Clinical Nutrition Open Science 36 (2021) 26–34

Contents lists available at ScienceDirect

Clinical Nutrition Open Science

journal homepage: www.clinicalnutritionopenscience.com

Original Article

Allele a of the rs16147 variant of neuropeptide Y predicts early metabolic improvements after bariatric surgery with biliopancreatic diversion in morbid obese subjects

David Pacheco, Olatz Izaola, David Primo, Daniel de Luis*

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

ARTICLE INFO

Article history:
Received 14 December 2020
Accepted 6 February 2021
Available online 16 February 2021

Keywords:
Biliopancreatic diversion
Morbid obesity
rs16147
NPY gene
SNPs

SUMMARY

Background and aims: Few studies have assessed the effect of rs16147 on metabolic response after weight loss interventions. We evaluated the effect of the genetic variant *rs16147 NPY* gene on biochemical changes after biliopancreatic diversion surgery in morbidly obese subjects during 4 years follow up.

Material and methods: One hundred and forty seven patients with morbid obesity without diabetes mellitus type 2 were enrolled. Biochemical and anthropometric evaluation were registered before and after 1, 2, 3 and 4 years follow up. Genotype of *rs16147 NPY* gene has been studied.

Results: Fasting glucose, insulin, HOMA-IR and lipid profile improved in both genotype groups. Although the improvement in glucose, insulin and HOMA-IR was significant in both genotypes, this change was earlier in the A allele carriers and as soon as 1 year after surgery, and we only detected the improvement at 3 years in non A allele carriers. So, basal glucose improvements the first year (non-A allele vs A allele carriers) (delta:-6.2 ± 2.1 mg/dL *vs.* −8.5 ± 0.8 mg/dL; $p = 0.02$) and the second year (delta: -9.0 ± 2.1 mg/dL *vs.* −15.4 ± 2.1 mg/dL; $p = 0.01$) were higher in A allele carriers than non-A allele carriers. The decrease of fasting insulin were better in A allele carriers the first year (delta:-2.0 ± 1.1 mUI/L *vs.* −4.8 ± 0.6 mUI/L; $p = 0.03$) and the second year (delta: -2.4 ± 0.2 mUI/L *vs.* −5.7 ± 0.2 mUI/L; $p = 0.01$). Finally, the improvement of HOMA-IR levels was earlier in A allele carriers than non-A allele carriers as reported; at year one (delta:-0.4 ± 0.2

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

https://doi.org/10.1016/j.nutos.2021.02.003

mUI/L *vs.* −1.7 ± 0.2 mUI/L; $p = 0.03$), year two (delta: -1.1 ± 0.2 mUI/L *vs.* −1.8 ± 0.2 mUI/L; $p = 0.02$), too.
Conclusion: The presence of A allele of rs16147 genetic variant produces an early metabolic response secondary to weight loss after bariatric surgery.

1. Introduction

Obesity is a worldwide health problem with many comorbidities such as diabetes mellitus type 2, high blood pressure and hyperlipidemia, reaching pandemic diffusion. Weight loss treatments targeting the decrease of caloric intake have proven limited effectiveness; moreover, bariatric surgery guarantees an important weight loss, with an important effect in reducing comorbidities [1]. However, the metabolism response to bariatric surgery is not always the same, mainly due to non-surgical causes [2]. There are a lot of factor imply in the response after surgery, many peripheral and central parameters have been implicated in the regulation of metabolism and energy homeostasis. For example, the *arcuate nucleus* (ARC) of the *hypothalamus* contains two sets of neurons that express either the neuropeptide pro-opiomelanocortin (POMC) or coexpress agouti-related protein (AGRP) and neuropeptide Y (NPY), which have opposite effects on the feeding behavior: the anorexigenic pro-opiomelanocortin (POMC) neurons and the orexigenic neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons [3]. Some studies have indicated a main role of neuropeptides Y (NPY) in determining obesity and its comorbidities, too [4,5].

In the NPY metabolic pathway, some genetic variants have been described as polymorphism (SNP) in *NPY* gene. One of the most important variant within the promoter region upstream of the *NPY* is rs16147 (G-399 A) [6] and this genetic variant a substitution G to A (G as major allele and A as minor allele). This genetic variant has been related with serum levels of NPY [7], and it is related with more than half of the variation in the expression of this peptide [8] and this genetic variants of *NPY* gene could produce metabolic disorders associated with obesity [9,10].

As we previously mentioned, weight loss with different strategies, including bariatric surgery is the main objective to achieve in the obese patient, to improve their metabolic comorbidities. As far as we know, few studies that evaluate the effect of rs16147 on metabolic response after weight loss have been realized. The study with the largest number of patients and the longest in time is the POUNDS LOST trial [11]. In this study [11], rs16147 variant of NPY *gene* was related with the change in abdominal adiposity in response to four different hypocaloric interventions. In other short-term intervention trial of 3 months with only one diet [12], it has been reported that the *rs16147* genotype affected abdominal adiposity measured as waist circumference and other metabolic parameters, too. Moreover, in other studies, no effect was found on anthropometric parameters, but a positive effect was reported on biochemical variables. For example, in A small intervention trial of 8 weeks [13], it has been reported the influence of this genetic variant on the response to *plantago ovata* husk without a caloric restriction on C reactive protein plasma levels. In other intervention trial of 12 weeks with two different hypocaloric diets (low in carbohydrate vs low in fat amount) showed similar weight loss, with a lack of reduction in insulin resistance in subjects with major allele [14]. To date, there is no study evaluating the effect of this polymorphism on metabolic respect secondary to bariatric surgery in patients with morbid obesity.

Therefore, we evaluated the effect of the genetic variant *rs16147 NPY* gene on biochemical changes after biliopancreatic diversion surgery in morbidly obese subjects during 4 years follow up.

2. Materials and methods

2.1. Population and bariatric surgery procedure

One hundred and forty seven morbid obese subjects were consecutively enrolled among those referring to the obesity clinic of our hospital (Table 1). Exclusion criteria were having age above 60 years and presence of diabetes mellitus type 2, systemic inflammatory diseases, malignancies, coagulopathy, severe liver or chronic renal diseases. The Local Ethical Committee (HURH-Committee- 3/2016) approved the study. All subjects signed a written informed consent. All procedures performed in this study were in accordance with the Declaration of Helsinki.

All participants underwent biliopancreatic diversion (BPD) surgery. The BPD surgery consisted in the set-up of 70-cm common limb and 175-cm alimentary limb with the addition of a partial gastrectomy. In addition, the final step was a transection of the small bowel half way form the Treitz angle to the ileocecal valve followed by a Roux on Y gastroenterostomy on the distal bowel loop and an end-to-side enteroileostomy of the proximal bowel loop on the ileum 50–75 cm before the ileocecal valve. After 30 days of bariatric surgery, all patients followed the same diet based on the intake of 1200–1400 calories, non-protein calories were distributed among fats (35%, divided into 10% saturated, 20% monounsaturated and 5% polyunsaturated) and carbohydrates (65%). Protein consumption was 1.4 g per Kg of ideal weight (Body Mass Index (BMI) 23 kg/m^2).

2.2. Design of study

After 10 h of fasting, all individuals underwent clinical examination; biochemical parameters, blood pressure and anthropometric parameters (body weight, waist circumference and percent excess weight loss (EWL%)). Among biochemical parameters, we measured; serum lipid levels (total cholesterol, Low-density lipoprotein cholesterol, High-density lipoprotein cholesterol, triglycerides), insulin, fasting glucose, (homeostasis model assessment of insulin resistance) HOMA-IR. We determined associated morbidities (percentage of patients with hypertension or hyperlipidemia), too. All these parameters were obtained at the last visit prior to surgery (baseline) and at each later postoperative visit at 1, 2, 3 and 4 years following the surgery. Genotype of *rs16147 NPY* gene has been studied.

2.3. Anthropometric measurements, blood pressure and comorbidities

Body weight, height and waist circumference (WC) were determined in the morning before breakfast at baseline, years 1, 2, 3 and 4. Later body mass index was computed as body weight in Kg/(height in m^2). WC was determined in the narrowest diameter between xiphoid process and iliac crest. Percent excess weight loss (EWL%) was calculated using the formula; (preoperative weight − current weight x100/preoperative weight − ideal weight). Ideal weight was calculated with an ideal BMI 22 kg/m^2. Bipolar body electrical bioimpedance (Akern, EFG, Pisa, It) was used to determine total fat mass with an accuracy of 50 g [15]. Blood pressure was determined twice and averaged after a 5 min rest with a random zero mercury sphygmomanometer, (Omrom, LA,CA, USA).

Table 1
Preoperative and postoperative characteristics of the patients.

	Basal time	1 year	2 years	3 years	4 years
	n = 147	n = 145	n = 140	n = 138	n = 134
Morbid obese	135	70	51	23	5
Super-obese	12	6	3	**0**	**0**
Gender (female/male)	113/34	112/33	109/31	107/31	104/30
Age (years)	48.7 ± 5.1	49.3 ± 4.9	49.9 ± 6.1	50.4 ± 5.9	51.1 ± 5.8
BMI (kg/m^2)	47.9 ± 5.0	38.0 ± 4.3	34.3 ± 3.9	32.9 ± 4.0	31.0 ± 3.9

Morbid: BMI>40 kg/m^2 and <50 kg/m^2. Superobese >50 kg/m^2

The presence of comorbidities was defined as hypertriglyceridemia (triglycerides > 150 mg/dL), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg, respectively) and low HDL cholesterol (<40 mg/dL and 50 mg/dL for men and women, respectively).

2.4. Assays

General lipid profile (total cholesterol and triglyceride levels) was measured by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N·Y., USA), while HDL cholesterol was determined in the supernatant after precipitation of other lipoproteins by enzymatic methods. LDL cholesterol was calculated using Friedewald formula (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/5) [16].

Glucose metabolism was investigated as followed; glucose levels were determined by an automated glucose oxidase method (Glucose analyser 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) [17] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values [18].

Genomic DNA from each obese individuals was purified from peripheral blood leucocytes using a commercial kit extraction (Quantum prep, Biorad, LA, CA). Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc. San Diego, CA, USA). The polymerase chain reaction (PCR) was carried out with 20–25 ng of genomic DNA and 0.1–0.15 μl each of oligonucleotide primer for rs16147 (primer forward: 5′- ACGTTGGATGCACAAAGAGGATTCAGGTGC -3′ and reverse 5′- ACGTTGGATGAGCCCAGACGATTCTTGTC -3′ in a 2-μl final volume (Termociclador Lifetecnologies, LA, CA,USA) by chain reaction real time analysis. DNA was denatured at 85 °C for 5 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 58.1 °C for 45 s). The PCR was run in a 2-μl final volume containing 0.1 μl of iPLEx Termination mix (Bio-Rad®, San Diego, CA, USA) with hot start Taq DNA polymerase. Hardy Weinberg equilibrium was determined with a statistical test (Chi-square). The variant of *NPY* gene was in Hardy Weinberg equilibrium ($p = 0.46$).

2.5. Statistical analysis

IBM SPSS Statistics version 23.0 (IL, USA) was used for statistical analysis. Power analysis suggested at least 140 subjects with the change in weight of 30% EWL% using polymorphism frequency (40%) in morbid obese subjects, with a type I error of 0.05 and type II error of 0.10 (power = 0.9). The statistical analysis was performed for the combined GG and GA as a group and AA genotype as second group, with a dominant model. The results were expressed as average ±standard deviation. The normal distribution of variables was studied with the Kolmogorov-Smirnov test. Non-parametric variables were analyzed with the Mann-Whitney test and Wilcoxon test. Parametric test was analyzed with ANOVA test and Bonferroni post hoc test. Qualitative variables and the presence of comorbidities were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test as necessary. A *p*-value under 0.05 was considered statistically significant.

3. Results

147 morbid obese subjects were enrolled. Table I reports the main characteristics of the patients included in the study. As expected, during the four-year follow-up period there was a gradual loss of patients follow up. Of this all group, 109 of whom were females (74.1%) and 38 were males (25.9%). The allelic frequency was found to be subjects carrying 47.0% G and 53.0% A alleles, respectively. The genotypic frequency was found to be 23.1% (34 patients) in GG genotype, 49.0% (72 patients) in GA genotype and 27.9% (41 patients) in AA genotype.

With the aim of analyzing the effect of this polymorphism on a dominant model, subjects were classified in two groups; those carriers A allele (AA + AG, 76.9%) and non-carriers A allele (GG, 23.1%). The gender distribution was similar in all genotypes (GG; 29.4% (n = 10) males and 70.6% (n = 24) females), (GA; 27.7% (n = 20) males and 72.3% (n = 52) females) and (AA; 20.0% (n = 8) males and 80.0%

(n = 33) females). Average age was similar in all genotype groups (GG: 48.4 ± 6.2 years *vs.* GA + AA 48.9 ± 9.1 years: ns), too.

Table 2 reports the adiposity parameters and blood pressure of obesity subjects before surgery and during the duration of the study. Anthropometric parameters and blood pressure were similar between genotypes. When the evolution of adiposity parameters over time was revised, we detected similar pattern between genotypes. Body weight, waist circumference and fat mass showed a statistically significant reduction after surgery at 1, 2, 3 and 4 years. Blood pressures decreased in both groups, too. Improvements in these parameters were similar in both genotypes. Percentage of excess of weight loss (EWL%) showed a significant improvement during the duration of the study in A-allele and non-A allele carriers.

Table 3 shows the improvements in all biochemical parameters. No significant preoperative differences in glucose parameters (fasting glucose levels, HOMA-IR and insulin) and lipid profile (total cholesterol, HDL-cholesterol, LDL cholesterol and triglyceride levels) were observed between genotypes. Fasting glucose, insulin, HOMA-IR, total cholesterol, LDL-cholesterol and triglyceride levels decreased in both genotype groups during the study. Although the improvement in glucose, insulin and HOMA-IR was significant in both genotypes, this change was earlier in the A allele carriers and as soon as 1 year after surgery, and we only detected the improvement at 3 years in non A allele carriers. So, basal glucose improvements the first year (non-A allele vs A allele carriers) (delta:-6.2 ± 2.1 mg/dL *vs.* −8.5 ± 0.8 mg/dL; $p = 0.02$) and the second year (delta: -9.0 ± 2.1 mg/dL *vs.* −15.4 ± 2.1 mg/dL; $p = 0.01$) were higher in A allele carriers than non-A allele carriers, and the improvement was similar in the rest of annual visits; third year (delta:-19.0 ± 3.4 mg/dL *vs.* −16.8 ± 2.5 mg/dL; $p = 0.23$) and the fourth year (delta:-19,1 ± 3.2 mg/dL *vs.* −18.8 ± 4.9 mg/dL; $p = 0.36$. The decrease of fasting insulin levels showed a similar pattern after the third year (delta:-7.1 ± 1.8 mUI/L *vs.* −8.8 ± 1.3 mUI/L; $p = 0.42$) and fourth year (delta:-7.2 ± 1.9 mUI/L *vs.* −9.0 ± 1.7 mUI/L; $p = 0.41$), but these improvements were better in A allele carriers the first year (delta:-2.0 ± 1.1 mUI/L *vs.* −4.8 ± 0.6 mUI/L; $p = 0.03$) and the second year (delta: -2.4 ± 0.2 mUI/L *vs.* −5.7 ± 0.2 mUI/L; $p = 0.01$). On the other hand, the improvement of HOMA-IR levels was earlier in A allele carriers than non-A allele carriers as reported; at year one (delta:-0.4 ± 0.2 mUI/L *vs.* −1.7 ± 0.2 mUI/L; $p = 0.03$), year two (delta: -1.1 ± 0.2 mUI/L *vs.* −1.8 ± 0.2 mUI/L; $p = 0.02$), year three (delta:-1.4 ± 0.4 mUI/L *vs.* −2.0 ± 0.5 mUI/L; $p = 0.53$) and at year four (delta:-1.5 ± 0.8 mUI/L *vs.* −2.1 ± 0.9 mUI/L; $p = 0.53$). Finally, HDL-cholesterol levels increased in both genotype groups during follow up to the same extent.

Table 4 reports the improvement in obese comorbidities (percentage of hypertriglyceridemia, hypertension and low-HDL levels). These rates (frequencies) were similar in both genotype groups.

4. Discussion

In this study, we evaluated the contribution of variant rs16147 of *NPY gene* on biochemical changes in non-diabetic morbid obese subjects undergoing bariatric surgery. Our data shows an early effect of A

Table 2
Changes in anthropometric variables rs16147 (mean±S.D).

Characteristics	rs16147					rs16147				
	GG (n = 34)					*GA or AA* (n = 113)				
	0 time	At 1 years	At 2 year	At 3 years	At 4 years	0 time	At 1 year	At 2 years	At 3 years	4 years
BMI	48.0 ± 6.1	38.1 ± 5.3[a]	34.5 ± 5.9[a]	33.1 ± 5.4[a]	31.1 ± 4.4[a]	47.6 ± 4.0	37.9 ± 3.8[a]	34.1 ± 4.0[a]	32.6 ± 4.0[a]	30.7 ± 3.1[a]
Weight (kg)	122.1 ± 24.6	93.9 ± 19.9[a]	86.1 ± 8.9[a]	80.1 ± 5.9[a]	75.1 ± 5.1[a]	121.8 ± 20.2	93.1 ± 7.2[a]	86.1 ± 6.1[a]	81.1 ± 5.9[a]	79.2 ± 5.1[a]
Fat mass (kg)	46.9 ± 8.4	34.7 ± 9.2[a]	33.1 ± 5.2[a]	31.4 ± 4.1[a]	29.9 ± 3.1[a]	46.7 ± 8.0	34.4 ± 7.1[a]	32.9 ± 5.9[a]	31.3 ± 3.9[a]	29.8 ± 3.7[a]
WC (cm)	119.2 ± 9.0	113.1 ± 5.0[a]	103.1 ± 5.9[a]	98.1 ± 6.0[a]	93.9 ± 4.9[a]	118.2 ± 6.0	112.1 ± 4.0[a]	102.9 ± 3.7[a]	97.9 ± 4.3[a]	92.9 ± 3.2[a]
EWL%	-	**58.9**	62.2	67.1	71.9	–	59.0	62.5	66.7	71.5
SBP (mmHg)	148.0 ± 7.1	132.1 ± 6.0[a]	129.1 ± 7.1[a]	129.2 ± 6.9[a]	129.1 ± 5.8[a]	149.0 ± 5.1	134.1 ± 4.2[a]	131.9 ± 4.1	129.4 ± 3.1[a]	128.9 ± 3.0[a]
DBP (mmHg)	89.1 ± 4.1	87.1 ± 4.1[a]	84.0 ± 6.2[a]	83.8 ± 5.1[a]	82.9 ± 5.0[a]	90.1 ± 3.1	84.2 ± 3.0[a]	84.2 ± 3.1	82.9 ± 2.2[a]	82.7 ± 3.1[a]

DBP: Diastolic blood pressure. SBP: Systolic blood pressure. WC: Waist circumference. EWL%: Percent excess weight loss.
[a] $p < 0.05$, in each genotype group with basal values. There are no statistical differences in demographic, anthropometric and metabolic characteristics between the two-genotype groups.

Table 3
Biochemical parameters (mean ± S.D).

Characteristics	rs16147					rs16147				
	GG (n = 34)					*GA or AA* (n = 113)				
	0 time	At 1 year	At 2 years	At 3 years	At 4 years	0 time	At 1 year	At 2 years	At 3 years	4 years
Glucose (mg/dl)	106.1 ± 5.9	95.9 ± 6.1[a]	89.1 ± 8.1[a]	87.1 ± 4.2[a]	87.0 ± 3.1[a]	104.5 ± 4.9	96.9 ± 5.1[a]	90.1 ± 4.2 [a]	88.7 ± 5.0[a]	86.7 ± 5.1[a]
Total ch. (mg/dl)	199.3 ± 21.2	139.4 ± 15.9[a]	128.1 ± 12.1[a]	126.2 ± 9.1	124.1 ± 8.0[a]	200.9 ± 10.1	137.2 ± 11.3 [a]	129.9 ± 9.0[a]	125.9 ± 8.1[a]	124.1 ± 7.1[a]
LDL-ch. (mg/dl)	121.2 ± 12.1	72.5 ± 9.3[a]	62.7 ± 8.4[a]	62.1 ± 7.0[a]	60.0 ± 6.9[a]	120.9 ± 13.0	71.0 ± 8.1 [a]	63.1 ± 8.1[a]	60.8 ± 7.2[a]	59.9 ± 4.8[a]
HDL-ch. (mg/dl)	49.5 ± 8.0	50.1 ± 7.1[a]	51.3 ± 9.2[a]	52.1 ± 8.3[a]	53.7 ± 9.2	50.9 ± 7.2[a]	51.8 ± 6.2[a]	52.1 ± 6.0[a]	52.0 ± 6.1[a]	53.0 ± 6.0[a]
TG (mg/dl)	168.9 ± 20.1	130.1 ± 18.2[a]	110.9 ± 15.6[a]	91.1 ± 10.1[a]	90.2 ± 8.1[a]	170.1 ± 12.8	128.2 ± 11.0[a]	111.1 ± 9.8[a]	90.7 ± 8.1[a]	90.1 ± 7.1[a]
Insulin (mUI/L)	19.2 ± 4.4	17.2 ± 5.0	16.8 ± 4.8	11.2 ± 4.0[a]	11.0 ± 3.8[a]	19.9 ± 2.1	15.1 ± 2.0[a]	14.2 ± 3.1[a]	11.1 ± 3.9[a]	10.9 ± 2.9[a]
HOMA-IR	4.3 ± 1.8	3.9 ± 1.9	3.2 ± 1.2	2.9 ± 0.4[a]	2.8 ± 0.4[a]	4.7 ± 0.8	3.0 ± 0.8[a]	2.9 ± 0.9[a]	2.7 ± 0.6[a]	2.6 ± 0.5[a]

LDL. Low density lipoprotein. HDL High density lipoprotein. Chol: Cholesterol. TG: Triglycerides. HOMA-IR (homeostasis model assessment), $p < 0.05 between genotypes.
[a] p<0.05, in each group with basal values.

Table 4
Preoperative and postoperative comorbidities of the patients.

Parameters	Baseline	1 year	2 year	3 year	4 year
	n = 147	N = 145	n = 140	N = 138	n = 134
Low levels HDL Cholesterol					
GG	44.1%	20.5%	17.6%[a]	11.9%[a]	11.7%[a]
GA + AA	42.4%	21.2%[a]	19.4%[a]	14.2%[a]	15.0%[a]
High Levels TG					
GG	47%	28.5%[a]	20.5%[a]	11.9%[a]	5.4%[a]
GA+ÁA	46.9%	23.4%[a]	18.6%[a]	15.9%[a]	13.3%[a]
Blood Hypertension					
GG	29.4%	14.7%[a]	11.7%[a]	11.7%	8.8%[a]
GA + AA	27.4%	18.7%	10.6%	9.7%[a]	8.6%[a]

HyperTG Hypertriglyceridemia (triglycerides > 150 mg/dL), hypertension (systolic and diastolic blood pressures higher than 130 and 85 mmHg respectively), low HDL cholesterol (<40 mg/dL and 50 mg/dL for men and women, respecti.
[a] p<0.05, in each group with basal values.

allele of this genetic variant in modulating changes of glucose, insulin levels and HOMA-IR levels after a huge weight loss during 4 years follow up.

To our knowledge, this is the first time that an evaluation of a genetic variant in *NPY* gene in a cohort of morbid obese patients is carried out in a long-term study of 4 years after a bariatric surgery. The *NPY* gene is located on chromosome 7p15.1 [19,20], the variant rs16147 alters NPY expression. There are two hypothesis that could explain this modification in the levels of the peptide, by the interaction of G/A allele with another regulatory genomic DNA regions different than Sp1 [21] or loss of a transcriptional factor (Sp1) binding consensus by substitution G to A [22].

In our study, we have observed how the A allele modulates the metabolic response, but there is no effect of this genetic variant on total weight loss, fat mass or waist circumference. Two studies in the literature have shown an effect of rs16147 on adiposity parameters. The duration of the first study [11] was 4 years and the average basal BMI was 32 kg/m^2 and in the second study [12] the duration of the intervention was only 3 months and the weight of the patients was similar to the previous one. In other words, there is an important difference between the morbidly obese patients evaluated in our study, with a basal BMI >40 kg/m^2 and the populations from the two previous studies. This may explain the lack of a clear effect on the weight of this polymorphism in our present design.

The effects on the glucose metabolism found in our study of intervention with bariatric surgery have already been described in other investigations using low calorie diets. For example, a short term study [12] with a hypocaloric low fat diet (Mediterranean pattern) demonstrated a significant decrease of HOMA-IR, insulin, CRP and IL-6 levels in response to weight loss diet in A allele carriers. Our present study didn't investigate the inflammatory status with biomarkers such as CRP or IL-6. Perhaps, the inflammatory response is not related with weight loss, and some nutrients such as fiber could influence

this improvement as indicated by Crescenti et al. [13], too. In this short-term study of 8 weeks [13], a decrease of CRP levels in A allele carriers was reported after 14 g/day of *plantago ovata* husk.

The main finding of our study is that A allele carriers showed a faster improvement of glucose, insulin and HOMA-IR after weight loss than non-A allele carriers. This better metabolic response has been reported in the previous mentioned study with a Mediterranean diet [12] and in other recent study with two different hypocaloric diets (low in carbohydrate vs. low in fat amount) [14]. The pathophysiological mechanisms that may explain this improvement in the metabolic parameters of patients carrying the A allele are unknown. We could hypothesize that the A allele produces an alteration in the synthesis, release and action of insulin in different tissues, as a cornerstone. To support this theory, some cross-sectional studies in the literature have demonstrated the relationship between this SNP and diabetes mellitus or its comorbidities. For example, Patel et al. [23] have described in a cross-sectional study a strong association between this genetic variant. Besides, Aller et al. [24] reported a lower percentage of liver damage in A allele carriers with non-alcoholic fatty liver disease. Moreover, it have reported [25–27] an association of *rs16147* variant with metabolic syndrome and its related phenotypes, such as central obesity and hyperglycemia. A possible physiological explanation for these relationships is that NPY neurones in the arcuate nucleus of the hypothalamus are critical control centers for insulin's central action and glucose homeostasis regulation. This colocalisation of insulin receptors with NPY neurones is also found in other brain areas, with unknown interactions and control functions. There is also a peripheral interaction of the NPY system with insulin release, thereby closing the loop between these two energy and glucose homeostasis controlling system [28].

On the other hand, the interrelationships in these metabolic pathways are complex, and different adipocytokines and hormones could be involved. For example, it has been reported that NPY can modulate white adipose tissue via the nerve endings situated in adipose tissue [29]. In addition, leptin forms a feedback loop with NPY and sends signals to the brain of body fat levels [30], supporting the interactions between NPY pathway, dietary habits and adiposity.

Several limitations need to be highlighted in the present study. Firstly, we did not measure serum NPY levels in the study population, which does not allow us to assess a relationship between the SNP, the peptide and the metabolic findings. Secondly, we only analyzed one genetic variant of NPY gene, so other SNPs in this or other NPY gene could be interacted with our observations. Thirdly, other non-genetic factors could modify the relationships in our design (smoke habit, exercise, hormone status, and so on) and epigenetic factors, too. Fourthly, the lack of a dietary assessment throughout the study in order to measure for example dietary fibre or fat intakes might be a bias [31]. Fifth, the lack of adipokine determination could be a bias, too. Finally, our group of patients is a morbid obese adult sample without diabetes mellitus; so the data is not generalizable to non-morbid obese patients or obese patients with diabetes mellitus type 2.

5. Conclusion

In conclusion, during the first two years the improvement of glucose, insulin levels and HOMA-IR was already significant in carriers of the A allele. It may be necessary in patients undergoing bariatric surgery to perform a previous genotyping and in those who do not carry the A allele to maintain the treatments for glycemic control for a longer time.

Statement of ethics

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 2/2018) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding sources

No funding sources.

Author contributions

D A de Luis wrote the article and made statistical analysis.
D Primo and O Izaola made anthropometric evaluation.
D Primo made biochemical evaluation.
D Pacheco realized surgeries and made statistical analysis.

Conflict of interest statement

No conflict of interest.

Acknowledgement

No applicable.

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