Brief Genetic Analysis

Effects of β2-Adrenergic Receptor Gene Variants on Adiposity: The HERITAGE Family Study

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Abstract

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We investigated whether the Arg16Gly and Gln27Glu polymorphisms of the β2-adrenergic receptor gene were associated with body-fat and fat-distribution phenotypes measured before and in response to a 20-week endurance-training program. BMI, fat mass (FAT), percentage of body fat (%FAT), sum of eight skinfolds (SF8), and abdominal fat areas assessed by computed tomography were measured in adult sedentary white and black participants of the HERITAGE Family Study. Evidence of gene-by-obesity interaction was found in whites for several adiposity phenotypes measured before training. Analyses performed separately in nonobese and obese subjects revealed that obese men carrying the *Glu27* allele have lower fat accumulation (BMI, FAT, and %FAT) than noncarriers. Among white obese women, Gly16Gly homozygotes had a lower fat accumulation (BMI, FAT, and SF8) than Arg16Gly and Arg16Arg carriers. In response to endurance training, white women with the Arg16Arg genotype exhibited a greater reduction in BMI, FAT, and %FAT. Results observed in blacks were mostly negative. These results suggest that polymorphisms in the β2-adrenergic receptor gene influence the amount of body fat in white obese men (Gln27Glu) and women (Arg16Gly), as well as the changes in adiposity in response to endurance training in white women (Arg16Gly).

Key words: β2-adrenergic receptor gene, polymorphism, HERITAGE Family Study, adiposity, exercise

It is now established that lipolytic activity of human adipocytes is influenced by obesity and gender and varies among the different fat depots (1). Abdominal subcutaneous fat cells of obese subjects are characterized by a resistance to catecholamine-induced lipolysis (2,3). Furthermore, the β-adrenergic stimulation is more marked in the abdominal subcutaneous fat than in the gluteal fat depot, and this difference is more marked in women than in men (4).

During exercise, high levels of catecholamines favor binding to β-adrenergic receptors, as compared with the resting state where α2-adrenergic receptors are primarily stimulated. In vivo, the lipolysis response to exercise is higher in abdominal subcutaneous than in gluteal adipose tissue and is more pronounced in women than in men (5). Also, catecholamine-induced lipolysis is more pronounced in subcutaneous adipocytes of endurance-trained women than in sedentary controls (6).

Since the first report using the *Ban*I polymorphism in the β2-adrenergic receptor gene (7), several studies have tested the association between Arg16Gly and Gln27Glu polymorphisms and obesity-related phenotypes. Some studies have shown positive associations (8–13), whereas others have reported negative results (14–16); very often, gender dif-

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ferences have been observed in the association between these polymorphisms and adiposity (9,12,17). One study also reported an association between the Arg16Gly polymorphism and body-weight loss in response to a 3-month treatment with a combined low-energy diet and exercise regimen (18).

The present report shows that obese men bearing the Glu27Glu genotype and obese women with the Gly16Gly genotype have lower fat accumulation than carriers of other genotypes. Also, white women who are Arg16Arg homozygotes exhibit a greater fat reduction in response to endurance training. These results suggest that polymorphisms in the β2-adrenergic receptor gene influence the amount of body fat in white obese subjects, as well as the changes in adiposity in response to endurance training in white women.

In whites, the *Gly16* and *Glu27* allele frequencies were 0.64 and 0.47, respectively, whereas the corresponding values in blacks were 0.47 and 0.17, respectively (results not shown). All genotype frequencies were in Hardy–Weinberg equilibrium. Genotype frequencies differed strongly between races for Arg16Gly [χ^2: (2*df*) = 39.1, $p < 0.0001$] and Gln27Glu [χ^2 (2*df*) = 132.4, $p < 0.0001$) polymorphisms. Strong linkage disequilibrium was observed between the Arg16Gly and Gln27Glu polymorphisms in whites ($D' = 0.965 \pm 0.014$, $\chi^2 = 463.6$, $df = 1$, $p < 0.0001$) and in blacks ($D' = 0.970 \pm 0.025$, $\chi^2 = 113.7$, $df = 1$, $p < 0.0001$).

In white men, no association was observed between the Arg16Gly polymorphism and body-fat phenotypes measured before and in response to training, except for a moderate effect ($p = 0.03$) on the difference between pre- and posttraining values for the sum of eight skinfolds (SF8)[1] (ΔSF8; see Table 1). A significant gene-by-obesity interaction (Gln27Glu polymorphism) effect was observed in white men for BMI, fat mass (FAT), and percentage of body fat (%FAT) (Table 1). Analyses performed separately in obese and nonobese subjects revealed that obese white men who were homozygotes for the *Glu27* allele were characterized by lower values of BMI ($p = 0.04$), FAT ($p = 0.0009$; Figure 1), and %FAT ($p = 0.002$) compared with obese men who were homozygotes for the *Gln27* allele. No effects of the polymorphism were observed in nonobese men. The variance attributable to the Gln27Glu polymorphism reached 10% (BMI), 21.4% (FAT), and 18.2% (%FAT). A significant interaction between the Gln27Glu polymorphism and obesity was also observed for ΔFAT ($p = 0.02$) in white men. When analyses were performed separately in nonobese and obese subjects, the effects of the Gln27Glu polymorphism were observed only in obese men, with a greater reduction in ΔFAT ($p = 0.01$) for Glu27Glu homozygotes (-2.79 ± 0.78 kg) as compared with Gln27Gln homozygotes (0.19 ± 0.76 kg).

In white women, no significant association was observed between the Arg16Gly polymorphism and %FAT, abdominal visceral (AVF), abdominal subcutaneous (ASF), or total abdominal (ATF) fat (Table 2). However, a significant gene-by-obesity interaction was observed for the BMI, FAT, and SF8 values that were measured before training. Results of analyses performed separately in obese and nonobese white women revealed that obese women homozygotes for the *Gly16* allele had the lowest amount of BMI, FAT (Figure 2), and SF8 as compared with Arg16Gly heterozygotes (BMI: $p = 0.02$; SF8: $p = 0.02$) and Arg16Arg homozygotes (BMI: $p = 0.003$; FAT: $p = 0.02$; SF8: $p = 0.01$). The variance attributable to the Arg16Gly polymorphism reached 22.2% (BMI), 16.8% (FAT), and 14.8% (SF8). In white women, the response to training for BMI, FAT, and %FAT was more important in Arg16Arg homozygotes as compared with homozygotes for the *Gly16* allele (Table 2). Finally, no significant association was observed between the Gln27Glu polymorphism and obesity-related phenotypes in white women, either before or in response to endurance training, with the exception of a moderately significant effect ($p = 0.04$) for Δ%FAT (Table 2).

Significant gene-by-obesity interaction was observed in black men for ΔASF with both Arg16Gly ($p = 0.03$) and Gln27Glu ($p = 0.01$) polymorphisms and for ΔAVF with the Gln27Glu polymorphism ($p = 0.006$). Analyses performed separately in nonobese and obese subjects showed that only obese black men carriers of *Glu27* allele exhibited the most important changes in ΔASF (-40.3 ± 8.3 cm^2) as compared with noncarriers (-18.8 ± 4.8 cm^2; $p = 0.04$) (Data not shown).

Results of the present study, summarized in Table 3, indicate that the β2-adrenergic receptor Arg16Gly and Gln27Glu polymorphisms are associated with adiposity phenotypes in white, but not in black, subjects participating in the HERITAGE Family Study. Two principal findings emerge from these results. First, the impact of these polymorphisms is gender- and race-specific, with the effects of the Arg16Gly polymorphism observed mainly in white women, and the effects of Gln27Glu polymorphism mainly found in white men, whereas no effects were found in blacks. Second, the presence of gene-by-obesity interactions was observed mainly for the pretraining phenotypes.

Results obtained with pretraining sedentary-state phenotypes indicate that β2-adrenergic receptor polymorphisms are associated with adiposity, but principally in white obese subjects. Results showed that white obese women who were homozygotes for the rare *Gly16* allele have, on average, 10 kg less body fat than obese homozygotes for the *Arg16* allele, suggesting that the *Gly16* allele may prevent fat accumulation in obese women. Similar results have been

[1] Nonstandard abbreviations: SF8, sum of eight skinfolds; FAT, fat mass; %FAT, percentage of body fat; AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat; ATF, total abdominal fat; PCR, polymerase chain reaction; ANCOVA, analysis of covariance.

Table 1. Associations of β2-adrenergic receptor gene Arg16Gly and Gln27Glu polymorphisms with body composition and fat distribution measured before and in response to training in white men

				p value					*p* value	
	Arg16Arg	**Arg16Gly**	**Gly16Gly**	**Asso**	**Int**	**Gln27Gln**	**Gln27Glu**	**Glu27Glu**	**Asso**	**Int**
Before training										
	$25 \leq N \leq 29$	$94 \leq N \leq 103$	$102 \leq N \leq 111$			$61 \leq N \leq 68$	$101 \leq N \leq 114$	$56 \leq N \leq 60$		
BMI (kg/m^2)	26.8 ± 0.8	26.9 ± 0.4	26.5 ± 0.4	0.78	0.19	27.5 ± 0.5	26.3 ± 0.4	26.7 ± 0.6	0.20	0.007
FAT (kg)	19.0 ± 1.9	20.8 ± 1.0	19.9 ± 0.9	0.64	0.07	21.0 ± 0.1	19.8 ± 0.9	20.1 ± 1.3	0.73	0.0001
%FAT (%)	21.5 ± 1.5	23.2 ± 0.7	22.8 ± 0.7	0.58	0.26	23.3 ± 0.9	22.7 ± 0.7	22.9 ± 1.0	0.94	0.02
SF8 (mm)	120 ± 10	136 ± 5	130 ± 5	0.35	0.55	133 ± 6	129 ± 5	132 ± 6	0.89	0.09
AVF (cm^2)	109 ± 7	109 ± 4	108 ± 4	0.95	0.35	109 ± 4	110 ± 3	105 ± 5	0.68	0.09
ASF (cm^2)	229 ± 8	223 ± 4	227 ± 4	0.67	0.39	226 ± 5	223 ± 4	228 ± 5	0.74	0.11
ATF (cm^2)	337 ± 9	332 ± 5	334 ± 5	0.82	0.51	334 ± 6	334 ± 5	334 ± 6	0.99	0.99
Response to training										
	$22 \leq N \leq 28$	$85 \leq N \leq 95$	$96 \leq N \leq 107$			$59 \leq N \leq 66$	$91 \leq N \leq 105$	$53 \leq N \leq 58$		
ΔBMI (kg/m^2)	−0.12 ± 0.13	−0.051 ± 0.068	−0.20 ± 0.06	0.28	0.35	−0.14 ± 0.08	−0.10 ± 0.07	−0.16 ± 0.09	0.84	0.23
ΔFAT (kg)	−0.72 ± 0.38	−0.66 ± 0.19	−1.15 ± 0.18	0.15	0.46	−0.78 ± 0.23	−0.82 ± 0.18	−1.14 ± 0.25	0.51	0.02
Δ%FAT (%)	−0.83 ± 0.35	−0.72 ± 0.18	−1.20 ± 0.17	0.15	0.82	−0.87 ± 0.22	−0.86 ± 0.17	−1.21 ± 0.23	0.42	0.20
ΔSF8 (mm)	−2.54 ± 2.73	−5.64 ± 1.45	−9.48 ± 1.34[a]	0.03	0.17	−5.30 ± 1.77	−6.88 ± 1.42	−9.07 ± 1.80	0.33	0.38
ΔAVF (cm^2)	−10.9 ± 3.4	−5.42 ± 1.68	−8.25 ± 1.63	0.26	0.33	−6.13 ± 2.08	−8.47 ± 1.63	−5.85 ± 2.22	0.54	0.58
ΔASF (cm^2)	−13.6 ± 3.4	−8.27 ± 1.66	−11.5 ± 1.6	0.22	0.23	−11.34 ± 2.07	−8.57 ± 1.62	−12.03 ± 2.19	0.37	0.05
ΔATF (cm^2)	−24.1 ± 5.0	−14.0 ± 2.5	−19.4 ± 2.4	0.12	0.13	−17.6 ± 3.1	−17.1 ± 2.4	−17.1 ± 3.3	0.99	0.68

Data are least-square means ± SEM (see text for details).

N, number of subjects.

Asso *p* values are from testing the effect of the polymorphism in the ANCOVA. Int *p* values are from the effect of the gene-by-obesity interaction term.

[a] From multiple comparison test of least-square means: Arg16Gly is significantly different from Gly16Gly; $p = 0.02$.

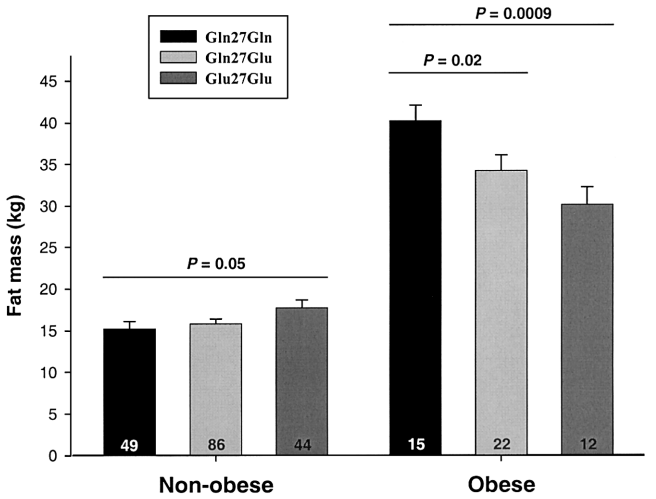

Figure 1: Association of the Gln27Glu polymorphism in the *ADRB2* gene with the pretraining FAT in nonobese and obese white men. Data are least-square means ± SEM; *p* values were obtained using ANCOVA.

reported regarding the effects of the Arg16Gly polymorphism on body fat. A study of women with a wide range of body fatness (BMI 17.8 kg/m^2 to 60.0 kg/m^2) showed that the Arg16Gly polymorphism was not associated with obesity, but *Gly16* carriers were found to have an altered β2-adrenergic receptor function, with a five-fold increase in agonist sensitivity (10). In Japanese subjects, the *Gly16* allele was less frequent in obese women as compared with nonobese women, whereas no association was observed in men (9). Finally, contradictory results have been reported in the French population with the *Gly16* allele being associated with lower body fatness in men, but not in women (12).

In whites, we observed a significant association between the Gln27Glu polymorphism and body fat in obese men and a marginal effect in nonobese men. In white obese men, homozygotes for the *Glu27* allele exhibited a lower accumulation of body fat (~10 kg) as compared with homozygotes for the *Gln27* allele. Our results are in agreement with those reported previously showing that men who are carriers of the *Gln27* allele have an increased risk to develop obesity (8,12). In contrast, in Swedish women, the *Glu27* allele was associated with a 20-kg excess of body fat, but had no effect in men (8). In this latter report, no difference in the β2-adrenergic receptor function was found among genotypes. In the Japanese population, the *Glu27* allele was more frequent in obese subjects (9,11). Other studies in Danish men (15), German men and women (14), and Australian women (16) did not show any association of the Gln27Glu polymorphism with obesity.

Recently, physical activity was proposed as a factor influencing the association of the *Gln27* allele with obesity in men (17). The categorization of physical activity levels described in the latter report is not fully comparable to the HERITAGE Family study in which subjects were initially sedentary and were asked to follow a standardized endurance-training program for 20 weeks. In our study, the associations between the Gln27Glu polymorphism and training-induced changes in body fat measured in white men were marginal, whereas obese women who were homozygotes for the *Arg16* allele seemed to benefit more from the exercise-training program because they exhibited a greater reduction in body fat in response to training compared with the *Gly16* carriers.

In summary, results of the present study reveal that the Arg16Gly and Gln27Glu polymorphisms of the β2-adrenergic receptor gene are associated with obesity, but only in white obese subjects. Because no effects were found in blacks, except for a weak association with training-induced changes in abdominal fat, the effects of these polymorphisms were race-specific. Finally, the β2-adrenergic receptor gene was also found to influence the changes of adiposity in response to exercise training in white women.

Research Methods and Procedures

Subjects

The HERITAGE Family Study aims, design, methods, and subjects have already been described elsewhere (19). Sedentary-state (pretraining) and response-to-training (difference between pre- and posttraining) phenotypes were analyzed. Among the four race-by-gender groups, 592 subjects had a BMI <30 kg/m^2 (67 black men, 120 black women, 186 white men, and 219 white women), whereas 184 subjects had a BMI ≥30 kg/m^2 (22 black men, 65 black women, 58 white men, and 39 white women). The total number of subjects with both pretraining and training-response phenotypes was 482 among whites (231 men and 251 women) and 260 among blacks (89 men and 171 women). The Institutional Review Board of each university of the HERITAGE Family Study research consortium approved the study protocol. Written informed consent was obtained from each participant.

The Endurance-Training Program

Details of the training program are given elsewhere (20). Briefly, the subjects exercised on a cycle ergometer (Universal Aerobicycle IV, Cedar Rapids, IA) three times per week under supervision and followed a standardized protocol using a duration and intensity that were gradually increased over 20 weeks of training.

Phenotype Measurements

The measurement methods for BMI, SF8, %FAT, and FAT have been described in detail previously (21). AVF, ASF, and ATF areas were assessed by computed tomography as described previously (22).

Genotype Determination

DNA was extracted from lymphoblastoid cell lines after digestion by proteinase K and purification with phenol-

Table 2. Associations of β2-adrenergic receptor gene Arg16Gly and Gln27Glu polymorphisms with body composition and fat distribution measured before and in response to training in white women

				p value					*p* value	
	Arg16Arg	**Arg16Gly**	**Gly16Gly**	**Asso**	**Int**	**Gln27Gln**	**Gln27Glu**	**Glu27Glu**	**Asso**	**Int**
Before training	$38 \leq N \leq 40$	$104 \leq N \leq 110$	$95 \leq N \leq 96$			$67 \leq N \leq 74$	$120 \leq N \leq 128$	$54 \leq N \leq 56$		
BMI (kg/m^2)	25.2 ± 0.70	24.8 ± 0.4	25.1 ± 0.4	0.80	0.002	24.9 ± 0.5	25.1 ± 0.4	25.1 ± 0.6	0.98	0.12
FAT (kg)	21.5 ± 1.5	20.8 ± 0.9	21.0 ± 1.0	0.92	0.01	20.4 ± 1.2	21.4 ± 0.9	21.3 ± 1.3	0.81	0.20
%FAT (%)	30.0 ± 1.3	30.0 ± 0.7	30.1 ± 0.9	0.99	0.21	29.3 ± 1.0	30.4 ± 0.7	30.2 ± 1.2	0.66	0.32
SF8 (mm)	156 ± 8	168 ± 5	164 ± 5	0.49	0.05	155 ± 6	170 ± 5	163 ± 7	0.17	0.28
AVF (cm^2)	75 ± 5	76 ± 3	71 ± 3	0.48	0.74	79 ± 4	74 ± 3	68 ± 4	0.15	0.07
ASF (cm^2)	269 ± 8	289 ± 5	284 ± 5	0.11	0.28	284 ± 6	281 ± 5	292 ± 7	0.42	0.91
ATF (cm^2)	344 ± 9	365 ± 5	356 ± 5	0.10	0.55	363 ± 7	355 ± 5	360 ± 8	0.61	0.45
Response to training	$34 \leq N \leq 41$	$99 \leq N \leq 110$	$91 \leq N \leq 96$			$61 \leq N \leq 72$	$115 \leq N \leq 124$	$52 \leq N \leq 55$		
ΔBMI (kg/m^2)	−0.34 ± 0.13	0.034 ± 0.078	−0.039 ± 0.083[a]	0.04	0.71	−0.06 ± 0.10	−0.11 ± 0.07	0.06 ± 0.11	0.45	0.28
ΔFAT (kg)	−1.56 ± 0.28	−0.34 ± 0.17	−0.48 ± 0.18[b]	0.0008	0.57	−0.77 ± 0.21	−0.76 ± 0.16	−0.13 ± 0.24	0.07	0.20
Δ%FAT (%)	−1.90 ± 0.30	−0.50 ± 0.18	−0.63 ± 0.19[c]	0.0003	0.36	−0.96 ± 0.23	−0.98 ± 0.18	−0.21 ± 0.26[d]	0.04	0.51
ΔSF8 (mm)	−12.5 ± 2.7	−6.05 ± 1.60	−5.72 ± 1.67	0.08	0.85	−8.32 ± 2.09	−7.72 ± 1.51	−2.90 ± 2.24	0.14	0.28
ΔAVF (cm^2)	−1.42 ± 2.05	−3.83 ± 1.15	−3.50 ± 1.22	0.59	0.30	−3.12 ± 1.46	−3.64 ± 1.09	−3.0 ± 1.6	0.93	0.28
ΔASF (cm^2)	−6.08 ± 3.68	−9.15 ± 2.07	−7.91 ± 2.19	0.75	0.49	−7.78 ± 2.71	−8.36 ± 2.02	−8.18 ± 3.01	0.98	0.65
ΔATF (cm^2)	−7.63 ± 4.17	−13.2 ± 2.35	−11.2 ± 2.5	0.51	0.35	−11.3 ± 3.1	−12.0 ± 2.3	−10.7 ± 3.4	0.95	0.96

Data are least-square means ± SEM (see text for details).

N, number of subjects.

Asso *p* values are from testing the effect of the polymorphism in the ANCOVA. Int *p* values are from the effect of the gene-by-obesity interaction term.

Superscript letters (a, b, c, d) represent multiple comparison tests of least-square means: [a], Arg16Arg is significantly different from Arg16Gly ($p = 0.01$) and Gly16Gly ($p = 0.05$); [b], Arg16Arg is significantly different from Arg16Gly ($p = 0.0002$) and Gly16Gly ($p = 0.001$); [c], Arg16Arg is significantly different from Arg16Gly ($p = 0.0001$) and Gly16Gly ($p = 0.0005$); [d], Glu27Glu is significantly different from Gln27Glu ($p = 0.02$) and Gln27Gln ($p = 0.03$).

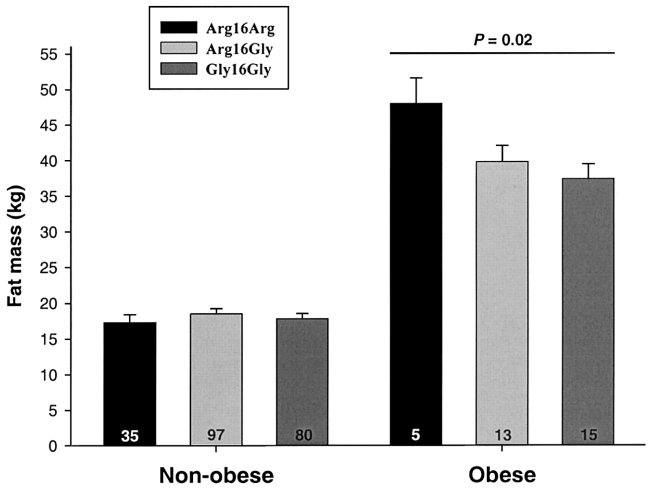

Figure 2: Association of the Arg16Gly polymorphism in the *ADRB2* gene with the pretraining FAT in nonobese and obese white women. Data are least-square means ± SEM; *p* values were obtained using ANCOVA.

chloroform (23). Polymerase chain reaction (PCR) amplification of the Arg16Gly polymorphism was carried out in a volume of 20 μL containing 150 ng of DNA, 200 μM each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, 1× buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol; pH = 7.5 at 25 °C), 300 nM of each primer and 1 U of *Taq* polymerase (Perkin Elmer Cetus, Boston, MA). Primers generated a product of 201 bp, which was cut into fragments of 14, 56, and 131 bp in the presence of the *Bsr*DI cutting site (*Arg16* allele) and in the absence of the cutting site into fragments of 14, 23, 56, and 108 bp (*Gly16* allele). PCR amplification of the Gln27Glu was carried out in the same way as the Arg16Gly polymorphism, but with the addition of 10% dimethyl sulfoxide. Primers generated a product of 353 bp, which was cut into fragments of 27, 55, 97, and 174 bp in the presence of the *Ita*I cutting site (*Gln27* allele) and in the absence of the cutting site into fragments of 27, 97, and 229 bp (*Glu27* allele). For both polymorphisms, primers were similar to those described by Large et al. (10). A negative control without DNA was performed in every run of amplification. After each amplification, PCR product was digested overnight at 37 °C after adding 4 U of the restriction enzyme *Bsr*DI (Arg16Gly) or 1 U of the restriction enzyme *Ita*I (Gln27Glu) to the PCR mixture. Resulting fragments were separated by electrophoresis in 2% agarose gels. Each gel was run for 2 hours at 150 V, stained with ethidium bromide, and photographed under ultraviolet-transmitted light. The ΦX174 DNA digested with the restriction enzyme *Hae*III was used as the length marker to estimate the size of the digested DNA fragments. Mendelian inheritance was confirmed within families for both markers.

Statistical Analysis

All statistical analyses were performed using the SAS software (Statistical Analysis System, Cary, NC). The distribution of each variable was tested for normality using the Shapiro–Wilk *W* test, and all variables were normally distributed. A χ^2 test was performed to determine whether the genotype frequencies of Arg16Gly and Gln27Glu polymorphisms were in Hardy–Weinberg equilibrium or presented race differences. Haplotype frequencies under the assumption of no allelic and allelic association were computed using the EH (estimating haplotype-frequencies) program (Rockefeller University, NY). Linkage disequilibrium between Arg16Gly and Gln27Glu polymorphisms was estimated in each race using the 2LD (two-locus linkage dis-

Table 3. Summary of the effects of β2-adrenergic receptor gene Arg16Gly and Gln27Glu polymorphisms on body fat and fat distribution measured before and in response to training

	Whites		Blacks	
Polymorphism	**Men**	**Women**	**Men**	**Women**
Arg16Gly				
Before training		BMI, FAT, SF8 in obese subjects		
Response to training	SF8	BMI, FAT, %FAT		
Gln27Glu				
Before training	BMI, FAT, %FAT in obese			
Response to training	FAT in obese	%FAT	ASF in obese	

Entries in the table indicate phenotypes showing significant ($p < 0.05$) evidence of association for each polymorphism.

equilibrium) program (Section of Genetic Epidemiology & Biostatistics, Institute of Psychiatry, London).

Associations between the Arg16Gly and Gln27Glu polymorphisms and each phenotype were investigated separately in each of the four race-by-gender groups using an analysis of covariance (ANCOVA) (general linear model) procedure that included the effects of age (age, age^2, age^3) as covariates. ASF, AVF, and ATF were further adjusted for the effects of FAT. Response phenotypes were further adjusted for pretraining values. Gene-by-obesity (cutoff: BMI = 30 kg/m^2) interaction was also modeled. In the presence of a significant gene-by-obesity interaction effect, analyses were repeated separately in obese and nonobese subjects.

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