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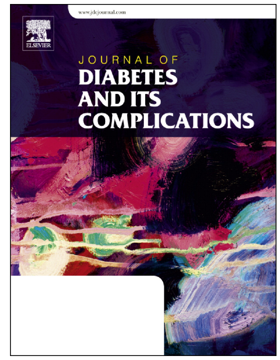

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A circadian rhythm-related *MTNR1B* genetic variant (rs10830963) modulate body weight change and insulin resistance after 9 months of a high protein/low carbohydrate vs a standard hypocaloric diet

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Running title: rs10830963 polymorphism and dietary intervention

The authors declare that they have no conflict of interests”

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Summary

Background & aims: The risk allele (G) of rs10830963 in the melatonin receptor 1 B (*MTNR1B*) gene presents an association with biochemical parameters and obesity. We study the effect of this SNP on insulin resistance and weight loss secondary to two hypocaloric diets.

Methods: 270 obese subjects were randomly allocated during 9 months (Diet HP: a high protein/low carbohydrate vs. Diet S: a standard severe hypocaloric diets)). Anthropometric parameters, fasting blood glucose, C-reactive protein (CRP), insulin concentration, insulin resistance (HOMA-IR), lipid profile and adipocytokines levels were measured. Genotype of *MTNR1B* gene polymorphism (rs10830963) was evaluated.

Results: All adiposity parameters, systolic blood pressure and leptin levels decreased in all subjects after both diets. This improvement of adiposity parameters was higher in non-G allele carriers than G allele carriers. After weight loss with Diet HP, (CC vs. CG+GG at 9 months); total cholesterol (delta:-9.9±2.4 mg/dl vs. -4.8±2.2 mg/dl:p<0.05), LDL-cholesterol (delta:-8.3±1.9 mg/dl vs. -5.1±2.2 mg/dl: p<0.05), insulin (delta:-4.7±0.8 UI/L vs. -0.9±1.0 UI/L: p<0.05), triglycerides (delta:-17.7±3.9 mg/dl vs. -6.1±2.8 mg/dl: p<0.05) and HOMA IR (delta:-0.8±0.2 units vs. -0.2±0.1 units: p<0.05) improved only in no G allele carriers. After weight loss with Diet S in non G allele carriers, insulin levels (delta (CC vs. CG+GG): -3.4±0.6 UI/L vs. -1.2±0.4 UI/L: p<0.05), triglycerides (delta:-29.2±3.4 mg/dl vs. -8.2±3.8 mg/dl: p<0.05), HOMA-IR (delta (CC vs. CG+GG): -1.1±0.2 units vs. -0.1±0.1 units: p<0.05), total cholesterol (delta:-15.9±7.4 mg/dl vs. -5.8±2.9 mg/dl:ns) and LDL-cholesterol (delta:-13.7±5.9 mg/dl vs. -6.0±2.9 mg/dl: ns) decreased, too.

Conclusions: our study detected a relationship of rs10830963 variant of *MTNR1B* gene with adiposity changes, cholesterol changes and insulin resistance modification induced by two different hypocaloric during 9 months.

Keywords:

rs10830963

Standard diet,

High protein diet

MTNR1B

INTRODUCTION

It has been recognized that circadian system is involved in regulation of energy balance and body weight (1). In human beings, disruption of this circadian rhythm by means of social jet lag, shift work, and consumption of a high amount of fat along the day has and metabolic complications (type 2 diabetes mellitus, glucose intolerance, hyperlipemia, hypertension or cardiovascular disease) (2-4). One of the most well-known chronobiotics is melatonin, a hormone produced by pineal gland that shows a main role in the control of these circadian rhythms (5).

The action of melatonin is realized by two membrane receptors; melatonin receptor 1 (MT1, encoded by *MTNR1A*) and melatonin receptor 2 (MT2, encoded by *MTNR1B*). *MTNR1B* is the ubiquitous receptor of both and it is located in diencephalon, pancreatic islets and retin tissue. Recent genome-wide association studies have identified common variants in the *MTNR1B* gene (6). One of this SNPs (single nucleotide polymorphisms) (rs10830963) in the melatonin receptor type 1B (*MTNR1B*) gene, has been related with altered rhythm and signal of melatonin (7). Interestingly, this genetic variant has also been related to diabetes mellitus type 2 (8-9), lipoproteins (10-11) and weight (12). Moreover, evidence has indicated that melatonin plays a key role in the regulation of adipose tissue (lipogenesis and lipolysis), the participation un the browning process of withe adipose tissue, the activation of brown adipose tissue and the maintenance of an energy balance (13-14)

Despite these above-mentioned relationships, investigations studying the effect of this polymorphism on response to weight loss strategies are scarce. Goni et al (15) reported that rs10830963 variant could be related with weight loss induced by a caloric restriction. The same authors (16) have detected a relationship of this genetic variant with lipid response after 2-year weight loss diet. A significant interaction was detected between rs10830963 genotypes (17) and the dietary intervention with a hypocaloric diet based in Mediterranean style on body weight loss and insulin resistance, too. Therefore, we hypothesized that the MTNR1B genotype might influence changes in body weight and metabolic parameters in response to different hypocaloric strategies.

In the present study, we evaluate the effect of this SNP on changes in body weight and insulin resistance in response to two different weight-loss diets (a high protein/low carbohydrate vs. a standard severe hypocaloric diets) during 9 months.

MATERIALS AND METHODS

Subjects and procedure:

Two hundred and eighty one patients were randomly assigned to one of two energy-reduced diets during 9 months follow-up time (a high protein/low carbohydrate vs. a standard severe hypocaloric diets) by a consecutive method of sampling among subjects send from Primary Care Physicians. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, the local ethics committee approved all procedures involving patients and all subjects provided informed consent.

Major exclusion criteria were the presence of a dietary intervention during the 6 months prior to the study, unstable cardiovascular or cerebrovascular diseases, insufficient motivation as well as the use of any of these drugs; dipeptidyl type IV inhibitors drugs, thiazolidinedione, metformin, GLP-1 analogs, sGLT2 inhibitors, insulin, glucocorticoids, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs. The inclusion criteria were the following; body mass index $\geq$ 30 kg/m^2 and an adult age ranged from 18 to 70 years.

Fasting blood samples (15 ml) were obtained at routine times in clinical settings at baseline, 3 months and 9 months. Levels of basal glucose, C-reactive protein (CRP), insulin, insulin resistance as homeostasis model assessment (HOMA-IR), total cholesterol, LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration and serum adipokines (leptin, adiponectin and resistin) were analyzed within the start of the trial and repeated after 3 and 9 months of both hypocaloric diets. Anthropometric parameters (weight, height, waist circumference and fat mass by bioimpedance) and blood pressure were measured in the morning before breakfast. Genotype of *MTNR1B* gene polymorphism (rs10830693) was evaluated. The results were analyzed for the combined *CG* and *GG* as a group and CC genotype as second group.

Dietary Intervention:

270 obese patients were randomly allocated to one of the next two diets. Diet HP (n=137) (severe hypocaloric diet, high protein-low carbohydrate) consisted in a diet of 1050 cal/day, 33% of fats (39.0 g/day), 33% of carbohydrates (86.1 g/day) and 34% of proteins (88.6 g/day). The distribution of fats was; 63.8% of monounsaturated fats, 23.5% of saturated fats, and 12.6% of polyunsaturated fats. Diet S (n=133) (severe hypocaloric diet, standard protein) consisted in a diet of 1093 cal/day, 27% fats (32.6 g), 53% carbohydrates (144.3 g/day), and 20% proteins (55.6 g/day). The distribution of fats was; 67.4% of monounsaturated fats, 20.9% of saturated fats, and 11.6% of polyunsaturated fats. The exercise recommendations for patients of both groups were the completion of aerobic physical activities at least 3 times per week (60 min each). The adherence of both diets was recorded each week with a phone call in order to improve both diets with a dietitian. National composition food tables were used as reference (18). Records of daily dietary intake for three days at basal time and at 9 months' time including a weekend day were evaluated with a computer-based data evaluation system (Dietosource ®, Gen, Sw).

Measurements

Body mass index was calculated as body weight in kilograms/(height ^{2} in meters). Waist circumference was measured in the narrowest diameter between xiphoid process and iliac crest. Electrical bioimpedance was used to measure body composition with an accuracy of 50 g (19). Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged (Omrom, LA,CA).

Insulin was analized by radio-immunoanalysis (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5mUI/L (normal range 0.5-30 mUI/L) (20), plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California) and the homeostasis model assessment for insulin resistance (HOMA-IR) was obtained using these values (21). Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay

(Technicon Instruments, Ltd., New York, N.Y., USA). HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula (LDL cholesterol= total cholesterol-HDL cholesterol-triglycerides/5) (22).

CRP was determined by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a CV% 2.8%. Adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, USA) (DRP300) with a CV% 3.8% (23). Leptin was by Enzyme-Linked Immunosorbent Assay (ELISA) (Diagnostic Systems Laboratories, Inc., Texas, USA) with a CV% 3.5% (24). Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a CV% 3.2% (25).

Genotyping of *MTNR1B* gene polymorphism

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood by using commercial kit extraction (Biorad, LA, CA). Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc.San Diego, California CA). Genotyping for the rs10830963 polymorphism was performed by polymerase chain reaction real time analysis. This polymerase chain reaction (PCR) was carried out with 20–25 ng of genomic DNA, 0.1–0.15 μl each of oligonucleotide primer for rs10830963 (primer forward: 5′-ACGTTGGATGCCCCCAGTGATGCTAAGAAT -3′ and reverse 5′-ACGTTGGATGGCATAGGCAGAATATTCCC -3′ in a 2-μl final volume (Termociclador Lifetecnologies, LA, CA). Hardy Weinberg equilibrium was calculated with a statistical test (Chi-square). The variant of *MTNR1B* gene was in Hardy Weinberg equilibrium (p=0.28).

Statistical analysis:

Sample size was calculated to detect differences over 2.5 kg in body weight loss with 90% power and 5% significance (n=140 in each group of diet). The statistical analysis were realized by intention to treat. Comparison of categorical variables were assessed by using chi-square test. Numerical variables with normal distribution were analyzed with a two-tailed Student's t-test. Non-parametric variables were analyzed with the Wilcoxon test. The statistical analysis to evaluate the gene –diet interaction was an univariate ANCOVA with Bonferroni test post Hoc The statistical analysis was performed for the combined *CG* and *GG* as a group and CC genotype as second group, with a dominant model. A p-value <0.05 was considered significant. SPSS version 15.0 has been used to realize statistical analysis.

RESULTS

Two hundred and eighty obese subjects were included in the study and 270 followed up and finalized the survey (figure 1), only 10 patients were not included in the trial. The mean age was 49.4±6.2 years (range: 28-66), the mean body mass index 35.1±4.2 kg/m^2 (range: 30.1-40.3) and the mean weight was 91.8±5.1 kg (range: 86.3-96.9). 143 patients (52.9%) had the genotype CC, 105 patients CG (38.9%) and 22 patients *GG* (8.2%). Age was similar in the three-genotype groups (CC; 49.5±5.1 years vs CG; 48.8±7.2 years vs GG; 49.2±6.3 years: ns).

In the group of (Diet HP) 137 obese patients (72 CC genotype and 65 G allele carriers), basal evaluation of nutritional intake with a 3 days written food record showed a calorie intake of 2018.7±236.1 kcal/day, a carbohydrate intake of 200.2±28.3 g/day (43.4 % of calories), a fat intake of 62.0±9.2 g/day (33.7% of calories) and a protein intake of 77.3±12.1 g/day (23.9% of calories). During the dietary intervention, these patients reached the right recommendations of the diet HP; 1023.9 cal/day, 32.5% of fats (38.6 g/day), 33.5% of carbohydrates (87.9 g/day) and 34.5% of proteins (89.6 g/day).

In the group of (Diet S) 133 subjects (71 CC genotype and 62 G allele carriers), basal evaluation of nutritional intake with a 3 days written food record showed a calorie intake of 2017.4±493.0 kcal/day, a carbohydrate intake of 208.2±48.9 g/day (43.1% of calories), a fat intake of 82.9±28.3 g/day (36.5% of calories) and a protein intake of 88.3±32.2 g/day (20.4% of calories). During the intervention, these subjects reached the recommendations of diet S; 1028.7 cal/day, 27.2% fats (37.9 g), 52.9% carbohydrates (144.2 g/day), and 19.9% proteins (55.5 g/day).

Table 1 shows anthropometric parameters and blood pressure characteristics of participants at baseline and at months 3 and 9 of intervention.

In both genotype groups, adiposity parameters and systolic blood pressure decreased. After weight loss with a severe hypocaloric diet, high protein-low carbohydrate (Diet HP; CC vs. CG+GG at 9 months); BMI (delta:-3.3±0.2 kg/m^2 vs. -3.1±0.2 kg/m^2:p=0.02), weight (delta:-8.6±1.1 kg vs. -6.2±0.9 kg: p=0.01), fat mass (delta:-6.2±1.8 kg vs. -3.7±1.2 kg: p=0.01) and waist circumference (delta:-11.7±2.1 cm vs. -6.7±1.9 cm: p=0.02) decreased. The improvement of these variables was higher in non-G allele carriers. After weight loss with a standard protein severe hypocaloric diet, (Diet S; CC vs. CG+GG al 9 months), BMI (delta:-3.1±0.2 kg/m^2 vs. -2.7±0.3 kg/m^2:p=0.04), weight (delta:-7.6±1.4 kg vs. -5.1±1.2 kg: p=0.03), fat mass (delta:-6.3±1.2 kg vs. -4.2±1.1 kg: p=0.03) and waist circumference (delta:-10.7±1.4 cm vs. -6.3±1.8 cm: p=0.01) decreased, too. This improvement of anthropometric parameters was higher in non-G allele carriers. Systolic blood pressure improved after both hypocaloric diets independently of the genotype (table 1).

Table 2 reports biochemical variables. After weight loss with Diet HP, (CC vs. CG+GG at 9 months); total cholesterol (delta:-9.9±2.4 mg/dl vs. -4.8±2.2 mg/dl:p=0.01), LDL-cholesterol (delta:-8.3±0.9 mg/dl vs. -10.1±0.2 mg/dl: p=0-01), insulin (delta:-4.7±0.8 UI/L vs. -0.9±1.0 UI/L: p=0.03), triglycerides (delta:-17.7±3.9 mg/dl vs. -6.1±2.8 mg/dl: p=0.04) and HOMA IR (delta:-0.8±0.2 units vs. -0.2±0.1 units: p=0.03) improved only in no G allele carriers. After weight loss with Diet S, in the group of subjects without G allele, insulin levels (delta (CC vs. CG+GG): -3.4±0.6 UI/L vs. -1.2±0.4 UI/L: p=0.02), triglycerides (delta:-29.2±3.4 mg/dl vs. -8.2±3.8 mg/dl: p=0.03), HOMA-IR (delta (CC vs. CG+GG): -1.1±0.2 units vs. -0.1±0.1 units: p=0.01), total cholesterol (delta:-15.9±7.4 mg/dl vs. -5.8±2.9 mg/dl:ns) and LDL-cholesterol (delta:-13.7±5.9 mg/dl vs. -6.0±2.9 mg/dl: ns) decreased, too.

Table 3 reports changes of serum adipokines. After weight loss with diet HP, both genotype groups showed a significant decrease on leptin levels (CC vs. CG+GG at 9 months) (delta: -22.1±7.1: ng/ml vs. -22.2±9.2 ng/ml:ns). After dietary intervention with Diet S, both genotypes showed a significant decrease on leptin levels (delta: -24.9±8.1: ng/ml vs. -26.2±8.8 ng/ml:ns). The effect on leptin levels were independently of dietary intervention. Resistin and adiponectin levels remained unchanged after both diets and in both genotypes.

DISCUSSION

In this randomized dietary intervention trial of 9 months, we detect a relationship among the rs10830963 variant of *MTNR1B* gene and changes of adiposity parameters, lipid profile and insulin resistance. Our data show that the G allele was associated with a worse weight loss, lipid and insulin resistance improvements secondary to both hypocaloric diets.

Otherwise, the common genetic SNP rs10830963 of MTNR1B has been associated with obesity and fasting glucose levels in different cross sectional studies (26-27). Moreover, there are few studies evaluating the relationship between a dietary intervention and a genetic variant located in *MTNR1B* (15-17). Our findings of adiposity parameters analysis suggested that the *MTNR1B* variant (rs10830963) may affect total body weight and fat mass response, even specific fat composition as waist circumference (trunk fat). According to our results, a previous study found an association between other genetic variant of *MTNR1B* (rs4753425) and total body fat (28). Goni et al (29) have reported that the rs10830963 was related with body composition changes after 6 months with two hypocaloric diets (low-fat diet vs high fat diet), although this gene-diet interaction became less significant at 24 months of follow-up. A recent study reported that this common genetic variant was associated with the timing of the melatonin rhythm (30). G allele carriers showed a later melatonin offset and longer duration of elevated melatonin levels. The authors proposed that the disruption of melatonin rhythm among carriers of the risk allele might produce an increase of food intake. Other hypothesis, it is possible that rs10830963 may be involved in the regulation of *MTNR1B* gene expression or other gene expression that might influence the role of melatonin on energy storage.

The second finding of our study is the relationship of this genetic variant with the modification of cholesterol levels after both diets. The circadian system, melatonin is one of the chronobiotics, has a main role in coordinating lipid metabolic pathways through activation or repression of genes imply in metabolism (31-32). On the other hand, it has been observed that melatonin administration can decrease lipid levels in both human and animal studies (33). In a recent study, administration of melatonin decreased LDL cholesterol in obese subjects (32) and in type 2 diabetic patients poorly controlled with metformin (34), too. In our design, we observed a relationship between the rs10830963 variant of *MTNR1B* gene and LDL-cholesterol response after weight loss with both hypocaloric diets. Given that melatonin appears to be involved in various lipid phenotypes, it can be speculated that the effect of MTNR1B genetic variant on dynamics of melatonin expression thereby could influence cholesterol levels. For example, Tuomi et al (35) reported that rs10830963 variant might affect MTNR1B mRNA expression in other cell types related to cholesterol metabolism. Finally Goni et al (16) have showed that G allele was associated with lower decrease in total cholesterol and LDL cholesterol in response to a high-fat diet and opposite effect was found in a low-fat diet. A meta-analysis has reported significant interactions between MTNR1B genotype and fat intake on cholesterol levels (36), too These results are in line with the “differential susceptibility hypothesis”, which proposes that risk alleles may function like plasticity genes because genetic risk could be modified by environmental factors including nutrients (37).

The third important finding of our study is that G allele carriers showed less improvement of insulin and HOMA-IR after weight loss than non-G allele carriers independently of the type of diet. The mechanisms by which the MTNR1B rs10830963 affects insulin resistance remains unknown. It could be speculated that the effect of the *MTNR1B* genetic variant on dynamics of melatonin expression thereby could influence glucose metabolism The effect of feeding on the rhythmic mRNA expression of clock genes (38) or circadian rhythmic balance (39) have also been reported in animals. Grotenfeld et al (40) have reported the relationship of this genetic variant with glucose metabolism. This investigation showed that among females at risk for gestational diabetes mellitus, non-G allele carriers seem to benefit from lifestyle intervention. In other studies, the risk G allele has been related with decreased insulin secretion in response to glucose (41) and decreased insulin sensitivity (42), too. An in vitro study (43) observed that the G-allele of rs10830963 that leads to increase glucose level was associated with reduced pancreatic cell function (HOMA-B). Sparso et al (44) reported that G-allele carriers had reduced suppression of hepatic glucose production during a hyperinsulinemic-euglycemic clamp indicating hepatic insulin resistance.

Our study has limitations. Firstly, we only analysed one SNP of *MTNR1B* gene, so other genetic variants in this or other genes could be related with our observations. Secondly, we did not measure circulating melatonin levels in the study population, which prevented the potential analysis of the relationship between serum melatonin levels and the genetic variant. Finally, it is difficult to evaluate which macronutrient played the main role of the detected effect of both diets on metabolic parameters and gene-diet interaction.

In summary, our design showed the association of rs10830963 *MTNR1B* polymorphism with body weight loss induced by two different hypocaloric diet and provided additional evidence on metabolic response such as cholesterol, insulin resistance and fasting insulin levels.

Conflict of Interest: All authors (Author 1, Author 2, Author 3 and Author 4) declare that they have no conflict of interest.

No funding

Statement of Informed Consent: "Informed consent was obtained from all individual participants included in the study."

Ethical approval: The study was approved by our local Ethical Committee (n46/2017)

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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TABLE 1: CHANGES IN ANTHROPOMETRIC PARAMETERS rs10830963 **(mean±S.D)**

Characteristics	DIET HP (n=137)						DIET S (n=133)					
	CC (n=72)			*CG+ GG* (n=65)			*CC* (n=71)			*CG+ GG (n=62)*		
	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	35.3±5.1	33.2±4.1*	32.0±4.0*	35.1±5.0	33.4±4.0*	32.0±5.0*	35.2±5.0	33.6±4.1*	32.1±5.0*	35.1±4.0	33.5±4.1*	32.4±4.0*
Weight (kg)	92.6±7.3	86.8±7.2*	84.0±9.1*	89.3±10.4	85.0±8.1*	83.1±8.2*	92.1±10.6	87.6±8.1*	84.5±7.2*	90.9±12.3	87.6±11.2*	85.9±7.1*
Fat mass (kg)	36.4±3.0	32.6±4.0*	30.2±5.0*	34.4±4.1	32.1±5.1*	30.7±7.1*	36.3±5.0	32.8±4.0*	30.0±4.1*	37.6±7.0	34.4±7.1*	32.4±6.1*
WC (cm)	114.1±9.0	107.1±5.2*	102.4±6.1*	109.9±7.1	106.2±5.1*	103.1±7.0*	112.4±8.1	108.3±7.0*	101.7±8.2*	111.2±9.0	106.7±7.1*	104.9±8.0*
SBP (mmHg)	126.2±8.1	123.4±6.2*	123.0±6.0*	127.0±9.2	124.2±7.0*	123.1±8.2*	125.1±9.1	121.5±8.1*	121.0±8.1*	129.0±8.0	126.2±7.1*	124.6±7.0*
DBP (mmHg)	81.1±8.0	79.9±7.1	77.2±9.1	80.1±9.0	79.9±8.1	78.9±7.1	80.2±9.0	78.2±4.5	78.4±5.0	80.2±5.0	79.8±4.0	79.2±4.3

HP: high protein/low carbohydrate. S: standard. DBP: Diastolic blood pressure. Mths: Months BMI: body mass index. SBP: Systolic blood pressure. DBP: Diastolic blood pressure WC: Waist circumference. (*) p<0.05, in each genotype group with basal values. No differences between genotypes groups. .

TABLE 2: BIOCHEMICAL PARAMETERS (mean±S.D)

Characteristics	DIET HP (n=137)						DIET S (n=133)					
	GG(n=72)			*GG+ GT* (n=65)			*GG* (n=71)			*GG+ GT (n=62)*		
	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)	104.5±8.1	102.2±8.0	99.4±7.0	102.9±8.2	101.9±8.3	100.6±5.1	99.7±8.1	99.8±7.0	97.2±8.1	101.7±9.1	98.7±6.5	98.3±7.1
Total ch. (mg/dl)	207.5 ±9.0	201.3±8.1*	197.7±9.7*	210.2±22.0	205.1±11.0	204.4±9.1	215.3±11.9	202.1±6.4*	193.3±10.4*	208.5±10.2	203.5±9.9	203.8±10.9
LDL-ch. (mg/dl)	130.3±9.1	124.7±8.1*	122.0±9.3*	130.4±13.2	125.9±11.1	125.1±13.1	127.1±10.5	117.3±10.2*	114.8±11.0*	123.1±10.1	119.5±8.2	117.6±9.1
HDL-ch. (mg/dl)	54.8±9.0	54.6±8.0	53.9±7.0	55.6±8.1	54.3±9.1	53.8±7.1	55.8±9.2	53.2±9.1	52.1±9.0	55.0±7.3	54.1±8.9	55.2±8.0
TG (mg/dl)	122.8±11.1	106.3±9.4*	105.1±9.2*	126.9±12.8	123.1±13.2	120.9±20.3	138.1±12.6	119.4±10.1*	109.1±10.1*	116.1±11.3	110.3±12.3	108.9±21.9
Insulin (mUI/L)	11.7±5.0	9.5±3.0*	7.0±4.1*	11.2±7.1	9.6±5.1	9.3±7.4	11.2±4.1	8.8±4.1*	7.8±3.0*	10.6±5.0	9.9±4.2	9.4±3.1
HOMA-IR	2.6±0.9	2.5±0.5	1.8±0.8*	2.2±1.2	2.0±1.1	2.0±1.4	2.5±1.1	2.2±1.0*	1.5±1.0*	2.1±1.0	2.3±1.1	2.1±1.2
CRP (mg/dl)	5.1±3.0	4.9±2.8	4.8±3.2	5.3±3.1	5.2±3.0	5.1±3.3	4.1±2.1	4.3±3.1	4.8±4.0	5.0±4.1	5.1±3.8	5.0±3.1

HP: high protein/low carbohydrate. S: standard. Ch: Cholesterol. TG: Triglycerides CRP: c reactive protein. HOMA-IR: Homeostasis model assessment. LDL: low density lipoprotein,. HDL: High density lipoprotein. 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 3: CIRCULATING ADYPOCITOKINES (mean+S.D)

Characteristics	DIET HP (n=137)						DIET S (n=133)					
	GG(n=72)			*GG+ GT* (n=65)			*GG* (n=71)			*GG+ GT (n=62)*		
	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)	10.0±3.9	11.6±2.8	12.1±3.2	10.9±4.1	12.0±3.1	13.8±4.3	11.2±4.3	12.9±3.1	13.1±4.2	10.8±5.0	11.2±4.2	12.4±5.0
Resistin (ng/ml)	6.0+2.0	6.1+2.1	6.0+4.1	6.1+3.2	6.0+3.3	6.0+3.1	6.1+2.1	6.2+3.0	5.8+2.9	5.9+3.5	5.6+4.1	5.2+4.1
Leptin (ng/ml)	34.1±11.1	12.9±9.3*	12.0±5.1*	35.1±8.0	13.8±4.1*	12.9±5.3*	36.9±9.0	15.1±6.2*	12.0±2.1 *	38.1±5.9	17.1±4.2*	12.3±4.1*

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

Highlights

- All adiposity parameters, systolic blood pressure and leptin levels decreased in all subjects after both diets (Diet HP: a high protein/low carbohydrate vs. Diet S: a standard severe hypocaloric diets).
- This improvement of adiposity parameters was higher in non-G allele carriers than G allele carriers.
- After weight loss with Diet HP, total cholesterol, LDL-cholesterol, insulin, triglycerides and HOMA IR improved only in no G allele carriers.
- After weight loss with Diet S in non G allele carriers, insulin levels, triglycerides, HOMA-IR, total cholesterol and LDL-cholesterol decreased.