The Trp64Arg Polymorphism of the β3-Adrenergic Receptor Gene Is Not Associated with Training-Induced Changes in Body Composition: The HERITAGE Family Study

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Abstract

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Objective: To investigate the association between the Trp64Arg polymorphism of the β3-adrenergic receptor gene and changes in body composition in response to endurance training.

Research Methods and Procedures: Adult sedentary white and black subjects participating in the HERITAGE Family Study were measured before and after 20 weeks on endurance training for the body mass index, fat mass, percentage of body fat, fat-free mass, sum of eight skinfolds, and subcutaneous, visceral, and total abdominal fat areas. The association between the Trp64Arg polymorphism and the response phenotypes, computed as the difference between pre- and post-training values, was tested by analysis of covariance separately in men and women. The gene by race interaction term was also tested.

Results: No race differences were observed for allelic and genotype frequencies. Training resulted in significant reduction of body fat in both men and women. No association of the Trp64Arg polymorphism was observed with training-induced changes for any of the body composition phenotypes in both men and women.

Discussion: These results suggest that the Trp64Arg polymorphism of the β3-adrenergic receptor gene is not related to changes in body composition in response to exercise training.

Key words: β3-adrenergic receptor gene, Trp64Arg polymorphism, body composition, abdominal fat, endurance training

Introduction

Several candidate genes have been tested for their association with obesity-related phenotypes (1). Among these genes, the β3-adrenergic receptor gene (ADRB3) has been the object of many studies particularly since the publication of three studies that showed that the Trp64Arg polymorphism was associated with weight gain (2), insulin resistance (3), and time of onset of noninsulin-dependent diabetes mellitus (4). The majority of association studies between obesity and the Trp64Arg polymorphism have yielded negative results. This was supported by a meta-analysis, which showed no effect of the ADRB3 Trp64Arg polymorphism on body mass index (BMI) (5). A second meta-analysis reported a moderate effect (6). The effect of this polymorphism differs according to the population studied and the phenotype investigated. Some studies in obese subjects showed that the Trp64Arg polymorphism was associated

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with BMI (7), body weight gain (2), and weight gain during pregnancy (8). Significant association has been reported with fasting insulin and glucose in some studies (9,10) but not in others (11–14). The ADRB3 gene polymorphism has also been tested for association with visceral obesity (10,15,16), but results remained controversial.

Very few studies have investigated the impact of this polymorphism on weight changes after intervention studies. One study showed that in response to a 3-month low-calorie diet and an exercise regimen, obese women carriers of the Arg64 allele exhibited a resistance to weight loss compared with noncarriers (9). In another study, changes in body weight after a 16-week treatment program, including a very-low-calorie diet regimen and exercise in markedly obese subjects, were not associated with the Trp64Arg polymorphism (12). Despite evidence showing that there are no functional and pharmacological differences between mutant and wild-type ADRB3 (11,17–19), it has been proposed that maximal cyclic adenosine 5′-monophosphate (cAMP) accumulation in response to various β3-adrenergic agonists was reduced in transfected cells carrying the mutation (19), suggesting a diminished coupling of the receptor with Gs proteins. Because endurance training improves maximal lipolysis after stimulation with post-β-adrenergic agents (20) and the ability of cAMP to stimulate hormone-sensitive lipase (21), we can speculate that the lower cAMP accumulation in carriers of the Arg64 allele will result in a lower abdominal fat mobilization in response to endurance training.

The purpose of the present study was, therefore, to determine whether the Trp64Arg polymorphism in the ADRB3 gene was associated with changes in body mass and body composition in response to a supervised 20-week endurance-training program in participants of the HERITAGE Family Study.

Research Methods and Procedures

Subjects

The HERITAGE Family Study aims, design, and methods have been described elsewhere (22). All subjects had to be sedentary and in good health to participate in the study protocol and meet a set of inclusion criteria (22). The population of the present study includes 480 (234 men and 246 women) white and 271 (89 men and 182 women) black adult subjects. They were tested for a battery of measurements before and after an endurance-training program of 20 weeks. 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 HERITAGE Endurance-Training Program

Briefly, subjects exercised under supervision on a cycle ergometer (Universal Aerobicycle IV, Cedar Rapids, IA) three times per week for 20 weeks following a standardized protocol. The cycle ergometer was connected to a computer system (Universal Mednet, Cedar Rapids, IA), which adjusted the power output to ensure that the target heart rates were maintained. For the first 2 weeks, subjects trained at a heart rate associated with 55% of their V_{O_2}max for 30 minutes. This was gradually increased to 50 minutes and a heart rate associated with 75% of their V_{O_2}max by the end of 14 weeks. These conditions were maintained through the remaining 6 weeks of the program (22).

Phenotype Measurements

Anthropometric and Body Density Measurements. These measurements have been described in detail previously (23). BMI was calculated as weight (kg)/height^2 (m^2). The sum of eight skinfolds (subscapular, suprailiac, abdominal, midaxillary, biceps, triceps, medial calf, and thigh) was used as an indicator of the amount of subcutaneous fat. Hydrostatic weighing was used to assess body density. Percentage of body fat was estimated from body density as described elsewhere (24) and fat mass and fat-free mass were derived.

Abdominal Visceral, Subcutaneous, and Total Fat Areas. Abdominal fat was assessed by computed tomography, as described previously (25). Scans were obtained between the fourth and fifth lumbar vertebrae. The abdominal visceral fat area was defined by drawing a line within the inner portion of the muscle walls surrounding the abdominal cavity. The abdominal subcutaneous fat area was derived by calculating the difference between total abdominal fat and abdominal visceral fat.

Genotype Determination

Polymerase Chain Reaction (PCR) Amplification of the Trp64Arg Polymorphism. DNA was extracted from lymphoblastoid cell lines after digestion by proteinase K and purification with phenol-chloroform (26). PCR amplification was carried out in a volume of 10 μL containing 250 ng DNA, 200 μM of each 2′-deoxyadenoside triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, 1X buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH = 7.5 at 25 °C), 2X of Q-solution, 300 nM of each primer, and 1 U of Taq polymerase (Perkin-Elmer/Cetus, Norwalk, CT). The forward primer was 5′-CCAGTGGGCTGCCAGGGG-3′ and the reverse primer was 5′-GCCAGTGGCGCCAACGG-3′. These primers generated a product of 255 bp, which was cut into fragments of 158 + 97 bp in the presence of the MspI cutting site in the presence of the Arg64 allele (2). The amplification protocol was one cycle of denaturation at 94 °C for 3 minutes, annealing at 65 °C and extension at 72 °C; 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 65 °C for 30 seconds and extension at 72 °C for 45 seconds; and one final elongation cycle at 72 °C for 10

minutes. 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 7.5 U of the restriction enzyme MspI 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 HaeIII was used as length marker to estimate the size of the digested DNA fragments. The allele without the MspI restriction site is designated here as Trp64 allele (255 bp), whereas the allele with the MspI restriction site is the Arg allele (158 bp + 97 bp).

Statistical Analysis

All statistical analyses were performed using SAS (27) software. A χ^2 test was performed to determine whether genotype frequencies were in Hardy–Weinberg equilibrium and to test for race differences in allelic frequencies. For all phenotypes, the response to training was computed as the difference between the post-training and baseline values. The resulting scores were adjusted as described below.

Two genotype groups were considered, i.e., carriers (genotypes Trp64Arg and Arg64Arg) and noncarriers (genotype Trp64Trp) of the Arg64 allele. Associations between the Trp64Arg polymorphism and each phenotype were investigated separately in men and women. We used an analysis of covariance (general linear model) procedure that included the effects of age (age, age^2, and age^3), baseline values, and race as covariates as well as the gene by race interaction term. For the abdominal fat phenotypes, the response phenotypes were further adjusted for changes in fat mass.

Results

Genotype and allele frequencies are presented in Table 1. Genotype frequencies were in Hardy–Weinberg equilibrium in both races. There were no race differences in genotype ($\chi^2 = 3.1$; $df = 2$; $p > 0.05$) and allele ($\chi 2 = 0.12$; $df = 1$; $p > 0.05$ frequencies. The frequency of the Arg64 allele was 8% and 10% in whites and blacks, respectively.

Descriptive statistics have been presented elsewhere for baseline and responses phenotypes (23). The endurance-training program resulted in significant reduction of body fat in men and women (23). Changes in fat-free mass were not significant. Results of associations with the ADRB3 Trp64Arg polymorphism and the response phenotypes in men and women are presented in Table 2. There were no significant evidence of gene by race interaction and, thus, association was tested in both races combined. No significant evidence of association was observed for any of the phenotypes investigated. In men, only a trend was observed for BMI ($p = 0.08$) with a tendency for carriers of the Arg64 allele to exhibit a greater reduction than did noncarriers.

Discussion

The major goal of the present study was to determine whether the Trp64Arg ADRB3 polymorphism was associated with changes in body composition after endurance training in sedentary subjects. To the best of our knowledge, this is the first study to test the role of this polymorphism on changes in body fat and body composition induced by exercise training performed under standardized laboratory conditions. The endurance-training program resulted in a significant reduction of percentage of body fat in both men ($-0.94\% \pm 0.10$) and women ($-0.78\% \pm 0.10$), but these changes were not significantly different between carriers and noncarriers of the Arg64 allele. These results suggest that the Trp64Arg ADRB3 polymorphism does not play a role in determining changes in body composition after exercise training. These results are concordant with those reported by Oksanen et al. (12), who found no association between the Trp64Arg ADRB3 polymorphism and weight loss in response to a hypocaloric diet in morbidly obese subjects. However, in obese Japanese women with type 2 diabetes, the Arg64 allele was found to be associated with changes in body weight after a low-calorie diet and an exercise regimen for 3 months (9). The design and population of these two intervention studies (9,12) were quite different from this one and the results are, thus, hardly comparable. Moreover, weight loss was not a main objective in the HERITAGE Family Study and, therefore, the magnitude of changes in body composition phenotypes is not as large as in actual weight loss studies.

Another goal of the present study was to investigate whether the Trp64Arg polymorphism in the ADRB3 gene was associated with abdominal obesity, after adjustment for fat mass, in both men and women. Unlike the results obtained in previous studies (10,16), we found no significant association between the Trp64Arg polymorphism and abdominal obesity. The discrepancy observed between the present study and the Japanese studies could be attributable to the higher frequency of the Arg64 allele in the Japanese population (~20%) compared with the white population (~10%). Another explanation is that the abdominal obesity phenotypes are not comparable between this study and the two other studies (10,16). The only other study with direct measure of visceral fat reported no effect of the Trp64Arg polymorphism (15). Our results also indicated that the Trp64Arg polymorphism has no significant effect on the training-induced changes of abdominal fat levels measured by computed tomography. Thus, despite an increased β-adrenergic tonus induced by exercise in the adipose tissue and the high distribution of the ADRB3 gene in abdominal visceral fat, our study suggests that the Trp64Arg polymorphism of ADRB3 gene has no effect on abdominal obesity.

Table 1. Genotype and allele frequencies of the ADRB3 Trp64Arg polymorphism in sedentary white and black subjects of the HERITAGE Family Study

		Allele frequency		Genotype frequencies		
	n*	**Trp64**	**Arg64**	**Trp64Trp**	**Trp64Arg**	**Arg64Arg**
Whites	480	0.92	0.08	0.85	0.14	0.01
Blacks	271	0.90	0.10	0.80	0.19	0.01

* Number of subjects.

Table 2. Associations of the Trp64Arg polymorphism with training-induced changes in body composition measured in men and women

	Noncarriers of the Arg64 allele	**Carriers of the Arg64 allele**	***p* value**
In men			
N	228–252	45–52	
BMI (kg/m^2)	−0.11 ± 0.05*	−0.32 ± 0.11*	0.08
Fat mass (kg)	−0.82 ± 0.14*	−1.25 ± 0.28*	0.17
Percentage body fat (%)	−0.87 ± 0.12*	−1.10 ± 0.26*	0.41
Fat-free mass (kg)	0.48 ± 0.10*	0.31 ± 0.21	0.47
Sum of 8 skinfolds (mm)	−6.07 ± 1.03*	−7.79 ± 2.27*	0.49
Subcutaneous abdominal fat (cm^2)	−9.64 ± 1.18*	−12.2 ± 2.4*	0.34
Visceral abdominal fat (cm^2)	−9.74 ± 2.26*	−6.85 ± 1.10*	0.25
Total abdominal fat (cm^2)	−16.0 ± 1.7*	−21.6 ± 3.5*	0.15
In women			
N	282–338	55–70	
BMI (kg/m^2)	−0.071 ± 0.051	−0.13 ± 0.11	0.63
Fat mass (kg)	−0.51 ± 0.13*	−0.77 ± 0.27*	0.38
Percentage body fat (%)	−0.65 ± 0.12*	−0.78 ± 0.26*	0.65
Fat-free mass (kg)	0.44 ± 0.07*	0.26 ± 0.15	0.27
Sum of 8 skinfolds (mm)	−5.18 ± 1.12*	−5.75 ± 2.44*	0.83
Subcutaneous abdominal fat (cm^2)	−7.53 ± 1.30*	−7.48 ± 2.76*	0.99
Visceral abdominal fat (cm^2)	−2.12 ± 0.74*	−2.61 ± 1.57	0.78
Total abdominal fat (cm^2)	−9.66 ± 1.47*	−9.85 ± 3.11*	0.95

Data are least square mean ± SEM (see text for details): *N*: number of subjects.
* Training-induced changes significantly ($p < 0.05$) different from zero.

We conclude that the Trp64Arg polymorphism of the ADRB3 gene is not associated with changes in body composition resulting from endurance training in sedentary men and women.

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References

1. **Chagnon YC, Pérusse L, Weisnagel SJ, Rankinen T, Bouchard C.** The human obesity gene map: the 1999 update. *Obes Res.* 2000;8:89–117.
2. **Clement K, Vaisse C, Manning BS, et al.** Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med.* 1995;333:352–4.
3. **Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC.** Association of a polymorphism in the β3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med.* 1995;333:348–51.
4. **Walston J, Silver K, Bogardus C, et al.** Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic-receptor gene. *N Engl J Med.* 1995;333:343–7.
5. **Allison DB, Heo M, Faith MS, Pietrobelli A.** Meta-analysis of the association of the Trp64Arg polymorphism in the β3-adrenergic receptor with body mass index. *Int J Obes Relat Metab Disord.* 1998;22:559–66.
6. **Fujisawa T, Ikegami H, Kawaguchi Y, Ogihara T.** Meta-analysis of the association of Trp64Arg polymorphism of β3-adrenergic receptor gene with body mass index. *J Clin Endocrinol Metab.* 1998;83:2441–4.
7. **Clement K, Manning BS, Basdevant A, Strosberg AD, Guy-Grand B, Froguel P.** Gender effect of the Trp64Arg mutation in the β3-adrenergic receptor gene on weight gain in morbid obesity. *Diabetes Metab.* 1997;23:424–7.
8. **Festa A, Krugluger W, Shnawa N, Hopmeier P, Haffner SM, Schernthaner G.** Trp64Arg polymorphism of the β3-adrenergic receptor gene in pregnancy: association with mild gestational diabetes mellitus. *J Clin Endocrinol Metab.* 1999;84:1695–9.
9. **Sakane N, Yoshida T, Umekawa T, Kogure A, Takakura Y, Kondo M.** Effects of Trp64Arg mutation in the β3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. *Diabetes Care.* 1997;20:1887–90.
10. **Sakane N, Yoshida T, Umekawa T, Kondo M, Sakai Y, Takahashi T.** β3-adrenergic-receptor polymorphism: a genetic marker for visceral fat obesity and the insulin resistance syndrome. *Diabetologia.* 1997;40:200–4.
11. **Li LS, Lönnqvist F, Luthman H, Arner P.** Phenotypic characterization of the Trp64Arg polymorphism in the β3-adrenergic receptor gene in normal weight and obese subjects. *Diabetologia.* 1996;39:857–60.
12. **Oksanen L, Mustajoki P, Kaprio J, et al.** Polymorphism of the β3-adrenergic receptor gene in morbid obesity. *Int J Obes Relat Metab Disord.* 1996;20:1055–61.
13. **Nagase T, Aoki A, Yamamoto M, et al.** Lack of association between the Trp64 Arg mutation in the β3-adrenergic receptor gene and obesity in Japanese men: a longitudinal analysis. *J Clin Endocrinol Metab.* 1997;82:1284–7.
14. **Büettner R, Schäffler A, Arndt H, et al.** The Trp64Arg polymorphism of the β3-adrenergic receptor gene is not associated with obesity or type 2 diabetes mellitus in a large population-based Caucasian cohort. *J Clin Endocrinol Metab.* 1998;83:2892–7.
15. **Gagnon J, Mauriège P, Roy S, et al.** The Trp64Arg mutation of the β3-adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish obese subjects cohorts. *J Clin Invest.* 1996;98:2086–93.
16. **Kim-Motoyama H, Yasuda K, Yamaguchi T, et al.** A mutation of the β3-adrenergic receptor is associated with visceral obesity but decreased serum triglyceride. *Diabetologia.* 1997;40:469–72.
17. **Tataranni PA, Pratley R, Shuldiner A, Ravussin E.** β3-adrenergic receptor gene variant and lipid metabolism in Pima Indians. *Diabetologia.* 1997;40:123–4.
18. **Candelore MR, Deng L, Tota LM, Kelly LJ, Cascieri MA, Strader CD.** Pharmacological characterization of a recently described human β3-adrenergic receptor mutant. *Endocrinology.* 1996;137:2638–41.
19. **Pietri-Rouxel F, St John Manning B, Gros J, Strosberg AD.** The biochemical effect of the naturally occurring Trp64→Arg mutation on human β3-adrenoceptor activity. *Eur J Biochem.* 1997;247:1174–9.
20. **Mauriège P, Prud'Homme D, Marcotte M, Yoshioka M, Tremblay A, Després JP.** Regional differences in adipose tissue metabolism between sedentary and endurance-trained women. *Am J Physiol.* 1997;273:E497–E506.
21. **Arner P.** Impact of exercise on adipose tissue metabolism in humans. *Int J Obes Relat Metab Disord.* 1995;19(suppl 4):S18–S21.
22. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J.** The HERITAGE Family Study: aims, design, and measurement protocol. *Med Sci Sports Exerc.* 1995;27:721–9.
23. **Wilmore JH, Després JP, Stanforth PR, et al.** Alterations in body weight and composition consequent to 20 wk of endurance training: the HERITAGE Family Study. *Am J Clin Nutr.* 1999;70:346–52.
24. **Wilmore JH, Stanforth PR, Domenick MA, et al.** Reproducibility of anthropometric and body composition measurements: the HERITAGE Family Study. *Int J Obes Relat Metab Disord.* 1997;21:297–303.
25. **Ferland M, Després JP, Tremblay A, et al.** Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr.* 1989;61:139–48.
26. **Dionne FT, Turcotte L, Thibault MC, Boulay MR, Skinner JS, Bouchard C.** Mitochondrial DNA sequence polymorphism, VO_2max, and response to endurance training. *Med Sci Sports Exerc.* 1991;23:177–85.
27. **SAS Institute.** *Statistical Analysis System, Version 6.* 12th ed. Cary, NC: SAS Institute; 1996.