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Trabajo Original

Obesidad y síndrome metabólico

Association of the TNF-alpha -308 G/A polymorphisms with metabolic responses secondary to a high protein/low carbohydrate *versus* a standard hypocaloric diet

Asociación del polimorfismo TNF-alpha -308 G/A con los cambios metabólicos secundarios a una dieta hipocalórica rica en proteínas/baja en hidratos de carbono versus *una dieta hipocalórica estándar*

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Abstract

Background: Mutation analysis has identified a G-> A transition in the promoter region of TNF-alpha gene at position -308 (rs1800629).

Objective: The aim of our study was to investigate the influence of polymorphism in -308 GA promoter variant of the TNF alpha gene on metabolic response and weight loss secondary to two hypocaloric diets.

Method: A sample of 283 obese subjects was enrolled in a consecutive prospective way. In the basal visit, patients were randomly allocated during 9 months to diet HP (high protein/low carbohydrate hypocaloric diet) and diet S (standard hypocaloric diet).

Results: There were no significant differences between the positive effects on weight loss in either genotype group with both diets. With both diets and only in wild genotype (diet HP *vs.* diet S), total cholesterol (-9.1 ± 3.4 mg/dL *vs.* -6.9 ± 2.0 mg/dL; $p > 0.05$), LDL cholesterol (-9.0 ± 2.9 mg/dL *vs.* -6.5 ± 2.1 mg/dL; $p > 0.05$) and triglycerides (-23.1 ± 5.1 mg/dL *vs.* -12.3 ± 4.8 mg/dL; $p < 0.05$) decreased. The improvement in triglycerides was higher in subjects without A allele. With diet HP and only in wild genotype, insulin levels (-3.1 ± 1.8 UI/L; $p < 0.05$) and HOMA-R (-0.8 ± 0.1 units; $p < 0.05$) decreased.

Conclusion: Carriers of -308 GG promoter variant of TNF-alpha gene have a better metabolic response than -308 GA obese with a high protein hypocaloric diet.

Key words:

G308 TNF-alpha polymorphism. Hypocaloric diet. Obesity.

Resumen

Antecedentes: los diferentes estudios genéticos han identificado un cambio G-> A en la región promotora del gen de TNF-alfa en la posición -308 (rs1800629).

Objetivo: el objetivo de nuestro estudio fue investigar la influencia del polimorfismo en -308 GA del gen TNF-alfa en la respuesta metabólica y la pérdida de peso secundarias a dos dietas hipocalóricas.

Método: una muestra de 283 sujetos obesos fue evaluada en forma prospectiva consecutiva. En la visita basal, los pacientes fueron asignados al azar durante 9 meses a una dieta HP (dieta de alto valor proteico/baja en hidratos de carbono) y dieta S (dieta hipocalórica estándar).

Resultados: no hubo diferencias significativas entre los efectos positivos sobre la pérdida de peso entre los grupos genotipo con ambas dietas. Con ambas dietas y solo en el genotipo salvaje (dieta HP *vs.* dieta S), el colesterol total (-9,1 ± 3,4 mg/dl *vs.* -6,9 ± 2,0 mg/dl; $p > 0,05$), colesterol LDL (-9,0 ± 2,9 mg/dl *vs.* -6,5 ± 2,1 mg/dl; $p > 0,05$) y los triglicéridos (-23,1 ± 5,1 mg/dl *vs.* -12,3 ± 4,8 mg/dl; $p < 0,05$) disminuyeron significativamente. La mejora de los triglicéridos fue mayor en sujetos sin el alelo A. Con la dieta HP y solo en el genotipo salvaje, los niveles de insulina (-3,1 ± 1,8 UI/l; $p < 0,05$) y HOMA-R (-0,8 ± 0,1 unidades; $p < 0,05$) disminuyeron.

Conclusión: los obesos portadores de la variante -308 GG del gen TNF tienen una mejor respuesta metabólica que los sujetos con genotipo -308 GA con una dieta hipocalórica rica en proteínas.

Palabras clave:

Polimorfismo G308 TNF-alpha. Dieta hipocalórica. Obesidad.

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INTRODUCTION

Obesity-related diseases are major public health problems. Hypocaloric diets are known to be an effective treatment for overweight and obese subjects (1). Individual responses to lifestyle modification vary and it is clear that they are partially genetically determined. Apart from the role of environmental determinants, also genetic factor predispose to obesity and may influence the response to intervention on lifestyle, Heterogeneous responses to lifestyle interventions should be at least in part controlled by genetic factors (2). Single nucleotide polymorphisms (SNPs) were described in association with the risk for obesity and metabolic diseases (3).

Mutation analysis has identified a G-> A transition in the promoter region of TNF-alpha gene at position -308 (rs1800629). This polymorphic variant has been shown to affect the promoter region of the TNF-alpha gene leading to a higher rate of transcription compared to the wild allele (4). Fernández Real et al. (5) have reported a significant association between the -308 GA variant and increased insulin resistance, BMI and increased production of leptin. However, other studies have reported negative results, with no correlation between TNF-alpha mutation and insulin resistance (6).

An accumulating body of evidence shows that modest weight loss through dietary changes is an effective means for managing obesity (7). As far as we known, few studies (8-10) have described the effect of different hypocaloric diets on weight loss and metabolic parameters, analyzing -308 GA promoter variant of TNF-alpha gene in obese subjects. De Luis et al. (8) have shown, with a standard hypocaloric diet, that carriers of the -308 GG genotype were associated with larger improvements in serum glucose, HOMA-R and leptin levels. Also, in a different study (9), A allele carriers were associated with a lack of improvement on metabolic parameters after two different hypocaloric diets (low fat *vs* low carbohydrate). Finally, carriers of the -308 GG promoter variant of TNF-alpha gene have a better metabolic response than A allele carriers with a high polyunsaturated fat hypocaloric diet (10). On the other hand, recent studies have suggested no major differences between the effects of various dietary approaches, including low-carbohydrate and low-fat diets, on body weight outcomes (11,12). However, other studies have reported that very low-carbohydrate ketogenic diets and the Mediterranean diet are superior to low-fat diets in reducing body weight (13,14).

Therefore, in light of previous findings, the aim of our study was to investigate the influence of polymorphism (rs1800629) in -308 GA promoter variant of the TNF alpha gene on metabolic response and weight loss in a medium-term intervention study secondary to a high protein/low carbohydrate diet *vs.* a standard hypocaloric diet (1,000 kcal/day).

SUBJECTS AND METHODS

SUBJECTS

A sample of 283 obese subjects was enrolled in a consecutive prospective way, from May 2013 to December 2014 in a tertiary hospital. These patients were studied in a Nutrition Clinic Unit and signed an informed consent (the Ethical Committee of our hospital approved this protocol). This study was conducted according to the guidelines laid down by the World Medical Association 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

Patients were randomly allocated to one of two diets for a period of 9 months. Diet HP (high protein-low carbohydrate hypocaloric diet) consisted in a diet of 1,050 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 1,093 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 polyunsaturaded fatty acids, w3 from fish and w6 from vegetable seeds; and of saturated fatty acids, beef, chicken and pork. Adherence to these diets was assessed each 7 days with a phone call by a dietitian in order to improve compliment of calorie restriction and macronutrient distribution. National composition food tables were used as reference (15).

Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and adypokines (leptin, adiponectin, and resistin) levels were measured at basal time and at 9 months, after both hypocaloric diet. Genotype of -308 GA promoter variant of the tumor necrosis factor-alpha gene was studied.

GENOTYPING OF G308A PROMOTER VARIANT OF THE TNF-ALPHA GENE

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 50 ng of genomic DNA from peripheral blood, 0.5 uL of each oligonucleotide primer (primer forward: 5'-CTG TCT GGA AGT TAG AAG GAA AC-3'; primer reverse: 5'-TGT GTG TAG GAC CCT GGA G-3'), and 0.25 uL of each probes (wild probe: 5'-Fam-AAC CCC GTC CTC ATG CCC-Tamra-3') and (mutant probe: 5'-Hex-ACC CCG TCT TCA TGC CCC- Tamra -3') in a 25 uL final volume (Termociclador iCycler IQ [Bio-Rad®], Hercules, CA). DNA was denaturized at 95 °C for 3 min; this was followed by 50 cycles of denaturation

at 95 °C for 15 sec, and annealing at 59.3 °C for 45 sec). 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. One probe was labeled at the 5-end with a LightCycler-Red fluorophore (Fam) and the other probe was labeled at the 3'-end with Hex. Only after hybridization to the template DNA the two probes do come in close proximity, resulting in fluorescence resonance energy transfer between the two fluorophores. The emitted fluorescence of the LyghtCycler fluorophore was measured (Real Time polymerase chain reaction). Hardy Weimberger equilibrium was assessed.

BIOCHEMICAL ASSAYS

Plasma hormone levels were evaluated using the multiplex Biorad© 10 plex assay following manufacturer's instructions (Bio-Rad®, Hercules, CA). This system allows for the quantitative measurement of different hormones (resistin, leptin and adiponectin), while consuming a small amount of biological material. Limits of detection were as follows (pg/ml): leptin (1.8), resistin (1.4) and adiponectin (3.8).

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 values (16). 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.

ANTHROPOMETRIC MEASUREMENTS

Body weight was measured to an accuracy of 0.1 kg and body mass index computed as body weight/(height^2) (kg/m^2). Waist circumference was measured in the midway between the lowest rib and the iliac crest using an anthropometric tape. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g (17). 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. Precautions taken to insure valid BIA measurements were no alcohol within 24 hours of taking the test and 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.

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 the Kolmogorov-Smirnov test. Quantitative variables were analyzed with a 2-way ANOVA model. Qualitative variables were analyzed with the Chi-squared test, with Yates correction as necessary, and Fisher's test. A Chi-squared test was used to evaluate the Hardy-Weinberg equilibrium. Non-parametric variables were analyzed with the Wilcoxon test. The statistical analysis was designed for the combined *G-308A* and *A-308A* as a mutant type group and wild type group G-*308G* as second group. Dominant model was used to analyze the results. A p-value under 0.05 was considered to be statistically significant.

RESULTS

Two hundred and eighty three patients gave informed consent and were enrolled in the study. The mean age was 48.9 ± 13.4 years and the mean BMI was 35.5 ± 4.6, with 77 males (26.5%) and 208 females (73.5%). All patients completed the 9-month follow-up period without drop-outs in both branches (diet HP *vs.* diet S). Two hundred and five patients (72.4%) had the genotype -308 GG and 78 (27.6%) patients had the genotype -308 GA (77 patients [27.2%] -308 GG and 1 patient [0.4%] -308 AG). Age was similar in both groups (-308 GG genotype: 48.7 ± 11.1 years *vs.* -308 GA genotype: 49.9 ± 12.0 years: p = 0.12). Sex distribution was similar in both groups, males (27.4% *vs.* 24.8%) and females (72.6% *vs.* 75.2%).

Regarding the 146 subjects (106 -308 GG genotype and 40 -308 GA genotype) treated with diet HP basal, basal assessment of nutritional intake with a 3 days written food record showed a basal calorie intake of 2,178.1 ± 391.2 kcal/day, carbohydrate intake of 227.91 ± 12.1 g/day (47.8% of calories), fat intake of 88.1 ± 23.1 g/day (31.0% of calories) and protein intake of 81.1 ± 38.2 g/day (21.2% of calories). During the intervention, these subjects reached the recommendations of the diet; 1,004.3 +/- 87.2 calories (29.6% of carbohydrates, 32.8% of lipids and 37.6% of proteins). On the other hand, in the 137 subjects (99 -308 GG genotype and 38 -308 GA genotype) treated with diet S, basal assessment of nutritional intake with a 3 days written food record showed a basal calorie intake of 2,181.8 ± 292.1 kcal/day, carbohydrate intake of 211.1 ± 28.1 g/day (45.5% of calories), fat intake of 82.1 ± 15.1 g/day (38.2% of calories) and protein intake of 87.1 ± 8.1 g/day (20.1% of calories). During the intervention, these patients reached the recommendations of diet; 1,003.9 +/- 70.1 calories (51.7% of carbohydrates, 30.1% of lipids and 19.2% 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 GG *vs.* GA genotypes at 9 months) (-3.6 ± 1.4 kg/m^2 *vs.* -3.4 ± 1.9 kg/m^2, p > 0.05),

weight (-8.1 ± 5.0 kg *vs.* -9.0 ± 3.9 kg, p > 0.05), fat mass (-5.4 ± 4.3 kg *vs.* -5.9 ± 3.9 kg, p > 0.05), waist circumference (-7.1 ± 2.1 cm *vs.* -6.5 ± 4.1 cm, p < 0.05) and systolic blood pressure (-7.3 ± 3.1 mmHg *vs* -6.9 ± 3.8 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 G/G *vs.* G/A genotypes at 9 months) (-2.9 ± 2.0 kg/m^2 *vs.* -2.5 ± 1.9 kg/m^2, p > 0.05), weight (-9.1 ± 4.1 kg *vs* -9.4 ± 4.4 kg, p > 0.05), fat mass (-4.6 ± 3.3 kg *vs.* -4.5 ± 2.3 kg, p > 0.05), waist circumference (-4.1 ± 2.1 cm *vs.* -4.2 ± 3.0 cm, p > 0.05) and systolic blood pressure (-6.0 ± 2.6 mmHg *vs.* -6.1 ± 2.2 mmHg, p > 0.05) decreased, too. No differences were detected among basal of anthropometric variables between subjects with both genotypes. BMI, weight, fat mass and waist circumference improvement was similar in subjects with both genotypes. Finally, 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 II shows the classic cardiovascular risk factors. With both diets and only in wild genotype (diet HP *vs.* diet S), total cholesterol (-9.1 ± 3.4 mg/dl *vs.* -6.9 ± 2.0 mg/dl; p > 0.05), LDL cholesterol (-9.0 ± 2.9 mg/dl *vs.* -6.5 ± 2.1 mg/dl; p > 0.05) and triglycerides (-23.1 ± 5.1 mg/dl *vs.* -12.3 ± 4.8 mg/dl; p <0.05) decreased. The improvement in total and LDL cholesterol was similar in subjects with diet HP and HS. The improvement in triglycerides was higher in subjects without A allele. With diet HP and only in wild genotype, insulin levels (-3.1 ± 1.8 UI/L; p < 0.05) and HOMA-R (-0.8 ± 0.1 units; p < 0.05) decreased.

Table III shows adipocytokines levels. With both diets and in both genotypes (G/G *vs* G/A genotypes), leptin levels (diet HP: -28.1 ± 5.1 ng/mL *vs.* -22.1 ± 5.0 ng/mL, p > 0.05) and (diet S: -25.1 ± 3.0 ng/mL *vs.* -26.9 ± 5.0 ng/mL, p > 0.05) decreased. The amount of leptin decrease was similar with both diets. Resistin and adiponectin levels remained unchanged along 9 months. No differences were detected among basal and post-treatment values of adipocytokines among genotypes.

DISCUSSION

Our data showed diverse response to both diets when analyzing the subgroups stratified according to -308 GG genotype of promoter of the TNF-alpha gene. We observed a significant decrease in weight, BMI, fat mass, waist circumference, systolic blood pressure and leptin levels with both diets regardless of the genotype. Our study shows that non-A allele carriers had a different metabolic response with a significant decrease in LDL cholesterol, total cholesterol and tryglicerides after both diets. HP diet produces a significant decrease in insulin and HOMA in non-A allele carriers, too.

In our study the frequencies of genotypes did not markedly differ from data previously reported in the literature (18-20). Contrasting with numerous reports on the frequencies of -308 G/A SNP and association with body adiposity and metabolic disturb-

Table I. Changes in anthropometric variables (mean ± SD)

Characteristics	Diet HP		Diet S	
	GG	*GA*	*GG*	*GA*
	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	35.1 ± 6.1 / 33.1 ± 5.2* / 32.1 ± 5.1*	35.7 ± 4.2 / 34.2 ± 5.2* / 32.3 ± 6.1*	34.9 ± 5.3 / 33.4 ± 5.5* / 32.1 ± 5.0*	35.3 ± 5.3 / 33.6 ± 5.6* / 32.7 ± 4.1*
Weight (kg)	90.5 ± 10.6 / 85.3 ± 12.0* / 83.3 ± 10.1*	94.0 ± 10.4 / 90.2 ± 11.1* / 85.9 ± 11.1*	91.53 ± 10.2 / 87.4 ± 11.2* / 82.6 ± 9.4*	93.9 ± 9.1 / 88.8 ± 7.6* / 82.7 ± 4.2*
Fat mass (kg)	36.0 ± 10.2 / 32.6 ± 9.0* / 30.6 ± 8.1*	37.8 ± 8.1 / 33.1 ± 7.1* / 31.9 ± 6.1*	37.7 ± 9.0 / 32.9 ± 10.1* / 32.3 ±7.2*	39.8 ± 9.0 / 35.9 ± 10.0* / 33.0 ± 8.0*
WC (cm)	111.8 ± 9.6 / 106.8 ± 10.2* / 104.1 ± 8.1*	113.5 ± 8.1 / 109.6 ± 7.1* / 104.0 ± 9.0*	110.3 ± 9.0 / 107.8 ± 6.0* / 106.1 ± 10.3	110.8 ± 10.1 / 108.5 ± 8.1* / 106.2 ± 7.3*
WHR	0.95 ± 0.06 / 0.94 ± 0.05 / 0.93 ± 0.1	0.94 ± 0.01 / 0.92 ± 0.1 / 0.92 ± 0.03	0.95 ± 0.07 / 0.94 ± 0.061 / 0.93±0.09	0.93 ± 0.1 / 0.92 ± 0.10 / 0.91 ± 0.10
SBP (mmHg)	129.1 ± 10.8 / 124.1 ± 10.7* / 122.1 ± 9.1*	127.6 ± 11.3 / 123.2 ± 8.4* / 122.5 ± 7.2*	127.1 ± 8.2 / 122.5 ± 5.0* / 121.9 ± 9.1*	127.8 ± 9.1 / 123.6 ± 9.0* / 122.0 ± 7.2*
DBP (mmHg)	82.8 ± 8.1 / 79.1 ± 7.1 / 79.8 ± 9.2	80.1 ± 7.1 / 79.7 ± 5.9 / 77.8 ± 6.1	80.8 ± 8.2 / 79.9 ± 7.1 / 78.9 ± 9.2	80.1 ± 6.1 / 78.3 ± 6.2 / 78.3 ± 6.9

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. Classical cardiovascular risk factors (mean ± SD)

Characteristics	Diet HP		Diet S	
	GG	*GA*	*GG*	*GA*
	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*
Glucose (mg/dL)	103.4 ± 7.2 / 100.1 ± 8.0 / 101.5 ± 9.2	101.2 ± 5.1 / 102.2 ± 8.1 / 100.8 ± 9.3	101.7 ± 8.0 / 98.8 ± 5.1 / 99.9 ± 5.2	98.9 ± 5.2 / 96.7 ± 6.0 / 99.1 ± 8.1
Total ch (mg/dL)	209.8 ± 26.8 / 200.4 ± 9.1* / 200.4 ± 19.3*	203.1 ± 10.1 / 199.7 ± 20.2 / 200.1 ± 19.9	205.2 ± 30.1 / 197.7 ± 22.3* / 199.3 ± 20.7*	208.1 ± 11.2 / 205.1 ± 18.4 / 207.1 ± 11.4
LDL-ch (mg/dL)	130.1 ± 22.1 / 123.1 ± 18.9* / 120.1 ± 11.1*	118.6 ± 11.1 / 119.5 ± 10.1 / 120.3 ± 8.2	125.6 ± 11.2 / 121.1 ± 12.2* / 120.8 ± 10.9*	123.7 ± 11.1 / 124.4 ± 11.1 / 125.2 ± 11.9
HDL-ch (mg/dL)	56.1 ± 9.5 / 55.3 ± 10.1 56.8 ± 4.1	55.1 ± 9.1 / 54.9 ± 8.1 / 54.9 ± 8.1	57.1 ± 9.0 / 55.8 ± 8.0 / 54.9 ± 11.0	55.3 ± 11.3 / 55.5 ± 7.2 / 54.8 ± 9.1
TG (mg/dL)	125.1 ± 430.1 / 106.8 ± 11.9 / 102.7 ± 10.1*	127.7 ± 19.1 / 122.3 ± 18.2 / 120.1 ± 30.3	126.1 ± 38.6 / 117.8 ± 22.1 / 112.8 ± 20.1*	126.1 ± 37.1 / 122.1 ± 13.2 / 124.1 ± 20.1
Insulin (mUI/L)	10.9 ± 6.7 / 8.8 ± 4.1* / 7.8 ± 4.0*	10.7 ± 5.1 / 11.9 ± .5.8 / 10.5 ± 5.5	10.9 ± 5.1 / 9.8 ± 5.2 / 9.0 ± 4.4	10.6 ± 5.1 / 9.8 ± 7.2 / 8.7 ± 7.2
HOMA-R	2.3 ± 2.1 / 2.0 ± 1.6* / 1.8 ± 1.5*	3.0 ± 0.9 / 2.9 ± 1.0 / 2.8 ± 1.4	2.1 ± 1.2 / 2.2 ± 1.9 / 2.0 ± 1.1	2.2 ± 1.3 / 2.1 ± 1.9 / 1.9 ± 1.7
CRP (mg/dL)	4.9 ± 5.1 / 4.8 ± 5.0 / 4.9 ± 4.1	5.1 ± 3.2 / 4.9 ± 4.01 / 5.0 ± 3.1	5.0 ± 3.0 / 5.2 ± 3.5 / 5.1 ± 4.0	5.5 ± 4.1 / 5.9 ± 4.1 / 5.1 ± 3.3

HP: High protein/low carbohydrate; S: Standard; Chol: Cholesterol; TG: 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 adypocitokines (mean ± SD)

Characteristics	Diet HP		Diet S	
	GG	*GA*	*GG*	*GA*
	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*
Adiponectin (ng/mL)	13.1 ± 5.0 / 9.9 ± 6.2 / 11.8 ± 7.4	11.9 ± 4.0 / 11.8 ± 7.0 / 12.1 ± 7.0	11.1 ± 4.2 / 11.9 ± 5.1 / 9.9 ± 4.2	11.8 ± 5.1 / 12.1 ± 6.1 / 12.9 ± 5.1
Resistin (ng/mL)	7.3 ± 3.2 / 7.5 ± 5.0 / 6.9 ± 4.1	7.2 ± 3.2 / 7.1 ± 3.9 / 7.8 ± 3.0	8.1 ± 2.9 / 8.2 ± 3.1 / 8.4 ± 4.0	7.6 ± 3.2 / 7.7 ± 4.2 / 7.1 ± 4.2
Leptin (ng/mL)	38.1 ± 18.4 / 15.6 ±1 4.1* / 10.1 ± 9.2*	40.0 ± 17.3 / 24.1 ± 10.2* / 18.1 ± 9.1*	39.7 ± 10.2 / 15.1 ± 9.2* / 14.1 ± 7.0 *	32.1 ± 10.2 / 20.1 ± 6.0* / 18.2 ± 4.1*

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

ance, their association with response to intervention on lifestyle has been rarely investigated. The results of different interventional studies with this polymorphism gene have shown unclear data. In one study (8), no carriers of the A allele had a greater reduction in glucose levels, insulin levels, leptin levels and weight than A allele carriers in response to 3-month hypocaloric diet. Other interventional study (9) carried out during 3 months with a low fat hypocaloric diet showed a better metabolic improvement secondary to weight loss in -308 GG genotype. In this previous study (9), the improvement of glucose, insulin levels, HOMA-R, LDL cholesterol, total cholesterol and triglyceride levels was statistically significant in -308 GG subjects with a low fat hypocaloric diet and only insulin levels improved in the same genotype with a low carbohydrate diet. Finally, A allele carriers did not show metabolic improvements after weight loss (9). On the other hand, leptin levels decreased more in -308 GG genotype subjects than in A allele carriers with both diets (9). Carriers of the -308 GG promoter variant of TNF-alpha gene have a better metabolic response than A allele carriers with a high polyunsaturated fat hypocaloric diet (10). Only one study has been designed with a surgical intervention; a surgical study with bariatric procedure (21) has shown that this polymorphism is not related to biochemical outcomes after a massive weight loss with a biliopancreatic diversion procedure. All previously cited studies have been conducted in Caucasian subjects, and only one study has evaluated a dietary intervention in a different ethnicity (22). In this design, -308 G/A SNP predisposed a better response of glucose metabolism to a dietary intervention. Certainly, the genetic base of the population studied modulated response to intervention because these results are not in agreement with previous literature data.

In our study, the metabolic benefits in lipid metabolism were not detected in variant allele carriers. The deleterious effect of this TNF-alpha variant allele was previously suggested in a meta-analysis in which individuals carrying -308 GA polymorphism had greater comorbidities related to obesity (23). Further, our design also shows an improvement in insulin concentrations and HOMA-R after weight loss secondary to a high protein/low carbohydrate hypocaloric diet only in subjects with -308 GG genotype. Some previous studies have proposed that the effects of the -308 GA promoter variant of the TNF-alpha gene on insulin action may depend on body weight (24). The A allele was associated with high RQ (resting quotient), indicating high rates of glucose oxidation and lipid synthesis, depending of BMI (24). On the other hand, the promoter variant at position -308 leads to a higher rate of TNF alpha gene transcription, followed by raised TNF-alpha concentrations and decreased insulin resistance (25). Nevertheless, a recent meta-analysis has reported lack of associations between this SNP and circulating levels of TNF-alpha (26).

Some specific reasons could explain these contradictory results on metabolic response. Firstly, the distribution of macronutrients in the prescribed diets and the type of dietary fat may influence secondary metabolic responses and weight loss. Secondly, duration of dietary intervention may influence secondary metabolic responses to weight loss as a function of this polymorphism. Perhaps the interaction between -308 GA polymorphism and weight loss secondary to diet is modulated during time. As far as we know, our present study is the longest designed so far: 9 months. Thirdly, the basal dietary intake could interact with the SNP and modulate the metabolic response after dietary intervention (27). Finally, the small sample size of all studies could be responsible for the absence of detection of biochemical changes in some sense.

In summary, the -308 GG SNP genotype may predispose a better response of insulin metabolism and HOMA-R after weight loss with a high protein hypocaloric diet. Both diets produced an improvement in anthropometric parameters in all patients. However, this weight decrease was accompanied by an improved lipid profile in patients non-carriers of the A allele.

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