THE INFLUENCE OF THE ADRB2 GLN27GLU AND ADRB3 TRP64ARG POLYMORPHISMS ON BODY WEIGHT AND BODY COMPOSITION CHANGES AFTER A CONTROLLED WEIGHT LOSS INTERVENTION

Barbara Szendrei, Domingo González-Lamuño, Teresa Amigo, Guan Wang, Yannis Pitsiladis, Pedro J. Benito, Carmen Gomez-Candela, Francisco J. Calderón and Rocío Cupeiro, on behalf of the PRONAF Study Group

Corresponding author:

Barbara Szendrei

Laboratory of Exercise Physiology Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain

Ciudad Universitaria de Madrid, Calle Martín Fierro 7. 28040 Madrid, Spain

barbara.szendrei@upm.es

Phone: +34 91 336 40 70

Fax: +34 91 336 40 26

Authors:

Barbara Szendrei

Laboratory of Exercise Physiology Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain, Ciudad Universitaria de Madrid, Calle Martín Fierro 7. 28040 Madrid, Spain

barbara.szendrei@upm.es

Domingo González-Lamuño

Laboratory of Pediatrics School of Medicine University of Cantabria, Santander, Spain

Avenida Cardenal Herrera Oria s/n, 39011, Santander, Spain

Department of Medical and Surgery Sciences, Faculty of Medicine, University of Cantabria - IDIVAL, Marqués de Valdecilla Research Institute, Santander, Spain

Avenida Cardenal Herrera Oria s/n, 39011, Santander, Spain

domingo.gonzalez-lamuno@unican.es

Teresa Amigo

Laboratory of Pediatrics School of Medicine University of Cantabria, Santander, Spain

Avenida Cardenal Herrera Oria s/n, 39011, Santander, Spain

teresa.amigo@unican.es

Guan Wang

FIMS Reference Collaborating Centre of Sports Medicine for Anti-Doping Research, University of Brighton

Welkin Science Laboratories

30 Carlisle Road, Eastbourne BN20 7SN United Kingdom

G.Wang2@brighton.ac.uk

Yannis Pitsiladis

FIMS Reference Collaborating Centre of Sports Medicine for Anti-Doping Research, University of Brighton

Welkin Science Laboratories

30 Carlisle Road, Eastbourne BN20 7SN United Kingdom

Y.Pitsiladis@brighton.ac.uk

Pedro J. Benito

Laboratory of Exercise Physiology Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain, Ciudad Universitaria de Madrid, Calle Martín Fierro 7. 28040 Madrid, Spain

pedroj.benito@upm.es

Carmen Gomez-Candela

Nutrition Department, Hospital La Paz Health Research Institute, University Autonoma of Madrid, Madrid, Spain

Paseo de la Castellana, 261.28046, Madrid, Spain

carmengomezcandela@telefonica.net

Francisco J. Calderón

Laboratory of Exercise Physiology Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain, Ciudad Universitaria de Madrid, Calle Martín Fierro 7. 28040 Madrid, Spain

franciscojavier.calderon@upm.es

Rocío Cupeiro

Laboratory of Exercise Physiology Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain, Ciudad Universitaria de Madrid, Calle Martín Fierro 7. 28040 Madrid, Spain

rocio.cupeiro@upm.es

ABSTRACT

Background β-2 and β-3 adrenergic receptors *(ADRB2, ADRB3)* are considered to play a role in energy expenditure and lipolysis. However the effects of the *ADRB2* Gln27Glu and the *ADRB3* Trp64Arg polymorphisms on weight loss are controversial. The aim of this study was to see the effect of these polymorphisms on the change of weight and body composition during a controlled weight loss program.

Methods 173 healthy overweight and obese participants (91 women, 82 men) aged 18-50 years participated in a 22 week-long intervention based on hypocaloric diet and exercise. They were randomized into four groups: strength, endurance, combined and physical activity recommendations. 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.

Results No effect of the *ADRB2* gene was shown on final weight, BMI or body composition, though Glu27 carriers of the supervised men group tended to reduce weight (p=0.019, 2.5 kg) and BMI (p=0.019, 0.88 kg) more than non-carriers. There seems to be an individual effect of the *ADRB3* polymorphism on fat mass (p=0.004) and fat percentage (p=0.036), besides an interaction with exercise for fat mass (p=0.038). Carriers of the Arg64 allele had higher fat mass and fat percentage than non-carriers after the intervention (p=0.004, 2.8 kg).

Conclusions The *ADRB2* Gln27Glu and the *ADRB3* Trp64Arg polymorphisms might influence weight loss and body composition, though the present 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

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 weight loss processes in order to prevent and treat this complex disease. Energy balance is regulated by the adrenergic system (Blaak et al. 1993; Monroe et al. 2001), since both β-2 and β-3 adrenergic receptors (*ADRB2, 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; Arner 1995). Common polymorphisms, the Gln27Glu (Gln - Glutamine; Glu - Glutamic acid/ Glutamate) of the *ADRB2* and the Trp64Arg (Trp – Tryptophan; Arg – Arginine) of the *ADRB3* genes, imply structural and functional differences among the protein versions thus can influence the control of body weight (Gagnon et al. 1996; Garenc et al. 2003; Green et al. 1995). Considering epidemiological studies of the *ADRB2* gene, some have not found 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). While some 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), on the contrary others reported that the Glu27 is the favorable allele, protective against obesity (Meirhaeghe et al. 2000; Pereira et al. 2003). As for the *ADRB3* gene, no association was shown in some cases between the Trp64Arg polymorphism and obesity (Bea et al. 2010; Gagnon et al. 1996), other studies showed the Trp64 allele is protective against the obesity (Clement et al. 1995; Corella et al. 2001; Ukkola et al. 2000; Widen et al. 1995).

The interaction of the gene, physical activity and obesity has been suggested for both polymorphisms. Arner et al. found different effects of the Glu27 allele in sedentary and active

people (Arner 2000); differences in fat oxidation were reported between Glu27 and Gln27 allele carriers in two studies (Macho-Azcarate et al. 2002; Rosado et al. 2015); Meirhaeghe et al. raised that physical activity can counteract the effect of the Gln27Glu polymorphism in weight control (Meirhaeghe et al. 1999), while for the ADRB3 gene Marti et al. observed different risk for obesity with the Trp64Arg polymorphism (Marti et al. 2002).

Regarding interventional studies, as far as we know, none of them included both controlled exercise and diet program and the polymorphisms analyzed by us. 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 resistance training in women (Bea et al. 2010), neither Rauhio et al. with a diet intervention, including weight maintenance (Rauhio et al. 2013). However, in the HERITAGE study Glu27 carriers lost more body fat mass (Garenc et al. 2003). Applying only a diet intervention Ruiz et al. reported that the Glu27 allele carriers had greater reduction in body weight, body mass index (BMI) and lean mass in women (Ruiz et al. 2011). For the *ADRB3* gene, previous studies showed no main individual effect of this polymorphism (Bea et al. 2010; Ukkola et al. 2003), however Bea et al. reported that within non-exercisers the carriers of the Arg64 allele gained greater percent body fat (Bea et al. 2010). No differences were found in response to weight loss between Arg64 allele carriers and non-carriers in body weight and body fat, but the loss of visceral adipose tissue (VAT) was 43% lower in the Arg64 allele carriers (Tchernof et al. 2000). On the contrary, Phares et al. concluded that the Arg64 allele carriers have two times greater loss of percentage body fat (Phares et al. 2004).

In contrast to the candidate gene studies, no genome-wide association studies (GWAS) showed associations with these polymorphisms on BMI, adiposity or fat distribution (Fox et al. 2012; Locke et al. 2015; Shungin et al. 2015; Speliotes et al. 2010), which questions the previous findings. To the best of our knowledge, there has been no GWA study carried out on

body composition changes during a weight loss program. Despite these controversies both polymorphisms are of interest in connection with obesity and weight loss. Consequently, the aim of the present study was to analyze the effect of two common polymorphisms, the *ADRB2* Gln27Glu and the *ADRB3* Trp64Arg on the changes of 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 mentioned parameters.

METHODS

The present study is the part of the RCT (ClinicalTrials.gov ID: NCT01116856), Nutrition and Physical Activity Programs for Obesity Treatments, PRONAF (the PRONAF study according to its Spanish initials). The aim of the RCT was to assess the usefulness of different types of physical activity and nutrition programs for the treatment of adult obesity, and it 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 non-smokers, sedentary (i.e., two hours 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 this study. Women with any disturbances in menstrual cycle were not eligible. Flow diagram of the participants and details on drop outs 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 two waves (overweight and obese phase), and were split into four randomly assigned groups, stratified by age and sex: strength (S), endurance (E), combined strength and endurance (SE) and the control groups (C) with physical activity recommendations. The measurements took place for all participants 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). 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). Additionally, participants were asked to report physical activity habits and the amount of any food undertaken 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, thus they followed an individualized hypo-caloric diet with a 25-30% caloric restriction (National Institutes of Health 1998). Macronutrient distribution was set according to the 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 three times per week exercise sessions during 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 from the C group followed the dietary intervention and the physical activity recommendations of the American College of Sports Medicine (Donnelly et al. 2009), 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 (kcal) of the diet divided by the real kcal intake in percentage ([estimated kcal of diet/real kcal intake]x100), being 100% the highest adherence to it, 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]x100). Assistance over 90% of the training sessions (Hunter et al. 2000), and an adherence to diet over to 80% were required (Del Corral et al. 2009).

Body composition

Anthropometric measures included height (SECA stadiometer, Valencia, Spain, 0.01 m) and body weight (TANITA BC-420MA balance, Bio Lógica Tecnología Médica S.L, Barcelona, Spain, 0.1 kg). 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 DXA (GE Lunar Prodigy; GE Healthcare, Madison, WI, GE Encore 2002, version 6.10.029

software) with an accuracy of 0.001 kg. Percentage body fat (% body fat) was calculated as [fat (kg)/ body weight (kg)*100%].

Genetic analysis

Whole blood samples (5 ml) were collected in ethylene-diaminetetraacetic acid (EDTA) from each participant and sent to the laboratory for the analysis. Deoxyribonucleic acid (DNA) was extracted from each sample using the "QIAamp® DNA Blood Mini Kit" (QIAGEN Hilden, Germany) and the genotyping was performed for each single nucleotide polymorphism (SNP). For the overweight phase, the analysis of the *ADRB2* Gln27Glu (rs1042714) and the *ADRB3* Trp64Arg (rs4994) polymorphisms was done using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques described before (Clement et al. 1995; Large et al. 1997); and for the obese phase, the genotyping of the two polymorphisms was carried out using the corresponding TaqMan® SNP Genotyping Assays (Applied Biosystem, Foster City, CA, USA) with StepOne™ Real Time PCR System (Applied Biosystem, Foster City, CA, USA).

Statistical analysis

The statistical analysis was performed using IBM® SPSS® Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY). Chi-square test was used to assess whether observed genotype frequencies were in the Hardy–Weinberg equilibrium (HWE). Normal distribution of each dependent variable was tested using Qunatile-Quantile plots, when needed Box-Cox transformation was applied using the optimal lambda value. Three genetic models (additive, dominant, recessive) were tested for the *ADRB2* gene, however for the *ADRB3* two groups (carriers and non-carriers Arg64 allele) were analyzed because of the low number of the homozygotes for the Arg64 allele. Based on exercise, the sample was divided in two groups: supervised (S, E, SE groups) as all protocols had the same characteristics (intensity, duration

and frequency) and non-supervised group (C group). Three-way (genotype x exercise type x sex) analysis of covariance (ANCOVA) was conducted using 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 x sex) analysis of covariance (ANCOVA) was performed adjusted by age for BMI and percentage body fat at baseline to see initial differences between genotype groups and sexes. Statistical significance for post intervention comparisons was defined at the corrected alpha 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.

RESULTS

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

Table 1 about here.

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

Table 2 about here.

ADRB2 Gln27Glu post-intervention comparisons

Results for the dominant model are shown as no significant results were found with the other models. According to this, it seems that this model is the one which fits the most to the behavior of this polymorphism. No main effect of the Gln27Glu polymorphism was observed for final body composition values adjusted by initial measurements, neither interaction with exercise, sex or both. Post hoc analyses revealed that within supervised men group, carriers of the Glu27 allele reduced weight and BMI more than non-carriers (p=0.019, 2.52 kg and p=0.019, 0.881 kg/m^2 respectively; Figure 1). No differences were found for the other variables. (Figure 1 and 2).

Figure 1 about here.

Figure 2 about here.

ADRB3 Trp64Arg post-intervention comparisons

Regarding the Trp64arg polymorphism, no differences were seen for weight, BMI, android fat or VAT (Figure 3 and 4). The 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 higher fat mass and fat percentage than non-carriers 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 the non-carriers (p=0.004), observing more specific differences depending on the genotype and type of exercise. The pair-wise comparison showed that the women carrying the Arg64 allele in the non-supervised exercise group had a higher final fat mass (p=0.004, 7.22 kg) (Figure 4). Accordingly, among the Arg64 carriers in the whole sample and in women, the

supervised group reduced fat mass more than the non-supervised group (p=0.019, 4.31 kg and p=0.010, 6.59 kg respectively).

Figure 3 about here

Figure 4 about here

Baseline

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

DISCUSSION

In the present work we studied the role of two polymorphisms of the beta-adrenergic receptors on body composition changes after a 24 week long diet and exercise intervention (supervised or non-supervised exercise) in healthy overweight and obese subjects. Our results suggest no strong influence of the *ADRB2* Gln27Glu (rs1042714) and the *ADRB3* Trp64Arg (rs4994) polymorphisms on these variables, but some associations were found, which could encourage us for further studies.

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

As it was mentioned before, previous studies with Caucasians only included exercise protocol or diet but not both, therefore they are not fully comparable with our intervention. Even

though it is proved that in a weight loss program, diet drives to a higher loss than only exercise (Franz et al. 2007), yet the best protocols include both for further benefits (Clark 2015; Curioni and Lourenco 2005). Moreover, the interaction between genes and physical activity was suggested by previous authors in connection with adrenergic receptor genes (Corbalan et al. 2002; Meirhaeghe et al. 1999; Phares et al. 2004).

ADRB2 Gln27Glu post-intervention comparisons

No main effect or interactions with other between-subject factors of the polymorphism were shown in our analyses for body composition parameters similarly to other studies based on weight loss programs (Bea et al. 2010; Phares et al. 2004; Rauhio et al. 2013). However, posthoc analyses revealed that supervised men carrying the Glu27 allele lost more weight and lowered BMI more (<0.05) than the Gln27Gln group, suggesting that supervised exercise and the Glu27 allele together in men can be beneficial to lose more weight. In previous studies of potential effect of this polymorphism on weight or BMI, also performed in Caucasian men subjects, was negative. Phares et al. applied a previous diet stabilization (but no caloric restriction) and aerobic training with subjects (age 50-75 years) with mean BMI of 27.8 kg/m^2, while the HERITAGE study only aerobic training with obese subjects, PRONAF consisted of a hypocaloric diet and exercise program. All three programs were between 20-24 weeks long, our intensity was a bit lower than the other two programs´ (PRONAF 50%-60% of the HRR, Garenc et al. 55%-75% of the maximal oxygen consumption (VO2max), Phares et al. 50%-70% of the VO2max), however our sessions were longer (PRONAF 51.15-60 min, Garenc et al. 30-50 min, Phares et al. 20-40 min)(Garenc et al. 2003; Phares et al. 2004). Garenc et al. reported bigger fat mass changes in obese Glu27 homozygote men in the same study (Garenc et al. 2003), which were not confirmed in our study. As for women most of the studies did not confirm the importance of this polymorphism with only exercise or with only

diet (Bea et al. 2010; Rauhio et al. 2013; Rosado et al. 2015), which are in line with our negative results. Nevertheless Ruiz et al. reported after a low energy mixed diet with a very similar sample to ours that Glu27 carriers reduced more weight and BMI (Ruiz et al. 2011). The added feature in our study compared to this is the exercise, which could balance the differences reported by them, as no differences were found for weight or BMI. On the contrary the HERITAGE study found that Glu27Glu women reduced less the percentage fat than the other two 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 have been reported in the literature to the contrary (Bea et al. 2010; Rauhio et al. 2013; Ukkola et al. 2003).

ADRB3 Trp64Arg post-intervention comparisons

The individual effect of the Arg64 allele was observed for fat mass and percentage fat reached the 0.05 level, for fat mass even the corrected threshold. Moreover there was an interaction with exercise was found for fat mass. However the main effect of the Trp64Arg polymorphism was observed for the change of other body composition variables or any interaction 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 were seen between carriers and non-carriers for weight or BMI what are in agreement with the other studies reporting the same after 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 the carriers of the Arg64 allele lost less fat mass and reduced percentage fat less than non-carriers during the intervention contrary to previous studies with 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 non-supervised group had higher final fat mass values than the supervised group, which can suggest that supervised exercise is beneficent for the these genotypes. The interaction of this gene and physical activity was raised before. Marti et al. reported that this polymorphism means higher obesity risk in sedentary people than in active people (Marti et al. 2002), which was in line with the results of other group (Phares et al. 2004). Within women the carriers of the Arg64 allele lost less fat mass than the non-carriers and non-supervised less fat mass than supervised subjects. Non-supervised Arg64 carrier women had smaller reduction in fat mass than non-carriers, though this should be cautiously judged because this group was very limited, but it should be pointed out that it is in line with the findings of Marti et al (Marti et al. 2002). Similarly, Bea et al. reported that after a 12 month-long resistance training program within the sedentary postmenopausal women (from normal weight to obese), Arg64 carriers gained significantly more percentage fat than non-carriers (Bea et al. 2010). As for android fat and VAT, differences were seen between carriers and non-carriers of the Arg64 allele. Conversely, Tchernof reported through a 13 month long diet program that Arg64 carriers lost 43% less VAT than the Trp64Trp group (Tchernof et al. 2000). This intervention was much longer and from the dietary point of view stricter than ours which could have been determinant.

The physiological changes of the receptor function caused by the gene variations together with the diet and exercise program could lead to divergent lipolysis rate and divergent amount of fat loss as a response to our program. However it is hard to state and confirm the underlying mechanisms of these differences for fat with our data as the project did not include deep physiological or molecular investigation. As it is well established 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; Arner 1995). The interaction of these polymorphisms and physical activity has been studied too and it seems to exist, but still not clear (Arner 2000; Meirhaeghe et al. 1999; Rosado et al. 2015). Our and previous studies' results are encouraging to further studies on this interaction.

Baseline – Gln27Glu and Trp64Arg polymorphisms

Our analyses showed no effect of the polymorphisms on BMI or percentage body fat or interaction of the polymorphisms and age or sex at baseline. Several studies, in agreement with our results, did not find 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 the Glu27 allele contributes to obesity risk (Clement et al. 1995; Gonzalez Sanchez et al. 2003; Lange et al. 2005; Large et al. 1997). 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 the 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 it is shown studies are not concordant. Part of the explication can be the study protocol, age and obesity status differences. Moreover previously it was suggested, 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 associations with obesity and the *ADRB2* Gln27Glu (Allison et al. 1998; Jalba et al. 2008), or *ADRB3* Trp64Arg,(Kurokawa et al. 2008) in Europeans, though do confirm the importance in other races, Asians, Pacific Islanders, and American Indians. Studies using new techniques

(GWAS) also confirm the same (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, as the main objective of the study was not the exploration of the genetic background of the 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, exercise groups and sexes, yet all results below 0.05 are reported and discussed. Another limitation of our study that there was no real control group for ethical reasons; all groups followed the individualized diet and the 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 of the field.

The conclusions of our study are that during an exercise plus diet program male carriers of the Glu27 allele of the *ADRB2* Gln27Glu polymorphism might have advantage in lowering weight and BMI, and carriers of the Arg64 allele of the *ADRB3* Trp64Arg polymorphism (especially women) might have difficulty in losing fat mass and percentage fat than the non-carriers in Spanish overweight and obese population. However for Arg64 allele carriers supervised exercise can help to lose more fat mass compensating the effect of the allele, which gives promising practical use. Nevertheless the evidence is weak, thus more research is needed on this field with larger sample sizes and controlled protocols, taking into account all possible interactions among diet, exercise, genetic background and other factors. Finally, physical activity seems influence the effect of these polymorphisms during weight loss as it was suggested before.

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Table 1. Baseline characteristics of the subjects

BASELINE WOMEN	***ADRB2***		***ADRB3***	
	Glu27 non-carrier (n=37)	**Glu27 carrier (n=54)**	**Arg64 non-carrier (n=76)**	**Arg64 carrier (n=15)**
Age (years)	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	***ADRB2***		***ADRB3***	
	Glu27 non-carrier (n=28)	**Glu27 carrier (n=54)**	**Arg64 non-carrier (n=73)**	**Arg64 carrier (n=9)**
Age (years)	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

Data presented as Mean ± Standard Deviation
ADRB2, β-2 adrenergic receptor; ADRB3, β-3 adrenergic receptor; BMI, body mass index; VAT, visceral adipose tissue

Table 2. Genotype distribution and allele frequency of the ADRB2 and ADRB3 genes

ADRB2					
	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)*
ADRB3					
	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)*
Data presented as n (%) for genotypes and n (frequency) for alleles. *ADRB2, β-2 adrenergic receptor; ADRB3, β-3 adrenergic receptors*					

Figure 1. *β-2 adrenergic receptor (ADRB2)* Gln27Glu polymorphism and weight and body composition variables after the intervention in men and women. Means of final values of Weight (a), Body mass index (BMI; b), Percentage body fat (fat %; c) are presented after adjustment for baseline values and age with 95% confidence intervals. * $p < 0.05$

Figure 2. *β-2 adrenergic receptor (ADRB2)* Gln27Glu polymorphism and body composition variables after the intervention in men and women. Means of final values of Fat mass (a), Android fat (b), Visceral adipose tissue (VAT; c) are presented after adjustment for baseline values and age with 95% confidence intervals.

Figure 3. *β-3 adrenergic receptor (ADRB3)* Trp64Arg polymorphism and weight and body composition variables after the intervention in men and women. Means of final values of Weight (a), Body mass index (BMI; b), Percentage body fat (fat %; c) are presented after adjustment for baseline values and age with 95% confidence intervals.

Figure 4. *β-3 adrenergic receptor (ADRB3)* Trp64Arg polymorphism and body composition variables after the intervention in men and women. Means of final values of Fat mass (a), Android fat (b), Visceral adipose tissue (VAT; c) are presented after adjustment for baseline values and age with 95% confidence intervals. ** $p<0.00625$