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Basal fat oxidation and after a peak oxygen consumption test in obese women with a β2 adrenoceptor gene polymorphism

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Abstract

The Glu27Glu genotype in the β2-adrenergic receptor (ADRB2) has been linked to a higher fat deposition and obesity in females. Also, in our population, it has been described that physically active women carrying the Glu allele had a higher BMI as compared to non-carriers performing the same level of activity. Since exercise may counterbalance a gene predisposition to obesity, we tested the hypothesis of a potential different metabolic response among ADRB2 Gln27Gln versus Glu27Glu obese women when submitted to a peak oxygen consumption test on a treadmill. In our study, 10 obese women with the Gln27Gln genotype were compared to 9 matched obese women bearing the Glu27Glu genotype. The ADRB2 polymorphism was identified by PCR-RFLP, fat oxidation was determined by indirect calorimetry and blood measurements were carried out following conventional procedures. The ADRB2 Glu27Glu subjects had lower plasma glycerol levels ($P = 0.026$), while plasma triglycerides ($P < 0.001$) and the insulin:glucose ratio were higher ($P = 0.046$) as compared to the Gln27Gln group along the peak oxygen consumption trial intervention. There was a significantly lower fat oxidation ($P = 0.024$) in the Glu27Glu obese women during the recovery compared to Gln27Gln obese individuals. These data suggest that exercise would not benefit equally the two ADRB2 polymorphism homozygous groups, since both lipolysis and fat oxidation promoted by a peak oxygen consumption test appear to be blunted in the polymorphic Glu27Glu obese group.

Keywords: Beta2 adrenoceptor; Gln27Glu polymorphism; Obesity; VO2max; Women

1. Introduction

The hydrolysis of triglyceride stores (lipolysis) releases non-esterified fatty acids (NEFAs) and glycerol from adipose tissue, which is a key step in the metabolic process leading to a decrease on body fat mass. In white human adipose tissue, the β2-adrenoceptor is the dominating lipolytic receptor [1], but β2-adrenoceptor-mediated increases in thermogenesis and lipid utilization may be altered in the obese [2].

Different polymorphisms in the promoter and the coding region for the β2-adrenoceptor gene have been described [3]. When some of these polymorphisms are present in recombinant cells, the function of the β2-adrenergic receptor (ADRB2) is markedly impaired [4,5].

Available evidence supports that Gln27Glu polymorphism in the ADRB2 is associated with higher BMI, fat mass and larger fat cells [6,7]. Indeed, the Glu27 allele has been linked to obesity in several male and female adult populations [8-16]. Moreover, in a French population, it has been reported that Gln27Glu polymorphism is strongly associated with obesity in sedentary patients, but physical activity might counterbalance the effect of the Gln27Glu genetic predisposition to increase body weight, body fat, and obesity [13]. Furthermore, a strong dissociation was recently found between Glu27Glu genotype and elite endurance runners in postmenopausal women [7]. Also, in our Spanish population, physically active women carrying the Glu27 allele had a higher BMI as compared to non-carriers performing the same level of activity [17].

Given that physical activity seems to be involved on the Gln27Glu polymorphism-obesity association, Gln27Gln obese women were matched to other Glu27Glu and all of

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Table 1
Anthropometric and fitness variables of subjects participating in the VO2max test

Variable	Glu27Glu (n=9)	Gln27Gln (n=10)	Statistical Analysis	P
Age (y)	42 (4)	43 (3)	T	0.817
Weight (m)	80.4 (2.4)	86.8 (3.2)	U	0.182
BMI (kg/m2)	31.8 (1.1)	34.4 (1.1)	T	0.127
Fat mass (kg)	34.4 (1.5)	37.8 (2.6)	U	0.549
Waist (cm)	95.9 (2.1)	97.0 (4.5)	T	0.831
VO2max (ml/kg/min)	23.5 (1.7)	21.2 (0.6)	U	0.842
Maximal HR (beat/min)	170 (5)	168 (3)	T	0.740
Maximal RQ	1.34 (0.05)	1.31 (0.02)	U	0.720

Values are means (SEM). BMI = Body mass index, VO2max = peak oxygen consumption. T = Students's t-test. U = Mann Whitney's U-test.

them performed a peak oxygen consumption test in order to detect potentially different metabolic responses that could explain the possible interaction of exercise on a genetic influence to develop obesity.

2. Methods

2.1. Subjects

In our study, 9 Glu27Glu obese women were compared to 10 Gln27Gln obese women, matched by age, weight, BMI, percentage of body fat mass and waist circumference (Table 1). These groups were selected after having genotyped 159 obese subjects (BMI >30 kg/m, age 20-60) from the Hospital of Navarra, Spain. Diabetes mellitus, hypothyroidism, hepatic or renal dysfunction, hypertension (systolic pressure over 160 mm Hg and/or diastolic pressure over 90 mm Hg, measured in two different occasions), cardiovascular diseases, asthma, bronchitis, EPOC, bone or joint disturbances, alcohol or drug addiction and smoking were considered exclusion criteria. The study was approved by the ethical committee of Navarra and informed consent was signed by all volunteers before the study.

2.2. Analysis of PCR-RFLP

Genomic DNA was extracted from leukocytes in samples of whole blood by proteinase K digestion followed by phenol/chloroform extraction. PCR amplification was performed on a Perkin Elmer, Gene Amp PCR system 2400, (Applied Biosystems, Foster City, CA).

The 30 μl reaction volume contained 150-250 ng DNA, 0,2 mM of each deoxynunucleoside triphosphate, 1× buffer (10mM Tris-HCl, 1.5 mM MgCl2, 5mM KCl, pH8.3), 20 pmol of each primer, and 1U of Biotaq DNA Polymerase. The following primers were used to amplify a 310 base pair (bp) fragment: 5′-ccgccgtgggtccgcc-3′ (forward) and 5′-CCATGACCAGATCAGCAC-3′(reverse). The PCR program was a modification of Large et al. [6]: each step of the cycle lasted 30 s and the annealing temperature was 64°C. The 310 bp PCR product was digested with 5 units of *Ita* I or with its isoschizomer *Fnu* 4HI overnight. The obtained fragments sized 84, 55 and 171 bp for the Gln27Gln allele and 84 and 226 bp for the Glu27Glu allele. They were observed on an ethydium bromide stained 1.35% agarose gel, under UV illumination, as previously described.

2.3. Peak oxygen consumption test

Subjects arrived at 8.00 a.m. to the Hospital unit and weight, together with height, were measured to calculate BMI. Body fat (BF) was estimated by bioimpedance (TANITA TBF-300, Bio Logica, Japan). Energy and macronutrient intake throughout the year was estimated by a validated semiquantitative food frequency questionnaire [18]. All participants were studied with a multistage exercise treadmill test according to the Bruce protocol [19] using a breath-by-breath MMC Horizon System 4400 tc, (SensorMedics, Anaheim, CA) which collected respiratory gases through a nose and mouth mask. Respiratory exchange ratio was measured constantly during the whole study, this means, during 20 min of basal rest, during the Bruce protocol and 60 min after the maximal effort. Peak oxygen consumption was reached (mean time 7.5 ± 1.3 min) when at least three of the four following criteria were fulfilled: a) Heart rate (HR) over 85% of maximal HR calculated by the age predicted formula, b) Respiratory Exchange Ratio (RER) over 1.2, c) A plateau in O2 consumption (VO2), despite an increase in workload, and d) An inability of the subject to continue despite urging by the testing staff. Data from the VO2max test were used to screen for evidence of cardiovascular disease and therefore exclude them from the study, given that obese patients have an increased risk of developing such diseases [20,21]. An intravenous catheter was inserted in the right arm and three samples of blood were extracted: a) at baseline, b) during the maximal effort, and c) five minutes after the maximal effort (recovery). Urine was collected in the basal state after an overnight fast (12 h) and at the end of the study.

Basal and post-exercise urinary urea was used to estimate nitrogen excretion in order to calculate basal and post-exercise protein oxidation, respectively [Urea (mg/dl)×0.0055 = Urinary nitrogen (g/L)]. After correction for protein oxidation, lipid oxidation rates were calculated from O2 consumption and CO2 production as described by Ferrannini [22]. Because of the possible interference of an excess of CO2, the first 20 min of the recovery period were excluded, and only the 40 last minutes were used to calculate the lipid oxidation [23].

2.4. Analytical methods

Plasma glycerol, triglycerides and glucose as well as urinary urea were determined with a Cobas Mira S apparatus, by spectrophotometric methods, whereas serum insulin

Table 2
Energy, alcohol and macronutrient distribution intake as estimated by a validated semi-quantitative food frequency questionnaire

Variable	Glu27Glu (n=9)	Gln27Gln (n=10)	Statistical Analysis	P
Carbohydrate (% E)	45.1 (2.5)	42.9 (1.9)	U	0.400
Protein (% E)	21.3 (1.2)	21.0 (0.8)	U	0.720
Fat (% E)	31.6 (2.7)	34.6 (1.8)	U	0.356
Alcohol (% E)	2.0 (1.0)	1.5 (0.7)	U	0.640
Energy intake (kcal/day)	2085 (263)	2559 (180)	U	0.159

Values are means (SEM). U = Mann Whitney's U-test.

(Coat-A-Count, Insulin, DPC, LA, CA) was measured by radioimmunoassay (DPC Gambyt, LA, CA).

2.5. Data analysis

All data are presented as the mean (SEM). Variables listed in Table 1 and fat oxidation were analyzed by either unpaired Student's *t*-test or Mann Whitney's *U*-test, as appropriate, depending on normality test results. Time-group interaction, time effect, and differences between groups were analyzed with a two way repeated measures ANOVA (TWRMA) in both biochemical and respiratory quotient values. When ANOVA for group was significant, unpaired Student's *t* test was performed [2]. The SPSS 7.5 version for WINDOWS was used for the statistical analysis.

3. Results

The measured anthropometric and fitness variables from subjects participating in the VO2max test did not differ statistically between both experimental groups (Table 1) and nor did the energy nutrients intake and the macronutrient distribution (Table 2).

In one hand, the polymorphic Glu27Glu group (case) had a lower plasma glycerol, which was significantly lower in this group at baseline (Fig. 1a), and along the whole study (ANOVA for group, $P = 0.026$).

On the other hand, the Glu27Glu group had significantly higher (ANOVA for group $P < 0.001$) plasma triglycerides (Fig. 1b) and higher values of the insulin:glucose ratio (ANOVA for group, $P = 0.046$) than the control group through the study, though no differences were detected at any particular point in both measures.

Finally, the respiratory quotient was significantly higher (ANOVA for group, $P = 0.001$) in the Glu27Glu group throughout the whole study (data not shown). Lipid oxidation did not differ at baseline, but was significantly lower (Student's unpaired *t* test, $P = 0.024$) in this group than in the Gln27Gln group during the recovery period (Fig. 2).

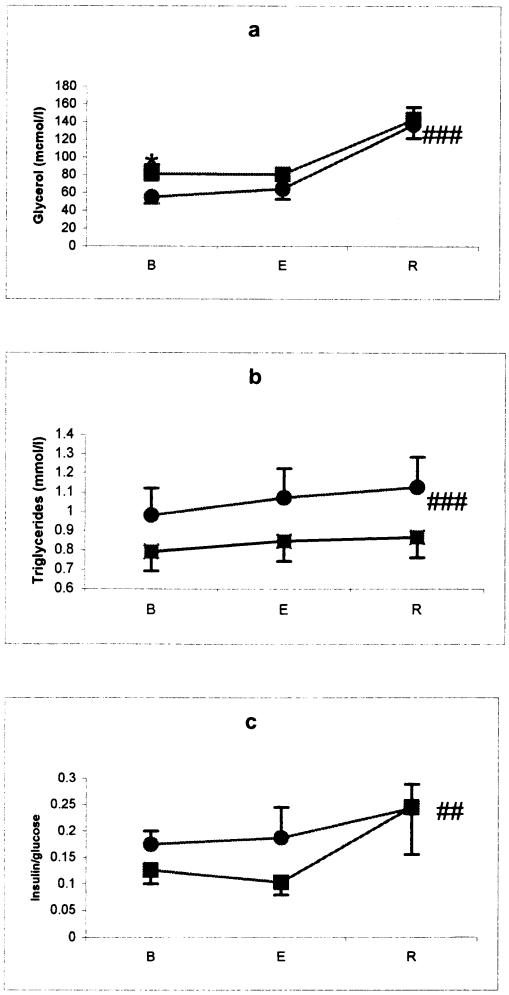

Fig. 1. Plasma glycerol (a), triglycerides (b), and insulin:glucose ratio (c) in Gln27Gln (■) subjects and Glu27Glu (●) subjects along the peak oxygen consumption study. Values are the mean (SEM). ###, $P < 0.001$ (by TWRMA for time), ##, P[r] < 0.05 (by TWRMA for time), *, $P < 0.05$ (by unpaired Student's *t* test). B = Baseline. E = Effort. R = Recovery.

4. Discussion

The main aims pursued by the maximal effort trial were:; a) To determine any possible energy metabolism difference between groups that could explain the already observed gene-environment interaction in our population [17]; b) To observe if the Gln27Glu polymorphism predisposes to a lower VO2max, as it was suggested in a recent study [7]; c) To detect possible cardiovascular impairments under strenuous conditions in our obese subjects and to exclude them from the study

The Bruce protocol is sufficient to measure adequately the aerobic capacity, the maximal cardiac frequency and ventilation in sedentary women, and is commonly used to detect CVD [24].

The groups were matched for some anthropometry and fitness related parameters. Particular interest was paid to

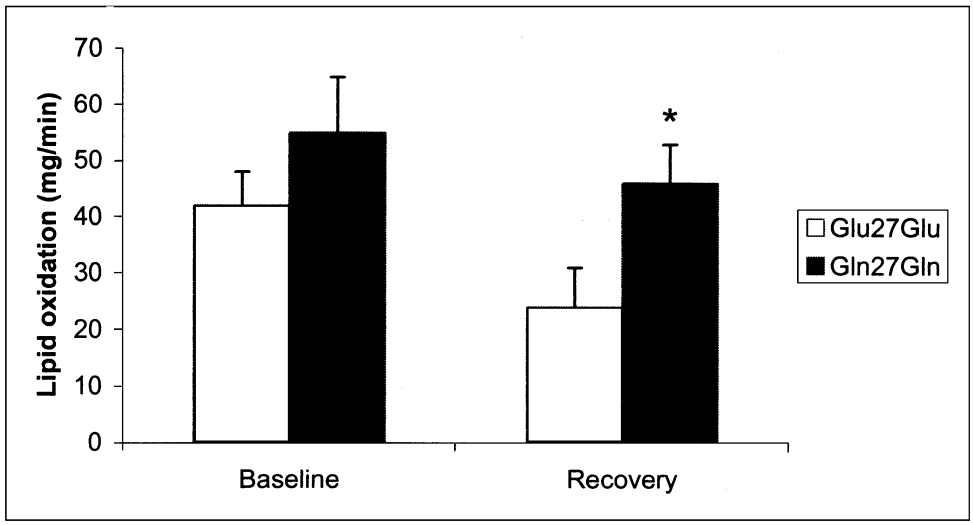

Fig. 2. Lipid oxidation (mg/min) measured at baseline and recovery. *, $P = 0.024$ (by unpaired Student's t test, Glu27Glu *vs.* Gln27Gln).

some variables, such as age, VO2max and percentage of fat mass, since recent findings revealed that Glu27Glu postmenopausal women reached a lower VO2max as compared to the other genotypes [7], probably due to their excess of fat mass. Therefore, our groups were matched for both VO2max and fat mass in order to make both groups comparable, so that the subsequent measures were not phenotype-dependent. Furthermore, no differences in macronutrient and energy intake were detected.

A good parameter of lipid mobilization is given by the measurement of plasma glycerol concentration. Once glycerol has been hydrolyzed from triglycerides (TG), in contrast to free fatty acids, it cannot be re-uptaken by adipocytes, nor by muscular cells, because they lack the glycerol kinase enzyme, which is only present in the liver [25,26].

The fact that lipid mobilization appeared to be blunted (lower lipolysis, as assessed by plasma glycerol levels) and fat oxidation was decreased in the polymorphic Glu27Glu group *vs.* the Gln27Gln group could be associated to a resistance to utilize fat stores during exercise in the Glu27Glu individuals. Therefore, the Gln27Gln genotype seems to benefit more from physical activity than the polymorphic Glu27Glu group. This finding is in accordance with the available literature for three main reasons: a) An impairment in β2- adrenoceptor-mediated increases in thermogenesis and lipid utilization has been shown in the obese [2], b) An association of the Glu27 allele and obesity has been repeatedly documented [8-16] and c) Physical activity may counterbalance the effect of the Gln27Glu polymorphism predisposition to increase body weight, body fat and obesity [13]. In this context, a statistically significant interaction was detected between the Gln27Glu polymorphism and physical activity for body weight in 255 French men, whereas no effect was found in men who took regular physical activity [13]. Interestingly, it has been suggested a strong dissociation between endurance elite runners and Glu27Glu ADRB2 genotype, since sedentary and active women were in Hardy-Weinberg equilibrium, whereas athletes were underrepresented for Glu27Glu [7]. Furthermore, Glu27Glu women had a higher weight, BMI and fat mass, with a lower VO2max than other genotypes [7].

Also, the Glu27Glu group had significantly higher plasma triglycerides (TG) and showed higher values for the insulin:glucose ratio than the control group throughout the study. The normal role of adipose tissue is to "buffer" the influx of dietary fat entering the circulation [27] and the same could be applied to exercise-induced fat mobilization. The increased flux of fatty acids (both as NEFA and TG) in the circulation has acute adverse effects on insulin sensitivity, but also produces in the longer term a deposition of TG in glucose-metabolizing tissues such as skeletal muscle, liver and the pancreatic β-cell. Accumulation of TG in these tissues may further drive to an impairment of the normal sensitivity of glucose metabolism to insulin [27]. Moreover, in the Glu27Glu group, both a low lipolysis and a low fat oxidation could cause an impairment in the adipose tissue buffering and partitioning capacity, specially in obesity, which could cause a variety of signs such as insulin resistance, hyperinsulinemia or hypertension. This explanation is supported by a number of studies that found an association of the Glu allele with higher triglyceridemia [10,16, 28,29,30], a higher frequency of the Glu allele among patients with NIDDM than in non-diabetic subjects [10] and higher fasting plasma insulin levels in Glu27Glu women than in other genotypes [6].

In conclusion, these results suggest that the ADRB2 Glu27Glu obese women had a different metabolic response when submitted to a VO2max test, from their Gln27Gln counterparts. Both the lower lipolysis and decreased fat oxidation found in the Glu27Glu group as well as the higher plasma triglycerides could lead to the observed impairment in insulin sensitivity. Therefore, this study provides infor-

mation about the possible role of physical activity on the metabolic differences involved in two different ADRB2 Gln27Glu homozygous groups of obese women, which can be of help at designing therapies for obesity and/or the metabolic syndrome.

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