European Review for Medical and Pharmacological Sciences 2020; 24: 5573-5581

RS9939609 FTO gene variant modified weight loss and insulin resistance after a partial meal-replacement hypocaloric diet

D.A. DE LUIS, O. IZAOLA, D. PRIMO, J.J. LOPEZ GOMEZ, R. ALLER

Center of Investigation of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain

Abstract. – OBJECTIVE: Some studies have demonstrated that the allele A of *FTO rs9939609* is related to both higher waist circumference and body mass index. Subsequently, some designs related biochemical variables and body weight changes with this genetic variant. We decide to analyze the effects of *rs9939609* genetic variant of *FTO* gene on metabolic parameters and weight loss secondary to partial meal replacements hypocaloric diets (pMRHDs) in obese subjects.

PATIENTS AND METHODS: This was a non-randomized, single-treatment study with a formula-diet in 44 obese subjects. The patients received nutritional education and a pMRHDs with two intakes of normocaloric hyperproteic formula during 12 weeks. Anthropometric parameters and biochemical profiles were measured at basal time and after 12 weeks. The variant of *FTO* gene *rs9939609* was determined.

RESULTS: Genotype distribution (n=44) was (16 TT (36.4%), 17 TA (38.6%) and 11 AA (25.0%)). After the pMRHD, body weight, body mass index (BMI), fat mass, waist circumference, serum leptin levels and systolic blood pressure improved in both genotypes without statistical differences in both branches. After dietary intervention with pMRHD, subjects with A allele showed a significant improvement in total cholesterol levels (TT *vs.* TA+AA) (-3.8±1.4 md/dL *vs.* -12.6±1.7 mg/dl: *p*=0.01), LDL-cholesterol (-0.2±1.5 md/dL *vs.* -10.5±1.9 mg/dl: *p*=0.02), insulin levels (-1.9±0.2 mU/L vs. -3.8±0.3 mU/L: *p*=0.02) and HOMA-IR (-0.6±0.2 units *vs.* -1.1±0.1 units: *p*=0.01).

CONCLUSIONS: Our data suggest that the genetic variant (*rs9939609*) of *FTO* gene showed better improvement of LDL-cholesterol, insulin and HOMA-IR in subjects with A allele.

Key Words:
Partial meal-replacement, Insulin resistance, *rs9939609*, Obesity, Weight loss.

Abbreviations

ILDL: Low-density lipoprotein; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model assessment; pMRHDs: partial meal replacements hypocaloric diets; FTO: fat mass and obesity associated gene.

Introduction

Obesity is a major public health problem that is estimated to affect a high percentage of general population and has been linked as risk factors for many common diseases[1]. Nevertheless, independently from the diet and physical activity, also genetics play a key role in the development of obesity. In the human genome, several numbers of genes are involved in obesity and Fat mass and obesity-associated gene (FTO) is one of the most important. Common polymorphisms of this gene have been associated with obesity in some populations[2,3]. One of these genetic variants (*rs9939609*) has been related to an increased risk for both obesity and metabolic disorders[4].

This gene (*FTO*) is expressed in several fetal and adult tissues and it is located on chromosome 16 (16q12.2) and encodes for the enzyme alpha-ketoglutarate-dependent dioxygenase[5]. This enzyme is involved in the regulation of both differentiation and the control of adipocyte thermogenesis, contributing to the body fat accumulation[6]. In addition, it contributes to the regulation of energy homeostasis, metabolic rate[7] and increasing the food intake[8]. Among the several variants of this gene, FTO *rs9939609* is one of the best-known. It is located in the first intron of the gene and it was associated with the body mass[9]. Further studies[10,11] demonstrated that the allele A of FTO *rs9939609* is related to both higher waist circumference and body mass index. Subsequent-

Corresponding Author: Daniel A. de Luis, MD; e-mail: dadluis@yahoo.es

ly, some studies are based on cross-sectional data and related lifestyle variables, biochemical parameters and body weight changes with this genetic variant[12,13]. Some intervention studies have explored the interaction between dietary intervention and FTO gene variant (*rs9939609*) on weight loss or metabolic changes. Razquin et al[14] reported that, although at baseline the A allele was associated with higher body weight, after 3 years of nutritional intervention with a Mediterranean-style-diet, A-allele carriers had lower body weight gain than wild type subjects. In Zhang et al[15], carriers of the risk allele A had a greater reduction in weight in response to 2-year high-protein diet. Other short-term interventional study[16] with a low fat hypocaloric diet (Mediterranean Pattern) showed a better metabolic improvement secondary to weight loss in A carriers. In addition, the genotype of this SNP was associated with metabolic response after bariatric surgery in morbid obese subjects[17].

Finally, there is evidence that partial meal replacements hypocaloric diets (pMRHDs) are useful in inducing weight loss. An interesting meta-analysis[18] comparing partial replacement programs *vs.* traditional energy restricted food based diets demonstrated that pMRHDs resulted in a 7% loss in body weight compared to 3% in traditional diets.

In attempting to understand the role of *rs9939609* DNA variant in weight and metabolic homeostasis, we decided to analyze the effects of *rs9939609* genetic variant of *FTO* gene on metabolic parameters and weight loss secondary to pMRHDs in obese subjects.

Patients and Methods

Patients

We attempted to obtain written informed consent from each eligible patients from January 2018 to September 2019. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the Hospital Clinic University Ethics Committee approved all procedures. A sample of 44 obese subjects was recruited in a prospective way with a consecutive method of sampling among subjects send to our Nutrition Unit from Primary Care. Inclusion criteria were body mass index (BMI) greater than 30 kg/m^2 and age between 30 and 70 years. Exclusion criteria were the following: history of thyroid disease, heart attack, ictus, severe renal or hepatic disorders, diabetes mellitus, active alcoholism, malignant tumor. And finally as exclusion criteria the following were used to receive within three months prior to study any medication known that affects lipid blood levels (hormonal therapy, glucocorticoids and anti-inflammatory drugs) and/or drugs related with diabetic treatment (metformin, sulfonylureas, thiazolidinedione, insulin, GLP-1 receptor antagonists, S-GLT2 or DPP-IV inhibitors).

Procedures

After the inclusion of the patient in the study, all subjects were advised on ways to change their habitual diet to a partial meal-replacement hypocaloric diet (pMRHD) with two bricks of normocaloric-hyperproteic formula (Table I) during 12 weeks. This dietary intervention was structured in six meals (breakfast, morning snack, lunch, afternoon snack, dinner, after dinner snack). The lunch and dinner were replaced by a normocaloric-hyperproteic formula (VEGEStart Complete®, Extremadura, Spain) (Table I). A dietitian assessed the adherence of these diets each 7 days with a phone call in order to improve compliment of the calorie restriction and macronutrient distribution. The records were reviewed by a dietitian and analysed by the program (Dietsource®, Nestlé, Geneve, Switzerland). National composition food tables were used as reference[19].

Table I. Dietary composition of five intakes diet (three intakes as natural food and two intakes as artificial formula) as indicated in Materials and Methods section.

Parameters	Diet + formula	Normocaloric hyperproteic formula (200 ml)
Caloric value (kcal)	1035	200
Proteins g (% TCV)	64.4 (25%)	15.4 (31%)
Lipids g (% TCV)	19.1 (17%)	5.2 (23%)
Carbohydrates g (% TCV)	151.6 (59%)	21 (42%)
Fiber (g)	15.9	4.2

Normocaloric hyperproteic formula is Vegestart® (% TCV: ,Total Caloric Value Percentage).

The exercise program consisted of an aerobic exercise at least 3 times per week (60 min each). Anthropometric parameters (weight, body mass index, waist circumference and fat mass) and biochemical parameters (fasting glucose, insulin, HOMA-IR, lipid profile and adipokines – leptin and adiponectin –) were carried out before the start of the dietary intervention and at 12 weeks after the beginning of intervention. Genotype of *rs9939609* FTO gene polymorphism was evaluated.

Anthropometric Measurements and Blood Pressure Determination

Body weight was measured to an accuracy of 0.1 kg and body mass index computed as body weight/(height^2). Waist circumference (WC) was measured in the narrowest diameter between xiphoid process and iliac crest Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g[20]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Blood pressure was measured twice after a 10 minutes rest with a simple mercury sphygmomanometer, and averaged (Omrom, Los Angeles, CA, USA).

Biochemical Assays

Lipid profile (total cholesterol and triglyceride levels) were measured by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), while HDL cholesterol was analysed enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula[21].

Plasma glucose levels were analysed using a 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 0.05 mUI/L (normal range 0.5-30 mUI/L)[22]. Homeostasis model assessment for insulin resistance (HOMA-IR) was obtained using these values with this equation: (fasting plasma insulin (mU/L)*glucose (mmol/L)/22.5)[23]. Adipokines were measured by enzyme-linked immunosorbent assay (ELISA), leptin (Diagnostic Systems Laboratories, Inc., Texas, TX, USA) with a sensitivity of 0.05 ng/ml and a normal range of 11-100 ng/ml[24] and adiponectin (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.24 ng/ml and a normal range of 8.63-21.42 ng/ml[25].

Genotyping of rs9939609 FTO Gene Polymorphism

Genotyping (rs7756992) was realized by using customized assays with the TaqMan® OpenArray™ Genotyping platform (Thermo Fisher Scientific, Pittsburgh, PA, USA). Samples were loaded using the AccuFill system, and amplification performed on the QuantStudio 12K Flex Real-Time qPCR instrument (Thermo Fisher Scientific, Waltham, MA, USA). A total volume of 7.5 µl with 2.5 µl TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, CA, USA) and 2.5 µl human DNA sample were loaded and amplified on arrays following the manufacturer's instructions. Genotype calling and sample clustering for Open Array assays were performed in TaqMan Genotyper (Life Technologies, Carlsbad, CA, USA). The variant of *FTO* gene was in Hardy Weinberg equilibrium (p=0.29).

Statistical Analysis

Data were analyzed with the SPSS/win program (version 19.0, SPSS IBM, Armonk, NY, USA). Sample size was calculated to detect differences over 3 kg in body weight with 90% power and 5% significance (n=40). Each variable was examined for normality with the Kolmogorov-Smirnov test. In within-groups, we conducted paired *t*-tests for biochemical parameters at baseline and after dietary intervention. In between-groups, independent *t*-test was used to compare the differences in both times. Non-parametric variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. The statistical analysis to evaluate the interaction between the gene and the intervention was performed using covariance analysis (ANCOVA) modeling the dependent variable with the starting values. Tables II, IV and V are indicated with *p*-values (first *p*, significance of dietary intervention after 12 weeks in TT genotype, second *p*, significance between TT genotypes *vs.* TA + AA baseline values, third *p*, significance of dietary intervention after 12 weeks in TA + AA genotype, fourth *p*, significance between TT genotypes *vs.* TA + AA post-treatment values).

The significance of interaction between clinical/biochemical characteristics and SNPs was assessed using a two-way ANOVA with Bonfer-

Table II. Blood pressure and anthropometric parameters (mean ± SD).

Parameters	TT (n = 16)		TA+AA (n = 28)		*p.*-Time TT - Basal Genotype - Time TA+AA - 12 weeks genotype
	Basal	12 weeks	Basal	12 weeks	
BMI	36.6 ± 2.2	33.6 ± 2.0*	36.8 ± 2.9	33.7 ± 1.8*	$p = 0.005$ $p = 0.35$ $p = 0.005$ $p = 0.35$
Weight (kg)	96.1 ± 6.5	88.3 ± 9.0$^{\$}$	96.4 ± 9.0	87.9 ± 9.1$^{\$}$	$p = 0.002$ $p = 0.33$ $p = 0.003$ $p = 0.39$
Fat mass (kg)	40.8 ± 5.1	34.4 ± 4.0$^{\#}$	41.1 ± 5.0	34.5 ± 4.0$^{\#}$	$p = 0.006$ $p = 0.34$ $p = 0.006$ $p = 0.51$
WC (cm)	113.2 ± 7.1	105.6 ± 6.0$^{\&}$	116.0 ± 7.0	107.3 ± 5.0$^{\&}$	$p = 0.02$ $p = 0.19$ $p = 0.01$ $p = 0.48$
SBP (mmHg)	138.4 ± 7.0	124.7 + 5.1**	135.5 + 9.0	121.6 + 5.0**	p =0.01 $p = 0.22$ $p = 0.02$ $p = 0.35$
DBP (mmHg)	77.7 ± 6.0	76.9 ± 3.2	79.4 ± 4.1	76.4 ± 2.3	$p = 0.12$ $p = 0.51$ $p = 0.09$ $p = 0.23$

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, ***DBP) No statistical differences between genotype groups. first p, significance of dietary intervention after 12 weeks in TT genotype, second *p*, significance between TT genotypes *vs*. TA + AA baseline values, third p, significance of dietary intervention after 12 weeks in TA + AA genotype, fourth *p*, significance between TT genotypes *vs*. TA + AA post-treatment values).

roni test Post Hoc. A Chi square test was used to evaluate categorical parameters. The statistical analysis was performed for the combined *TA* and *AA* as a group (mutant group) and wild type TT as second group, with a dominant model. A *p*-value <0.05 was considered significant.

Results

We recruited 44 subjects with the following allelic distribution (16 TT (36.4%), 17 TA (38.6%) and 11 AA (25.0%)). All patients completed the 12 weeks follow-up period without dropouts and no adverse events related with dietary intervention were reported. The mean age of the all group was 60.9±5.1 years (range: 35-70 years) and the mean BMI 36.7±2.4 kg/m^2 (range: 34.3- 39.1 kg/m^2). Age was similar in both groups (non-A allele carriers (TT) *vs*. A allele carriers (TA+AA)) (61.3±6.0 years *vs*. 60.6±5.9 years: ns). In the total group, gender distribution was 34 females (77.3%) and 10 males (22.7%). Gender distribution was similar in both genotype groups, males (18.5% *vs*. 24.6%) and females (81.5% *vs*. 75.4%). Baseline characteristics of both genotype groups were similar (Table III).

Basal evaluation of dietary intakes (previously obese subjects received a pMRHD) with a 3 days written food record showed the following data; total calorie intake of 1812.9±471.80 cal/day, carbohydrate intake of 162.2±50.9 g/day (40.9% of calories), fat intake of 65.0±18.8 g/day (37.0% of calories), protein intake of 74.8±14.1 g/day (23.1% of calories) and dietary fibre intake 14.1±6.5 g/day. After the 12 weeks of pMRHD, these subjects reached the recommendations: 1032.1 calories per day, 142.1±38.0 g/day of carbohydrates (64.0% of calories), 27.1±15.2 g/day of lipids (23.3% of calories), 62.0±9.0 g/day of proteins (23.7%. of calories) and 18.2±2.4 g/day of dietary fibre. The quality of fats was the next; 32.4%

Table III. Baseline characteristics of the population by genotype.

Characteristics	TT (n = 16)	TA+AA (n = 28)	
Age	61.3 ± 6.0	60.6 ± 5.9	$p = 0.34$
Gender (Mae/female)	3/13	7/21	$p = 0.45$
BMI	36.6 ± 2.2	36.8 ± 2.9	p = 0.35
Weight (kg)	96.1 ± 6.5	96.4 ± 9.0	$p = 0.43$
Fat mass (kg)	40.8 ± 5.1	41.1 ± 5.0	$p = 0.36$
WC (cm)	113.2 ± 7.1	116.0 ± 7.0	$p = 0.31$
SBP (mmHg)	138.4+7.0	135.5 + 9.0	$p = 0.35$
DBP (mmHg)	77.7 + 6.0	79.4 + 4.1	$p = 0.23$
Fasting Glucose (mg/dl)	98.5 + 5.1	98.1 + 4.0	$p = 0.34$
Total cholesterol (mg/dl)	186.9 + 12.7	195.4 + 7.0	$p = 0.42$
LDL-cholesterol (mg/dl)	105.1 + 7.2	120.3 + 8.0	$p = 0.45$
HDL-cholesterol (mg/dl)	59.9 + 4.1	57.3 +5 .0	$p = 0.63$
Triglycerides (mg/dl)	111.9 + 27.1	117.4 + 9.2	$p = 0.30$
Insulin (mUI/l)	13.4 + 5.1	17.2 + 4.1	$p = 0.39$
HOMA-IR	3.3 + 2.0	4.3 +2 .0	$p = 0.45$

Table IV. Blood pressure and anthropometric parameters (mean ± SD).

Parameters	TT (n = 16)		TA+AA (n = 28)		*p.*-Time TT - Basal Genotype - Time TA+AA - 12 weeks genotype
	Basal	12 weeks	Basal	12 weeks	
Fasting Glucose (mg/dl)	98.5 ± 5.1	98.4 ± 3.1	98.1 ± 4.0	96.9 ± 3.1	$p = 0.12$ $p = 0.41$ $p = 0.19$ $p = 0.60$
Total cholesterol (mg/dl)	186.9 ± 12.7	183.1 ± 9.2	195.4 ± 7.0	182.8 ± 6.1*	$p = 0.21$ $p = 0.39$ $p = 0.03$ $p = 0.19$
LDL-cholesterol (mg/dl)	105.1 ± 7.2	104.2 ± 4.9^{+}	120.3 ± 8.0	109.8 ± 7.0^{+}	$p = 0.18$ $p = 0.35$ $p = 0.01$ $p = 0.13$
HDL-cholesterol (mg/dl)	59.9 ± 4.1	57.9 ± 3.8	57.3 ± 5.0	54.9 ± 4.1	$p = 0.44$ $p = 0.71$ $p = 0.21$ $p = 0.51$
Triglycerides) (mg/dl	111.9 ± 27.1	99.6 ± 12.2	117.4 ± 9.2	114.8 ± 8.2	$p = 0.28$ $p = 0.60$ $p = 0.13$ $p = 0.32$
Insulin (mUI/l)	13.4 ± 5.1	11.4 ± .2	17.2 ± 4.1	13.4 ± 3.2$^{\&}$	$p = 0.11$ $p = 0.33$ $p = 0.01$ $p = 0.11$
HOMA-IR	3.3 ± 2.0	2.7 ± 1.1	4.3 ± 2.0	3.3 ± 1.8**	$p = 0.13$ $p = 0.28$ $p = 0.01$ $p = 0.16$

HOMA-IR (homeostasis model assessment). Statistical differences $p < 0.05$, in each genotype group (glucose$^{\#}$, insulin$^{\&}$, total cholesterol*, LDL Cholesterol+, HOMA IR **) % statistical differences between genotype groups in fasting glucose levels No statistical differences between genotype groups first p, significance of dietary intervention after 12 weeks in TT genotype, second *p*, significance between TT genotypes *vs*. TA + AA baseline values, third p, significance of dietary intervention after 12 weeks in TA + AA genotype, fourth p, significance between TT genotypes vs. TA + AA post-treatment values).

of saturated fats, a 50.5% of monounsaturated fats and a 17.1% of polyunsaturated fats. Time of physical exercise was similar in both allele groups (128.0±14.3 min/week *vs.* 121.2±12.2 min/week: *p*=0.28).

As showed in Table II, the baseline values and after the intervention including the changes were similar in both genotypes in blood pressure and adiposity parameters. After the pMRHD, body weight, body mass index (BMI), fat mass, waist circumference and systolic blood pressure improved in both genotypes. The percentage of weight reduction at 12 weeks was 7.8 (5.8-9.1) % in non A allele carriers and a similar weight loss in A allele carriers 8.7 (6.2-10.1) %. The changes of body weight (-7.8±2.1 kg *vs*. -8.4±1.8 kg: *p*=0.23), BMI (-3.0±0.6 kg/m^2 *vs.* -3.1±0.7 kg/m^2: *p*=0.32), fat mass (-6.4±1.3 kg *vs*.-6.6±1.2 kg: *p*=0.30) and waist circumference (-7.6±1.3 cm *vs*. -7.7±1.2 cm: *p*=0.41) were similar in both genotype groups. The improvement in systolic blood pressure was similar in both genotypes (Table III).

Table IV showed changes on serum biochemical parameters. Baseline biochemical values were similar in both genotypes. After dietary intervention with pMRHD, subjects with A allele showed a significant improvement in total cholesterol levels (TT *vs.* TA+AA) (-3.8±1.4 md/dL *vs.* -12.6±1.7 mg/dl: *p*=0.01), LDL-cholesterol (-0.2±1.5 md/dL *vs.* -10.5±1.9 mg/dl: *p*=0.02), insulin levels (-1.9±0.2 mU/L *vs.* -3.8±0.3 mU/L: *p*=0.02) and HOMA-IR (-0.6±0.2 units *vs.* -1.1±0.1 units: *p*=0.01).

Table V showed changes on serum adipokine levels. After dietary intervention, subjects of both genotypes showed a significant improvement in total leptin levels without statistical differences with A allele (TT *vs.* TA+AA) (-35.3±10.4 ng/dL *vs*. -24.6±14.7 mg/dl: *p*=0.18).

Discussion

Our results showed an association between the *FTO* variant *rs9939609* and improvement in lipid profile and insulin resistance after a partial meal replacement hypocaloric diets (pMRHDs).

The results of cross-sectional studies with this polymorphism of *FTO* gene have shown unclear data. Some cross-sectional studies confirmed the association between dietary intakes and *rs9939609* genetic variant with metabolic or adiposity parameters[26,27], and other studies[14,15] showed interesting data with interventional designs. For example, Razquin et al[14] reported that, after 36 months of nutritional intervention with a Mediterranean-style-diet, A-allele carriers had lower body weight gain than non-A allele carriers. In another interventional design[15], carriers of the A allele had a greater reduction in weight in response to 24 months high-protein diet, whereas an opposite genetic effect was observed on changes in fat distribution in response to a low-protein diet. In a short-term study[16] during 12 weeks with a low-fat hypocaloric diet showed a better metabolic improvement secondary to weight loss in A carriers, as our results. However, the effects on metabolic parameters and even weight loss are different among these previously mentioned studies.

In another study with an intervention of 3 months[28], after a more moderate caloric restric-

Table V. Adipokine levels (mean+SD).

Parameters	TT (n = 16)		TA+AA (n = 28)		*p*.-Time TT - Basal Genotype - Time TA+AA - 12 weeks genotype
	Basal	12 weeks	Basal	12 weeks	
Adiponectin (ng/dl)	11.8 ± 4.1	15.0 ± 3.0	11.0 ± 4.0	12.9 ± 3.1	*p* = 0.19 *p* = 0.44 *p* = 0.18 *p* = 0.56
Leptin (ng/dl)	73.6 ± 17.7	38.3 ± 8.2*	73.8 ± 16.0	40.8 ± 6.0*	*p* = 0.01 *p* = 0.19 *p* = 0.03 *p* = 0.13

First *p*, significance of dietary intervention after 12 weeks in TT genotype, second p, significance between TT genotypes *vs*. TA + AA baseline values, third *p*, significance of dietary intervention after 12 weeks in TA + AA genotype, fourth *p*, significance between TT genotypes *vs*. TA + AA post-treatment values).

tion than that used in our current study (only a target of 1500 cal/day), similar results were obtained with an improvement of HOMA-IR and insulin levels in the carriers of the A allele. However, in another study with a longer intervention (9 months) than our design[29], the improvement in insulin and HOMA-IR levels was similarly achieved in both genotypes with better lipid response in A allele carriers than non-carriers. Moreover, the distribution of macronutrients was different from that of our current study, with an intervention branch with 33% of the caloric intake from proteins, 33% carbohydrates and 34% lipids and another intervention branch with 20% of proteins, 53% carbohydrates and 27% lipids. Both branches of intervention move away from those achieved in our current study (23% protein, 64% carbohydrates and 23% lipids). Undoubtedly, the distribution of macronutrients could interact with the genotype of the obese patient and obtain different responses depending on the presence or not of the A allele and the percentage of nutrients in the diet. For example, Daya et al[30] have demonstrated that A allele carriers had higher preferences for high dietary fat intake than those with A allele.

Regarding weight loss, in our work we did not find differences in response depending on the genotype. This lack of association is in line with a recent meta-analysis[31]. However, there are also works that have demonstrated a different response in adiposity parameters, with a smaller decrease in the waist circumference in patients with allele A[32], however this is a work in children. These contradictory data had already been demonstrated in previous studies[14,15].

In our study, the improvement of LDL-cholesterol, insulin levels and HOMA index was statistically significant only in A allele carriers with the dietary intervention (pMRHDs). This better metabolic result in A allele carriers with diet intervention could be secondary to an interaction of the macronutrient distribution of the diet with the A variant of this *FTO* SNP or could be due other genetic interactions. First, an interaction between rs*9939609* and insulin sensitivity has been described, too[33].

Andreasen et al[33] found that insulin sensitivity was significantly decreased in homozygous carriers (AA) and that the impact of the *rs9939609* DNA variant on BMI levels was highly influenced by insulin sensitivity. Second, the presence of other types of polymorphisms in *FTO* gene could be explained by these results. For example, Dlouha et al[27] demonstrated that in overweight healthy females, *FTO* gene variants (*rs17817449*) and (*rs17818902*) have been related with potential interactions between diet intervention and metabolic response. Recently, Younus et al[34] demonstrated that the FTO gene polymorphisms *rs9939609* and rs17817449 play a role in the development of insulin resistance and hence occurrence of type 2 DM in obese patients.

The limitations of our study included, firstly, that we analysed only one SNP of the *FTO* gene, so other genetic variants in this gene or others could be implied with our outcomes. Second, the absence of a control group without dietary intervention might be a bias. Third, the self-reported dietary intake with a questionnaire is not reliable and it might include bias of under – or over – reporting. The major limitation of our study is the lack of a control group with different (or no intervention), that would have allowed for a proper test of the gene (SNP) by environment interaction. The strength of our study was its design as an interventional trial with high adherence to a partial-meal replacement.

Conclusions

In summary, our data suggest that the genetic variant (*rs9939609*) of *FTO* gene showed better improvement of LDL-cholesterol, insulin and HOMA-IR in subjects with A allele. The novelty of our study is that it is the first in the literature to evaluate a pMRHDs diet and the effect of this genetic variant, and the pMRHDs diet strategy is increasingly used to treat obese patients. The exact molecular mechanisms of this genetic variant of *FTO* gene changing the lipid and glucose metabolism are still unclear and need to be clarified in further studies to evaluate the role in diabetes mellitus[34] and with different designs using a no intervention branch.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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 3/2017) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding Sources
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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

Authors' Contribution
Daniel Antonio de Luis designed the study an wrote the article; Olatz Izaola realized nutritional evaluation; R Aller realized laboratory analysis; JJ Lopez Gomez realized statistical study and realized statistical study; D Primo realized nutritional evaluation.

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