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Independent and combined influence of the *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms on hypocaloric diet induced changes in body mass and composition and energy metabolism in non-morbid obese premenopausal women

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Abstract

Purpose: **To examine the independent and combined influence of the *FTO*rs9939609 and the *MC4R*rs17782313 polymorphisms on changes in fat mass (FM), resting energy expenditure (REE), leptin, and thyrotropin (TSH) levels, after a 12-week energy-restricted diet intervention in non-morbid premenopausal obese women.**

Methods: **Fat mass (dual X-ray absorptiometry), REE (indirect calorimetry) and plasma leptin and thyrotropin levels were measured (before and after the intervention) in 77 obese (BMI: 33.9±2.8kg/m^2) women (age: 36.8±7.0y).**

Results*:* There were no significant differences across *FTO*rs9939609 genotype groups (TT vs. A allele carriers, Ps>0.1) on changes in body mass (-8.6±3.2% vs. -8.7±3.3 %), FM (12.8±4.7% vs. –12.9±6.3%), REE (-11.3±4.7 vs. -9.4±8.1%), leptin (-34.1±25.1% vs. -43.5±24.1%) or TSH (5.2±34.5% vs. -1.7±27.1%) levels. Moreover, it was not observed any significant difference on changes in body mass (-8.6±3.6% vs. -8.9±2.6%), FM (-12.7±6.1% vs. -13.4±5.3%), REE (-9.8±7.4% -9.4±9.4%), leptin (-39.0±26.9% vs. -44.8±18.4%) or TSH (-1.0±30.0% vs. 1.5±26.5%) levels between non-C allele carriers and C allele carriers of the *MC4R*rs17782313 (Ps>0.3). Finally, there were no significant difference on changes in body mass and composition, REE, leptin or TSH levels

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INFLUENCIA INDIVIDUAL Y COMBINADA DE LOS POLIMORFISMOS GENÉTICOS *FTO* RS9939609 Y *MC4R* RS17782313 SOBRE LOS CAMBIOS EN LA MASA Y COMPOSICIÓN CORPORAL Y EL METABOLISMO ENERGÉTICO INDUCIDOS POR UN TRATAMIENTO CON DIETA HIPOCALÓRICA EN MUJERES PRE-MENOPÁUSICAS CON OBESIDAD NO MÓRBIDA

Resumen

Objetivo: **Examinar la influencia individual y combinada de los polimorfismos genéticos *FTO* rs9939609 y *MC4R* rs17782313 en los cambios en la masa grasa (MG), gasto energético en reposo (GER), leptina y tirotropina (TSH) tras una intervención de 12 semanas de duración con dieta hipocalórica en mujeres pre-menopáusicas con obesidad no mórbida.**

Métodos: **Se evaluaron al inicio y al final de la intervención la MG (absorciometría dual de rayos X), el GER (calorimetría indirecta) y los niveles de leptina y TSH en sangre en 77 mujeres (edad: 36.8±7.0 años) obesas (IMC: 33.9±2.8kg/m^2).**

Resultados: **No se observaron diferencias estadísticamente significativas (Ps>0.1) entre las portadoras y las no portadoras del alelo A del *FTO*rs9939609 (TT vs. portadores del alelo) en los cambios en la masa corporal (-8.6±3.2% vs. -8.7±3.3 %), MG (12.8±4.7% vs. –12.9±6.3%), GER (-11.3±4.7 vs. -9.4±8.1%), leptina (-34.1±25.1% vs. -43.5±24.1%) y TSH (5.2±34.5% vs. -1.7±27.1%). Tampoco se observaron diferencias estadísticamente significativas en los cambios en la masa corporal (-8.6±3.6% vs. -8.9±2.6%), MG (-12.7±6.1% vs. -13.4±5.3%), GER (-9.8±7.4% -9.4±9.4%), leptina (-39.0±26.9% vs. -44.8±18.4%) y TSH (-1.0±30.0% vs. 1.5±26.5%) entre las participantes portadoras y no portadoras del alelo C del *MC4R*rs17782313 (Ps>0.3). Finalmente, no se encontraron diferencias estadísticamente significativas en los cambios en la masa y composición corporal, el GER, o los niveles de leptina y TSH entre**

among non-risk allele carriers, carriers of the C allele risk of the *MC4R*rs17782313, carriers of the A allele of the *FTO*rs9939609 and carriers of both risk alleles after the 12-week energy-restricted diet intervention (Ps>0.1).

Conclusion: **Carrying the A risk allele of the *FTO*rs9939609 and/or the C risk allele of the *MC4R*rs17782313 did not influence body mass and FM loss, or REE decrease in obese women after a 12-week energy-restricted diet intervention.**

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mujeres no portadoras de alelos de riesgo, portadoras del alelo C del *MC4R*rs17782313, portadoras del alelo A del *FTO*rs9939609 y portadoras de los dos alelos de riesgo (A y C) al final de las 12 semanas de intervención con dieta hipocalórica (Ps>0.1).

Conclusión: **Ser portador del alelo de riesgo A del *FTO*rs9939609 y/o del alelo de riesgo C del *MC4R*rs17782313 no influye en la pérdida de masa grasa o en el descenso del GER en mujeres obesas tras 12 semanas de intervención con dieta hipocalórica.**

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Palabras clave: FTO. MC4R. *SNP. Obesidad. Gasto energético en reposo. Pérdida de peso. Dieta.*

Introduction

The literature shows that successfully maintaining body mass loss achieved through low caloric diet treatment is very low in obese individuals. However, there are considerable inter-individual differences in body mass and regain that may be partially explained by genetic predisposition[1,2].

Evidence exists that energy-restricted diet interventions lead to reductions in resting energy expenditure (REE) beyond that explained by body mass, lean mass (LM) and fat mass (FM) losses. This phenomenon has been described as ''metabolic adaptation''[3,4]. Metabolic adaptation occurs when the body countervails energy restriction by decreasing REE[5]. This decrease in REE could account in part for the common cessation of body mass loss observed after 12–20 weeks of energy restriction[6].

Genome-wide association studies have identified 52 genetic loci unequivocally associated with obesity-related traits[7]. Among them, genetic variants influencing energy balance control are strong candidates to explain the resistance for body mass loss and maintenance. Single nucleotide polymorphisms (SNPs) in the fat mass and obesity associated (*FTO*) and the melanocortin-4 receptor (*MC4R*) genes have been associated with lower energy expenditure in overweight and obese children[8] and adults[9-11]. Therefore, it is likely that obese individuals carrying risk alleles of *FTO* and *MC4R* polymorphisms might have lower body mass loss when participating in a low caloric diet intervention. However, most of the studies focused on the influence of SNPs on body mass, instead of changes in body mass. In addition, studies examining the influence of *FTO* and *MC4R* polymorphisms on changes in REE are lacking.

The most robust associations with adiposity in populations of European descents have been observed for *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms. In a recently published report[10], we observed that the A allele of the *FTO* rs9939609 was associated with lower REE. In contrast, the *MC4R rs17782313* polymorphism did not significantly influence on REE; however, we observed that it might exert an additive effect together with the *FTO* rs9939609 polymorphism lowering REE. We also found that women carrying the A allele of the *FTO* rs9939609 polymorphism had higher levels of leptin and thyrotropin (TSH) that, in turn, were associated with higher resistance to mass loss[12]. Given that obese women carrying the A allele of the *FTO* rs9939609 had lower REE, as well as higher leptin and TSH levels, we hypothesized that they might achieve lower diet induced body mass and FM loss and maintenance than non-A allele carriers, and that the response to the treatment might be worse in women carrying both the *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms.

Objectives

The present study aimed to examine the independent and combined influence of the *FTO* rs9939609 and the *MC4R* rs17782313 polymorphisms on body mass and body composition changes, after a 12-week energy-restricted diet period in a well characterized sample of Spanish obese women. In addition, we explored the influence of these polymorphisms on REE, leptin, and TSH levels after the low caloric diet intervention.

Methods

Design and participants

Design, inclusion and exclusion criteria have been published elsewhere[13-16]. Briefly, eligible participants had to be non-morbid obese ((body mass index (BMI): 30-39.9 kg/m^2), premenopausic and sedentary women (≤20 minutes on <3 days/week) and showed body mass stability over the last 3 months (body mass changes <3kg). Exclusion criteria included history of cardiovascular disease or diabetes, pregnancy, total cholesterol levels >300 mg/dL, triglycerides >300 mg/dL and blood pressure >140/90 mmHg. Women

under medication (except oral contraceptives) for hypertension, hyperlipidemia, hiperuricemia or other illness were not included in the study. The present study was designed as a 12-week controlled body mass loss intervention. All the study examinations were performed before and after the weight loss program in the Clinical Trials Unit of TECNALIA (Vitoria-Gasteiz). Body mass reduction was induced by a low energy mixed (55% carbohydrates, 30% lipids and 15% proteins) diet providing 600 kcal less than individually estimated energy requirements based on measured REE and multiplied by a factor of 1.3, as corresponds to a low physical activity level. Energy content and macronutrient composition of diets were according to the American Diabetes Association nutrition recommendations[17,18]. Diets were designed to achieve mass losses of 0.5 to 1 kg per week, and are considered as a low risk intervention[18,19]. To optimize compliance, dietary instructions were reinforced weekly by a dietician.

A total of 83 obese women, aged between 19 and 49 years volunteered to participate in this study underwent a comprehensive medical examination. Seventy-eight women completed the 12-week diet intervention program (four participants left the study due to inability to follow the research protocol and one due to pregnancy). Only data from women who finished the 12-week diet intervention program (N=78) and whose *FTO* and *MC4R* data were available (N=1 missing data) were included in this study.

This study was in accordance with the Helsinki II Declaration and was approved by the Ethical Committee in Hospital of Txagorritxu (Vitoria-Gasteiz, Spain). All women received verbal and written information about the nature and purpose of the study, and all of them gave written consent for participation in the study.

Body composition

Body mass (±10g) was measured after voiding, and using a digital integrating scale (SECA 760). Height was measured to the nearest 5 mm using a stadiometer (SECA 220) at the start of the study. Body mass index (BMI) was calculated as body mass (kg)/height (m^2). Fat mass (FM) and bone free lean tissue mass (LM) was measured with Dual Energy X-ray Absorciometry scanner 140 (HOLOGIC, QDR 4500W, v12.4).

Indirect calorimetry

Respiratory exchange measurements were done by indirect calorimetry (Vmax, Sensormedics, Germany) to estimate fasting resting energy expenditure (REE) and non protein respiratory quotient (NPRQ), following the recommended measurements conditions[20] and as described elsewhere[21]. REE was expressed as kJ/ day. Fasting urine was collected (between the ~09-10 p.m. of the day before and the 08-09 a.m. of the examination day) to determine nitrogen output, and non-protein respiratory quotient was thereafter calculated[22].

Biochemical variables

Fasting (≥12-h overnight fast) blood samples were taken from an antecubital vein after gas exchange measurement. Samples were processed after collection and stored at -80ºC for later analysis. Serum levels of leptin (ng/mL) was measured by ELISA kits (EZHL-80SK, LINCO Research, Missouri, USA) and TSH by immunoradiometric assay (Immunotech, Beckman Coulter). All the samples were prepared according to the manufacturer's recommendation and were measured in duplicate and the mean scored.

Genotyping

Genomic DNA was isolated from the buffy coat of centrifuged whole blood using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. Genotyping was carried out using Taqman probes and Applied Biosystems 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Genotyping success rate was 100% and no discordant genotypes were observed in duplicate samples.

Statistical analysis

Values are shown as means and standard deviation. Analyses were performed using the SPSS, *v.* 21.0 (SPSS Inc, Chicago). Variables with skewed distribution were logarithmically transformed to obtain a more symmetrical distribution.

Pre vs. post body mass loss program changes in the study measures were assessed using paired *t* tests. Percentage of changes of the outcome variables after the body mass loss program was calculated as: Δ (%): [(Post-intervention – Pre-intervention)/Pre-intervention] x 100. We used ANCOVA to analyze differences in changes on body mass and composition, energy and substrate metabolism (REE and NPRQ), and biochemical variables (i.e., leptin and TSH levels) after the 12-week energy-restricted diet intervention across *FTO* rs9939609 (TT vs. A-carriers) and *MC4R* rs17782313 (TT vs. C-carriers) genotype groups adjusting for age. All the analyses were repeated after further adjustment for body mass loss. To test for the existence of an interaction effect between the two polymorphisms ANCOVA was also used. When significant interaction effects were found (P<0.1), the combined influence of both polymorphisms was examined.

For this purpose, women were categorized as non-risk allele carriers, carriers of the C allele risk of the *MC4R* rs17782313 polymorphism, carriers of the A allele of the *FTO* rs9939609 polymorphism and carriers of both risk alleles, i.e. women carrying the A allele of the *FTO* rs9939609 and the C allele of the *MC4R* rs17782313 polymorphisms.

Results

Genotype frequencies of the *FTO* rs9939609 were 22 (28.6%), 43 (55.8%) and 12 (15.6%) for the TT, TA and AA genotypes, respectively; and 53 (68.8%), 21 (27.3%) and 3 (3.9%) for the TT, TC and CC genotypes, respectively, of the *MC4R* rs17782313. Genotype distributions of the two polymorphisms did not deviate from Hardy-Weinberg expectations (Ps>0.2).

Independent influence of the FTO *rs9939609 and the* MC4R *rs17782313*

Though post-intervention body mass, FM, LM and waist circumference reduction occurred, there were no significant differences across genotype groups of the *FTO* rs9939609 (Table I) and *MC4R* rs17782313 (Table II) polymorphisms.

REE was significantly reduced after the intervention (Ps<0.001) in all the genotype groups, while we did not find significant changes in NPRQ (Table I and Table II). However, we did not find any significant difference on changes in energy and substrate metabolism between A allele carriers and non-carriers of the *FTO* rs9939609 (Table I) and between C allele carriers and non-carriers of the *MC4R* rs17782313 (Table II).

The results also showed that there were no statistically significant differences in diet effects on leptin

Table I
FTO *rs9939609 polymorphism and body mass and composition, energy and substrate metabolism, leptin and thyrotropin changes after a 12-week energy-restricted diet intervention on obese Spanish women (N=77)*

	TT (n=22)	*A carriers (n=55)*					*P dominant (TT vs. AT+AA)*
	Mean (SD)	*Change percent (SD)*	*P pre-post*	*Mean (SD)*	*Change percent (SD)*	*P pre-post*	
Body mass (kg)							
Pre	91.8 (11.1)			87.0 (9. 6)			
Post	83.9 (10.9)	-8.6 (3.2)	<0.001	79.0 (9.6)	-8.7 (3.3)	<0.001	0.924
Lean mass (kg)							
Pre	51.6 (6.0)			47.0 (4.5)			
Post	48.8 (5.9)	-5.4 (3.0)	<0.001	44.5 (4.3)	-5.3 (2.8)	<0.001	0.981
Body fat (%)							
Pre	40.8 (4.0)			42.9 (3.6)			
Post	38.8 (4.1)	-5.0 (2.9)	<0.001	40.8 (4.0)	-5.0 (2.9)	<0.001	0.987
Fat mass (kg)							
Pre	37.4 (6.6)			37.4 (6.2)			
Post	32.7 (6.3)	-12.8 (4.7)	<0.001	32.6 (6.3)	-12.9 (6.3)	<0.001	0.929
Waist (cm)							
Pre	107.8 (1.4)			104.0 (6.8)			
Post	104.0 (1.3)	-3.4 (3.8)	<0.001	99.8 (5.9)	-3.9 (3.5)	<0.001	0.575
Leptin (ng/mL)							
Pre	42.0 (14.4)			50.2 (17.8)			
Post	27.4 (13.1)	-34.1 (25.1)	<0.001	28.5 (15.6)	-43.5 (24.1)	<0.001	0.123
TSH (μU/mL)*							
Pre	1.55 (0.51)			1.96 (0.79)			
Post	1.56 (0.54)	5.2 (34.1)	0.571	1.89 (0.99)	-1.7 (27.1)	0.162	0.398
REE (kJ/day)							
Pre	6985 (858)			6370 (613)			
Post	6192 (601)	-11.3 (4.7)	<0.001	5769 (553)	-9.4 (8.1)	<0.001	0.312
NPRQ							
Pre	0.76 (0.04)			0.77 (0.05)			
Post	0.78 (0.05)	-1.7 (5.0)	0.081	0.77 (0.06)	0.1 (5.7)	0.904	0.123

BMI: body mass index; REE: resting energy expenditure; NPRQ: non protein respiratory quotient; TSH: Thyrotropin.
% Change calculated as: [(Post-intervention-Pre)/Pre] x 100. Pre: before diet intervention; Post: after diet intervention.

Table II
MC4R *rs17782313 polymorphism and body mass and composition, energy and substrate metabolism, leptin and thyrotropin changes after a 12-week energy-restricted diet intervention (Post) on obese Spanish women (N=77)*

	TT (n=53)			*C carriers (n=24)*			*P dominant (TT vs. TC+CC)*
	Mean (SD)	*Change percent (SD)*	*P pre-Post*	*Mean (SD)*	*Change percent (SD)*	*P pre-Post*	
Body mass (kg)							
Pre	89.3 (10.3)			86.5 (10.1)			
Post	81.3 (10.3)	-8.6 (3.6)	<0.001	78.4 (9.8)	-8.9 (2.6)	<0.001	0.698
Lean mass (kg)							
Pre	48.7 (5.0)			47.5 (6.1)			
Post	46.1 (4.8)	-5.3 (2.9)	<0.001	45.0 (6.0)	-5.3 (2.7)	<0.001	0.941
Body fat (%)							
Pre	42.4 (4.1)			42.1 (3.3)			
Post	40.4 (4.4)	-4.8 (3.8)	<0.001	39.9 (3.3)	-5.3 (3.8)	<0.001	0.633
Fat mass (kg)							
Pre	37.9 (6.7)			36.2 (5.1)			
Post	33.2 (6.7)	-12.7 (6.1)	<0.001	31.4 (5.0)	-13.4 (5.3)	<0.001	0.593
Waist (cm)							
Pre	105.8 (7.0)			103.6 (6.4)			
Post	101.3 (6.7)	-4.2 (3.5)	<0.001	100.6 (6.2)	-2.8 (3.6)	0.001	0.111
REE (kJ/day)							
Pre	6585 (685)			6418 (815)			
Post	5914 (542)	-9.8 (7.4)	<0.001	5784 (717)	-9.4 (9.4)	<0.001	0.835
NPRQ							
Pre	0.77 (0.05)			0.77 (0.05)			
Post	0.78 (0.06)	0.9 (5.3)	0.179	0.77 (0.04)	0.3 (6.6)	0.728	0.399
Leptin (ng/mL)							
Pre	47.95 (17.49)			47.57 (17.06)			
Post	29.06 (15.75)	-39.0 (26.9)	<0.001	26.24 (12.74)	-44.8 (18.4)	<0.001	0.344
TSH (µU/mL)*							
Pre	1.91 (0.82)			1.79 (0.54)			
Post	1.90 (1.00)	-1.0 (30.0)	0.922	1.80 (0.67)	1.5 (26.5)	0.971	0.747

BMI: body mass index; REE: resting energy expenditure; NPRQ: non protein respiratory quotient; TSH: thyrotropin.
% Change calculated as: [(Post-intervention-Pre)/Pre] x 100. Pre: before diet intervention; Post: after diet intervention.

and TSH levels between women carrying the TT genotype and A allele carriers of the *FTO* rs9939609 polymorphism (Table I) or across genotype groups of the *MC4R* rs17782313 (TT vs. C carriers, Table II) after the 12-week intervention period. The results did not significantly differ when the analyses were adjusted with body mass loss (data not shown).

Combined influence of the FTO *rs9939609 and the* MC4R *rs17782313*

When the combined influence of both polymorphisms was tested, the results showed that there were interaction effects between *FTO* rs9939609 and *MC4R* rs17782313 on body mass (P=0.030) and FM (P=0.079) loss after the 12-week of dietary intervention program. However, no significant differences were observed in body mass reduction and FM loss across genotype groups (Fig. 1).

In contrast, we did not observe any significant interaction effect between the *FTO rs9939609* and the *MC4R rs17782313* polymorphisms on changes in LM (P=0.358), body fat percentage (P=0.317), REE (P=0.772), NPRQ (P=0.647), waist circumference (P=0.692), leptin (P=0.102) and TSH (0.544) levels. These findings were not significantly influenced after adjustment for body mass loss (data not shown).

Discussion

The present study aimed to examine whether two SNPs previously associated with adiposity and reduced REE, *FTO* rs9939609 and *MC4R* rs17782313[8-11], predict lower body mass and FM loss after a 12-week energy-restricted diet intervention period in obese women. Our results did not support that obese women carrying the A allele of the *FTO* rs9939609 and/or the C allele of the *MC4R* rs17782313 are disad-

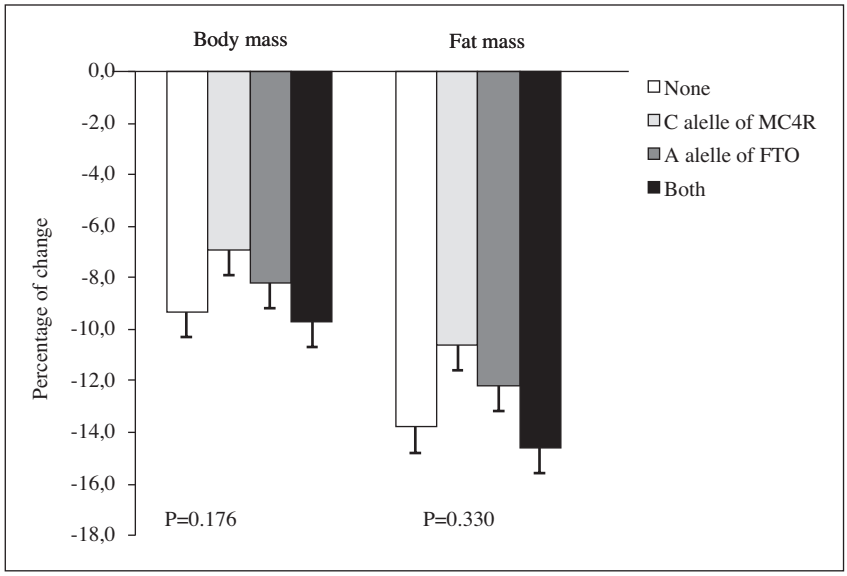

Fig. 1—Body mass (%) and fat mass loss (%) in obese women non-carrying risk alleles of the FTO rs9939609 and MC4R rs17782313 polymorphisms (none, N=15), carrying the C risk allele of the MC4R rs17782313 polymorphism (N=7), carrying the A allele of the FTO rs9939609 polymorphism (N=38) and carrying both the A risk allele of the FTO rs9939609 and the C risk allele of the MC4R rs17782313 polymorphisms (both, N=17) after 12-week of hypocaloric diet intervention. Analyses were adjusted for age. Values are means ± standard errors.

vantaged to loosing body mass and FM. In addition, to the best of our knowledge, this is the first study exploring the associations of these two SNPs with changes in REE, leptin and TSH levels that could predispose obese women to later body mass regain during body mass maintenance period. Likewise, previous studies showed lower long-time body mass loss and/or greater body mass regain in obese participants carrying the A allele of the *FTO* rs9939609[23] or the C allele of the *MC4R* rs17782313[24]. However, we did not find any significant individual or combined influence of these two polymorphisms in the reduction of REE and on changes in leptin and TSH levels after the intervention period, even after adjusting with body mass loss.

Some authors previously examined the individual influence of the *FTO* rs9939609[25-27] and the *MC4R* rs17782313[28] on body mass loss in overweight or obese adults. Likewise, Lappalainen et al[26] and De Luis et al.[25] did not observe any influence of the A allele of the *FTO* rs9939609 polymorphism on changes in body mass in overweight individuals after a long term lifestyle intervention or in obese patients after a 3 months of energy restricted diet intervention, respectively. Haupt et al[29] and Franks et al[27] reported that the *FTO* rs9939609 had no influence on body mass loss after a lifestyle intervention in subjects with high diabetes risk. Similarly, Haupt et al.[28] examined the influence of the *MC4R* rs17782313 on the success after a lifestyle intervention in the same high diabetes risk cohort, and they did not find any effect of the C allele on changes in body mass and fat distribution. In the same line, Verhoef et al[24] did not find any significant association of the *MC4R* rs17782313 on changes in body mass after a 8-weeks of very low energy diet intervention in obese individuals. The results of the present study confirm all these findings in obese premenopausal women who underwent an individually calculated energy restricted diet intervention based on measured REE by indirect calorimetry. We also add to these observations the lack of the combined influence of the two polymorphisms on changes in body mass, FM and LM measured by DEXA.

Studies focused on genetic mechanisms responsible of body mass regain after successful body mass loss intervention programs are clinically relevant, since they may help indentify susceptible individuals and improve long-term effects of body mass loss interventions. Genetic susceptibility to greater reductions in REE after body mass loss has been proposed as a potential mechanism explaining genetic predisposition to body mass regain during body mass maintenance period[30]. Two previous studies reported that carriers of the A risk allele of the *FTO* rs9939609[23] and the C risk allele of the *MC4R* rs17782313[24] polymorphisms were more likely to regain body mass during the maintenance period in severe obese and overweight adults, respectively. However, they did not examine potential mechanisms that could explain these observations. The most relevant finding of the present investigation was that carriers and non-carriers of the A allele of the *FTO* rs9939609 and/or the C allele of the *MC4R* rs17782313 experienced similar decrease in REE, even after adjustment for body mass loss. Moreover, we did not observe any significant difference in changes in leptin and TSH levels between carriers and non-carriers of risk alleles of the two polymorphisms that could predispose these individuals to higher later body mass regain[12]. Therefore, present data suggest that other mechanisms affecting energy balance would be implicated in the genetic susceptibility to body mass regain. As far as we are aware, there is only one previous study examining the association of the *FTO* rs9939609 polymorphism on changes in REE after a body mass loss program[31], and no one focused on either the influence of the

MC4R rs17782313 polymorphism or the combined influence of *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms on changes in REE. Grau et al[31] did not find any significant association of the *FTO* rs9939609 with changes in body mass and REE after 10 weeks of hypocaloric diet intervention in the whole sample of obese individuals, which concurs with our findings; however, they observed an interaction effect between diet composition and *FTO* rs9939609 on changes in REE. The authors reported that carriers of the TT genotype had lower decrease in REE than A allele carriers after a low fat hypocaloric diet (20-25% of energy from fat). In contrast, there was no significant influence of the *FTO* rs9939609 on changes in REE in individuals who consumed high fat diet hypocaloric diets (40-45% of energy from fat). In the present study, women consumed a mixed balanced hypocaloric diet providing 30% of energy from fat which could explain the different results reported by the two studies. On the other hand, two previous reports have examined the cross-sectional association of the *FTO* rs9939609 with TSH levels[10,32]. However, there are no previous studies reporting the influence of the *FTO* rs9939609 and/or the *MC4R* rs17782313 on changes in TSH levels after a body mass loss program which hampers comparisons.

Due to the relatively small sample size of our investigation, these findings should be taken as preliminary. The homogeneity of our well-characterized study sample and the well controlled intervention (energy content of the hypocaloric diet individually calculated from measured REE, with similar macronutrient composition based on Mediterranean dietary habits) are strengths of our study. However, we need to be very cautious before extrapolating our conclusions to other populations, and replication of our findings is essential.

In conclusion, the results of the present study suggest that obese women carrying the A risk allele of the *FTO* rs9939609 and/or the C risk allele of the *MC4R* rs17782313 might obtain the same benefit from an energy restricted diet intervention. In addition, our findings did not provide any data supporting that the *FTO* rs9939609 and the *MC4R* rs17782313 polymorphisms increase genetic susceptibility to greater body mass regain during the maintenance period. Likewise, the *FTO* rs9939609 and/or the *MC4R* rs17782313 did not influence changes in REE, leptin and TSH after body mass loss. Further studies with larger sample size are needed to confirm these results.

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