No Effect of the Trp64Arg β_3-Adrenoceptor Gene Variant on Weight Loss, Body Composition, or Energy Expenditure in Obese, Caucasian Postmenopausal Women

Eric S. Rawson, Amy Nolan, Kristi Silver, Alan R. Shuldiner, and Eric T. Poehlman

The Trp64Arg polymorphism in the β_3-adrenoceptor gene has been associated with increased prevalence of obesity, type 2 diabetes, and low rates of energy expenditure, although these findings are not unanimous. It is currently unknown if the presence of the Trp64Arg gene variant impedes the loss of body weight in obese, postmenopausal women via a reducing effect on energy expenditure. The objective of this study was to compare body composition and energy expenditure in carriers and noncarriers of the Trp64Arg variant in the β_3-adrenoceptor before and after weight loss. We measured body composition, total daily energy expenditure (TEE), resting metabolic rate (RMR), physical activity energy expenditure (PAEE), thermic effect of feeding (TEF), and respiratory quotient (RQ) in 34 obese, postmenopausal women (19 carriers and 15 noncarriers for the Trp64Arg variant) before and after a weight loss intervention. There were no differences in body composition or daily energy expenditure and its components between the 2 groups at baseline. There were significant reductions in body mass, body mass index (BMI), percent body fat, fat-free mass, and fat mass (main effect, all $P < .0001$) when analyzed with the 2 genotypes combined, but no significant differences between carriers and noncarriers with respect to change in these variables (group × time interaction term, all $P > .05$). Total energy expenditure tended to be reduced (490 kJ · d^{-1}, $P = .13$) in both groups following weight loss, but there was no significant group × time interaction term ($P = .78$), indicating no difference in the response of the 2 genotypes. There was a 9% reduction in RMR (611 kJ · d^{-1}, $P < .001$) when both groups were considered together, but no significant group × time interaction term ($P = .84$), suggesting that both groups responded in a similar manner to the weight loss intervention. PAEE and the TEF were not different following weight loss (both $P > .60$). There was a trend for RQ to be reduced after weight loss ($P = .07$), but there was no difference between carriers or noncarriers of the Trp64Arg variant ($P = .58$). In summary, we found that obese postmenopausal women who carry the Trp64Arg variant in the β_3-adrenoceptor had similar changes in body composition and energy expenditure to noncarriers of the variant in response to prolonged caloric restriction. These results suggest that the presence of the Trp64Arg variant in the β_3-adrenoceptor should not be a hindrance to weight reduction.

THE TrpP64Arg POLYMORPHISM in the β_3-adrenoceptor gene has been associated with increased prevalence of obesity, type 2 diabetes, and low rates of energy expenditure.[1-5] However, these findings are not unanimous, as approximately half of the studies demonstrate an association between the Trp64Arg gene variant and obesity phenotypes and others find no meaningful effect. Two recent meta-analyses have even provided discordant findings regarding the effects of the Trp64Arg polymorphism on body fatness.[6,7] Discordant findings among investigators may result in part from cohort differences in ethnicity, gender, age, and degree of adiposity and body composition assessment techniques.

Cross-sectional studies draw inferences from the presence of the Trp64Arg polymorphism and its association with obesity. A more rigorous experimental approach is to examine the interaction between the Trp64Arg polymorphism and an environmental perturbation (ie, weight loss on obesity-related phenotypes). It is currently unknown if the presence of the Trp64Arg gene variant influences the magnitude of weight loss, although one group reported in a letter that Japanese carriers of the gene variant lost less weight than noncarriers following a diet and exercise intervention program.[5] There is a suggestion that a lower resting metabolic rate (RMR) at baseline in carriers of the gene variant may have blunted their ability to lose weight, but these data were published in letter form and few methodological and experimental details are provided. Because daily energy expenditure and its components ultimately influence weight loss, it is logical to hypothesize that the Trp64Arg polymorphism may impede the loss of body weight in postmenopausal women via a reducing effect on energy expenditure. We recently reported that carriers of the Trp64Arg variant in the β_3-adrenoceptor lose less visceral fat following weight loss than noncarriers.[9] Additionally, we reported that never-obese individuals who harbor the Trp64Arg variant in the β_3-adrenoceptor have decreased rates of energy expenditure compared to obese subjects who are carriers of the gene variant after adjustment for age, fat mass, and fat-free mass.[8] This indicates that the obese state may mask the effect of the Trp64Arg gene variant on energy expenditure, and that a weight loss intervention may be necessary for the effects of the gene variant to be detected. We tested the hypothesis that older obese women with the Trp64Arg variant in the β_3-adrenoceptor will lose less body fat during a medically supervised weight loss program compared to women without the variant due to lower rates of resting and physical activity energy expenditure (PAEE).

From the Department of Medicine, University of Vermont, Burlington, VT; the Division of Endocrinology, Diabetes, and Nutrition, Departments of Medicine, University of Maryland School of Medicine, and Geriatrics Research and Education Clinical Center, Veterans Administration Center, Baltimore, MD.

Submitted September 28, 2001; accepted December 31, 2001.

Supported by the General Clinical Research Center of the University of Vermont (RR-00109) and National Institutes of Health Grant No. DK 52754 (to E.T.P.).

Address reprint requests to Eric S. Rawson, PhD, Preventive and Behavioral Medicine, University of Massachusetts Medical School, 55 Lake Ave N Shaw SH2-229, Worcester, MA 01655.

0026-0495/02/5106-0015$35.00/0

doi:10.1053/meta.2002.32781

MATERIALS AND METHODS

Experimental Design

Initially, subjects were screened for the Trp64Arg gene variant. After genotyping, subjects were tested during an overnight stay in the General Clinical Research Center at the University of Vermont. On the first day, body mass and body mass index (BMI) were determined, and subjects were dosed with doubly labeled water to measure total energy expenditure over 10 days. The morning after treatment, following an overnight fast, RMR was determined from indirect calorimetry, and following a standardized meal, postprandial energy expenditure was determined from indirect calorimetry. All tests were repeated in an identical sequence following the weight loss intervention. Prior to each testing session, volunteers were weight stabilized for at least 1 month.

Subjects

Obese, postmenopausal women were recruited by local advertisement from the Burlington, VT area. Initially, 491 women were screened, of which 38 were heterozygous for the Trp64Arg variant. Of this initial cohort 34 obese women (19 carriers for the Trp64Arg variant, 15 noncarriers) completed all pre- and post-testing and were included in this analysis. Baseline data on energy expenditure, free fatty acid kinetics (palmitate infusion), and intra-abdominal fat from some of these subjects have been previously reported.[8] Changes in intra-abdominal fat, insulin sensitivity, and cardiovascular risk factors in some of these subjects following weight loss have been reported elsewhere.[9] Inclusion criteria for the study were (1) a BMI $\geq$ 27 kg/m^2, (2) the absence of a menstrual period for at least 1 year, and (3) a follicle-stimulating hormone (FSH) level greater than 30 U $\cdot$ L^{-1}. All participants were apparently healthy and had no history or evidence on physical examination of (1) cardiovascular disease, peripheral vascular disease, or stroke; (2) diabetes; (3) moderate to severe hypertension (resting blood pressure $>$ 170/100 mm Hg); (4) orthopedic limitations or history of pathologic fracture; (5) body weight fluctuation greater than 5 kg in the previous 6 months; (6) thyroid or pituitary disease; and (7) medication that could affect cardiovascular function or metabolism. Participants also had to be sedentary ($<$2 times a week of exercise participation), nonsmokers, and low to moderate alcohol consumers. All participants signed an informed consent document prior to the study, and the University of Vermont Medical Sciences Committee on Human Research approved this study.

Genotyping

Genotyping for the Trp64Arg variant in the β_3-adrenoceptor gene was performed as previously described[10] by polymerase chain reaction–restriction fragment length polymorphism analysis.

Weight Loss Protocol

Subjects were entered into a medically supervised weight loss program aimed at reducing body mass to less than 120% of ideal as determined from the Metropolitan Life Insurance Tables. The program consisted of a 5,000 kJ $\cdot$ d^{-1} American Heart Association Step 2 Diet. Throughout the study volunteers consulted with a registered dietitian at the General Clinical Research Center regarding energy and macronutrient composition. Food was self-selected with or without the use of a modified fasting supplement (Medifast, Take Shape; Jason Pharmaceuticals, Baltimore, MD). Before and after the weight loss protocol, volunteers were submitted to a weight stabilization period ($\pm$2 kg of body mass) that lasted on average 43 $\pm$ 27 days before pre-testing and 84 $\pm$ 44 days before post-testing. Subjects were in the weight loss program for an average of 13.5 $\pm$ 2.6 months, including weight stabilization prior to metabolic testing.

Diet Stabilization Period

Three days before metabolic testing, dietary intake was provided and standardized for all subjects ($\approx$30% of energy as fat, 58% as carbohydrate, and 12% as protein) by the metabolic kitchen of the General Clinical Research Center.

Body Composition

Body mass was measured to the nearest 0.1 kg on a calibrated balance. Using the values of total body water determined by the doubly labeled water technique, and a hydration constant of 0.73 for fat-free mass, percent body fat, fat-free mass, and fat mass were calculated.[11]

Total Daily Energy Expenditure

Total daily energy expenditure (TEE; kJ $\cdot$ d^{-1}) was determined from doubly labeled water over a 10-day period. During that period, subjects were asked to maintain their normal daily physical activity routines. These individuals, however, were not participating in any structured exercise training program. Between noon and 4 PM, a premixed-dose containing 0.078 g of ^2H_2O and 0.092 g of $H_2^{18}O$ per kilogram of body mass was orally consumed by each subject to measure TEE over 10-day period using the method of Schoeller and van Santen.[12] One urine sample was collected prior to dosing, 2 the following morning, and 2 samples 10 days later. Samples were frozen at -20°C in Vacutainers (Becton Dickinson, Franklin Lakes, NJ) until later analysis for ^2H and ^{18}O enrichments by isotope ratio mass spectrometry. ^{18}O isotopic enrichments were determined from the CO_2 equilibration techniques and ^2H enrichments were determined by the zinc catalyst method of Wong et al.[13] Rate of CO_2 production (r_{CO2}, mol/d) was calculated using equation 3 of Speakman et al[14]: $r_{CO2} = N/2.196 \times (c_O k_O - c_H k_H)$, where k_O and k_H are the elimination rates of ^{18}O and ^2H tracers from the body, and c_O and c_H are the dilution spaces for ^{18}O and ^2H tracers as recommended by Racette et al.[15] Assuming a respiratory quotient (RQ) of the food consumed of 0.85,[16] total CO_2 production was converted to TEE (kJ $\cdot$ d^{-1}) using the Weir formula.[17]

Resting Metabolic Rate

RMR (kJ $\cdot$ d^{-1}) was measured by indirect calorimetry using the ventilated hood technique,[18] following an overnight, 12-hour fast in the General Clinical Research Center. Respiratory gas analysis was performed using a Deltatrac metabolic cart (Sensormedics, Yorba Linda, CA). RMR (kJ $\cdot$ d^{-1}) was calculated from the equation of Weir.[17] The test-retest correlation coefficient within 1 week has been shown to be 0.90 for RMR in our laboratory. Additionally, we calculated the RQ from indirect calorimetry.

Thermic Effect of Feeding

Thermic effect of feeding (TEF; kJ $\cdot$ d^{-1}) was measured by indirect calorimetry using the ventilated hood technique. After a standardized liquid meal (Ensure; Abbott Laboratories, Abbott Park, IL), of 30% of daily energy requirements, measurements of indirect calorimetry were performed for 3 hours. Percentage of energy expended relative to energy consumed was calculated, extrapolated to 24 hours, and used as the energy expenditure of feeding.

Physical Activity Energy Expenditure

Doubly labeled water in conjunction with indirect calorimetry was used to measure PAEE. PAEE was calculated using the following equation: PAEE (kJ $\cdot$ d^{-1}) = TEE – (RMR + TEF).

Statistical Analysis

All data were analyzed using Statistica for Windows (StatSoft Inc, Tulsa, OK). Body mass, BMI, percent body fat, fat-free mass, fat mass, TEE, RMR, TEF, PAEE, and RQ were analyzed with a repeated-

Table 1. Descriptive Characteristics of the Subjects Prior to Weight Loss

	Trp64Arg Carriers	Noncarriers
No.	19	15
Age (yr)	57.8 ± 6.6	57.5 ± 4.2
Body mass (kg)	94.4 ± 17.0	92.7 ± 7.7
BMI (kg m^2)	35.6 ± 6.3	35.3 ± 3.1
Percent body fat	48.6 ± 4.3	49.8 ± 4.1
Fat-free mass (kg)	48.0 ± 6.0	46.4 ± 3.2
Fat mass (kg)	46.4 ± 11.9	46.3 ± 6.8

NOTE. Data are presented as the mean ± SD.

measures analysis of variance (ANOVA) with main effects of group and time. Tukey's post hoc tests were conducted when the ANOVA revealed a significant interaction. The frequency of subjects with the Trp64Arg gene variant who dropped out of the study versus subjects who completed the study was compared with a chi-square analysis. Data are presented as means ± SD. Significance was set a priori at $P \leq .05$.

RESULTS

Since it is possible that differences between subjects who did not complete the study and those who did complete the dietary intervention and subsequent metabolic testing could potentially confound our results, we examined whether subjects who dropped out of the study were different from subjects who completed the study in terms of age, body composition, energy expenditure, and genotype. Subjects cited a variety of reasons for dropping out of the study, including 18 who stated they were unhappy with their rate of weight loss, 8 were noncompliant with the protocol and were dropped by the investigators, 3 had medical problems unrelated to the study, 2 moved, 2 cited job stress, 1 cited family problems, and 1 cited extended periods of travel. Of those who dropped out, 19 were carriers of the Trp64Arg gene variant and 16 were noncarriers. There were no differences in the frequency of the Trp64Arg gene variant between subjects who dropped out of the study and subjects who completed the study (χ^2, $P = .40$). Subjects who dropped out were significantly younger (54.7 *v* 57.7 years, $P = .02$) and had significantly lower RQ values (0.83 *v* 0.85, $P = .02$); they also tended to have a lower percent body fat (46.9% *v* 49.1%, $P = .07$) and higher RMR (6,987 *v* 6,544 kJ · d^{-1}, $P = .07$), but were not different in terms of body mass, BMI, fat mass, fat-free mass, TEE, PAEE, or TEF.

Descriptive characteristics of subjects who completed the study are presented in Table 1. There were no differences in body composition or daily energy expenditure and its components between the 2 groups (carriers *v* noncarriers) at baseline. Changes in body composition are presented in Fig 1. There were significant reductions in body mass, BMI, percent body fat, fat-free mass, and fat mass (main effect of time, all $P < .0001$) when analyzed with the 2 genotypes combined, indicating that the weight loss protocol was an effective intervention. When both groups were combined, subjects lost on average 16% of their body mass, 16% of their BMI, 11% body fat, 7% fat-free mass, and 25% fat mass. There were no significant differences between carriers and noncarriers with respect to change in these variables (group × time interaction term, all $P > .05$). It should be noted that despite significant weight loss, the average subject could still be classified as obese (post–weight loss BMI and percent body fat, 30.3 ± 5.9 kg/m^2 and

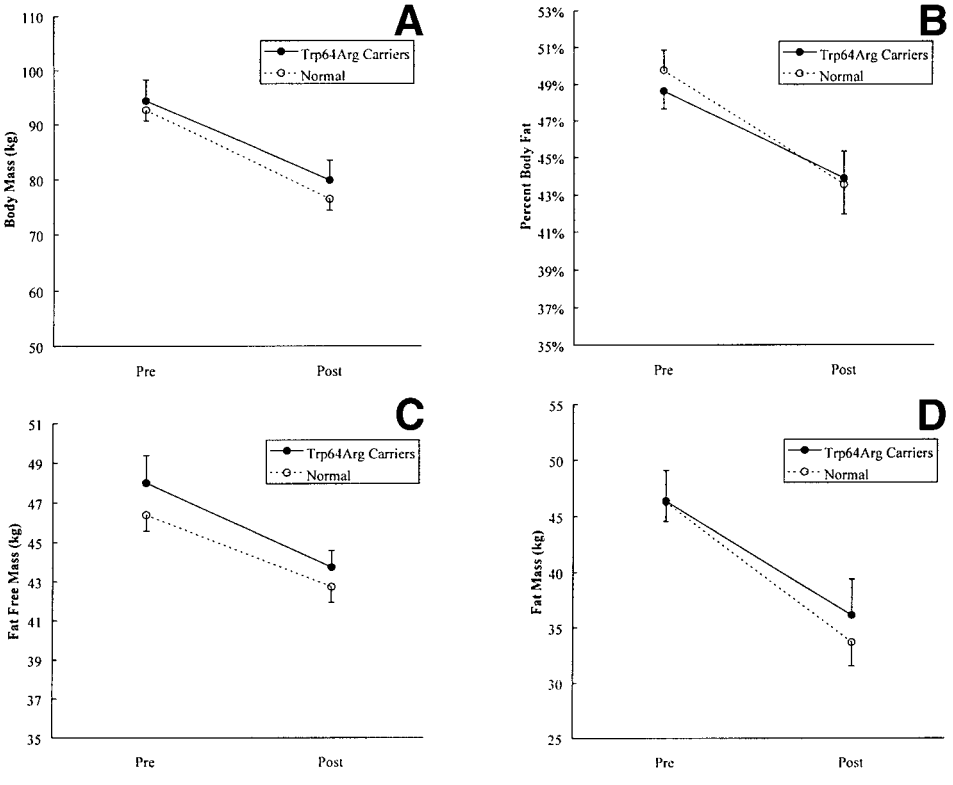

Fig 1. (A) Body mass, (B) percent body fat, (C) fat free mass, and (D) fat mass pre– and post–weight loss in carriers and noncarriers of the Trp64Arg adrenoceptor gene variant. Body mass, BMI, percent body fat, fat-free mass, and fat mass were significantly reduced from baseline when analyzed with the 2 genotypes combined (main effect of time, all $P < .0001$). There were no significant differences between carriers and noncarriers with respect to change in these variables (group × time interaction term, all $P > .05$).

Table 2. Energetic Adaptations to Weight Loss in Carriers and Noncarriers of the Trp64Arg Adrenoceptor Gene Variant

	Trp64Arg Carriers		*Noncarriers*		*P Values*	
	Pre	Post	Pre	Post	Main Effect of Time	(Gene × Time Interaction)
TEE	11,514 ± 2146	11,100 ± 2071	11,042 ± 1582	10,447 ± 1820	.13	.78
RMR	6565 ± 1121	5941 ± 874	6514 ± 623	5925 ± 477	<.0001	.84
PAEE	3615 ± 1502	3845 ± 1971	3184 ± 1503	3230 ± 1753	.68	.78
TEF	1335 ± 565	1318 ± 552	1343 ± 247	1293 ± 402	.69	.84
RQ	0.85 ± 0.05	0.83 ± 0.04	0.85 ± 0.04	0.84 ± 0.04	.07	.58

NOTE. Units are kJ × d^{-1}. Data are presented as the mean ± SD.

43.9% ± 8.4% in carriers and 29.1 ± 3.8 kg/m^2 and 43.5% ± 6.9% in noncarriers, respectively).

Daily energy expenditure and its components are presented in Table 2. TEE tended to be reduced (490 kJ · d^{-1}, $P = .13$) in both groups following weight loss, but there was no significant group × time interaction term ($P = .78$), indicating no difference in the response of the 2 genotypes. The weight loss protocol resulted in a 9% reduction in RMR of 611 kJ · d^{-1} ($P < .001$) when both groups were considered together. However, there was no significant genotype × time interaction term ($P = .84$), as both Trp64Arg carriers and noncarriers responded in a similar manner to the weight loss intervention. PAEE and the TEF were not different following weight loss (main effect of time $P = .68$ and $P = .69$, respectively). There was a trend for RQ to be reduced after weight loss ($P = .07$), but there was no difference between carriers or noncarriers of the Trp64Arg variant ($P = .58$).

DISCUSSION

In the present study, we tested the hypothesis that older obese women with the Trp64Arg variant in the β_3-adrenoceptor would lose less body fat during a medically supervised weight loss program compared to women without the variant, due potentially to lower rates of RMR and PAEE. Contrary to our hypothesis, although there were significant changes in body composition, we found no difference between carriers and noncarriers in the loss of body mass, percent body fat, fat-free mass, and fat mass following the weight loss intervention. Similarly, there was no difference in the changes in total daily energy expenditure and its components in response to weight reduction. Our results suggest that the energetic adaptation to weight loss is similar between carriers and noncarriers of the Trp64Arg variant.

The majority of studies that have examined an association of the Trp64Arg variant in the β_3-adrenoceptor and obesity are observational, and data are discrepant among investigations.[6,7] Independently, the effects of the Trp64Arg variant in the β_3-adrenoceptor on obesity may be modest, and an observational study design thus may not be a robust approach to detect the modest effects of the gene. The weakness of the observational study design is apparent when one considers that 2 recent meta-analyses have reached opposite conclusions regarding the role of the Trp64Arg variant on BMI.[6,7] Allison et al[7] concluded the Trp64Arg polymorphism is not significantly associated with BMI, whereas Fujisawa et al[6] reported a significant association between the Trp64Arg polymorphism and BMI. We believe that an intervention-weight loss paradigm may represent a more rigorous test to examine adaptive changes in fat mass and energy expenditure based on a genetic strategy. In addition to our experimental design, our conclusions are strengthened by our methodological approaches that included direct measures of energy expenditure with doubly labeled water. Also, prior to metabolic testing all subjects underwent diet and weight stabilization periods, which is critical to accurately assess changes in energy expenditure following weight loss. The weight stabilization period ensures that subjects were not tested during the acute phase of energy restriction, during which the residual effects of the calorically restricted state can influence energy expenditure. Recently, Weinsier et al[19] reported that energy restriction produces transient decreases in energy expenditure that normalizes upon return to energy-balanced conditions. Thus, the failure to establish energy balance, after weight loss, gives the misleading impression that weight-reduced persons are energy conservative and predisposed to weight regain. One disadvantage of this prospective interventional research design is that it is only practical to study a relatively small number of subjects. Thus we may not have adequate power to detect modest gene effects on the phenotypes. However, we do not believe that the negative findings in the current study are due to a type 2 error. Differences in energy expenditure between genotypes never approached significance; thus, the addition of a greater number of subjects would unlikely change our interpretation. Additionally, with our sample size we were unable to study Trp64Arg homozygotes, and therefore no conclusions can be drawn regarding their response to caloric restriction.

We have recently reported that the Trp64Arg variant in the β_3-adrenoceptor results in an impaired ability to lose visceral fat in response to weight loss. Tchernof et al[9] assessed the effects of the Trp64Arg variant on total and visceral adipose tissue loss, insulin sensitivity, and cardiovascular disease risk factors in response to weight reduction in 24 obese, postmenopausal women. In response to weight loss, carriers and noncarriers of the Trp64Arg allele had similar reductions in body mass, fat-free mass, fat mass, and percent body fat. However, carriers of the Trp64Arg gene variant lost 43% less visceral fat compared to noncarriers. When considered in the context of previous work from our laboratory, we would suggest that the Trp64Arg variant in the β_3-adrenoceptor gene may blunt regional, but not total fat loss during caloric restriction. The observation that the Trp64Arg variant in the β_3-adrenoceptor gene influences visceral but not total fat loss is also supported by previous studies that indicate a small role for the β_3-adrenoceptor in lipolysis in subcutaneous fat stores.[20-22]

There are 2 potential reasons for the absence of an effect of the Trp64Arg variant in the β_3-adrenoceptor on body compo-

sition and energy expenditure reported in the current study. First, the role of the Trp64Arg variant in the β_3-adrenoceptor gene on lipolysis in subcutaneous fat may be minor compared to its effects on lipolysis in visceral fat.[20-22] Thus, in the presence of significant fat loss (25% of total fat mass) it could be difficult to detect the modest effect of this gene variant. Second, it is possible that such an aggressive weight loss program may mask the modest effects of the gene variant. That is, the environmental challenge may overwhelm any modest gene effects of the Trp64Arg variant on body composition and energy expenditure. To our knowledge, only one other study has examined the influence of the Trp64Arg variant in the β_3-adrenoceptor on energy expenditure and fat loss in response to caloric restriction.[5] Yoshida et al[5] reported that carriers of the gene variant (homozygotes and heterozygotes) lost less weight (5 kg) than noncarriers (8 kg) following a diet and exercise intervention program. There is a suggestion that a lower RMR at baseline in carriers of the gene variant may have blunted their ability to lose weight. Direct comparison with the present study is difficult because their data were published in letter form and few methodological and experimental details are provided. Furthermore, these studies were performed in Japanese subjects whose genetic background almost certainly differs from Caucasians and their study contained a higher proportion of Trp64Arg β_3-adrenoceptor homozygotes than our study. Nonetheless, we find no differences at baseline in energy expenditure or in response to significant caloric restriction. It is possible that the discrepancy between our findings and those of Yoshida et al[5] resulted from the large difference in the amount of weight lost between the 2 studies. Mean weight loss in carriers of the Trp64Arg variant in our study was approximately 15 kg, whereas obese subjects in the study of Yoshida et al[5] lost 5 kg. Thus, it is possible that the modest effects of the gene in our study were overwhelmed by the magnitude of the weight loss. Clearly, further studies are needed to examine the interaction between gene variants and environment challenges such as caloric restriction on body composition and energy expenditure.

In summary, we found that obese postmenopausal women who carry the Trp64Arg variant in the β_3-adrenoceptor responded similarly to noncarriers of the variant in response to prolonged caloric restriction. That is, changes in body composition and energy expenditure were similar between carriers and noncarriers of the gene variant following the weight loss intervention. These results suggest that the presence of the Trp64Arg variant in the β_3-adrenoceptor should not be a hindrance to weight reduction.

ACKNOWLEDGMENT

We would like to thank all participants for their time and effort in this study. We also thank Denise DeFalco-McGeein, RNC, NP for her clinical management of our volunteers, Christine Potter, BA for her technical assistance with the doubly labeled water analysis, and Demian Lewis for performing the genotype analysis.

REFERENCES

1. Walston J, Silver K, Bogardus C et al: Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the beta 3-adrenergic-receptor gene. N Engl J Med 333:343-347, 1995

2. Valve R, Heikkinen S, Rissanen A, et al: Synergistic effect of polymorphisms in uncoupling protein 1 and β_3-adrenergic receptor genes on basal metabolic rate in obese Finns. Diabetologia 41:357-361, 1998

3. Sakane N, Yoshida T, Umekawa T, et al: Effects of Trp64Arg mutation in the beta 3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. Diabetes Care 20:1887-1890, 1997

4. Sipilainen R, Uusitupa M, Heikkinen S, et al: Polymorphism of the beta3-adrenergic receptor gene affects basal metabolic rate in obese Finns. Diabetes 46:77-80, 1997

5. Yoshida T, Sakane N, Umekawa T, et al: Mutation of beta 3-adrenergic-receptor gene and response to treatment of obesity. Lancet 346:1433-1434, 1995

6. Fujisawa T, Ikegami H, Kawaguchi Y, et al: Meta-analysis of the association of Trp64Arg polymorphism of beta 3-adrenergic receptor gene with body mass index. J Clin Endocrinol Metab 83:2441-2444, 1998

7. Allison DB, Heo M, Faith MS, et al: Meta-analysis of the association of the Trp64Arg polymorphism in the β_3 adrenergic receptor with body mass index. Int J Obes Relat Metab Disord 22:559-566, 1998

8. Tchernof A, Starling RD, Walston JD, et al: Obesity-related phenotypes and the beta3-adrenoceptor gene variant in postmenopausal women. Diabetes 48:1425-1428, 1999

9. Tchernof A, Starling RD, Turner A, et al: Impaired capacity to lose visceral adipose tissue during weight reduction in obese postmenopausal women with the Trp64Arg beta3-adrenoceptor gene variant. Diabetes 49:1709-1713, 2000

10. Widen E, Lehto M, Kanninen T, et al: Association of a polymorphism in the β_3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333:348-351, 1995

11. Wang Z, Deurenberg P, Wang W, et al: Hydration of fat-free body mass: Review and critique of a classic body-composition constant. Am J Clin Nutr 69:833-841, 1999

12. Schoeller DA, van Santen E: Measurement of energy expenditure in humans by doubly labeled water method. J Appl Physiol 53:955-959, 1982

13. Wong WW, Lee LS, Klein PD: Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. Am J Clin Nutr 45:905-913, 1987

14. Speakman JR, Nair KS, Goran MI: Revised equations for calculating CO_2 production from doubly labeled water in humans. Am J Physiol 264:E912-917, 1993

15. Racette SB, Schoeller DA, Luke AH, et al: Relative dilution spaces of 2H- and 18O-labeled water in humans. Am J Physiol 267: E585-590, 1994

16. Black AE, Prentice AM, Coward WA: Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. Hum Nutr Clin Nutr 40:381-391, 1986

17. Weir JB: New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1-9, 1949

18. Poehlman ET, McAuliffe TL, Van Houten DR, et al: Influence of age and endurance training on metabolic rate and hormones in healthy men. Am J Physiol 259:E66-72, 1990

19. Weinsier RL, Nagy TR, Hunter GR, et al: Do adaptive changes in metabolic rate favor weight regain in weight-reduced individuals? An examination of the set-point theory. Am J Clin Nutr 72:1088-1094, 2000

20. Rosenbaum M, Malbon CC, Hirsch J, et al: Lack of β_3-adrenergic effect on lipolysis in human subcutaneous adipose tissue. J Clin Endocrinol Metab 77:352-355, 1993

21. Krief S, Lonnqvist F, Raimbault S, et al: Tissue distribution of β_3-adrenergic receptor mRNA in man. J Clin Invest 91:344-349, 1993

22. Tavernier G, Barbe P, Galitzky J, et al: Expression of β_3-adrenoceptors with low lipolytic action in human subcutaneous white adipocytes. J Lipid Res 37:87-97, 1996