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PAPER

Interactions among the β2- and β3- adrenergic receptor genes and total body fat and abdominal fat level in the HERITAGE Family Study

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OBJECTIVE AND SUBJECTS: Interactions between markers in the β2- and β3-adrenergic receptor (ADR) genes and total body fat and computerized tomography-measured abdominal fat phenotypes were studied in the HERITAGE Family Study cohort of Black ($n=205$; 81 males and 124 females) and White ($n=415$; 198 males and 217 females) subjects before and after an endurance training program.
RESULTS: In Black subjects, β2- and β3-ADR gene variants showed evidence of interactions on changes in total body fat mass and abdominal fat area ($P<0.005$ and $=0.010$, respectively). Black subjects who were carriers of both β2-ADR Arg16 and β3-ADR Arg64 alleles had a greater decrease in total fat mass as well as abdominal total and subcutaneous, but not visceral fat areas in response to endurance training than subjects with other genotype combinations (P from 0.011 to 0.047). After correction for multiple tests, the findings remained essentially unchanged for total body fat mass and abdominal fat area, but became nonsignificant for subcutaneous fat area. The changes in abdominal fat correlated positively with the changes in fat mass ($P<0.0001$). The interactions between β2 and β3-ADR gene markers accounted for a maximum of 3% of the variances in the response of total fat mass and abdominal fat area to endurance training in Black subjects but it was not significant in White subjects.
CONCLUSION: Interactions between sequence variants in the β2–β3-ADR gene contributed to the changes in fat mass and abdominal adiposity in response to endurance training in Black subjects.
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Keywords: genetics; adiposity; gene-gene interactions; adrenergic receptors

Introduction

Total body adiposity and abdominal fat levels are influenced by genetic factors[1,2] although behavioral factors and their interactions with genes are undoubtedly involved.[3] Earlier studies have found an association between some genetic variants and adiposity phenotypes.[4–7] However, these variants account for only a small fraction of the phenotypic variance. Given the complexity of the genetic determinants of body fat level, it is likely that gene–gene interactions play some role. For instance, an interaction effect of β3- and α2B-adrenergic receptor (ADR) genes on fat mass in Caucasian women has been observed.[8] In addition, gene–gene interaction effects between α2A-, β2- and β3-ADR gene markers on the phenotypic variability of abdominal obesity and plasma lipid levels were observed in the Quebec Family Study cohort.[7] In the present study, we investigated the interactions among polymorphisms in the β2- and β3-ADR genes on

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total body adiposity and abdominal fat levels in the sedentary state and in response to endurance training in the HERITAGE Family Study cohort.

Methods

Subjects

The HERITAGE Family Study is a multicenter clinical trial conducted at five institutions. The specific aims, design, inclusion and exclusion criteria, and methodology of the study have been described in detail elsewhere.[9] The sample for the present study consists of 620 subjects, including 415 **(**198 males and 217 females) Whites and 205 (81 males and 124 females**)** Black subjects. To be enrolled in the study, the individuals were required to be in good health (ie, free of diabetes, cardiovascular diseases, or other chronic diseases) and to be sedentary at baseline (defined as no regular strenuous physical activity over the previous 6 months). The study protocol had been previously approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

Endurance exercise training program

The exercise training program was conducted on cycle ergometers and lasted 20 weeks. The exercise intensity was customized for each participant based on the heart rate–oxygen consumption (VO_2) relation measured at baseline. The training frequency was three times per week, starting at a heart rate representing an intensity of 55% of the subjects' initial VO_{2max} for 30 min during the first 2 weeks of training. The intensity and/or duration of the training program was adjusted every 2 weeks, such that the subjects were working at the heart rate associated with 75% VO_{2max} for 50 min during the last 6 weeks. Heart rate was monitored during all training sessions, and a computerized cycle ergometer system (Universal Gym Mednet, Cedar Rapids, IA, USA) adjusted power output (W) to maintain the target heart rate. Exercise sessions were supervised by trained exercise leaders. The program has been described in detail elsewhere.[10]

Phenotype measurements

A complete battery of tests was administered both prior to (baseline) and after (post) training. Body composition measures included hydrostatic weight, residual volume, and body density, from which fat mass and fat-free mass were estimated.[11] Abdominal fat was quantified before and after training by computerized tomography as described by Sjöström *et al*.[12]

Genotype determinations

Genomic DNA was isolated from lymphoblastoid cell lines by the proteinase K and phenol/chloroform technique. PCR analyses of the Arg16Gly polymorphism of the β2-ADR gene, as well as the Trp64Arg polymorphism of the β3-ADR gene, have been described earlier.[7]

Statistical analysis

All analyses were performed with the SAS Statistical Software Package (SAS Institute Inc., Cary, NC, USA). Pearson's correlation coefficients between fat mass and abdominal fat changes were also calculated. Abdominal total fat areas before and after training, as well as their changes in response to training, were normally distributed. Fat mass and fat-free mass were adjusted for age and sex. As a result of significant correlations between fat mass and abdominal fat phenotypes, the latter were adjusted for total fat mass, age and sex. Training response phenotypes (delta values) were adjusted for age, sex, baseline fat mass (except fat mass response) and baseline value of the phenotypes. Gene–gene interactions were analyzed using a MIXED procedure in the SAS software package. Nonindependence among family members was adjusted for using a 'sandwich estimator', which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the dependencies. The method is general, assuming the same degree of dependency among all members within a family. The analyses were performed separately for Black and White subjects. *P*-values were adjusted for multiple tests using the modified Bonferroni test.[13]

Results

None of the polymorphisms were independently associated with total body fat or abdominal fat level. Interactions between β2- and β3-ADR gene markers on training response phenotypes are depicted in Figures 1 and 2. Black subjects who were carriers of the β2-ADR Arg16 (Arg16+) and β3-ADR Arg64 (Arg64+) alleles showed a greater decrease in fat mass in response to endurance training than subjects with other allelic combinations (Arg16−/Arg64+($P=0.047$) or Arg16+/Arg64− ($P=0.011$) ($P<0.005$ for the $\beta2\times\beta3$-ADR marker interaction) (Figure 1). Abdominal total fat area changes in response to training followed the same trend as the changes in total fat ($P=0.010$ for the $\beta2\times\beta3$-ADR marker interaction) (Figure 2). Also the decrease in abdominal subcutaneous, but not visceral fat, was greatest in Black subjects with Arg16+/Arg64+ than in those with Arg16−/Arg64+($P=0.039$) or Arg 16+/Arg64− ($P=0.057$) ($P=0.023$ for the $\beta2\times\beta3$-ADR marker interaction). However, after adjustment for the change in fat mass, the associations for abdominal fat changes previously detected were no longer significant (not shown). After correction for multiple tests, the interactions remained statistically significant for the changes in total body fat mass and abdominal fat area, but became nonsignificant for the subcutaneous fat area. The

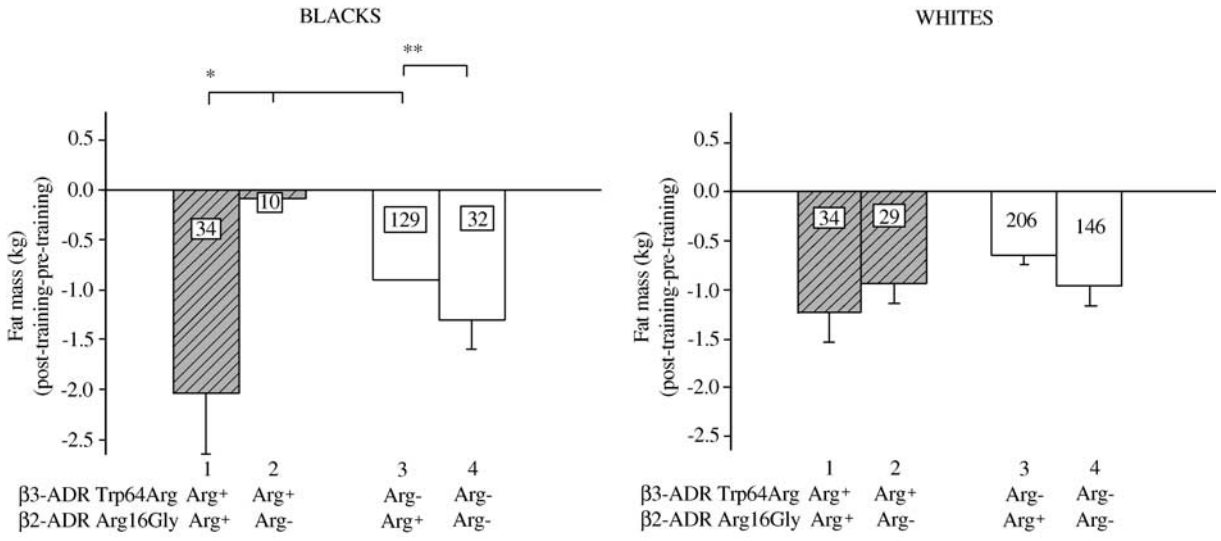

Figure 1 Total fat mass change (post-training−pretraining) by β3-ADR Trp64Arg and β2-ADR Arg16Gly genotypes in Black (left panel) and White (right panel) subjects. For the $\beta3 \times \beta2$-ADR marker interaction, $P<0.005$ among Black subjects. *$P=0.047$ between 1 and 2 and $P=0.011$ between 1 and 3. **$P=0.029$ between 3 and 4. The number of subjects embedded into columns.

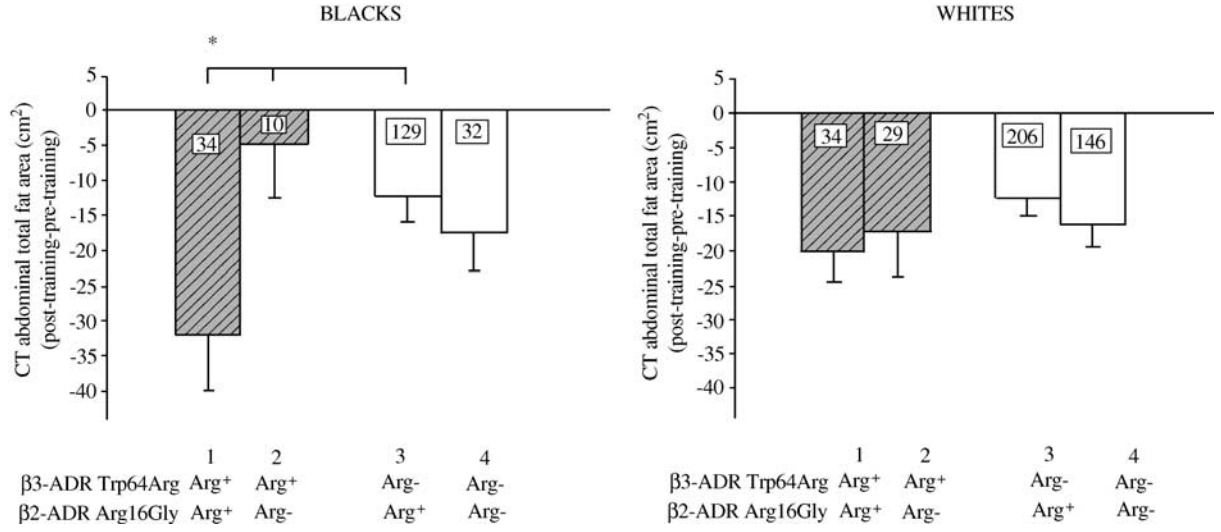

Figure 2 Total abdominal fat area changes (post-training−pretraining) in Black (left panel) and in White (right panel) subjects by β3-ADR Trp64Arg and β2-ADR Arg16Gly genotypes. For the $\beta3 \times \beta2$-ADR marker interaction, $P=0.010$ among Blacks. *$P=0.023$ between 1 and 2, $P=0.014$ between 1 and 3.

changes in total ($r=0.74$), subcutaneous ($r=0.73$) and visceral ($r=0.39$) abdominal fat correlated positively ($P<0.0001$) with changes in fat mass.

A similar trend, although it did not reach statistical significance, was observed among White subjects for fat mass (Figure 1) and abdominal total fat (Figure 2) and subcutaneous fat. Therefore, the relations between the genotype groups and adiposity changes in Black subjects were not significantly different from those in White subjects. The responses were also characterized by the same trends in men and women in both ethnic groups.

In Black subjects, 2.9 % of the total variation in the change of total fat mass and 1.7 % of total variation in the change of abdominal fat area in response to endurance training were explained by the β2- and β3-ADR marker interactions.

Discussion

β2-ADRs modulate lipolysis during physical exercise.[14] In the present investigation, an interaction effect between β2- and β3-ADR gene markers on total fat mass changes in response

to endurance training was demonstrated. The joint presence of the β2-ADR Arg16 and β3-ADR Arg64 alleles was associated with the largest total fat mass decrease in response to training among Black subjects. In addition, abdominal total and subcutaneous fat changes followed the same pattern as fat mass changes, consistent with the notion that the changes in abdominal fat are strongly dependent on the changes in total fatness. The interaction effects between β2- and β3-ADR gene markers on abdominal fat changes in Black subjects disappeared after adjusting for fat mass changes. Thus, abdominal fat training response seems to follow the training-induced changes in total fat mass. The latter is supported by the notion that the change in abdominal fat correlated positively with the change in fat mass. Reports from the Quebec Family Study[15] and HERITAGE Family Study[16] cohorts have provided evidence for the presence of genetic factors with pleiotropic effects on abdominal fat and overall body adiposity.

In cell culture studies and transfected cell systems,[17] the Arg16 allele carriers of the β2-ADRs showed increased responsiveness to endogenous catecholamines, which was thought to be dependent on the lack of downregulation of the receptor. In contrast, in human native adipocytes, β2-ADRs in Gly16 allele carriers showed an increased agonist affinity among female subjects.[18] Therefore, subjects carrying the Gly16 allele could be expected to display increased lipolysis and thus increased fat loss. However, Gly16 was not associated with any obesity-related phenotypes.[18] To summarize, the functional significance of β2-ADR Arg16Gly polymorphism is as yet unknown.

The β3-ADR Trp64Arg polymorphism has been shown to reduce β3 agonist-induced lipolysis in human omental fat.[19] Therefore, one would expect, as has been shown in a recent study,[20] that the presence of a β3-ADR Trp64Arg polymorphism would limit the ability of the subjects to lose intra-abdominal fat. However, although the Trp64Arg polymorphism of the β3-ADR gene has been associated with some obesity-related phenotypes in various populations,[21–27] its association with obesity remains controversial.[28,29] Furthermore, there was no association of the Trp64Arg β3-ADR variant with training-induced changes in body composition in the HERITAGE Family Study[30] neither among Black nor White subjects.

In the present study, the interaction between β2- and β3-ADR genes seems to affect training-induced changes in total fat in Black subjects but not White subjects. The interaction was characterized by the largest abdominal total fat decrease in response to training among subjects who carried both the β2-ADR Arg16 and β3-ADR Arg64 alleles. One could hypothesize that β2-ADR Arg16 and β3-ADR Arg64 allele carriers of the present study experienced enhanced exercise-induced adipose cell lipolysis that could explain a greater decrease in fatness with training. This interaction effect was not dependent on the initial value of the phenotype since training response phenotypes were adjusted for baseline value of the phenotypes. In addition, it is important to point out that the various genotype combinations were all trained with the same exercise program.

We have tested gene–gene interactions earlier in the Quebec Family Study cohort[7] although not on training response phenotypes. It was observed that gene–gene interactions among the ADR genes contributed to the phenotypic variability in abdominal obesity in White subjects from Caucasian origin in the latter study. In the present study, significant results were observed on Black subjects. Thus, for an unknown reason, there seems to be ethnic differences in the gene–gene interaction effects. In addition, it must be pointed out that the genetic factors underlying baseline phenotypes may be different from those modulating responses to endurance training. A focus of our current study was on β-ADR genes since they have been suggested to modulate lipolysis during physical exercise.[14]

In conclusion, the data of this study showed that β2- and β3-ADR gene–gene interactions seemed to be involved in the regulation of total fat mass changes in response to endurance training in Black subjects but not in White subjects. The abdominal fat training response seems to follow the training-induced changes in total fat mass. The interactions between β2- and β3-ADR gene markers of this study accounted for up to 3% of the variance in total fat mass change in response to endurance training in Black subjects.

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