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Different effects of high-protein/low-carbohydrate versus standard hypocaloric diet on insulin resistance and lipid profile: Role of rs16147 variant of neuropeptide Y

Daniel Antonio de Luis *, *Olatz Izaola*, *David Primo*, *Rocio Aller*

Center of Investigation of Endocrinology and Nutrition, School of Medicine, Department of Endocrinology and Nutrition, Hospital Clinico Universitario, University of Valladolid, Valladolid, Spain

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ABSTRACT

Background and aims: Few studies have assessed the effect of the NPY gene rs16147 variant on metabolic response following a dietary intervention. We evaluated the effect of rs16147 on body weight and biochemical changes after a high-protein/low-carbohydrate hypocaloric diet compared with a standard severe hypocaloric diet over 9 months.

Materials and methods: A population of 270 obese individuals was enrolled. At baseline, participants were randomly allocated to one of two hypocaloric diets, high protein (Diet HP) or standard (Diet S), for a period of 9 months.

Results: After both diets, all genotypes showed decreased body mass index, weight, fat mass, waist circumference, and leptin levels. Participants with the minor allele (A) assigned to the HP diet showed decreases in total cholesterol (−6.5 ± 4.8 vs 10.1 ± 4.1 mg/dL; $p < 0.05$), LDL cholesterol (−5.9 ± 3.8 vs 9.6 ± 2.4 mg/dL; $p < 0.05$), triglycerides (−1.0 ± 4.8 vs 16.2 ± 4.1 mg/dL; $p < 0.05$), insulin (−0.5 ± 2.8 vs 1.7 ± 2.1 UI/L; $p < 0.05$), HOMA–IR (−0.2 ± 2.1 vs 0.5 ± 2.0 units; $p < 0.05$), and CRP (−0.3 ± 0.4 vs 1.3 ± 0.2 mg/dL; $p < 0.05$). Participants with the minor allele assigned to diet S also showed decreases in total cholesterol (−6.1 ± 4.1 vs 14.4 ± 3.1 mg/dL; $p < 0.05$), LDL-cholesterol (−3.1 ± 2.8 vs 15.0 ± 3.1 mg/dL; $p < 0.05$), triglycerides (−6.9 ± 4.1 vs 13.2 ± 4.0 mg/dL; $p < 0.05$), insulin (−0.3 ± 2.1 vs. −1.2 ± 0.2 UI/L: $p < 0.05$), HOMA–IR (−0.3 ± 2.1 vs. −1.6 ± 1.1 units: $p < 0.05$), and CRP (−0.4 ± 0.1 vs 1.1 ± 0.2 mg/dL; $p < 0.05$).

Conclusion: In obese Caucasians, the presence of the A allele of the rs16147 genetic variant produces a better metabolic response that is secondary to weight loss with two different hypocaloric diets.

* Corresponding author at: Center of Investigation of Endocrinology and Nutrition, School of Medicine, Valladolid University, C/Los perales 16 Simancas, 47130 Valladolid, Spain.
E-mail address: dadluis@yahoo.es (D.A. de Luis).
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1. Introduction

Obesity is a major health problem that has many associated comorbidities such as type 2 diabetes mellitus, hypertension, and dyslipidemia. Weight loss treatments and drugs that reduce energy intake have been shown to be relatively ineffective, as many peripheral and central parameters have been implicated in the regulation of metabolism and energy homeostasis. Previous studies have indicated a pivotal role of neuropeptide Y (NPY) in obesity and cardiovascular disease [1,2]. There are two major neural populations within the arcuate nucleus of the hypothalamus that function to regulate feeding: expression of neuropeptide Y (NPY) and agouti-related protein increases appetite, while pro-opiomelanocortin (POMC) neurons have anorexigenic effects [3].

Various single-nucleotide polymorphisms (SNPs) have been reported in the NPY gene. Among these, rs16147 (G-399A) is the main genetic variant [4], and is located within the promoter region upstream of the NPY gene (where G is the major allele and A is the minor allele). This SNP has been associated with serum levels of NPY [5] and is responsible for more than half of the variation in the expression of NPY [6]. Studies have reported an association between genetic variants of the NPY gene and metabolic disorders [7,8]. Hypocaloric diets are the principal strategy of treating obesity; however, few studies have investigated the effect of rs16147 on metabolic response and weight change after a dietary intervention. In The POUNDS LOST trial [9], the rs16147 variant of the NPY gene modulated changes in abdominal adiposity in response to four different hypocaloric interventions. Furthermore, a small intervention trial lasting 8 weeks [10] reported that the rs16147 variant of the NPY gene modulated the response to *Plantago ovata* husk without caloric restriction in terms of plasma C-reactive protein (CRP) levels and systolic blood pressure. These results show the effect of the qualitative composition of the diet. Another short-term intervention trial lasting 12 weeks [11] showed that the rs16147 genotype was associated with changes in waist circumference (WC), insulin resistance, and interleukin levels in response to a standard hypocaloric diet. In another study, two different hypocaloric diets with different macronutrient percentages (low carbohydrate vs low fat) led to similar weight loss, but showed no reduction in insulin resistance in participants with the major allele [12].

The present study evaluated the effect of the rs16147 genetic variant of the NPY gene on biochemical changes and weight loss after a high-protein/low-carbohydrate diet compared with a standard severe hypocaloric diet over 9 months.

2. Materials and methods

2.1. Participants

Our study population included 276 obese Caucasian individuals who were enrolled using a non-probabilistic consecutive method of sampling from 24 Primary Care Centers in our health area. Obesity was defined as body mass index (BMI) $\geq$ 30 kg/m^2. Recruitment was based on referral of patients from their primary care physician to our Nutrition Unit. All participants provided informed consent and the study protocol was approved by the relevant local ethical review boards. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Hospital Clinico Universitario Valladolid ethics committee.

Inclusion criteria were BMI > 30 kg/m^2 and age 18–70 years. Exclusion criteria were: weight loss of more than 5% of body weight in the past three months, history of stroke or cardiovascular disease during the previous 12 months, history of cancer requiring active treatment or terminal cancer situation, total cholesterol $\geq$ 200 mg/dL, triglycerides $\geq$ 200 mg/dL, blood pressure $\geq$ 140/85 mmHg, or use of drugs including sulfonylureas, metformin, dipeptidyl type IV inhibitors, thiazolidinedione, sodium glucose co-transporter 2 receptor inhibitors, glucagon-like peptide-1 analogs, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins, or other anti-dyslipidemic drugs.

2.2. Procedures

Venous blood specimens (10 mL) were collected in EDTA-treated tubes following an 8-h fast. At 0 (baseline), 3, and 9 months of both dietary interventions, the following parameters were measured: basal fasting glucose, insulin, homeostasis model assessment–insulin resistance (HOMA–IR), triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, serum adipokines (leptin, adiponectin, resistin), and CRP. At the same time points, height, weight, BMI, fat mass by bioimpedance, and blood pressure were also measured. For each participant, rs16147 NPY genotype was also determined.

The 270 obese participants who met the selection criteria were randomly assigned to one of two dietary interventions for a period of 9 months: diet S (standard protein severe hypocaloric diet, n = 133) or diet HP (high-protein/low-carbohydrate hypocaloric diet, n = 137) (Table 1). Completion of both diets was confirmed each week via a phone call to improve compliance. Data regarding dietary intake over three days including a weekend day were analyzed using a computer-based data evaluation system (Dietosource,

Table 1 – Composition of the two hypocaloric diets.

	Diet S (n = 133)	Diet HP (n = 137)
Energy (kcal/day)	1093	1050
Carbohydrates (g/day)	144.3	88.1
Carbohydrates (%TCV)	53	33
Fats (g/day)	32.6	39.0
Fat (%TCV)	27	33
Monounsaturated fat (%)	67.4	63.8
Polyunsaturated fat (%)	20.9	23.5
Saturated fat (%)	11.6	12.6
Proteins (g/day)	55.6	88.6
Proteins (%TCV)	20	34

Diet HP, high-protein/low-carbohydrate hypocaloric diet; Diet S, standard hypocaloric diet; TCV, total caloric value.

Geneva, Switzerland) using composition food tables as a Ref. [13]. The exercise intervention consisted of completion of aerobic physical activities at least twice per week (45–60 min each time). Recommended exercises included running, walking, cycling, and swimming, and activities were recorded by the patient using a self-reported questionnaire.

2.3. Anthropometric and blood pressure measurements

Body weight, height, and WC were measured in the morning before breakfast at 0 (baseline), 3, and 9 months. BMI was calculated as body weight (kg) divided by height (m^2). WC was determined as the narrowest diameter between the xiphoid process and iliac crest. Tetrapolar body electrical bioimpedance (Akern, EFG, Pisa, Italy) was used to measure total fat mass, with an accuracy of 50 g [14]. Blood pressure was measured as the average of three measurements using a random zero mercury sphygmomanometer, (Omrom, LA, CA, USA), with a 5-min rest in between measurements.

2.4. Biochemical measurements

Serum total cholesterol and triglyceride levels were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA) and HDL cholesterol was measured in the supernatant after precipitation of other lipoproteins using enzymatic methods. LDL cholesterol was calculated using the Friedewald formula (LDL cholesterol = total cholesterol – HDL cholesterol – triglycerides/5) [15]. Glucose levels were measured using an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured 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) [16], and HOMA–IR was calculated using these values [17].CRP was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0–7 mg/dL and analytical sensitivity of 0.5 mg/dL. The following adipokines were measured by enzyme-linked immunosorbent assay: resistin (Biovendor Laboratory, Inc., Brno, Czech Republic; sensitivity, 0.2 ng/mL and normal range, 4–12 ng/mL) [18], adiponectin (R&D systems, Inc., Mineapolis, USA; sensitivity, 0.24 ng/mL and normal range, 8.63–21.42 ng/mL) [19], and leptin (Diagnostic Systems Laboratories, Inc., Texas, USA; sensitivity, 0.05 ng/mL and normal range, 11–100 ng/mL) [20].

2.5. NPY gene polymorphism genotyping

Genomic DNA from each participant was isolated from peripheral blood leukocytes using a commercial extraction kit (Quantum prep, BioRad, LA, CA). Primers were designed using Sequenom Assay Design v4 (SEQUENOM, Inc. San Diego, CA, USA) as follows: forward, 5′- ACGTTGGAT GCACAAAGAGGATTCAGGTGC -3′ and reverse, 5′- ACGTTGGATGAGCCCAGACGATTCTTGTC -3′. Genotyping for the rs16147 polymorphism was performed using real-time polymerase chain reaction (PCR) analysis using 20–25 ng of genomic DNA and 0.1–0.15 μL each of oligonucleotide primer for rs16147 in a thermal cycler (Life Technologies, LA, CA, USA) with a final volume of 2 μL containing 0.1 μL of iPLEx Termination mix (Bio-Rad, San Diego, CA, USA) and hot-start Taq DNA polymerase. DNA was denatured at 85 °C for 5 min followed by 45 cycles of denaturation at 95 °C for 15 s and annealing at 58.1 °C for 45 s. Hardy–Weinberg equilibrium was determined using a statistical test (chi-square), and the variant of *NPY* gene was in Hardy–Weinberg equilibrium ($p = 0.36$).

2.6. Statistical analysis

The sample size was calculated to detect differences > 5 kg in body weight after dietary intervention with 90% power and 5% significance (n = 130 for each diet group). The Kolmogorov–Smirnov test was used to determine variable distribution. The results were expressed as mean ± standard deviation. Differences in baseline characteristics by genotype were tested using a two-tailed Student's *t* test for continuous variables with normal distribution and chi-square test with Yates correction as necessary, with categorical variables. Non-parametric variables were analyzed using the Mann–Whitney *U* test. Differences in anthropometric and biochemical variables between the SNP genotypes were assessed using analysis of covariance (ANCOVA) adjusted for age and sex. Correction with multiple testing was realized. Statistical analyses were performed using GG and GA genotypes as one group and AA as a second group, with a dominant model. Chi-square test was used to evaluate the Hardy–Weinberg equilibrium. All p-values < 0.05 were considered statistically significant. All analyses were conducted using SPSS version 19.0 (IL, USA).

3. Results

Among the 270 participants who consented to be included in the study, there were 70 (25.9%) males and 200 (74.1%) females with a mean age of 47.1 ± 0.1 years and mean BMI of 34.3 ± 4.1 kg/m^2. All participants completed the 9-month follow-up period and there was no drop-out for either dietary intervention (diet HP and diet S). Seventy nine (29.2%) participants had the GG genotype (major allele group) while 191 (70.8%) participants had GA (147 participants, 54.5%) or AA (44 participants, 16.3%) genotypes (minor allele group). Sex distribution was similar in the two groups: 27.7% vs. 24.1% for males and 72.3% vs. 75.9% for females. Allelic frequency was G (0.66) and A (0.34). Age was similar between the two groups (major allele group = 47.3 ± 9.1 years vs. minor allele group = 46.5 ± 8.9 years; not statistically significant).

The diet HP group comprised 137 obese participants (43 with the GG genotype and 94 A allele carriers). In this group, the basal calorie intake was 1781.1 ± 402.8 kcal/day, with the following macronutrient distribution: fat, 90.9 ± 12.3 g/day (40.3% of calories); carbohydrate, 201.3 ± 20.0 g/day (40.7% of calories); and protein, 77.2 ± 28.2 g/day (27.0% of calories). During the 9-month diet, these participants reached the objectives of the dietary intervention. Physical activity was similar for both genotype groups (61.1 ± 12.2 min/week vs. 60.9 ± 8.3 min/week; $p = 0.42$). The diet S group comprised

133 participants (36 with the GG genotype and 97 A allele carriers). In this group, the basal calorie intake was 1830.1 ± 210.2 kcal/day, with the following macronutrient distribution: fat, 94.1 ± 12.3 g/day (39.1% of calories); carbohydrate, 198.2 ± 20.9 g/day (42.3% of calories); and protein, 87.1 ± 7.0 g/day (19.6% of calories). Finally, physical activity was similar for both genotype groups (62.8 ± 12.1 min/week vs 61.9 ± 10.3 min/week; p = 0.36).

Table 2 shows the mean and standard deviation of blood pressure and anthropometric parameters at baseline and months 3 and 9. There were no significant differences between the two dietary groups at baseline or after months 3 and 9 of dietary intervention. For both genotypes (GG vs GA + AA), there was a similar decrease in BMI (−1.9 ± 0.8 vs −2.1 ± 0.8 kg/m^2; p = 0.23), weight (−6.2 ± 1.3 vs −6.4 ± 1.9 kg; p = 0.34), fat mass (−3.4 ± 1.0 vs −3.1 ± 1.1 kg; p = 0.36), and WC (−5.7 ± 2.1 vs − 5.2 ± 1.9 cm; p = 0.39) after the 9-month dietary intervention (diet HP). The decrease in systolic blood pressure (−6.5 ± 2.1 vs −7.1 ± 2.0 mmHg; p = 0.34) was also similar for both genotype groups. After the standard hypocaloric diet (diet S), improvements in BMI (−2.1 ± 1.0 vs −1.8 ± 0.9 kg/m^2; p = 0.32), weight (-6.0 ± 2.0 vs −5.9 ± 1.3 kg; p = 0.45), fat mass (−3.3 ± 2.1 vs −3.2 ± 1.1 kg; p = 0.53), WC (−5.1 ± 3.1 vs −5.8 ± 2.9 kg; p = 0.43), and systolic blood pressure (−6.8 ± 2.0 vs −5.9 ± 2.9 mmHg; p = 0.45) were similar for both genotypes (GG vs GA + AA).

Table 3 shows changes in biochemical parameters. Participants with the minor allele (A) who received diet HP showed decreases in total cholesterol (−6.5 ± 4.8 vs 10.1 ± 4.1 mg/dL; p = 0.01), LDL cholesterol (−5.9 ± 3.8 vs 9.6 ± 2.4 mg/dL; p = 0.01), triglycerides (−1.0 ± 4.8 vs 16.2 ± 4.1 mg/dL; p = 0.02), insulin (−0.5 ± 2.8 vs 1.7 ± 2.1 UI/L; p = 0.01), HOMA–IR (−0.2 ± 2.1 vs 0.5 ± 2.0 units; p = 0.03), and CRP (−0.3 ± 0.4 vs 1.3 ± 0.2 mg/dL; p = 0.02). Participants with the minor allele receiving diet S showed decreases in total cholesterol (−6.1 ± 4.1 vs 14.4 ± 3.1 mg/dL; p = 0.01), LDL cholesterol (−3.1 ± 2.8 vs 15.0 ± 3.1 mg/dL; p = 0.01), triglycerides (−6.9 ± 4.1 vs 13.2 ± 4.0 mg/dL; p = 0.01), insulin (−0.3 ± 2.1 vs −1.2 ± 0.2 UI/L; p = 0.02), HOMA–IR (−0.3 ± 2.1 vs −1.6 ± 1.1 units; p = 0.01), and CRP (−0.4 ± 0.1 vs 1.1 ± 0.2 mg/dL; p = 0.02). After both diets, these parameters remained unchanged in participants with the major allele. No differences in biochemical parameters were detected at baseline or at months 3 and 9 between the two genotypes (GG and GA + AA).

Table 4 shows levels of serum adipokines. For both genotypes (GG vs GA + AA genotypes), diet HP led to decreased leptin levels (−25.6 ± 8.1 vs −27.6 ± 7.2 ng/mL; p = 0.15). For both the major and minor allele groups, diet S also led to decreased leptin levels (−25.0 ± 9.1 vs −25.8 ± 8.9 ng/mL; p = 0.33). Leptin levels were not significant different between the two diets for either group. Resistin and adiponectin levels also showed no statistical differences.

4. Discussion

The present study revealed that the A allele of the NPY rs16147 variant was associated with improvements in lipid

Table 2 – Changes in anthropometric parameters according to diet and NPY rs16147 genotype.

	Diet HP (n = 137)						Diet S (n = 133)					
	GG (n = 43)			AA + AG (n = 94)			GG (n = 36)			AA + AG (n = 97)		
	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months
BMI	34.2 ± 3.1	32.7 ± 2.0*	32.3 ± 3.1*	34.4 ± 3.1	32.7 ± 4.0*	32.3 ± 4.0*	34.5 ± 3.0	33.8 ± 4.0*	32.4 ± 4.0 *	34.8 ± 4.1	33.9 ± 4.1*	33.0 ± 3.0*
Weight (kg)	86.8 ± 7.0	82.8 ± 6.0*	80.6 ± 4.1*	90.5 ± 4.1	85.3 ± 4.0*	84.1 ± 4.1*	90.4 ± 7.1	86.1 ± 7.0*	84.4 ± 7.0*	89.8 ± 12.3	86.2 ± 7.4*	83.9 ± 6.1*
Fat mass (kg)	32.8 ± 3.0	30.1 ± 2.1*	29.4 ± 5.0*	34.3 ± 3.1	31.6 ± 2.9*	29.2 ± 4.1*	34.8 ± 4.0	31.9 ± 3.1*	30.5 ± 4.3*	37.2 ± 7.0	34.8 ± 6.1*	34.0 ± 4.1*
WC (cm)	107.8 ± 5.1	103.1 ± 4.0*	102.1 ± 3.0*	109.6 ± 4.1	105.1 ± 4.2*	104.3 ± 3.0*	110.1 ± 4.0	106.9 ± 5.1*	105.1 ± 7.0*	110.2 ± 9.0	107.1 ± 8.3*	104.4 ± 7.1*
SBP (mmHg)	126.5 ± 5.1	121.1 ± 4.1*	120.0 ± 5.1*	128.2 ± 8.3	123.2 ± 4.3*	121.9 ± 4.2*	128.3 ± 8.1	123.9 ± 7.1*	121.5 ± 6.3*	124.9 ± 7.3	120.3 ± 3.9*	119.1 ± 3.9*
DBP (mmHg)	79.1 ± 3.0	76.9 ± 4.1	76.1 ± 3.0	80.3 ± 3.1	79.1 ± 7.0	78.5 ± 4.1	82.1 ± 9.1	80.8 ± 6.2	79.1 ± 5.1	81.0 ± 5.1	79.9 ± 4.3	79.8 ± 4.9

Data represent mean ± SD.
*p < 0.05 for each genotype group compared with basal values. No differences were found between the genotype groups.
BMI, body mass index; DBP, diastolic blood pressure; Diet HP, high-protein/low-carbohydrate hypocaloric diet; Diet S, standard hypocaloric diet; SBP, systolic blood pressure; WC, waist circumference.

Table 3 – Changes in biochemical parameters according to diet and NPY rs16147 genotype.

	Diet HP (n = 137)						Diet S (n = 133)					
	GG (n = 43)			AA + AG (n = 94)			GG (n = 36)			AA + AG (n = 97)		
	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months
Glucose (mg/dL)	107.2 ± 6.1	102.9 ± 6.0	99.1 ± 4.1	104.7 ± 8.2	101.9 ± 8.0	100.1 ± 5.2	103.8 ± 7.0	99.3 ± 7.1	98.4 ± 7.0	100.6 ± 6.7	97.7 ± 6.2	96.1 ± 4.1
Total chol (mg/dL)	209.8 ± 8.0	203.2 ± 3.1	202.3 ± 9.1	208.1 ± 9.1	198.1 ± 7.1*	196.1 ± 6.0*	201.2 ± 7.1	196.2 ± 6.1	194.3 ± 7.4	210.9 ± 8.1	200.5 ± 6.1*	194.5 ± 8.2*
LDL chol (mg/dL)	131.3 ± 7.0	125.2 ± 4.1	124.9 ± 9.1	130.6 ± 8.1	122.4 ± 8.0*	121.1 ± 7.3*	120.2 ± 8.0	119.0 ± 6.2	117.9 ± 7.2	126.1 ± 8.0	116.9 ± 8.2*	111.1 ± 7.2*
HDL chol (mg/dL)	55.1 ± 9.1	54.7 ± 8.1	53.9 ± 7.1	54.3 ± 3.2	55.2 ± 6.1	55.4 ± 7.3	56.4 ± 4.1	55.9 ± 5.8	54.8 ± 6.0	57.0 ± 4.1	56.9 ± 7.2	57.0 ± 6.3
TG (mg/dL)	105.9 ± 4.1	106.2 ± 9.1	104.1 ± 9.1	131.1 ± 21.8	116.7 ± 12.2*	115.1 ± 11.3*	132.8 ± 9.1	127.8 ± 7.9	125.1 ± 9.9	121.1 ± 10.0	109.2 ± 10.9*	108.1 ± 13.0*
Insulin (mUI/L)	10.5 ± 5.2	10.1 ± 3.2	10.0 ± 4.0	10.7 ± 3.2	9.4 ± 2.3*	9.0 ± 4.1*	10.2 ± 4.9	10.1 ± 3.8	9.9 ± 4.3	10.0 ± 4.1	9.1 ± 4.3*	8.8 ± 3.2*
HOMA–IR	2.5 ± 0.3	2.3 ± 0.4	2.3 ± 0.1	2.5 ± 1.2	2.2 ± 1.0*	2.0 ± 1.1*	2.8 ± 1.1	2.7 ± 1.3	2.5 ± 1.3	2.7 ± 1.2	2.3 ± 1.9*	2.1 ± 1.2*
CRP (mg/dL)	4.8 ± 3.0	4.4 ± 2.1	4.5 ± 3.1	5.1 ± 3.0	4.0 ± 1.2*	3.8 ± 1.1*	4.8 ± 2.0	4.7 ± 1.1	4.4 ± 2.1	4.9 ± 2.1*	4.1 ± 1.1*	3.8 ± 1.2*

Data represent mean ± SD.

* $p < 0.05$ for each group compared with basal values. # $p < 0.05$ among genotypes for each diet.

Chol, cholesterol; CRP, C-reactive protein; HOMA–IR, homeostasis model assessment–insulin resistance; HDL, high-density lipoprotein; Diet HP, high-protein/low-carbohydrate hypocaloric diet; LDL, low-density lipoprotein; Diet S, standard hypocaloric diet; TG, triglyceride.

Table 4 – Changes in circulating adipokines according to diet and NPY rs16147 genotype.

	Diet HP (n = 137)						Diet S (n = 133)					
	GG (n = 43)			AA + AG (n = 94)			GG (n = 36)			AA + AG (n = 97)		
	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months	Baseline	3 months	9 months
Adiponectin (ng/mL)	11.1 ± 3.2	13.9 ± 2.0	14.7 ± 3.2	10.5 ± 4.2	12.8 ± 3.0	14.3 ± 4.1	11.1 ± 4.1	12.9 ± 3.3	14.9 ± 4.0	11.1 ± 4.1	12.9 ± 4.1	13.4 ± 5.1
Resistin (ng/mL)	5.1 ± 2.1	5.0 ± 2.1	5.0 ± 3.1	5.3 ± 3.0	5.1 ± 2.9	5.0 ± 3.1	5.4 ± 2.0	5.3 ± 3.1	5.2 ± 2.3	5.5 ± 3.1	5.4 ± 4.1	5.3 ± 3.0
Leptin (ng/mL)	38.1 ± 7.0	14.7 ± 5.7*	12.5 ± 6.1*	39.9 ± 4.1	14.2 ± 3.1*	12.3 ± 5.0*	37.1 ± 5.2 1	5.4 ± 4.1*	12.1 ± 2.4 *	37.9 ± 4.1	14.0 ± 4.0*	12.1 ± 3.1*

Data represent mean ± SD.

* $p < 0.05$ for each group compared with basal values. No statistical differences were found among genotypes for each diet or among the different diet groups.

Diet HP, high-protein/low-carbohydrate hypocaloric diet; Diet S, standard hypocaloric diet.

profile, CRP levels, insulin levels, and HOMA–IR following two different hypocaloric diets.

The NPY gene is located on chromosome 7p15.1 [21–22]. The rs16147 SNP is the most important genetic variant in this gene as it alters NPY expression secondary to the loss of a transcriptional factor (Sp1) binding consensus via a substitution of G to A [23] or via interaction of the G/A allele with another regulatory genomic DNA regions other than Sp1 [24]. The allele frequencies found in the present study were similar to those previously described [23,24].

A recent study [11] using a hypocaloric low-fat diet (Mediterranean pattern) reported that the rs16147 genetic variant of the NPY gene modulated reductions in WC, HOMA–IR, insulin, CRP, and IL-6 levels in response to a weight-loss diet in obese individuals. Consequently, obese carriers of the A allele showed greater improvements compared with non-A allele carriers. While the present study showed no association between genotype and response in terms of adiposity markers such as WC, Crescenti et al. [10] reported an association between the A allele of the rs16147 polymorphism and inflammatory markers (CRP and IL-6 levels), as well as decreased CRP levels in A allele carriers after consumption of 14 g/day of *P. ovata* husk during a short-term (8-week) low-fat diet intervention. In a recent cross-sectional study, Patel et al. [25] reported a strong association between this genetic variant and up-regulation of IL-B transcript levels and susceptibility to type 2 diabetes mellitus. The relationship between the NPY pathway and pro-inflammatory status [26] was explained by a high release and synthesis of pro-inflammatory cytokines [27]. Furthermore, Aller et al. [28] reported that the A allele was associated with a lower index of liver inflammation. The authors reported a lower percentage of steatohepatitis and lobular inflammation in liver biopsies from non-alcoholic fatty liver disease patients carrying the A allele.

In the present study, we found an association between the rs16147 SNP and changes in lipid profile (LDL cholesterol and triglycerides), insulin, and HOMA–IR after both dietary interventions. A allele carriers showed a better response compared with non-A allele carriers, but the same weight loss with both diets. Similar findings were reported in a previous study conducted over three months using a Mediterranean diet [11], as well as another study using two different short-term dietary interventions (low carbohydrate vs low fat) [12]. Previous cross-sectional studies have reported [29] an association between the rs16147 variant and various cardiovascular risk factors, including increased risk of metabolic syndrome and its related phenotypes, such as central obesity and hyperglycemia in obese individuals without the A allele in a Caucasian population [30]. Another study [8] also reported this relationship in a non-Caucasian population [31]. Insulin resistance is a major component of metabolic syndrome and is associated with dyslipidemia. Therefore, it is possible that the A allele may alter the synthesis, release, and action of insulin in different tissues.

The present study show no association between the rs16147 variant and adiposity parameters or weight loss secondary to either of the two diets. A previous meta-analysis [31] reported that the minor alleles of this genetic variant were associated with increased risk of obesity in 942 children. In a previous study using a pediatric cohort [32], a longitudinal relationship between rs16147 and BMI during childhood was also demonstrated. However, the results in adult studies are contradictory. Mutschler et al. [33] reported that the rs16147 variant of the NPY gene was significantly associated with WC, while another adult study showed no association [34]. It was postulated that this contradictory association between adiposity parameters and this SNP may be due to modulation of white adipose tissue via the nerve endings situated in adipose tissue by NPY [35]. Adipokines such as leptin form a feedback loop with NPY and inform the brain of body fat levels [36]. Dietary fat intake may regulate NPY gene expression, supporting the interaction between the NPY pathway, dietary habits, and adiposity. Finally, Barth et al. [37] reported that NPY levels were not elevated in obese individuals. Expression of NPY in adipose tissue specimens was comparable between obese individuals and controls, but the overall production rate of adipose tissue-derived factors varied due to different amounts of adipose tissue in the body.

Our biochemical results could be explained by the hypothesis of differential susceptibility, which postulates that risk alleles may function more like plasticity genes, thereby allowing some individuals to be more responsive to environmental factors than others [38]. Consistent with this, our data supported that the A allele may act as a protective factor, depending on the dietary intervention.

The present study has some limitations. First, we did not measure circulating NPY levels. Second, we only analyzed one SNP of the NPY gene, and other low-frequency genetic variants in this or other genes may also be associated with adipose tissue. Third, our results can be extrapolated to a Caucasian population with obesity, and other ethnicities or obese subjects with comorbidities may have other associations with this genetic variant. Finally, other unknown factors, such as sex hormone status, that were not measured may have influenced our results.

In conclusion, the presence of the A allele of the rs16147 variant was shown to be associated with a better metabolic response secondary to weight loss in response to two different hypocaloric diets in obese Caucasian individuals. LDL cholesterol, triglycerides, insulin resistance, insulin levels, and CRP improved in individuals with the minor allele.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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D A de Luis wrote the article and performed the statistical analyses.

R. Aller and O Izaola performed the anthropometric evaluation.

D Primo performed the biochemical evaluation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.107825.

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