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Brain derived neurotrophic factor (BDNF) polymorphism rs 10767664 affects metabolic parameters after weight loss secondary to high-fat hypocaloric diet with Mediterranean pattern

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Abstract. – OBJECTIVE: We evaluated the effect of the genetic variant rs10767664 of BDNF gene on anthropometric and biochemical changes after weight loss secondary to a high-fat hypocaloric diet with a Mediterranean dietary pattern.

PATIENTS AND METHODS: A sample of 277 obese subjects was recruited. After subjects met the inclusion criteria, they received a nutritional intervention with a high-fat hypocaloric diet [36% of carbohydrates, 40% of fats (60.0% of monounsaturated fats, 25.0% of saturated fats and 15.0% of polyunsaturated fats) and 24% of proteins]. Biochemical and anthropometric parameters were measured at basal and 3 months.

RESULTS: One hundred and seventy-nine subjects (64.6%) had the genotype AA (wild group) and 98 (35.4%) subjects had the next genotypes; AT (81 patients, 29.2%) or TT (17 patients, 6.2%) (Mutant group). The improvement of BMI, weight, fat mass, waist circumference, systolic blood pressure, leptin, total cholesterol and LDL cholesterol was similar in both genotypes after dietary intervention. Secondary to weight loss and only in non-T allele, insulin levels (AA vs. At+TT) (-5.2+0.2 UI/L *vs.* -2.9+0.3 UI/L: *p*=0.02) and HOMA-IR (-2.1+0.2 units *vs.* -1.1+0.1 units: *p*=0.02) decreased significantly.

CONCLUSIONS: T allele carriers of the BDNF variant rs10767664 may be an independent predictor of the lack of improvement induced by weight loss on insulin levels and insulin resistance after a high-fat hypocaloric diet with a Mediterranean dietary pattern.

Key Words:
Rs10767664, BDNF, Hypocaloric diet, Mediterranean pattern.

Abbreviations
BMI: body mass index; BDNF: brain derived neurotrophic factor; HOMA-IR: Homeostasis model assessment; LDL: low density lipoprotein; HDL: High density lipoprotein; CRP: C reactive protein; SNPs Single nucleotide polymorphism.

Introduction

Overweight and obesity are important worldwide health problems associated with an increased risk of cardiovascular disease, diabetes mellitus type 2, cancer and metabolic disorders[1]. Obesity prevalence has increased fast over recent decades, and it is secondary to a decreased physical activity and an increased calorie intake. Obesity can be considered a serious health concern not restricted to developed nations but in developing countries as well. Moreover, obesity susceptibility loci identified through genome-wide association studies and replicated in multiple cohorts have provided new insights into the genetic factors that contribute to the development of this entity[2].

Therefore, the single nucleotide polymorphisms (SNPs) may be relevant to explain genetic susceptibility to obesity. Many of these SNPs of genes are expressed in the brain, emphasizing the role of the central nervous system and potentially dietary intake in obesity predisposition, for example, loci include FTO (fat mass and obesity associated gene), Mc4R (melanocortin subtype 4 receptor), NPY (neuropeptide Y) and BDNF (brain-derived neurotrophic factor)[3]. Although these associations of many genetic variant have been realized until now, only few studies reported the association of BDNF with dietary interventions in obese subjects.

BDNF is expressed in an important area of central nervous system called hypothalamus,

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where this neurotrophic factor has an important role in the control of metabolism, energy homeostasis and dietary intakes[4,5]. Some SNPs located in the gene of *BDNF* have been related with obesity markers, too. Of note, three studies[6-8] showed a positive association between Val66Met polymorphism (rs6265) and body mass index (BMI). Recently, another genetic variant (rs10767664) has shown an important-effect association with obesity in a non-Caucasian population[9]. There are also studies in the literature with a nutritional intervention design in samples of obese patients. Two reseraches[10,11] with a dietary intervention have shown an influence of this SNP on the modification of the weight and metabolic changes. The effect of this polymorphism on the metabolic response secondary to weight loss has also been demonstrated in an intervention study with bariatric surgery (biliopancreatic diversion)[12]. Obese patients with the T allele of this genetic variant showed less weight loss and improvement in some biochemical markers, such as triglycerides, insulin resistance and basal insulin levels[10]. The relationship of the T allele with a worse weight response was not detected in the following study of dietary intervention[11] or bariatric surgery[12]; however, the lack of an adequate metabolic response was detected again in obese patients with the allele T. Even in the second study with nutritional intervention[11], when comparing two hypocaloric diets, only the hypocaloric diet with a balanced distribution of macronutrients but rich in monounsaturated fats showed beneficial metabolic effects on glucose metabolism in obese non-carriers of the T allele.

Taking into account all the previously mentioned data, we evaluated the effect of the genetic variant rs10767664 of *BDNF* gene on anthropometric and biochemical changes after weight loss secondary to a high-fat hypocaloric diet with a Mediterranean dietary pattern.

Patients and Methods

Patients

277 Caucasians subjects with obesity were enrolled with an age range between 20 and 65 years. Adult obesity was defined by a Body Mass Index (BMI) $\geq$30 kg/m^2. The recruitment of subjects was realized with a consecutive method of sampling among patients send from Primary Care Physicians with obesity. All participants signed informed consent to a protocol approved by the local Ethical Review boards. This study was realized with the guidelines laid down in the Declaration of Helsinki. The local Ethics Committee (HCUVA) approved all procedures involving patients (HCUVA 1/2016 code register).

The inclusion criteria were as follows BMI> 30 kg/m^2 and aged 20-65 years. The exclusion criteria were to meet any of the following mentioned situations; weight loss of more than 5% of body weight in the past 6, a previous history of cardiovascular disease or stroke during the previous 24 months, history of cancer undergoing active treatment, total cholesterol $\geq$ 200 mg/dl, triglycerides $\geq$ 250 mg/dl, blood pressure $\geq$ 140/90 mmHg, diagnosis of diabetes mellitus, as well as the use of any drug to treat diabetes mellitus, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and fibrates.

Procedures

Peripheral venous blood (15 ml) was obtained in EDTA-treated tubes after overnight fast. Basal fasting insulin. glucose, Homeostasis model assessment-insulin resistance (HOMA-IR), triglycerides concentration total cholesterol, (low density lipoprotein), LDL-cholesterol, (high density lipoprotein), HDL-cholesterol, plasma adipokines levels (leptin, adiponectin, resistin), C reactive protein were determined within the start of the trial and repeated after 12 weeks. Anthropometric parameters were evaluated with body weight, height, BMI, fat mass by bioimpedance at the same times (basal and after 12 weeks). Systolic and diastolic blood pressure were also measured. Genotype of BDNF gene (rs10767664) was determined in all subjects with polymerase chain reaction (PCR).

Nutritional intervention

This intervention was realized to achieving a calorie restriction of 500 daily calories. The obese patients during trial (12 weeks) received counseling on a high-fat hypocaloric diet with a Mediterranean dietary pattern diet and physical exercise. The nutrition intakes of subjects were assessed by using 3-day food records. Tables were used to explain the intervention with a Mediterranean dietary pattern including (legumes, vegetables, poultry, whole grains, fish, fresh fruit, using olive oil and limit unhealthy fats such as margarines, fatty meats, snacks, industrial pastries)[13]. The distribution of macronutrients was

36% from carbohydrates, 40% from fats and 24% from proteins. Distribution of fats was 60.0% from monounsaturated fats, 25.0% from saturated fats and 15.0% from polyunsaturated fats. All patients had two individual sessions (90 minutes with diet sheets and example menu plans) with the dietitian at the start of the intervention to explain the diet and solve doubts. Compliance with the diet was checked every 7 days. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day, before the dietary intervention and after 12 weeks of the intervention. Dietary intakes were evaluated with a computer-based data evaluation system (Dietosource®, Ge, Swi) with national composition food tables[13]. The physical exercise program was a conventional aerobic intervention with at least 3 times per week (60 min each, reaching a total of 180 minutes each week). The patients were training to record the physical activity with a self-reported questionnaire.

Assays

Lipid profile (total cholesterol and triglyceride levels) was determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), while HDL cholesterol was analyzed enzymatically in the supernatant after precipitation of other lipoproteins. LDL cholesterol was calculated using Friedewald equation (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/5)[14]. Fasting glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was analyzed by radioimmunoassay RIA (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L)[15] and the homeostasis model assessment for insulin resistance (HOMA) was calculated using these values[16] and the next equation (fasting plasma insulin (mU/L)*glucose (mmol/L)/22.5). CRP was analyzed by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl. All adipokines were determined by ELISA; resistin (Biovendor Laboratory, Inc., Brno, Czech Republic) RD191016100 with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml[17], adiponectin (R&D Systems, Inc., Minneapolis, MN, USA) DRP300 with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml[18] and leptin (R&D systems, Inc., Minneapolis, MN, USA) RD191001100 with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml[19].

Adiposity Parameters and Blood Pressure

Body composition was determined by the next parameters; body weight was measured to an accuracy of 0.1 Kg and BMI computed as body weight in Kg/(height in m^2). Waist circumference was determined in the narrowest diameter between xiphoid process and iliac crest. Bioimpedance was used to measure body composition with an accuracy of 50 g[20] (Akern BIA 101.EFG, Florence, Italy). Blood pressure was determined three times after a 10-minute rest with a random zero mercury sphygmomanometer, and averaged (Omron, Los Angeles, CA, USA).

Genotyping of Rs10767664 BDNF Gene Variant

The polymerase chain reaction (PCR) was realized with an amount of 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'- ACGTTGGATGTAAAATGCTTATAGTGATAG-3' and reverse 5'- ACGTTGGATGTGAGAACAGAGGTAGTAGGC-3') in a 2 uL of total volume (Termociclador Life Technologies, Los Angeles, CA, USA). Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International®, Los Angeles, CA, USA). DNA was denaturated at 95ºC for 3 min; this was followed by 45 cycles of denaturation at 95ºC for 15 s and annealing at 59.3°C for 45 s. The reaction was realized in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase. Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed counts. This genetic variant was in Hardy Weinberg equilibrium (p=0.36).

Statistical Analysis

Sample size was realized to detect differences over 6 kg in body weight after diets with 90% power and 5% significance (n=270). The Kolmogorov-Smirnov test was used to determine variable distribution. The results were showed as average+/-standard deviation. Numerical variables with normal distribution were analyzed with a two-tailed Student's t-test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary. Non-parametric variables were analyzed with the Mann-Whitney U test. The

differences in anthropometric and biochemical variables between the SNP genotypes were tested with analysis of the covariance (ANCOVA) adjusted age and sex. Correction with multiple testing was realized. The statistical analysis was performed for the combined *TT* and *TA* genotypes as a group and AA genotype as second group, with a dominant model. A p-value <0.05 was considered statistically significant. All analysis was conducted using SPSS version 23.0 (IBM, Armonk, NY, USA).

Results

277 Caucasian obese patients gave informed consent and were enrolled. All patients followed the 12-weeks intervention period without drop-outs. The average age was 46.3±8.2 years and the mean BMI 36.1±5.1, with 75 males (27.1%) and 202 females (72.9%). One hundred and seventy-nine subjects (64.6%) had the genotype *AA* (wild group) and 98 (35.4%) subjects had the next genotypes; *AT* (81 patients, 29.2%) or *TT* (17 patients, 6.2%) (Mutant group). Average ages were similar in both genotypes (wild type: 46.8±9.0 years *vs.* mutant group: 46.1±8.0 years: ns). Sex distribution was similar in both groups, males (25.7% *vs.* 29.6%) and females (74.3% *vs.* 70.4%).

Following the food recommendations and sessions of the dietitian, the dietary recommendations were reached as indicated in method section. A total caloric amount of 1451.2±121.2 calories, the percentage of macronutrients, was the following: 36.1% of carbohydrates, 40.9% of fats and 23.0% of proteins, without statistical differences between genotypes. The distribution of macronutrient in both groups (AA *vs.* AT *vs.* TT) was similar (carbohydrates; 36.0±2.3% *vs.* 36.4±2.1%; p=0.33), (fats; 41.1±3.3% *vs.* 40.8±2.2%; p=0.36) and (proteins; 22.9±2.1% *vs.* 22.8±1.9%; p=0.43).

Percentage of dietary fats was 59.5% of monounsaturated fats, 25.2% of saturated fats and 14.8% of polyunsaturated fats, without statistical differences between genotype groups. The distribution of dietary fats in both groups (AA *vs.* AT *vs.* TT) was equal: (monounsaturated fats; 59.8±4.3% *vs.* 59.3±2.9%; p=0.42), (saturated fats; 25.0±3.1% *vs.* 25.4±2.0%; p=0.37) and (polyunsaturated fats; 16.2±1.0% *vs.* 15.3±1.3%; p=0.47).

Basal physical activity was similar in both groups (AA *vs.* AT *vs.* TT) (128.2±16.3 min/week *vs.* 131.1±21.9 min/week; p=0.33). In addition, after the intervention, the physical activity increased without statistical differences with basal values. Both genotype groups had the same final physical activity (148.2±21.1 min/week *vs.* 151.9±23.1 min/week; p=0.32) or deltas (20.1±1.9 min/week *vs.* 20.3±2.9 min/week; p=0.42).

Table I reports the adiposity parameters and blood pressure on both genotypes from basal through 12 weeks. The decreases of BMI (AA *vs.* AT+TT genotypes) (-2.4±0.8 kg/m^2 *vs.* -2.4±0.9 kg/m^2: p=0.23), weight (-3.9±1.3 kg *vs.* -3.2±1.2 kg: p=0.32), fat mass (-2.6±0.7 kg *vs.* -2.3±0.6 kg: p=0.13) and waist circumference (-4.0±2.1 cm *vs.* – 4.6±2.0 cm: p=0.22) were similar in both genotypes. The decrease in systolic blood pressure (-7.4±2.1 mmHg *vs.* -8.2±2.2 mmHg: p=0.15) was similar in both genotype groups, too. Diastolic blood pressure remained without statistical changes after weight loss.

Table II reports the cardiovascular risk factors. After 12 weeks with the high-fat hypocaloric diet and in both genotypes, total cholesterol (AA *vs.* AT+TT genotypes) (-10.1±5.1 mg/dl *vs.* -13.8±4.9mg/dl; p=0.36) and LDL-cholesterol (-10.3±2.2 mg/dl *vs.* -11.2±2.9 mg/dl; p=0.45) improved similarly. Secondary to weight loss and only in non-T allele, insulin levels (-5.2±0.2 UI/L *vs.* -2.9±0.3 UI/L: p=0.02) and HOMA-IR (-2.1±0.2 units *vs.* -1.1±0.1 units: p=0.02) decreased.

Table III reports changes of adipocytokines levels after dietary intervention. After weight loss, leptin levels (-22.3±7.4 ng/ml *vs.* -20.8±7.2 ng/ml: p=0.19) decreased in both genotypes. Resistin and adiponectin levels didn't change after weight loss in both groups.

Discussion

Our results demonstrate that the T allele of the *BDNF* variant rs10767664 may act as an independent predictor to the lack of improvement induced by weight loss in glycemic metabolism (insulin levels and HOMA-IR) secondary to a high-fat hypocaloric diet enriched in monounsaturated fatty acids.

In the literature, some polymorphisms of *BDNF* gene have been related with adiposity parameters[6-8]. For example, Alharrbi et al[9] have demonstrated a huge effect in the risk of obesity for rs10767664 (BDNF) (OR = 1.923)[9]. However, this relationship in cross-sectional studies is more interesting if any effect of this SNP is demonstrated in the weight or secondary metabolic range. Few

Table I. Anthropometric parameters of obesity and blood pressure measurement (mean ± SD).

	AA (n=179)		AT+TT(n=98)		
Parameters	**Basal**	**12 weeks**	**Basal**	**12 weeks**	***p-values***
					-Time AA - Basal Genotype - Time AT+TT - 3 months genotype
BMI	36.2±6.0	34.8±6.2*	36.1±5.4	34.7±5.9*	$p = 0.01$ $p = 0.39$ $p = 0.02$ $p = 0.33$
Weight (kg)	94.8±10.6	90.9±9.9$	93.6±9.2	90.5±6.9$	$p = 0.02$ $p = 0.33$ $p = 0.03$ $p = 0.45$
Fat mass (kg)	41.3±.2	38.7±10.1#	39.3±.2	37.0±7.9#	$p = 0.01$ $p = 0.30$ $p = 0.02$ $p = 0.43$
WC (cm)	110.1±14.1	106.1±9.1&	107.7±10.9	103.1±9.1&	$p = 0.03$ $p = 0.43$ $p = 0.02$ $p = 0.41$
SBP (mmHg)	131.4±9.2	123.0±8.9**	132.3±.2	124.6±6.8**	$p = 0.01$ $p = 0.34$ $p = 0.01$ $p = 0.41$
DBP (mmHg)	83.3±8.1	81.7±5.1	80.9±6.0	80.2±7.1	$p = 0.59$ $p = 0.61$ $p = 0.62$ $p = 0.5$!

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; Statistical differences p<0.05, in each genotype group (* BMI, $ Weight, #fat mass, & WC, **SBP).
First p, significance of dietary intervention after 12 weeks in AA genotype, second p, significance between AA genotypes vs. AG + GG baseline values, third p, significance of dietary intervention after 12 weeks in AG + GG genotype, fourth p, significance between AA genotypes vs. AG + GG post-treatment values.

studies have been realized to investigate this relationship. A first group of trials is the one that used surgical treatments to lose weight and another group of trials have used hypocaloric diets, with different caloric restrictions, distribution of macronutrients and duration of the intervention. For example, one study rs10767664 variant of the *BDNF* gene demonstrates a lack of association between weight loss after a biliopancreatic diversion during a follow-up of 3 years[12]. Moreover, morbid obese subject's carriers of T allele had a lower improvement in fasting glucose, insulin levels and HOMA-IR after a huge weight loss than non-T allele carriers. In other bariatric surgery study[21], genetic variants of *BDNF* gene were not evaluated, but it was detected that the decrease in serum BDNF levels was greater in subjects who had bypass surgery than banding. The authors used a potential interaction between the environment and the genetic background of these obese subjects to explain this metabolic difference.

Two previous interventional studies[10,11] have demonstrated a higher decrease of HOMA IR and basal insulin levels in non-T allele carriers than T-carriers after different hypocaloric diets. The first interventional study of three months[10] reported a higher decrease in weight, fat mass, waist

Table II. Lipid profile and glucose metabolism (mean±SD).

	AA (n=179)		AT+TT(n=98)		
Parameters	**Basal**	**12 weeks**	**Basal**	**12 weeks**	***p-values***
					-Time AA - Basal Genotype - Time AT+TT - 3 months genotype
Glucose (mg/dl)	100.2±9.3	97.8±8.0	101.7±8.1	96.5±7.3	$p = 0.11$ $p = 0.36$ $p = 0.12$ $p = 0.43$
Total cholesterol (mg/dl)	203.1±21.7	193.2±14.2$^{\$}$	202.2±23.1	188.4±18.2$^{\$}$	$p = 0.03$ $p = 0.50$ $p = 0.01$ $p = 0.34$
LDL-cholesterol (mg/dl)	124.4±18.3	114.1±.1$^{\#}$	119.5±10.1	119.5±10.1	$p = 0.02$ $p = 0.49$ $p = 0.01$ $p = 0.34$
HDL-cholesterol (mg/dl)	54.1±4.1	52.1±6.2	52.9±5.1	53.8±4.1$^{\&}$	$p = 0.21$ $p = 0.47$ $p = 0.59$ $p = 0.45$
Triglycerides (mg/dl)	124.6±27.9	118.8±21.4	11.1±23.2	110.8±17.2 *	$p = 0.02$ $p = 0.61$ $p = 0.21$ $p = 0.45$
Insulin (mUI/l)	17.3±6.1	12.1±4.9&	17.5±6.2	15.6±6.7	$p = 0.02$ $p = 0.39$ $p = 0.13$ $p = 0.40$
HOMA-IR	5.3±1.1	3.3±1.0*	5.4±2.1	4.3±1.3	$p = 0.01$ $p = 0.33$ $p = 0.22$ $p = 0.49$
CRP	5.9±2.2	6.0±1.1	5.6±2.0	5.8±2.1	$p = 0.21$ $p = 0.39$ $p = 0.37$ $p = 0.44$

HOMA-IR (homeostasis model assessment). CRP (C reactive protein) Statistical differences $p<0.05$, in each genotype group (total cholesterol $, LDL cholesterol #, insulin &, HOMA IR *, CRP&&). First p, significance of dietary intervention after 12 weeks in AA genotype, second p, significance between AA genotypes vs. AT + TT baseline values, third p, significance of dietary intervention after 12 weeks in AT + TT genotype, fourth p, significance between AA genotypes vs. AT + TT post-treatment values.

circumference and metabolic parameters such as HOMA-IR, insulin levels and triglycerides in no T allele carriers than in obese T allele carriers. In this first trial[10], a moderate caloric restriction of 1500 calories per day with a Mediterranean diet pattern was used with a distribution of macronutrients of 52% carbohydrates, 25% fats and 23% proteins with a 50.7% of fats as monounsaturated. In the second trial[11], two hypocaloric diets have been used for 3 months with an average intake of 1500 calories per day and two different lipid profiles, one of which is rich in monounsaturated fatty acids (46.0% carbohydrates, 34.4% fats and 19.6% proteins with a 67.7% of fats as monounsaturated). The second diet (rich in polyunsaturated fatty acids) had a distribution of nutrients as indicated: 45.9% carbohydrates, 34.3% fats and 19.8% proteins with a 55.3% of fats as monounsaturated.

Only in patients treated with a diet enriched in monounsaturated fats was demonstrated the interaction between the improvement in insulin resistance levels and basal insulin with the presence of the T allele found. Perhaps the total amount of monounsaturated fats is the cause of this interaction, in the first study[1] it was (41 g/day) and in the present study it is close to the 43 g/day. Recently, Chung et al[22] have demonstrated that BDNF levels had a positive association with monounsaturated fatty acids.

The role of the BDNF system on the metabolism of carbohydrates is controversial. Cross-sectional studies reported a relationship between BDNF and glucose metabolism in humans[23]. BDNF gene encodes a neurotrophic factor related with neuronal differentiation during the development of the nervous system, and subsequent synaptic efficiency[24]. In addition, BDNF is a mediator in the neuronal responses to external environmental factors such as dietary restriction[25] and this action could explain the effect of this SNP on glucose metabolism and adiposity. Notably, Ribasés et al[26] have been reported a strong relationship between a variant of BDNF gene (rs6265) and low body mass index. This association could be explained by a potential effect of this SNP variant on functional changes in hippocampal neurons by attenuating the regulated pathway that secretes BDNF[21]. In the literature, low levels of circulating BDNF in individuals with obesity and type 2 diabetes mellitus have been described, implying a potential role for this neurotrophin in mediating metabolism of glucose[27,28]. Recently, other design showed that serum BDNF levels were higher in naive diabetic patients than healthy subjects[29]. In animal models, it has been shown that BDNF might reduce insulin resistance and thus has antidiabetic effect by PPAR-alpha pathway[30]. In addition to the possible direct effects of BDNF levels, the BDNF gene itself may also influence. Such as, rs4074134-BDNF minor allele has been associated in one cross sectional study with diabetes mellitus[31] and other SNP in this gene (*rs6265)* showed an association with metabolic syndrome[32].

The association of BDNF genetic variants with weight loss after dietary interventions is controversial, and we did not find this data in our present study. In some studies, the genetic variant rs10767664 had been related with weight loss[10] and Delahanty et al[33] showed that *rs 6265* variant and two other different SNPs were associated with weight regain. Perhaps the type of diet and

Table III. Serum Adipokine levels (mean± SD).

	AA (n=179)		AT+TT (n=98)		
Parameters	**Basal**	**12 weeks**	**Basal**	**12 weeks**	***p-values***
					-Time AA - Basal Genotype - Time AT+TT - 3 months genotype
Resistin (ng/dl)	3.7±1.5	3.8±1.6	3.9±1.8	3.8±1.7	$p = 0.51$ $p = 0.69$ $p = 0.42$ $p = 0.44$
Adiponectin (ng/dl)	31.1±9.1	37.8±8.0	30.8±7.1	38.3±3.2	$p = 0.22$ $p = 0.51$ $p = 0.23$ $p = 0.56$
Leptin (ng/dl)	99.5±19.6	77.2±.5*	93.8±18.1	63.8±12.1*	$p = 0.02$ $p = 0.42$ $p = 0.03$ $p = 0.43$

Statistical differences p<0.05, in each genotype group (* leptin). First p, significance of dietary intervention after 12 weeks in AA genotype, second p, significance between AA genotypes s. AT + TT baseline values, third p, significance of dietary intervention after 12 weeks in AT + TT genotype, fourth p, significance between AA genotypes vs. AT + TT post-treatment values.

distribution of macronutrient also modulate this relationship. In particular, BDNF expression is mediated by dietary fat[34] by activation of AMPK and down-regulation of mTOR. Recently, other genetic variant of *BDNF* gene (rs2030323) was associated with 100 to 150 greater total caloric intakes per allele[35]. It has been demonstrated that central administration of BDNF suppresses appetite, induces weight loss and protects against high-fat diet obesity in animals' models[36]. Finally, risk alleles at BDNF rs10767664 and rs 6265 predicted a pattern of dietary variables, including servings of eggs, meats and servings of dairy products associated with total caloric intake[37]. All these data could explain the relationship between genetic variants of this gene and metabolic responses after weight loss during therapeutic interventions. On the other hand, we must take into account the complex metabolic relationships that the BDNF system, for example BDNF acts through its receptors and it is produced mainly by the central nervous system, but also by peripheral tissues (skeletal muscles, endothelial cells, liver, adipose tissue and activated immune cells). However, leptin induces BDNF expression in the dorsomedial part of the ventromedial hypothalamic nucleus, with what this relationship with leptin can pose in the obese patient[38] and the exercise could be implied in this relationship.

The limitations of the study are as follows: firstly, the possibility of racial ethnic differences is not evaluated in our design because only Caucasian subjects were evaluated. Secondly, we only analyzed one SNP of *BDNF* gene, so other genetic variants could be associated with glucose metabolism. Thirdly, there are many uncontrolled factors that could explain our conclusions for example epigenetic or hormonal status. Fourthly, the caloric restriction was similar in males and females. Finally, we did not determine serum levels of BDNF, that could explain our metabolic and adiposity results.

Conclusions

T allele carriers of the *BDNF* variant rs10767664 may be an independent predictor to the lack of improvement induced by weight loss on insulin levels and insulin resistance. These results should make us think about the application of personalized diets in the treatment of obesity and its comorbidities such as diabetes depending on the genetic background of each patient[39]. These findings allow new ways of working in more personalized medicine and clinical nutrition, taking into account that the diet with a Mediterranean profile is one of the ones that presents more scientific evidence in the literature.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the Ethical standards of the institutional and/or National Research Committee (HVUVA Committee 6/2017) and with the 1964 Helsinki Declaration and its later amendments or comparable Ethical standards.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Author's contribution

DA de Luis designed the study and realized statistical analysis. O Izaola anthropometric evaluation and control of dietary intake. D Primo realized biochemical evaluation and genotype. JJ Lopez anthropometric evaluation and control of dietary intake

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