Leońska-Duniec, A, Jastrzębski, Z, Jażdżewska, A, Moska, W, Lulińska-Kuklik, E, Sawczuk, M, Gubaydullina, SI, Shakirova, AT, Cięszczyk, P, Maszczyk, A and Ahmetov, II

Individual responsiveness to exercise-induced fat loss and improvement of metabolic profile in young women is associated with polymorphisms of adrenergic receptor genes

INDIVIDUAL RESPONSIVENESS TO EXERCISE-INDUCED FAT LOSS AND IMPROVEMENT OF METABOLIC PROFILE IN YOUNG WOMEN IS ASSOCIATED WITH POLYMORPHISMS OF ADRENERGIC RECEPTOR GENES

Agata Leońska-Duniec[1], Zbigniew Jastrzębski[1], Aleksandra Jażdżewska[1], Waldemar Moska[1], Ewelina Lulińska-Kuklik[1], Marek Sawczuk[1], Svetlana I. Gubaydullina[2], Alsu T. Shakirova[3], Pawel Cięszczyk[4], Adam Maszczyk[5], Ildus I. Ahmetov[6,7]

[1]Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

[2]Sport Technology Research Centre, Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russia

[3]Department of Propaedeutics of Childhood Diseases, Kazan State Medical University, Kazan, Russia

[4]Faculty of Physical Education, Gdansk University of Physical Education and Sport, Gdansk, Poland

[5]Department of Theory and Practice of Sport; Academy of Physical Education in Katowice; Poland.

[6]Sports Genetics Laboratory, St Petersburg Research Institute of Physical Culture, St Petersburg, Russia

[7]Laboratory of Molecular Genetics, Kazan State Medical University, Kazan, Russia

Corresponding author: Dr. Ildus I. Ahmetov, Kazan State Medical University, 6/30, Tolstoy Street, 420015, Kazan, Russia; genoterra@mail.ru; Tel. +79625586002

Running head: ADRs genes and fat loss in response to training

Abstract

The effectiveness of physical exercise on fat loss and improvement of aerobic capacity varies considerably between individuals. A strong linkage exists between common allelic variants of the adrenergic receptor genes and weight gain, as well as changes in body composition. Therefore we aimed to check if body composition and metabolic variables were modulated by the *ADRB2* (Gly16Arg and Glu27Gln), *ADRB3* (Trp64Arg) and *ADRA2A* (rs553668 G/A) gene polymorphisms in 163 Polish sedentary women (age 19-24; body mass index (BMI) 21.7±0.2 kg/m^2) involved in a 12-week aerobic training program. Only 74.8% of participants lost fat mass. On average, participants lost 5.8 (10.4)% of their relative fat mass with training (range: +28.3 to -63.6%). The improvement of VO_{2max} was significantly greater in women who could lose their fat mass compared to women who were unsuccessful in fat loss (4.5 (5.6)% vs. 1.5 (3.8)%; P = 0.0045). The carriers of a low number (0-3) of obesity-related risk alleles (*ADRB2* Gly16, *ADRB2* Glu27, *ADRA2A* rs553668 G) were more successful in fat mass loss compared to the carriers of a high number (5-6) of risk alleles (7.7 (9.8) vs 4.0 (9.4)%, P=0.0362). The presented results support the assumption that variation within adrenergic receptor genes contributes to interindividual changes of body composition in response to physical exercise.

Key words: *ADRB2*, *ADRB3, ADRA2A,* polygenic, obesity, fat, HDL

Key points

- There is a wide range of individual variability in the change of relative fat mass and BMI in response to a 12-week aerobic training program.
- The efficiency of fat loss was inversely correlated with the improvement of VO_{2max} in response to a 12-week aerobic training.
- The carriers of a low number of obesity-related risk alleles were more successful in fat mass loss compared to the carriers of a high number of risk alleles.

Introduction

Unhealthy lifestyle habits like lack of physical activity and excessive energy intake may result in overweight and obesity (Rank et al., 2012). The latter is one of the major and growing health problems of the XXI century. The presence of the elevated adipose tissue (increased adiposity) increases the likelihood of various medical conditions, such as hypertension, coronary heart disease, type 2 diabetes mellitus, and certain types of cancer (Masuo and Lambert, 2011). Thus, it is at least partly preventable by developing healthy diet and regular physical exercises that, in consequence, could help participants stay at a healthy weight (Greenway, 2015). However, a wide range of inter-individual variability in weight gain and changes in body composition induced by physical exercises and diets is seen in human populations which indicates the role of non-environmental factors such as genetic modifiers (Masuo et al., 2001; Garenc et al., 2003; Wolfarth et al., 2005; Bouchard, 2008; Eynon et al., 2013; Leońska-Duniec et al., 2016).

Various epidemiological and clinical studies indicate that strong linkage exists between common allelic variants of the adrenergic receptor genes and weight gain as well as changes in body composition (Phares et al., 2004; Masuo et al., 2005*a*; Bea et al., 2010; Szendrei et al., 2016). These adrenergic receptors (ADRs) encoded by the *ADRA* (α-adrenergic receptors - inhibitory) and *ADRB* (β-adrenergic receptors - stimulatory) genes are part of the sympathetic nervous system and exert their actions via coupling with the catecholamines. Because catecholamines are important regulators of lipolysis and energy expenditure during both energy restriction as well as exercise, it is clearly understandable that sympathetic nerve activation may play a role in modifying weight gain and changes in body composition. Reduced energy expenditure and

lowered resting metabolic rate are predictive of overweight and obesity (Ahles and Engelhardt, 2014; O'Dell et al., 2015).

Recent studies have shown that allelic variation in the ADRs family exists, with the single nucleotide polymorphisms (SNPs) as the most common genetic polymorphisms (Green et al., 1993; Ikegami et al., 1996). Genetic diversity of the ADRs influence receptor expression, activity, and agonist regulation, in consequence contribute to the variable changes in body composition as well as weight gain and obesity (Large et al., 1997; Hellstrom et al., 1999). Within the β-adrenergic receptor family genes, the *ADRB2* and the *ADRB3* are of particular interest. β2-adrenergic receptors (β2-ADRs) encoded by the *ADRB2* gene are the dominant lipolytic receptors in white adipose tissue and skeletal muscle (Hagstrom-Toft et al., 1998; Enoksson et al., 2000). β2-ADRs are also expressed throughout the smooth muscles of the cardiovascular and respiratory tracts and in the heart. Therefore, they play a pivotal role in the metabolic and musculoskeletal systems, promoting gluconeogenesis and glycogenolysis in the liver and skeletal muscles. They also influene insulin secretion and regulate energy expenditure through lipid mobilization from white adipose tissue (Brodde, 2008; Sarpeshkar and Bentley, 2010).

Gly16Arg (rs1042713,46G>A, G285A) and Glu27Gln (rs1042714, 79G>C, G318C) are the most common investigated polymorphisms of the *ADRB2* gene (Meirhaeghe et al., 2000; Petrone et al., 2006; Gjesing et al., 2009; Masuo and Lambert, 2011; Szendrei et al., 2016). Studies of agonist stimulation in cultured cells revealed that neither Gly16Arg nor Glu27Gln affected the function of the β2-ADRs in terms of ligand binding or adenylyl cyclase activity. However, transfected cells expressing the Gly16 variant of the receptor were shown to have greater reduction in numbers or

undergo significantly enhanced agonist-promoted downregulation when compared to Arg16. In contrast to Gly16, the Glu27 receptor form appears to be resistant to downregulation when compared to Gln27 variant (Green et al., 1994, 1995).

Numerous studies have investigated the impact of these polymorphic variants on changes in body composition, weight gain and obesity, as well as physical activity and athletic performance and conflicting results have been obtained (Ahmetov et al., 2016; Leońska-Duniec et al., 2016). In some studies it was found, that subjects carrying the Gly16 or Glu27 alone or both had increased risk of obesity (Large et al., 1997; Gonzalez Sanchez et al., 2003; Lange et al., 2005; Masuo et al., 2005*a*; Kawaguchi et al., 2006). Specifically, it was observed that Glu27 polymorphism interacts with physical activity influencing obesity risk among female subjects (Corbalan et al., 2002). Some research groups on the contrary, reported that the Gln27 is the risk allele (Meirhaeghe et al., 2000; Pereira et al., 2003). However, others found no relationship between Gly16Arg and Gln27Glu polymorphisms and obesity-related phenotypes (Echwald et al., 1998; Kortner et al., 1999; Bea et al., 2010; Gjesing et al., 2009; Rosado et al., 2015).

The β3-adrenergic receptors (β3-ADRs) that are encoded in human by *ADRB3* gene are mainly expressed in adipose tissue and differ from the β2-ADRs in terms of a lower affinity for catecholamines, and resistance to desensitisation and downregulation (Masuo and Lambert, 2011). These differences lead to the different effects of catecholamine on β3-ADRs - they exert their effects mainly by lipolysis in white adipose tissue and thermogenesis in brown adipose tissue (Hoffstedt et al., 1999; Collins et al., 2004; Kirstein and Insel, 2004). It seems that decreased function of β3-ADRs in white adipose tissue could slow the mobilization of lipids from the white

adipose tissue and, in consequence cause the retention of lipids in adipocytes. It could also affect thermogenesisin brown adipose tissue influencing body weight in humans. Within the *ADRB3* gene the Trp64Arg (rs4994, T387C) polymorphism exists in human population. It was shown that adipose cells carrying at least one mutated Arg64 allele exhibit 2/3-fold reduced ability to produce cyclic adenosine monophosphate (cAMP) and lypolitic glycerol when compared to Trp64 homozygotes (Pietri-Rouxel et al., 1997; Umekawa et al., 1999; Ahles and Engelhardt, 2014). These results suggest that the Arg64 allele carriers have less ability to stimulate adenylyl cyclase, and in consequence lipolytic activity through the β3-adrenergic might be suppressed. Many studies have confirmed increased BMI (average 0.28 kg/m^2) and body fat in carriers of the Arg64 allele (Ahles and Engelhardt, 2014; Leońska-Duniec et al., 2016). Polymorphic variants of the Trp64Arg are associated in many studies with abdominal obesity, weight gain, difficulty in losing weight, and lower resting metabolic rate as well as changes in body weight in response to exercise pointing at the Arg64 allele as risk allele or showed that the Trp64 allele is protective against obesity (Clement et al., 1995; Walston et al., 1995; Widen et al., 1995; Yoshida et al., 1995; Kim-Motoyama et al., 1997, Ukkola et al., 2000; Corella et al., 2001). In some cases no association was observed between the Trp64Arg polymorphism and obesity-phenotypes (Gagnon et al., 1996; Bea et al., 2010) or even contradictory results have been obtained (Phares et al., 2004).

As opposed to *ADRB* genes, there is less information about relationship between polymorphisms in *ADRA* genes and changes in body composition as well as weight gain and obesity-related phenotypes. However there are evidences that polymorphisms of the *ADRA2B* and the *ADRA2A* genes could be involved (Phares et al., 2004; Bea et al., 2010). Specifically, it was observed that the A allele of the G1780A (rs553668)

polymorphism localized in the 3'UTR of the *ADRA2A* gene is associated with obesity and type 2 diabetes, as well as body mass index (BMI) and percentage of body fat (Lima et al., 2007; Langberg et al., 2013).

Considering the aforementioned facts we have decided to check if body mass and body composition, as well as metabolic variables observed in physically active participants will be modulated by the *ADRB2*, *ADRB3* and *ADRA2A* gene polymorphisms. To test this hypothesis, we have performed a genetic association study that aimed to detect a correlation between the Gly16Arg and Glu27Gln of the *ADRB2* gene, Trp64Arg of the *ADRB3* gene as well as G1780A of the *ADRA2A* gene polymorphisms and selected body composition measurements as well as obesity-related metabolic traits in response to a 12-week aerobic training program.

Materials and Methods

Ethics Statement

All the procedures followed in the study were approved by the Ethics Committee of the Regional Medical Chamber in Szczecin (Approval number 09/KB/IV/2011) and were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. The experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the Strengthening the Reporting of Genetic Association studies (STREGA) Statement (Little et al., 2009).

Participants

One hundred and sixty three Polish European Caucasian women mean age 21 ± 1 years (range 19–24) met the inclusion criteria and were included in the study. None of these individuals had engaged in regular physical activity in the previous 6 months. They had no history of any metabolic, cardiovascular diseases or previous musculoskeletal injuries. Participants were nonsmokers and refrained from taking any medications or supplements known to affect metabolism. Prior to the start of the training phase, participants were included into a dietary program and on the basis of individual dietary plan they were asked to keep a balanced diet, of approximately 2000 kcal a day. The participants were asked to keep a food diary every day. Weekly consultations were held on which the quality and quantity of meals were analyzed and, if necessary, minor adjustments were made.

Physical exercise training protocol

The training stage was preceded by a week-long familiarization stage, when the examined women exercised 3 times a week for 30 min, at an intensity of about 50% of their maximum heart rate (HR_{max}) (Zarębska et al., 2016). After the week-long familiarization stage, the main training started. Each training unit consisted of a warm-up routine (10 min), the main aerobic routine (43 min), and cool-down phase (stretching and breathing exercise for 7 min). The main aerobic routine was a combination of 2 alternating styles – low and high impact. Music of variable rhythm intensity (tempo) was incorporated into both styles. A 12-week program of low-high impact aerobics was divided as follows: (i) 3 weeks (9 training units), 60 min each, at about 50%–60% of HR_{max}, tempo 135–140 beats per minute (BPM), (ii) 3 weeks (9 training units), 60 min

each, at 60%–70% of HR_{max}, tempo 140–152 BPM, (iii) 3 weeks (9 training units), 60 min with the intensity of 65%−75% of HR_{max}, tempo 145–158 BPM, and (iv) 3 weeks (9 training units), 60 min with an intensity of 65%−80% of HR_{max}, tempo 145–160 BPM. All 36 training units were administered and supervised by the same instructor.

Body Composition Measurements

All participants were measured for selected body mass and body composition variables before and after the completion of a 12-week training period. Body composition tests took place after fasting for at least 8 hours. Body mass and body composition were assessed with the bioimpedance method using a Tanita TBF 300M electronic scale (Arlington Heights, Illinois, USA). Body mass and body composition measurements taken with the use of the Tanita electronic scale are as follows: total body mass (kg), fat free mass (FFM, kg), fat mass (kg), fat mass percentage (FM, %), BMI (kg/m^2), tissue impedance (Ohm), total body water (TBW, kg) and basal metabolic rate (BMR, kJ or kcal).

Biochemical and Hematological Analyses

Fasting blood samples were obtained in the morning from the elbow vein. Blood samples from each participant were collected in 2 tubes. Biochemical and hematological analyses were performed before the start of the aerobic fitness training programme and repeated at the 12th week of this training programme (after the 36th training unit). The analyses were performed immediately after the blood collection. Complete blood count, including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin

(MCH), mean corpuscular hemoglobin concentration (MCHC), and total platelet level (PLT) were obtained using Sysmex K-4500 Haematology Analyzer (TOA SYSMEX, Kobe, Japan). All biochemical analyses were conducted using Random Access Automatic Biochemical Analyzer for Clinical Chemistry and Turbidimetry A15 (BIO-SYSTEMS S.A., Barcelona, Spain). Blood plasma was used to determine lipid profile: triglycerides (Tg), cholesterol (Chol), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations. Plasma Tg and Chol concentrations were determined using diagnostic colorimetric enzymatic method according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). HDL plasma concentration was determined using human anti-ß-lipoprotein antibody and colorimetric enzymatic method according to the manufacturer's protocol (BioMaxima S.A.). Plasma concentrations of LDL were determined using a direct method according to the manufacturer's protocol (PZ Cormay S.A., Lomianki, Poland). All analysis procedures were verified with the use of multiparameteric control serum (BIOLABO S.A.S, Maizy, France), as well as control serum of normal level (BioNormL) and high level (BioPathL) lipid profiles (BioMaxima S.A.).

VO_{2max} measurement

Subjects performed a continuous graded exercise test on an electronically braked cycle ergometer (VIAsprint™ 150P Bicycle, CareFusion Germany GmbH, Hoechberg, Germany) with an automatically calibrated volume sensor and a breath-by-breath gas analyzer (Oxycon Pro, Erich JAEGER GmbH, Hoechberg, Germany) to determine their maximal oxygen uptake (VO_{2max}) before and after the completion of a 12-week training period. The device was calibrated in accordance with the manufacturer's instructions.

The test began by 5 min continuous pedaling, with a frequency of 60 revolutions per minute (RPM) and a relative load of 1.2 W/kg. After this phase, the workload was systematically increased by 15 watts every minute until voluntary exhaustion. The effort was interrupted when pedaling frequency declined by 10%, that is, when the pedalling frequency fell below 54 RPM. All of the participants reached RER greater than 1.0. The highest value of oxygen uptake was considered to be VO_{2max}.

Genetic Analyses

The buccal cells donated by the subjects were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit; Sigma, Steinheim, Germany) with the use of sterile foam-tipped applicators (Puritan, Guilford, Maine, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) according to the manufacturer's protocol. Obtained concentration of genomic DNA was 30–50 ng/1 μl. All samples were genotyped in duplicate using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Carlsbad, California, USA) on CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). Freshly purified/sterile water was used as a negative control for PCR. To discriminate the *ADRB2* Gly16Arg and Glu27Gln, *ADRB3* Trp64Arg as well as *ADRA2A* G1780A alleles, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C___2084764_20, C___2084765_20, C___2215549_20 and C____996424_20, respectively), including appropriate primers and fluorescently labelled (FAM and VIC) MGB™ probes to detect the alleles.

Statistical Analyses

Hardy-Weinberg equilibrium was tested by comparing the observed genotype frequencies with the expected ones using the Chi-square test with one degree of freedom in Microsoft EXCEL. Training responses were expressed as percentage change from baseline. The percentage changes were compared across genotypes using either parametric (t-test or one-way ANOVA) or non-parametric (Mann-Whitney or Kruskal-Wallis) tests for normally and non-normally distributed data, respectively. Spearman's (non-parametric) correlations were used to assess the relationships between different phenotypes. Differences in phenotypes between groups were analysed using unpaired *t* tests. Power was calculated using non-central chi-square distribution (pchisq function in R, https://cran-r.projec.org) assuming alpha 0.05 and a variance explained of 0.01, 0.05, and 0.10 by additive effects at the marker of interest. The power for a stated sample size (n=163) was 24.8%, 81.5% and 98.1% for variances of 0.01, 0.05 and 0.10, respectively, Normality of the distribution was evaluated using the Kolmogorov-Smirnov test. The association of *ADRB2* haplotypes with training responses was analysed using haplo.stats package for R . The regression of percentage change of body composition parameters, lipids and glucose on *ADRB2* haplotypes was conducted using haplo.glm function assuming the additive model and minimum haplotype frequency of 5%.

Power for a stated sample size (n=163) was calculated for a given set of haplotypes, their population frequencies and a specified genetic effect size (additive model of haplotype effects) in terms of a regression model R squared value (a haplo.power.qt function of the haplo.stats package). The power for the R squared values 1%, 5% and 10% was 18.9%, 74.1% and 97.3%, respectively. Linear regression coefficients corresponding to R squared 1%, 5% and 10% were 0.14, 0.32, 0.45, respectively

Gene-gene interactions among *ADRB2*, *ADRB3* and *ADRA2A* polymorphisms were analysed using non-parametric model-free method of reducing genotype combinations called multifactor dimensionality reduction (MDR) using he MDR software package (version 3.0.2, http://sourceforge.net) (Ritchie et al., 2001; Gui et al., 2013). The 10-fold cross-validation training scores, cross-validation testing scores (the t-test statistic for the unequal variance computed by comparing phenotype between high- and low-level genotypes for the ten pooled testing sets), as well as cross validation consistency (CVC, the number of times the same model was chosen in the training set) were determined. P values for interactions were calculated using permutation tests. Single-locus analyses were carried out in STATISTICA data analysis software system, version 12 (StatSoft, Inc. 2014, www.statsoft.com).

Results

Individual variability in the change of relative fat mass and BMI is shown in Figures 1-2. Only 74.8% of participants could lose their relative fat mass in response to a 12-week aerobic training program, which was not dependent on their initial relative fat mass (p=0.744) or BMI (p=0.988). On average, participants lost 5.8 (10.4)% of their relative fat mass with training (range: +28.3 to -63.6%). The improvement of VO_{2max} was significantly greater in women who could lose their fat mass compared to women who were unsuccessful in fat loss (4.5 (5.6)% vs. 1.5 (3.8)%; P = 0.0045). The efficiency of fat loss was inversely correlated with the improvement of VO_{2max} in response to a 12-week aerobic training (r = – 0.37; P < 0.0001).

FIGURES 1-2

All investigated polymorphisms conformed to Hardy-Weinberg expectations (χ^2=0.92, p=0.337; χ^2=2.36, p=0.124; χ^2=2.31, p=0.129; χ^2=0.17, p=0.680, for Gly16Arg, Glu27Gln of the *ADRB2*, Trp64Arg of the *ADRB3* and G1780A of the *ADRA2A*, respectively. Owing to the low number of the *ADRB3* Arg64/Arg64 (n=3) and *ADRA2A* AA (n=2) homozygotes, they were pooled together with corresponding heterozygous genotypes. There were no differences in percentage changes across genotypes for any of the analysed polymorphisms (Tables 1-4). However, polygenic analysis has shown that the carriers of a low number (0-3) of obesity-related risk alleles (*ADRB2* Gly16, *ADRB2* Glu27, *ADRA2A* rs553668 G) were more successful in fat mass loss compared to the carriers of a high number (5-6) of risk alleles (7.7 (9.8)% vs 4.0 (9.4)%, *P*=0.0362).

TABLE 1

TABLE 2

TABLE 3

TABLE 4

Three haplotypes (Gly16;Glu27, 45.1%; Arg16; Gln27, 34.4%; Gly16; Gln27, 20.6%) were reconstructed. The most prevalent *ADRB2* haplotype was Gly16;Glu27 (45.1%) and it was used as a reference in haplotype-based association analysis. The Arg16; Gln27 haplotype was associated with significantly smaller percentage change of BMI (-0.57 per copy of haplotype, p=0.036) compared with reference Gly16;Glu27 haplotype (Table 5). Additionally, a two-way *ADRB3* x *ADRA2A* interaction was detected for HDL percentage change (cross-validation consistency 10/10, cross-validation testing score 3.67, p=0.018**,** Table 6). The double heterozygotes (*ADRB3* Trp64Arg64/*ADRA2A* AG) and double homozygotes (*ADRB3* Trp64Trp64/*ADRA2A*

GG) exhibited a decrease while single heterozygotes (*ADRB3* Trp64Arg64/*ADRA2A* GG and *ADRB3* Trp64Trp64/*ADRA2A* AG) showed an increase in HDL serum concentration in response to training (Figure 3).

TABLE 5

TABLE 6

FIGURE 3

Discussion

Our genetic association study was designed to test whether variation in the *ADRB2*, *ADRB3* and *ADRA2A* genes can modulate changes in selected body mass, body composition and metabolic variables following 12 weeks of supervised aerobic exercise training in women. Despite the fact that there were no differences in percentage changes across genotypes for any of the analysed polymorphisms of the *ADRB2* (Gly16Arg, Glu27Gln), *ADRB3* (Trp64Arg) and *ADRA2A* (G1780A), the polygenic analysis has shown that the carriers of a low number (0-3) of obesity-related risk alleles (*ADRB2* Gly16, *ADRB2* Glu27, *ADRA2A* rs553668 G) were more successful in fat mass loss compared to the carriers of a high number (5-6) of risk alleles (7.7 (9.8)% vs 4.0 (9.4)%, P=0.0362). Moreover, *ADRB3*x*ADRA2A* interaction was detected for HDL percentage change. Therefore the presented results support the assumption that, i) genetic variation contributes to interindividual changes of selected body mass, body composition and metabolic variables in response to physical exercise as well as that ii) complex interaction of multiple genetic polymorphisms rather than an individual effect of a

single polymorphic site have an influence on individual variation in responsiveness to exercise training (Jensen et al., 2009; Rankinen and Bouchard, 2012).

The ADRs gene family members have been extensively studied in the obesity field because of their participation in the regulation of energy expenditure (Ochoa et al., 2004; Marti et al., 2008). Particularly, the role of the lipolytic receptors genes, *ADRB2,* with its Gly16Arg and Glu27Gln polymorphisms, alone or in haplotype combination, in weight gain, obesity and changes in body composition have been investigated by many scientists. It has been shown that the Gly16 allele may influence the propensity to higher BMI, because the Gly16 allele is associated with lower receptor density, and in consequence reduced efficiency, when compared to Arg16 allele (Chou et al., 2012). A higher frequency of the Gly16 allele in men resistant to weight loss and those who regained body weight after successful initial weight loss at 6 months was noticed in a study of overweight men who participated in a 24-month weight loss programme consisting of a low-calorie diet and everyday aerobic exercise (Masuo et al., 2005*b*). Numerous studies have also focused on the second polymorphic site in the *ADRB2* gene. Some studies showed that the Glu27 allele may limit *ADRB2* downregulation and thus affect body weight (Lange et al., 2005; Kawaguchi et al., 2006). Corbalan et al. (2002) reported that women who were more active during their free time and were carriers of the Glu27 allele had higher body weight compared to non-carriers, suggesting that these women may be more resistant to losing weight.

In contrast, the study by Phares et al. (2004) and Szendrei et al. (2016) showed that Glu27 carriers had a tendency for a greater loss of percent total body fat, greater weight and BMI reductions compared with noncarriers. What is more, the study of Bea et al. (2010) showed gene x exercise interactions for *ADRB2* Glu27Gln on change in

lean soft tissue (LST). There was a significant LST gain with exercise of the Glu27 allele carriers compared to loss among controls and no intervention effect of the Glu27 allele noncarriers (Bea et al., 2010).

In our study we have observed only a tendency of association of Gly16Arg and Glu27Gln alone with changes of selected body mass and body composition variables. However, we found that the *ADRB2* risk alleles (Gly16 and Glu27) in combination with another risk allele of the *ADRA2A* gene (rs553668 G) were associated with significantly smaller change of fat mass following 12 weeks of supervised aerobic exercise training in women. The results of Jensen et al. (2009) were focused on analyzing the haplotype structure of the *ADRB2* gene in Danish Caucasian subjects and association with BMI. The investigation clearly suggested that when multiple SNPs from a single gene were analyzed, unique interactions in specific haplotype pairs rather than individual SNPs may affect BMI.

Because the main role of catecholamines in human fat cells depends on the balance between lipolytic ADRB and antilipolytic ADRA receptors activities, gene-gene interactions in genes involved in the reciprocal regulation of lipolysis is inevitable (Phares et al., 2004). Indeed, such polygenic interactions have been spotted by some researchers. It has been reported that interactive effect of the *ADRA2B* Glu12/Glu9 and the *ADRB3* Trp64Arg polymorphisms on obesity-related phenotypes in healthy white women exist (Dionne et al., 2001). It is worth noting that when the Glu12/Glu9 *ADRA2B* polymorphism did not associate with obesity-related phenotypes alone, subjects that carried the Arg64 *ADRB3* and Glu9 *ADRA2B* variants had 9.3 kg greater fat mass and 4.8% greater percent body fat compared with subjects carrying only the Arg64 *ADRB3* variant. Phares et al. (2004) found that the combined effects of the

Glu12/Glu9, *ADRA2B*, Trp64Arg *ADRB3*, and Gln27Glu *ADRB2* gene polymorphisms and their gene-gene interactions contribute significantly to explaining interindividual variability in body fat responses to exercise training. However, *ADRA2B* and *ADRB3* interaction was the most significant source of variation for change in total body fat, trunk fat, and fat mass.

In the current study, we also observed interaction between ADRs genes; specifically, *ADRB3*x*ADRA2A* interaction was detected for HDL percentage change. Our study showed that only single heterozygotes (*ADRB3* Trp64Arg64/*ADRA2A* GG and *ADRB3* Trp64Trp64/*ADRA2A* AG) had an increase in HDL serum concentration in response to training; the double heterozygotes (*ADRB3* Trp64Arg64/*ADRA2A* AG) and double homozygotes (*ADRB3* Trp64Trp64/*ADRA2A* GG) exhibited a decrease in HDL serum concentration after completion of 12 weeks of supervised aerobic exercise training.

It is widely accepted that regular aerobic exercise increases HDL-Chol. It was showed that beneficial adaptations in lipoprotein profile is achieved with moderate training intensities below the anaerobic threshold and training above the anaerobic threshold has no or even negative effects on blood lipoprotein profiles (Aellen et al., 1993; Drygas et al., 2000). Meta-anlysis by Kodama et al. (2007) showed that the minimum aerobic exercise volume for an increase in HDL level exist - minimal weekly exercise volume for HDL level increase was 900 kcal of energy expenditure or 120 minutes of exercise per week (Kodama et al., 2007). The intensity of our 12-week exercise program gradually increased from 50-60% HR_{max} to 65%−80% of HR_{max} in the last 3 weeks of the training programme and the weekly exercise volume was 180 minutes. Each training unit consisted of a warm-up, the main aerobic routine which was

a combination of 2 alternating styles – low and high impact, and cool-down phase. Despite that, low-high impact aerobics in general refer to cardio with moderate training intensities zones, one may speculate that low- and high-impact workouts are at least partly similar to interval training with the high-intensity periods that are typically at or close to anaerobic exercise, while the recovery periods involve activity of lower intensity.

With respect to our findings, there is a reason for us to hypothesise that subjects with combination of *ADRB 3*Arg64 / *ADRA2A* G and *ADRB3* Trp64 / *ADRA2A* A rather than *ADRB3* Trp64 / *ADRA2A* G respond better to our physical exercise program in terms of larger increase in HDL. It seems that duration, intensity as well as exercise frequency of our physical exercise programme were appropraite stimuli for *ADRB3* Arg64 / *ADRA2A* G and *ADRB3* Trp64 / *ADRA2A* A carriers to increase the HDL level. On the other hand, the *ADRB3* Trp64 / *ADRA2A* G rather than *ADRB3* Arg64 / *ADRA2A* A carriers respond with lowered HDL serum concentration in response to our 12 weeks of supervised aerobic exercise training. Therefore, there is a reason to hypothesise that low- and high- aerobic exercise training is not suitable for *ADRB3* Trp64 / *ADRA2A* G carriers in terms of lower HDL levels, and the observed effect can be explained by an increase in energy consumption and achieving an ‘energy expenditure threshold’ during physical effort (Gibala and McGee, 2008; Kostrzewa-Nowak et al., 2015). It is also, higly probable, that other, rhythmic and repeated, aerobic exercises with moderate training intensities below the anaerobic threshold such as bicycling, jogging, or swimming would be more appropriate for this group of participants.

In summary, our findings suggest that the carriers of a low number of obesity-related risk alleles were more successful in fat mass loss compared to the carriers of a high number of risk alleles, as well as *ADRB3*x*ADRA2A* gene interaction modifies the effects of aerobic exercise training in women on HDL levels. However, when we consider all aforementioned facts, the impact of genetic markers on determination of obesity-related traits is still unclear. Thus, the true level and the nature of the genotype x physical activity interactions in the field of obesity-related traits deserves to be further investigated. One of the possibile ways is using a composite score of genetic markers that have been identified in GWAS as an obesity risk SNPs in the gene × physical activity interaction analyses (Li et al., 2010). However, only a comprehensive understanding of the underlying genetic and epigenetic mechanisms will enable us to uncover the "missing heritability" of the obesity-related traits (Herrera et al., 2011, Rankinen and Bouchard, 2012).

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AUTHOR BIOGRAPHY

Agata LEOŃSKA-DUNIEC

Employment

Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Genetics, Genomics

E-mail: leonska.duniec@gmail.com

Zbigniew JASTRZĘBSKI

Employment

Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Sport Exercise, Physiology, Genetics

E-mail: zb.jastrzebski@op.pl

Aleksandra JAŻDZEWSKA

Employment

Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Sport Exercise, Physiology, Genetics

E-mail: olazarebska@o2.pl

Waldemar MOSKA

Employment

Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Sport Exercise, Sport Managements

E-mail: waldemarmoska@wp.pl

Ewelina LILIŃSKA-KUKLIK

Employment

Faculty of Physical Education, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Traumatology, Physical Exercise, Physiology

E-mail: e.lulinska-kuklik@osw2wejherowo.pl

Marek SAWCZUK

Employment

Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Genetics, Genomics

E-mail: sawczuk_marek@wp.pl

Svetlana I. GUBAYDULLINA

Employment

Sport Technology Research Centre, Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russia

Degree

MS

Research interests

Anthropology, nutrition, genetics

E-mail: gubajdullina_svetlana@mail.ru

Alsu T. SHAKIROVA

Employment

Department of Propaedeutics of Childhood Diseases, Kazan State Medical University, Kazan, Russia

Degree

PhD, MD

Research interests

Nutrition, Genetics

E-mail: a.t.shakirova@mail.ru

Paweł CIĘSZCZYK

Employment

Faculty of Physical Education, Gdansk University of Physical Education and Sport, Gdansk, Poland

Degree

PhD, MD

Research interests

Genetics, Genomics

E-mail: cieszczyk@poczta.onet.pl

Adam MASZCZYK

Employment

Department of Theory and Practice of Sport; Academy of Physical Education in Katowice; Poland.

Degree

PhD, MD

Research interests

Statistics, Physical Exercise, Physiology

E-mail: a.maszczyk@awf.katowice.pl

Ildus I. AHMETOV

Employment

Head of the Laboratory of Molecular Genetics, Kazan State Medical University, Kazan, Russia.

Degree

Dr. Med. Sci., PhD, MD

Research interests

Exercise and sports genomics

E-mail: genoterra@mail.ru

Table 1. The *ADRB2* Gly16Arg genotypes and response to training

Percentage change from baseline	Gly16/Gly16 (n=73)	Gly16/Arg16 (n=68)	Arg16/Arg16 (n=22)	p
body mass (kg)	-0.86±2.52	-1.42±2.51	-1.67±2.16	0.259
BMI	-0.54±2.25	-1.40±2.42	-1.48±1.74	0.051
BMR	-0.33 (-0.82, 0.19)	-0.43 (-0.82, 0.0)	-0.55 (-0.79, -0.13)	0.214†
%FM	-3.80 (-7.14, 2.73)	-5.45 (-9.53, -1.77)	-6.94 (-10.98, -0.29)	0.088†
FFM	0.63 (-0.65, 1.36)	1.07 (-0.33, 2.29)	1.27 (-0.70, 2.06)	0.524†
TBW	0.61 (-0.58, 1.67)	0.77 (-0.92, 2.35)	1.51 (-0.63, 2.24)	0.813†
TC	0.10±12.30	-0.64±11.67	0.54±14.67	0.904
TGL	10.06±40.55	8.25±35.40	20.73±40.06	0.410
HDL	-4.58±17.06	-5.40±18.41	-5.29±15.92	0.959
LDL	4.89±20.89	4.06±20.54	3.20±22.59	0.938
glucose	-4.55 (-11.25, 2.86)	-2.74 (-9.97, 4.98)	-0.69 (-6.10, 2.50)	0.512†

Mean ± SD or median with interquartile range (in brackets), † Kruskal-Wallis test; BMI – body mass index; BMR – basal metabolic rate; %FM – fat mass percentage; FFM – fat free mass; TBW – total body water; TC – total cholesterol; TGL – triglycerides; HDL – high density lipoprotein, LDL –low density lipoprotein

Table 2. The *ADRB2* Glu27Gln genotypes and response to training

Percentage change from baseline	Glu27/Glu27 (n=38)	Glu27/Gln27 (n=71)	Gln27/Gln27 (n=54)	p
body mass, kg	-0.98±2.85	-1.10±2.38	-1.50±2.32	0.545
BMI	-0.59±2.32	-1.03±2.44	-1.33±2.05	0.310
BMR	-0.24 (-0.99, 0.20)	-0.42 (-0.83, 0.07)	-0.47 (-0.79, -0.12)	0.450†
%FM	-3.31 (-7.04, 0.98)	-4.33 (-8.33, -0.82)	-6.09 (-10.71, -0.29)	0.412†
FFM	0.77 (-0.42, 1.93)	0.64 (-0.88, 1.92)	1.15 (-0.69, 2.18)	0.955†
TBW	0.60 (-0.31, 1.73)	0.67 (-0.90, 2.06)	1.19 (-0.63, 2.29)	0.967†
TC	-1.35±11.02	1.94±12.49	-2.05±12.75	0.159
TGL	3.69±35.67	14.07±40.31	11.34±37.67	0.404
HDL	-4.11±16.60	-6.35±19.13	-3.90±15.65	0.693
LDL	3.71±18.83	7.48±21.30	0.59±21.33	0.184
glucose	-5.26 (-11.27, 3.95)	-2.70 (-9.64, 4.35)	-2.62 (-9.41, 3.23)	0.787†

Mean ± SD or median with interquartile range (in brackets), † Kruskal-Wallis test; BMI – body mass index; BMR – basal metabolic rate; %FM – fat mass percentage; FFM – fat free mass; TBW – total body water; TC – total cholesterol; TGL – triglycerides; HDL – high density lipoprotein, LDL –low density lipoprotein

Table 3. The *ADRB3* Trp64Arg genotypes and response to training

Percentage change from baseline	Trp64/Trp64 (n=136)	Arg64/Arg64+Trp64/Arg64 (n=27)	p
body mass, kg	-1.14±2.45	-1.56±2.62	0.416
BMI	-0.99±2.35	-1.23±2.02	0.620
BMR	-0.42 (-0.83, 0.04)	-0.39 (-0.80, 0.23)	0.551†
%FM	-4.34 (-9.03, 0.0)	-5.08 (-7.04, -0.82)	0.986†
FFM	0.65 (-0.67, 2.13)	1.08 (-0.44, 1.71)	0.915†
TBW	0.74 (-0.63, 2.32)	1.11 (-0.60, 1.67)	0.787†
TC	0.08±12.51	-1.28±11.44	0.603
TGL	11.46±39.36	7.14±33.44	0.594
HDL	-6.10±17.58	0.44±15.61	0.074
LDL	5.13±21.10	0.21±19.40	0.263
glucose	-2.84 (-9.61, 3.82)	-3.95 (-11.25, 4.29)	0.844†

Mean ± SD or median with interquartile range (in brackets), † Mann-Whitney test ; BMI – body mass index; BMR – basal metabolic rate; %FM – fat mass percentage; FFM – fat free mass; TBW – total body water; TC – total cholesterol; TGL – triglycerides; HDL – high density lipoprotein, LDL –low density lipoprotein

Table 4. The *ADRA2A* G1780A genotypes and response to training

Percentage change from baseline	GG (n=124)	AA+AG (n=39)	p
body mass,kg	-1.18±2.45	-1.30±2.59	0.796
BMI	-1.10±2.40	-0.80±1.92	0.470
BMR	-0.40 (-0.84, 0.08)	-0.48 (-0.79, 0.0)	0.782†
%FM	-4.44 (-8.65, -0.15)	-5.11 (-10.98, 0.0)	0.472†
FFM	0.64 (-0.57, 1.92)	1.08 (-0.93, 2.40)	0.547†
TBW	0.85 (-0.59, 2.11)	0.81 (-1.24, 2.29)	0.849†
TC	-0.51±12.10	0.99±13.08	0.509
TGL	11.08±38.44	9.67±38.68	0.841
HDL	-6.47±16.42	-0.39±19.71	0.057
LDL	4.33±20.64	4.27±21.79	0.987
glucose	-2.74 (-9.50, 4.32)	-3.95 (-11.59, 2.38)	0.610†

Mean ± SD or median with interquartile range (in brackets), † Mann-Whitney test ; BMI – body mass index; BMR – basal metabolic rate; %FM – fat mass percentage; FFM – fat free mass; TBW – total body water; TC – total cholesterol; TGL – triglycerides; HDL – high density lipoprotein, LDL –low density lipoprotein

Table 5. Regression of percentage change of body composition parameters, lipids and glucose on *ADRB2* haplotypes

Haplotype /intercept	body mass, kg	BMI	BMR	%FM	FFM	TBW	TC	TGL	HDL	LDL	glucose
Intercept	-0.93 (-2.67) p=0.008	-0.65 (-2.04) p=0.043	-0.28 (-1.15) p=0.251	-4.70 (-3.23) p=0.001	1.0 (2.50) p=0.013	1.01 (2.45) p=0.015	0.58 (0.36) p=0.738	7.18 (1.33) p=0.184	-5.39 (-2.21) p=0.029	6.54 (2.24) p=0.027	-2.06 (-1.03) p=0.306
[Arg16; Gln27]	-0.43 (-1.49) p=0.138	-0.57 (-2.11) p=0.036	-0.36 (-1.71) p=0.088	-1.86 (-1.51) p=0.132	0.12 (0.35) p=0.723	0.001 (0.005) p=0.996	-0.33 (-0.23) p=0.821	3.92 (0.86) p=0.390	-0.18 (-0.09) p=0.929	-1.53 (-0.62) p=0.537	1.19 (0.70) p=0.485
[Gly16; Gln27]	0.05 (0.13) p=0.893	0.03 (0.08) p=0.937	-0.16 (-0.60) p=0.548	0.37 (0.24) p=0.814	-0.23 (-0.53) p=0.595	-0.04 (-0.10) p=0.920	-1.22 (-0.66) p=0.509	2.12 (0.37) p=0.713	1.22 (0.47) p=0.640	-2.85 (-0.91) p=0.363	-2.34 (-1.09) p=0.277

Regression coefficients and t statistic (in brackets); minimum frequency for a haplotype to be included 5%; the most common haplotype [Gly16;Glu27] (45.1%) was the reference haplotype; BMI – body mass index; BMR – basal metabolic rate; %FM – fat mass percentage;

FFM – fat free mass; TBW – total body water; TC – total cholesterol; TGL – triglycerides; HDL – high density lipoprotein, LDL –low density lipoprotein

Table 6. Analysis of the two-way and three-way interactions between *ADRB2*, *ADRB3* and *ADRA2A* genes using quantitative multifactor dimensionality reduction for body composition parameters, lipids and glucose.

Parameter	Best model*	Cross-validation training score	Cross-validation testing score	CVC*	p†
body mass (kg)	Gly16Arg	1.57	0.61	9/10	0.557
BMI (kg/m^2)	Gly16Arg	2.35	1.06	9/10	0.435
%FM (%)	Gly16Arg,G1780A	2.45	-0.75	6/10	0.875
FFM (kg)	G1780A	1.18	-2.29	6/10	0.995
TBW (kg)	Gly16Arg, Glu27Gln,Trp64Arg	2.20	-1.86	6/10	0.986
TC (mg/dL)	Glu27Gln	1.81	1.91	10/10	0.213
TGL (mg/dL)	Glu27Gln	2.11	-1.56	6/10	0.969

HDL (mg/dL)	Trp64Arg, G1780A	3.69	3.67	10/10	0.018
LDL (mg/dL)	Glu27Gln	1.76	0.55	7/10	0.557
Glucose (mg/dL)	Gly16Arg,Glu27Gln, Trp64Arg	2.45	-1.25	9/10	0.960

* the best gene-gene interaction model was determined using cross-validation consistency (CVC) and cross-validation testing score; † permuted p value (1000 permutations)

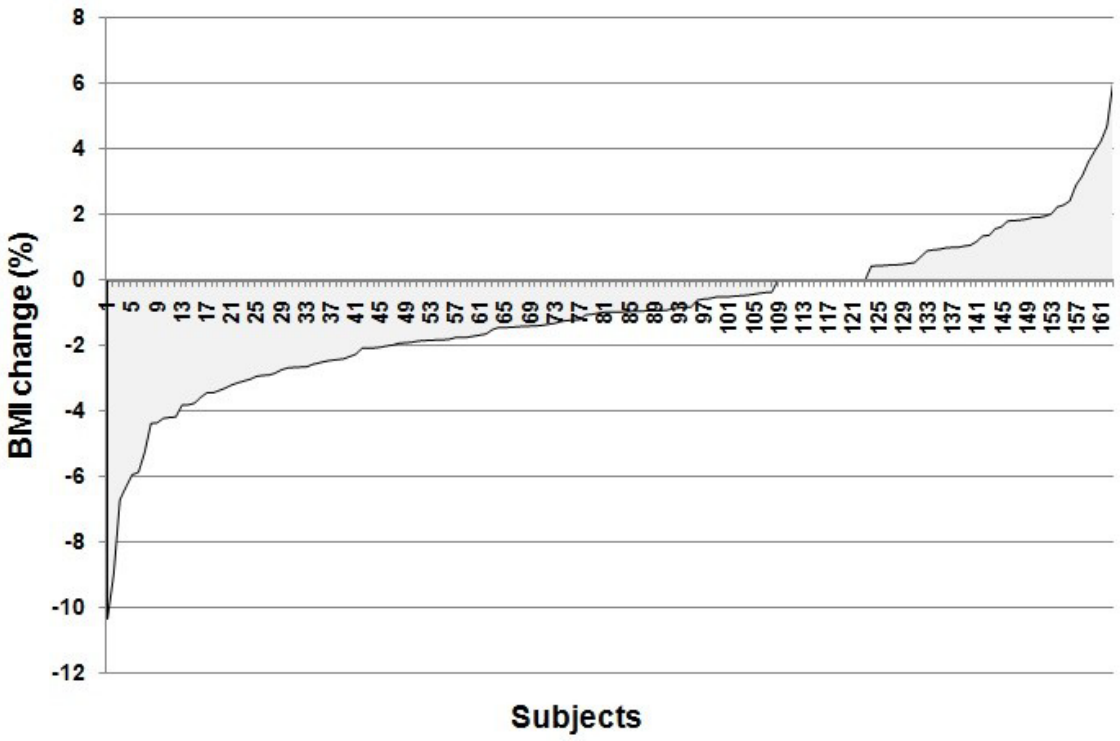
BMI change (%)
8
6
4
2
0
-2
-4
-6
-8
-10
-12
1
5
9
13
17
21
25
29
33
37
41
45
49
53
57
61
65
69
73
77
81
85
89
93
97
101
105
109
113
117
121
125
129
133
137
141
145
149
153
157
161
Subjects

Figure 1.

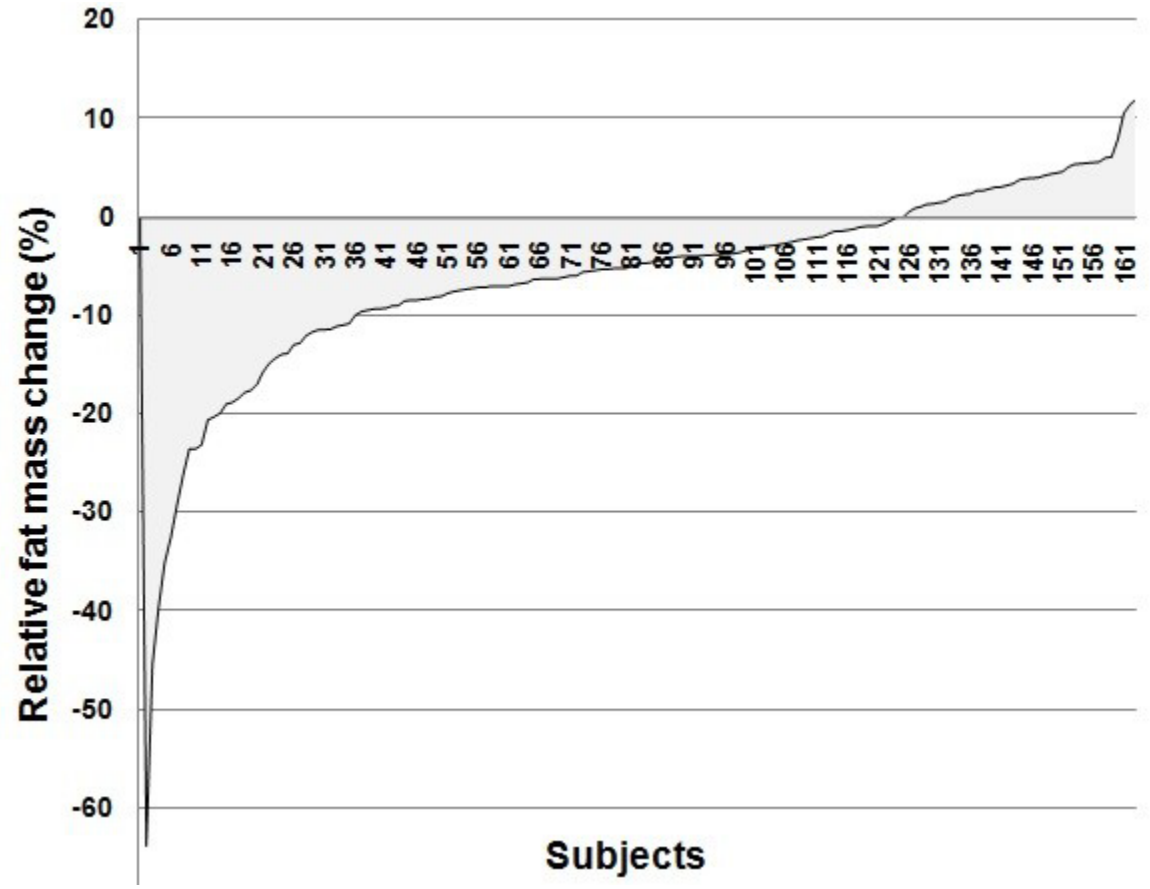
20
10
0
-10
-20
-30
-40
-50
-60
Relative fat mass change (%)
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
101
106
111
116
121
126
131
136
141
146
151
156
161
Subjects

Figure 2

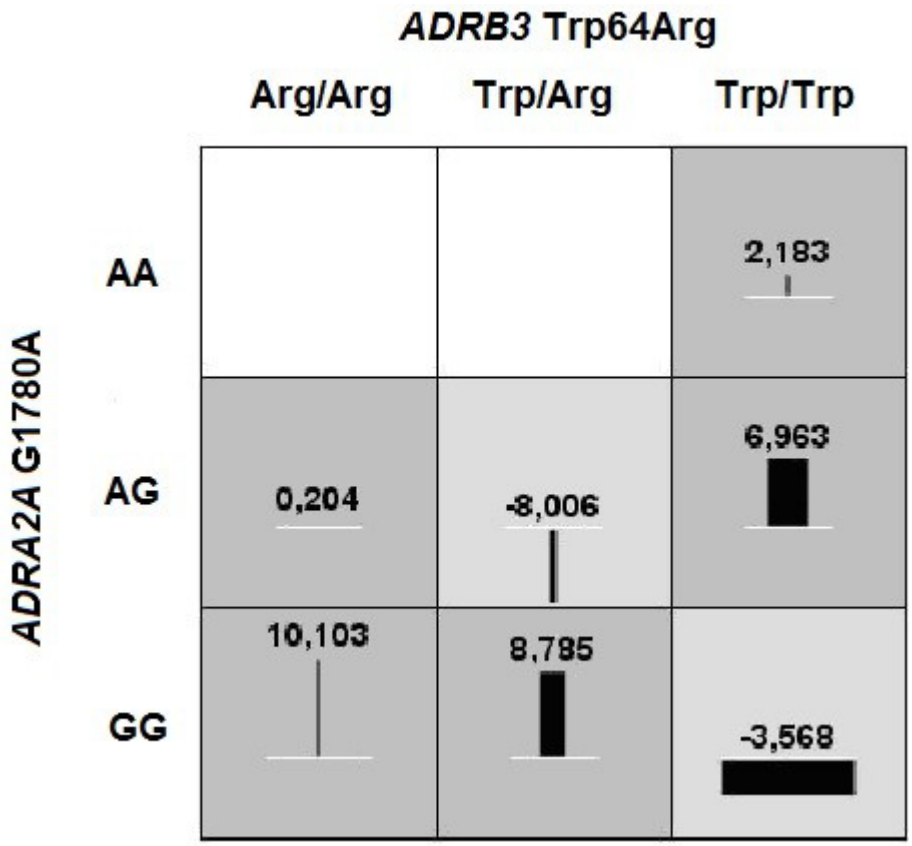
ADRB3 Trp64Arg
Arg/Arg
Trp/Arg
Trp/Trp
ADRA2A G1780A
AA
AG
GG
2,183
0,204
-8,006
6,963
10,103
8,785
-3,568

Figure 3.