



Artículo especial

Genetic variation in the beta-3-adrenoreceptor gene (Trp64arg polymorphism) and their influence on anthropometric parameters and insulin resistance after a high protein/low carbohydrate versus a standard hypocaloric diet

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Abstract

Introduction: the Trp64Arg variant in Beta receptor has been reported to be associated with increased body weight and insulin resistance

Objective: the aim of our study was to investigate the influence of polymorphism (rs 4994) in Beta-3-adrenoreceptor gene on metabolic response and weight loss in a medium-term intervention study secondary's to a high protein/low carbohydrate vs. a standard hypocaloric diets (1000 kcal/day).

Material and methods: a population of 284 obese subjects was analyzed in a randomized trial. A nutritional evaluation was performed at the beginning and at the end of a 9-month period in which subjects received 1 of 2 diets (diet HP: high protein/low carbohydrate vs diet S: standard diet).

Results: there were no significant differences between the positive effects (on weight, BMI, waist circumference, fat mass, systolic blood pressure and leptin levels) in either genotype group with both diets. With both diets and only in wild genotype (diet HP vs diet S), total cholesterol (-10.1 ± 3.9 mg/dl vs -10.1 ± 2.2 mg/dl; $p>0.05$), LDL cholesterol (-9.5 ± 2.1 mg/dl vs -8.5 ± 2.3 mg/dl; $p>0.05$) and triglycerides (-19.1 ± 2.1 mg/dl vs -14.3 ± 2.1 mg/dl; $p>0.05$) decreased. The improvement in these parameters was similar in subjects with diet HP than HS. With diet HP and only in wild genotype, insulin levels (-3.7 ± 1.9 UI/L; $p<0.05$) and HOMA-R (-0.7 ± 0.1 units; $p<0.05$) decreased.

Conclusion: metabolic effect of weight reduction by two hypocaloric diets is the greatest in subjects with normal homozygous beta 3-AR gene. Improvement in total cholesterol, LDL-cholesterol, triglyceride, glucose, insu-

VARIACIÓN GENÉTICA EN EL GEN BETA-3-ADRENORRECEPTOR (TRP64ARG POLIMORFISMO) Y SU INFLUENCIA EN PARÁMETROS ANTROPOMÉTRICOS Y RESISTENCIA A LA INSULINA DESPUÉS DE UNA DIETA CON ALTO CONTENIDO EN PROTEÍNAS/BAJA EN CARBOHIDRATOS VERSUS UNA DIETA ESTÁNDAR HIPOCALÓRICA

Resumen

Introducción: la variante Trp64Arg del receptor Beta ha sido relacionada con un aumento del peso corporal y resistencia a la insulina.

Objetivo: el objetivo de nuestro estudio fue investigar la influencia del polimorfismo (rs 4994) del gen del receptor adrenérgico-Beta-3 en la respuesta metabólica y la pérdida de peso en un estudio de intervención a medio plazo con una dieta con alto contenido en proteínas/baja en carbohidratos vs una dieta hipocalórica estándar (1.000 kcal / día).

Material y métodos: se evaluó una muestra de 284 sujetos obesos con un diseño de ensayo aleatorio. Se realizó una evaluación nutricional al inicio y al final de un período de 9 meses en el que los sujetos recibieron una de las dos dietas (dieta HP: alta en proteínas/baja en carbohidratos vs dieta S: dieta estándar).

Resultados: no hubo diferencias significativas entre los efectos positivos (sobre el peso, el índice de masa corporal, la circunferencia de la cintura, la masa grasa, la presión arterial sistólica y los niveles de leptina) en los dos genotipos con ambas dietas. Con ambas dietas y solo en el genotipo salvaje (dieta HP vs dieta S), colesterol total ($-10,1 \pm 3,9$ mg / dl vs $-10,1 \pm 2,2$ mg / dl; $p> 0,05$), colesterol LDL ($-9,5 \pm 2,1$ mg / dl vs $-8,5 \pm 2,3$ mg / dl; $p> 0,05$) y los triglicéridos ($-19,1 \pm 2,1$ mg / dl vs $-14,3 \pm 2,1$ mg / dl; $p> 0,05$) disminuyeron. La mejoría de estos parámetros fue similar en sujetos con dieta HP vs dieta HS. Con la dieta HP y solo en el genotipo salvaje, los niveles de insulina ($-3,7 \pm 1,9$ UI / L; $p<0,05$) y HOMA-R ($-0,7 \pm 0,1$ unidades; $p<0,05$) disminuyeron.

Conclusión: el efecto metabólico de reducción de peso por dos dietas hipocalóricas es mayor en los sujetos homocigotos. La mejoría en el colesterol total, LDL-colesterol,

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lin and HOMA-R levels is better than in the heterozygous group.

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Key words: *Trp64arg beta-3-adrenoreceptor. Hypocaloric diet. Metabolic parameters. Obesity.*

Introduction

Many diseases and health conditions have been consistently associated with obesity such as hypertension, type 2 diabetes mellitus and cardiovascular risk factors¹. Several candidate genes have been investigated in relation to human obesity, one such gene is Trp64Arg polymorphism of the Beta3-adrenergic receptor gene (Beta3-AR). Beta3-AR is the principle mediator of catecholamine-stimulated thermogenesis and lipolysis, which mainly occur at subcutaneous and visceral sites². The Trp64Arg variant in this receptor has been reported to be associated with increased body weight and insulin resistance³⁻⁴.

Social and environmental factors are important mediators for weight control, and the environment may affect the control of food intake. For instance, appetite is regulated by physiological mechanisms, but an obesogenic environment may lead do overconsumption of food, thus affecting weight gain. Additionally, the individual response to an obesogenic environment and to different diets may be related to gene polymorphisms. Some studies have shown that the mutation of tryptophan by arginine at position 64 in the Beta3-AR gene (rs 4994) affected obesity treatment in humans⁵. In other study, De Luis et al⁶ have shown that carriers of the normal homozygote genotype were associated with larger improvements in metabolic parameters such as fasting glucose levels and insulin resistance after a conventional hypocaloric diet. Moreover, the heterozygote genotype of Beta3-AR was associated with a lack of improvement on metabolic parameters (fasting glucose levels and insulin resistance) after two different hypocaloric diets (low fat vs low carbohydrate diet)⁷. However, other studies did not demonstrate this effect on metabolic parameters⁸. Also, the effect of this polymorphism on weight response secondary to different hypocaloric diets has been evaluated in a panel of obesity-related candidate genes, showing a minor role⁹. Perhaps the distribution of macronutrients, considering previous studies, may influence secondary metabolic responses to weight loss as a function of this polymorphism. Recent studies have suggested no major differences between the effects of various dietary approaches, including between low-carbohydrate and low-fat diets on body weight outcomes¹⁰⁻¹². However, other studies have reported that very low-carbohydrate ketogenic diets and the Mediterranean diet are superior to low-fat diets in reducing body weight¹³⁻¹⁴.

triglicéridos, glucosa, insulina y los niveles de HOMA-R es superior en los pacientes sin el alelo Arg.

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Palabras clave: *Trp64Arg beta-3-adrenérgicos. Dieta hipocalórica. Parámetros metabólicos. Obesidad.*

Furthermore, in light of previous findings, the aim of our study was to investigate the influence of polymorphism (rs 4994) in Beta3-adrenergic receptor gene on metabolic response and weight loss in a medium-term intervention study secondary's to a high protein/low carbohydrate vs. a standard hypocaloric diets (1000 kcal/day).

Subjects and methods

Subjects

A population of 284 obesity subjects (body mass index >30) was recruited in a prospective way. These patients were studied in a Nutrition Clinic Unit and they gave written informed consent. Local ethical committee (CEIC-HURH) approved the protocol (4-2014 CEIC HURH) and patients approved the use of their genetic material for this study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Exclusion criteria included a history of cardiovascular disease or stroke during the previous 36 months, total cholesterol > 300 mg/dl, triglycerides > 400 mg/dl, blood pressure > 140/90 mmHg, fasting plasma glucose >110 mg/dl, as well as the use of sulfonylurea, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit.

Procedure

Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, insulin resistance (HOMA-R), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and adipokines (leptin, adiponectin, resistin) levels were measured at basal time and at the end of the 3 months on both hypocaloric diets branches. A tetrapolar bioimpedance and a prospective serial assessment of nutritional intake with 3 days written food records were realized. Genotype of Beta3 adrenoreceptor gene polymorphism was studied.

Patients were randomly allocated to one of two diets for a period of nine months. Diet HP (high protein-low carbohydrate hypocaloric diet) consisted in a diet of 1050 cal/day, 33% of carbohydrates (86.1 g/day), 33% of fats (39.0 g/day) and 34% of proteins (88.6 g/day). The distribution of fats was; 23.5% of saturated fats,

63.8% of monounsaturated fats and 12.6% of polyunsaturated fats. Diet S (standard protein hypocaloric diet) consisted in a diet of 1093 cal/day, 53% carbohydrates (144.3 g/day), 27% fats (32.6 g), and 20% proteins (55.6 g/day). The distribution of fats was; 20.9% of saturated fats, 67.4% of monounsaturated fats and 11.6% of polyunsaturated fats. The main food source of monounsaturated fatty acids was olive oil, of polyunsaturated fatty acids (w3 from fish) and (w6 from vegetable) seeds and saturates fatty acids were beef, chicken and pork. The adherence of these diets was assessed each 7 days with a phone call by a dietitian in order to improve compliance of the calorie restriction and macronutrient distribution. National composition food tables were used as reference¹⁵.

Biochemical assays and Genotyping of Beta3 adrenoceptor gene polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA). The polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'-CAA CCT GCT GGT CAT CGT-3'; primer reverse: 5'-AGG TCG GCT GCG GC-3'), and 0.25 uL of each probes (wild probe: 5'-Fam-CCA TCG CCT GGA CTC CG-BHQ-1-3') and (mutant probe: 5'-Hex-CAT CGC CCG GAC TCC G- BHQ-1-3') in a 25 uL final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA). DNA was denaturated at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3° for 45 s). The PCR were run in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase. Hardy Weinberger equilibrium was assessed.

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin resistance (HOMA-R) was calculated using these values¹⁶. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensitivity 0.5 mg/dl. Plasma hormone levels were evaluated using the multiplex Biorad® 10 plex assay following manufacturer's instructions (Bio-Rad®, Hercules, CA). This system allows for quantitative measurement of

different hormones, while consuming a small amount of biological material; resistin, leptin and adiponectin. Limits of detection were as follows (pg/ml): leptin (1.8), resistin (1.4) and adiponectin (3.8).

Anthropometric measurements and blood pressure

Body weight was measured to an accuracy of 0.1 Kg and body mass index computed as body weight/(height²). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences were measured also to derive waist-to hip ratio (WHR). Tetrapolar body electrical bioimpedance was used to determine body composition¹⁷. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. The same investigator measured patients and controls. Precautions taken to insure valid BIA measurements were; no alcohol within 24 hours of taking the test, no exercise or food for four hours before taking the test.

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Dietary intake and habits

All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a dietitian and analysed with a computer-based data evaluation system. National composition food tables were used as reference¹⁵.

Statistical analysis

Sample size was calculated to detect differences over 3 kg in body weight with 90% power and 5% significance (n=140, in each diet group). The results were expressed as average±standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables were analyzed with a 2-way ANOVA model. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A Chi square test was used to evaluate the Hardy-Weinberg equilibrium. Non-parametric variables were analyzed with the Wilcoxon test. The statistical analysis was performed for the combined *Trp64/Arg64* and *Arg64/Arg64* as a mutant type group and type *Trp64/Trp64* as wild type group, as a dominant model. A p-value <0.05 was considered statistically significant.

Results

Two hundred and eighty four patients gave informed consent and were enrolled in the study. The mean age was 51.9±11.2 years and their mean BMI 36.1±3.2 kg/m², with 75 males (26.4%) and 209 females (73.6%). Two hundred and forty eight (61 males/187 females) (87.3%) had the genotype *Trp64/Trp64* and 36 patients (14 males/22 females) *Trp64/Arg64* (12.7%).

The 144 subjects (129 *Trp/Trp* genotype and 15 *Arg* allele carriers) treated with diet HP basal, basal assessment of nutritional intake with a 3 days written food record showed a basal calorie intake of 2110.1±511.2 kcal/day, a carbohydrate intake of 223.21±19.1 g/day (47.6% of calories), a fat intake of 89.1±28.9 g/day (31.1% of calories) and a protein intake of 82.1±41.2 g/day (21.3% of calories). During the intervention, these subjects reached the recommendations of diet; 1009.1±90.2 calories (29.8% of carbohydrates, 32.8% of lipids and 37.4% of proteins). In the other hand, the 140 subjects (119 *Trp/Trp* genotype and 21 *Arg* allele carriers) treated with diet S, basal assessment of nutritional intake with a 3 days written food record showed a basal calorie intake a calorie intake of 2083.8±372.1 kcal/day, a carbohydrate intake of 216.0±20.1 g/day (45.2% of calories), a fat intake of 89.1±15.1 g/day (38.3% of calories) and a protein intake of 89.1±8.1 g/day (20.5% of calories). During the intervention, these patients reached the recommendations of diet; 1013.1±71.1 calories (52.7% of carbohydrates, 29.0% of lipids and 18.3% of proteins).

Anthropometric characteristics of participants at baseline and at 3-9 months of intervention are shown in table I. With the diet type HP and in both genotypes, BMI (values in *Trp/Trp* vs *Trp/Arg* genotypes at 9 months) (-2.7±1.2 kg/m² vs. -2.8±1.1 kg/m²; p>0.05), weight (-8.7±5.2 kg vs. -7.3±3.4 kg; p>0.05), fat mass (-6.0±4.1 kg vs. -5.1±2.9 kg; p>0.05), waist circumference (-8.1±3.1 cm vs. -5.5±3.1 cm; p<0.05) and systolic blood pressure (-3.3±2.1 mmHg vs -3.4±2.8 mmHg; p>0.05) decreased. BMI, weight, fat mass and waist circumference improvement was similar in subjects with both genotypes.

With the diet type S and in both genotypes at 9 months (Table I), BMI (values in *Trp/Trp* vs *Trp/Arg* genotypes at 9 months) (-2.8±2.1 kg/m² vs. -2.1±1.7 kg/m²; p>0.05), weight (-8.9±4.2 kg vs. -9.0±4.4 kg; p>0.05), fat mass (-4.7±3.1 kg vs. -4.0±2.2 kg; p>0.05), waist circumference (-5.4±2.1 cm vs. -5.2±3.1 cm; p>0.05) and systolic blood pressure (-3.2±2.1 mmHg vs. -3.1±2.9 mmHg; p>0.05) decreased, too. BMI, weight, fat mass and waist circumference improvement was similar in subjects with both genotypes. No differences were detected among basal of anthropometric variables between subjects with both genotypes. There were no significant differences between the effects on weight, BMI, waist circumference, fat mass and systolic blood pressure of the different diets in either genotype group.

TABLE I
changes in anthropometric variables (mean±S.D)

Characteristics	DIET HP						DIET S					
	Trp/Trp			Trp/Arg			Trp/Trp			Trp/Arg		
	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths
BMI	35.5±6.1	33.5±5.9*	32.8±5.4*	34.9±4.1	33.3±5.1*	32.1±5.0*	35.2±5.3	33.7±5.7*	32.4±5.1*	35.3±5.3	33.6±5.6*	32.2±4.0*
Weight (kg)	92.4±12.6	87.3±17.0*	83.7±13.1*	89.7±11.4	84.2±10.1*	82.4±13.1*	91.5±11.2	87.7±11.7*	82.6±9.4*	91.4±9.6	87.7±9.6*	81.9±5.2*
Fat mass (kg)	36.6±10.1	33.1±9.1*	30.5±9.1*	35.5±8.1	32.4±7.1*	31.1±6.9*	36.9±9.1	33.8±10.3*	32.3±7.2*	36.9±9.1	33.7±10.1*	32.9±8.1*
WC (cm)	112.8±10.6	107.8±11.2*	104.7±8.7*	109.5±8.1	105.6±7.2*	104.0±9.0*	111.6±9.1	107.3±7.0*	106.1±10.3	111.5±11.1	107.5±9.6*	105.1±8.3*
WHR	0.96±0.06	0.94±0.05	0.93±0.1	0.93±0.01	0.92±0.1	0.92±0.03	0.95±0.07	0.94±0.06	0.93±0.09	0.93±0.1	0.92±0.10	0.91±0.10
SBP (mmHg)	127.7±12.8	124.3±11.7*	124.4±10.1*	126.6±12.3	122.8±9.4*	122.5±7.2*	127.4±8.1	123.5±6.1*	124.2±10.0*	127.3±9.0	123.5±10.0*	124.1±7.2*
DBP (mmHg)	81.9±8.1	79.0±7.0	79.2±9.1	80.3±7.0	78.7±6.9	77.8±6.1	80.9±8.1	78.9±7.0	78.9±7.2	80.9±8.1	78.9±6.7	77.9±7.0

HP: high protein/low carbohydrate. S: standard. DBP: Diastolic blood pressure. Mths: Months SBP: Systolic blood pressure. WHR: Waist to hip ratio. WC: Waist circumference. (*) p<0.05, in each genotype group with basal values. No statistical differences between genotypes in each diet.

Table II shows the classic cardiovascular risk factors. With both diets and only in wild genotype (diet HP vs diet S), total cholesterol (-10.1+3.9 mg/dl vs -10.1+2.2 mg/dl;p>0.05), LDL cholesterol (-9.5+2.1 mg/dl vs -8.5+2.3 mg/dl;p>0.05) and triglycerides (-19.1+2.1 mg/dl vs -14.3+2.1 mg/dl;p>0.05) decreased. The improvement in these parameters was similar in subjects with diet HP than HS. With diet HP and only in wild genotype, insulin levels (-3.7+1.9 UI/L;p<0.05) and HOMA-R (-0.7+0.1 units;p<0.05) decreased.

Table III shows levels of adipocytokines. With the diet HP and in both genotypes (Trp/Trp vs Trp/Arg genotypes) and leptin levels (-26.1+3.1 ng/ml vs. -22.9+5.1 ng/ml: p>0.05) decreased. With the diet S, leptin levels (-25.1+7.2 ng/ml vs. -24.1+9.1 ng/ml: p>0.05) decreased in both genotypes, too. Resistin and adiponectin levels remained unchanged along 9 months. The amount of leptin decrease was similar with both diets. No differences were detected among basal and post-treatment values of adipocytokines among genotypes.

Discussion

In obese subjects with both genotypes of Trp64Arg polymorphism of the beta adrenoreceptor gene treated with two different hypocaloric diets during 9 months (high protein “HP” and standard “S” hypocaloric diets), we observed a significant decrease of weight, BMI, fat mass, waist circumference, blood pressure and leptin levels. Our study show that carriers of Arg allele had a different metabolic response, with a lack of effect on total cholesterol, LDL cholesterol, glucose, insulin, HOMA-R and triglyceride levels. The improvement of HOMA-R and insulin levels appeared after HP diet and in non carriers of Arg allele.

Firstly, our present finding of frequency of the Arg64 allele in obese subjects is in agreement with previous reports, less than 15%¹⁸. Secondly, the lack of association of this polymorphism with obesity has been described in some metanalysis¹⁹. Contradictories results are present in the literature, for example a meta-analysis assessing quantitative phenotypes in relation to a genetic polymorphism, and the results support the association of Trp64Arg polymorphism with BMI across diverse populations²⁰. Perhaps, the variations reported in the literature may partially be explained by differences in ethnic background, baseline BMI, gender distribution, interaction with other polymorphisms, weight loss, and experimental design.

In our study, fasting glucose levels, lipid profile, insulin levels and HOMA-R improved in the subjects with Trp64Trp genotype after a HP hypocaloric diet without change in Arg allele carriers. After S hypocaloric, an improvement in lipid profile was observed in non Arg allele carriers, without a significant

Table II
Classical cardiovascular risk factors (mean+S.D)

Characteristics	DIET HP						DIET S					
	Trp/Trp			Trp/Arg			Trp/Trp			Trp/Arg		
	0 time	At 9 mths	At 3 mths	0 time	At 9 mths	At 3 mths	0 time	At 9 mths	At 3 mths	0 time	At 9 mths	At 3 mths
Glucose (mg/dl)	104.8±7.2	100.6±9.1	100.4±9.0	103.2±7.1	102.7±8.0	102.7±8.0	102.0±9.0	99.9±5.2	99.9±5.2	101.9±5.3	98.2±6.1	98.1±8.2
Total ch. (mg/dl)	209.5±26.8	199.4±20.3*	199.5±8.1*	201.3±12.1	197.6±21.2	202.1±12.9	209.2±30.1	199.3±20.7*	200.7±12.1*	209.3±12.2	200.6±28.4	209.0±15.4
LDL-ch. (mg/dl)	128.6±27.1	119.1±18.1*	121.5±20.9*	121.6±16.1	121.5±11.1	124.3±9.2	128.7±21.1	120.8±10.1*	122.7±13.2*	124.7±19.1	122.7±12.1	126.1±13.1
HDL-ch. (mg/dl)	54.6±10.5	56.6±8.1	54.3±11.1	55.6±9.2	54.7±8.2	55.6±8.2	57.2±11.0	54.8±9.0	54.8±9.0	55.8±10.3	54.5±9.2	53.8±8.1
TG (mg/dl)	123.1±30.1	112.8±10.9	112.8±10.9	118.7±20.1	109.3±28.2	113.1±31.3	126.1±39.6	117.8±22.1	117.8±22.1	126.3±39.1	117.9±18.2	123.9±21.9
Insulin (mUI/L)	11.5±6.3	7.8±5.0*	9.6±6.1*	10.7±6.0	7.9±4.3	8.5±4.8	10.8±5.2	8.9±4.3	9.5±5.8	10.8±5.7	9.7±7.1	8.6±7.1
HOMA-R	2.6±2.1	1.9±1.5*	2.3±1.9	2.1±0.9	1.8±1.1	1.9±1.3	2.2±1.2	2.0±0.7	2.3±1.5	2.2±1.9	2.3±1.5	1.9±1.6
CRP (mg/dl)	4.9±5.3	4.7±3.1	4.6±5.2	5.1±3.2	5.0±4.1	5.0±3.2	4.9±4.0	5.2±4.1	5.2±3.8	5.3±4.1	5.2±4.0	5.0±3.9

HP: high protein/low carbohydrate. S: standardChol: CholesterolTG: Triglycerides CRP: c reactive protein. HOMA: Homeostasis model assessment. Mths: months (*) p<0.05, in each group with basal values. No statistical differences among genotypes in each diet or in different diet groups.

Table III
circulating adipocytokines (mean±S.D)

Characteristics	DIETHP						DIETS					
	Trp/Trp		Trp/Arg		Trp/Trp		Trp/Arg		Trp/Trp		Trp/Arg	
	0 time	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	0 time	At 3 mths	At 9 mths	At 9 mths
Adiponectin (ng/ml)	12.1±6.0	11.1±7.1	11.3±5.0	11.9±8.0	11.0±7.1	11.0±4.1	10.9±5.2	9.7±4.3	10.8±5.0	12.0±6.2	14.9±5.0	14.9±5.0
Resistin (ng/ml)	7.3±3.2	6.8±4.2	7.1±3.1	7.0±3.1	7.3±3.0	8.0±2.9	8.1±3.2	8.0±4.0	7.2±3.1	7.0±4.1	6.8±4.1	6.8±4.1
Leptin (ng/ml)	37.1±19.4	15.8±7.1*	40.1±18.3	25.2±11.2*	18.2±10.1*	39.8±11.2	15.0±9.4*	14.3±7.1*	32.5±11.2	20.8±6.1*	18.1±4.2*	18.1±4.2*

(*) p<0.05, in each group with basal values. No statistical differences among genotypes in each diet or in different diet groups.

effect on glucose metabolism. This metabolic response could be explained with previous data observed in animal models. For example, mice with knockout of the Beta 3-AR gene showed marked reductions in lipolysis stimulated by Beta 3 agonists²¹, and omental adipocyte Beta 3-AR sensitivity was related to waist hip ratio and insulin resistance²². Perhaps, defects in Beta3-AR signal transduction or regulatory mechanism may result in a diminished lipolytic response in visceral adipose tissue, aggravating the response to insulin action. A second hypothesis could explain these differences in diet response of glucose and lipid metabolisms depending of genotype, it has been postulated that those individuals with beta 3-AR Trp64Arg variant may have increased delivery of portal fatty acids due to their higher visceral fat²³.

This different effect in the improvement in glucose metabolism, it is in agreement with the previous reports that Trp64Arg mutation of the Beta3-AR gene was associated with difficulties in improving glyce-mic control and insulin concentrations during oral glucose tolerance test⁵. Moreover, the results of different interventional studies with this polymorphism gene have shown different data. De Luis et al⁶ have shown with a conventional hypocaloric diet that carriers of the normal homozygous genotype were associated with larger improvements in metabolic parameters such as fasting glucose levels and insulin resistance. Also, the heterozygous genotype of Beta3-AR was associated with a lack of improvement on fasting glucose levels and insulin resistance after two different hypocaloric diets (low fat vs low carbohydrate)⁷. The amount of monounsaturated fatty acids in a hypocaloric diet has a role in glucose response after weight loss, too²⁴. A recent study²⁵ showed that the Arg allele of this SNP is associated with long term changes on body weight in obese individuals during a follow up of 4 years. This polymorphism may become an indicator in personalized weight loss programs in obese subjects.

These contradictory results in metabolic changes secondary to weight loss in the literature could be due to the duration and style of dietary intervention in the protocols such as distribution of macronutrients in diet, percentage of dietary fat and duration of intervention or differences on background characteristics in the study populations such as basal weight, sex distributions, average and age.

Further studies will be designed to elucidate this controversial area and to clarify the role of this polymorphism in metabolic response secondary to weight loss in obese patients and the relationship among glucose metabolism, lipid profile and Trp64Arg polymorphism.

In conclusion, this study shows that the metabolic effect of weight reduction by two hypocaloric diets is not affected by this polymorphism of Beta3-AR gene. Carriers of Arg allele had a lack of effect on total cholesterol, LDL cholesterol, glucose, insulin,

HOMA-R and triglyceride levels. The improvement of HOMA-R and insulin levels appeared after HP diet and in non carriers of Arg allele.

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