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ARTICLE

Influence of ADRB2 Gln27Glu and ADRB3 Trp64Arg polymorphisms on body weight and body composition changes after a controlled weight-loss intervention

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Abstract: The β-2 and β-3 adrenergic receptors (*ADRB2* and *ADRB3*) are thought to play a role in energy expenditure and lipolysis. However, the effects of the *ADRB2* glutamine (Gln) 27 glutamic acid (glutamate) (Glu) and *ADRB3* tryptophan (Trp) 64 arginine (Arg) polymorphisms on weight loss remain controversial. The aim of this study was to investigate the effect of these polymorphisms on changes in weight and body composition during a controlled weight-loss program. One hundred seventy-three healthy overweight and obese participants (91 women, 82 men) aged 18–50 years participated in a 22-week-long intervention based on a hypocaloric diet and exercise. They were randomly assigned to 1 of 4 groups: strength, endurance, strength and endurance combined, and physical activity recommendations only. Body weight, body mass index (BMI), and body composition variables were assessed before and after the intervention. Genetic analysis was carried out according to standard protocols. No effect of the *ADRB2* gene was shown on final weight, BMI, or body composition, although in the supervised male group, *Glu27* carriers tended to have greater weight ($p = 0.019$, 2.5 kg) and BMI ($p = 0.019$, 0.88 kg/m^2) reductions than did noncarriers. There seems to be an individual effect of the *ADRB3* polymorphism on fat mass ($p = 0.004$) and fat percentage ($p = 0.036$), in addition to an interaction with exercise for fat mass ($p = 0.038$). After the intervention, carriers of the *Arg64* allele had a greater fat mass and fat percentage than did noncarriers ($p = 0.004$, 2.8 kg). In conclusion, the *ADRB2* Gln27Glu and *ADRB3* Trp64Arg polymorphisms may influence weight loss and body composition, although the current evidence is weak; however, further studies are necessary to clarify their roles.

Key words: β-adrenergic receptors, weight loss, body composition, Gln27Glu and Trp64Arg, exercise and diet.

Résumé : Les récepteurs β-2 et β-3 adrénergiques (*ADRB2, ADRB3*) jouent un rôle, selon des études, dans la dépense d'énergie et la lipolyse. Toutefois, les effets des polymorphismes *ADRB2* Gln27Glu et *ADRB3* Trp64Arg sur la perte de poids soulèvent la controverse. Cette étude a pour objectif d'examiner l'effet de ces polymorphismes sur la modification de la masse et de la composition corporelles au cours d'un programme de perte de poids sous supervision. Cent soixante-treize sujets en surpoids et obèses (91 femmes, 82 hommes) âgés de 18 à 50 ans participent à un programme supervisé d'une durée de 22 semaines et comprenant un régime hypocalorique et de l'exercice physique. On répartit aléatoirement les sujets dans 4 groupes : force, endurance, force et endurance, recommandations en matière d'activité physique. Avant et après les 22 semaines d'intervention, on évalue la masse corporelle, l'indice de masse corporelle (IMC) et la composition corporelle. On effectue une analyse génétique au moyen des protocoles usuels. On n'observe aucun effet du gène *ADRB2* sur la masse corporelle, l'IMC et la composition corporelle en fin d'intervention; toutefois, les porteurs de *Glu27* dans le groupe masculin supervisé présentent une tendance à une perte de poids plus grande ($p = 0{,}019$, 2,5 kg) et à un abaissement de l'IMC ($p = 0{,}019$, 0,88 kg/m^2) comparativement aux sujets non porteurs. On observe possiblement un effet individuel du polymorphisme *ADRB3* sur la masse adipeuse ($p = 0{,}004$) et le pourcentage de gras ($p = 0{,}036$), indépendamment de l'interaction de la masse adipeuse avec l'exercice physique ($p = 0{,}038$). Les porteurs de l'allèle *Arg64* présentent des valeurs plus élevées de masse adipeuse et de pourcentage de gras que les non-porteurs à la fin de l'intervention ($p = 0{,}004$, 2,8 kg). In conclusion, les polymorphismes *ADRB2* Gln27Glu et *ADRB3* Trp64Arg pourraient avoir un effet sur la perte de poids et la composition corporelle, mais les données de cette étude sont peu probantes; il faut donc effectuer d'autres études pour élucider leurs rôles. [Traduit par la Rédaction]

Mots-clés : récepteurs β-adrénergiques, perte de poids, composition corporelle, Gln27Glu, Trp64Arg, exercice physique, diète.

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Introduction

The response to weight-loss programs is influenced by genetic factors (Bouchard 2008; Loos and Rankinen 2005; Ordovas and Shen 2008); therefore, it is essential to understand the genetic and biological background of obesity and the weight-loss processes to prevent and treat this complex disease. Energy balance is regulated by the adrenergic system (Blaak et al. 1993; Monroe et al. 2001); both the β-2 and the β-3 adrenergic receptor (*ADRB2* and *ADRB3*) promote lipolysis and fat mobilization and modify glucose metabolism (Arner 1992; Enoksson et al. 2000; Hagstrom-Toft et al. 1998; Lafontan et al. 1997). The lipolysis function is even more important during exercise and energy restriction (Arner 1992, 1995). Common polymorphisms, the glutamine (Gln) 27 glutamic acid (glutamate) (Glu) of the *ADRB2* gene and the tryptophan (Trp) 64 arginine (Arg) of the *ADRB3* gene, imply structural and functional differences among the protein versions and thus can influence body weight (Gagnon et al. 1996; Garenc et al. 2003; Green et al. 1995). In epidemiological studies of the *ADRB2* gene, some researchers found no relationship between the Gln27Glu polymorphism and obesity-related phenotypes (Bea et al. 2010; Echwald et al. 1998; Kortner et al. 1999; Rosado et al. 2015). However, other researchers found that the *Glu27* is the risk allele for obesity (Clement et al. 1995; Gonzalez Sanchez et al. 2003; Lange et al. 2005; Large et al. 1997), and still others, on the contrary, reported that the *Glu27* is the favorable allele, protective against obesity (Meirhaeghe et al. 2000; Pereira et al. 2003). As for the *ADRB3* gene, although in some cases no association was shown between the Trp64Arg polymorphism and obesity (Bea et al. 2010; Gagnon et al. 1996), other studies showed that the *Trp64* allele is protective against obesity (Clement et al. 1995; Corella et al. 2001; Ukkola et al. 2000; Widen et al. 1995).

An interaction among the gene, physical activity, and obesity has been suggested for both polymorphisms. Arner (2000) found different effects of the *Glu27* allele in sedentary and active people, and differences in fat oxidation were reported between *Glu27* and *Gln27* allele carriers in 2 studies (Macho-Azcarate et al. 2002; Rosado et al. 2015). Meirhaeghe and colleagues (1999) suggested that physical activity can counteract the effect of the Gln27Glu polymorphism in weight control, whereas for the *ADRB3* gene, Marti and colleagues (2002) observed a different risk of obesity with the Trp64Arg polymorphism.

As far as we know, no interventional studies have included both controlled exercise and a diet program and the polymorphisms that we have analyzed. In the case of the *ADRB2* gene, no significant main effect of the Gln27Glu polymorphism on changes in body weight or body composition after a program based on resistance training was found in women (Bea et al. 2010); similarly, Rauhio and colleagues (2013) found no main effect with a diet intervention including weight maintenance. However, in the HERITAGE study, *Glu27* carriers lost more body fat mass (Garenc et al. 2003). Applying only a diet intervention, Ruiz et and colleagues (2011) reported that the female *Glu27* allele carriers had greater reductions in body weight, body mass index (BMI), and lean mass. For the *ADRB3* gene, previous studies showed no main individual effect of this polymorphism (Bea et al. 2010; Ukkola et al. 2003); however, Bea and colleagues (2010) reported that in nonexercisers, the carriers of the *Arg64* allele gained a greater percentage of body fat. No differences between *Arg64* allele carriers and noncarriers were found in body weight and body fat in response to weight loss, but the loss of visceral adipose tissue (VAT) was 43% lower in the *Arg64* allele carriers (Tchernof et al. 2000). On the contrary, Phares and colleagues (2004) concluded that the *Arg64* allele carriers had a 2 times greater loss of percentage body fat.

In contrast to the candidate gene studies, no genome-wide association studies (GWASs) have shown an association between these polymorphisms and BMI, adiposity, or fat distribution (Fox et al. 2012; Locke et al. 2015; Shungin et al. 2015; Speliotes et al. 2010), which casts doubt on the previous findings. To the best of our knowledge, there has been no GWAS carried out on body composition changes during a weight-loss program. Despite these controversies, both polymorphisms are of interest regarding obesity and weight loss. Consequently, the aim of the current study was to analyze the effect of 2 common polymorphisms, *ADRB2* Gln27Glu and *ADRB3* Trp64Arg, on changes in body weight, BMI, and fat distribution during a highly controlled exercise and diet weight-loss program and to examine the influence of these polymorphisms on baseline values for the aforementioned parameters.

Materials and methods

This study is part of the randomized controlled trial (RCT) Nutrition and Physical Activity Programs for Obesity Treatments (the PRONAF study according to its Spanish initials) (ClinicalTrials.gov ID: NCT01116856). The RCT, whose aim was to assess the usefulness of different types of physical activity and nutrition programs in the treatment of adult obesity, was conducted in 2010 and 2011 following the ethical guidelines of the Declaration of Helsinki. The Human Research Review Committee of the University Hospital La Paz reviewed and approved the study design and the research protocol (code of approval PI-643). Further details of the study are described elsewhere (Zapico et al. 2012).

Subjects

The study participants were recruited through advertisements covering a wide variety of media (television, radio, press, and Internet). A total of 2319 potential participants were informed about the nature of the study, and those who were 18 to 50 years old, had a BMI between 25 and 34.9 kg/m^2, were nonsmokers, were sedentary (i.e., 2 h or less of structured exercise per week) (Brochu et al. 2009), and had glucose values <5.6 mmol/L (<100 mg/dL) (Rutter et al. 2012) were invited to participate in the study. Women with any disturbances in menstrual cycle were not eligible. A flow diagram of the participants and details on dropouts can be found elsewhere (Zapico et al. 2012). Participants provided written informed consent prior to joining the study and completed a baseline assessment at the medical center, after which they were randomly assigned to groups.

Study design

The intervention was a 6-month diet and exercise-based program focusing on a behavior change. Participants entered into the study in 2 phases, in the first year overweight, in the second year obese subjects, and were split into 4 randomly assigned groups, stratified by age and sex: strength (S), endurance (E), combined strength and endurance (SE), and the control group with physical activity recommendations only (C). For all participants, the measurements took place before starting, in week 1, and after 22 weeks of intervention, in week 24. Physical activity was assessed by a SenseWear Pro3 Armband accelerometer (Body Media, Pittsburgh, Pa., USA). Participants wore the monitor continuously for 5 days, including weekends and weekdays, following general recommendations (Murphy 2009). Daily energy expenditure was calculated using the Body Media propriety algorithm (Interview Research Software, version 6.0; BodyMedia Inc., Pittsburgh,. Pa., USA). Additionally, participants were asked to report physical activity habits and the amount of any food consumed during the intervention through a personal diary.

Diet intervention

Before the intervention, negative energy balance was calculated for all participants, taking into account their own daily energy expenditure, based on accelerometry data and the 3-day food record; they followed an individualized hypocaloric diet with a 25%–30% caloric restriction (National Institutes of Health 1998). Macronutrient distribution was set according to Spanish Society of Community Nutrition recommendations (Dapcich et al. 2004).

Exercise intervention

All exercise training groups (S, E, and SE) followed an individualized training program, which consisted of exercise sessions 3 times per week for 22 weeks, carefully supervised by certified personal trainers. Details of the different protocols developed by the groups are described elsewhere (Zapico et al. 2012).

Control group

Participants in the C group followed the dietary intervention and the physical activity recommendations of the American College of Sports Medicine (Donnelly et al. 2009) and thus were advised to undertake at least 200–300 min of moderate-intensity physical activity per week.

Adherence to diet was calculated as the estimated kilocalories of the diet divided by the real kilocalorie intake in percentage ((estimated kcal of diet/real kcal intake) × 100), 100% being the greatest adherence, following a methodology used previously (Acharya et al. 2009). Moreover, adherence to exercise was calculated by the number of sessions completed in regard to the theoretical sessions ((sessions performed/total sessions) × 100). Participants achieving 90% adherence to exercise (Hunter et al. 2000) and 80% to diet (Del Corral et al. 2009) were included in the analysis.

Body composition

Anthropometric measures included height measured to 0.01 m (SECA stadiometer, Seca Ltd., Valencia, Spain) and body weight measured to 0.1 kg (TANITA BC-420MA balance, Bio Lógica Tecnología Médica S.L, Barcelona, Spain). BMI was calculated as body weight (kg)/(height (m^2). Body composition (fat mass (kg), abdominal fat (kg), VAT (kg)) was assessed by dual-energy X-ray absorptiometry (GE Lunar Prodigy, GE Healthcare, Madison, Wis., USA) and GE Encore 2002 (version 6.10.029) software (GE Healthcare) with an accuracy of 0.001 kg. Percentage body fat was calculated as (fat (kg)/body weight (kg)) × 100%.

Genetic analysis

Whole blood samples (5 mL) from each participant were collected in EDTA and were sent to the laboratory for analysis. DNA was extracted from each sample using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), and genotyping was performed for each single nucleotide polymorphism (SNP). For the overweight participants, analysis of the *ADRB2* Gln27Glu (rs1042714) and the *ADRB3* Trp64Arg (rs4994) polymorphisms was done using PCR and restriction fragment length polymorphism techniques described previously (Clement et al. 1995; Large et al. 1997). For the obese participants, genotyping of the 2 polymorphisms was carried out using the corresponding TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, Calif., USA) with the StepOne Real Time PCR System (Applied Biosystems).

Statistical analyses

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). A χ^2 test was used to assess whether observed genotype frequencies were in the Hardy–Weinberg equilibrium. Normal distribution of each dependent variable was tested using quantile-quantile plots, and when needed, a Box–Cox transformation was applied using the optimal λ value. Three genetic models (additive, dominant, and recessive) were tested for the *ADRB2* gene; however, for the *ADRB3*, 2 groups (carriers and noncarriers of the *Arg64* allele) were analyzed because of the low number of homozygotes for the *Arg64* allele. Based on exercise, the sample was divided into 2 groups: supervised (S, E, SE groups) with all protocols having the same characteristics (intensity, duration, and frequency) and nonsupervised (C group). Three-way (genotype × exercise type × sex) analysis of covariance (ANCOVA) was conducted, using the final values of weight and body composition parameters adjusted by age and initial values to reveal differences between genotype groups, men and women, and exercise groups, as well possible interactions. Two-way (genotype × sex) ANCOVA was performed, adjusted by age for BMI and percentage body fat at baseline to determine the initial differences between genotype groups and sexes. Statistical significance for postintervention comparisons was defined at the corrected α of 0.00179 for the *ADRB2* and 0.00625 for the *ADRB3* polymorphism, for baseline comparisons at 0.00357 for the *ADRB2* and 0.0125 for the *ADRB3* polymorphism, with correction for repeated tests across the levels of the ANCOVA model factors, where appropriate.

Table 1. Baseline characteristics of the subjects.

	ADRB2		*ADRB3*	
	Glu27 noncarrier	Glu27 carrier	Arg64 noncarrier	Arg64 carrier
Baseline women				
n	37	54	76	15
Age (y)	38.24±8.45	39.72±8.16	39.61±7.93	36.67±9.75
Body weight (kg)	79.16±9.86	81.82±10.96	81.08±10.58	79.03±10.57
BMI (kg/m^2)	29.81±2.67	30.93±3.48	30.64±3.08	29.67±3.78
Fat mass (kg)	34.17±5.19	35.63±7.1	35.3±6.37	33.78±6.73
Fat percentage (%)	44.93±3.53	45.29±4.37	45.3±3.99	44.41±4.35
Android fat (kg)	2.82±0.57	2.94±0.81	2.92±0.72	2.74±0.72
VAT (kg)	0.71±0.34	0.79±0.38	0.77±0.35	0.69±0.44
Baseline men				
n	28	54	73	9
Age (y)	39.39±7.15	39.78±8.82	39.88±8.09	37.78±9.74
Body weight (kg)	97.02±11.47	95.45±10.39	95.86±10.24	97.03±14.84
BMI (kg/m^2)	31.27±2.46	30.78±2.85	30.91±2.6	31.25±3.67
Fat mass (kg)	33.28±7.59	33.75±6.72	33.63±6.84	33.23±8.5
Fat percentage (%)	35.48±4.78	36.82±4.75	36.49±4.73	35.29±5.32
Android fat (kg)	3.43±0.96	3.44±0.93	3.46±0.88	3.24±1.31
VAT (kg)	1.76±0.69	1.74±0.73	1.79±0.68	1.38±0.94

Note: Data presented as means ± SD. ADRB2, β-2 adrenergic receptor; ADRB3, β-3 adrenergic receptor; Glu, glutamic acid (glutamate); Arg, arginine; BMI, body mass index; VAT, visceral adipose tissue.

Table 2. Genotype distribution and allele frequency of the *ADRB2* gene.

	Gln27Gln	Gln27Glu	Glu27Glu	Allele *Gln27*	Allele *Glu27*
All	65 (37.57)	82 (47.40)	26 (15.03)	212 (0.61)	134 (0.39)
Women	37 (40.65)	40 (43.95)	14 (15.4)	114 (0.63)	68 (0.37)
Men	28 (34.14)	42 (51.22)	12 (14.64)	98 (0.60)	66 (0.40)

Note: Data presented as *n* (%) for genotypes and *n* (frequency) for alleles. ADRB2, β-2 adrenergic receptor; Gln, glutamine; Glu, glutamic acid (glutamate).

Results

The baseline characteristics of the 173 subjects who participated in the study (after dropouts, exclusions because of low adherence, and missing data) are shown in Table 1.

Genotype distributions and allele frequencies of the *ADRB2* Gln27Glu and *ADRB3* Trp64Arg polymorphisms are shown in Tables 2 and 3. Both genotype distributions were found to be in Hardy–Weinberg equilibrium ($p = 0.987$ for the *ADRB2* and $p = 0.980$ for the *ADRB3* polymorphism).

ADRB2 Gln27Glu postintervention comparisons

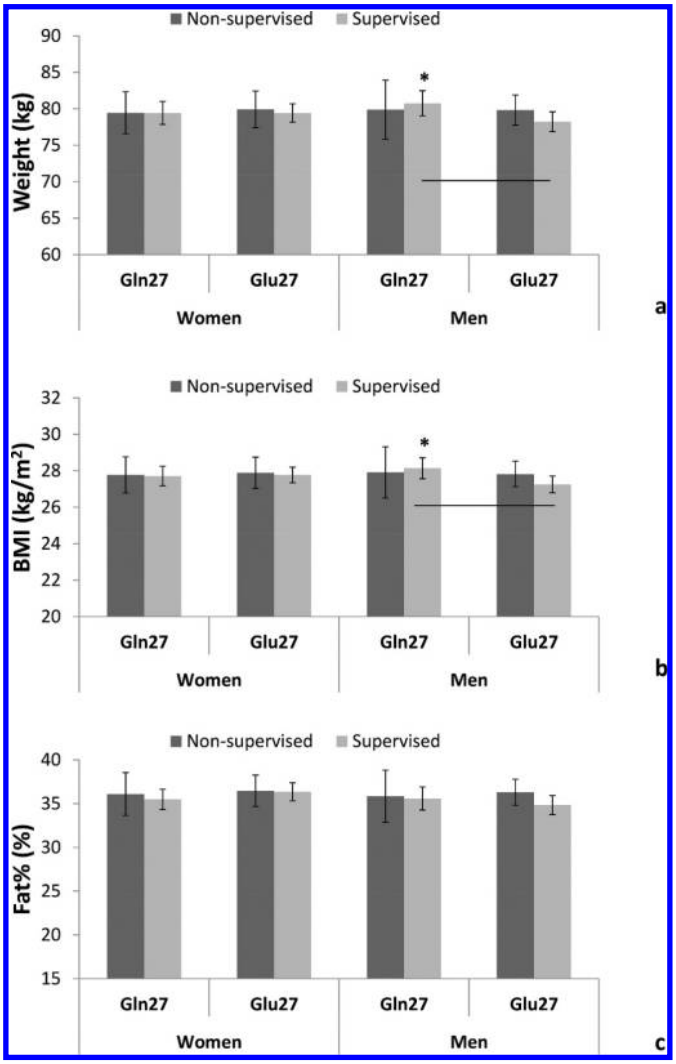
Results for the dominant model are shown; no significant results were found with the other models. Based on these data, it appears that this model is the one that best fits the behavior of this polymorphism. We observed no main effect of the Gln27Glu polymorphism for final body composition values, which were adjusted by initial measurements, or for interaction with exercise, sex, or both. Post hoc analyses revealed that within the supervised men group, carriers of the *Glu27* allele reduced weight and BMI more than did noncarriers ($p = 0.019$, 2.52 kg, and $p = 0.019$, 0.881 kg/m^2 respectively) (Fig. 1). No differences were found for the other variables. (Figs. 1 and 2).

Table 3. Genotype distribution and allele frequency of the *ADRB3* gene.

	Trp64Trp	Trp64Arg	Arg64Arg	Allele *Trp64*	Allele *Arg64*
All	148 (85.55)	24 (13.87)	1 (0.58)	320 (0.92)	26 (0.08)
Women	76 (83.52)	15 (16.48)	0 (0)	167 (0.92)	15 (0.08)
Men	72 (87.80)	9 (10.98)	1 (1.22)	153 (0.93)	11 (0.07)

Note: Data presented as *n* (%) for genotypes and *n* (frequency) for alleles. ADRB3, β-3 adrenergic receptors; Trp, tryptophan; Arg, arginine.

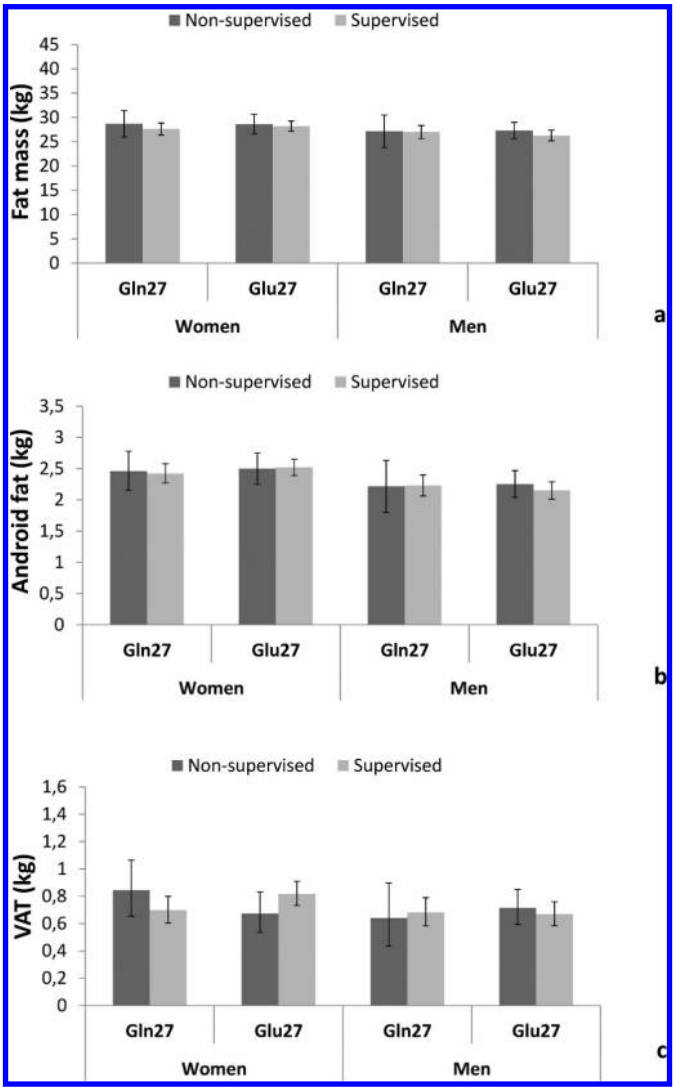
Fig. 1. β-2 adrenergic receptor glutamine (Gln) 27 glutamic acid (glutamate) (Glu) polymorphism and weight and body composition variables after the intervention in men and women. Means of final values of (*a*) weight, (*b*) body mass index (BMI), and (*c*) percentage body fat (Fat%) are presented after adjustment for baseline values and age, with 95% confidence intervals. *, $p < 0.05$.



ADRB3 Trp64Arg postintervention comparisons

Regarding the Trp64arg polymorphism, no differences were seen for weight, BMI, android fat, or VAT (Figs. 3 and 4). An individual effect of the polymorphism was found for fat mass ($p = 0.004$, $F = 8.519$ (1)) and percentage fat ($p = 0.036$, $F = 4.457$ (1)), for fat mass reaching the corrected significance level. Moreover, an interaction with exercise was observed for fat mass ($p = 0.038$, $F = 4.383$ (1)). Post hoc analyses indicated that *Arg64* carriers had greater fat mass and fat percentages than did noncarriers after the intervention ($p = 0.004$, 2.82 kg and $p = 0.036$, 1.83%, respectively). Moreover, the final fat mass of the female *Arg64* carriers was 3.9 kg higher than that of the noncarriers ($p = 0.004$); more specific differences were observed depending on the genotype and type of exercise. The pairwise comparison showed that the women carrying the *Arg64* allele in the nonsupervised exercise group had a greater final fat mass ($p = 0.004$, 7.22 kg) (Fig. 4). Accordingly, among the *Arg64* carriers in the whole sample and in women, the supervised group reduced fat mass more than did the nonsupervised group ($p = 0.019$, 4.31 kg and $p = 0.010$, 6.59 kg, respectively).

Fig. 2. β-2 adrenergic receptor glutamine (Gln) 27 glutamic acid (glutamate) (Glu) polymorphism and body composition variables after the intervention in men and women. Means of final values of (*a*) fat mass, (*b*) android fat, and (*c*) visceral adipose tissue (VAT) are presented after adjustment for baseline values and age, with 95% confidence intervals.



Baseline

Neither of the analyzed polymorphisms, the Gln27Glu of the *ADRB2* gene or the Trp64Arg of the *ADRB3* gene, showed a main effect or interaction with sex or age for BMI or percentage body fat at baseline.

Discussion

In this work, we studied the role that 2 polymorphisms of the β-adrenergic receptors play in body composition changes after a 24-week-long diet and exercise intervention (supervised or nonsupervised exercise) in healthy, overweight, and obese subjects. Our results suggest no strong influence of the *ADRB2* Gln27Glu

Fig. 3. β-3 adrenergic receptor tryptophan (Trp) 64 arginine (Arg) polymorphism and weight and body composition variables after the intervention in men and women. Means of final values of (*a*) weight, (*b*) body mass index (BMI), and (*c*) percentage body fat (Fat%) are presented after adjustment for baseline values and age, with 95% confidence intervals.

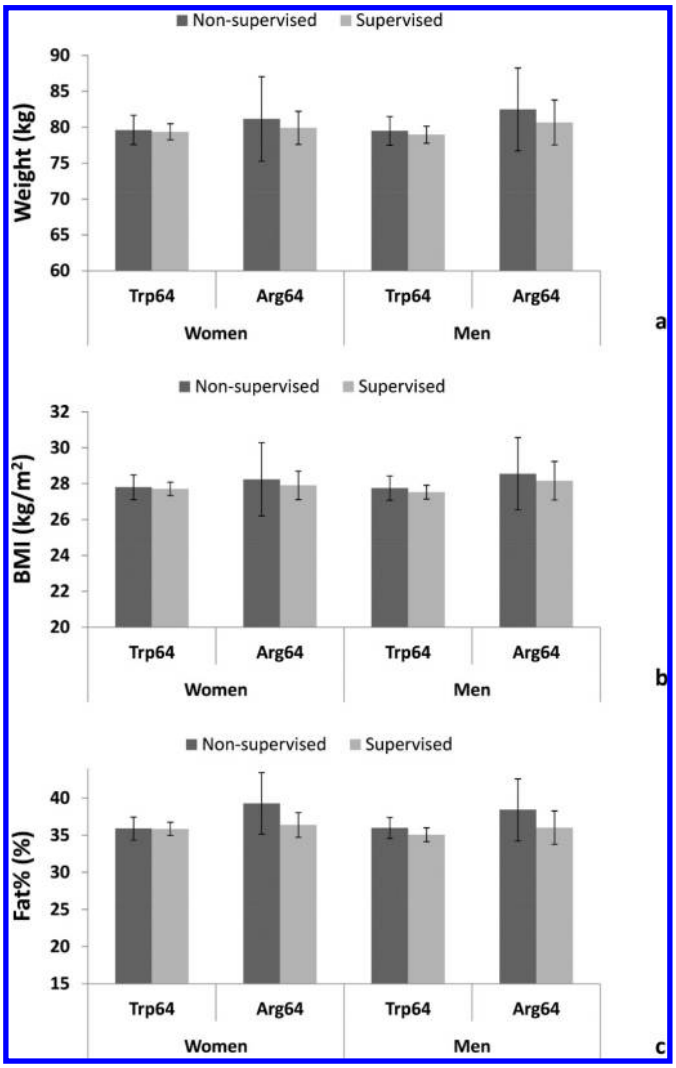
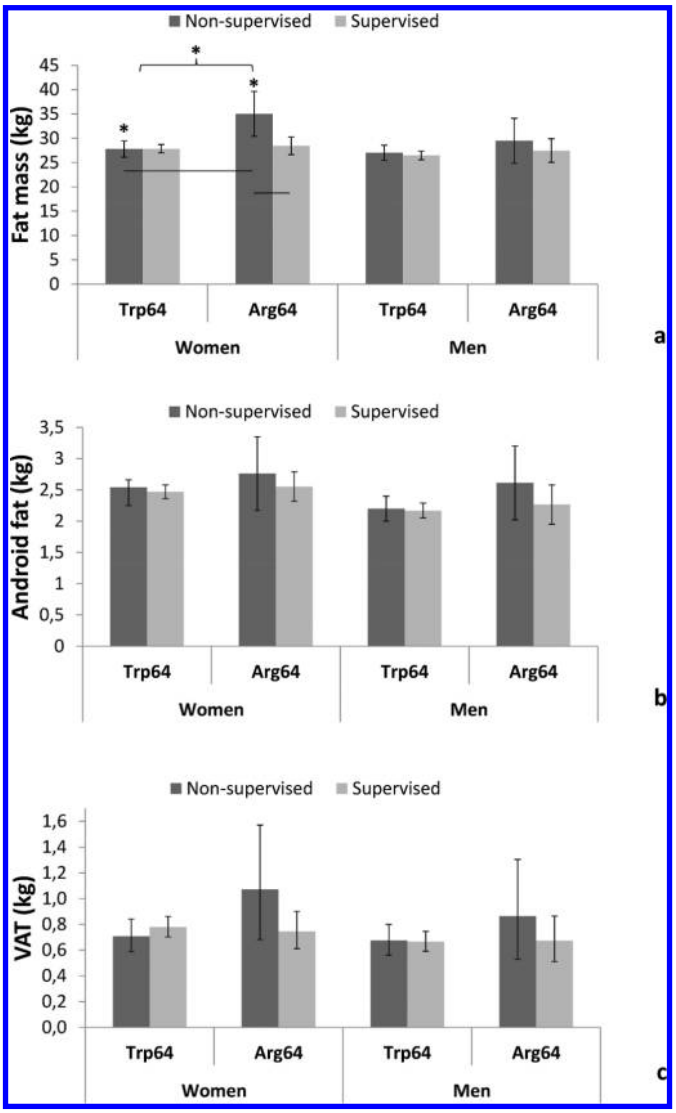


Fig. 4. β-3 adrenergic receptor tryptophan (Trp) 64 arginine (Arg) polymorphism and body composition variables after the intervention in men and women. Means of final values of (*a*) fat mass, (*b*) android fat, and (*c*) visceral adipose tissue (VAT) are presented after adjustment for baseline values and age, with 95% confidence intervals. *, $p < 0.00625$.



(rs1042714) or the *ADRB3* Trp64Arg (rs4994) polymorphisms on these variables, but some associations were found, which encourages further studies.

The genotype distribution and allele frequencies of both polymorphisms in our sample were similar to those of the Iberian European population of the 1000 Genomes Project (Abecasis et al. 2012) and to previous Spanish studies on the *ADRB2* gene (Gonzalez Sanchez et al. 2003; Martinez et al. 2003) and the *ADRB3* gene (Corella et al. 2001).

As mentioned before, previous studies with Caucasians included an exercise protocol or diet only but not both; therefore, they are not fully comparable with our intervention. Even though it has been proven that in a weight-loss program, diet results in a higher loss than does exercise alone (Franz et al. 2007), the best protocols include both for the greatest benefit (Clark 2015; Curioni and Lourenco 2005). Moreover, the interaction between genes and physical activity has been suggested by previous authors in connection with adrenergic receptor genes (Corbalan et al. 2002; Meirhaeghe et al. 1999; Phares et al. 2004).

ADRB2 Gln27Glu postintervention comparisons

No main effect or interactions of the polymorphism with other between-subject factors were shown in our analyses for body composition parameters, which is similar to the findings of other studies based on weight-loss programs (Bea et al. 2010; Phares et al. 2004; Rauhio et al. 2013). However, post hoc analyses revealed that supervised men carrying the *Glu27* allele lost more weight and lowered BMI more (<0.05) than did the men in the Gln27Gln group, suggesting that supervised exercise and the *Glu27* allele together in men can be beneficial for losing more weight. In previous studies, the potential effect of this polymorphism on the weight or BMI of Caucasian men was negative. Phares and colleagues (2004) used diet stabilization (but no caloric restriction) and aerobic training in subjects (aged 50–75 years) with a mean BMI of 27.8 kg/m², whereas the HERITAGE study investigated only aerobic training with obese subjects, and PRONAF consisted of a hypocaloric diet and exercise program. Although all 3 programs were between 20 and 24 weeks long, our intensity was a bit lower than that of the other 2 programs (PRONAF, 50%–60% of the heart rate reserve;

Garenc and colleagues (2003), 55%–75% of the maximal oxygen consumption; Phares and colleagues (2004), 50%–70% of the maximal oxygen consumption), and our sessions were longer (PRONAF, 51.15–60 min; Garenc and colleagues (2003), 30–50 min; Phares and colleagues (2004), 20–40 min). Garenc and colleagues (2003) reported greater fat mass changes in obese Glu27 homozygote men, results that were not confirmed in our study. As for women, most studies have not confirmed the importance of this polymorphism with exercise only or with diet only (Bea et al. 2010; Rauhio et al. 2013; Rosado et al. 2015), findings that are in line with our negative results. Nevertheless, Ruiz and colleagues (2011) reported that after a low-energy mixed diet in a sample very similar to ours, *Glu27* carriers lost more weight and had a lower BMI. The added feature in our study compared with this study is the exercise, which could balance the differences reported by them, because no differences were found for weight or BMI. On the contrary, the HERITAGE study found that Glu27Glu women reduced percentage fat by less than the other 2 groups in response to endurance training (Garenc et al. 2003). No differences were found for android fat or VAT in our analyses, but to the best of our knowledge, no antecedents to the contrary have been reported in the literature (Bea et al. 2010; Rauhio et al. 2013; Ukkola et al. 2003).

ADRB3 Trp64Arg postintervention comparisons

The individual effect of the *Arg64* allele was observed for fat mass and percentage fat that reached the 0.05 level; for fat mass this was observed at even the corrected threshold. Moreover, an interaction with exercise was found for fat mass. However, the main effect of the Trp64Arg polymorphism was observed in the change in other body composition variables and in any interactions with exercise or sex. Previous negative results disagree with our results on fat mass and fat percentage, but support the negative results of the other variables (Garenc et al. 2001; Phares et al. 2004; Rawson et al. 2002; Ukkola et al. 2003). No differences in weight or BMI were seen between carriers and noncarriers, which is in agreement with other studies reporting different weight-loss interventions (hypocaloric diet, aerobic training, resistance training) with sedentary obese participants (Bea et al. 2010; Rawson et al. 2002; Tchernof et al. 2000; Ukkola et al. 2003). Nevertheless, post hoc analyses showed that carriers of the *Arg64* allele lost less fat mass and reduced percentage fat less than did noncarriers during the intervention, contrary to previous studies using a wide variety of protocols (Garenc et al. 2001; Phares et al. 2004; Rawson et al. 2002; Tchernof et al. 2000; Ukkola et al. 2003). Among the *Arg64* carriers, the nonsupervised group had higher final fat mass values than did the supervised group, which may suggest that supervised exercise is beneficial for these genotypes. The interaction between this gene and physical activity has been raised before. Marti and colleagues (2002) reported that this polymorphism means higher obesity risk in sedentary people than in active people, which is in line with the results of other studies (Phares et al. 2004). In women, the carriers of the *Arg64* allele lost less fat mass than did noncarriers, and nonsupervised subjects lost less fat mass than did supervised subjects. Nonsupervised female *Arg64* carriers had a smaller reduction in fat mass than did noncarriers, but this should be judged cautiously because this group was very small. However, it should be pointed out that this finding is in line with the findings of Marti and colleagues (2002). Similarly, Bea and colleagues (2010) reported that after a 12-month-long resistance training program in sedentary postmenopausal women (from normal weight to obese), *Arg64* carriers gained a significantly greater percentage of fat than did noncarriers. As for android fat and VAT, differences were seen between carriers and noncarriers of the *Arg64* allele. Conversely, Tchernof and colleagues (2000) reported that through a 13-month-long diet program, *Arg64* carriers lost 43% less VAT than did the Trp64Trp group. This intervention was much longer and from a dietary point of view, stricter, than ours, which could have been a determinant.

The physiological changes of the receptor function caused by the gene variations, together with the diet and exercise program, could lead to a divergent lipolysis rate and a divergent amount of fat loss as a response to our program. However, it is hard to confirm the underlying mechanisms of these differences for fat with our data because the project did not include a deep physiological or molecular investigation. It is well established that adrenergic receptors play a role in fat mobilization and lipolysis (Arner 1992; Enoksson et al. 2000; Hagstrom-Toft et al. 1998; Lafontan et al. 1997), and this influence can be greater with exercise or diet (Arner 1992, 1995). In addition, the interaction between these polymorphisms and physical activity has been studied, but its existence is still not clear (Arner 2000; Meirhaeghe et al. 1999; Rosado et al. 2015). Our results and those of previous studies encourage further research.

Baseline Gln27Glu and Trp64Arg polymorphisms

Our analyses showed no effect of the polymorphisms on BMI or percentage body fat or the interaction between the polymorphisms and age or sex at baseline. Several studies, in agreement with our results, did not find an association between the Gln27Glu polymorphism of the *ADRB2* gene and obesity or related parameters (Bea et al. 2010; Echwald et al. 1998; Kortner et al. 1999). Most positive findings with the Gln27Glu polymorphism suggest that the favorable allele is the *Gln27* allele, and that the *Glu27* allele contributes to obesity risk (Clement et al. 1995; Gonzalez Sanchez et al. 2003; Lange et al. 2005, 1997). On the contrary, other researchers showed that the *Gln27* allele enhances the risk of obesity (Meirhaeghe et al. 2000; Pereira et al. 2003). As for the Trp64Arg polymorphism of the *ADRB3* gene, no association was found in various studies in different populations (Bea et al. 2010; Gagnon et al. 1996); nevertheless, a favorable feature of the *Trp64* allele was demonstrated in the HERITAGE study and others (Clement et al. 1995; Corella et al. 2001; Ukkola et al. 2000; Widen et al. 1995).

As we have shown, study findings are not in agreement. This can be explained partly by study protocol, age of the subjects, and obesity status differences. Moreover, it was suggested previously that most candidate gene studies are underpowered, mainly because of the sample size and the lack of adjustment for multiple testing (Bray et al. 2009). Meta-analyses are in line with our results, reporting no association between obesity and the *ADRB2* Gln27Glu (Allison et al. 1998; Jalba et al. 2008) or *ADRB3* Trp64Arg (Kurokawa et al. 2008) in Europeans, although they do confirm the importance in other races including Asians, Pacific Islanders, and American Indians. Studies using new techniques (GWAS) also confirm these findings (Fox et al. 2012; Locke et al. 2015; Shungin et al. 2015; Speliotes et al. 2010).

A limitation of our work is the sample size. The main objective of our study was not to explore the genetic background of weight loss; thus, this part of the study is underpowered. A correction for multiple testing was applied, taking into account the polymorphisms, the genetic models, the exercise groups, and the sexes, yet all results below 0.05 were reported and discussed. Another limitation of our study is that for ethical reasons there was no real control group; all groups followed the individualized diet and exercise programs or received physical activity recommendations. However, the strength of our study is that both exercise and diet were included in the weight-loss program and were controlled by experts in the field.

The conclusions of our study are that during an exercise and diet program in the Spanish overweight and obese population, male carriers of the *Glu27* allele of the *ADRB2* Gln27Glu polymorphism may have an advantage in lowering weight and BMI, and the carriers of the *Arg64* allele of the *ADRB3* Trp64Arg polymorphism (especially women) may have more difficulty in losing fat mass and percentage fat than do noncarriers. However, for *Arg64* allele carriers, supervised exercise can help individuals lose more fat mass (thus compensating for the effect of the allele), which

gives promising practical use. Nevertheless, the evidence is weak; thus, more research is needed in this field, with larger sample sizes and controlled protocols that take into account all possible interactions among diet, exercise, genetic background, and other factors. Finally, physical activity seems to influence the effect of these polymorphisms during weight loss, as was suggested previously.

Conflict of interest statement

No conflicts of interest or funding are declared.

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