UNIVERSIDAD POLITÉCNICA DE MADRID

FACULTAD DE CIENCIAS DE LA ACTIVIDAD FÍSICA Y EL DEPORTE (INEF)

POLITÉCNICA

**Influence of diverse genetic
polymorphisms on body weight and body
composition during a weight loss program
/ Influencia de diversos polimorfismos
genéticos sobre el peso y la composición
corporal durante un programa de pérdida
de peso**

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DEPARTAMENTO DE SALUD Y RENDIMIENTO HUMANO
FACULTAD DE CIENCIAS DE LA ACTIVIDAD FÍSICA Y DEL DEPORTE
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Life

Life is an opportunity, benefit from it.

Life is beauty, admire it.

Life is a dream, realize it.

Life is a challenge, meet it.

Life is a duty, complete it.

Life is a game, play it.

Life is a promise, fulfill it.

Life is sorrow, overcome it.

Life is a song, sing it.

Life is a struggle, accept it.

Life is a tragedy, confront it.

Life is an adventure, dare it.

Life is luck, make it.

Life is too precious, do not destroy it.

Life is life, fight for it.

(Mother Teresa)

Utam során kalandjaim magam vagyok

tulajdonságaim összessége,

tovább kell mennem. Messze a cél.

A cél: a kaland, én vagyok.

(Varga Dániel)

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In two words: happy and grateful.

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%FFM: percentage fat free mass

ACSM: American College of Sports Medicine

ADRB2: beta-2 adrenergic receptor

ADRB3: beta-3 adrenergic receptor

AEPap: Asociación Española de Pediatría de Atención Primaria

AEPap: Spanish Association of Primary Care Pediatrics, Asociación Española de Pediatría de Atención Primaria

AHO: Albright hereditary osteodystrophy

Ala: alanine

AMP: adenosine monophosphate

ANCOVA: analysis of covariance

Arg: arginine

Asn: asparagine

Asp: aspartic acid

BBS: Bardet–Biedl Syndrome

BMI: body mass index

CI: confidence interval

DNA: deoxyribonucleic acid

DXA: dual-energy x-ray absorptiometry

EDTA: ethylene-diaminetetraacetic acid

ES: effect size

FFM: fat free mass

FTO: fat mass and obesity associated gene

GLM: general lineal model

Gln: glutamine

Glu: glutamic acid

GWAS: genome wide association studies

HWE: Hardy–Weinberg equilibrium

kg: kilogram

l: liter

LEP: leptin

LEPR: leptin receptor

Lys: lysine

m: meter

MCT1: monocarboxylates transporter 1

mRNA: messenger ribonucleic acid

NIH: National Institutes of Health

OMIM: Online Mendelian Inheritance in Man

PCR: polymerase chain reaction

PPARG: peroxisome proliferator activated receptor gamma

Pro: proline

PRONAF: Programas de Nutrición y Actividad Física para el tratamiento del sobrepeso y la obesidad

PWS: Prader–Willi Syndrome

QTLs: Quantitative Trait Loci

RCT: randomized control trial

RFLP: restriction fragment length polymorphism

SE: standard error

SEPEAP: Spanish Society of Outpatient Pediatrics and Primary Care, Sociedad Española de Pediatría Extrahospitalaria y Atención Primaria

SNP: single nucleotide polymorphism

Trp: tryptophan

VAT: visceral adipose tissue

WHO: World Health Organization

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Do common polymorphisms influence body weight and fat regain after a weight loss intervention?

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ABSTRACT

Introduction. Obesity is defined as an abnormal or excessive accumulation of fat that may impair health according to World Health Organization (WHO). Around the world it is every time more and more extended implying public health, economical and aesthetic problems. Researchers are trying to find the solution for the treatment or the prevention from different approaches such as diet, exercise, surgery, psychology, sociology what also shows its multifactorial causation. The genetic background of obesity has been proved by family and twin studies and up to date hundreds of genes have been identified to be related. However, today's knowledge explains only a small part of the development of obesity far from the 40-70% estimated for genetic influence on BMI. Obviously environmental factors are essential for weight loss and the prevention of obesity, but genetic information might help designing interventions in the future.

Objectives. The objective of the present thesis was to study the influence of previously suggested candidate genes related to obesity on body weight and body composition variables, during and after a weight loss intervention. The main objective of the studies I and II was to examine if there were differences among genotype groups of the *ADRB2* (Gln27Glu), *ADRB3* (Trp64Arg), *LEPR* (Gln223Arg and Lys656Asn), and *PPARG* (Pro12Ala) genes during a controlled intervention in body weight, body mass index (BMI), fat mass, percentage fat, android fat or visceral adipose tissue. The secondary objective of the studies I and II was to evaluate the influence of these polymorphisms on baseline variables, body mass index and percentage fat. The objective of study III was to examine differences between *MCT1* (Glu490Asp/T1470T) genotype groups in

body weight, BMI, percentage fat and percentage fat free mass. The objective of the study IV was to observe differences among genotype groups of the *ADRB2* (Gln27Glu), *ADRB3* (Trp64Arg), *LEPR* (Gln223Arg and Lys656Asn), *MCT1* (Glu490Asp/T1470T) and *PPARG* (Pro12Ala) genes 3 years after a free living follow up in body weight, BMI, fat mass and percentage fat.

Research design. This thesis is part of de *Programas de Nutrición y Actividad Física para el Tratamiento de la Obesidad* (PRONAF) project carried out in Spain. The PRONAF project was a clinical trial including 22-week long controlled exercise and diet program. The weight loss intervention for overweight and obese participants applied a hypocaloric diet and aimed to compare different exercise protocols (supervised: strength, endurance, combined strength and endurance and non-supervised: physical activity recommendations) in order to see which is the most effective. Strengthening the project, genetic polymorphisms related to obesity phenotypes were incorporated too.

Results. Glu27 carriers of the *ADRB2* gene in the supervised men group reduced weight and BMI more than the non-carriers ($p=0.019$, 2.52 kg and $p=0.019$, 0.88 kg/m²). Non-supervised women carrying of the Arg64 allele of the *ADRB3* gene ended up with higher fat mass values than non-carriers after the intervention ($p=0.004$, 7.22 kg). No differences were seen for body weight and body composition changes for the *LEPR* polymorphisms. Women non-supervised carriers of the Ala12 allele of the *PPARG* gene reduced visceral adipose tissue (VAT) less than non-carriers ($p=0.024$, 0.32 kg). No differences were found at baseline comparisons. Female non-carriers of the Glu490 allele of the *MCT1* gene reduced weight and BMI more than Glu490 allele carriers

($p=0.004$, 3.88 kg, effect size (ES) moderate and most likely positive and $p=0.00037$, 1.48 kg/m², ES large and most likely positive). In the female non-supervised group Glu490 allele carriers of the *MCT1* gene ended up with higher values of percentage fat ($p=0.025$, 1.94%), while the contrary in men, Glu490 allele carriers reduced fat more ($p=0.037$, 1.87%). In women non-carriers of the Glu490 allele of the *MCT1* gene finished the program with higher percentage fat free mass values than the carriers of the Glu490 allele ($p=0.005$, 2.76%, ES small and possibly negative). In the follow up study, carriers of the Arg64 allele of the *ADRB3* gene in men regained more weight and increased BMI more than non-carriers, however effect size was unclear. Male carriers of the Arg223 allele of the *LEPR* gene had higher percentage fat values than non-carriers, however effect size also was unclear. No other differences were seen between polymorphisms and follow up phenotypes.

Conclusions. Conclusions of study I: The Gln27Glu polymorphism of the *ADRB2* gene does not seem to influence weight or body composition changes. Arg64 allele of the *ADRB3* gene in women might be disadvantageous in reducing fat mass and fat percentage during a weight loss program. Conclusions of study II: The Gln223Arg polymorphism and the Lys656Asn polymorphism of the *LEPR* gene does not seem have effect on influence weight or body composition changes. Ala12 allele might imply difficulty in losing visceral adipose tissue. The *ADRB2* Gln27Glu, the *ADRB3* Trp64Arg, *LEPR* Gln223Arg and Lys656Asn polymorphisms are not likely to influence baseline obesity phenotypes. Conclusions of study III: Carrying the Glu490 allele of the *MCT1* gene might be related to smaller reductions in weight and BMI after a weight loss program. The Glu490 allele might be disadvantageous for women to lower percentage fat, but advantageous for men. Finally, the conclusions of the study IV: The Arg64 allele

of the *ADRB3* gene might be disadvantageous in weight maintenance in men. The Arg223 allele of the *LEPR* gene might facilitate body fat recovery in free-living conditions in men. The *ADRB2* Gln27Glu, the *LEPR* Lys656Asn, *MCT1* Glu490Asp, *PPARG* Pro12Ala polymorphisms do not seem to influence body weight or fat regain after a 3 year follow up.

Our results suggest that some of these polymorphism might affect body weight and body composition during a weight loss intervention and during subsequent 3-year long follow up, however these effects are not determinant and results should be replicated.

RESUMEN

Introducción. La obesidad se define como una acumulación anormal o excesiva de grasa que puede perjudicar la salud según la Organización Mundial de la Salud. En todo el mundo se extiende cada vez más, lo que implica problemas de salud pública, económicos y estéticos. Los investigadores están tratando de encontrar la solución para el tratamiento y la prevención de diferentes enfoques como la dieta, el ejercicio, la cirugía, la psicología, la sociología, lo que también muestra su causalidad multifactorial. Las bases genéticas de la obesidad han sido demostradas por estudios de familias y gemelos y hasta la fecha se han identificado cientos de genes relacionados. Sin embargo, el conocimiento de hoy sólo explica una pequeña parte del desarrollo de la obesidad, lejos del 40-70% estimado para la influencia genética en el índice de masa corporal. Obviamente, los factores ambientales son esenciales para la pérdida de peso y la prevención de la obesidad, pero la información genética podría ayudar a diseñar intervenciones en el futuro.

Objetivos. El objetivo de la presente tesis fue estudiar la influencia de genes candidatos previamente sugeridos relacionados con la obesidad en el peso corporal y las variables de composición corporal, durante y después de una intervención de pérdida de peso. El objetivo principal de los estudios I y II fue examinar si había diferencias entre los grupos de genotipo de los genes *ADRB2* (Gln27Glu), *ADRB3* (Trp64Arg), *LEPR* (Gln223Arg y Lys656Asn) y *PPARG* (Pro12Ala) durante una intervención controlada en peso corporal, índice de masa corporal, masa grasa, porcentaje de grasa, grasa androide o tejido adiposo visceral. El objetivo secundario de estos estudios fue evaluar la influencia de estos polimorfismos sobre las variables

basales, el índice de masa corporal (IMC) y el porcentaje de grasa. El objetivo del estudio III fue examinar diferencias entre grupos genéticos del polimorfismo *MCT1* (Glu490Asp/T1470T) en peso corporal, IMC, porcentaje de grasa y porcentaje de masa magra. El objetivo principal del estudio IV fue observar las diferencias entre los grupos genéticos de los genes *ADRB2* (Gln27Glu), *ADRB3* (Trp64Arg), *LEPR* (Gln223Arg y Lys656Asn), *MCT1* (Glu490Asp) y *PPARG* (Pro12Ala) durante un seguimiento de 3 años en peso corporal, IMC, masa grasa y porcentaje de grasa.

Diseño de la investigación. Esta tesis es parte del proyecto de *Programas de Nutrición y Actividad Física para el Tratamiento de la Obesidad* (PRONAF) realizado en España. El proyecto PRONAF fue un ensayo clínico que incluyó un programa controlado de ejercicio y dieta de 22 semanas de duración. La intervención de pérdida de peso para participantes con sobrepeso y obesidad aplicó una dieta hipocalórica y tuvo como objetivo comparar diferentes protocolos de ejercicio (supervisado: fuerza, resistencia, fuerza combinada y resistencia y no supervisado: recomendaciones de actividad física) para ver cuál es el más eficaz. Fortaleciendo el proyecto, se incorporaron polimorfismos genéticos relacionados con la obesidad.

Resultados. Portadores del Glu27 del gen *ADRB2* en el grupo supervisado de hombres redujeron el peso corporal y el IMC más que los no-portadores ($p=0.019$, 2.52 kg y $p=0.019$, 0.88 kg/m²). Mujeres no-supervisadas con el alelo Arg64 del gen *ADRB3* terminaron el programa con valores mayores en masa grasa que los no portadores ($p=0.004$, 7.22 kg). No había diferencias en cambio de peso o composición corporal en los polimorfismos de *LEPR*. Mujeres no-supervisadas con el alelo Ala12 del gen *PPARG* bajaron el tejido adiposo visceral menos que los no-portadores ($p=0.024$, 0.32 kg). No se encontraron diferencias en las comparaciones de los valores basales. Mujeres no-

portadores del alelo A del gen *MCT1* bajaron el peso y el IMC más que los portadores ($p=0.004$, 3.88 kg, tamaño del efecto moderado y lo más probable positivo y $p=0.00037$, 1.48 kg/m², tamaño del efecto grande y lo más probable positivo). Dentro de las mujeres no-supervisadas portadoras del alelo A del gen *MCT1* terminaron el programa con valores mayores de porcentaje de grasa ($p=0.025$, 1.94%), pero el contrario en hombres, los portadores del alelo A bajaron más el porcentaje de grasa ($p=0.037$, 1.87%). Mujeres no portadoras del alelo A del gen *MCT1* acabaron la intervención con más porcentaje de masa magra que los portadores ($p=0.005$, 2.76%, tamaño del efecto pequeño y posible negativo). En el estudio de seguimiento, portadores del alelo Arg64 del gen *ADRB3* en hombres recuperaron más peso e incrementaron el IMC más que los no-portadores, aunque el tamaño del efecto fue dudoso. Hombres con el alelo Arg223 del gen *LEPR* tuvieron valores de porcentaje de grasa mayores que los no-portadores aunque el tamaño del efecto fue dudoso también. No se encontraron otras diferencias entre polimorfismos y fenotipos del seguimiento.

Conclusiones. Conclusiones del estudio I: El polimorfismo Gln27Glu del gen *ADRB2* no parece influir los cambios de peso corporal o composición corporal. El alelo Arg64 del gen *ADRB3* en mujeres puede ser desventajoso para reducir masa grasa y porcentaje de grasa durante un programa de pérdida de peso. Conclusiones del estudio II: Los polimorfismos Gln223Arg y Lys656Asn del gen *LEPR* no parecen influir en los cambios de peso corporal o composición corporal. El alelo Ala12 del gen *PPARG* podría implicar dificultad en perder tejido adiposo visceral. No es probable que los polimorfismos *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *LEPR* Gln223Arg y Lys656Asn influyen fenotipos basales relacionados a la obesidad. Conclusiones del estudio III: Tener el alelo Glu490

del gen *MCT1* puede ser relacionada a reducciones menores en peso corporal y IMC después de un programa de pérdida de peso. El alelo Glu490 puede ser desventajoso para mujeres para bajar porcentaje de grasa, pero ventajoso para hombres. Finalmente, las conclusiones del estudio IV: El alelo Arg64 del gen *ADRB3* puede ser desventajoso en mantener el peso corporal. El alelo Arg223 del gen *LEPR* puede facilitar la recuperación de grasa corporal durante el seguimiento en hombres. Los polimorfismos *ADRB2* Gln27Glu, *LEPR* Lys656Asn, *MCT1* Glu490Asp y *PPARG* Pro12Ala no parecen influir la recuperación de peso corporal o masa grasa después de un seguimiento de 3 años.

Nuestros resultados sugieren que algunos de estos polimorfismos pueden afectar el peso corporal y la composición corporal durante una intervención de pérdida de peso y durante el seguimiento posterior de 3 años, sin embargo estos efectos no son determinantes y deben ser replicados.

1. INTRODUCTION

1. INTRODUCTION

1.1. Obesity

1.1.1. What is obesity?

The definition of the World Health Organization (WHO) of obesity is: “Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health” (WHO 2016). The epidemic of our era is getting frightening around the world. Figure 1 shows obesity prevalence in different countries and it is still increasing. It is not any more the problem of the developed, high-income countries, but developing, medium- and low-income countries are suffering too. It is well known that it is not only an aesthetic problem, but goes together with serious comorbidities ,such as cardiovascular diseases, diabetes, musculoskeletal disorders, some cancers and psychological problems (Costa-Font and Gil 2005; Varela-Moreiras et al. 2013), poorer quality of life and reduced life expectancy (WHO 2016). Overweight and obesity are linked to more deaths worldwide than underweight (WHO 2016).

Figure 1. Overweight and obesity prevalence in 2013 (both sexes, age +20 years)

Source: Institute for Health Metrics and Evaluation (<http://vizhub.healthdata.org/obesity/#>)

1.1.2. Obesity prevalence in Spain

Spain is in a leading position in Europe considering overweight and obesity prevalence in adults (Gallus et al. 2015), and statistics are even worse for children (Smith and Smith 2016). In fact, approximately 2 out of 3 people are overweight and 1 out of 6 people are obese (Aranceta et al. 2007; Gutiérrez - Fisac et al. 2012). Spanish children have been proved to be first in childhood obesity according to the Spanish Society of Outpatient Pediatrics and Primary Care (SEPEAP) and the Spanish Association of Primary Care Pediatrics (AEPap) (Schröder et al. 2014).

1.2. Genetics and obesity

1.2.1. Toward personalized medicine

Every time it seems to be clearer that personalized treatment is the best option in medicine to cure diseases and every day we get closer to this. It is well established that there are many influencing factors: social, cultural, genetic, physiological, metabolic, psychological and behavioral (McPherson, Marsh, and Brown 2007). The fact that prevalence of obesity has been rapidly increasing around the world in the past few decades and our genome has not changed much during this time, could make us think that only the “obesogenic environment” (ie. consumption of large portion size meals, high-fat diets, high sugar intake, many hours spent watching TV, playing video games or sitting at the computer) is responsible for this disease (Bouchard 2007). However our genes do allow for obesity to occur. The “thrifty gene” theory was already suggested in 1962 by James Neel, which includes these genes which helped the

families where these were inherited to survive. We are prone to weight gain, because the past it was an advantage to be able to store energy for harder times, i. e. famine. Nevertheless it was proposed that this theory is insufficient to explain all (Bouchard 2007; Speakman 2006). According to Bouchard, for sustained positive energy balance our present gene variants should be included in these categories: (1) a thrifty genotype: low metabolic rate and insufficient thermogenesis; (2) a hyperphagic genotype: poor regulation of appetite and satiety and propensity to overfeed; (3) a sedens genotype: propensity to be physically inactive; (4) a low lipid oxidation genotype: propensity to be a low lipid oxidizer; and (5) an adipogenesis genotype: ability to expand complement of adipocytes and high lipid storage capacity (Bouchard 2007). Based on his review on the genes suggested in the 2005 version of the human obesity gene map, do not confirm the “thrifty gene” theory (Bouchard 2007).

For certain diseases, when only one (or a few) gene is affected, based on the genetic background doctors can prescribe one or another medicine which works better achieving greater effect with less side effects (pharmacogenetics/ pharmacogenomics). Specific treatments can be ineffective for some people, effective for others and harmful for others. Hopefully science reaches this point soon for complex diseases (such as obesity) too; however, there is still a long way to go to understand the information of the potential genes, interaction between genes and interaction with the environment etc. Even though the genetic testing of multifactorial diseases has been questioned (Holtzman and Marteau 2000; Vineis, Schulte, and McMichael 2001), with advances might refute these doubts and distrust. Which in turn seems clear is that current gene tests on complex phenotypes are not trustworthy.

1.2.2. Evidence for the role of genetics in obesity

There is always an interaction between both genes and environment (de Castro 2004). Environment is significant, although the importance of the genetic background on obesity phenotypes (body mass index (BMI) and fatness) has been proved beyond doubt (as it can be read below). Borjeson according to the results of his twin study suggested that genetic factors play a decisive role in the origin of obesity and this was supported with images; in figure 2. some of these can be seen (Börjeson 1976).

Figure 2. Monozygotic (up) and dizygotic twins (down)

Source: (Börjeson 1976)

Ten years later, in a twin and an adoptee study Stunkard et al. found clear relation between adoptee weight class (ie. thin, normal, overweight, obese) and the body mass index of the biologic parents but not with the adoptive parents (Figure 3) (Stunkard et al. 1986).

Figure 3. Mean body-mass index of parents of for weight classes of adoptees

(BF denotes biologic father, BM biologic mother, AF adoptive father, AM adoptive mother)

Source: (Stunkard et al. 1986)

Stunkard et al. confirmed that genetic influences on BMI are substantial with a twin study reporting that the estimate of heritability is about 66% for women and 70% for men. (Stunkard et al. 1990; Stunkard et al. 1986) Several calculations have been made since this one, most of these heritability estimates are between 40% and 70% (Herrera and Lindgren 2010).

1.2.3. Types of obesity from the genetics point of view

Obesity first was thought to be the result of a monogenic mutation, but based on the research of the past decades it has been established that its common form rather has polygenic origins. Hereinafter the different genetic causes of obesity are explained.

Monogenic obesity

Only a few hundred cases have been described to be associated with a single gene mutation (however the number is much bigger) (Bouchard 2008). These lie in one of 11 genes which most of the cases encode proteins participating in appetite regulation and energy homeostasis, such as leptin (LEP), leptin receptor (LEPR), melanocortin 4 receptor (MC4R) or prohormone convertase 1 (POMC) (Bell, Walley, and Froguel 2005; Bouchard 2008). Figure 4 shows the location of these genes on the chromosomes. Almost always these patients have extreme obesity. Overall, about 5% of the obesity cases belong here (Bouchard 2008).

Figure 4. Chromosomal location of obesity genes

Ideogram of human karyotype with loci for Prader–Willi Syndrome (PWS), Albright hereditary osteodystrophy (AHO), Bardet–Biedl Syndrome (BBS) (green line), monogenic mutations (green triangle), selected obesity candidate genes (red triangle) and QTLs identified by genome-wide linkage scans (red line)

Source: (Loos and Bouchard 2003)

Syndromic obesity

Some of the rare syndromes are also characterized by obesity. The most frequent of these syndromes (1 in 25,000 births) is Prader–Willi syndrome (PWS), accompanied by obesity, hyperphagia, diminished foetal activity, muscular hypotonia, mental retardation, short stature and hypogonadotropic hypogonadism. Other examples are the pseudohypoparathyroidism type 1A syndrome, Albright hereditary osteodystrophy (AHO) or the Bardet–Biedl syndrome (BBS), these are less common (Bell, Walley, and Froguel 2005). The location of these syndromes are shown in the chromosome map in Figure 4. The aetiology of syndromic obesity is more complex than the monogenic cases thus understanding them is more complicated too and the treatment of these cases is even more complicated.

Polygenic obesity

In most cases obesity is a complex phenotype as a sum of countless factors. Among them there are numerous gene variants (even hundreds or thousands we still do not know) with small but still significant effects and they are very common in the population (the opposite of the monogenic obesity) (Mutch and Clément 2006).

There are three strategies to identify these genes:

- **Candidate gene studies:** This approach relies on the current understanding of the pathophysiology of obesity, candidate genes are selected based on their biological / physiologic function in the regulation of energy balance or adipose tissue and so on (Loos and Bouchard 2003). The last update of the Human Obesity Gene Map reported 127 candidate genes associated with obesity

phenotypes summarizing the findings of these studies (Rankinen et al. 2006).

Some of these genes are shown in Figure 4. However, it was suggested that results of these association studies are quite heterogeneous and at times inconsistent (Bouchard 2008).

- **Genome linkage scans:** The aim is to identify chromosomal regions of interest and then genes within this region.
- **Genome wide association studies (GWAS):** This approach, however, is studying the whole genome / most of the genes comparing cases and controls (for example obese and normal weight people) to identify gene variants which differ. GWAS made it possible to find the most influential gene associated with obesity, the Fat Mass and Obesity Associated gene (FTO) and other new potential genes. Adults homozygous for the risk allele of the FTO polymorphism weighted 3 kg more and had 1.67-fold increased odds of obesity when compared with the non-carriers in the first study identifying this gene (Frayling 2007), which was confirmed with the subsequent ones (Speliotes, Willer, Berndt, Monda, Thorleifsson, Jackson, Lango Allen, et al. 2010; Thorleifsson et al. 2009).

These two second approaches did not confirm the results of the candidate gene studies. Nevertheless, the most studied candidate genes (including the ones we examined in our study) are registered as genes related to obesity phenotypes in the most recognized databases like OMIM (Online Mendelian Inheritance in Man, An Online Catalog of Human Genes and Genetic Disorders).

1.2.4. Genetics of body weight response to diet or exercise

On the other hand, it is not enough to know the influencing factors of the development of obesity but also the influencing factors of the response to a treatment (to exercise in our case), which can be completely different. Some experimental studies were seeking for answers about heritability through overfeeding or negative energy balance with diet or exercise. Bouchard et al. overfed twelve identical twin pairs (during 100 days by 1000 kcal) and showed that the change in body weight differed considerably between the twin pairs but was similar within twin pairs (Figure 5), which confirms the influence of the genetic background (Bouchard et al. 1990).

Figure 5. Similarity within pairs with respect to changes in body weight in 12 pairs of male twins in response to 100 days of overfeeding

Each point represents one pair of twins (A and B). The closer the points are to the diagonal line, the more similar the twins are to each other.

Source: (Bouchard et al. 1990)

In a subsequent study of Bouchard et al. seven male identical twin pairs completed a 93 day negative energy balance program with exercise. In weight loss (mean 5.0 kg; equal fat loss as fat free mass was unchanged) there were large individual differences

in response to the program, but with the same genotype responses were more alike (Bouchard and Tremblay 1997). The F ratio of the between-pairs to the within-pairs variances reached about 6 for the body weight changes (Bouchard and Tremblay 1997). In line with these findings, Hainer et al. studied 14 obese premenopausal female identical twin pairs, who followed a 28 day weight loss intervention including a very low calorie diet and exercise. Mean body weight loss was 8.8 kg (SD=1.9). Variability was about 13 times more between identical twin pairs than within identical twin pairs for the changes of body weight (Hainer et al. 2000; Hainer et al. 2001). Body weight and fat loss for the monozygotic twins are illustrated in Figure 6.

Figure 1 Similarity within pairs with respect to decrease in body weight in 14 pairs of obese female twins in response to 28 days of very low calorie diet (1.6MJ). Each point represents one pair of twins (A and B).

Figure 2 Similarity within pairs with respect to decrease in body fat in 14 pairs of obese female twins in response to 28 days of very low calorie diet (1.6MJ). Each point represents one pair of twins (A and B).

Figure 6. Similarity within pairs with respect to decrease in body weight (left) and body fat (right) in 14 pairs of obese twins

Source: (Hainer et al. 2000)

Bouchard also suggested that the heterogeneity of responsiveness to regular exercise (not everybody responds the same way/ same equally to exercise or any environmental impact) is trait specific. In other words, someone who is low responder

for one phenotype can be a better responder for other traits (Bouchard, Blair, and Haskell 2012). This means that an obese patient may not lose much weight (preferably fat mass), but it is still likely that he will achieve health benefits from a physically active lifestyle (for example improving quality of life), which we have to keep in mind always.

Therefore, looking through these studies, the role of genes is unquestionable in obesity and weight loss, though at present we do not possess many details about it.

1.3. Polymorphisms, genes and their corresponding proteins included in the thesis

This present thesis is part of the PRONAF study, which aimed to discover which is the most effective training protocol in combination with caloric restriction for overweight and obese subjects. In order to see the genetic influence on the response to the treatment, based on the literature available at the time of designing the project genetic single nucleotide polymorphisms (SNPs) were selected. A SNP is an alteration in deoxyribonucleic acid (DNA) sequence that is present in the population with a frequency of at least 1% (Bouchard, Blair, and Haskell 2012). The SNPs give the genetic basis for the uniqueness of each individual, at least one in 1000 base pairs of human DNA differs among individuals (Passarge 1995). These SNPs are located in genes involved in metabolic pathways related to adiposity and in genes involved in energy metabolism and the production of energy during physical activity.

1.3.1. Beta adrenergic receptors

Beta-2 adrenergic receptor (ADRB2) and beta-3 adrenergic receptor (ADRB3) proteins

ADRB2 and ADRB3 belong to the G-protein coupled receptor 1 family, adrenergic receptor subfamily, ADRB2/ ADRB3 sub-subfamily. The structure of the proteins are shown in Figure 7 and 8.

Figure 7. Structure and reported polymorphisms of ADRB2 protein

Source: (Taylor and Bristow 2004)

Figure 8. The beta-3 adrenoceptor structure

Source: (Coman et al. 2009)

Function of the adrenergic receptors (together ADRB2 and ADRB3)

Adrenoceptors play a major role in several processes of our body, including fat cell metabolism as they are functionally expressed in fat cells. These receptors bind catecholamines (Arner 1992).

The principal role of adrenoceptors in brown fat cells is to regulate thermogenesis (Arner 1992). About 80% of heat is produced via beta receptors. The beta effect is by stimulating adenylate cyclase and cyclic adenosine monophosphate (AMP) production through the G_s protein, while the opposite effect is on the same pathway through the G_i protein (Figure 9). On the other hand, the role of the adrenergic receptors in white fat cells is to regulate the breakdown of the triglycerides to free fatty acids and glycerol through lipolysis, besides they influence lipid synthesis and the transport and metabolism of glucose (Figure 13). Beta receptors increase lipolysis too (Arner 1992).

Figure 9. Catecholamine receptor signal transduction in human fat cells

Source: (Arner 2001)

As contributors in physiological processes, the adrenergic receptors interact with hormones and other endogenous substances. Estrogens reduce the lipolytic function of the catecholamines in fat cells (Arner 1992) through adenylyl cyclase (Pecquery, Leneuve, and Giudicelli 1987), while androgens increase the lipolytic sensitivity of catecholamines (Arner 1992) through an increase in the number of beta receptors in fat cells (Xu, Pergola, and Björntorp 1990). Glucocorticoids have multiple effects on the beta receptor-adenylyl cyclase complex, though the exact explication is not known (Arner 1992). The increase in the number of the beta receptors and the modified function of the adenylyl cyclase was described before (Lacasa, Agli, and Giudicelli 1988). Catecholamines, through beta receptors, can inhibit insulin action in fat cells and therefore cause insulin resistance (Arner and Lonnqvist 1987). Moreover insulin can directly reduce beta receptor number in fat cells through translocation mechanism which reduces catecholamine sensitivity (Engfeldt et al. 1988). Lactate is another

factor which can alter the function of beta receptors, which occurs with the decrease in the lipolytic sensitivity of catecholamines (De Pergola et al. 1989).

Beta adrenergic receptors might operate altered in different physiological conditions. Fasting increases the in vivo lipolytic sensitivity of norepinephrine and epinephrine (Arner 1992), which leads to increased lipolytic activity (Jensen et al. 1987; Arner, Engelfeldt, and Nowak 1981). Caloric deficit might increase beta receptor number in fat cells (Östman et al. 1984). In turn, other studies suggest that overfeeding (high carbohydrate or high fat diets) does not seem to alter adrenergic receptor function in fat cells (Poehlman et al. 1986; Kather et al. 1987). Physical exercise causes similar adaptations to fasting, increases lipolytic action of catecholamines which adaptation is very rapid (Arner 1992). The explanation probably is in the effectiveness of hormone-sensitive lipase (Wahrenberg et al. 1987). In addition, a difference in adrenergic receptor function in fat cells has been observed between sexes. Indirect evidence suggests that there is an increased catecholamine-induced lipolysis in women (Arner et al. 1990). The background of it is not clear, might be the difference in the balance of alpha and beta adrenergic receptors in males compared with females (Leibel and Hirsch 1987).

The relation between obesity and adrenergic receptors is not solved yet. Circulating fatty acid levels are increased in obese people, which indicates alterations in the lipolysis (Arner 2005), which could be due to the adrenergic receptor / catecholamines, but studies are not concordant. Decreased lipolytic effect of catecholamines was reported in obese rats, however these studies had the confusing effect of age (as lean young rats were compared with old obese ones), therefore the real cause of these

changes is doubtful (Arner 1989). Stimulation of lipolysis (beta agonist) was observed in genetically obese mice too (Shepherd et al. 1977), though it is hard to compare monogenic obese mouse models with multifactorial human cases (Arner 1992). Studies on obese dogs showed that obese dogs might have higher number of alpha and lower number of beta receptors but it is counterbalanced with the increased activity of beta receptors (Arner 1992). On the other hand, in humans normal lipolytic sensitivity of catecholamines was found in vitro (Jacobsson et al. 1976) in agreement with an in vivo study reporting normal catecholamine activity through beta adrenergic receptors (Zahorska-Markiewicz, Kucio, and Piskorska 1986). The size of fat cells of the obese subjects larger than the non-obese subjects, and it was reported that larger fat cells have increased beta receptor activity (Arner 1992) and decreased alpha receptor activity (Arner et al. 1987), which suggest catecholamines might have an increased lipolytic activity in human obesity (Arner 1992), which contradicts the previous hypothesis on this topic.

ADRB2 Gln27Glu polymorphism

The *ADRB2* gene is located on the long arm of the chromosome 5 as it can be seen on Figure 10 (red line).

Figure 10. *ADRB2* Gene in genomic location: bands according to Ensembl, locations according to GeneLoc

Source: <http://www.genecards.org/cgi-in/carddisp.pl?gene=ADRB2&keywords=ADRB2>

Gln27Glu (rs1042714) polymorphism is a missense mutation, a Cytosine-Guanine base change, which results in a Glutamine-Glutamic acid amino acid change. Missense is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene (NIH 2017).

Generally there is little information about the functional alteration of the proteins with these polymorphisms, some studies aimed to investigate at the molecular level and find possible differences, which will be summarized at each polymorphism of the study.

An alteration at the amino acid sequence in the extracellular N-terminus of the ADRB2, the Gln27Glu polymorphism was suggested to alter ADRB2 function (Reihnsaus et al. 1993). Namely, altered degrees of agonist-promoted down-regulation of receptor expression were described (Green et al. 1994; Hesse and Eisenach 2008).

ADRB3 Trp64Arg polymorphism

The *ADRB3* gene is located on the short arm of the chromosome 8, illustrated in Figure 11 (red line).

Figure 11. *ADRB3* Gene in genomic location: bands according to Ensembl, locations according to GeneLoc

Source: <http://www.genecards.org/cgi-in/carddisp.pl?gene=ADRB3&keywords=ADRB3>

Trp64Arg (rs4994) polymorphism of the *ADRB3* gene is a missense mutation, a Thymine-Cytosine base change, which results in a Tryptophan-Arginine amino acid change.

Research groups of Snitker and Li found no functional differences between variants (Snitker et al. 1997; Li et al. 1996), however Piétri-Rouxel suggested that this mutant could be important in pathophysiological situations related to obesity (Pietri-Rouxel et al. 1997), in agreement with Umekawa, who reported that is associated with lower lipolytic activities, Arg64Arg homozygotes had lower median effective concentration, lower responsiveness compared with Trp64Trp (Umekawa et al. 1999).

1.3.2. Leptin receptor, LEPR

LEPR protein

The structure of the leptin receptor is shown in Figure 12.

Figure 12. Model for leptin and leptin receptor interaction

Source: (Peelman et al. 2014)

LEPR is the receptor of leptin. It is member of the cytokine receptor family. LEPR is involved in appetite control and the regulation of fat metabolism (beside other functions) (Schulz and Widmaier 2006).

Leptin

Leptin is mainly expressed in adipose tissue (Koerner, Kratzsch, and Kiess 2005) and it has an important role in the maintenance of homeostasis (Friedman and Halaas 1998; Zhang et al. 2005). As a proof of this 9-year old girl with extreme obesity was found to lack leptin, who reduced her weight to the normal range with a leptin treatment just like her cousin with similar symptoms (Montague et al. 1997; Farooqi et al. 2002).

Leptin reports to the central nervous system the abundance of available energy stores in order to prevent food intake and induce energy expenditure, therefore in lack of leptin hunger arises which leads to excess food intake and can result in morbid obesity, though only a handful of cases have been associated with leptin deficiency (Montague et al. 1997; Strobel et al. 1998). Leptin-induced inhibition of food intake is through both suppression of orexigenic neuropeptides (neuropeptide Y and agouti-related peptide) and the induction of anorexigenic neuropeptides (alpha melanocyte-stimulating hormone) (Strobel et al. 1998).

Leptin expression is increased by overfeeding, insulin, glucocorticoids, endotoxin and cytokines and is decreased by fasting, testosterone, thyroid hormone and exposure to cold temperature (Yang and Barouch 2007; Fried et al. 2000). However, acute changes in food intake do not induce short-term increases in plasma leptin levels (Jequier 2002; Considine et al. 1996), in contrast to rodents, where leptin gene expression is stimulated by eating and leptin administration acutely reduces food intake (Campfield

et al. 1995). Leptin secretion has a circadian rhythm, plasma leptin concentrations are low around noon and begin to rise around 3 in the afternoon and reach maximal values during the night (Jequier 2002). Women have markedly higher leptin concentrations than man independently of the amount of fat mass (Jequier 2002; Saad et al. 1997).

The complex function and interactions of leptin are illustrated in Figure 13.

Figure 13. Systemic leptin function

Chronic hyperleptinemia impairs the centrally mediated metabolic actions of the hormone, although its activation of sympathetic outflow is preserved. Selective central leptin resistance results in obesity and adverse effects on the cardiovascular system including hypertension, atherosclerosis, and left ventricular hypertrophy (LVH). Although leptin can protect against ectopic lipid deposition in non-adipose tissue, whether this effect is abolished because of (selective) peripheral leptin resistance requires further examination.

Source: (Yang and Barouch 2007)

Leptin and obesity

During continuous administration of leptin to ob/ob mice (obese mice without leptin) these animals lose weight or maintain their weight loss (Jequier 2002; Campfield et al.

1995; Halaas et al. 1995). Administration of leptin also reduces food intake and body weight in diet induced obese mice (Jequier 2002). In humans, leptin gene expression is increased in the adipose tissue of obese individuals (Lönnqvist et al. 1995) and in most cases their blood leptin levels are higher too, moreover a strong correlation between blood leptin levels and body fat mass has been showed (Considine et al. 1996).

Leptin receptor

The leptin receptor is expressed primarily in the brain, mainly in the choroid plexus and hypothalamic regions, but is also widely distributed in peripheral tissues including lung, kidneys, spleen, testes, uterus, lymph nodes, liver, pancreas and adipocytes (Tartaglia et al. 1995; Lee et al. 1996; Wauters et al. 2001) thereby referring to its multiple functions. Six different isoforms of the leptin receptor have been identified (Schulz and Widmaier 2006), but the two major isoforms are the long form (ObRb) and the short form (ObRa) (Lee et al. 1996).

Leptin receptors form heterodimers, which are capable of activating Janus kinases. Then the Janus kinase is able to start activators of the transcription family (Paracchini, Pedotti, and Taioli 2005; Watowich et al. 1996). Leptin receptors have been found to play a role in several aspects of human health. Not surprisingly, they are most associated with energy homeostasis, but there are other conditions in which leptin receptor function appears to be important (Schulz and Widmaier 2006). A mutation in the *LEPR* gene was reported to be associated with morbid obesity and infertility (Clement et al. 1998). Polymorphisms in the leptin receptor gene which typically affect the extracellular portion of the receptor and these do not result in such severe loss of function (Schulz and Widmaier 2006). Its involvement in obesity has been widely

studies, however results are controversial, besides it has been associated with sleep apnea (Manzella et al. 2002), bone mineral density (Koh et al. 2002) and lymphoma (Skibola et al. 2004).

LEPR Gln223Arg and Lys656Asn polymorphisms

The *LEPR* gene is located on the short arm of the chromosome 1 as it is shown in Figure 14 (red line).

Figure 14. *LEPR* Gene in genomic location: bands according to Ensembl, locations according to GeneLoc

Source: <http://www.genecards.org/cgi-bin/carddisp.pl?gene=LEPR&keywords=LEPR>

Gln223Arg (rs1137101) polymorphism of the *LEPR* gene is a missense mutation, a Adenine-Guanine base change, which results in a Glutamine-Arginine amino acid change.

Lys656Asn (rs1805094) is a missense mutation, a Guanine-Cytosine base change, which results in a Lysine-Asparagine amino acid change.

Amino acid substitutions result in changes of the electric charge from neutral to positive for Lys223Arg and from positive to neutral for Lys656Asn polymorphism, therefore, they are likely to have functional consequences resulting in altered receptor structure, function, signaling capacity and, in the broader perspective, an affected response to energy restriction (Gajewska et al. 2016; Chung et al. 1997; Yang et al. 2016).

1.3.3. Peroxisome Proliferator Activated Receptor Gamma, PPARG

PPARG protein

Figure 15. The structure of the PPARG protein

Source: <http://www.rcsb.org/pdb/explore.do?structureId=2PRG>

The structure of the PPARG protein is illustrated in Figure 15. PPARG is a transcription factor, member of the nuclear hormone receptor superfamily. It is mainly expressed in adipocytes, but also can be found in vascular smooth muscle cells and macrophages (Chawla et al. 1994). It is the master regulator of fat cell formation and lipid metabolism, modulates insulin sensitivity, cell proliferation and inflammation thus it contributes to obesity (Lehrke and Lazar 2005). PPARG may also act as a tumor suppressor gene, since it inhibits the growth of different cell types and induces apoptosis (Meirhaeghe and Amouyel 2004).

Due to the alternative slicing of the PPARG gene there are four messenger ribonucleic acid (mRNA) isoforms of PPARG1, PPARG2 and PPARG4, which encode the same

protein product and PPARG2 which contains an additional N-terminal 28 amino acid axon (Meirhaeghe and Amouyel 2004).

Function of the PPARG

PPARG has a key role in fat cell maturation (i.e. turning from a fibroblast-like preadipocyte to a mature, lipid-enriched adipocyte). It was seen that the expression and activation of PPARG was sufficient to induce adipogenesis (Tontonoz, Hu, and Spiegelman 1994; Lehrke and Lazar 2005), and the importance of this protein was confirmed in other functional and genetic knockdown experiments (Barak et al. 1999). PPARG also has a major role in regulating mature adipocytes. Great part of the knowledge on the underlying pathways came with the discovery that thiazolidinedione antidiabetic drugs are high-affinity ligands for PPARG (Lehmann et al. 1995; Lehrke and Lazar 2005). It controls several genes directly in these biological processes, such as lipoprotein lipase, fatty-acid transport protein, oxidized low-density lipoprotein receptor 1, which all favor adipocyte uptake of circulating fatty acids; phosphoenolpyruvate carboxykinase, glycerol kinase, and the glycerol transporter aquaporin 7, which promote recycling rather than export of intracellular fatty acids (Lehrke and Lazar 2005).

PPARG takes part in glucose metabolism. It was seen that mice with increased PPARG activity, due to a mutation, are protected from obesity-associated insulin resistance (Rangwala and Lazar 2004; Lehrke and Lazar 2005), while mice lacking PPARG in fat, muscle or liver are predisposed to developing insulin resistance (He et al. 2003; Lehrke and Lazar 2005; Norris et al. 2003). In humans, few patients with rare mutation in PPARG are severely insulin resistant (Barroso et al. 1999; Lehrke and Lazar 2005) and

carriers of the Pro12Ala polymorphism were found to have improved glucose homeostasis (Deeb, Fajas, Nemoto, Pihlajamaki, et al. 1998; Lehrke and Lazar 2005).

Triglyceride storage causes increased production of the hormone leptin in the adipose tissue. During long-term overfeeding leptin's expression is increased in order to limit further food intake and excessive weight gain. The production of leptin in adipose tissue is under negative control by PPARG as it promotes lipogenesis and tries to control wasteful lipolysis and fatty acid oxidation (Wang, Lee, and Unger 1999; Kersten, Desvergne, and Wahli 2000). Therefore, expression of both PPARG and leptin is reduced by fasting and increased by feeding (Kersten, Desvergne, and Wahli 2000).

Figure 16 shows the diverse and far-reaching effects of the PPARG protein.

Figure 16. Activation of PPARG results in beneficial effects (green arrows) as well as adverse side effects (red arrows)

Source: (Ahmadian et al. 2013)

Furthermore, PPARG can play a role in the development other diseases like polycystic ovary syndrome (Korhonen et al. 2003), cardiovascular diseases atherogenesis (Bishop - Bailey 2000), different cancers (Meirhaeghe and Amouyel 2004).

PPARG monogenic mutations

Rare mutations of the PPARG were found in patients, who had a complex clinical phenotype called 'PPARG ligand resistance syndrome' with partial lipodystrophy (subcutaneous fat is lost from the limbs and gluteal region but accumulated in the abdominal area), early-onset severe insulin resistance, type 2 diabetes, dyslipidemia (high triglycerides, low high-density lipoprotein HDL cholesterol), early-onset hypertension and hepatic steatosis (Barroso et al. 1999; Meirhaeghe and Amouyel 2004; Savage et al. 2003) This indicates that PPARG has a key role in insulin action, glucose homeostasis and fat mass control (Meirhaeghe and Amouyel 2004).

PPARG Pro12Ala polymorphism

The *PPARG* gene is located on the short arm of the chromosome 3 marked with a red line in Figure 17.

Figure 17. *PPARG* Gene in genomic location: bands according to Ensembl, locations according to GeneLoc

Source: <http://www.genecards.org/cgi-bin/carddisp.pl?gene=PPARG&keywords=PPARG>

Pro12Ala (rs1801282) polymorphism of the PPARG gene is a missense mutation, a Cytosine-Guanine base change, which results in a Proline-Alanine amino acid change.

In vitro it was seen that the PPARG Ala12 isoform has two-fold lower affinity for its PPAR response element and reduced ability to its promoters such as the lipoprotein lipase promoter (Deeb, Fajas, Nemoto, Pihlajamaki, et al. 1998; Meirhaeghe and Amouyel 2004). Other studies confirmed that the Ala12 allele causes a poorer function of the protein (Schneider et al. 2002; Masugi et al. 2000).

In line with the in vitro studies mentioned before, in vivo studies also confirmed decreased expression level of PPARG (Schneider et al. 2002; Simon et al. 2002). Three other in vivo studies with Caucasian obese and non-obese subjects independently from each other showed that insulin sensitivity was improved in Ala12 carriers (Stumvoll et al. 2001; Jacob et al. 2000; Koch et al. 1999). In addition, insulin-sensitivity of lipolysis (inhibited) was greater in Ala12 carriers (Stumvoll et al. 2001).

1.3.4. Monocarboxylate Transporter 1, MCT1

MCT1 protein

Figure 18. Closed and open structural models of the MCT1 protein

Source: (Wilson et al. 2009)

MCT1 belongs to the monocarboxylate transporters MCTs family (Halestrap and Wilson 2012). MCT1 is ubiquitous, can be found in muscle, heart, liver, white adipose tissue, intestine, red blood cells, placenta and brain and other tissues. MCT1 can transport several monocarboxylates such as lactate, pyruvate, ketone bodies, acetate, butyrate and propionate (Carneiro and Pellerin 2015; Halestrap 2012). The structure of the MCT1 protein can be seen in Figure 18.

Function of the MCT1

MCT1 is rather found on glia cells supporting a potential lactate shuttling mechanism between different cell types (Carneiro and Pellerin 2015). Moreover, indirectly MCTs might be involved in various key processes for body energy homeostasis (for example

fuel sensing). MCT isoforms has been shown to be overexpressed during high fat diet-induced obesity and hyperglycemia (Canis et al. 2009; Carneiro and Pellerin 2015; Pierre et al. 2007), which confirms a specific sensitivity to the metabolic status and the role of these proteins. Lactate, one of the substrates of the MCTs, has been showed to affect glucose-inhibited and glucose-excited neurons in the hypothalamus, which suggests importance in glucose metabolism (Song and Routh 2005; Carneiro and Pellerin 2015). Besides, other studies demonstrated that central exposure to lactate suppresses food intake and stimulates insulin secretion (Kokorovic et al. 2009; Cha and Lane 2009).

Muscle is involved in glucose homeostasis and insulin resistance and it is a major source and user of monocarboxylates (Carneiro and Pellerin 2015). A study demonstrated that food restriction reduces muscle lactate and glycogen content and authors described an increased lactate transport capacity in the muscle without altered MCT1 or MCT4 expression (Lambert et al. 2003). Obesity and exercise have been proved to alter MCT1 expression, both acute and chronic exercise increase MCT1 expression which seems to be correlated with energy demand (Thomas et al. 2012).

Lactate production in adipose tissue depends on nutritional state (Lovejoy, Mellen, and DiGirolamo 1990). This lactate serves as substrate in the liver for gluconeogenesis in fasting or for glycogen synthesis after eating. In obesity, lactate production in the adipose tissue is increased (which is correlated with insulin resistance) (DiGirolamo, Newby, and Lovejoy 1992). Hypoxia can also modify MCT 1,2 and expression and lactate production (De Heredia, Wood, and Trayhurn 2010).

MCT1 seems to have a key role in lipid and glucose homeostasis as it was identified as an antagonist of PPARG (Carneiro and Pellerin 2015). In addition, increased MCT1 and decreased MCT2 levels in liver were associated with glucagon and insulin sensitivity in underfed state. This can be explained as an adaptation for food restriction, the use of alternative substrates (such as ketone bodies) in order to maintain the normal function of the cells (Lizarraga-Mollinedo et al. 2012). In addition, monocarboxylates transporters seem to have important function in other organs such as the intestines or the pancreas, modifying energy homeostasis (Carneiro and Pellerin 2015).

Mouse model

A knockin mouse for *mct1* gene was created and its phenotype was characterized (Lengacher, Nehiri-Sitayeb, Steiner, Carneiro, Favrod, Preitner, Thorens, Stehle, Dix, and Pralong 2013). Homozygote for mutated alleles was not viable, only wildtype homozygotes and heterozygotes survived and lived normally. Mice of these genotypes were very similar; differences only came up when mice were fed a high-sugar, high-fat diet for several weeks. During high fat diet wildtype homozygote mice (both male and female) gained weight and became obese, these animals seemed to be sensitive to diet-induced obesity, while heterozygotes were resistant. It was revealed that heterozygote mice reduced food intake compared with wild type during the high-sugar, high-fat diet. This can be explained by the role of MCT1 in the hypothalamic neurons and nutrient sensing suggested before (Ainscow et al. 2002). Intestinal nutrient absorption was reduced in heterozygotes and their basal metabolism was higher compared to wildtype too. Of course, heterozygotes had a reduced fat mass compared

to homozygote wildtype mice (Lengacher, Nehiri-Sitayeb, Steiner, Carneiro, Favrod, Preitner, Thorens, Stehle, Dix, and Pralong 2013).

Glu490Asp polymorphism

The *MCT1* gene is located on the short arm of the chromosome 1 as it can be seen on Figure 19 marked with red line.

Figure 19. *MCT1* Gene in genomic location: bands according to Ensembl, locations according to GeneLoc

Source: <http://www.genecards.org/cgi-in/carddisp.pl?gene=SLC16A1&keywords=MCT1>

Glu490Asp (rs1049434) polymorphism of the *MCT1* gene leads to a Thymine -Adenine base change, which results in a Aspartic acid-Glutamic acid amino acid change.

A 40% reduction of lactate transport rate was identified in the erythrocytes carriers of this variant (Merezhinskaya et al. 2000). A recent functional study, reported that the Glu490Asp polymorphism caused an observable change in lactate transport via *MCT1*, since lactate uptake via the *MCT1* of the Glu490 allele was increased compared with the Asp490 allele (Sasaki et al. 2015).

2. OBJECTIVES

2. OBJECTIVES

This present thesis is structured in four studies giving the whole of the analyses of the six polymorphisms throughout the PRONAF study. The general objective of the present thesis was to observe the influence of the *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *LEPR* Gln223Arg and Lys656Asn, *MCT1* Glu490Asp and *PPARG* Pro12Ala polymorphisms on weight and body composition changes during a controlled diet and exercise weight loss program and a subsequent 3-year follow up.

Objective of Study I.

Objective 1: Analyze the effect of two common polymorphisms, the *ADRB2* Gln27Glu and the *ADRB3* Trp64Arg on the changes of body weight, body mass index and fat distribution during a highly controlled exercise and diet weight loss program.

Objective 2: Examine the influence of *ADRB2* Gln27Glu and the *ADRB3* Trp64Arg polymorphisms on baseline values of body mass index and percentage fat.

Objective of Study II.

Objective 1: Analyze the effect of three common polymorphisms, Gln223Arg and Lys656Asn of the *LEPR* and Pro12Ala of the *PPARG* on changes in body mass, body mass index, fat mass, percentage fat, android fat and visceral adipose tissue during a highly controlled exercise and diet weight-loss program.

Objective 2: Evaluate the effect of the Gln223Arg and Lys656Asn of the *LEPR* and Pro12Ala of the *PPARG* polymorphisms on baseline body mass index and fat percentage.

Objective of Study III.

Objective 1: Analyze the influence of the *MCT1* Glu490Asp polymorphism on changes in body mass, body mass index, percentage fat and percentage fat free mass during a highly controlled exercise and diet weight-loss program.

Objective of Study IV.

Objective: Analyze whether the *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *LEPR* Gln223Arg and Lys656Asn, *PPARG* Pro12Ala, *MCT1* Glu490Asp polymorphisms influence weight and body fat maintenance after the completion of a controlled diet and exercise weight loss program.

3. MATERIALS, METHODS, RESULTS & DISCUSSION

3. MATERIALS, METHODS, RESULTS AND DISCUSSION

3.1. Study I. Influence of *ADRB2* Gln27Glu and *ADRB3* Trp64Arg polymorphisms on body weight and body composition changes after a controlled weight-loss intervention

Abstract

The Beta-2 and Beta-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/m²) 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 non-carriers ($p = 0.004$, 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.

Introduction

The response to weight loss programs is influenced by genetic factors (Loos and Rankinen 2005; Ordovas and Shen 2008; Bouchard 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 beta-2 and beta-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, 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 (Kortner et al. 1999; Echwald et al. 1998; Bea et al. 2010; Rosado, Bressan, and Martinez 2015). While some researchers found that the Glu27 is the risk allele for obesity (Clement et al. 1995; Large et al. 1997; Gonzalez Sanchez et al. 2003; Lange et al. 2005), 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 (Widen et al. 1995; Clement et al. 1995; Corella et al. 2001; Ukkola et al. 2000).

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, Bressan, and Martinez 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 (Ukkola et al. 2003; Bea et al. 2010), 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; Speliotes, Willer, Berndt, Monda, Thorleifsson, Jackson, Allen, et al. 2010; Locke et al. 2015; Shungin et al. 2015), 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/m², were non-smokers, sedentary (i.e., two hours or less of structured exercise per week) (Brochu, Malita, 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). Finally, 173 subjects were included (91 women, 82 men) in this study. 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, endurance, combined strength and endurance and the control groups 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 (NIH 1998). Macronutrient distribution was set according to the Spanish Society of Community Nutrition recommendations (Dapcich et al. 2004).

Exercise intervention

All exercise training groups 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 control group followed the dietary intervention and the physical activity recommendations of the American College of Sports Medicine (ACSM) (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 ($[\text{estimated kcal of diet}/\text{real kcal intake}] \times 100$), 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 ($[\text{sessions performed}/\text{total sessions}] \times 100$). 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 $[\text{body weight (kg)}]/[\text{height (m}^2\text{)}]$. 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 $[\text{fat (kg)} / \text{body weight (kg)} * 100\%]$.

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 (Large et al. 1997; Clement et al. 1995); 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 Quantile-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 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) are shown in Table 1.

Table 1. Baseline characteristics of the subjects in Study I

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, beta-2 adrenergic receptor; ADRB3, beta-3 adrenergic receptor; BMI, body mass index; VAT, visceral adipose tissue				

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. Genotype distribution and allele frequency of the *ADRB2* and *ADRB3* genes in Study I

<i>ADRB2</i>					
	<i>Gln27Gln</i>	<i>Gln27Glu</i>	<i>Glu27Glu</i>	<i>Allele Gln27</i>	<i>Allele Glu27</i>
<i>All</i>	65 (37.57%)	82	26 (15.03%)	212 (0.61)	134 (0.39)
<i>Women</i>	37 (40.65%)	40	14 (15.4%)	114 (0.63)	68 (0.37)
<i>Men</i>	28 (34.14%)	42	12 (14.64%)	98 (0.60)	66 (0.40)
<i>ADRB3</i>					
	<i>Trp64Trp</i>	<i>Trp64Arg</i>	<i>Arg64Arg</i>	<i>Allele Trp64</i>	<i>Allele Arg64</i>
<i>All</i>	148	24	1 (0.58%)	320 (0.92)	26 (0.08)
<i>Women</i>	76 (83.52%)	15	0 (0%)	167 (0.92)	15 (0.08)
<i>Men</i>	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. <i>ADRB2</i> , beta-2 adrenergic receptor; <i>ADRB3</i> , beta-3 adrenergic receptors					

***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/m² respectively; Figure 20). No differences were found for the other variables (Figure 20).

Figure 20. Beta-2 adrenergic receptor (ADRB2) Gln27Glu polymorphism and weight and body composition variables after the intervention in men and women

Gln27Gln (Gln27), Gln27Glu and Glu27Glu (Glu27). Means of final values of Weight (a), Body mass index (BMI; b), Fat mass (c) Percentage body fat (%fat; d), Android fat (e), Visceral adipose tissue (VAT; f) are presented after adjustment for baseline values and age with Standard error (SE). * $p < 0.05$

ADRB3 Trp64Arg post-intervention comparisons

Regarding the Trp64arg polymorphism, no differences were seen for weight, BMI, android fat or VAT (Figure 21). The individual effect of the polymorphism was found for fat mass ($p=0.004$, $F=8.519$ (1), $\eta^2=0.51$) and percentage fat ($p=0.036$, $F=4.457$ (1),

$\eta p=0.028$), 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), $\eta p=0.027$). 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 21). 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).

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.

Figure 21. Beta-3 adrenergic receptor (*ADRB2*) Gln27Glu polymorphism and weight and body composition variables after the intervention in men and women

Gln27Gln (Gln27), Gln27Glu and Glu27Glu (Glu27). Means of final values of Weight (a), Body mass index (BMI; b), Fat mass (c) Percentage body fat (%fat; d), Android fat (e), Visceral adipose tissue (VAT; f) are presented after adjustment for baseline values and age with Standard error (SE). * $p < 0.05$

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, European population of the 1000 Genomes Project (Abecasis et al. 2012) and to previous studies on Spanish samples for the *ADRB2* gene (Martinez et al. 2003; Gonzalez Sanchez 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/m², 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, Bressan, and Martinez 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; Ukkola et al. 2003; Rauhio et al. 2013).

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 (Tchernof et al. 2000; Bea et al. 2010; Rawson et al. 2002; 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; Ukkola et al. 2003; Rawson et al. 2002; Tchernof et al. 2000). 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, 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, Bressan, and Martinez 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 (Gonzalez Sanchez et al. 2003; Lange et al. 2005; Large et al. 1997; Clement et al. 1995). 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 (Jalba, Rhoads, and Demissie 2008; Allison et al. 1998), 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; Speliotes, Willer, Berndt, Monda, Thorleifsson, Jackson, Allen, et al. 2010; Locke et al. 2015; Shungin et al. 2015).

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.

3.2. Study II. *LEPR* and *PPARG* gene polymorphisms: associations with body composition changes in response to a weight loss intervention

Abstract

The aim of this present study was to analyze the effect of common polymorphisms of the leptin receptor (*LEPR*, *Gln223Arg*, *Lys656Asn*) and the peroxisome proliferator-activated receptor gamma (*PPARG*, *Pro12Ala*) genes on the changes of body mass and body composition after a weight loss intervention. 173 healthy overweight and obese volunteers (91 women, 82 men) aged 18-50 years participated in a 22 week-long intervention. They were randomized into four groups: strength, endurance, combined and physical activity recommendations while hypocaloric diet was prescribed for everybody. Body mass and body composition variables were assessed before and after the program using a scale and dual-energy X-ray absorptiometry. Genetic analysis was carried out according to standard protocols. ANCOVA was used to assess differences between genotype groups. Genotype distributions were in Hardy–Weinberg equilibrium. No differences were found at the baseline comparison, neither for final values in the case of the *LEPR* polymorphisms. However a possible interaction was seen between the Ala12 allele (*PPARG*) and sex ($p=0.041$) for visceral adipose tissue (VAT), but not for the other phenotypes. Post hoc analysis showed that carriers of the Ala12 allele finished the intervention with higher VAT levels than the non-carriers ($p=0.036$; 0.89 kg vs. 0.71 kg). Our results suggest that these polymorphisms of the *LEPR* or *PPARG* genes have no effect on body composition variables during a controlled

intervention or at baseline, except the Pro12Ala polymorphism on VAT changes in women, where carriers of the Ala12 allele might have difficulty to lose VAT.

Introduction

Obesity is one of the major problems of our era. The root of obesity cannot be described simply with the well-known energy balance, it is much more complicated, the combination of social, cultural, genetic, physiological, metabolic, psychological and behavioral and other factors (McPherson, Marsh, and Brown 2007). On the other hand obesity is associated with common comorbidities such as diabetes, hypertension, heart disease, hypercholesterolemia, and directly related to increased mortality and reduced life expectancy according to the World Health Organization (Organization 2000). Great amount of research has been done on this topic, but it seems that there is still a long way to find the solution to treat obesity.

Leptin receptor (LEPR) is a component of the leptin-insulin pathway, binds the leptin which is a protein mainly produced in adipose tissue (Yang, Kelly, and He 2007). Leptin jointly with other neuropeptides regulates food intake, appetite control, energy homeostasis and thermogenesis (Jequier 2002; Dulloo et al. 2002). Peroxisome proliferator-activated receptor gamma (PPARG) is a nuclear receptor (specifically expressed in adipose tissue) that influences the transcriptional processes regulating adipocyte differentiation, lipid storage, fat-specific gene expression and insulin signaling (Auwerx 1999; Lehrke and Lazar 2005; Meirhaeghe and Amouyel 2004). Common single nucleotide polymorphisms (SNP) of *LEPR* (Gln223Arg, Lys656Asn) and *PPARG* (Pro12Ala) genes have been suggested to be associated with obesity (body mass and body composition phenotypes) related phenotypes and have been widely

studied from the epidemiological aspect, however just a handful of studies can be found including diet and exercise intervention. To the best of our knowledge none of the previous studies included diet together with comparing supervised and non-supervised exercise. In any case, results of these candidate gene studies for all three polymorphisms are very controversial and genome-wide association studies (GWAS) did not confirm their importance in relation with obesity phenotypes (no GWAS has been carried out on response to interventions), which encourage us for further research.

Consequently, the aim of the current study was to analyze the effect of three common polymorphisms, Gln223Arg and Lys656Asn of the *LEPR* and Pro12Ala of the *PPARG* on changes in body mass, body mass index (BMI), fat mass, percentage fat, android fat and visceral adipose tissue (VAT) during a highly controlled exercise and diet weight-loss program and as a secondary objective to see their effect on baseline BMI and fat percentage.

Methods

Study design

This present study is the part of the Randomized Controlled Trial (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 project was to evaluate the usefulness of different types of physical activity and diet programs for the treatment of adult obesity through a 22 week long intervention. It was conducted in 2010 for overweight volunteers and in 2011 for obese volunteers (Zapico

et al. 2012). The measurements took place for all subjects during the week before training and during week 24 (after training).

Subjects

Subjects were overweight and obese [body mass index (BMI) 25–34.9 kg/m²] between 18-50 years from the Region of Madrid, Spain, and were recruited through diverse advertisements in the media. All of them was healthy nonsmoker, sedentary (one or less exercise bout per week) (Brochu, Malita, et al. 2009) and normoglycemic. Volunteers with any physical or psychological disease were excluded. Women had regular menstrual cycles. The voluntary subjects who fulfilled the inclusion criteria and passed the baseline physical examination were stratified by age and BMI and randomized into three training groups and a non-supervised group. After drop outs, 173 subjects were included (91 women, 82 men) in this study. In agreement with the guidelines of the Declaration of Helsinki regarding research on human subjects, before participating in this investigation, participants read and signed an institutionally approved informed consent document. All subjects were carefully informed about the possible risks and benefits of the project, which was approved by the Human Research Review Committee of the University Hospital La Paz (PI-643).

Diet intervention

The hypo-caloric diet (25-30% caloric restriction) of the participants was individualized based on a before-intervention negative energy balance calculation taking into account their own daily energy expenditure using on accelerometry data and the 3-day food record (NIH 1998). Macronutrient distribution was set according to the Spanish Society of Community Nutrition recommendations, approximately, 29–34% of the

energy came from fat, 14–18% from protein, and 50–55% from carbohydrates (Sacks et al. 2009). In the obesity group, energy provided from protein was increased up to 14–20% (Dapcich et al. 2004). The dietitian interviewed each participant at baseline, 3 months, and 6 months and reviewed a 3-day food diary.

Exercise intervention

Participants were randomized into a strength, endurance, combined strength and endurance training group (supervised groups) or non-supervised group. Subjects in the strength, endurance, combined strength and endurance training group performed training 3 times per week for 22 weeks at a sports center. All training sessions were carefully supervised by certified personal trainers. The exercise programs were designed taking into account each subject's muscle strength using the 15-repetition maximum and the heart rate reserve. Both volume and intensity of exercise was increased over the study period according to standard procedures (Hunter et al. 2008; Del Corral et al. 2009). Details of the different protocols developed by the groups are described elsewhere (Zapico et al. 2012).

Non-supervised group

Participants in the non-supervised group had the same dietary intervention as the rest of the groups and were encouraged to follow 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.

Body composition

Anthropometric measures included height (SECA stadiometer, Valencia, Spain, 0.01 m) and body mass (TANITA BC-420MA balance, Bio Lógica Tecnología Médica S.L, Barcelona, Spain, 0.1 kg). BMI was calculated as $[\text{body mass (kg)} / (\text{height (m)})^2]$. Fat mass (kg), android fat (kg) and visceral adipose tissue (VAT) 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 and percentage body fat (% body fat) was calculated as $[\text{fat (kg)} / \text{body mass (kg)} * 100\%]$.

Genetic analysis

Whole blood samples (5 ml) were collected in ethylene-diaminetetraacetic acid 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. For the overweight phase, the analysis of the Gln223Arg (rs1137101) and Lys656Asn (rs1805094) of the *LEPR* and the Pro12Ala of the *PPARG* (rs1801282) polymorphisms was done using polymerase chain reaction and restriction fragment length polymorphism techniques described before (Gotoda et al. 1997; Chung et al. 1997; Hara et al. 2000; Matsuoka et al. 1997); and for the obese phase, the genotyping of the three 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. Normal distribution of each dependent variable was tested using Quantile-Quantile plots, when needed Box-Cox transformation was applied using the optimal lambda value. Three genetic models (additive, dominant, recessive) were tested for the *LEPR* Gln223Arg polymorphism, however for the *LEPR* Lys656Asn and the *PPARG* Pro12Ala polymorphisms two groups (carriers and non-carriers of the minor allele) were analyzed because of the low number of the homozygotes for the minor allele. Based on exercise, the sample was divided in two groups: supervised (strength, endurance and combined strength and endurance training groups) as all protocols had the same characteristics (intensity, duration and frequency) and non-supervised group (C group). 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. Three-way (genotype x exercise group x sex) analysis of covariance (ANCOVA) was conducted adjusted by age and initial values to reveal interactions and differences between genotype groups, genders and exercise groups, for the final values of body mass, BMI, fat mass, percentage fat, android fat and VAT. Bonferroni test was used for post hoc comparisons. Statistical significance for post intervention comparisons was defined at the corrected alpha of 0.00179 for the *LEPR* Gln223Arg polymorphism and 0.00625 for the *LEPR* Lys656Asn and the *PPARG* Pro12Ala polymorphisms. For baseline comparisons statistical significance was set at 0.00357 for the *LEPR* Gln223Arg polymorphism and 0.0125 for the *LEPR* Lys656Asn and

the *PPARG* Pro12Ala polymorphisms with correction for repeated tests across the levels of the ANCOVA model factors, where appropriate. Correction was carried out taking into account sex, exercise groups, genetic models and genotype groups.

Results

Baseline characteristics of the subjects who participated in present study (after dropouts, exclusions because of low adherence, and missing data) are summarized in table 3.

Table 3. Baseline characteristics of the subjects in Study II

	Women (91)	Men (82)
Age (years)	39.12 ± 8.27	39.65 ± 8.24
Height (m)	1.62 ± 0.07	1.76 ± 0.06
Body weight (kg)	80.74 ± 10.55	95.99 ± 10.72
BMI (kg/m²)	30.48 ± 3,21	30.95 ± 2.71
Fat mass (kg)	35.04 ± 6.41	33.59 ± 6.98
Fat percentage (%)	45.15 ± 4.04	36.36 ± 4.78
Android fat (kg)	2.89 ± 0.72	3.44 ± 0.93
VAT (kg)	0.76 ± 0.37	1.74 ± 0.72
<i>Data presented as Mean ± Standard Deviation</i>		
<i>BMI, body mass index; VAT, visceral adipose tissue</i>		

Genotype distribution and allele frequencies

Genotype distributions and allele frequencies of the Gln223Arg and Lys656Asn polymorphisms of the *LEPR* gene and the Pro12Ala polymorphism of the *PPARG* gene

are shown in Table 2. All genotype distributions were found to be in Hardy–Weinberg equilibrium ($p=0.582$ for the Gln223Arg, $p=0.137$ for the Lys656Asn, $p=0.717$ for the Pro12Ala polymorphism).

Pre-intervention comparisons

No p value reached the corrected significance level, not even the permissive 0.05 level for any of the three polymorphisms for BMI or percentage fat before the intervention.

***LEPR* post-intervention comparisons**

No p value of main effects or interactions reached the corrected significance levels or the 0.05 level for two analyzed polymorphisms of the leptin receptor gene for any of the final values of body mass, BMI, fat mass, percentage fat, android fat or VAT. Final values of the body composition parameters in different groups are shown in Figure 22 and 23.

Table 4. Genotype distribution and allele frequency of the Gln223Arg and Lys656Asn polymorphisms of the LEPR gene and the Pro12Ala polymorphism of the PPARG gene in Study II

LEPR Gln223Arg					
	Gln223Gln	Gln223Arg	Arg223Arg	Gln223	Arg223
All	62 (35.84)	86 (49.71)	25 (14.45)	210 (0.61)	136 (0.39)
Women	31 (34.07)	47 (51.65)	13 (14.28)	109 (0.60)	73 (0.40)
Men	31 (37.80)	39 (47.56)	12 (14.64)	101 (0.62)	63 (0.38)
LEPR Lys656Asn					
	Lys656Lys	Lys656Asn	Asn656Asn	Lys656	Asn656
All	118 (68.21)	53 (30.64)	2 (1.15)	289 (0.84)	57 (0.16)
Women	65 (71.43)	25 (27.47)	1 (1.10)	155 (0.85)	27 (0.15)
Men	53 (64.63)	28 (34.15)	1 (1.22)	134 (0.82)	30 (0.18)
PPARG Pro12Ala					
	Pro12Pro	Pro12Ala	Ala12Ala	Pro12	Ala12
All	143 (82.66)	29 (16.76)	1 (0.58)	315 (0.91)	31 (0.09)
Women	77 (84.62)	14 (15.38)	-	168 (0.92)	14 (0.08)
Men	66 (80.49)	15 (18.29)	1 (1.22)	147 (0.90)	17 (0.10)
Data presented as n (%) for genotypes and n (frequency) for alleles. LEPR leptin receptor; PPARG peroxisome proliferator-activated receptor gamma; Gln, glutamine; Arg, arginine; Lys, lysine; Asn, asparagine; Pro, proline; Ala, alanine.

Figure 22. LEPR Gln223Arg polymorphism and body composition variables after the intervention in men and women

Gln223Gln (Arg223-), Gln223Arg and Arg223Arg (Arg223+). Means of final values of Body mass (a), BMI (b), Fat mass (c), Percentage fat (d), Android fat (e), Visceral adipose tissue (VAT; f) are presented after adjustment for baseline values and age with Standard error (SE)

Figure 23. LEPR Lys656Asn polymorphism and body composition variables after the intervention in men and women

Lys656Lys (Asn656-), Lys656Asn and Asn656Asn (Asn656+). Means of final values of Body mass (a), BMI (b), Fat mass (c), Percentage fat (d), Android fat (e), Visceral adipose tissue (VAT; f) are presented after adjustment for baseline values and age with Standard error (SE)

PPARG post-intervention comparisons

A possible interaction was found between the *PPARG* Ala12 allele and sex for VAT but did not reach the corrected significance level ($p=0.041$, $F=4.228$ (1), $\eta p=0.026$). Post hoc analyses showed a trend for difference ($p=0.036$; 0.89 kg vs. 0.71 kg) in women between carriers and non-carriers of the *PPARG* Ala12 allele, namely that carriers

ended up at higher final VAT values after the weight loss program. This was seen again in the non-supervised women subgroup with a lower but still not significant p value ($p=0.024$; 0.98 kg vs 0.66 kg, Figure 3(f)). However no other differences were observed for the other body composition parameters. All *PPARG* post-intervention results can be seen in Figure 24.

Figure 24. *PPARG* Pro12Ala polymorphism and body composition variables after the intervention in men and women

Pro12Pro (Ala12-), Pro12Ala and Ala12Ala (Ala12+) Means of final values of Body mass (a), BMI (b), Fat mass (c), Percentage fat (d), Android fat (e), Visceral adipose tissue (VAT; f) are presented after adjustment for baseline values and age with Standard error (SE) * $p < 0.05$

Discussion

We have studied the role that two polymorphisms of the leptin receptor (*LEPR*) and one of the peroxisome proliferator-activated receptor gamma (*PPARG*) genes might have on body composition changes after a 24-week-long diet and exercise intervention (supervised or non-supervised group) in healthy overweight and obese subjects. Our results do not support evidence of the influence of the Gln223Arg, Lys656Asn (*LEPR*) and Pro12Ala (*PPARG*) polymorphisms on body mass, BMI, fat mass, percentage fat, android fat or VAT changes except a possible interaction between the Pro12Ala polymorphism and sex on VAT changes.

Genotype distribution and allele frequencies

The genotype distribution and allele frequencies of all three polymorphisms were comparable with the those of the Iberian European population of the 1000 Genomes Project (Abecasis et al. 2012) and other previous studies (De Luis et al. 2011; Mammes et al. 2001; Goyenechea, Dolores Parra, and Alfredo Martinez 2006).

LEPR and *PPARG* polymorphisms pre-intervention comparisons

We have not found any associations at baseline between BMI or percentage fat and the any of the three polymorphisms. As we have mentioned before previous literature is not consistent on it. No association was observed in several studies for the Gln223Arg polymorphism (Gotoda et al. 1997; Silver et al. 1997; Wauters et al. 2001; Constantin et al. 2010; Mergen et al. 2007; Pyrzak et al. 2009), for the Lys656Asn polymorphism (Chagnon et al. 2000; Gotoda et al. 1997; Rosmond et al. 2000; Silver et al. 1997; De Luis et al. 2011; Mammes et al. 2001; Yiannakouris et al. 2001) and for the

Pro12Ala polymorphism (Goyenechea, Dolores Parra, and Alfredo Martinez 2006; Lindi et al. 2002; Nicklas et al. 2001; Zarebska et al. 2014). On the other hand advantageous or disadvantageous nature of the polymorphism regarding obesity (i.e. carrying the rare allele implied lower weight or BMI in some studies and higher weight or BMI in others) was observed for the Gln223Arg (Chagnon et al. 2000; Masuo et al. 2008; Portoles et al. 2006; Yiannakouris et al. 2001), Lys656Asn polymorphism (Liu et al. 2004; Masuo et al. 2008), for the Pro12Ala polymorphism (Vogels et al. 2005; Beamer et al. 1998). As it can be seen in the previous lines, most studies do not affirm the relevance of these polymorphisms on obesity related phenotypes. Inconsistency can be due to differences in project characteristics such as ethnicity (we have only considered studies including Caucasian subjects), age, obese/general population, health status, allele frequency, exercise and diet protocols (in interventional studies) etc. Moreover underpowered studies can suggest non-existing, false positive associations.

LEPR polymorphisms post-intervention comparisons

No significant association was observed between the Gln223Arg or Lys656Asn polymorphisms of the leptin receptor gene and body composition changes. The LEPR gene as candidate gene is justified because of its role in the regulation of food intake and energy expenditure (Dulloo et al. 2002; Jequier 2002), although there is not much functional data on these polymorphisms available and based on our data we cannot confirm their role in weight loss or body composition change either. In line with this, a study reported that the Gln223Arg polymorphism does not affect the affinity and binding kinetics of the leptin receptor (Verkerke et al. 2014) here the authors suggest

that previous positive results can be due to differences in study design and power, as it was concluded also in a systematic review (Bender et al. 2011). To the best of our knowledge, very few studies have been carried out with intervention and none of them with supervised exercise. Salopuro et al and Mammés et al did not find evidence for association between these polymorphisms and body composition changes (such as body mass, BMI or fat mass) after a diet plus physical activity recommendations and a low calorie diet respectively (Mammés et al. 2001; Salopuro et al. 2005) which is in line with our negative findings.

On the other hand, two studies of a Spanish research group observed that the carriers of the Asn656 allele have different response to a Mediterranean hypocaloric diet, both groups achieved to reduce body mass and BMI, but Asn656 carrier group did not reduce fat mass (Luis et al. 2015; de Luis Roman et al. 2006). In the case of these studies the statistical analyses did not include comparison between the genotype groups, only paired Student's t-test and then the outcomes were contrasted, hence it did not confirm the difference between the genotype groups (de Luis Roman et al. 2006).

The influence of the leptin receptor on the change of body mass, BMI, fat mass, percentage fat, android fat or VAT after a controlled diet and exercise weight loss program was not proven in our study, but neither we can reject the possibility of it, therefore we propose further investigation in the light of the previous results.

***PPARG* polymorphisms post-intervention comparisons**

We observed a possible interaction between the Ala12 allele and sex on the change of VAT, though the p value did not reach the corrected significance, therefore the strength of evidence is weak. It is complicated to compare our results with others, as earlier interventional studies have not analyzed the interaction of the Pro12Ala polymorphism and sex, many of them only included women participants and the others' statistical methods included the sex factor as covariate. We observed a trend showing that within non-supervised women participants, carriers of the Ala12 allele lost less VAT than non-carriers. This could mean that the Ala12 allele might make it more difficult to lose VAT. This is an important issue not only from the aesthetical point of view but because of health problems as VAT is associated with metabolic syndrome and cardiovascular diseases (Britton et al. 2013); however it seems that controlled exercise could counterbalance this effect of the Ala12 allele as we have not seen this different in supervised groups. Likewise Ala12 carriers lowered percentage fat less than non-carriers within non-supervised women group. However, all these results should be interpreted carefully because of the low number of participants in these groups. Our results are similar to the ones of the study of Zarebska et al, where carriers had smaller increase of fat mass and percentage fat than non-carriers; however their intervention included an endurance training program and participants were young normal weight women and were asked to keep a balanced diet of approximately 2000 kilocalories (Zarebska et al. 2014). On the other hand, an endurance training program with 50-75 year-old sedentary participants found no association with fat mass or percentage fat (Weiss et al. 2005), similarly to a 6-month-long hypocaloric diet with postmenopausal women (Nicklas et al. 2001).

Considering that PPARG has a key role on adipogenesis, it is responsible for fatty acid storage and the control of energy balance (Auwerx 1999; Lehrke and Lazar 2005; Meirhaeghe and Amouyel 2004), its influence on the change of VAT and percentage fat seems to be logical. The physiological and functional differences between the allelic variants are still not completely clear, however in vitro studies showed that the presence of the Ala12 allele causes a diminished affinity of the receptor for the peroxisome proliferator response element in target gene promoters (Zarebska et al. 2014; Deeb, Fajas, Nemoto, Pihlajamäki, et al. 1998) and that leads to decreased expression level, which was seen by in vivo studies too (Schneider et al. 2002; Simon et al. 2002). Moreover Ala12 allele has an insulin-sensitizing effect on the liver and skeletal muscles (Deeb, Fajas, Nemoto, Pihlajamäki, et al. 1998; Ek et al. 1999), which can result in the suppression of lipolysis in adipocytes and reduced release of free fatty acids (Stumvoll et al. 2001).

No other p values reached either the permissive significance 0.05 level, for the change of other body composition parameters (body mass, BMI, fat mass, android fat or VAT). In line with these findings, an endurance training program based study did not find training-induced genotype-specific differences in body mass, BMI in older sedentary participants (Weiss et al. 2005), neither the previously mentioned study of Nicklas et al (Nicklas et al. 2001). Similarly no influence of the Pro12Ala polymorphism was detected in two lifestyle changing programs for overweight and obese subjects with low energy diet and increased physical activity (Aldhoon, Zamrazilová, et al. 2010; Rittig et al. 2007). Nevertheless, Adamo suggested that the Pro12Ala polymorphism is a significant predictor of resistance to weight loss (Adamo et al. 2007). Furthermore studies from two big diabetes prevention projects confirm that the Ala12 allele carriers

lost more body mass during diet and physical activity (Delahanty et al. 2012; Franks et al. 2007; Lindi et al. 2002). Therefore again the role of this polymorphism is not clarified yet in connection with weight loss, there are signs suggesting that it could be important, but new studies are needed to confirm this.

Interventional studies including diet and supervised exercise (not only recommendations) and genetics or genomics are still scarce probably because it is complicated to carry out a controlled and complex interventional study with the adequate sample size to ensure the sufficient effect size, what is not ensured in most of the candidate gene studies. As the main objective of our study was not to explore the genetic background of body composition changes but the effect of different training protocols on weight loss; this part of the study is underpowered. A correction for multiple testing was applied (there is no consensus on the statistical correction for multiple testing in these kind of studies), taking into account the genetic models, the exercise groups and the sexes, yet results below 0.05 were reported and discussed. Other limitations of this study is that there was no real control group (for ethical reasons all groups followed the hypocaloric diet and exercise programs or physical activity recommendations); and the lack of a geographically independent cohort.

Although the evidence of candidate gene studies is weak, the doubt is present, as the role of genetic factors in obesity and the capability to lose body weight was determined before (Bouchard et al. 1994; Hainer et al. 2000; Loos and Bouchard 2003) and the biological/physiological function of the proteins encoded by these genes are strategically important, which encourages us for further research on this field. We also have to keep in mind that the individual effect of these genes are minor, complex

studies, new methods or approaches will be necessary to progress. Maybe 'omics' can be the solution including genomics, transcriptomics, metabolomics and proteomics. Studies should be carried out with meticulous methodology (controlled protocols taking into account diet, different types of exercise, genetic background, other influencing factors and their possible interactions, trying to control the seemingly endless number of collusive factors causing obesity, additionally placing extra emphasis on statistics) and if possible with collaborations between research groups to ensure sample size.

Conclusions

The conclusions of our study are that the Gln223Arg and Lys656Asn polymorphisms of the *LEPR* gene and the Pro12Ala polymorphism of the *PPARG* gene (except VAT) do not seem to influence the weight loss or body composition remodeling during a diet and exercise program. The Ala12 allele carrier (*PPARG* gene) women might have difficulty in reducing percentage fat and VAT; nevertheless supervised, well-designed exercise can compensate this effect. This result supports the concept that the best way to optimize the results of a weight loss program is to include both diet and supervised exercise in subjects with refractory weight loss.

3.3. Study III. Monocarboxylate transporter 1 in weight loss interventions: Role of the Glu490Asp polymorphism on body composition responses

Abstract

Background The Monocarboxylate Transporter 1 (MCT1) is a membrane transporter expressed in most of the human cells. In addition to its role regarding pH regulation and lactate transport during exercise, this protein has also been related with body energy homeostasis and with regulation of energy balance when exposed to an obesogenic diet. Therefore, the aim of this study was to investigate the influence of the Glu490Asp (T1470A, rs1049434) polymorphism of the MCT1 gene 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 (supervised) and physical activity recommendations (non-supervised). Body weight, body mass index (BMI), percentage fat and percentage fat free mass (FFM) were assessed before and after the intervention. Genetic analysis was carried out according to standard protocols.

Results Main effect of the Glu490Asp polymorphism was observed for final body weight and BMI values ($p=0.038$ and $p=0.029$ respectively), moreover interaction with sex and exercise was shown. The Asp490Asp genotype group decreased body weight ($p=0.038$, 1.79 kg) and BMI ($p=0.025$, 0.66 kg/m²) more than Glu490 allele carriers.

Interaction with sex and exercise was found for percentage fat. Asp490Asp women had less final percentage fat than Glu490 carriers ($p=0.025$, 1.94%). Finally, interaction with sex and exercise was seen for percentage FFM, among women the Asp490Asp group ended up with higher values than Glu490 carriers ($p=0.005$, 2.76%).

Conclusions Our results uncover a possible influence of the Glu490Asp polymorphism of the MCT1 gene on body composition responses. It seems that the absence of the Glu40 allele implies less weight, BMI and percentage fat at the end of the program, especially in non-supervised women. However, further studies are necessary to confirm these results and clarify the underlying mechanisms.

Introduction

The Monocarboxylate Transporter 1 (MCT1) is a membrane transporter expressed in most of the human cells (Fishbein, Merezhinskaya, and Foellmer 2002; Halestrap and Wilson 2012; Halestrap 2012), including hypothalamic neurons and glia and adipocytes (Hajdúch et al. 2000). This protein symports a proton together with a molecule of short chain monocarboxylate such L-lactate, pyruvate and the ketone bodies beta-hydroxybutyrate and acetoacetate (Poole and Halestrap 1993). In addition to its role regarding pH regulation (Halestrap and Meredith 2004) or lactate transport among muscle cells during exercise (Brooks 2009), the function of this protein has also been related with body energy homeostasis and cell-cell signaling (Carneiro and Pellerin 2015). In fact, lactate seems to play an important role on glucose homeostasis (Kokorovic et al. 2009) and food intake (Cha and Lane 2009) by means of hypothalamic lactate sensing. Moreover, several studies have reported changes in the expression of MCT1 induced by diet composition (Hargrave et al. 2015) or maternal obesity (Simar et

al. 2012), as well as diabetic status (Hajduch et al. 2000). In line with this results, it has been recently reported how a transgenic mouse for the MCT1 gene (*SLC16A1*), with one invalidated allele (*MCT1^{+/-}*), presented resistance to diet-induced obesity and related diseases when were fed a high fat diet (Lengacher, Nehiri-Sitayeb, Steiner, Carneiro, Favrod, Preitner, Thorens, Stehle, Dix, Pralong, et al. 2013). As these authors stated, the results uncover the critical role of MCT1 in the regulation of energy balance when exposed to an obesogenic diet, being tempting to hypothesized a key role of MCT1 in adaptations taking place during weight loss (Carneiro and Pellerin 2015).

Merezhinskaya et al. described a single-nucleotide polymorphism (SNP), Glu490Asp (rs1049434), for the MCT1 gene (Merezhinskaya et al. 2000). This SNP results in a glutamic acid aspartic acid change in codon 490, and has been related with lactate transport immediately after high intensity exercise (Cupeiro et al. 2016; Cupeiro et al. 2012; Cupeiro et al. 2010; Fedotovskaya et al. 2014), suggesting an impaired lactate transport capability into less active muscle cells for oxidation through the transporter in Asp490 male carriers. Furthermore, these *in vivo* results have recently been confirmed when oocytes expressing the Asp490 allele showed a lower lactate uptake (Sasaki et al. 2015). Regarding the above mentioned relation of MCT1 with energy metabolism and body composition, the cited SNP has been also related with body composition in athletes, observing a higher fat-free mass percentage in Asp490Asp or Asp490 carriers soccer players compared to Glu490Glu players (Massidda et al. 2015). Therefore, as well as an influence of MCT1 over weight loss process has been suggested (Carneiro and Pellerin 2015), the Glu490Asp polymorphism could be associated with body composition changes during a weight loss program.

Thus, the objective of the present study is to investigate the influence of the Glu490Asp (rs1049434) polymorphism of the MCT1 gene on body weight, body mass index (BMI), percentage fat and percentage fat free mass (FFM) changes after a 6-month weight loss program in obese and overweight people.

Methods

PRONAF study

The present RCT (ClinicalTrials.gov ID: NCT01116856) was conducted from January, 2010, through June, 2011, and followed the ethical guidelines of the Declaration of Helsinki.

A 6-months diet and exercise-based intervention was carried out aiming a behavior change. Participants entered into the study in two waves, in the first year the overweight, in the second the obese participants. Each wave was split into four randomly assigned groups, stratified by age and sex: strength group, endurance group, combined strength and endurance group and the control group, which followed only physical activity recommendations. The measurements took place in week 1 (pre-intervention values) for all participants before starting and after 22 weeks of intervention, in week 24 (post-intervention values). Before the intervention started, physical activity was assessed by a SenseWear Pro3 Armband™ accelerometer (Body Media, Pittsburgh, PA). Monitors were worn 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). Details of the study's theoretical rationale, protocol, and intervention are described elsewhere (Zapico et al. 2012).

Participants

Subjects were overweight and obese [body mass index (BMI) 25–34.9 kg/m²] between 18-50 years from the Region of Madrid, Spain, and were recruited through diverse advertisements in the media. All of them was healthy nonsmoker, sedentary (one or less exercise bout per week) (Brochu, Malita, et al. 2009) and normoglycemic. Volunteers with any physical or psychological disease were excluded. Women had regular menstrual cycles. After drop outs, in the present study 173 subjects were included (91 women, 82 men). In agreement with the guidelines of the Declaration of Helsinki regarding research on human subjects, before participating in this investigation, participants read and signed an institutionally approved informed consent document. All subjects were carefully informed about the possible risks and benefits of the project, which was approved by the Human Research Review Committee of the University Hospital La Paz (PI-643).

Diet intervention

At the beginning of the intervention, the negative energy balance was calculated for each participant taking into account the daily energy expenditure, and a 3-day food record, in order to decrease the energy intake of the diet by a 25-30% during the intervention. Macronutrient distribution was set according to the Spanish Society of Community Nutrition recommendations, approximately, 29–34% of the energy came from fat, 14–18% from protein, and 50–55% from carbohydrates (Sacks et al. 2009). In the obesity group, energy provided from protein was increased up to 14–20% (Dapcich et al. 2004). The dietitian interviewed each participant at baseline, 3 months, and 6 months and reviewed a 3-day food diary.

Exercise intervention

Participants were randomized into strength, endurance, combined strength and endurance training group (supervised groups) or the non-supervised group. Subjects in the supervised groups performed training 3 times per week for 22 weeks at a sports center. All training sessions were carefully supervised by certified personal trainers. The exercise programs were designed taking into account each subject's muscle strength using the 15-repetition maximum and the heart rate reserve. Both volume and intensity of exercise was increased over the study period according to standard procedures (Hunter et al. 2008; Del Corral et al. 2009). Details of the different protocols developed by the groups are described elsewhere (Zapico et al. 2012). On the other hand, participants from the control group followed the dietary intervention as the other groups and were encouraged to follow the recommendations about physical activity of the American College of Sports Medicine (Donnelly et al. 2009), namely to undertake at least 150-250 min of moderate-intensity physical activity per week.

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 $[\text{body weight (kg)} / (\text{height (m)})^2]$. Fat mass (kg), FFM were 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 FFM and percentage fat was calculated as $[\text{FFM (kg) or fat (kg)} / \text{body weight (kg)} * 100\%]$.

Genotyping

Whole blood samples (5ml) were collected in ethylene-diaminetetraacetic acid (EDTA) from each patient and sent to the laboratory for the analysis. DNA was extracted from each sample using the "QIAamp® DNA Blood Mini Kit" from QIAGEN (Hilden, Germany) and the genotyping was performed for each SNP afterwards. For the overweight phase, the analysis of the polymorphism was performed by polymerase chain reaction (PCR) amplification followed by direct sequencing as described previously (Cupeiro et al. 2010); and for the obese phase, the genotyping of the polymorphism was carried out using the corresponding TaqMan® SNP Genotyping Assays (Applied Biosystem, Foster City, CA, USA) in a 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. Normal distribution of each dependent variable was tested using Quantile-Quantile plots, when needed Box-Cox transformation was applied using the optimal lambda value. Three genetic models (additive, dominant, recessive) were tested. Based on exercise, the sample was divided in two groups: supervised (strength, endurance and combined strength and endurance training groups) as all protocols had the same characteristics (intensity, duration and frequency) and non-supervised group. Three-way (genotype x exercise group x sex) ANCOVA was conducted for the final values of weight, BMI, percentage fat and percentage fat free mass to see interactions and differences

between genotype groups, genders and exercise groups. Bonferroni test was used for post hoc comparisons. Statistical significance for post intervention comparisons was defined at the corrected alpha of 0.00179 with correction for repeated tests across the levels of the ANCOVA model factors. Correction was carried out taking into account sex, exercise groups, genetic models and genotype groups. Effects sizes and their respective 90% confidence intervals were calculated to show the magnitude of the effects (standardized differences in means: Cohen's units) of carrying the Glu490 allele (i.e. comparing Asp490Asp vs Glu490Asp plus Glu490Glu) over body weight, BMI, percentage fat and percentage FFM. These calculations were adjusted by the baseline scores of the dependent variables, and they were performed separately by gender and by exercise groups (supervised and non-supervised). Interpretation of effect sizes was based on the following criteria: 0-0.2 trivial, >0.2-0.6 small, >0.6-1.2 moderate, >1.2-2 large, and >2 very large (Hopkins 2006). Probabilities that the true effect of carrying the Glu490 allele was harmful, trivial, and beneficial were also calculated and interpreted as follows (Hopkins et al. 2009): <0.5%, most unlikely, almost certainly not; >0.5- 5%, very unlikely; >5-25%, unlikely, probably not; >25-75%, possibly; >75-95%, likely, probably; >95-99.5%, very likely; >99.5%, most likely, almost certainly. These calculations were performed using Microsoft Excel spreadsheets (Microsoft, Redmond, WA).

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 5.

Table 5. Baseline characteristics of the subjects in Study III

BASELINE	WOMEN		MEN	
	Asp490Asp (n=17)	Glu490 carriers (n=74)	Asp490Asp (n=23)	Glu490 carriers (n=59)
Age (years)	40.18±10.66	38.88±7.68	40.96±9.0	39.14±7.95
Body weight (kg)	79.1±9.20	81.19±10.86	96.57±12.48	95.77±10.07
BMI (kg/m ²)	30.62±3.04	30.45±3.26	31.14±3.12	30.87±2.56
Fat mass (kg)	33.85±5.6	35.32±6.6	33.91±7.65	33.46±6.77
Percentage fat (%)	44.98±3.6	45.19±4.16	36.41±4.39	36.34±4.95
Fat free mass (kg)	41.09±4.12	42.48±5.06	58.15±4.76	58.04±5.68
Percentage FFM (%)	52.16±3.79	52.6±3.9	60.67±4.47	60.84±4.77
<i>Data presented as Mean ± Standard Deviation</i>				

Genotype distribution and allele frequencies of the Glu490Asp polymorphism of the MCT1 gene are shown in Table 6. Genotype distributions were found in Hardy-Weinberg equilibrium ($p=0.44$).

Table 6. Genotype distribution and allele frequency of the MCT1 gene in Study III

	Glu490Glu	Glu490Asp	Asp490Asp	Glu490	Asp490
All	52 (30.05)	81 (46.82)	40 (23.12)	185 (0.54)	161 (0.46)
Women	32 (35.16)	42 (46.15)	17 (18.68)	106 (0.58)	76 (0.42)
Men	20 (24.39)	39 (47.56)	23 (28.05)	79 (0.48)	85 (0.52)
<i>Data presented as n (%) for genotypes and n (frequency) for alleles.</i>					

Post-intervention comparisons

Main effect of the Glu490Asp polymorphism was observed for final body weight and BMI values adjusted by initial measurements ($p=0.038$, $F=4.363$ (1), $\eta p=0.026$ for weight and $p=0.029$, $F=4.864$, $\eta p=0.029$ for BMI), moreover interaction with sex ($p=0.015$, $F=6.029$ (1), $\eta p=0.036$ for body weight and $p=0.006$, $F=7.824$ (1), $\eta p=0.046$ for BMI) and exercise group (supervised/ no supervised, $p=0.028$, $F=4.888$ (1), $\eta p=0.029$ for body weight and $p=0.016$, $F=5.917$ (1), $\eta p=0.035$ for BMI) and a triple interaction among the polymorphism, sex and exercise group ($p=0.043$, $F=4.167$ (1), $\eta p=0.025$ for body weight and $p=0.019$, $F=5.659$ (1), $\eta p=0.034$ for BMI) were found. Post hoc analyses revealed same tendency for body weight as well as for BMI. Subjects with Asp490Asp genotype decreased these variables more than Glu490 allele carriers (body weight, $p=0.038$, 1.79 kg; BMI, $p=0.025$, 0.66 kg/m²). This was also observed in the subgroups of women (body weight, $p=0.001$; BMI, $p=0.0004$), of non-supervised subjects (body weight, $p=0.014$; BMI, $p=0.08$ for BMI), and of non-supervised women (body weight, $p=0.0002$; BMI, $p=.00003$) (Figure 25a and 25b).

Figure 25. Weight and BMI values before and after the intervention in women and men in MCT1 genotype and exercise groups

Asp490Asp (Glu490-), Glu490Asp and Glu490Glu (Glu490+). Means of pre and post values of Body mass in women (a), BMI in women (b), Body mass in men (c), BMI in men (d).

Moreover within Glu490 carrier and Glu490 carrier female participants the supervised group finished with lower weight ($p=0.045$, 1.70 kg and $p=0.031$, 2.65 kg) and BMI ($p=0.03$, 0.64 kg/m² and $p=0.018$, 1.0 kg/m²) than the non-supervised. However the female Asp490Asp group showed the contrary, supervised female Asp490Asp group ended up with 4.61 kg more than non-supervised ($p=0.023$) and 1.84 kg/m² of BMI ($p=0.009$) (Figure 25). The effect sizes determined for body weight and BMI indicated similar effects of the Glu490 allele over both parameters, in accordance with the results observed with the ANCOVA. Effects were trivial or unclear in all the groups except for women in the non-supervised groups, where is certainly likely that the Glu490 allele exhibit a large (for body weight) and moderate (for BMI) positive effect (Table 7).

Table 7. Differences during the intervention on the studied variables for gender and exercise groups in carriers vs non-carriers

Variables	Groups	ES (CI 90%)	Chance of being +/trivial/-	Qualitative Inference
Weight	Men supervised	-0.03 (-0.21, 0.15)	2/92/6	likely trivial
	Men non-supervised	0.02 (-0.39, 0.43)	22/61/17	unclear
	Women supervised	0.01 (-0.21, 0.24)	8/87/5	unclear
	Women non-supervised	1.24 (0.79, 1.68)	100/0/0	most likely positive
BMI	Men supervised	-0.04 (-0.28, 0.2)	5/81/14	likely trivial
	Men non-supervised	0.03 (-0.45, 0.51)	26/54/20	unclear
	Women supervised	0.02 (-0.26, 0.32)	15/74/11	unclear
	Women non-supervised	1.04 (0.67, 1.42)	100/0/0	most likely positive
%Fat	Men supervised	-0.25 (-0.52, 0.02)	0/37/63	possibly negative
	Men non-supervised	-0.46 (-0.91, -0.01)	1/15/84	likely negative
	Women supervised	-0.19 (-0.45, 0.08)	1/52/47	possibly negative
	Women non-supervised	1.15 (0.42, 1.15)	98/2/1	very likely positive
%FFM	Men supervised	0.15 (0.04, 0.26)	24/76/0	likely trivial
	Men non-supervised	0.26 (0.08, 0.45)	0/27/73	possibly negative
	Women supervised	-0.03 (-0.14, 0.08)	1/99/0	very likely trivial
	Women non-supervised	0.25 (-0.12, 0.62)	3/37/60	possibly negative
<i>ES, effect size; CI, confidence interval</i>				

On the other hand, the Glu490Asp polymorphism seems to have a slightly different influence on body composition variables. Interaction with sex ($p=0.002$) and a triple interaction with sex and exercise ($p=0.017$) came out in the ANCOVA. Based on post hoc analyses, Asp490Asp women had less final percentage fat in their whole group ($p=0.025$, 1.94%) as well as within the non-supervised women group ($p=0.002$, 4.49%), than Glu490 carriers (Figure 26a). The effect size associated with the latter difference indicates that the Glu490 allele has a moderate and very likely positive effect over percentage fat response only in women within the non-supervised group (Table 7).

Furthermore, the post-hoc analysis showed opposite results for men, where the Glu490 carriers ended up with less percentage fat than Asp490Asp subjects ($p=0.037$, 1.87%) (Figure 26c), being the effect sizes small and possibly negative in the supervised group or likely in non-supervised group (Table 7).

Figure 26. Percentage fat and percentage fat free mass values before and after the intervention in women and men in MCT1 genotype and exercise groups

Asp490Asp (Glu490-), Glu490Asp and Glu490Glu (Glu490+). Means of pre and post values of percentage fat in women (a), percentage fat free mass in women (b), percentage fat in men (c), percentage fat free in men (d).

As for the comparison of the exercise groups within genetic groups, in female Asp490Asp group non-supervised participants lowered greater percentage fat than supervised ($p=0.036$, 3.07%) while in the Glu490 allele carrier group the contrary ($p=0.002$, 1.55%, in women $p=0.032$, 2.02%).

For percentage FFM interaction with sex ($p=0.002$), and interaction among polymorphism, sex and exercise groups (0.017) were found. Differences were seen for

Asp490Asp and Glu490 carrier women ($p=0.005$, 2.76%), and more specifically in non-supervised women ($p=0.001$, 5.61%) (Figure 26b). For this variable, the effect sizes were similar in men and women, showing a possibly small protective effect of carrying the Glu490 allele in the non-supervised groups (Table 7).

Finally comparing the exercise groups in each genetic group, we observed that Glu490 carriers ended up with higher percentage FFM than Asp490Asp homozygotes only in the supervised group (in the whole group $p=0.002$, 2.23%; in women $p=0.01$, 2.62%), but with a trivial effect (Table 7).

Discussion

Besides its key role in lactate metabolism, recent works have suggested that MCT1 might have an influence over the regulation of energy balance and body composition parameters (Carneiro & Pellerin, 2015; Hajduch et al., 2000; Hargrave et al., 2015; Lengacher et al., 2013; Massidda et al., 2016; Simar et al., 2012). This makes interesting to analyze the role of MCT1 in body composition responses after a weight loss program. Our results uncover an influence of the Glu490Asp genetic variant in the MCT1 gene on these responses, showing that the absence of the Glu490 allele implies less weight, BMI and percentage fat at the end of the program, especially in non-supervised women. The effects of the genotype in these cases are large or moderate, indicating that differences between genetic groups are considerable.

The impact of the Glu490Asp polymorphism over the MCT1 function has been proved both in vitro (Sasaki et al., 2015) and in vivo (Cupeiro et al., 2012, 2016, 2010; Fedotovskaya et al., 2014), evidencing a reduced lactate transport for the Asp allele. Given these differences in lactate transport and that role of MCT1 in body weight

regulation was suggested, possible differences in body weight reduction and in body composition changes were expected in our study.

Therefore our results would support the involvement of MCT1 in body composition since a different functionality of MCT1 caused by the SNP (i.e. the Asp490Asp group) somehow benefit the responses to a traditional weight loss program (i.e. hypocaloric diet plus exercise recommendations) in healthy overweight and obese women. These results are in line with those by Lengacher (Lengacher et al., 2013), who observed a resistance to obesity in genetically modified mice for *MCT1*. These authors reported that heterozygotes mice with only one valid *MCT1* allele, which displayed a reduce expression for MCT1, were resistant to increase their body fat, insulin resistance, glucose intolerance and other metabolic dysregulations associated with obesity, when the mice were feed with a high fat diet. Although their work differs from ours in the body weight change induced (their intervention increased body weight while our intervention reduce it), both studies showed a protective or beneficial effect of a lower MCT1 function. In our case, however, we detected this effect mainly in women within the non-supervised group. One plausible explanation for this result lies on a different MCT1 expression due to the different exercise programs and the different male and female muscle metabolisms. The non-supervised group followed a non-specific and non-compulsory recommendation of at least 150 min of moderate exercise per week, without any kind of supervision (as it happens in the actual treatment that most of the obese and overweight people receive at Spain). This probably resulted in a less intense exercise intervention compared with the supervised group, which would have produced higher MCT1 expression in the supervised groups, which has been stated by several authors (Bickham et al. 2006; Dubouchaud et al. 2000; Juel, Holten, and Dela

2004). Moreover, the higher anaerobic metabolism reported for men (Isacco, Duché, and Boisseau 2012) might promote the non-supervised exercise as an anaerobic stimulus enough for eliminating the differences seen for women. However, this is only a theoretical hypothesis since we did not measure MCT1 expression, and further studies analyzing weight loss, Glu490Asp polymorphism and MCT expression are needed to elucidate the physiological basis of the results observed in our sample.

On the other hand, we also seen that within the women carrying the Glu490 allele, those who exercised under supervision lost more weight, BMI and percentage fat than those in the non-supervised group, and the opposite occurs for the Asp490Asp women. This divergent behavior implies that with a normal MCT1 functionality the most beneficial intervention seems to be hypocaloric diet plus supervised exercise at moderate intensity (i.e. 50%-60% of the maximum), which has been reported to be the most successful program for losing percentage fat in overweight subjects in a paper being under review from our project. Therefore, the Glu490 carrier women would behave as the overweight subjects, supporting the idea of a different response of the Asp490Asp female mediated by MCT1 function.

Based on the mouse study of Lengacher et al. we expected less Asp490 allele presence in our overweight and obese sample, because the Glu490 allele would imply more susceptibility to be obese in obesogenic environment (Lengacher, Nehiri-Sitayeb, Steiner, Carneiro, Favrod, Preitner, Thorens, Stehle, Dix, Pralong, et al. 2013). However the frequency of the Asp490 allele in our sample was 0.54, while other studies report in non-athletic populations 0.30-0.35 (Ben-Zaken et al. 2015; Merezhinskaya et al. 2000; Lean and Lee 2009; Sawczuk et al. 2015). The explanation for this might be that

our participants were all healthy, in contrast to the impaired insulin sensitivity and fatty liver of the affected mice, thus it is possible that that Glu490 allele inflicts health problems.

Finally we also observed a small effect of the Glu490Asp polymorphism over the change in percentage FFM during our intervention, in both men and women following the non-supervised group. Within these groups, those with the Asp490Asp genotype lost less percentage FFM than the Glu490 carrier groups. Although with a little effect, these results are in accordance with those seen by Massidda et al., where the Asp490Asp genotype had a significantly higher percentage FFM than those with the Glu490Glu genotype (Massidda et al., 2016). The authors explain their results suggesting an elevated expression of genes associated with muscle hypertrophy due to the accumulation of lactate in Glu490Glu subjects. However, their sample was composed of highly trained athletes (young soccer players competing in the National Level of the Italian soccer Championship) whereas our result only appears in the non-supervised group, uncover a potential key interaction with exercise. It seems that the differences in percentage FFM among genetic groups appears when the training load is high (soccer players) or very low (exercise recommendations), but in medium ranges the exercise could compensate the effect of the polymorphism in starting practitioners, where other stimuli apart from lactate accumulation could lead to muscle protein synthesis (Schoenfeld, 2013).

A limitation of our work is the small sample size, as the main project was designed taking into account the main objective (weight loss) and not the future genetic analyses. Moreover we could not measure MCT1 expression, it would have been good

to see direct relation with the genotypes and get information on the underlying mechanisms, which results in the differences observed in our study. However, the strength of our project was the controlled exercise and diet during the intervention, which should be followed in future studies. Such quality (controlled programs and extended variable number) with larger sample sizes could be carried out with multi-center collaborations.

In conclusion, MCT1 Glu490Asp polymorphism seems to influence weight and body composition changes during a diet and exercise intervention, our novel results should be replicated though.

3.4. Do common polymorphisms influence body weight and fat regain after a weight loss intervention?

Abstract

Purpose: To test whether polymorphisms in previously suggested obesity genes are associated with weight regain, BMI and body fat changes over 3 years of follow-up after a controlled weight loss intervention of overweight and obese subjects. **Methods:** A total of 98 subjects (49 men, 49 women) were available for analysis who completed a 22-week long weight loss intervention and a subsequent 3-year long follow up. Polymorphisms from six genes were included: *ADRB2*, *ADRB3*, *LEPR*, *PPARG* and *MCT1*. General lineal model (GLM) was used to analyze associations between the polymorphisms and weight, BMI, fat mass and percentage fat at the end of the intervention and 3 years later. Effect size was calculated (standardized mean differences and 90% confidence intervals). **Results:** All genotype distributions were found to be in Hardy–Weinberg equilibrium. *ADRB3* Arg64 allele showed an interaction with sex for weight and BMI (weight: $F=4.013$, $p=0.048$; BMI: $F=4.814$, $p=0.031$) 3 years after the program. In men, carriers of the Arg64 allele increased weight and BMI more than the non-carriers. In addition, main effect of the *LEPR* Arg223 allele was observed for percentage fat 3 year follow up point ($F=4.830$, $p=0.031$). Non-carriers of the Arg223 had less percentage fat than the carriers. Nevertheless, no p-values reached the corrected alpha level. No other differences were found between genotype groups of the other polymorphisms for changes of weight, BMI, fat mass or percentage fat. **Conclusion:** A tendency was seen for the

influence of the Trp64Arg and Gln223Arg polymorphisms on body composition changes over 3 years follow up after a diet and exercise weight loss intervention. However, we did not find strong evidence that these polymorphisms are associated with weight regain or body fat changes. They cannot be discarded either, further studies are needed to clarify if they have any role in obesity.

Introduction

Overweight and obesity are today considered to be amongst the major public health problems of our society. Nicklas et al. found (according to a population survey) that in the United States 63% of obese participants reported trying to lose weight in the past 12 months, among these 40% achieved to lose >5% and 20% achieved to lose >10% weight (Nicklas et al. 2012). However, the fact that the prevalence is still rising suggests that most of them do not succeed and only 20% of them is able to maintain the achieved weight with struggles and hard work (Wing and Phelan 2005; Kraschnewski et al. 2010), which turns out to be a bigger challenge than weight loss.

It is undoubted that weight, overweight, weight loss and weight maintenance are complex phenotypes and phenotype changes, that results of the effect of many dependent and independent factors (social, cultural, genetic, physiological, psychological etc.) (McPherson 2007). Many candidate gene studies and a few genome-wide association studies (GWAS) set the goal to see the association between certain polymorphisms and obesity related phenotypes such as weight, body mass index (BMI), fat mass, but the findings are not concordant. Nevertheless, there are less candidate gene studies on weight loss and even less with weight maintenance; and to the best of our knowledge, no GWAS has been carried out with these variables.

The *ADRB2* and *ADRB3* genes encode proteins that influence energy expenditure by promoting lipolysis and fat mobilization and modifying glucose metabolism (Arner 1992; Hagstrom-Toft et al. 1998; Lafontan et al. 1997). The protein of the *LEPR* gene is a component of the leptin-insulin pathway, binds the leptin which interacts with other neuropeptides regulating food intake, appetite control, energy homeostasis and thermogenesis (Dulloo et al. 2002; Jequier 2002). The protein expressed from the *PPARG* gene plays a role in the transcriptional processes regulating adipocyte differentiation, lipid storage, fat-specific gene expression and insulin signaling (Auwerx 1999; Lehrke and Lazar 2005; Meirhaeghe and Amouyel 2004). MCT1 is a membrane transporter which symports a proton together with a monocarboxylate molecule such L-lactate, pyruvate or the ketone bodies beta-hydroxybutyrate and acetoacetate (Poole and Halestrap 1994), and lately it has been mentioned in relation to obesity and body composition (Carneiro and Pellerin 2015; Massidda et al. 2015).

Polymorphisms in the genes of these proteins have been suggested to affect obesity related genotypes, among them the beta-2 and beta-3 adrenergic receptors (*ADRB2*, *ADRB3*) (Clement et al. 1995; Lange et al. 2005; Large et al. 1997; Ukkola et al. 2003; Ukkola et al. 2000), the leptin receptor (*LEPR*) (Bender et al. 2011; Chagnon et al. 1999; Mammes et al. 2001), the peroxisome proliferator-activated receptor gamma (*PPARG*) (Clement et al. 2000; Deeb, Fajas, Nemoto, Pihlajamäki, et al. 1998; Nicklas et al. 2001) and recently has appeared a possible new candidate gene in this field, the monocarboxylic acid transporter (*MCT1*) (Massidda et al. 2015; Carneiro and Pellerin 2015).

Therefore, the objective of this study was to analyze whether the *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *LEPR* Gln223Arg and Lys656Asn, *PPARG* Pro12Ala, *MCT1* Glu490Asp polymorphisms influence weight and body fat maintenance after the completion of a controlled diet and exercise weight loss program.

Methods

Design

The present Randomized Control Trial (RCT; ClinicalTrials.gov ID: NCT01116856) was conducted from January, 2010, through June, 2011, and followed the ethical guidelines of the Declaration of Helsinki. The Institutional Review Board of the La Paz University Hospital (PI-643) reviewed and approved the study design and research protocol. Details of the study's theoretical rationale, protocol, and intervention are described elsewhere (Zapico et al. 2012).

Participants

The study participants were recruited through several advertisements campaigns 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. Those who were 18 to 50 years old, had a BMI between 25 and 34.9 kg/m², were non-smokers, were sedentary (i.e., two hours or less of structured exercise per week) (Brochu, Wagner, et al. 2009; Brochu, Malita, 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 to participate in the study. The 239 eligible participants who were willing to participate provided written informed consent

prior to joining the study and then completed a baseline assessment at the medical center. Subjects were randomly assigned (computer-generated) to intervention groups. After dropouts, finally, 98 subjects were included in the present study (having completed the 3 year follow up assessment).

Procedures

A 6-months diet and exercise-based intervention, focusing on a behavior change. Participants entered into the study in two waves, one of overweight participants and the other of obese participants. Each wave was split into four randomly assigned groups, stratified by age and sex: strength group, endurance group, combined strength and endurance group and the control group, who follow the physical activity recommendations. The measurements took place in the first week (pre-intervention values) for all participants before starting and after 22 weeks of intervention, in week 24 (post-intervention values). After the intervention period, all participants were under a free-living condition, and they were required to inform about their body weight and their nutritional and physical activity behaviors every 6 months, by e-mail or telephone call. In addition, a third measurement took place 3 years after the intervention period.

Before the intervention physical activity was assessed by a SenseWear Pro3 Armband™ accelerometer (Body Media, Pittsburgh, PA). Daily energy expenditure was calculated using the Body Media propriety algorithm (Interview Research Software Version 6.0).

Diet intervention

All participants followed an individualized hypo-caloric diet, with a 25-30% caloric restriction from their own daily energy expenditure (NIH 1998), which was measured by using the SenseWear Pro3 Armband™ (Body Media, Pittsburgh, PA), that underestimates by a mean value of 8.8% (Papazoglou et al. 2006). Then, the macronutrient distribution was according to the Spanish Society of Community Nutrition recommendations (Dapcich et al. 2004).

Exercise intervention

All exercise groups (Strength, Endurance and combined Strength and Endurance groups) followed an individualized training program, which consisted on 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 control group followed the dietary intervention and received recommendations about physical activity from the ACSM (Donnelly et al. 2009). Thus, the C subjects were advised to undertake at least 200-300 min of moderate-intensity physical activity per week (30–60 min on most, if not all, days of the week).

Body composition

Body weight was measured in kilograms with a Tanita scale (TANITA BC-420MA. Bio Lógica. Tecnología Médica SL). Height was measured using a SECA stadiometer (range 80-200 cm). The BMI was calculated as $[\text{body weight (kg)} / (\text{height (m)})^2]$. Body

composition (fat mass and fat-free mass) was assessed by Dual-energy X-ray Absorptiometry (DXA, GE Encore 2002, version 6.10.029, GE Lunar Prodigy; GE Healthcare, Madison, WI).

Follow up

Participants were contacted each year via telephone to answer a short questionnaire including current weight. In addition, 3 years after the end of the intervention participants took part in an evaluation of weight and body composition (DXA).

Genetic analyses

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. For the overweight phase, the analysis was done using polymerase chain reaction and restriction fragment length polymorphism techniques described before; *ADRB2* Gln27Glu (rs1042714) (Large et al. 1997); *ADRB3* Trp64Arg (rs4994) (Clement et al. 1995); *LEPR* Gln223Arg (rs1137101) and Lys656Asn (rs1805094) (Gotoda et al. 1997; Matsuoka et al. 1997); *PPARG* Pro12Ala (rs1801282) (Hara et al. 2000) and *MCT1* Glu490Asp (rs1049434) (Cupeiro et al. 2010) polymorphisms. For the obese phase, the genotyping of the six 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 analyses

Normal distribution of each dependent variable was tested using Qunatile-Quantile plots. Descriptive analysis was performed using mean and standard deviations. Chi-square test was used to assess whether observed genotype frequencies were in the Hardy–Weinberg equilibrium. We divided the sample into carriers and non-carriers of the certain alleles of the polymorphisms based on literature and previous results from our study. General lineal model (GLM, multivariate model) was performed for weight, BMI, fat mass and percentage body fat (final values of the intervention and 3 years) for the follow up adjusted by change values during the intervention and age to see differences between genotype groups and sexes. Statistical significance was defined at the corrected alpha of 0.00417 with correction for repeated tests across the analyses. Afterwards, the effect sizes for the presence of each polymorphism were assessed via standardized mean differences computed with pooled variance, and the respective 90% confidence intervals were calculated. Interpretation of effect sizes was based on the following criteria: 0-0.2 trivial, >0.2-0.6 small, >0.6-1.2 moderate, >1.2-2 large, and >2 very large (Hopkins 2006). Probabilities that the true effect of carrying the Glu490 allele was harmful, trivial, and beneficial were also calculated and interpreted as follows (Hopkins et al. 2009): <0.5%, most unlikely, almost certainly not; >0.5- 5%, very unlikely; >5-25%, unlikely, probably not; >25-75%, possibly; >75-95%, likely, probably; >95-99.5%, very likely; >99.5%, most likely, almost certainly. The statistical analyses were performed using IBM SPSS statistics for windows, Version 24.0 (Armonk, NY, USA: IBM Corp.) and Microsoft Excel spreadsheets (Microsoft, Redmond, WA).

Results

Baseline characteristics (time point after the weight loss intervention, before the follow up) of the 98 subjects who participated in present study (after dropouts, exclusions because of low adherence, and missing data) are summarized in table 8.

Table 8. Baseline (after the weight loss intervention) characteristics of the subjects in Study IV

BASELINE	WOMEN (49)	MEN (49)
Age (years)	38.73±8.53	39.61±7.71
Height (m)	1.61±0.07	1.76±0.07
Body weight (kg)	71.37±9.55	86.00±10.92
BMI (kg/m²)	27.47±2.81	27.84±2.87
Fat mass (kg)	28.21±5.92	25.49±7.29
Fat percentage (%)	40.30±4.29	30.18±6.07
<i>Data presented as Mean ± Standard Deviation</i>		

Genotype distributions and allele frequencies of the six gene polymorphisms are shown in Table 9 and 10. All genotype distributions were found to be in Hardy–Weinberg equilibrium ($p>0.05$).

Table 9. Genotype distribution and allele frequency of the polymorphisms of *ADRB2*, *ADRB3* and *LEPR* genes in Study IV

<i>ADRB2</i>					
	Gln27Gln	Gln27Glu	Glu27Glu	Gln27	Glu27
All (98)	36 (36.7%)	44 (45%)	18 (18.3%)	116 (59.2%)	80 (40.8%)
Women (49)	19 (38.8%)	26 (53.1%)	4 (8.1%)	64 (65.3%)	34 (34.7%)
Men(49)	17 (34.7%)	18 (36.7%)	14 (28.6%)	52 (53.1%)	46 (46.9%)
<i>ADRB3</i>					
	Trp64Trp	Trp64Arg	Arg64Arg	Trp64	Arg64
All (98)	88 (89.8%)	10 (10.2%)	0	186 (94.9%)	10 (5.1%)
Women (49)	43 (87.8%)	6 (12.2%)	0	92 (93.9%)	6 (6.1%)
Men(49)	45 (91.8%)	4 (8.2%)	0	94 (95.9%)	4 (4.1%)
<i>LEPR</i>					
	Gln223Gln	Gln223Arg	Arg223Arg	Gln223	Arg223
All (98)	34 (34.7%)	51 (52%)	13 (13.3%)	119 (60.71%)	77 (39.3%)
Women (49)	17 (34.7%)	28 (57.1%)	4(8.2%)	62 (63.3%)	36 (36.7%)
Men(49)	17 (34.7%)	23 (46.9%)	9 (18.4%)	57 (58.2%)	41 (41.8%)
Data presented as n (%) for genotypes and n (frequency) for alleles.					
<i>ADRB2</i> , beta-2 adrenergic receptor; <i>ADRB3</i> , beta-3 adrenergic receptor; <i>LEPR</i> , leptin receptor.					

Table 10. Genotype distribution and allele frequency of the polymorphisms of *LEPR*, *MCT1* and *PPARG* genes in Study IV

LEPR					
	Lys656Lys	Lys656Asn	Asn656Asn	Lys656	Asn656
All (98)	64 (65.3%)	33 (33.7%)	1 (1%)	161 (82.1%)	35 (17.9%)
Women (49)	36 (73.5%)	12 (24.5%)	1 (2%)	84 (85.7%)	14 (14.3%)
Men(49)	28 (57.1%)	21 (42.9%)	0	77 (78.6%)	21 (21.4%)
MCT1					
	Glu490Glu	Glu490Asp	Asp490Asp	Glu490	Asp490
All (98)	28 (28.57%)	45 (45.92%)	25 (25.52%)	101 (51.5 %)	95 (48.5 %)
Women (49)	16 (32.7%)	24 (49%)	9 (18.3%)	98 (57.1%)	42 (42.9%)
Men(49)	12 (24.5%)	21 (42.9%)	16 (32.7%)	45 (45.9 %)	53 (54.1%)
PPARG					
	Pro12Pro	Pro12Ala	Ala12Ala	Pro12	Ala12
All (98)	81 (82.7%)	16 (16.3%)	1 (1%)	178 (90.8%)	18 (9.2%)
Women (49)	39 (79.6%)	9 (18.4%)	1 (2%)	87 (88.8%)	11 (11.2%)
Men(49)	42 (85.7%)	7 (14.3%)	0	91 (92.9%)	7 (7.1%)
Data presented as n (%) for genotypes and n (frequency) for alleles.					
<i>LEPR</i> , leptin receptor; <i>PPARG</i> , peroxisome proliferator-activated receptor gamma; <i>MCT1</i> , monocarboxylic acid transporter.					

An interaction between the Arg64 allele and sex was observed for weight and BMI at the 3 years follow up time point (weight: $p=0.048$, $F=4.013$ (1) ; BMI: $p=0.031$, $F=4.814$ (1)), and it was revealed that difference was within men, carriers of the Arg64 allele regained more weight than non-carriers (Figure 27b), although the associated effect size in both parameters was unclear (Table 11).

Figure 27. BMI and percentage fat in non-carriers and carriers for each polymorphism at the end of the intervention and after 36 months in men

Table 11. Differences during the follow up on the studied variables in the carrier vs non-carrier groups in men

Allele (gene)	Variables	ES (CI 90%)	Chance of being +/trivial/-	Qualitative Inference
Glu27 (ADRB2)	weight	-0.02 (-0.26, 0.22)	6/83/11	unclear
	BMI	-0.03 (-0.33, 0.27)	10/73/17	unclear
	fat	-0.09 (-0.48, 0.30)	11/57/32	unclear
	fat%	-0.12 (-0.56, 0.32)	12/50/38	unclear
Arg64 (ADRB3)	weight	0.37 (-0.49, 1.23)	69/21/10	unclear
	BMI	0.37 (-0.49, 1.23)	69/21/10	unclear
	fat	0.79 (0.24, 1.33)	96/3/1	very likely positive
	fat%	0.78 (-0.21, 1.78)	89/6/5	unclear
Arg223 (LEPR)	weight	-0.03 (-0.34, 0.27)	10/73/18	unclear
	BMI	-0.04 (-0.42, 0.33)	14/62/24	unclear
	fat	0.25 (-0.12, 0.63)	59/38/3	possibly positive
	fat%	0.42 (-0.03, 0.86)	80/19/1	likely positive
Asn656 (LEPR)	weight	0.07 (-0.19, 0.33)	20/76/4	unclear
	BMI	0.09 (-0.23, 0.41)	28/65/7	unclear
	fat	0.15 (-0.18, 0.48)	40/56/4	possibly positive
	fat%	0.17 (-0.24, 0.57)	44/49/7	unclear
Glu490 (MCT1)	weight	0.00 (-0.28, 0.29)	12/76/12	unclear
	BMI	0.00 (-0.35, 0.36)	18/65/17	unclear
	fat	0.27 (-0.12, 0.67)	62/35/3	possibly positive
	fat%	0.26 (-0.16, 0.67)	59/37/4	possibly positive
Ala12 (PPARG)	weight	0.25 (0.09, 0.41)	71/29/0	possibly positive
	BMI	0.31 (0.11, 0.52)	83/17/0	likely positive
	fat	-0.42 (-1.17, 0.34)	8/22/70	unclear
	fat%	-0.38 (-1.11, 0.34)	8/24/68	unclear
<i>ES, effect size; CI, confidence interval</i>				

Moreover, main effect of the Arg223 was found for percentage fat at the 3 year follow up time point ($F=4.830$, $p=0.031$). Carriers of the Arg223 allele ended up with higher percentage fat (figure 27c). The effect size of the Arg223 allele for percentage fat (and fat mass) in both men and women was small and possibly positive (Tables 11 and 12).

Values for BMI and percentage fat before and after the follow up in women is shown in

Figure 28.

Figure 28. BMI and percentage fat in non-carriers and carriers for each polymorphism at the end of the intervention and after 36 months in women

Table 12. Differences during the follow up on the studied variables in the carrier vs non-carrier groups in women

Allele (gene)	Variables	ES (CI 90%)	Chance of being +/trivial/-	Qualitative Inference
Glu27 (ADRB2)	weight	0.12 (-0.24, 0.49)	36/57/7	unclear
	BMI	0.16 (-0.31, 0.63)	44/46/10	unclear
	fat	-0.03 (-0.52, 0.46)	22/50/28	unclear
	fat%	-0.18 (-0.75, 0.38)	13/39/48	unclear
Arg64 (ADRB3)	weight	-0.01 (-2.52, 2.50)	35/30/35	unclear
	BMI	-0.01 (-3.24, 3.23)	37/24/39	unclear
	fat	-0.37 (-0.89, 0.16)	4/25/71	possibly negative
	fat%	-0.29 (-0.88, 0.29)	8/31/61	unclear
Arg223 (LEPR)	weight	0.11 (-0.29, 0.51)	36/55/9	unclear
	BMI	0.03 (-0.09, 0.15)	2/98/0	very likely trivial
	fat	0.31 (-0.15, 0.77)	66/31/3	possibly positive
	fat%	0.31 (-0.18, 0.79)	65/31/4	possibly positive
Asn656 (LEPR)	weight	0.06 (-0.29, 0.41)	25/64/11	unclear
	BMI	0.08 (-0.37, 0.53)	32/53/15	unclear
	fat	0.03 (-0.42, 0.47)	26/54/20	unclear
	fat%	-0.12 (-0.62, 0.39)	15/46/39	unclear
Glu490 (MCT1)	weight	-0.06 (-0.38, 0.26)	9/68/23	unclear
	BMI	-0.08 (-0.49, 0.33)	13/56/31	unclear
	fat	-0.10 (-0.82, 0.63)	24/36/40	unclear
	fat%	-0.13 (-0.96, 0.71)	25/31/44	unclear
Ala12 (PPARG)	weight	0.22 (-0.39, 0.83)	53/36/11	unclear
	BMI	0.28 (-0.50, 1.06)	58/29/13	unclear
	fat	0.27 (-1.02, 1.55)	54/21/25	unclear
	fat%	0.04 (-1.55, 1.64)	43/19/38	unclear
<i>ES, effect size; CI, confidence interval</i>				

Weight regain, BMI, fat mass or percentage fat changes were similar during the 3-year follow up in carriers and non-carriers for the other polymorphisms. Calculated effect sizes showed the following. In women, a small and possibly negative effect of the Arg64 allele was seen for fat mass (Table 12), but the contrary was observed in men, a moderate, very likely positive effect for fat mass (Table 11). Furthermore, a possible harmful effect of the Asn656 allele was found for fat mass in men (Table 11). Moreover,

effect size showed a trivial and possibly positive effect on fat and fat mass in men (Table 11). Weight (small and possibly positive) and BMI (small and likely positive) seems to be associated with Ala12 allele in men according to standardized mean differences (Table 11).

Discussion

The aim of the present study was to see the effect of common polymorphisms of the *ADRB2*, *ADRB3*, *LEPR*, *PPARG* and *MCT1* genes on weight and body fat maintenance throughout 3 years after a 22-week diet and exercise intervention. Our results suggest no strong influence of the studied polymorphisms and body composition changes.

Though we can find a wide range of epidemiological studies on obesity phenotypes in connection with these polymorphisms, just a handful of studies has investigated the role of these in weight loss or even less in weight regain. The comparison of studies is also complicated because the methodologies of the interventions are diverse.

Based on our results, men carriers of the Arg64 allele of the *ADRB3* gene had higher weight and BMI values 3 years after the intervention than the non-carriers, however the difference due to the presence of the allele was unclear. A previous study analyzed the effect of Trp64Arg polymorphism in an intervention program with weight maintenance, but found no differences among genetic groups (Masuo et al. 2005). However, this study included Japanese participants and subjects of this intervention followed a low calorie diet and aerobic exercise; however, counseling sessions were continuous all long the 24 months (not a free living maintenance as in our study) (Masuo et al. 2005). It was previously suggested that there might be big differences among races and a meta-analysis confirmed the heterogeneity of the effect of the

Trp64Arg polymorphism according to race, namely, it might be associated in East Asians, but not in Europeans (Kurokawa et al. 2008). Larsen et al. found no evidence for the influence of the Trp64Arg polymorphism on European populations (different European centers), after an 8-week long low calorie diet during 6-month long follow up, but still applying different diets (comparing different dietary protein and glycemic index proportions) again not free living condition as in our study and no exercise was included (Larsen et al. 2012). Another research group found that a combination of the Trp64Arg polymorphism and A-G mutation in the UCP-1 (uncoupled protein-1) gene may be associated with faster weight gain after a very low calorie diet (Fogelholm et al. 1998). Arg64 allele's implication in weight regain and BMI increase can be explained by its original proposal as a candidate gene, since the ADRB3 protein influences energy expenditure, lipid and glucose metabolism (Arner 1992; Hagstrom-Toft et al. 1998; Lafontan et al. 1997). Differences in these roles (lower lipolysis) were reported by functional studies (Pietri-Rouxel et al. 1997; Umekawa et al. 1999), which could explain, at least in part, why men carrying this allele regain more weight than no-carriers during the follow-up.

On the other hand, carriers of the Arg223 allele of the *LEPR* gene had higher percentage fat than the non-carriers 3 years after the intervention, with small sizes in both genders. To the best of our knowledge, only the previously mentioned study of Larsen et al. included the *LEPR* gene studying only the Gln223Arg polymorphisms reporting no differences (Larsen et al. 2012). As mentioned before, it was not a free living follow up study, therefore the different results obtained by us could be due to the different protocols. However, the fact that the Arg223 allele caused altered leptin

receptor function (food intake, appetite control, energy homeostasis) (Dulloo et al. 2002; Jequier 2002) support the differences between genetic groups seen in our study.

The previously mentioned Japanese study included the Gln27Glu polymorphism, showing that groups which failed to lose weight at 6 months had higher frequencies of the Glu27 allele compared to the groups succeeded in significant weight loss at 6 months. In addition, subjects carrying the Glu27 allele had greater total body fat mass, but no difference in BMI (Masuo et al. 2005). No differences in weight and BMI phenotypes are concordant with our results, however differences in fat mass are contradictory. Race differences were suggested for the Gln27Glu polymorphism too, specifically it seems to be a risk factor for obesity in Asians, Pacific Islanders, and American Indians, but not in Europeans (Jalpa, Rhoads, and Demissie 2008).

In line with our results, Larsen et al. found no differences between carriers and non-carriers of the *PPARG* Pro12Ala polymorphism. Aldhoun et al. during a low energy diet and increased physical activity intervention and follow up in Czech obese women revealed no difference either (Aldhoun, Zamrazilova, et al. 2010), nor Vogels et al. during a 1 year maintenance following a 6-week very low calorie diet with Dutch subjects (Vogels et al. 2005). Nevertheless, Delanthy et al. during a long follow up in overweight and obese adults (pooling placebo, metformin treated and lifestyle modification groups, however adjusting later for age, sex, ethnicity, treatment and baseline values) reported that the Ala12 allele was associated with weight regain (Delahanty et al. 2012). Nicklas et al. found that weight regain during 12-month follow up of a 6-month hypocaloric diet was greater in Ala12 allele carrier women than in Pro12Pro homozygotes and *PPARG* genotype was the best predictor of weight regain.

On the other hand, Goyenechea et al. 1 year after a low energy diet found that the protective effect of the 174G-C polymorphism of the IL-6 gene was strengthened by the Arg64 allele (Goyenechea, Dolores Parra, and Alfredo Martinez 2006).

Due to the interventional nature of the study, relatively limited number of participants can be involved in individualized and supervised exercise and diet counseling. On the other hand, number of subjects in different genotype groups is low in some cases, as genotyping took place after selecting the subjects, although with several genes and polymorphisms it would be hard to randomize equally for each gene. One of the strengths of our study is that both diet and exercise parts of the weight loss intervention were controlled. Moreover, at the 3-year time point again not only weight was measured, but body composition with DXA was assessed.

In conclusion, our results do not clearly support the role of the studied polymorphisms in weight and body fat regain after the completion of a weight loss intervention. The exceptions are the Trp64Arg polymorphism of the *ADRB3* and the Gln223Arg polymorphism of the *LEPR*, where the Arg64 and Arg223 alleles might imply easier weight and fat regain respectively. Similar controlled interventions with larger sample size should be carried out to study and discard or confirm the role of these polymorphisms taking into account gene-gene, gene-environment interactions and many other factors. On the other hand, it has been suggested, that weight loss maintenance is mainly influenced by psychobehavioral factors and even those factors are frequently genetically determined (Hainer et al. 2008). This raises the idea that these genes might be important in the development of obesity and weight loss, but not in weight maintenance, and that we should look for other potential genes and

polymorphisms that might have importance in the psychobehavioral side of obesity, which might influence more weight maintenance and regain.

4. CONCLUSIONS

4. CONCLUSIONS

Conclusions of Study I

- The Gln27Glu polymorphism of the ADRB2 gene does not seem to have major effect on weight changes.
- Arg64 carriers (especially women) might have difficulty in reducing fat mass and fat percentage during a diet and exercise intervention.
- Supervised exercise might counterbalance the harmful effect of the Arg64.

Conclusions of Study II

- The Gln223Arg polymorphism and the Lys656Asn polymorphism of the LEPR gene does not seem influence body weight or body composition variables after the weight loss intervention.
- The Pro12Ala polymorphism of the PPARG gene is not likely to influence body weight changes; however Ala12 allele might imply higher visceral adipose tissue values a weight loss intervention.

Conclusion of Study III

- Carrying the Glu490 allele might lead to difficulty in decreasing body weight and BMI.
- The Glu490 allele might be disadvantageous for women to reduce percentage fat, but advantageous for men.
- Carrying the Glu490 might be unfavorable for maintaining fat free mass during a weight loss program.

Conclusion of Studies I and II (Secondary objective)

Conclusion 1: None of the studied polymorphisms (the *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, *LEPR* Gln223Arg and Lys656Asn, *PPARG* Pro12Ala) seem to influence baseline obesity phenotypes.

Conclusion of Study IV

- Carrying the Arg64 allele of the *ADRB3* gene might be disadvantageous during weight maintenance.
- The Arg223 allele of the *LEPR* gene might imply easier body fat recovery in free-living conditions after a diet and exercise intervention.
- Gln27Glu polymorphism of the *ADRB2*, Lys656Asn polymorphism of the *LEPR*, Glu490Asp polymorphism of the *MCT1* and Pro12Ala polymorphism of the *PPARG* genes do not seem to influence body weight or fat regain after a 3 year follow up.

5. LIMITATIONS OF THE STUDY

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After the implementation of the project, data analysis, discussion and comparison of our results with other studies, we present a critical view of our work, listing the main limitations below:

- Sample size and power was calculated for the main objective of the project i.e. weight loss, therefore genetic study part could be underpowered.
- The balanced genotype distribution was not ensured as genotyping was carried out after choosing and randomizing participants in intervention groups.
- During the follow up we have no information about eating of physical activity habits of the participants

However the strengths of the PRONAF study should be highlighted too:

- Double blinded study.
- Those who completed the intervention and are included in present thesis, all had a adherence of 90% for exercise and 80% for diet .
- The intervention was conducted as a collaboration of different centers by a multidisciplinary group.
- In the supervised groups, through the 22 weeks, during all the training sessions participants were monitored by certified trainers to ensure the correct execution of the exercises and the completion of each session.

6. FUTURE RESEARCH LINES

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This project has given the possibility to study the influence of genetic polymorphisms on body composition variables during a controlled weight loss intervention. Learning from it and having a wider view of similar projects, future studies should take into consideration the following points:

- Include subjects with pathologies.
- Include novel candidate genes from genome wide association studies.
- Organize multi-center collaborations in order to obtain a large sample size to ensure power.
- Take into account gene-gene interactions.
- Add further variables: direct or almost direct ligands, proteins, hormones etc. in order to see a closer relationship between polymorphisms and phenotypes and let us gain an insight to the physiological processes running in the background.

7. PRACTICAL APPLICATION

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Our study implies that these polymorphisms individually do not influence decisively weight loss or weight regain or body composition changes during an exercise and diet intervention. Therefore, we believe that currently available gene tests (including these polymorphisms) promising to support an individualized treatment based on genetic background should be skeptically examined. However scientific advances might lead us to accurate gene tests which help us designing weight loss interventions (diet, exercise even psychology) for each individual and implement the personalized medicine for patients with obesity in the future.

8. REFERENCES

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- Abecasis, G. R., A. Auton, L. D. Brooks, M. A. DePristo, R. M. Durbin, R. E. Handsaker, H. M. Kang, G. T. Marth, and G. A. McVean.** 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* 491 (7422):56-65.
- Acharya, S. D., O. U. Elci, S. M. Sereika, E. Music, M. A. Styn, M. W. Turk, and L. E. Burke.** 2009. Adherence to a behavioral weight loss treatment program enhances weight loss and improvements in biomarkers. *Patient Prefer Adherence* 3:151-60.
- Adamo, K. B., R. Dent, C. D. Langefeld, M. Cox, K. Williams, K. M. Carrick, J. S. Stuart, S. S. Sundseth, M. E. Harper, R. McPherson, and F. Tesson.** 2007. Peroxisome proliferator-activated receptor gamma 2 and acyl-CoA synthetase 5 polymorphisms influence diet response. *Obesity (Silver Spring)* 15 (5):1068-75.
- Ahmadian, Maryam, Jae Myoung Suh, Nasun Hah, Christopher Liddle, Annette R Atkins, Michael Downes, and Ronald M Evans.** 2013. PPAR [gamma] signaling and metabolism: the good, the bad and the future. *Nature medicine* 99 (5):557-566.
- Ainscow, Edward K, Shirin Mirshamsi, Teresa Tang, Michael LJ Ashford, and Guy A Rutter.** 2002. Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP - independent control of ATP - sensitive K⁺ channels. *The Journal of physiology* 544 (2):429-445.
- Aldhoon, B., H. Zamrazilova, I. Aldhoon Hainerova, P. Sramkova, J. Spalova, M. Kunesova, B. Bendlova, and V. Hainer.** 2010. Role of the PPARalpha Leu162Val and PPARgamma2 Pro12Ala gene polymorphisms in weight change after 2.5-year follow-up in Czech obese women. *Folia Biol (Praha)* 56 (3):116-23.
- Allison, DB, M Heo, MS Faith, and A Pietrobelli.** 1998. Meta-analysis of the association of the Trp64Arg polymorphism in the beta3 adrenergic receptor with body mass index. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity* 22 (6):559.
- Aranceta, Javier, Carmen Pérez-Rodrigo, Lluís Serra-Majem, Diego Bellido, Martín López de la Torre, Xavier Formiguera, and Basilio Moreno.** 2007. Prevention of overweight and obesity: a Spanish approach. *Public health nutrition* 10 (10A):1187-1193.
- Arner, P, and F Lonnqvist.** 1987. Interactions between receptors for insulin and catecholamines in fat cells. *Recent advances in obesity research.*
- Arner, P, C Marcus, B Karpe, T Sonnenfeld, and P Blome.** 1987. Role of alpha - adrenoceptors for adipocyte size in man. *European journal of clinical investigation* 17 (1):58-62.

- Arner, P.** 1992. Adrenergic receptor function in fat cells. *Am J Clin Nutr* 55 (1 Suppl):228S-236S.
- Arner, P.** 1995. The beta 3-adrenergic receptor--a cause and cure of obesity? *N Engl J Med* 333 (6):382-3.
- Arner, P.** 2000. Obesity--a genetic disease of adipose tissue? *Br J Nutr* 83 Suppl 1:S9-16.
- Arner, Peter.** 2001. Genetic variance and lipolysis regulation: implications for obesity. *Annals of medicine* 33 (8):542-546.
- Arner, Peter.** 2005. Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best practice & research Clinical endocrinology & metabolism* 19 (4):471-482.
- Arner, Peter.** 1989. Metabolism of fatty acids. An overview. In *Obesity: towards a molecular approach* edited by R. D. Bray G, Spiegelman B, . UCLA symposia on molecular and cellular biology. New Series.
- Arner, Peter, Peter Engfeldt, and Jacek Nowak.** 1981. In vivo observations on the lipolytic effect of noradrenaline during therapeutic fasting. *The Journal of Clinical Endocrinology & Metabolism* 53 (6):1207-1212.
- Arner, Peter, Eva Kriegholm, Peter Engfeldt, and Jan Bolinder.** 1990. Adrenergic regulation of lipolysis in situ at rest and during exercise. *Journal of Clinical Investigation* 85 (3):893.
- Auwerx, J.** 1999. PPARgamma, the ultimate thrifty gene. *Diabetologia* 42 (9):1033-49.
- Barak, Yaacov, Michael C Nelson, Estelita S Ong, Ying Z Jones, Pilar Ruiz-Lozano, Kenneth R Chien, Alan Koder, and Ronald M Evans.** 1999. PPARγ is required for placental, cardiac, and adipose tissue development. *Molecular cell* 4 (4):585-595.
- Barroso, I, M Gurnell, VEF Crowley, M Agostini, JW Schwabe, MA Soos, G Li Maslen, TDM Williams, H Lewis, and AJ Schafer.** 1999. Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 402 (6764):880-883.
- Bea, J. W., T. G. Lohman, E. C. Cussler, S. B. Going, and P. A. Thompson.** 2010. Lifestyle modifies the relationship between body composition and adrenergic receptor genetic polymorphisms, ADRB2, ADRB3 and ADRA2B: a secondary analysis of a randomized controlled trial of physical activity among postmenopausal women. *Behav Genet* 40 (5):649-59.
- Beamer, Brock A, Chung-Jen Yen, Ross E Andersen, Denis Muller, Dariush Elahi, Lawrence J Cheskin, Reubin Andres, Jesse Roth, and Alan R Shuldiner.** 1998. Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor- 2 gene with obesity in two Caucasian populations. *DIABETES-NEW YORK-* 47:1806-1808.
- Bell, Christopher G, Andrew J Walley, and Philippe Froguel.** 2005. The genetics of human obesity. *Nature Reviews Genetics* 6 (3):221-234.

- Bender, N., N. Allemann, D. Marek, P. Vollenweider, G. Waeber, V. Mooser, M. Egger, and M. Bochud.** 2011. Association between variants of the leptin receptor gene (LEPR) and overweight: a systematic review and an analysis of the CoLaus study. *PLoS One* 6 (10):e26157.
- Bickham, Dale C, David J Bentley, Peter F Le Rossignol, and David Cameron-Smith.** 2006. The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. *European journal of applied physiology* 96 (6):636-643.
- Bishop-Bailey, David.** 2000. Peroxisome proliferator - activated receptors in the cardiovascular system. *British journal of pharmacology* 129 (5):823-834.
- Blaak, E. E., M. A. van Baak, K. P. Kempen, and W. H. Saris.** 1993. Role of alpha- and beta-adrenoceptors in sympathetically mediated thermogenesis. *Am J Physiol* 264 (1 Pt 1):E11-7.
- Bouchard, C.** 2008. Gene-environment interactions in the etiology of obesity: defining the fundamentals. *Obesity (Silver Spring)* 16 Suppl 3:S5-S10.
- Bouchard, C., A. Tremblay, J. P. Despres, G. Theriault, A. Nadeau, P. J. Lupien, S. Moorjani, D. Prudhomme, and G. Fournier.** 1994. The response to exercise with constant energy intake in identical twins. *Obes Res* 2 (5):400-10.
- Bouchard, Claude.** 2007. The biological predisposition to obesity: beyond the thrifty genotype scenario. *Int J Obes (Lond)* 31 (9):1337.
- Bouchard, Claude.** 2008. Gene–environment interactions in the etiology of obesity: defining the fundamentals. *Obesity* 16 (S3):S5-S10.
- Bouchard, Claude, Steven N Blair, and William Haskell.** 2012. *Physical activity and health:* Human Kinetics.
- Bouchard, Claude, and Angelo Tremblay.** 1997. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *The Journal of nutrition* 127 (5):943S-947S.
- Bouchard, Claude, Angelo Tremblay, Jean-Pierre Després, André Nadeau, Paul J Lupien, Germain Thériault, Jean Dussault, Sital Moorjani, Sylvie Pinault, and Guy Fournier.** 1990. The response to long-term overfeeding in identical twins. *New England Journal of Medicine* 322 (21):1477-1482.
- Börjeson, M.** 1976. The Aetiology Of Obesity In Children A Study of 101 Twin Pairs. *Acta Paediatrica* 65 (3):279-287.
- Bray, M. S., J. M. Hagberg, L. Perusse, T. Rankinen, S. M. Roth, B. Wolfarth, and C. Bouchard.** 2009. The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. *Med Sci Sports Exerc* 41 (1):35-73.

- Britton, Kathryn A, Joseph M Massaro, Joanne M Murabito, Bernard E Kreger, Udo Hoffmann, and Caroline S Fox.** 2013. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. *Journal of the American College of Cardiology* 62 (10):921-925.
- Brochu, C. A., J. R. Wagner, S. Jouve, C. D. Sumrall, and L. D. Densmore.** 2009. A correction corrected: consensus over the meaning of Crocodylia and why it matters. *Syst Biol* 58 (5):537-43.
- Brochu, M., M. F. Malita, V. Messier, E. Doucet, I. Strychar, J. M. Lavoie, D. Prud'homme, and R. Rabasa-Lhoret.** 2009. Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women. *J Clin Endocrinol Metab* 94 (9):3226-33.
- Brooks, G. A.** 2009. Cell-cell and intracellular lactate shuttles. *J Physiol* 587 (Pt 23):5591-600.
- Campfield, L Arthur, Francoise J Smith, Yves Guisez, Rene Devos, and Paul Burn.** 1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269 (5223):546.
- Canis, Martin, Martin H Maurer, Wolfgang Kuschinsky, Lutz Duembgen, and Roman Duelli.** 2009. Increased densities of monocarboxylate transporter MCT1 after chronic hyperglycemia in rat brain. *Brain research* 1257:32-39.
- Carneiro, L, and L Pellerin.** 2015. Monocarboxylate transporters: new players in body weight regulation. *obesity reviews* 16 (S1):55-66.
- Cha, Seung Hun, and M Daniel Lane.** 2009. Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway. *Biochemical and biophysical research communications* 386 (1):212-216.
- Chagnon, Y. C., W. K. Chung, L. Perusse, M. Chagnon, R. L. Leibel, and C. Bouchard.** 1999. Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. *Int J Obes Relat Metab Disord* 23 (3):278-86.
- Chagnon, Y. C., J. H. Wilmore, I. B. Borecki, J. Gagnon, L. Perusse, M. Chagnon, G. R. Collier, A. S. Leon, J. S. Skinner, D. C. Rao, and C. Bouchard.** 2000. Associations between the leptin receptor gene and adiposity in middle-aged Caucasian males from the HERITAGE family study. *J Clin Endocrinol Metab* 85 (1):29-34.
- Chawla, Ajay, Emily J Schwarz, Dwight D Dimaculangan, and Mitchell A Lazar.** 1994. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 135 (2):798-800.
- Chung, Wendy K, Loraine Power-Kehoe, Melvin Chua, and Florence Chu.** 1997. Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). *Diabetes* 46 (9):1509.

- Clark, J. E.** 2015. Diet, exercise or diet with exercise: comparing the effectiveness of treatment options for weight-loss and changes in fitness for adults (18-65 years old) who are overfat, or obese; systematic review and meta-analysis. *J Diabetes Metab Disord* 14:31.
- Clement, K., S. Hercberg, B. Passinge, P. Galan, M. Varroud-Vial, A. R. Shuldiner, B. A. Beamer, G. Charpentier, B. Guy-Grand, P. Froguel, and C. Vaisse.** 2000. The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. *Int J Obes Relat Metab Disord* 24 (3):391-3.
- Clement, K., C. Vaisse, B. S. Manning, A. Basdevant, B. Guy-Grand, J. Ruiz, K. D. Silver, A. R. Shuldiner, P. Froguel, and A. D. Strosberg.** 1995. Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 333 (6):352-4.
- Clement, Karine, Christian Vaisse, Najiba Lahlou, Sylvie Cabrol, Veronique Pelloux, Dominique Cassuto, Micheline Gormelen, Christian Dina, Jean Chambaz, and Jean-Marc Lacorte.** 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392 (6674):398-401.
- Coman, Oana Andreia, H Paunescu, Isabel Ghita, L Coman, Anca Badararu, and I Fulga.** 2009. Beta 3 adrenergic receptors: molecular, histological, functional and pharmacological approaches. *Rom J Morphol Embryol* 50 (2):169-79.
- Considine, Robert V, Madhur K Sinha, Mark L Heiman, Aidas Kriauciunas, Thomas W Stephens, Mark R Nyce, Joanna P Ohannesian, Cheryl C Marco, Linda J McKee, and Thomas L Bauer.** 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine* 334 (5):292-295.
- Constantin, A., G. Costache, A. V. Sima, C. S. Glavce, M. Vladica, and D. L. Popov.** 2010. Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects. *Biochem Biophys Res Commun* 391 (1):282-6.
- Corbalan, M. S., A. Marti, L. Forga, M. A. Martinez-Gonzalez, and J. A. Martinez.** 2002. The risk of obesity and the Trp64Arg polymorphism of the beta(3)-adrenergic receptor: effect modification by age. *Ann Nutr Metab* 46 (3-4):152-8.
- Corella, D., M. Guillen, O. Portoles, J. V. Sorli, V. Alonso, J. Folch, and C. Saiz.** 2001. Gender specific associations of the Trp64Arg mutation in the beta3-adrenergic receptor gene with obesity-related phenotypes in a Mediterranean population: interaction with a common lipoprotein lipase gene variation. *J Intern Med* 250 (4):348-60.
- Costa-Font, J., and J. Gil.** 2005. Obesity and the incidence of chronic diseases in Spain: a seemingly unrelated probit approach. *Econ Hum Biol* 3 (2):188-214.

- Cupeiro, R., P. J. Benito, N. Maffulli, F. J. Calderon, and D. Gonzalez-Lamuno.** 2010. MCT1 genetic polymorphism influence in high intensity circuit training: a pilot study. *J Sci Med Sport* 13 (5):526-30.
- Cupeiro, R., D. Gonzalez-Lamuno, T. Amigo, A. B. Peinado, J. R. Ruiz, F. B. Ortega, and P. J. Benito.** 2012. Influence of the MCT1-T1470A polymorphism (rs1049434) on blood lactate accumulation during different circuit weight trainings in men and women. *J Sci Med Sport* 15 (6):541-7.
- Cupeiro, R., R. Perez-Prieto, T. Amigo, P. Gortazar, C. Redondo, and D. Gonzalez-Lamuno.** 2016. Role of the monocarboxylate transporter MCT1 in the uptake of lactate during active recovery. *Eur J Appl Physiol* 116 (5):1005-10.
- Curioni, C. C., and P. M. Lourenco.** 2005. Long-term weight loss after diet and exercise: a systematic review. *Int J Obes (Lond)* 29 (10):1168-74.
- Dapcich, Verónica, Gemma Salvador Castell, Lourdes Ribas Barba, Carmen Pérez Rodrigo, Javier Aranceta Bartrina, and Lluís Serra Majem.** 2004. Guía de la alimentación saludable. In *Guía de la alimentación saludable*: Sociedad Española de Nutrición Comunitaria.
- de Castro, John M.** 2004. Genes, the environment and the control of food intake. *British Journal of Nutrition* 92 (S1):S59-S62.
- de Heredia, Fátima Pérez, I Stuart Wood, and Paul Trayhurn.** 2010. Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes. *Pflügers Archiv-European Journal of Physiology* 459 (3):509-518.
- de Luis, D. A., M. Gonzalez Sagrado, R. Aller, O. Izaola, R. Conde, and M. J. Castro.** 2011. Lys656Asn polymorphism of leptin receptor gene and metabolic syndrome in obese patients. *Eur Rev Med Pharmacol Sci* 15 (5):463-8.
- de Luis Roman, Daniel, Rocio Aller de la Fuente, Manuel Gonzalez Sagrado, Olatz Izaola, and Rosa Conde Vicente.** 2006. Leptin receptor Lys656Asn polymorphism is associated with decreased leptin response and weight loss secondary to a lifestyle modification in obese patients. *Archives of medical research* 37 (7):854-859.
- de Pergola, G, M Cignarelli, G Nardelli, Gabriella Garruti, Maria Corso, S Di Paolo, Felice Cardone, and R Giorgino.** 1989. Influence of lactate on isoproterenol-induced lipolysis and b-adrenoceptors distribution in human fat cells. *Horm Metab Res* 21:210-213.
- Deeb, S. S., L. Fajas, M. Nemoto, J. Pihlajamaki, L. Mykkanen, J. Kuusisto, M. Laakso, W. Fujimoto, and J. Auwerx.** 1998. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20 (3):284-7.

- Deeb, Samir S, Lluís Fajas, Masami Nemoto, Jussi Pihlajamäki, Leena Mykkänen, Johanna Kuusisto, Markku Laakso, Wilfred Fujimoto, and Johan Auwerx.** 1998. A Pro12Ala substitution in PPAR γ 2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nature genetics* 20 (3):284-287.
- del Corral, P., P. C. Chandler-Laney, K. Casazza, B. A. Gower, and G. R. Hunter.** 2009. Effect of dietary adherence with or without exercise on weight loss: a mechanistic approach to a global problem. *J Clin Endocrinol Metab* 94 (5):1602-7.
- del Corral, Pedro, Paula C Chandler-Laney, Krista Casazza, Barbara A Gower, and Gary R Hunter.** 2009. Effect of dietary adherence with or without exercise on weight loss: a mechanistic approach to a global problem. *The Journal of Clinical Endocrinology & Metabolism* 94 (5):1602-1607.
- Delahanty, Linda M, Qing Pan, Kathleen A Jablonski, Karol E Watson, Jeanne M McCaffery, Alan Shuldiner, Steven E Kahn, William C Knowler, Jose C Florez, and Paul W Franks.** 2012. Genetic predictors of weight loss and weight regain after intensive lifestyle modification, metformin treatment, or standard care in the Diabetes Prevention Program. *Diabetes Care* 35 (2):363-366.
- DiGirolamo, M, FD Newby, and J Lovejoy.** 1992. Lactate production in adipose tissue: a regulated function with extra-adipose implications. *The FASEB Journal* 6 (7):2405-2412.
- Donnelly, J. E., S. N. Blair, J. M. Jakicic, M. M. Manore, J. W. Rankin, and B. K. Smith.** 2009. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc* 41 (2):459-71.
- Donnelly, JE, SN Blair, JM Jakicic, MM Manore, JW Rankin, and BK Smith.** 2009. Appropriate Physical Activity Intervention Strategies for Weight Loss and Prevention of Weight Regain for Adults. *Medicine & Science in Sports & Exercise*:459-471.
- Dubouchaud, Hervé, Gail E Butterfield, Eugene E Wolfel, Bryan C Bergman, and George A Brooks.** 2000. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *American Journal of Physiology-Endocrinology And Metabolism* 278 (4):E571-E579.
- Dulloo, A. G., M. J. Stock, G. Solinas, O. Boss, J. P. Montani, and J. Seydoux.** 2002. Leptin directly stimulates thermogenesis in skeletal muscle. *FEBS Lett* 515 (1-3):109-13.
- Echwald, S. M., T. I. Sorensen, A. Tybjaerg-Hansen, T. Andersen, and O. Pedersen.** 1998. Gln27Glu variant of the human beta2-adrenoreceptor gene is not associated with early-onset obesity in Danish men. *Diabetes* 47 (10):1657-8.

- Ek, J., S. A. Urhammer, T. I. Sorensen, T. Andersen, J. Auwerx, and O. Pedersen.** 1999. Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* 42 (7):892-5.
- Engfeldt, Peter, Johan Hellmér, Hans Wahrenberg, and Peter Arner.** 1988. Effects of insulin on adrenoceptor binding and the rate of catecholamine-induced lipolysis in isolated human fat cells. *Journal of Biological Chemistry* 263 (30):15553-15560.
- Enoksson, S., M. Talbot, F. Rife, W. V. Tamborlane, R. S. Sherwin, and S. Caprio.** 2000. Impaired in vivo stimulation of lipolysis in adipose tissue by selective beta2-adrenergic agonist in obese adolescent girls. *Diabetes* 49 (12):2149-53.
- Farooqi, I Sadaf, Giuseppe Matarese, Graham M Lord, Julia M Keogh, Elizabeth Lawrence, Chizo Agwu, Veronica Sanna, Susan A Jebb, Francesco Perna, and Silvia Fontana.** 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *The Journal of clinical investigation* 110 (8):1093-1103.
- Fedotovskaya, O. N., L. J. Mustafina, D. V. Popov, O. L. Vinogradova, and Ahmetov, II.** 2014. A common polymorphism of the MCT1 gene and athletic performance. *Int J Sports Physiol Perform* 9 (1):173-80.
- Fishbein, W. N., N. Merezinskaya, and J. W. Foellmer.** 2002. Relative distribution of three major lactate transporters in frozen human tissues and their localization in unfixed skeletal muscle. *Muscle Nerve* 26 (1):101-12.
- Fogelholm, M., R. Valve, K. Kukkonen-Harjula, A. Nenonen, V. Hakkarainen, M. Laakso, and M. Uusitupa.** 1998. Additive effects of the mutations in the beta3-adrenergic receptor and uncoupling protein-1 genes on weight loss and weight maintenance in Finnish women. *J Clin Endocrinol Metab* 83 (12):4246-50.
- Fox, C. S., Y. Liu, C. C. White et al.** 2012. Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women. *PLoS Genet* 8 (5):e1002695.
- Franks, PW, KA Jablonski, L Delahanty, RL Hanson, SE Kahn, D Altshuler, WC Knowler, JC Florez, and Diabetes Prevention Program Research Group.** 2007. The Pro12Ala variant at the peroxisome proliferator-activated receptor γ gene and change in obesity-related traits in the Diabetes Prevention Program. *Diabetologia* 50 (12):2451-2460.
- Franz, M. J., J. J. VanWormer, A. L. Crain, J. L. Boucher, T. Histon, W. Caplan, J. D. Bowman, and N. P. Pronk.** 2007. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *J Am Diet Assoc* 107 (10):1755-67.

- Frayling, Timothy M.** 2007. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nature Reviews Genetics* 8 (9):657-662.
- Fried, Susan K, Matthew R Ricci, Colleen D Russell, and Blandine Laferrère.** 2000. Regulation of leptin production in humans. *The Journal of nutrition* 130 (12):3127S-3131S.
- Friedman, Jeffrey M, and Jeffrey L Halaas.** 1998. Leptin and the regulation of body weight in mammals. *Nature* 395 (6704):763-770.
- Gagnon, J., P. Mauriege, S. Roy, D. Sjostrom, Y. C. Chagnon, F. T. Dionne, J. M. Oppert, L. Perusse, L. Sjostrom, and C. Bouchard.** 1996. The Trp64Arg mutation of the beta3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish Obese Subjects cohorts. *J Clin Invest* 98 (9):2086-93.
- Gajewska, Joanna, Alina Kuryłowicz, Ewa Mierzejewska, Jadwiga Ambroszkiewicz, Magdalena Chęłchowska, Halina Weker, and Monika Puzianowska-Kuźnicka.** 2016. Complementary Effects of Genetic Variations in LEPR on Body Composition and Soluble Leptin Receptor Concentration after 3-Month Lifestyle Intervention in Prepubertal Obese Children. *Nutrients* 8 (6):328.
- Gallus, Silvano, Alessandra Lugo, Bojana Murisic, Cristina Bosetti, Paolo Boffetta, and Carlo La Vecchia.** 2015. Overweight and obesity in 16 European countries. *European journal of nutrition* 54 (5):679-689.
- Garenc, C., L. Perusse, Y. C. Chagnon, T. Rankinen, J. Gagnon, I. B. Borecki, A. S. Leon, J. S. Skinner, J. H. Wilmore, D. C. Rao, and C. Bouchard.** 2003. Effects of beta2-adrenergic receptor gene variants on adiposity: the HERITAGE Family Study. *Obes Res* 11 (5):612-8.
- Garenc, C., L. Perusse, T. Rankinen, J. Gagnon, A. S. Leon, J. S. Skinner, J. H. Wilmore, D. C. Rao, and C. Bouchard.** 2001. The Trp64Arg polymorphism of the beta3-adrenergic receptor gene is not associated with training-induced changes in body composition: The HERITAGE Family Study. *Obes Res* 9 (6):337-41.
- Gonzalez Sanchez, J. L., A. M. Proenza, M. T. Martinez Larrad, J. M. Ramis, C. Fernandez Perez, A. Palou, and M. Serrano Rios.** 2003. The glutamine 27 glutamic acid polymorphism of the beta2-adrenoceptor gene is associated with abdominal obesity and greater risk of impaired glucose tolerance in men but not in women: a population-based study in Spain. *Clin Endocrinol (Oxf)* 59 (4):476-81.
- Gotoda, T., B. S. Manning, A. P. Goldstone, H. Imrie, A. L. Evans, A. D. Strosberg, P. M. McKeigue, J. Scott, and T. J. Aitman.** 1997. Leptin receptor gene variation and obesity: lack of association in a white British male population. *Hum Mol Genet* 6 (6):869-76.
- Gotoda, Takanari, Brian S Manning, Anthony P Goldstone, Helen Imrie, Alison L Evans, A Donny Strosberg, Paul M McKeigue, James Scott, and Timothy J Aitman.** 1997. Leptin

- receptor gene variation and obesity: lack of association in a white British male population. *Human molecular genetics* 6 (6):869-876.
- Goyenechea, E., M. Dolores Parra, and J. Alfredo Martinez.** 2006. Weight regain after slimming induced by an energy-restricted diet depends on interleukin-6 and peroxisome-proliferator-activated-receptor-gamma2 gene polymorphisms. *Br J Nutr* 96 (5):965-72.
- Green, SA, J Turki, IP Hall, and SB Liggett.** 1995. Implications of genetic variability of human β 2-adrenergic receptor structure. *Pulmonary pharmacology* 8 (1):1-10.
- Green, Stuart A, Jamal Turki, Michael Innis, and Stephen B Liggett.** 1994. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 33 (32):9414-9419.
- Gutiérrez-Fisac, JL, P Guallar-Castillón, LM León-Muñoz, A Graciani, JR Banegas, and F Rodríguez-Artalejo.** 2012. Prevalence of general and abdominal obesity in the adult population of Spain, 2008–2010: the ENRICA study. *obesity reviews* 13 (4):388-392.
- Hagstrom-Toft, E., S. Enoksson, E. Moberg, J. Bolinder, and P. Arner.** 1998. beta-Adrenergic regulation of lipolysis and blood flow in human skeletal muscle in vivo. *Am J Physiol* 275 (6 Pt 1):E909-16.
- Hainer, V, A Stunkard, M Kunesova, J Parizkova, V Stich, and DB Allison.** 2001. A twin study of weight loss and metabolic efficiency. *Int J Obes (Lond)* 25 (4):533.
- Hainer, V, AJ Stunkard, M Kunesova, J Parizkova, V Stich, and DB Allison.** 2000. Intrapair resemblance in very low calorie diet-induced weight loss in female obese identical twins. *Int J Obes (Lond)* 24 (8):1051.
- Hainer, V., H. Zamrazilova, J. Spalova, I. Hainerova, M. Kunesova, B. Aldhoon, and B. Bendlova.** 2008. Role of hereditary factors in weight loss and its maintenance. *Physiol Res* 57 Suppl 1:S1-15.
- Hajdуч, E., R. R. Heyes, P. W. Watt, and H. S. Hundal.** 2000. Lactate transport in rat adipocytes: identification of monocarboxylate transporter 1 (MCT1) and its modulation during streptozotocin-induced diabetes. *FEBS Lett* 479 (3):89-92.
- Halaas, Jeffrey L, Ketan S Gajiwala, Margherita Maffei, and Steven L Cohen.** 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269 (5223):543.
- Halestrap, A. P.** 2012. The monocarboxylate transporter family--Structure and functional characterization. *IUBMB Life* 64 (1):1-9.
- Halestrap, A. P., and D. Meredith.** 2004. The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch* 447 (5):619-28.

- Halestrap, A. P., and M. C. Wilson.** 2012. The monocarboxylate transporter family--role and regulation. *IUBMB Life* 64 (2):109-19.
- Hara, Kazuo, Terumasa Okada, Kazuyuki Tobe, Kazuki Yasuda, Yasumichi Mori, Hiroko Kadowaki, Ryoko Hagura, Yasuo Akanuma, Satoshi Kimura, and Chikako Ito.** 2000. The Pro12Ala polymorphism in PPAR γ 2 may confer resistance to type 2 diabetes. *Biochemical and biophysical research communications* 271 (1):212-216.
- Hargrave, S. L., T. L. Davidson, T. J. Lee, and K. P. Kinzig.** 2015. Brain and behavioral perturbations in rats following Western diet access. *Appetite* 93:35-43.
- He, Weimin, Yaacov Barak, Andrea Hevener, Peter Olson, Debbie Liao, Jamie Le, Michael Nelson, Estelita Ong, Jerrold M Olefsky, and Ronald M Evans.** 2003. Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle. *Proceedings of the National Academy of Sciences* 100 (26):15712-15717.
- Herrera, Blanca M, and Cecilia M Lindgren.** 2010. The genetics of obesity. *Current diabetes reports* 10 (6):498-505.
- Hesse, C, and John H Eisenach.** 2008. Genetic variation in the β 2-adrenergic receptor: Impact on intermediate cardiovascular phenotypes. *Current Pharmacogenomics and Personalized Medicine (Formerly Current Pharmacogenomics)* 6 (3):160-170.
- Holtzman, Neil A, and Theresa M Marteau.** 2000. Will genetics revolutionize medicine? : Mass Medical Soc.
- Hopkins, Will G.** 2006. Spreadsheets for Analysis of Controlled Trials, with Adjustment for a Subject Characteristic. *Sportscience* 10:46-50.
- Hopkins, William, Stephen Marshall, Alan Batterham, and Juri Hanin.** 2009. Progressive statistics for studies in sports medicine and exercise science. *Medicine+ Science in Sports+ Exercise* 41 (1):3.
- Hunter, G. R., N. M. Byrne, B. Sirikul, J. R. Fernandez, P. A. Zuckerman, B. E. Darnell, and B. A. Gower.** 2008. Resistance training conserves fat-free mass and resting energy expenditure following weight loss. *Obesity (Silver Spring)* 16 (5):1045-51.
- Hunter, G. R., C. J. Wetzstein, D. A. Fields, A. Brown, and M. M. Bamman.** 2000. Resistance training increases total energy expenditure and free-living physical activity in older adults. *J Appl Physiol (1985)* 89 (3):977-84.
- Isacco, Laurie, Pascale Duché, and Nathalie Boisseau.** 2012. Influence of hormonal status on substrate utilization at rest and during exercise in the female population. *Sports Medicine* 42 (4):327-342.
- Jacob, S, M Stumvoll, R Becker, M Koch, M Nielsen, K Löblein, E Maerker, A Volk, W Renn, and B Balletshofer.** 2000. The PPAR γ 2 polymorphism Pro12Ala is associated with better insulin

- sensitivity in the offspring of type 2 diabetic patients. *Hormone and metabolic research* 32 (10):413-416.
- Jacobsson, B, G Holm, P Björntorp, and U Smith.** 1976. Influence of cell size on the effects of insulin and noradrenaline on human adipose tissue. *Diabetologia* 12 (1):69-72.
- Jalba, M. S., G. G. Rhoads, and K. Demissie.** 2008. Association of codon 16 and codon 27 beta 2-adrenergic receptor gene polymorphisms with obesity: a meta-analysis. *Obesity (Silver Spring)* 16 (9):2096-106.
- Jensen, Michael Dennis, MW Haymond, JE Gerich, PE Cryer, and John M Miles.** 1987. Lipolysis during fasting. Decreased suppression by insulin and increased stimulation by epinephrine. *Journal of Clinical Investigation* 79 (1):207.
- Jequier, E.** 2002. Leptin signaling, adiposity, and energy balance. *Ann N Y Acad Sci* 967:379-88.
- Juel, Carsten, Mads K Holten, and Flemming Dela.** 2004. Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. *The Journal of physiology* 556 (1):297-304.
- Kather, H, E Wieland, A Scheurer, G Vogel, U Wildenberg, and C Joost.** 1987. Influences of variation in total energy intake and dietary composition on regulation of fat cell lipolysis in ideal-weight subjects. *Journal of Clinical Investigation* 80 (2):566.
- Kersten, Sander, Beatrice Desvergne, and Walter Wahli.** 2000. Roles of PPARs in health and disease. *Nature* 405 (6785):421-424.
- Koch, M, K Rett, E Maerker, A Volk, K Haist, M Deninger, W Renn, and HU Häring.** 1999. The PPAR γ 2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 42 (6):758-762.
- Koerner, Antje, Jürgen Kratzsch, and Wieland Kiess.** 2005. Adipocytokines: leptin—the classical, resistin—the controversial, adiponectin—the promising, and more to come. *Best practice & research Clinical endocrinology & metabolism* 19 (4):525-546.
- Koh, Jung-Min, Duk J Kim, Jeong S Hong, Joong Y Park, Ki-Up Lee, Shin-Yoon Kim, and Ghi S Kim.** 2002. Estrogen receptor alpha gene polymorphisms Pvu II and Xba I influence association between leptin receptor gene polymorphism (Gln223Arg) and bone mineral density in young men. *European journal of endocrinology* 147 (6):777-783.
- Kokorovic, Andrea, Grace WC Cheung, Luciano Rossetti, and Tony KT Lam.** 2009. Hypothalamic sensing of circulating lactate regulates glucose production. *Journal of cellular and molecular medicine* 13 (11-12):4403-4408.
- Korhonen, S, S Heinonen, M Hiltunen, S Helisalmi, M Hippeläinen, Riitta Koivunen, JS Tapanainen, and M Laakso.** 2003. Polymorphism in the peroxisome proliferator-activated

- receptor- γ gene in women with polycystic ovary syndrome. *Human Reproduction* 18 (3):540-543.
- Kortner, B., A. Wolf, D. Wendt, U. Beisiegel, and D. Evans.** 1999. Lack of association between a human beta-2 adrenoceptor gene polymorphism (gln27glu) and morbid obesity. *Int J Obes Relat Metab Disord* 23 (10):1099-100.
- Kraschnewski, JL, J Boan, J Esposito, NE Sherwood, EB Lehman, DK Kephart, and CN Sciamanna.** 2010. Long-term weight loss maintenance in the United States. *Int J Obes (Lond)* 34 (11):1644-1654.
- Kurokawa, Naoyuki, Elizabeth H Young, Yoshitomo Oka, Hiroshi Satoh, Nicholas J Wareham, MS Sandhu, and Ruth JF Loos.** 2008. The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44 833 individuals. *International journal of obesity* 32 (8):1240-1249.
- Lacasa, Danièle, Brigitte Agli, and Yves Giudicelli.** 1988. Permissive action of glucocorticoids on catecholamine-induced lipolysis: direct "in vitro" effects on the fat cell β -adrenoreceptor-coupled-adenylate cyclase system. *Biochemical and biophysical research communications* 153 (2):489-497.
- Lafontan, M., P. Barbe, J. Galitzky, G. Tavernier, D. Langin, C. Carpenne, A. Bousquet-Melou, and M. Berlan.** 1997. Adrenergic regulation of adipocyte metabolism. *Hum Reprod* 12 Suppl 1:6-20.
- Lambert, K, G Py, N Eydoux, S Matecki, M Ramonatxo, C Prefaut, and J Mercier.** 2003. Effect of food restriction on lactate sarcolemmal transport. *Metabolism* 52 (3):322-327.
- Lange, L. A., J. M. Norris, C. D. Langefeld, B. J. Nicklas, L. E. Wagenknecht, M. F. Saad, and D. W. Bowden.** 2005. Association of adipose tissue deposition and beta-2 adrenergic receptor variants: the IRAS family study. *Int J Obes (Lond)* 29 (5):449-57.
- Large, V., L. Hellstrom, S. Reynisdottir, F. Lonnqvist, P. Eriksson, L. Lannfelt, and P. Arner.** 1997. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest* 100 (12):3005-13.
- Larsen, L. H., L. Angquist, K. S. Vimalaswaran, J. Hager, N. Viguerie, R. J. Loos, T. Handjieva-Darlenska, S. A. Jebb, M. Kunesova, T. M. Larsen, J. A. Martinez, A. Papadaki, A. F. Pfeiffer, M. A. van Baak, Tia Sorensen, C. Holst, D. Langin, A. Astrup, and W. H. Saris.** 2012. Analyses of single nucleotide polymorphisms in selected nutrient-sensitive genes in weight-regain prevention: the DIOGENES study. *Am J Clin Nutr* 95 (5):1254-60.
- Lee, Gwo-Hwa, R Proenca, JM Montez, and KM Carroll.** 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379 (6566):632.
- Lehmann, Jürgen M, Linda B Moore, Tracey A Smith-Oliver, William O Wilkison, Timothy M Willson, and Steven A Kliewer.** 1995. An antidiabetic thiazolidinedione is a high affinity ligand

- for peroxisome proliferator-activated receptor γ (PPAR γ). *Journal of Biological Chemistry* 270 (22):12953-12956.
- Lehrke, Michael, and Mitchell A Lazar.** 2005. The many faces of PPAR γ . *Cell* 123 (6):993-999.
- Leibel, Rudolph L, and Jules Hirsch.** 1987. Site-and sex-related differences in adrenoreceptor status of human adipose tissue. *The Journal of Clinical Endocrinology & Metabolism* 64 (6):1205-1210.
- Lengacher, S., T. Nehiri-Sitayeb, N. Steiner, L. Carneiro, C. Favrod, F. Preitner, B. Thorens, J. C. Stehle, L. Dix, F. Pralong, P. J. Magistretti, and L. Pellerin.** 2013. Resistance to diet-induced obesity and associated metabolic perturbations in haploinsufficient monocarboxylate transporter 1 mice. *PLoS One* 8 (12):e82505.
- Li, LS, F Lönnqvist, H Luthman, and P Arner.** 1996. Phenotypic characterization of the Trp64Arg polymorphism in the beta 3-adrenergic receptor gene in normal weight and obese subjects. *Diabetologia* 39 (7):857-860.
- Lindi, Virpi I, Matti IJ Uusitupa, Jaana Lindström, Anne Louheranta, Johan G Eriksson, Timo T Valle, Helena Hämäläinen, Pirjo Ilanne-Parikka, Sirkka Keinänen-Kiukaanniemi, and Markku Laakso.** 2002. Association of the Pro12Ala polymorphism in the PPAR- γ 2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* 51 (8):2581-2586.
- Liu, Y. J., S. M. Rocha-Sanchez, P. Y. Liu, J. R. Long, Y. Lu, L. Elze, R. R. Recker, and H. W. Deng.** 2004. Tests of linkage and/or association of the LEPR gene polymorphisms with obesity phenotypes in Caucasian nuclear families. *Physiol Genomics* 17 (2):101-6.
- Lizarraga-Mollinedo, Esther, Elisa Fernández-Millán, Juan de Toro Martín, Carlos Martínez-Hondurilla, Fernando Escrivá, and Carmen Álvarez.** 2012. Early undernutrition induces glucagon resistance and insulin hypersensitivity in the liver of suckling rats. *American Journal of Physiology-Endocrinology And Metabolism* 302 (9):E1070-E1077.
- Locke, A. E., B. Kahali, S. I. Berndt et al.** 2015. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 518 (7538):197-206.
- Loos, R. J., and T. Rankinen.** 2005. Gene-diet interactions on body weight changes. *J Am Diet Assoc* 105 (5 Suppl 1):S29-34.
- Loos, R.J. , and C. Bouchard.** 2003. Obesity—is it a genetic disorder? *Journal of internal medicine* 254 (5):401-425.
- Lovejoy, J, B Mellen, and M Digirolamo.** 1990. Lactate generation following glucose ingestion: relation to obesity, carbohydrate tolerance and insulin sensitivity. *Int J Obes (Lond)* 14 (10):843-855.

- Lönnqvist, Fredrik, Peter Arner, Louise Nordfors, and Martin Schalling.** 1995. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nature medicine* 1 (9):950-953.
- Luis, DA, R Aller, O Izaola, M Gonzalez Sagrado, R Conde, and D Primo.** 2015. Effect of Lys656Asn polymorphism of leptin receptor gene on cardiovascular risk factors and serum adipokine levels after a high polyunsaturated fat diet in obese patients. *Journal of clinical laboratory analysis* 29 (6):432-436.
- Macho-Azcarate, T., A. Marti, A. Gonzalez, J. A. Martinez, and J. Ibanez.** 2002. Gln27Glu polymorphism in the beta2 adrenergic receptor gene and lipid metabolism during exercise in obese women. *Int J Obes Relat Metab Disord* 26 (11):1434-41.
- Mammes, O., R. Aubert, D. Betoulle, F. Pean, B. Herbeth, S. Visvikis, G. Siest, and F. Fumeron.** 2001. LEPR gene polymorphisms: associations with overweight, fat mass and response to diet in women. *Eur J Clin Invest* 31 (5):398-404.
- Manzella, D, M Parillo, T Razzino, P Gnasso, S Buonanno, A Gargiulo, M Caputi, and G Paolisso.** 2002. Soluble leptin receptor and insulin resistance as determinant of sleep apnea. *Int J Obes (Lond)* 26 (3):370.
- Marti, A., M. S. Corbalan, M. A. Martinez-Gonzalez, and J. A. Martinez.** 2002. TRP64ARG polymorphism of the beta 3-adrenergic receptor gene and obesity risk: effect modification by a sedentary lifestyle. *Diabetes Obes Metab* 4 (6):428-30.
- Martinez, J. A., M. S. Corbalan, A. Sanchez-Villegas, L. Forga, A. Marti, and M. A. Martinez-Gonzalez.** 2003. Obesity risk is associated with carbohydrate intake in women carrying the Gln27Glu beta2-adrenoceptor polymorphism. *J Nutr* 133 (8):2549-54.
- Massidda, Myosotis, Nir Eynon, Valeria Bachis, Laura Corrias, Claudia Culigioni, Francesco Piras, Paolo Cugia, Marco Scorcu, and Carla M Calò.** 2015. Influence of the MCT1 rs1049434 on Indirect Muscle Disorders/Injuries in Elite Football Players. *Sports medicine-open* 1 (1):33.
- Masugi, Jiro, Yoshikazu Tamori, Hiroyuki Mori, Takashi Koike, and Masato Kasuga.** 2000. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor- γ 2 on thiazolidinedione-induced adipogenesis. *Biochemical and biophysical research communications* 268 (1):178-182.
- Masuo, K., T. Katsuya, H. Kawaguchi, Y. Fu, H. Rakugi, T. Ogihara, and M. L. Tuck.** 2005. Rebound weight gain as associated with high plasma norepinephrine levels that are mediated through polymorphisms in the beta2-adrenoceptor. *Am J Hypertens* 18 (11):1508-16.
- Masuo, K., N. E. Straznicky, G. W. Lambert, T. Katsuya, K. Sugimoto, H. Rakugi, F. Socratous, J. Hastings, E. A. Lambert, T. Ogihara, and M. D. Esler.** 2008. Leptin-receptor polymorphisms

relate to obesity through blunted leptin-mediated sympathetic nerve activation in a Caucasian male population. *Hypertens Res* 31 (6):1093-100.

Matsuoka, Naoki, Y Ogawa, K Hosoda, J Matsuda, H Masuzaki, T Miyawaki, N Azuma, K Natsui, H Nishimura, and Y Yoshimasa. 1997. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia* 40 (10):1204-1210.

McPherson, K., T. Marsh, and M. Brown. 2007. Foresight report on obesity. *Lancet* 370 (9601):1755; author reply 1755.

McPherson, R. 2007. Genetic contributors to obesity. *Can J Cardiol* 23 Suppl A:23A-27A.

Meirhaeghe, A., N. Helbecque, D. Cattel, and P. Amouyel. 1999. Beta2-adrenoceptor gene polymorphism, body weight, and physical activity. *Lancet* 353 (9156):896.

Meirhaeghe, A., N. Helbecque, D. Cattel, and P. Amouyel. 2000. Impact of polymorphisms of the human beta2-adrenoceptor gene on obesity in a French population. *Int J Obes Relat Metab Disord* 24 (3):382-7.

Meirhaeghe, Aline, and Philippe Amouyel. 2004. Impact of genetic variation of PPAR γ in humans. *Molecular genetics and metabolism* 83 (1):93-102.

Merezhinskaya, N., W. N. Fishbein, J. I. Davis, and J. W. Foellmer. 2000. Mutations in MCT1 cDNA in patients with symptomatic deficiency in lactate transport. *Muscle Nerve* 23 (1):90-7.

Mergen, H., C. Karaaslan, M. Mergen, E. Deniz Ozsoy, and M. Ozata. 2007. LEPR, ADRB3, IRS-1 and 5-HTT genes polymorphisms do not associate with obesity. *Endocr J* 54 (1):89-94.

Monroe, M. B., D. R. Seals, L. F. Shapiro, C. Bell, D. Johnson, and P. Parker Jones. 2001. Direct evidence for tonic sympathetic support of resting metabolic rate in healthy adult humans. *Am J Physiol Endocrinol Metab* 280 (5):E740-4.

Montague, CT, IS Farooqi, JP Whitehead, MA Soos, H Rau, NJ Wareham, CP Sewter, JE Digby, SN Mohammed, and JA Hurst. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in h.

Murphy, S. L. 2009. Review of physical activity measurement using accelerometers in older adults: considerations for research design and conduct. *Prev Med* 48 (2):108-14.

Mutch, David M, and Karine Clément. 2006. Unraveling the genetics of human obesity. *PLoS Genet* 2 (12):e188.

Nicklas, B. J., E. F. van Rossum, D. M. Berman, A. S. Ryan, K. E. Dennis, and A. R. Shuldiner. 2001. Genetic variation in the peroxisome proliferator-activated receptor-gamma2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50 (9):2172-6.

- Nicklas, Jacinda M, Karen W Huskey, Roger B Davis, and Christina C Wee.** 2012. Successful weight loss among obese US adults. *American journal of preventive medicine* 42 (5):481-485.
- NIH.** 6 Suppl 2. *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health* (1998/11/14). National Institutes of Health, Sep 19986 Suppl 2]. Available from <http://www.ncbi.nlm.nih.gov/pubmed/9813653>.
- NIH.** 2017. *What kinds of gene mutations are possible?* National Institutes of Health 20172017]. Available from <https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations>.
- Norris, Andrew W, Lihong Chen, Simon J Fisher, Ildiko Szanto, Michael Ristow, Alison C Jozsi, Michael F Hirshman, Evan D Rosen, Laurie J Goodyear, and Frank J Gonzalez.** 2003. Muscle-specific PPAR γ -deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *The Journal of clinical investigation* 112 (4):608-618.
- Ordovas, J. M., and J. Shen.** 2008. Gene-environment interactions and susceptibility to metabolic syndrome and other chronic diseases. *J Periodontol* 79 (8 Suppl):1508-13.
- Östman, Jan, Peter Arner, Hiroshi Kimura, Hans Wahrenberg, and Peter Engfeldt.** 1984. Influence of fasting on lipolytic response to adrenergic agonists and on adrenergic receptors in subcutaneous adipocytes. *European journal of clinical investigation* 14 (5):383-391.
- Papazoglou, D., G. Augello, M. Tagliaferri, G. Savia, P. Marzullo, E. Maltezos, and A. Liuzzi.** 2006. Evaluation of a multisensor armband in estimating energy expenditure in obese individuals. *Obesity (Silver Spring)* 14 (12):2217-23.
- Paracchini, Valentina, Paola Pedotti, and Emanuela Taioli.** 2005. Genetics of leptin and obesity: a HuGE review. *American journal of epidemiology* 162 (2):101-114.
- Passarge, Eberhard.** 1995. *Color atlas of genetics*: Georg Thieme Verlag.
- Pecquery, René, Marie-Christine Leneuve, and Yves Giudicelli.** 1987. Evidence that the lipolytic defect induced by estradiol-treatment in hamster adipocytes is related to an estrogen receptor-mediated defect in the adenylate cyclase catalytic subunit but not in *Ns*. *Biochemical and biophysical research communications* 145 (1):369-375.
- Peelman, Frank, Lennart Zabeau, Kedar Moharana, Savvas N Savvides, and Jan Tavernier.** 2014. 20 YEARS OF LEPTIN: Insights into signaling assemblies of the leptin receptor. *Journal of Endocrinology* 223 (1):T9-T23.
- Pereira, A. C., M. S. Floriano, G. F. Mota, R. S. Cunha, F. L. Herkenhoff, J. G. Mill, and J. E. Krieger.** 2003. Beta2 adrenoceptor functional gene variants, obesity, and blood pressure level interactions in the general population. *Hypertension* 42 (4):685-92.

- Phares, D. A., A. A. Halverstadt, A. R. Shuldiner, R. E. Ferrell, L. W. Douglass, A. S. Ryan, A. P. Goldberg, and J. M. Hagberg.** 2004. Association between body fat response to exercise training and multilocus ADR genotypes. *Obes Res* 12 (5):807-15.
- Pierre, Karin, Annabelle Parent, Pierre - Yves Jayet, Andrew P Halestrap, Urs Scherrer, and Luc Pellerin.** 2007. Enhanced expression of three monocarboxylate transporter isoforms in the brain of obese mice. *The Journal of physiology* 583 (2):469-486.
- Pietri-Rouxel, F., B. St John Manning, J. Gros, and A. D. Strosberg.** 1997. The biochemical effect of the naturally occurring Trp64-->Arg mutation on human beta3-adrenoceptor activity. *Eur J Biochem* 247 (3):1174-9.
- Poehlman, ET, A Tremblay, E Fontaine, JP Despres, A Nadeau, J Dussault, and C Bouchard.** 1986. Genotype dependency of the thermic effect of a meal and associated hormonal changes following short-term overfeeding. *Metabolism* 35 (1):30-36.
- Poole, R. C., and A. P. Halestrap.** 1993. Transport of lactate and other monocarboxylates across mammalian plasma membranes. *Am J Physiol* 264 (4 Pt 1):C761-82.
- Poole, Robert C, and Andrew P Halestrap.** 1994. N-terminal protein sequence analysis of the rabbit erythrocyte lactate transporter suggests identity with the cloned monocarboxylate transport protein MCT1. *Biochemical Journal* 303 (3):755-759.
- Portoles, O., J. V. Sorli, F. Frances, O. Coltell, J. I. Gonzalez, C. Saiz, and D. Corella.** 2006. Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. *Eur J Epidemiol* 21 (8):605-12.
- Pyrzak, B., A. Wisniewska, A. Kucharska, M. Wasik, and U. Demkow.** 2009. No association of LEPR Gln223Arg polymorphism with leptin, obesity or metabolic disturbances in children. *Eur J Med Res* 14 Suppl 4:201-4.
- Rangwala, Shamina M, and Mitchell A Lazar.** 2004. Peroxisome proliferator-activated receptor γ in diabetes and metabolism. *Trends in pharmacological sciences* 25 (6):331-336.
- Rankinen, Tuomo, Aamir Zuberi, Yvon C Chagnon, S John Weisnagel, George Argyropoulos, Brandon Walts, Louis Pérusse, and Claude Bouchard.** 2006. The human obesity gene map: the 2005 update. *Obesity* 14 (4):529-644.
- Rauhio, A., K. Uusi-Rasi, S. T. Nikkari, P. Kannus, H. Sievanen, and T. Kunnas.** 2013. Association of the FTO and ADRB2 genes with body composition and fat distribution in obese women. *Maturitas* 76 (2):165-71.
- Rawson, E. S., A. Nolan, K. Silver, A. R. Shuldiner, and E. T. Poehlman.** 2002. No effect of the Trp64Arg beta(3)-adrenoceptor gene variant on weight loss, body composition, or energy expenditure in obese, caucasian postmenopausal women. *Metabolism* 51 (6):801-5.

- Reihnsaus, Ellen, Michael Innis, Neil MacIntyre, and Stephen B Liggett.** 1993. Mutations in the gene encoding for the 132-adrenergic receptor in normal and asthmatic subjects. *American journal of respiratory cell and molecular biology* 8 (3):334-339.
- Rittig, K, C Thamer, F Machicao, R Rietig, N Stefan, A Fritsche, H-U Haring, and B Balletshofer.** 2007. The Pro12Ala polymorphism in PPARG2 increases the effectiveness of primary prevention of cardiovascular disease by a lifestyle intervention. *Diabetologia* 50 (6):1345-1347.
- Rosado, E. L., J. Bressan, and J. A. Martinez.** 2015. Environmental factors and beta2-adrenergic receptor polymorphism: influence on the energy expenditure and nutritional status of obese women. *Lipids* 50 (5):459-67.
- Rosmond, R., Y. C. Chagnon, G. Holm, M. Chagnon, L. Perusse, K. Lindell, B. Carlsson, C. Bouchard, and P. Bjorntorp.** 2000. Hypertension in obesity and the leptin receptor gene locus. *J Clin Endocrinol Metab* 85 (9):3126-31.
- Ruiz, J. R., E. Larrarte, J. Margareto, R. Ares, and I. Labayen.** 2011. Role of beta(2)-adrenergic receptor polymorphisms on body weight and body composition response to energy restriction in obese women: preliminary results. *Obesity (Silver Spring)* 19 (1):212-5.
- Rutter, M. K., J. M. Massaro, U. Hoffmann, C. J. O'Donnell, and C. S. Fox.** 2012. Fasting glucose, obesity, and coronary artery calcification in community-based people without diabetes. *Diabetes Care* 35 (9):1944-50.
- Saad, Mohammed F, Sidhharth Damani, Ronald L Gingerich, Maggy G Riad-Gabriel, Arshad Khan, Rima Boyadjian, Sujata D Jinagouda, Khalid El-Tawil, Robert K Rude, and Vikram Kamdar.** 1997. Sexual Dimorphism in Plasma Leptin Concentration 1. *The Journal of Clinical Endocrinology & Metabolism* 82 (2):579-584.
- Sacks, F. M., G. A. Bray, V. J. Carey, S. R. Smith, D. H. Ryan, S. D. Anton, K. McManus, C. M. Champagne, L. M. Bishop, N. Laranjo, M. S. Leboff, J. C. Rood, L. de Jonge, F. L. Greenway, C. M. Loria, E. Obarzanek, and D. A. Williamson.** 2009. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* 360 (9):859-73.
- Salopuro, T., L. Pulkkinen, J. Lindstrom, J. G. Eriksson, T. T. Valle, H. Hamalainen, P. Ilanne-Parikka, S. Keinanen-Kiukaanniemi, J. Tuomilehto, M. Laakso, and M. Uusitupa.** 2005. Genetic variation in leptin receptor gene is associated with type 2 diabetes and body weight: The Finnish Diabetes Prevention Study. *Int J Obes (Lond)* 29 (10):1245-51.
- Sasaki, Shotaro, Yuya Futagi, Masaki Kobayashi, Jiro Ogura, and Ken Iseki.** 2015. Functional characterization of 5-oxoproline transport via SLC16A1/MCT1. *Journal of Biological Chemistry* 290 (4):2303-2311.
- Savage, David B, Garry D Tan, Carlo L Acerini, Susan A Jebb, Maura Agostini, Mark Gurnell, Rachel L Williams, A Margot Umpleby, E Louise Thomas, and Jimmy D Bell.** 2003. Human

- metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor- γ . *Diabetes* 52 (4):910-917.
- Schneider, Jochen, Joerg Kreuzer, Andreas Hamann, Peter P Nawroth, and Klaus A Dugi.** 2002. The proline 12 alanine substitution in the peroxisome proliferator-activated receptor- γ 2 gene is associated with lower lipoprotein lipase activity in vivo. *Diabetes* 51 (3):867-870.
- Schröder, Helmut, Lourdes Ribas, Corinna Koebnick, Anna Funtikova, Santiago F Gomez, Montserrat Fito, Carmen Perez-Rodrigo, and Lluís Serra-Majem.** 2014. Prevalence of abdominal obesity in Spanish children and adolescents. Do we need waist circumference measurements in pediatric practice? *PLoS One* 9 (1):e87549.
- Schulz, Laura C, and Eric P Widmaier.** 2006. Leptin receptors. In *Leptin*: Springer.
- Shepherd, Raymond E, Craig C Malbon, Charles J Smith, and JN Fain.** 1977. Lipolysis and adenosine 3': 5'-monophosphate metabolism in isolated white fat cells from genetically obese-hyperglycemic mice (ob/ob). *Journal of Biological Chemistry* 252 (20):7243-7248.
- Shungin, D., T. W. Winkler, D. C. Croteau-Chonka et al.** 2015. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 518 (7538):187-96.
- Silver, K., J. Walston, W. K. Chung, F. Yao, V. V. Parikh, R. Andersen, L. J. Cheskin, D. Elahi, D. Muller, R. L. Leibel, and A. R. Shuldiner.** 1997. The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. *Diabetes* 46 (11):1898-900.
- Simar, D., H. Chen, K. Lambert, J. Mercier, and M. J. Morris.** 2012. Interaction between maternal obesity and post-natal over-nutrition on skeletal muscle metabolism. *Nutr Metab Cardiovasc Dis* 22 (3):269-76.
- Simon, I, J Vendrell, C Gutierrez, JM Fernandez-Real, I Vendrell, LI Gallart, R Fontova, and C Richart.** 2002. Pro12Ala substitution in the peroxisome proliferator-activated receptor- γ is associated with increased leptin levels in women with type-2 diabetes mellitus. *Hormone Research in Paediatrics* 58 (3):143-149.
- Skibola, Christine F, Elizabeth A Holly, Matthew S Forrest, Alan Hubbard, Paige M Bracci, Danica R Skibola, Christine Hegedus, and Martyn T Smith.** 2004. Body mass index, leptin and leptin receptor polymorphisms, and non-hodgkin lymphoma. *Cancer Epidemiology and Prevention Biomarkers* 13 (5):779-786.
- Smith, Kristy Breuhl, and Michael Seth Smith.** 2016. Obesity statistics. *Primary Care: Clinics in Office Practice* 43 (1):121-135.
- Snitker, S, OE Odeleye, J Hellmer, M Boschmann, MB Monroe, AR Shuldiner, and E Ravussin.** 1997. No effect of the Trp64Arg β 3-adrenoceptor variant on in vivo lipolysis in subcutaneous adipose tissue. *Diabetologia* 40 (7):838-842.

- Song, Z, and VH Routh.** 2005. Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* 54 (1):15-22.
- Speakman, John R.** 2006. Thrifty genes for obesity and the metabolic syndrome—time to call off the search? *Diabetes and Vascular Disease Research* 3 (1):7-11.
- Speliotes, E. K., C. J. Willer, S. I. Berndt et al.** 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 42 (11):937-48.
- Speliotes, Elizabeth K, Cristen J Willer, Sonja I Berndt, Keri L Monda, Gudmar Thorleifsson, Anne U Jackson, Hana Lango Allen, Cecilia M Lindgren, Jian'an Luan, and Reedik Mägi.** 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature genetics* 42 (11):937-948.
- Strobel, Andreas, Tarik Issad, Luc Camoin, Metin Ozata, and A Donny Strosberg.** 1998. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature genetics* 18 (3):213-215.
- Stumvoll, Michael, Hans Günther Wahl, Klaus Löblein, Regine Becker, Fausto Machicao, Stephan Jacob, and Hans Häring.** 2001. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor- γ 2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes* 50 (4):876-881.
- Stunkard, Albert J, Jennifer R Harris, Nancy L Pedersen, and Gerald E McClearn.** 1990. The body-mass index of twins who have been reared apart. *New England Journal of Medicine* 322 (21):1483-1487.
- Stunkard, Albert J, Thorkild IA Sørensen, Craig Hanis, Thomas W Teasdale, Ranajit Chakraborty, William J Schull, and Fini Schulsinger.** 1986. An adoption study of human obesity. *New England Journal of Medicine* 314 (4):193-198.
- Tartaglia, Louis A, Marlene Dembski, Xun Weng, Nanhua Deng, Janice Culpepper, Rene Devos, Grayson J Richards, L Arthur Campfield, Frederick T Clark, and Jim Deeds.** 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83 (7):1263-1271.
- Taylor, Matthew RG, and Michael R Bristow.** 2004. The Emerging Pharmacogenomics of the β -Adrenergic Receptors. *Congestive Heart Failure* 10 (6):281-288.
- Tchernof, A., R. D. Starling, A. Turner, A. R. Shuldiner, J. D. Walston, K. Silver, and E. T. Poehlman.** 2000. Impaired capacity to lose visceral adipose tissue during weight reduction in obese postmenopausal women with the Trp64Arg beta3-adrenoceptor gene variant. *Diabetes* 49 (10):1709-13.
- Thomas, Claire, David J Bishop, Karen Lambert, Jacques Mercier, and George A Brooks.** 2012. Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human

- skeletal muscles: current status. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 302 (1):R1-R14.
- Thorleifsson, Gudmar, G Bragi Walters, Daniel F Gudbjartsson, Valgerdur Steinthorsdottir, Patrick Sulem, Anna Helgadóttir, Unnur Styrkarsdóttir, Solveig Gretarsdóttir, Steinunn Thorlacius, and Ingileif Jonsdóttir.** 2009. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nature genetics* 41 (1):18-24.
- Tontonoz, Peter, Erding Hu, and Bruce M Spiegelman.** 1994. Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell* 79 (7):1147-1156.
- Ukkola, O., T. Rankinen, T. Rice, J. Gagnon, A. S. Leon, J. S. Skinner, J. H. Wilmore, D. C. Rao, and C. Bouchard.** 2003. Interactions among the beta2- and beta3- adrenergic receptor genes and total body fat and abdominal fat level in the HERITAGE Family Study. *Int J Obes Relat Metab Disord* 27 (3):389-93.
- Ukkola, O., T. Rankinen, S. J. Weisnagel, G. Sun, L. Perusse, Y. C. Chagnon, J. P. Despres, and C. Bouchard.** 2000. Interactions among the alpha2-, beta2-, and beta3-adrenergic receptor genes and obesity-related phenotypes in the Quebec Family Study. *Metabolism* 49 (8):1063-70.
- Umekawa, T., T. Yoshida, N. Sakane, A. Kogure, M. Kondo, and H. Honjyo.** 1999. Trp64Arg mutation of beta3-adrenoceptor gene deteriorates lipolysis induced by beta3-adrenoceptor agonist in human omental adipocytes. *Diabetes* 48 (1):117-20.
- Varela-Moreiras, Gregorio, Merino LF Alguacil, Aperte E Alonso, Bartrina J Aranceta, Torres JM Avila, Laín S Aznar, Cortés S Belmonte, García L Cabrerizo, Saavedra MÁ Dal Re, and Rubio A Delgado.** 2013. CONSENSUS DOCUMENT AND CONCLUSIONS-Obesity and sedentarism in the 21st century: what can be done and what must be done? *Nutricion hospitalaria* 28:1.
- Verkerke, Hans, Caitlin Naylor, Lennart Zabeau, Jan Tavernier, William A Petri Jr, and Chelsea Marie.** 2014. Kinetics of leptin binding to the Q223R leptin receptor. *PLoS One* 9 (4):e94843.
- Vineis, Paolo, Paul Schulte, and Anthony J McMichael.** 2001. Misconceptions about the use of genetic tests in populations. *The Lancet* 357 (9257):709.
- Vogels, Neeltje, Edwin CM Mariman, Freek G Bouwman, Arnold DM Kester, Kristel Diepvens, and Margriet S Westerterp-Plantenga.** 2005. Relation of weight maintenance and dietary restraint to peroxisome proliferator-activated receptor γ 2, glucocorticoid receptor, and ciliary neurotrophic factor polymorphisms. *The American journal of clinical nutrition* 82 (4):740-746.
- Wahrenberg, Hans, Peter Engfeldt, J Bolinder, and Peter Arner.** 1987. Acute adaptation in adrenergic control of lipolysis during physical exercise in humans. *American Journal of Physiology-Endocrinology And Metabolism* 253 (4):E383-E390.
- Wang, May-Yun, Young Lee, and Roger H Unger.** 1999. Novel form of lipolysis induced by leptin. *Journal of Biological Chemistry* 274 (25):17541-17544.

- Watowich, Stephanie S, Hong Wu, Merav Socolovsky, Ursula Klingmuller, Stefan N Constantinescu, and Harvey F Lodish.** 1996. Cytokine receptor signal transduction and the control of hematopoietic cell development. *Annual review of cell and developmental biology* 12 (1):91-128.
- Wauters, M., I. Mertens, M. Chagnon, T. Rankinen, R. V. Considine, Y. C. Chagnon, L. F. Van Gaal, and C. Bouchard.** 2001. Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women. *Int J Obes Relat Metab Disord* 25 (5):714-20.
- Weiss, E. P., O. Kulaputana, I. A. Ghuu, J. Brandauer, C. R. Wohn, D. A. Phares, A. R. Shuldiner, and J. M. Hagberg.** 2005. Endurance training-induced changes in the insulin response to oral glucose are associated with the peroxisome proliferator-activated receptor-gamma2 Pro12Ala genotype in men but not in women. *Metabolism* 54 (1):97-102.
- WHO.** 2000. *Obesity: preventing and managing the global epidemic*: World Health Organization.
- WHO.** 2017. *Obesity and overweight Fact sheet*. World Health Organization (WHO), 2016 June 20162017].
- Widen, Elisabeth, Markku Lehto, Timo Kanninen, Jeremy Walston, Alan R Shuldiner, and Leif C Groop.** 1995. Association of a polymorphism in the β 3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *New England Journal of Medicine* 333 (6):348-352.
- Wilson, Marieangela C, David Meredith, Chotirote Bunnun, Richard B Sessions, and Andrew P Halestrap.** 2009. Studies on the DIDS-binding site of monocarboxylate transporter 1 suggest a homology model of the open conformation and a plausible translocation cycle. *Journal of Biological Chemistry* 284 (30):20011-20021.
- Wing, Rena R, and Suzanne Phelan.** 2005. Long-term weight loss maintenance. *The American journal of clinical nutrition* 82 (1):222S-225S.
- Xu, Xuefan, Giovanni De Pergola, and Per Björntorp.** 1990. The effects of androgens on the regulation of lipolysis in adipose precursor cells. *Endocrinology* 126 (2):1229-1234.
- Yang, Jianhui, Heng Du, Jianguo Lv, and Lianhe Zhang.** 2016. Association of rs1137101 polymorphism in LEPR and susceptibility to knee osteoarthritis in a Northwest Chinese Han population. *BMC Musculoskeletal Disorders* 17 (1):311.
- Yang, Ronghua, and Lili A Barouch.** 2007. Leptin signaling and obesity. *Circulation research* 101 (6):545-559.
- Yang, W., T. Kelly, and J. He.** 2007. Genetic epidemiology of obesity. *Epidemiol Rev* 29:49-61.

- Yiannakouris, N., M. Yannakoulia, L. Melistas, J. L. Chan, D. Klimis-Zacas, and C. S. Mantzoros.** 2001. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab* 86 (9):4434-9.
- Zahorska-Markiewicz, B, C Kucio, and D Piskorska.** 1986. Adrenergic control of lipolysis and metabolic responses in obesity. *Hormone and metabolic research* 18 (10):693-697.
- Zapico, A. G., P. J. Benito, M. Gonzalez-Gross, A. B. Peinado, E. Morencos, B. Romero, M. A. Rojo-Tirado, R. Cupeiro, B. Szendrei, J. Butragueno, M. Bermejo, M. Alvarez-Sanchez, M. Garcia-Fuentes, C. Gomez-Candela, L. M. Bermejo, C. Fernandez-Fernandez, and F. J. Calderon.** 2012. Nutrition and physical activity programs for obesity treatment (PRONAF study): methodological approach of the project. *BMC Public Health* 12:1100.
- Zarebska, A., Z. Jastrzebski, P. Cieszczyk, A. Leonska-Duniec, K. Kotarska, M. Kaczmarczyk, M. Sawczuk, and A. Maciejewska-Karlowska.** 2014. The Pro12Ala Polymorphism of the Peroxisome Proliferator-Activated Receptor Gamma Gene Modifies the Association of Physical Activity and Body Mass Changes in Polish Women. *PPAR Res* 2014:373782.
- Zhang, Faming, Yanyun Chen, Mark Heiman, and Richard DiMarchi.** 2005. Leptin: structure, function and biology. *Vitamins & Hormones* 71:345-372.

9. APPENDIX

9. APPENDIX

9.1. Ethics committee report of the technical university of madrid

Hospital Universitario La Paz

J. Comunidad de Madrid

INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Don Antonio Gil Aguado, Presidente del Comité Ético de Investigación Clínica del Hospital Universitario La Paz

CERTIFICA

Que este Comité ha evaluado la propuesta de la Dra. Carmen Gómez Candela de la Unidad de Nutrición del Hospital General para que se realice el proyecto de investigación titulado **"PRONAF: PROGRAMAS DE NUTRICIÓN Y ACTIVIDAD FÍSICA PARA EL TRATAMIENTO DE LA OBESIDAD"**, código HULP: **PI-643**

y considera que teniendo en cuenta la respuesta a las aclaraciones solicitadas:

- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- La capacidad del investigador y los medios de disponibles son apropiados para llevar a cabo el estudio.
- Es adecuado el procedimiento para obtener el consentimiento informado y no interfiere con el respeto a los postulados éticos.

Y que este Comité acepta que dicho proyecto de investigación sea realizado en el Hospital Universitario La Paz por la Dra. Carmen Gómez Candela de la Unidad de Nutrición del Hospital General como investigador principal.

Lo que firmo en Madrid a 27 Marzo de 2008

Firmado: CLÍNICA
Don Antonio Gil Aguado
PRESIDENTE DE LA CLÍNICA

9.2. PRONAF methodology article

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http://www.biomedcentral.com/1471-2458/12/1100

STUDY PROTOCOL

Open Access

Nutrition and physical activity programs for obesity treatment (PRONAF study): methodological approach of the project

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Abstract

Background: At present, scientific consensus exists on the multifactorial etiopatogenia of obesity. Both professionals and researchers agree that treatment must also have a multifactorial approach, including diet, physical activity, pharmacology and/or surgical treatment. These two last ones should be reserved for those cases of morbid obesities or in case of failure of the previous ones. The aim of the PRONAF study is to determine what type of exercise combined with caloric restriction is the most appropriate to be included in overweight and obesity intervention programs, and the aim of this paper is to describe the design and the evaluation methods used to carry out the PRONAF study.

Methods/design: One-hundred nineteen overweight (46 males) and 120 obese (61 males) subjects aged 18–50 years were randomly assigned to a strength training group, an endurance training group, a combined strength + endurance training group or a diet and physical activity recommendations group. The intervention period was 22 weeks (in all cases 3 times/wk of training for 22 weeks and 2 weeks for pre and post evaluation). All subjects followed a hypocaloric diet (25–30% less energy intake than the daily energy expenditure estimated by accelerometry). 29–34% of the total energy intake came from fat, 14–20% from protein, and 50–55% from carbohydrates. The mayor outcome variables assessed were, biochemical and inflammatory markers, body composition, energy balance, physical fitness, nutritional habits, genetic profile and quality of life. 180 (75.3%) subjects finished the study, with a dropout rate of 24.7%. Dropout reasons included: personal reasons 17 (28.8%), low adherence to exercise 3 (5.1%), low adherence to diet 6 (10.2%), job change 6 (10.2%), and lost interest 27 (45.8%).

Discussion: Feasibility of the study has been proven, with a low dropout rate which corresponds to the estimated sample size. Transfer of knowledge is foreseen as a spin-off, in order that overweight and obese subjects can benefit from the results. The aim is to transfer it to sports centres. Effectiveness on individual health-related parameter in order to determine the most effective training programme will be analysed in forthcoming publications.

Trial registration: ClinicalTrials.gov NCT01116856

Keywords: Overweight, Obesity, Caloric restriction, Exercise, Weight loss

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Background

The increase in overweight and obesity rates is a major public health concern. In most countries it has affected all population groups regardless of sex, age, race, income or education level, but to varying extents [1]. Based on comparative studies with other European countries, Spain is at the forefront [1], one in four people is overweight or obese, ie 12.5 million people [2]. In fact, two out of three men are overweight and one of every six is obese, with a clear trend to increase in the forthcoming years [3].

Today it is accepted that obesity has a multifactorial origin [4,5] and is a known risk factor for numerous health problems, including high cholesterol, diabetes, hypertension, respiratory problems, musculoskeletal diseases, cardiovascular diseases, and some forms of cancer. Mortality also increases sharply once the overweight threshold is crossed [6,7].

Research has been very active in the last years trying to identify the most effective treatment for adult overweight and obesity [8-40]. A combination of caloric restriction with an increase in energy output by means of exercise has been proven in the hospital setting [41]. Results have been quite conclusive regarding aerobic exercise [9] and very restrictive caloric intake (less than 50% of kcal intake) [35]. But data are still scarce regarding the effect of a combination of strength and aerobic training and a much lesser restrictive hypocaloric diet on weight loss [35]. The PRONAF study wants to get deeper into the latter, also considering the Consensus Paper 2011 from the Spanish Society of Obesity Research (SEEDO), that has stated that the primary goal of treatment should not focus on achieving the ideal body weight, but to have smaller weight losses maintained in the long term [42].

Therefore, the main aim of the PRONAF study was to discover which is the most effective training protocol in combination with caloric restriction for overweight and obese subjects included in a specific intervention program. The aim of this paper is to describe the design and the evaluation methods used for the PRONAF study.

Methods/design

Study design

PRONAF is a 22 week-intervention study performed among Spanish overweight and obese adults, who were randomly assigned to one of the following intervention groups: strength training group (S), endurance training group (E), combined strength plus endurance training group (SE), and physical activity recommendations group (C), which are described in detail below. The study was performed twice, one year apart, first in the overweight group (year 2009/2010) and then in the obese group (year 2010/2011). In total, we had 8 interventions groups.

Population and sample size estimation

The estimation of the sample size was calculated to detect a main effect of the treatment on the percentage of body fat with a 80% statistical power at 5% significance, assuming a 0.80 correlation between repeated measures [43]. The initial calculated sample size per intervention group ($n = 22$) permits the detection of a large effect size (Cohen's $d = -0.8$), as observed in a previous investigation [43].

The initial adjustment of sample size for dropouts carried out used a maximum of 25% of dropouts in each group [37]. Adjusted dropouts sample = $n / (1 - R)$, where n = number of subjects not lost, and R = expected proportion for dropouts. With our data, the initial necessary sample size was: $88 (1 / 1 - 0.25) = 118$ subjects or 29/30 in each intervention group, this being applicable for both the overweight and the obese interventions groups. A total of two hundred thirty nine people initiated the study, completing it 180 (75.3%), which supposed 59 dropouts for different reasons. For personal reasons 17 (28.8%), low exercise adherence 3 (5.1%), low diet adherence 6 (10.2%), job change 6 (10.2%), and lost interest 27 (45.8%).

Recruitment of the subjects

Subjects were recruited by means of diverse advertisements published in the media and the applicants filtered using different criteria. The voluntary subjects who fulfilled the inclusion criteria and passed the baseline physical examination were stratified by age ranges and body mass index (BMI), and randomized into the different intervention groups (Figure 1).

Inclusion and exclusion criteria

Inclusion criteria included being male or female living in the Region of Madrid, being overweight (BMI 25–29.9 kg/m^2) or obese (BMI: 30–34.9 kg/m^2), middle-aged (from 18 to 50 years), having a sedentary lifestyles (< 30 min Physical Activity/day), being normoglycaemic, and non-smoker [37,41]. In the case of females, having regular menstrual cycles was required.

The exclusion criteria were suffering from a physical (orthopaedic limitations, stroke, etc.) and/or psychological (anorexia, bulimia, etc.) disease that may have precluded the performance of the requested strength or endurance training, intake of any medication (beta blockers, alcohol, etc.) known to influence physical performance or the interpretation of the results, having a history of systematic strength or endurance training (moderate to high intensity training more than once a week) in the year before the beginning of the study [37,40].

After the intervention period, participants who failed to comply with 90% assistance to the training sessions

and less than 80% adherence to the diet were excluded from further analysis. The characteristics of the final sample (completers) are shown in Table 1.

Ethical issues

The protocols and procedures of the PRONAF study were in agreement with the ethical guidelines on biomedical research on human subjects of the World Medical Association's Declaration of Helsinki (1964) and further

amendments. Before participating in this research, all subjects were carefully informed about the possible risks and benefits of the project, being required to read and sign an institutionally approved informed consent document. PRONAF study was approved by the Human Research Review Committee of the University Hospital La Paz (PI-643).

Access to the database was restricted to the researchers that participated in the PRONAF study. Therefore, the

Table 1 Baseline data (n = 180)

	Overweight n = 84		Obesity n = 96	
	Women n = 48	Men n = 36	Women n = 48	Men n = 48
Age (years)	37.29 ± 8.25	37.42 ± 8.02	39.02 ± 7.74	38.79 ± 7.99
Body Weight (kg)	73.5 ± 5.87	88.21 ± 8.13	88.33 ± 10.1	102.04 ± 8.94
Height (m)	1.62 ± 0.06	1.75 ± 0.07	1.64 ± 0.07	1.77 ± 0.06
BMI (kg/m ²)	28.01 ± 1.32	28.57 ± 1.12	32.41 ± 1.85	32.4 ± 1.9
Body fat (%)	43.28 ± 3.62	33.8 ± 4.63	47.11 ± 3.49	38.17 ± 4.02
Body fat free mass (kg)	40.25 ± 4.3	55.99 ± 5.47	44.3 ± 4.71	58.63 ± 8.96
Bone mineral density (g/cm ²)	1.18 ± 0.1	1.26 ± 0.09	1.21 ± 0.11	1.3 ± 0.1

Results are shown as Mean ± SD.

Note. BMI = Body Mass Index. Body fat, Body fat free mass and Bone mineral density calculated by Dual-energy x-ray absorptiometry (DXA).

data and information obtained in the project was considered as confidential following current Spanish legislation regulating personal data protection (Organic Law 15/1999 and Royal Decree 1720/2007).

This trial was registered at clinical trials.gov as NCT01116856. <http://clinicaltrials.gov/>.

Intervention

Time line intervention

The assessments took place for all subjects one week before and after 22 weeks of intervention. Additionally, body weight was measured every 15 days, training intensity and physical activity were controlled monthly, and dietary intake was evaluated every three months.

Exercise protocols

Four different intervention groups were involved in the study as stated above. The S, E and SE groups followed the corresponding training program plus the dietary intervention, and the C group followed the dietary intervention and was instructed about the general recommendations regarding physical activity for weight loss from the American College of Sports Medicine (ACSM) [44].

Subjects in the S, E and SE groups performed training 3 times/wk for 22 weeks at a Sports Centre in Madrid (Spain). All training sessions were carefully supervised by certified personal trainers. The exercise programs were designed taking into account each subject's muscle strength (MS) and the heart rate reserve (HRR). MS was measured using the 15-repetition maximum (15 RM) testing method [45] in the S and SE groups (both of which involved strength training). Both volume and intensity of exercise was increased over the study period (Figure 2) according to standard procedures [35,41].

Prior to each training session each subject started with a 5-minute aerobic warm-up routine, followed by the session routine, and concluded with a 5-minute cool down and stretching exercises routine. Moreover, an MP3 audio track was played for each participant to set the execution rhythm of the exercises during all the sessions. In this case, the heart rate (HR) was monitored by a pulse-meter (Polar RS800CX, Polar Electro, Kempele, Finland). The cadence for the resistance exercises was fixed at 1:2 (concentric-eccentric phase). A session routine consisted in the execution on eight scheduled exercises in the case of group S (i.e., shoulder press, squat, barbell row, lateral split, bench press, front split, biceps curl and French press for triceps). Regarding types of exercises, running, cycling or elliptical (self-selected) exercises were the main components of the session routine for group E, while the routine for the SE group consisted in a combination of cycle ergometry, treadmill or elliptical intercalated with squat, row machine, bench press and front split.

The volume and intensity of the three training programs increased progressively. During the adaptation period (cf. weeks 1–4) subjects were taught on the exercise technique. Between the fifth and eighth week, exercises were carried out at an intensity of 50% of 15RM and HRR, performing 2 laps to the circuit (cf. 51 minutes and 15 seconds), then between the 9th and 14th week the intensity was increased up to 60% of 15RM and HRR, and finally during the period between the 15th and 24th week the volume increased from 2 to 3 laps to the circuit (64 minutes). Then, a five-minute recovery period was set between the circuits. The S and SE participants performed 15 repetitions (45") for each exercise with a rest period of 15 seconds between them.

Diet intervention

Hypocaloric diets (between 5028 and 12570 KJ) were prescribed individually for all participants by expert dieticians at the Department of Nutrition, La Paz University Hospital, Madrid. The diet was designed to provide 25–30% less energy than the daily energy expenditure (DEE), as measured using SenseWear Pro3 Armband™ (Body Media, Pittsburgh, PA). Approximately, 29–34% of the energy came from fat, 14–18% from protein, and 50–55% from carbohydrates [46]. In the obesity group, energy provided from protein was increased up to 14–20%.

Before-after measurements

Physical fitness and health

Maximal aerobic power test The test was conducted on a computerized treadmill (H/P/COSMOS 3P® 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany). Peak oxygen uptake (VO_2 peak) was measured with the modified Bruce protocol used elsewhere with overweight and obese population [35,47]. Volume and composition of expired gas was analysed with a gas analyzer Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany) which validity and reliability has been demonstrated previously [48,49], and continuous 12-lead electrocardiographic monitoring. The exercise test was maintained until exhaustion. The mean of the three highest measurements was used as VO_2 peak. Identical procedure was carried out to calculate the maximal HR.

Resting metabolic rate

Subjects were asked to refrain from any exercise for at least 24 h before the test. To assess the resting metabolic rate (RMR), participants were cited between 7 am to 10 am, after a 9 h overnight fasted period. RMR was then measured by indirect calorimetry standing up (during 11 min) and in a lying position (additional 20 min). Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were recorded by a gas analyzer Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany), and resting heart rate (RHR) was measured using a heart rate monitor (Polar Electro Oy, Kempele, Finland). All these parameters were averaged during the last 5 min of the standing up position and the last 10 min of the lying position. Oxygen uptake and VCO_2 data were used to calculate RMR according to the formula of Weir [50].

Maximal dynamic strength

Muscle strength was measured with the 15RM testing method described elsewhere [51] for both strength training groups S and SE. The 15 RM for each exercise was tested twice on different days during the evaluation

periods. The intraclass correlation coefficient of reliability for all the exercises was $\text{ICCr} = 0,995$ and $\text{ICCr} = 0,994$ for men and women, respectively. All the assessments, the data collection sessions and the previous trainings were carried out with the same machines and free weights (Johnson Health Tech. Iberica, Matrix, Spain).

Static strength (dinamometry)

The general strength index (SI) was calculated using previous criteria [52]. This method measures leg and arm strength with respect to body weight via two exercises: the bench press and squat. The dynamometric strength index (DSI) was calculated by measuring muscular strength using a Tecsyp Tkk5002 hand dynamometer and a Tecsyp Tkk5401 leg and back dynamometer (Tecsyp, Barcelona, Spain). The DSI value was calculated as the sum of the values obtained with both apparatuses divided by subject's body weight.

Physical activity and daily energy expenditure

Habitual physical activity (PA) was assessed with a SenseWear Pro3 Armband™ (Body Media, Pittsburgh, PA). Subjects were instructed to wear the monitor continuously for 5 days including weekend days and weekdays following general recommendations [53]. Data was recorded by 15 min intervals. DEE was calculated using the Body Media propriety algorithm (Innerview Research Software Version 6.0).

Energy balance, adherence to diet and exercise

All subjects were instructed to continue their habitual daily activities as before the intervention period. They were required to report the kind, duration, and intensity of any physical activity and the amount of any food undertaken during the intervention period.

At the beginning of the intervention, negative energy balance was calculated taking into account the DEE, and a 3-day food record, in order to decrease the energy intake of the diet by a 25–30% during the intervention.

In one hand, adherence to diet was calculated as the estimated Kcal of the diet divided by the real Kcal intake in percentage ($[\text{estimated kcal of diet} / \text{real Kcal intake}] \cdot 100$), representing the highest adherence the value 100%. Higher values mean a higher restriction and viceversa. On the other hand, adherence to exercise was calculated by the number of sessions completed in regard to the theoretical sessions ($[\text{sessions performed} / \text{total sessions}] \cdot 100$).

Psychological and eating behavior assessment

Quality of life The impact of overweight and non-morbid obesity on Health-Related Quality of Life (HRQL) was assessed using two different questionnaires:

EUROQOL 5D (EQ-5D)

The EQ-5D, is a self-administered questionnaire capable of generating a score reflecting a value associated with a given health status. The EQ-5D™ descriptive system consists of five dimensions: 1. Mobility; 2. Self-care; 3. Usual activities; 4. Pain/discomfort; 5. Anxiety/depression. Each dimension has three levels: "level 1: no health problems", "level 2: moderate health problems" and "level 3: extreme health problems". Each unique health state described by the instrument has an associated 5-digit descriptor ranging from 11111 for perfect health to 33333 for the worst possible state. The resulting descriptive system defines 243 health states. The instrument also includes a 20-centimeter visual analogue scale for the self-assessment of current general health [54].

Short Form-36 Health Survey (SF-36) version 1.4, valid for the Spanish population was used [55]. This questionnaire evaluates the individual health status in two dimensions: functional and emotional. Functional status is divided in 6 subscales: Physical Functioning (PF), Role-Physical (RP), Bodily Pain (BP), Vitality (VT), Social Functioning (SF) and Role-Emotional (RE). Emotional status includes Mental Health (MH) and General Health (GH) subscales. Scores on all subscales range from 0 to 100 (higher scores indicate better health status) [56].

Eating behaviour At baseline and at the end of the intervention all food and beverages consumed by the participants were recorded using a food frequency questionnaire and a "3-day food and drink record", validated for the Spanish population [57]. This recorded all food and drinks consumed at home and away for three days, including 2 weekdays and 1 weekend day. Subjects were instructed to record the weights of food consumed if possible and to use household measurements (spoonfuls, cups, etc.) if not. The energy and nutritional content of the food consumed was then calculated using the DIAL software (Alce Ingeniería, 2004). Then, the values obtained were compared to the recommended dietary intakes for the Spanish population to determine dietary adequacy [58].

Body composition

Anthropometric measurements Body height was measured using a SECA stadiometer (range 80-200 cm, Valencia, Spain). Body weight (BW) was measured using a TANITA BC-420MA balance (Bio Lógica Tecnología Médica S.L, Barcelona, Spain). BMI was calculated as [body weight (kg)/(height (m))²]. Waist circumference (WC) was measured using a SECA 201 steel tape (Quirumed, Valencia, Spain). Body fat percentage was measured using bioelectrical impedance analysis (BIA)

with an OMRON BF 306® analyzer (OMRON HEALTH-CARE Co., Ltd, Ukyo-ku, Kyoto, Japan).

Dual-energy x-ray absorptiometry Dual-energy x-ray absorptiometry (DXA) was used to measure percentage of total fat mass (TFM%), percentage of android fat (AF%), the android gynoid ratio (A/GF) and lean mass (kg), using a GE Lunar Prodigy apparatus (GE Healthcare, Madison, Wisconsin, USA). All DXA scan analyses were performed at the Faculty of Physical Activity and Sport Sciences using GE Encore 2002 software v 6.10.029.

Nuclear magnetic resonance Nuclear Magnetic Resonance (MRI) was used to study the proportion and location of the adipose tissue. The MRI study measured changes at different abdominal adipose tissue compartments: SAT (superficial and deep depots) and VAT (intra-peritoneal and retroperitoneal compartments). Images were obtained with a GE Medical Systems 1.5 Tesla whole body scanner using a T1-weighted fast-spin echo pulse sequence at the University Hospital La Paz Research Institute. The subjects were examined in supine position with arms positioned parallel along the lateral sides of the body. The subjects were imaged in two half-body volumes. A breath-hold sequence during expiration (approximately 20 seconds per acquisition) was used to minimize the effects of respiratory motion on the images. The total research time was about 5-10 min. All images were acquired on a 256x192 matrix, 16 bits/pixel, with a field of view = 480 mm. Slice thickness was 6 mm and contiguous slices were obtained every 1.5 mm from the first sacral vertebra upwards, until to achieve 30 images per person. For each subject six reference levels were studied, corresponding to intervertebral disc levels L5-S1 to L2-L3, at umbilical and at caudal left kidney positions. The acquired axial MR images were retrieved from the scanner using the DICOM® protocol and after were transferred to an external personal computer running Windows XP. We used specially designed image analysis software Stereonauta for quantitative analysis of the images.

Biochemical, metabolic, inflammatory and genetic profile

Biomarkers Blood samples were taken early in the morning at the La Paz University Hospital Extraction Unit after a 12 h overnight fast by venipuncture from the antecubital vein. Samples were kept at 4- 6°C until analysis, which was always performed within 48 h following standard hospital procedures. The following parameters were measured:

- Hematology was performed in a Pentra 120 DX cell counter (ABX-Horiba, Montpellier, France).

- Serum calcium, phosphorus, total proteins, albumin, creatinine, uric acid, bilirubin, lipase, alkaline phosphatase, creatinase, LDH, GOT and GPT were analyzed by enzymatic-spectrophotometric assay (Olympus AU 5400, Izasa, Porto, Portugal).
- Blood lipid profile (total cholesterol, HDL and LDL cholesterol, triglycerides) and glucose were analyzed by means of a enzymatic-spectrophotometric assay (Olympus AU 5400, Izasa, Porto, Portugal). Apo A and Apo B were determined using a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Atherogenic risk ratios were calculated: total cholesterol/HDL, LDL/HDL and Apo B/Apo A1.
- Plasma fasting immunoreactive insulin was measured by enzymatic method in a fluoro-immunochemistry Analyzer (AIA-360, Tosoh Bioscience, Tessenderlo, Belgium). Insulin sensitivity was estimated using the HOMA-IR index [HOMA-IR = fasting glucose (mmol/l)/fasting immunoreactive insulin (mU/ml)/22.5].
- Inflammatory biomarkers: Plasma C-reactive protein concentrations (CRP) were determined using a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Plasma fibrinogen was measured by coagulometric methods (ACL TOP 700, Izaasa, Porto, Portugal). Plasma leptin, IL-6 and TNF- α plasma concentrations were determined using a Luminex[®]-LX200 Analyzer (Millipore Corp, Billerica, Massachusetts, USA) and a MILLIPLEX MAP human circulating cancer biomarker magnetic bead panel (HCCBP1MAG-58 K) (Millipore, St. Charles, Missouri, USA). All samples were analyzed in duplicate. Data were analyzed using 3.1 xPONENT Software (Millipore, St. Charles, Missouri, USA). The intra and inter-assay coefficients of variation for the cytokine assays all fell in the 5-10% range.
- 25-hydroxyvitamin D was measured by chemiluminescent immunoassay using the LIAISON[®] Analyzer family (Palex Medical, Barcelona, Spain).
- Serum TSH was measured by an electrochemiluminescence immunoassay (ECLIA) by using an E170 analyser (Roche Diagnostics, Mannheim, Germany).

Genetic profile We investigated the potential influence of different genetic single nucleotide polymorphisms (SNPs), highly prevalent in the general population, on the response to different training protocols in overweight and obesity treatment. These SNPs are located in genes involved in metabolic pathways related to adiposity

(Beta-Adrenergic and Leptin receptors, and Peroxisome Proliferator-Activated Receptor), and in genes involved in energy metabolism and the production of energy during physical activity (metabolism of lactate and fatty acids). In addition, we have studied the Angiotensin-Converting Enzyme (ACE) Insertion/Deletion (I/D) polymorphism, which has been associated with athletic performance and training adaptation. The potential role for most of the selected polymorphisms is well documented, both as overweight/obesity risk factors and as modulators for the response to different interventions.

With regard to the method used, we firstly reviewed published literature about the techniques for analyzing the polymorphisms [59-68]. Then, we adapted these protocols to the conditions in our laboratory. Whole blood (5 ml) from each patient was collected in EDTA and sent to the Metabolism, Genetics and Nutrition Research Group of the School of Medicine-IFIMAV, in Santander. DNA was extracted from each sample using the "QIAamp[®] DNA Blood Mini Kit" from QIAGEN (Hilden, Germany) and the genotyping was performed for each SNP afterwards. The DNA samples were preserved at -40°C. All subjects signed a specific informed consent which allows storage and genetic characterization in relation to the objectives of the PRONAF study by the Metabolism, Genetics and Nutrition Research Group of the IFIMAV following the protocols of confidentiality and clinical safety, ensuring the anonymity of the samples and their use for research only.

After selecting the most interesting SNPs for the project objectives, the analyses were performed using genotyping assays based on techniques of double PCR, PCR and RFLP or sequencing for Phase II, and real-time PCR (TaqMan[®] SNP Genotyping Assays, Applied Biosystem) for Phase III, which allows the semi-automation of these determinations. All these techniques proved to be reproducible and stable under the conditions of the study. The reliability of the techniques used for genotyping in the different phases of the study has been proved using internal quality controls through different methods: repeated measurements of randomized samples, double-blinded reading of random samples, introduction of positive and negative controls, and random doubled testing of samples by both techniques. We obtained consistent results for any of the techniques used.

Assessment during the follow-up

Training control

Feedbacks for training loads were done once a month with the Rate of Perceived Exertion (RPE) to subjectively evaluate each session and determine where the participant considered the intensity to be at, following a similar methodology previously used [69].

Physical activity control

PA was assessed as described above once a month. All subjects were instructed to continue their habitual daily activities as before the intervention period and were provided with a PA diary to log the type, duration, and intensity of any PA or exercise undertaken during the intervention.

Body weight control

BW was measured as described earlier every fifteen days at the Sports Center. Subjects included in group C used their own scales to monitor BW at home during the intervention.

Dietary intake control

A dietician interviewed each participant at baseline, and at 3 and 6 months after the start of the intervention. All subjects were instructed about how to record their dietary intake using a daily log, and given recommended portion sizes and information on possible food swaps. In addition, voluntary group nutrition education sessions were given by the dieticians. The goal was to equip the participants with the necessary knowledge and skills to achieve gradual, permanent behavioural changes.

Statistical analysis

The main independent variables of the study were: intervention group, age, gender, measurement (baseline – post) and BMI classification (overweight or obese). The delta percentage was calculated through the standard formula: $\text{change (\%)} = \frac{[(\text{post-test score} - \text{pre-test score}) / \text{pre-test score}] \times 100}{100}$. The power calculated previously to find significant differences between groups was 0.8 to fat mass (%).

Standard statistical methods will be used for the calculation of the means and standard deviation for all dependent variables. The statistical analysis will be as follows: 1) Univariate descriptive analysis, study of data distribution, basic statistics such as central and dispersion values. Pair comparison tests with previous analysis of the homogeneity of variance will be used. Pearson correlation coefficients, chi-squared tests and exact probability calculations will be also performed to study the relationship between quantitative and qualitative variables, respectively; 2) General lineal models, such as a multivariate analysis (MANOVA) or two way analysis of variance (ANOVA) for repeated measures, will be used to determine differences among the four interventions groups and between baseline and post-training values. Compound symmetry, or sphericity, will be verified by the Mauchly test. When the assumption of sphericity would not be met, the significance of F ratios will be adjusted according to the Greenhouse-Geisser procedure.

Multiple comparisons of ANOVAs will be made with the Bonferroni post-hoc test.

The analysis of the data will be done using statistical package SPSS (SPSS Inc., Chicago, Illinois, USA). The significant level will be set at $\alpha = 0.05$.

Assessment for quality control

Previous to the start of the intervention we developed a protocol book with all the tests carried out in the pre and post intervention, plus the intervention procedures. An external observer followed the pilot study and his observations were included in the protocols. Weekly meetings during the pilot study were made with all the people involved in the project to train them and perform the protocol book.

At the end of the overweight intervention, the subjects filled in a satisfaction questionnaire. The results were included in the design of the obese intervention.

Finally, in order to clean the final data base of possible errors, a double data entry procedure was performed. Also an assessment of missing data and the identification of potential outliers were carried out.

Discussion

The PRONAF study is the first randomized controlled intervention study performed in Spanish overweight and obese adults without any other associated disease with the aim of losing weight and improving several health-related parameters by means of combining caloric restriction and controlled training programs. One of the strengths of the PRONAF study is the multidisciplinary approach. A group of professionals and researchers with expertise in a specific field: physical activity and fitness, nutrition, body composition, genetics and biochemical markers have designed together the study protocols after an in-depth review of the state of the art in the literature [8-40]. Exercise protocols were tested in healthy subjects and study protocols harmonized in a pilot study [70,71].

The PRONAF study has both strengths and limitations. The main limitation is that the study did not include psychological control of the subjects. During the study and in the final questionnaire evaluation the subjects pointed out the need of some psychological support especially when the body weight changes were not so evident.

On the other hand, the PRONAF study has also several strengths: 1) the final sample size of our study was maintained according to the sample size estimation, keeping a low dropout rate similar to other studies [37,40,41]. It is important to note that there were no differences between groups in the number of dropouts. 2) During the training sessions the participants in the study were continuously monitored by certified trainers to ensure the correct execution of the exercises and the

completion of each training session. 3) Inclusion of additional variables as visceral fat, blood lipid profile, and genotyping broadens the dimension of the PRONAF study.

In summary, feasibility of the PRONAF protocol has been proven. Transfer of knowledge is foreseen as a spin-off, in order that overweight and obese subjects can benefit from the results. The aim is to transfer it to sports centers. Effectiveness on individual health-related parameter will be analyzed in forthcoming publications.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in the writing of the paper and provided comments on the drafts and approved the final version.

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References

- Berghofer A, Pischon T, Reinhold T, Apovian CM, Sharma AM, Willich SN: **Obesity prevalence from a European perspective: a systematic review.** *BMC Publ Health* 2008, **8**:200.

- Bellido A, Soto JM, García Almeida M, de la López T: **Foro ACTUA II (abordaje y recomendaciones de actuación útil sobre el exceso de peso en atención primaria).** *Revista Española de Obesidad* 2008, **6**(4):175–197.
- Salas-Salvado J, Rubio MA, Barbany M, Moreno B, Aranceta J, Bellido D, Blay V, Carraro R, Formiguera X, Foz M, et al: **Consenso SEEDO 2007 para la evaluación del sobrepeso y la obesidad y el establecimiento de criterios de intervención terapéutica.** *Rev Esp Obes* 2007, **3**:7–48.
- Gutin B: **Diet vs exercise for the prevention of pediatric obesity: the role of exercise.** *Int J Obes (Lond)* 2011, **35**:29–32.
- Maziak W, Ward KD, Stockton MB: **Childhood obesity: are we missing the big picture?** *Obes Rev* 2008, **9**(1):35–42.
- Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, Hill JO, Brancati FL, Peters A, Wagenknecht L: **Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes.** *Diabetes Care* 2011, **34**(7):1481–1486.
- Rodríguez A, López-Valcárcel BG: **El trasfondo económico de las intervenciones sanitarias en la prevención de la obesidad.** *Rev Esp Salud Publica* 2009, **83**:25–41.
- Hunter GR, Wetzstein CJ, Fields DA, Brown A, Bamman MM: **Resistance training increases total energy expenditure and free-living physical activity in older adults.** *J Appl Physiol* 2000, **89**(3):977–984.
- Poehlman ET, Dvorak RV, DeNino WF, Brochu M, Ades PA: **Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial.** *J Clin Endocrinol Metab* 2000, **85**(7):2463–2468.
- Ryan AS: **Insulin resistance with aging: effects of diet and exercise.** *Sports Med* 2000, **30**(5):327–346.
- Castaneda C, Layne JE, Munoz-Orians L, Gordon PL, Walsmith J, Foldvari M, Roubenoff R, Tucker KL, Nelson ME: **A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes.** *Diabetes Care* 2002, **25**(12):2335–2341.
- Dunstan DW, Daly RM, Owen N, Jolley D, De Courten M, Shaw J, Zimmet P: **High-intensity resistance training improves glycemic control in older patients with type 2 diabetes.** *Diabetes Care* 2002, **25**(10):1729–1736.
- Fahman MM, Boardley D, Lambert CP, Flynn MG: **Effects of endurance training and resistance training on plasma lipoprotein profiles in elderly women.** *J Gerontol A Biol Sci Med Sci* 2002, **57**(2):B54–B60.
- Hunter GR, Bryan DR, Wetzstein CJ, Zuckerman PA, Bamman MM: **Resistance training and intra-abdominal adipose tissue in older men and women.** *Med Sci Sports Exerc* 2002, **34**(6):1023–1028.
- Janssen I, Fortier A, Hudson R, Ross R: **Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women.** *Diabetes Care* 2002, **25**(3):431–438.
- Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D: **Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes.** *Diabetes Res Clin Pract* 2002, **56**(2):115–123.
- Poehlman ET, Denino WF, Beckett T, Kinaman KA, Dionne JJ, Dvorak R, Ades PA: **Effects of endurance and resistance training on total daily energy expenditure in young women: a controlled randomized trial.** *J Clin Endocrinol Metab* 2002, **87**(3):1004–1009.
- Baldi JC, Snowling N: **Resistance training improves glycaemic control in obese type 2 diabetic men.** *Int J Sports Med* 2003, **24**(6):419–423.
- Banz WJ, Maher MA, Thompson WG, Bassett DR, Moore W, Ashraf M, Keefer DJ, Zemel MB: **Effects of resistance versus aerobic training on coronary artery disease risk factors.** *Exp Biol Med (Maywood)* 2003, **228**(4):434–440.
- Park SK, Park JH, Kwon YC, Kim HS, Yoon MS, Park HT: **The effect of combined aerobic and resistance exercise training on abdominal fat in obese middle-aged women.** *J Physiol Anthropol Appl Human Sci* 2003, **22**(3):129–135.
- Reynolds TH, Supiano MA, Dengel DR: **Resistance training enhances insulin-mediated glucose disposal with minimal effect on the tumor necrosis factor-alpha system in older hypertensives.** *Metabolism* 2004, **53**(3):397–402.
- Tokmakidis SP, Zois CE, Volaklis KA, Kotsa K, Tourva AM: **The effects of a combined strength and aerobic exercise program on glucose control and insulin action in women with type 2 diabetes.** *Eur J Appl Physiol* 2004, **92**(4–5):437–442.
- Cauza E, Hanusch-Enserer U, Strasser B, Ludvik B, Metz-Schimmerl S, Pacini G, Wagner O, Georg P, Prager R, Kostner K, et al: **The relative benefits of endurance and strength training on the metabolic factors and muscle**

- function of people with type 2 diabetes mellitus. *Arch Phys Med Rehabil* 2005, **86**(8):1527-1533.
24. Fatouros IG, Tournis S, Leontsini D, Jamurtas AZ, Sxina M, Thomakos P, Manousaki M, Douroudos I, Taxildaris K, Mitrakou A: **Leptin and adiponectin responses in overweight inactive elderly following resistance training and detraining are intensity related.** *J Clin Endocrinol Metab* 2005, **90**(11):5970-5977.
 25. Ibanez J, Izquierdo M, Arguelles I, Forga L, Larrion JL, Garcia-Unciti M, Idoate F, Gorostiaga EM: **Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes.** *Diabetes Care* 2005, **28**(3):662-667.
 26. Kukkonen-Harjula KT, Borg PT, Nenonen AM, Fogelholm MG: **Effects of a weight maintenance program with or without exercise on the metabolic syndrome: a randomized trial in obese men.** *Prev Med* 2005, **41**(3-4):784-790.
 27. Arciero PJ, Gentile CL, Martin-Pressman R, Ormsbee MJ, Everett M, Zwicky L, Steele CA: **Increased dietary protein and combined high intensity aerobic and resistance exercise improves body fat distribution and cardiovascular risk factors.** *Int J Sport Nutr Exerc Metab* 2006, **16**(4):373-392.
 28. Fenkci S, Sarsan A, Rota S, Ardic F: **Effects of resistance or aerobic exercises on metabolic parameters in obese women who are not on a diet.** *Adv Ther* 2006, **23**(3):404-413.
 29. Ferrara CM, Goldberg AP, Ortmeyer HK, Ryan AS: **Effects of aerobic and resistive exercise training on glucose disposal and skeletal muscle metabolism in older men.** *J Gerontol A Biol Sci Med Sci* 2006, **61**(5):480-487.
 30. Shaibi GQ, Cruz ML, Ball GD, Weigensberg MJ, Salem GJ, Crespo NC, Goran MI: **Effects of resistance training on insulin sensitivity in overweight latino adolescent males.** *Med Sci Sports Exerc* 2006, **38**(7):1208-1215.
 31. Brooks N, Layne JE, Gordon PL, Roubenoff R, Nelson ME, Castaneda-Sceppa C: **Strength training improves muscle quality and insulin sensitivity in hispanic older adults with type 2 diabetes.** *Int J Med Sci* 2007, **4**(1):19-27.
 32. Olson TP, Dengel DR, Leon AS, Schmitz KH: **Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women.** *Int J Obes (Lond)* 2007, **31**(6):996-1003.
 33. Sigal RJ, Kenny GP, Boule NG, Wells GA, Prud'homme D, Fortier M, Reid RD, Tulloch H, Coyle D, Phillips P, et al: **Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial.** *Ann Intern Med* 2007, **147**(6):357-369.
 34. Camargo MD, Stein R, Ribeiro JP, Schwartzman PR, Rizzatti MO, Schaen BD: **Circuit weight training and cardiac morphology: a trial with magnetic resonance imaging.** *Br J Sports Med* 2008, **42**(2):141-145. discussion 145.
 35. Hunter GR, Byrne NM, Sirikul B, Fernandez JR, Zuckerman PA, Darnell BE, Gower BA: **Resistance training conserves fat-free mass and resting energy expenditure following weight loss.** *Obesity (Silver Spring)* 2008, **16**(5):1045-1051.
 36. Bouchard DR, Soucy L, Senechal M, Dionne IJ, Brochu M: **Impact of resistance training with or without caloric restriction on physical capacity in obese older women.** *Menopause* 2009, **16**(1):66-72.
 37. Brochu M, Malta MF, Messier V, Doucet E, Strychar I, Lavoie JM, Prud'homme D, Rabasa-Lhoret R: **Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women.** *J Clin Endocrinol Metab* 2009, **94**(9):3226-3233.
 38. Davidson LE, Hudson R, Kilpatrick K, Kuk JL, McMillan K, Janiszewski PM, Lee S, Lam M, Ross R: **Effects of exercise modality on insulin resistance and functional limitation in older adults: a randomized controlled trial.** *Arch Intern Med* 2009, **169**(2):122-131.
 39. Kirk EP, Donnelly JE, Smith BK, Jonas J, Lecheminant JD, Bailey BW, Jacobsen DJ, Washburn RA: **Minimal resistance training improves daily energy expenditure and fat oxidation.** *Med Sci Sports Exerc* 2009, **41**(5):1122-1129.
 40. Sillanpaa E, Laaksonen DE, Hakkinen A, Karavirta L, Jensen B, Kraemer WJ, Nyman K, Hakkinen K: **Body composition, fitness, and metabolic health during strength and endurance training and their combination in middle-aged and older women.** *Eur J Appl Physiol* 2009, **106**(2):285-296.
 41. Del Corral P, Chandler-Laney PC, Casazza K, Gower BA, Hunter GR: **Effect of dietary adherence with or without exercise on weight loss: a mechanistic approach to a global problem.** *J Clin Endocrinol Metab* 2009, **94**(5):1602-1607.
 42. Salas-Salvado J, Rubio MA, Barbany M, Moreno B, Aranceta J, Bellido D, Blay V, Canaro R, Formiguera X, Foz M, et al: **Recomendaciones nutricionales basadas en la evidencia para la prevención y el tratamiento del sobrepeso y la obesidad en adultos.** *Rev Esp Obes* 2011, **9**(Suplemento):6-78.
 43. Singhal A, Trilk JL, Jenkins NT, Bigelman KA, Cureton KJ: **Effect of intensity of resistance exercise on postprandial lipemia.** *J Appl Physiol* 2009, **106**(3):823-829.
 44. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK: **American college of sports medicine position stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults.** *Med Sci Sports Exerc* 2009, **41**(2):459-471.
 45. Morgan BWS, Tiidus PM: **Aerobic energy expenditure during recreational weight training in females and males.** *J Sports Sci Med* 2003, **2**(1303-2968):117-122.
 46. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, McManus K, Champagne CM, Bishop LM, Laranjo N, et al: **Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates.** *N Engl J Med* 2009, **360**(9):859-873.
 47. Ghroubi S, Elleuch H, Chikh T, Kaffel N, Abid M, Elleuch MH: **Physical training combined with dietary measures in the treatment of adult obesity. A comparison of two protocols.** *Ann Phys Rehabil Med* 2009, **52**(5):394-413.
 48. Carter J, Jeukendrup AE: **Validity and reliability of three commercially available breath-by-breath respiratory systems.** *Eur J Appl Physiol* 2002, **86**(5):435-441.
 49. Foss O, Hallen J: **Validity and stability of a computerized metabolic system with mixing chamber.** *Int J Sports Med* 2005, **26**(7):569-575.
 50. Weir JB: **New methods for calculating metabolic rate with special reference to protein metabolism.** *J Physiol* 1949, **109**(1-2):1-9.
 51. Morgan B, Woodruff SJ, Tiidus PM: **Aerobic energy expenditure during recreational weight training in females y males.** *J Sports Sci Med* 2003, **2**:117-122.
 52. Jurca R, Lamonte MJ, Church TS, Earnest CP, Fitzgerald SJ, Barlow CE, Jordan AN, Kampert JB, Blair SN: **Associations of muscle strength and fitness with metabolic syndrome in men.** *Med Sci Sports Exerc* 2004, **36**(8):1301-1307.
 53. Murphy SL: **Review of physical activity measurement using accelerometers in older adults: considerations for research design and conduct.** *Prev Med* 2009, **48**(2):108-114.
 54. Brooks R: **EuroQol: the current state of play.** *Health Policy* 1996, **37**(1):53-72.
 55. Alonso J, Prieto L, Anto JM: **The spanish version of the SF-36 health survey (the SF-36 health questionnaire): an instrument for measuring clinical results.** *Med Clin (Barc)* 1995, **104**(20):771-776.
 56. McHorney CA, Ware JE Jr, Raczek AE: **The MOS 36-item short-form health survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs.** *Med Care* 1993, **31**(3):247-263.
 57. Ortega R, Requejo A, Lopez-Sobaler A: **Questionnaires for dietetic studies and the assessment of nutritional status.** In *Nutriguia manual of clinical nutrition in primary care*. Edited by Requejo A, Ortega R. Madrid: Editorial Complutense; 2003:456-459.
 58. Ortega R, Requejo A, Navia B, López-Sobaler A: **Recommended daily intakes of energy and nutrients for the spanish population.** In *Food composition A basic tool for assessing nutritional status*. Edited by RM O, López-Sobaler A, Requejo A, Andrés P. Madrid: Complutense; 2004:82-85.
 59. Chung WK, Power-Kehoe L, Chua M, Chu F, Aronne L, Huma Z, Sothern M, Udall JN, Kahle B, Leibel RL: **Exonic and intronic sequence variation in the human leptin receptor gene (LEPR).** *Diabetes* 1997, **46**(9):1509-1511.
 60. Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD: **Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity.** *N Engl J Med* 1995, **333**(6):352-354.
 61. Cupeiro R, Benito PJ, Maifulli N, Calderon FJ, Gonzalez-Lamuno D: **MCT1 genetic polymorphism influence in high intensity circuit training: a pilot study.** *J Sci Med Sport* 2010, **13**(5):526-530.
 62. Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD, McKeigue PM, Scott J, Aitman TJ: **Leptin receptor gene variation and obesity: lack of association in a white british male population.** *Hum Mol Genet* 1997, **6**(6):869-876.
 63. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, et al: **The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes.** *Biochem Biophys Res Commun* 2000, **271**(1):212-216.

64. Hixson JE, Vernier DT: **Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI.** *J Lipid Res* 1990, **31**(3):545–548.
65. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, Arner P: **Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function.** *J Clin Invest* 1997, **100**(12):3005–3013.
66. Lindpaintner K, Pfeiffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH: **A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease.** *N Engl J Med* 1995, **332**(11):706–711.
67. Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y, et al: **Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity.** *Diabetologia* 1997, **40**(10):1204–1210.
68. Taroni F, Verderio E, Fiorucci S, Cavadini P, Finocchiaro G, Uziel G, Lamantea E, Gellera C, DiDonato S: **Molecular characterization of inherited carnitine palmitoyltransferase II deficiency.** *Proc Natl Acad Sci U S A* 1992, **89**(18):8429–8433.
69. Ball Geoff D, Crespo Noe C, Cruz Martha L, Goran Michael I, Salem George J, Shaibi Gabriel Q, Weigensberg Marc J: **Effects of resistance training on insulin sensitivity in overweight latino adolescent males.** *Med Sci Sports Exerc* 2006, **38**(7):1208–1215.
70. Benito PJ, Neiva C, Gonzalez-Quijano PS, Cupeiro R, Morencos E, Peinado AB: **Validation of the sensewear armband in circuit resistance training with different loads.** *Eur J Appl Physiol* 2012, **112**(8):3155–3159.
71. Cupeiro R, González-Lamuño D, Amigo T, Peinado AB, Ruiz JR, Ortega FB, Benito PJ: **Influence of the MCT1-T1470A polymorphism (rs1049434) on blood lactate accumulation during different circuit weight trainings in men and women.** *J Sci Med Sport* 2012, **15**(6):541–547.

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Publications

- Augusto García Zapico; Pedro José Benito Peinado; Marcela González-Gross; Ana Belén Peinado Lozano; Esther Morencos Martínez; Blanca Romero Moraleda; Miguel Ángel Rojo Tirado; Rocío Cupeiro Coto; Barbara Szendrei; Javier Butragueño Revenga; Maite Bermejo; María Álvarez Sánchez; Miguel García Fuentes; Carmen Gómez Candela; Laura M Bermejo; Ceila Fernández Fernández; Francisco Javier Calderón Montero.

Nutrition and physical activity programs for obesity treatment (PRONAF study). Methodological approach of the project.

BMC Public Health. 12 (1), 1100. 2012.

- Pedro José Benito Peinado, Laura M. Bermejo, Ana Belén Peinado Lozano, Bricia López-Plaza, Rocío Cupeiro Coto, Barbara Szendrei, Francisco Javier Calderón Montero, Eliane Aparecida Castro, Carmen Gómez-Candela.

Change in weight and body composition in obese subjects following a hypocaloric diet plus different training programs or physical activity recommendations.

Journal of Applied Physiology. 118(8), 1006-1013. 2015.

- Barbara Szendrei, Domingo González-Lamuño, Maria Teresa Amigo Lanza, Guan Wang, Yannis Pitsiladis, Pedro José Benito Peinado, Carmen Gomez-Candela,

Francisco Javier Calderón Montero and Rocío Cupeiro Coto, on behalf of the PRONAF Study Group.

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

Applied Physiology, Nutrition, and Metabolism. 41(3), 307-314. 2015.

Congresses

- July 2014 European College of Sport Science, 19th Annual Congress of the ECSS

Oral presentation: Impact of ADRB3 SNP on abdominal fat in overweight and obese women

Amsterdam, The Netherlands

- May 2014 American College of Sports Medicine, ACSM's 61st Annual Meeting

Poster: Controlled Exercise As A Tool For Preventing Obesity And Metabolic Diseases In Arg64 Carrier Women

ACSM, Orlando, Florida, USA

Medicine & Science in Sports & Exercise: May 2014 - Volume 46 - Issue 5S - p 101–114

doi: 10.1249/01.mss.0000451113.55137.dd

- July 2012 European College of Sport Science, 17th Annual Congress of the ECSS

Poster: Role of the ADRB2 GLN27GLU polymorphism on the effect of a weight loss program

ECSS, The Vrije Universiteit Brussel, Bruges, Belgium

Participation in projects

“Point by point breathing pattern recovery in professional tennis players”

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