 (33)	0.808 ± 0.232 (2)	4.53 ± 0.00 (1)	.022
Women	1.12 ± 0.60 (40)	1.08 ± 0.52 (26)	0.959 ± 0.343 (6)	1.04 ± 0.57 (4)	NS
Total cholesterol (mmol/L)					
Men	5.80 ± 0.81 (22)	5.94 ± 1.01 (33)	4.80 ± 0.20 (2)	7.32 ± 0.00 (1)	NS
Women	5.73 ± 1.00 (40)	5.47 ± 1.09 (26)	5.73 ± 0.55 (6)	5.65 ± 1.14 (4)	NS
HDL-cholesterol (mmol/L)					
Men	1.15 ± 0.26 (22)	1.25 ± 0.36 (33)	1.29 ± 0.33 (2)	1.27 ± 0.00 (1)	NS
Women	1.48 ± 0.35 (40)	1.45 ± 0.32 (26)	1.55 ± 0.22 (6)	1.33 ± 0.37 (4)	NS
LDL-cholesterol (mmol/L)					
Men	3.83 ± 0.79 (22)	3.98 ± 0.91 (33)	3.13 ± 0.42 (2)	3.98 ± 0.00 (1)	NS
Women	3.74 ± 0.87 (40)	3.53 ± 0.89 (26)	3.74 ± 0.32 (6)	3.84 ± 0.94 (4)	NS
Leptin (μg/L)					
Men	7.36 ± 6.10 (12)	4.66 ± 2.97 (24)	9.02 ± 0.00 (2)	± (0)	NS
Women	14.7 ± 8.3 (28)	18.8 ± 9.4 (15)	39.3 ± 20.8 (6)	26.8 ± 30.2 (4)	.003
Glucose (mmol/L)					
Men	5.35 ± 0.95 (22)	5.49 ± 0.92 (33)	4.44 ± 0.08 (2)	5.61 ± 0.00 (1)	NS
Women	5.23 ± 1.24 (40)	4.92 ± 0.43 (26)	4.88 ± 0.61 (6)	4.72 ± 0.74 (4)	NS
2h Glucose (mmol/L)					
Men	5.78 ± 2.11 (21)	5.80 ± 2.05 (28)	7.28 ± 3.69 (2)	± (0)	NS
Women	5.63 ± 1.63 (34)	5.11 ± 1.32 (23)	4.81 ± 0.91 (6)	4.22 ± 0.24 (4)	NS
Insulin (μU/mL)					
Men	13.4 ± 8.2 (22)	12.1 ± 6.6 (33)	5.02 ± 2.84 (2)	11.5 ± 0.0 (1)	NS
Women	11.5 ± 5.0 (40)	11.5 ± 4.8 (26)	14.3 ± 5.6 (6)	12.3 ± 2.0 (4)	NS

NOTE. Values are mean ± SD (n).

we did not find differences between groups either in genotype distribution or allele frequencies. There were no differences between genotype groups, either UCP1 or β_3-AR, regarding anthropometric measurements (Table 2), lipid profile, and other parameters studied such as glucose tolerance (Tables 3 and 4).

As regards to the leptin levels, obese women carrying the Arg64 allele showed higher leptin levels than noncarriers (Table 3). We also found higher leptin levels in women carrying the −3826G allele (Table 4). However, when the analysis was performed according to the degree of obesity, differences were found to be mainly due to the non-obese group. In contrast, differences in leptin levels between genotype groups in men did not reach statistical significance, even when stratified according to the degree of obesity.

Gender-stratified univariate analysis showed a positive correlation between leptin levels and WHR in both genders (r = 0.342, P = .000 and r = 0.356, P = .000 in men and women, respectively).

A multiple linear regression analysis (Table 5) showed that the Arg64 allele of the β_3-AR gene was associated with increased leptin levels after adjustment for gender, age, WHR, and the degree of glucose tolerance (adjOR, 1.16; 95% CI, 1.02 to 1.33; P = .026). In another additional model, although the Arg64 allele tended to be also associated with higher leptin levels after adjustment for gender, age, WHR, BMI, and glucose tolerance, this did not reach the level of statistical significance (adjOR, 1.10; 95% CI, 0.99 to 1.22; P = .058). However, neither the −3826A→G polymorphism nor the combination of both mutated alleles had an impact on leptin levels after adjustment in multivariate linear regression analysis (data not shown).

DISCUSSION

The present study provides evidence of the involvement of the β_3-AR Trp64Arg polymorphism in the impairment of the mechanisms that regulate blood leptin levels. Moreover, our results are in agreement with previously reported gender-related effects of the UCP1 −3826G allele on obesity in Australian and Japanese populations.[14,21]

The frequencies of the −3826G allele found in the whole population (0.22 in women and 0.31 in men) were similar to those previously reported in other Caucasian populations, which included a number of subjects similar to that in our

study,[8,11] and the same was true for the Arg64 β_3-AR allele.[22,23] We have found an association between the UCP1 −3826A→G polymorphism and obesity in women but not in men. Thus, the increase in BMI values according to the UCP1 genotype shown by women followed a significant linear trend—27.7 ± 4.7, 28.9 ± 4.5, and 32.2 ± 3.7, $P = .019$, for −3826A/−3826A, −3826A/−3826G, and −3826G/−3826G genotypes respectively—which was not observed in men. In addition, women carrying the −3826G allele showed increased leptin levels compared to noncarriers, which might reflect the changes in BMI values according to genotype (see Table 2).

The Arg64 allele appears to be associated with higher leptin levels in women as well, specifically in obese women. Because this polymorphism has been related to an alteration of the β_3-AR cell signaling,[24] we might consider this association a consequence of an impairment of the adrenergic inhibitory capacity on leptin secretion,[15] leading to increased leptin levels. The fact that we did not find such differences in men might be explained by the gender-related differences in leptin production.[25] Our results agree with those obtained in a study on Chinese women (n = 188) where, in the obese group, carriers of the Arg64 allele showed higher leptin levels than noncarriers,[26] but ours is the first study to find such an association in Caucasians. The lack of agreement with the results of other studies in Caucasoids[23,27,28] might be due to a lack of gender stratification[23] or to the preselected pathologic conditions, such as type 2 diabetes mellitus and coronary artery disease, of the studied populations.[27,28]

Moreover, our data show that the Arg64 allele is associated to increased leptin levels even after adjustment for gender and WHR. Therefore, while the effect on leptin levels of the UCP1 −3826G allele could not be considered apart from its association to obesity, the β_3-AR Arg64 allele here shows an independent effect on leptin levels. Another contributing factor to leptin levels in our population is WHR. Some studies have shown an inverse relationship between plasma leptin levels and WHR,[29,30] at variance with our results. In fact, gender-stratified univariate analysis showed a positive correlation between leptin levels and WHR in both genders. These differences could be due to the fact that our study included both lean and obese subjects, whereas in other studies subjects were obese and morbidly obese.[29,30] Indeed, WHR appears negatively correlated to leptin levels in our population ($r = -0.350$, $P = .013$) when subjects with BMI values lower than 30 kg/m^2 are excluded from the analysis and men and women are pooled together as in others studies.[29,30]

We did not find an additive effect of both polymorphisms on leptin levels, but these results must be cautiously interpreted due the small number of individuals in different genotype combinations (Table 6).

In conclusion, our results support the idea of an involvement of the β_3-AR Trp64Arg polymorphism in the impairment of the mechanisms that regulate plasma leptin levels, and reinforce the functional consequences of the polymorphism as well. Furthermore, we find an association between the UCP1 −3826G allele and obesity that is gender-dependent.

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