

# Obesity-Related Phenotypes and the $\beta_3$ -Adrenoceptor Gene Variant in Postmenopausal Women

André Tchernof, Raymond D. Starling, Jeremy D. Walston, Alan R. Shuldiner, Roman V. Dvorak, Kristi Silver, Dwight E. Matthews, and Eric T. Poehlman

We examined the hypothesis that postmenopausal women with the  $\beta_3$ -adrenoceptor gene variant (Trp64Arg) have reduced total daily energy expenditure (TEE), altered free fatty acid kinetics, and increased intra-abdominal fat. A secondary objective was to examine whether the obese state masks the effect of the variant on resting metabolic rate (RMR). There were 23 obese heterozygous women with the genetic variant (age  $58 \pm 6$  years; BMI  $36 \pm 7$  kg/m<sup>2</sup>) who were compared with 19 homozygous obese women with the normal allele (age  $56 \pm 4$  years; BMI  $36 \pm 3$  kg/m<sup>2</sup>). Daily energy expenditure was determined from doubly labeled water and indirect calorimetry, lipolysis from infusion of [<sup>1-<sup>13</sup>C</sup>]palmitate, and body fat distribution from computed tomography. No significant differences were found in TEE, RMR, energy expenditure of physical activity, the thermic effect of a meal, fat oxidation as estimated by fasting and postprandial respiratory quotients (RQs), or rate of lipolysis. Similarly, no difference was found in visceral adipose tissue and abdominal subcutaneous fat areas. When RMR was compared between obese ( $n = 23$ ) and never-obese women with the Trp64Arg variant ( $n = 16$ ), we found a 317 kcal/day lower RMR in never-obese women after controlling for fat mass, fat-free mass, and age ( $P < 0.0017$ ). These results do not support the hypothesis that already obese women with the Trp64Arg polymorphism of the  $\beta_3$ -adrenergic receptor gene have lower daily energy expenditure, altered lipolysis, and increased abdominal obesity. On the other hand, the lower RMR in never-obese women suggests that the obese state may mask a moderate effect of the Trp64Arg variant on energy expenditure. Although these results need to be confirmed in other populations, the obese state may have been a confounding factor in previous studies of the  $\beta_3$ -adrenoceptor Trp64Arg variant and energy expenditure. *Diabetes* 48:1425–1428, 1999

From the Department of Medicine (A.T., R.D.S., R.V.D., D.E.M., E.T.P.), College of Medicine, University of Vermont, Burlington, Vermont; the Division of Geriatric Medicine and Gerontology (J.D.W.), Johns Hopkins University; the Division of Diabetes, Obesity and Nutrition (A.R.S., K.S.), Department of Medicine, University of Maryland; and the Geriatric Research Education and Clinical Center (A.R.S.), Baltimore Veterans Administration Medical Center, Baltimore, Maryland.

Address correspondence and reprint requests to Eric T. Poehlman, PhD, Department of Medicine, University of Vermont, Burlington, VT 05405. E-mail: epoehlma@zoo.uvm.edu.

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FFA, free fatty acid; GCMS, gas chromatography–mass spectrometry; RMR, resting metabolic rate; RQ, respiratory quotient; TEE, total daily energy expenditure; TEM, thermic effect of the meal.

The variant of the  $\beta_3$ -adrenergic receptor gene coding for the replacement of tryptophan by arginine at codon 64 (Trp64Arg) has been related to lower resting metabolic rate (RMR) and increased visceral adipose tissue accumulation in different populations and ethnic groups (1–5). These results, however, are controversial, since several studies found no effect of the polymorphism on obesity-related phenotypes (6–9). Divergent results among investigators may be partially attributable to random sampling variations, cohort differences in ethnicity, sex, age, and degree of obesity, and methodologies to measure energy expenditure and fat distribution.

The present study examined total daily energy expenditure (TEE), fat oxidation, and lipolysis in postmenopausal obese women with and without the Trp64Arg variant. We specifically tested the hypothesis that older obese women with the Trp64Arg variant of the  $\beta_3$ -adrenoceptor gene have lower daily energy expenditure, reduced fat oxidation, and increased lipolysis and abdominal obesity.

A second objective of the study was to examine whether the presence or absence of the obese state, per se, influences RMR. The rationale underlying this hypothesis is that the obese state may be associated with a normalization of energy expenditure and fat oxidation through mechanisms that include increased fat-free mass and fat mass (10,11). Thus, phenotypes that predict the propensity to become obese may be masked by the obese state itself. In an attempt to test this hypothesis, we measured RMR in never-obese women who were heterozygous for the Trp64Arg variant.

## RESEARCH DESIGN AND METHODS

**Subjects.** Postmenopausal obese Caucasian women in the greater Burlington, VT, area were recruited by local advertisement. Inclusion criteria were the cessation of menstruation for at least 1 year, a BMI  $\geq 28$  kg/m<sup>2</sup>, and physical inactivity. Women also had to be nonsmokers, not taking hormone replacement therapy, and nondiabetic. Exclusion criteria included atherosclerosis, hypertension (diastolic blood pressure  $>90$  mmHg), orthopedic limitations or history of fractures, weight loss/gain over the previous 6 months, or thyroid or pituitary disease. There were also 16 Trp64Arg heterozygous never-obese women who were included in the study for a post hoc comparison of RMR. Other outcome variables were not measured in this subgroup. Exclusion/inclusion criteria used for this group of women were the same as those used to recruit obese women with the exception of BMI, which had to be  $<27$  kg/m<sup>2</sup>. Screening for the presence of the Trp64Arg variant was performed after subjects gave their informed consent. This study was approved by the Committee on Human Research and Medical Sciences of the University of Vermont. **Genotyping.** Genotyping for the Trp64Arg variant in the  $\beta_3$ -adrenoceptor gene was performed as previously described (12) by polymerase chain reaction–restriction fragment length polymorphism analysis.

**Standardization for the metabolic testing.** Participating volunteers were submitted to weight stabilization (within 2 kg of body weight) for a duration of 1 month before metabolic testing. Macronutrient intake was stabilized 3 days before testing with a standard diet. Subjects consumed, on average,  $70.5 \pm 1.5\%$  of energy intake as carbohydrate,  $12.5 \pm 0.9\%$  as fat, and  $17.0 \pm 1.0\%$  as protein.

**TEE and its components.** RMR was determined for 45 min in a resting and fasted state, and thermic effect of a liquid meal (10 kcal/kg body wt, Ensure; Ross Laboratories, Columbus, OH) was measured using indirect calorimetry for 3 h (13). Respiratory gas analysis was measured using a Deltatrac metabolic cart (Sensormedics, Yorba Linda, CA). RMR (kilocalories/day) was calculated from the equation of Weir (14). The intraclass correlation and coefficient of variation for RMR determined during test-retest on two occasions (within 1 week) in 17 volunteers from our laboratory were 0.90 and 4.3%, respectively. Fasting and postprandial respiratory quotients (RQs) were calculated as the ratio of CO<sub>2</sub> production to O<sub>2</sub> consumption. The thermic effect of the meal (TEM) was expressed as absolute calories expended over 180 min multiplied by 3, assuming three meals per day. Leisure time physical activity was estimated by the Minnesota questionnaire (15).

TEE was determined from doubly labeled water over a 10-day period in a subset of 18 subjects heterozygous and 16 homozygous for the wild-type allele using the method of Schoeller and van Santen (16) as previously described (17). Each subject drank a premeasured dose containing ~0.078 g <sup>2</sup>H<sub>2</sub>O and 0.092 g H<sub>2</sub><sup>18</sup>O per kilogram of body mass. One urine sample was collected before administration of <sup>2</sup>H<sub>2</sub><sup>18</sup>O; two samples were obtained the following morning, and two samples 10 days later. Samples were frozen at -20°C in vacutainers until later analysis for <sup>2</sup>H and <sup>18</sup>O enrichments by isotope ratio mass spectrometry. <sup>18</sup>O isotopic enrichment was determined from the carbon dioxide (CO<sub>2</sub>) equilibration technique, and <sup>2</sup>H enrichment was determined by the zinc catalyst method of Wong et al. (18). Rate of CO<sub>2</sub> production (rCO<sub>2</sub>; moles/day) was calculated using equation 3 from Speakman et al. (19): rCO<sub>2</sub> = N/2.196 × (c<sub>o</sub>k<sub>o</sub> - c<sub>H</sub>k<sub>H</sub>), where k<sub>o</sub> and k<sub>H</sub> are the elimination rates of <sup>18</sup>O and <sup>2</sup>H tracers from the body, N is total body water in moles, determined from the <sup>18</sup>O- and <sup>2</sup>H-water tracer dilutions at time zero, and c<sub>o</sub> and c<sub>H</sub> are the dilution spaces for <sup>18</sup>O and <sup>2</sup>H tracers as recommended by Racette et al. (20). Assuming an RQ of the food consumed of 0.85 (21), total CO<sub>2</sub> production was converted to TEE (kilocalories/day) using the Weir formula (14). The following equation was used to calculate physical activity energy expenditure (kilocalories/day): TEE = (TEM + RMR).

**Rate of lipolysis.** Lipolysis was determined in a subset of 18 Trp64Arg heterozygous and 12 normal homozygous subjects using an infusion of [1-<sup>13</sup>C]palmitate. Catheters were placed in an arm for infusion of [1-<sup>13</sup>C]palmitate and in a hand vein for collection of blood samples more than an hour before the measurement of the RMR. The hand was intermittently warmed in a heated-air box (50°C) to produce arterialized venous blood samples. [1-<sup>13</sup>C]palmitate was infused for 120 min at 50 ng · kg<sup>-1</sup> · min<sup>-1</sup> before and during the RMR measurement. Blood samples were obtained before the start of the infusion and at 90, 100, 110, and 120 min. Blood samples were placed on ice until the plasma was prepared by centrifugation at 4°C at the end of the infusion. The plasma samples were frozen at -60°C until later analysis for total free fatty acid (FFA) concentration and palmitate <sup>13</sup>C enrichment and concentration. Plasma FFA concentration was measured by colorimetric assay using kits purchased from Biochemical Diagnostics (Brentwood, NY). Plasma palmitate level was measured by gas chromatography-mass spectrometry (GCMS). An aliquot of [16,16,16-<sup>2</sup>H<sub>3</sub>]palmitate internal standard was added to each plasma sample before extraction of the FFAs. The FFAs were derivatized to form the t-butylidimethylsilyl (tBDMS), and were measured by GCMS using selected ion monitoring of masses at mass-to-charge ratios (m/z) of 313, 314, and 316 (unlabeled, [<sup>13</sup>C]), and [<sup>2</sup>H<sub>3</sub>]palmitate, respectively). The [<sup>13</sup>C]palmitate enrichment was determined as mole percent excess <sup>13</sup>C from the measurement of the samples during the infusion, after subtraction of the unlabeled 314:313 ratio of the preinfusion palmitate sample and correction using a standard curve of known [<sup>13</sup>C]palmitate enrichment. The palmitate concentration in plasma was determined against the [<sup>2</sup>H<sub>3</sub>]palmitate internal standard (the 313:316 ratio) using a standard curve of palmitate versus [<sup>2</sup>H<sub>3</sub>]palmitate. The palmitate rate of appearance (R<sub>a16:0</sub>; millimoles/minute) was calculated from the tracer dilution relationship of rate of tracer infusion (I; 50 ng · kg<sup>-1</sup> · min<sup>-1</sup> [<sup>13</sup>C]palmitate) divided by the mean of the plateau [1-<sup>13</sup>C]palmitate enrichment (E; mole percent excess): R<sub>a</sub> = 100 × I/E, where the factor of 100 converts mole percent to mole fraction of <sup>13</sup>C tracer. The rate of lipolysis was calculated from the overall rate of FFA appearance. FFA appearance (R<sub>aFFA</sub>) was assumed to be proportionate to the palmitate appearance: R<sub>aFFA</sub> = R<sub>a16:0</sub> × [FFA]/[16:0], where [FFA] and [16:0] are the total FFA and palmitate plasma concentrations (millimoles/liter), respectively.

**Body composition and fat distribution.** Body composition was determined by dual energy x-ray absorptiometry, using a Lunar DPX-L densitometer (Lunar Radiation, Madison, WI). Measurements included fat mass and fat-free mass. Percent body fat was calculated by dividing fat mass by body weight. Fat mass and fat-free mass were measured by underwater weighing in three women of the never-obese group, as previously described (22).

Intra-abdominal and subcutaneous adipose tissue was measured by computed tomography (23) using a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI). The subjects were examined in the supine position with both arms stretched above their head. The position of the scan was established at the L4-L5 level using a scout image of the body. Intra-abdominal adipose tissue area was quantified by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls sur-

TABLE 1  
Physical characteristics of women included in the study according to Trp64Arg genotype

	Trp64Arg heterozygous subjects	Normal homozygous subjects
n	23	19
Age (years)	57.9 ± 6.2	56.1 ± 4.3
Weight (kg)	97.3 ± 17.9	96.4 ± 10.3
BMI (kg/m <sup>2</sup> )	36.4 ± 7.2	35.6 ± 3.4
Percent body fat	46.6 ± 5.1	48.8 ± 3.7
Fat mass (kg)	42.3 ± 11.3	47.3 ± 14.1
Fat-free mass (kg)	47.7 ± 6.6	47.6 ± 5.4
Leisure-time activity (kcal/day)	308 ± 229	319 ± 189

Data are means ± SD. No significant differences were noted between genotypes.

rounding the cavity and the posterior aspect of the vertebral body, and adipose tissue was highlighted and computed using an attenuation range of -190 to -30 Hounsfield Units. The subcutaneous adipose tissue area was quantified by highlighting adipose tissue located between the skin and the external-most aspect of the abdominal muscle wall. Total abdominal adipose tissue area was obtained by adding subcutaneous and intra-abdominal adipose tissue areas.

**Statistical analyses.** Homogeneity of group variances was tested using the Levene test, accepting unequal variances at P < 0.10. Variances were unequal for the following variables: body weight, BMI, and abdominal subcutaneous and total adipose tissue areas. The Welch analysis of variance procedure was used to compare the means for these four variables. Means for other variables, including measures of body composition, body fat distribution, TEE, RMR, physical activity energy expenditure, thermic effect of a meal, and lipolysis were compared between the groups with a two-tailed unpaired Student's t test. RMR in never-obese women with the Trp64Arg variant was compared with that of obese women with or without the variant by analysis of covariance adjusting for age, total fat mass, and fat-free mass. Values are expressed as means ± SE.

**RESULTS**

Some 380 women were screened, of which 38 were heterozygous for the Trp64Arg variant (allelic frequency = 0.10). Of this initial cohort, 42 obese women (23 heterozygous, 19 normal homozygous) completed clinical and metabolic testing. Descriptive statistics for each genotype are shown in Table 1. Subjects were not significantly different with respect to age, body composition, or leisure-time physical activity.

Daily energy expenditure and its components for the two obese genotypes are shown in Fig. 1A. No significant differences were found in TEE (2,881 ± 116 vs. 2,941 ± 115 kcal/day), RMR (1,639 ± 44 vs. 1,631 ± 41 kcal/day), physical activity energy expenditure (1,133 ± 91 vs. 1,178 ± 81 kcal/day), or TEM (120 ± 9 vs. 138 ± 8 kcal/day) in Trp64Arg heterozygous versus normal homozygous subjects, respectively.

No significant difference in fasting (0.83 ± 0.04 vs. 0.84 ± 0.04) or postprandial RQ (0.89 ± 0.03 vs. 0.89 ± 0.03) was found between Trp64Arg women and normal homozygous women, respectively. The appearance rate of palmitate (lipolysis) was also similar between Trp64Arg women (1,114 ± 330 μmol/min, n = 18) and normal homozygous women (1,046 ± 344 μmol/min, n = 12).

No significant differences were found between genotypes in subcutaneous (485 ± 22 vs. 517 ± 15 cm<sup>2</sup>) or intra-abdominal adipose tissue areas (193 ± 16 vs. 199 ± 17 cm<sup>2</sup>) (Fig. 1B). Similarly, no significant differences were found in total abdominal adipose tissue area (677 ± 179 vs. 716 ± 80 cm<sup>2</sup>) or in the ratio of visceral to subcutaneous fat (0.41 ± 0.18 vs. 0.40 ± 0.16) between Trp64Arg women and normal homozygous women, respectively.

To test the hypothesis that the obese state may act as a confounding factor in the examination of the effects of the Trp64Arg variant, we compared RMR and RQ of the obese women to those of a group of 16 never-obese Trp64Arg heterozygous women. Women of the never-obese group were, on average,  $41.4 \pm 20.5$  years old, and had a mean BMI of  $22.8 \pm 2.4$  kg/m<sup>2</sup>. After adjustment for age, fat mass, and fat-free mass by analysis of covariance, RMR was 24% lower (317 kcal/day difference,  $P < 0.0017$ ) in never-obese women with the variant versus obese women with or without the variant (Fig. 2). Fasting respiratory quotient, however, was not significantly different among never-obese Trp64Arg women ( $0.83 \pm 0.01$ ), obese Trp64Arg women ( $0.84 \pm 0.01$ ), and normal homozygous women ( $0.84 \pm 0.01$ ).

## DISCUSSION

To our knowledge, this is the first study to examine TEE, substrate oxidation, and fatty acid appearance in individuals with and without the Trp64Arg variant. This study was prompted by previous findings suggesting that low rates of energy expenditure (24) and low fat oxidation (25) are two phenotypes that predict weight gain, which may be affected by the presence of the  $\beta_3$ -adrenoceptor gene variant. We found no significant difference between genotypes in TEE or its components (RMR and physical activity energy expenditure). Similarly, no differences in fasting and postprandial fat oxidation or lipolysis were found between obese carriers and noncarriers of the variant. Accumulation of visceral adipose tissue was also similar between the two genotypes. Collectively, these results do not support an association of the Trp64Arg variant with low rates of energy expenditure, fat oxidation, or intra-abdominal fat in individuals who are already obese.

There is controversy regarding the effects of the Trp64Arg variant on RMR. RMR was found to be lower in carriers, compared with noncarriers, of the polymorphism in some

(1–4,26), but not all, studies (8). A significant sib-pair linkage was found between the mutation and RMR in one study (27). The present study extends previous works by measuring TEE with doubly labeled water. The application of this methodology permits an integrated and unbiased assessment of TEE over a 10-day period in free-living individuals.

We hypothesized that differences in TEE and fatty acid kinetics may be associated with greater abdominal fat deposition in subjects with the Trp64Arg variant. No difference was found among carriers and noncarriers of the Trp64Arg polymorphism for this phenotype. The effect of the  $\beta_3$ -adrenoceptor variant on body fat distribution has been examined in several other studies; however, no consensus exists. A positive effect of the variant on abdominal adiposity was found in Japanese, Finnish, and Mexican-American populations (2,4,5,12). On the other hand, negative results were obtained by others in Caucasian, Swedish, Danish, Jamaican, and Finnish subjects (3,7,8,27,28). It is possible that the effects of the variant may be specific to certain ethnic groups. Similarly, it is also possible that the gene variant effect may be specific to certain age and sex groups, as previously suggested (29).

The absence of an association between the  $\beta_3$ -adrenoceptor genotype and energy expenditure, substrate oxidation, and fat distribution may be related to the possibility that heterozygosity for the Trp64Arg variant exerts only moderate effects on these phenotypes. We did not test individuals who were homozygous for polymorphism 64 of the  $\beta_3$ -adrenoceptor gene. One would intuitively predict a greater effect of this variant on the measured phenotypes in homozygous individuals. However, the rarity of the variant would limit its clinical significance. Moreover, previous data from Sakane et al. (5) and Yoshida et al. (4) do not support the notion of a stronger effect of homozygosity for the variant, since both heterozygous and homozygous subjects had similar visceral adipose tissue accumulation or visceral-to-subcutaneous fat ratio determined by computed tomography scan (4,5).

In our experimental design, we considered the possibility that the obese state per se may mask differences in energy expenditure between genotypes. The rationale for this hypothesis is based on the concept that the accumulation of fat mass and accretion of fat-free mass normalizes energy expenditure in individuals who become obese (10,11). Thus, the elucidation of phenotypes that may predict obesity are likely to be unmasked by studying pre- or never-obese individuals with the variant. To address this possibility, we com-

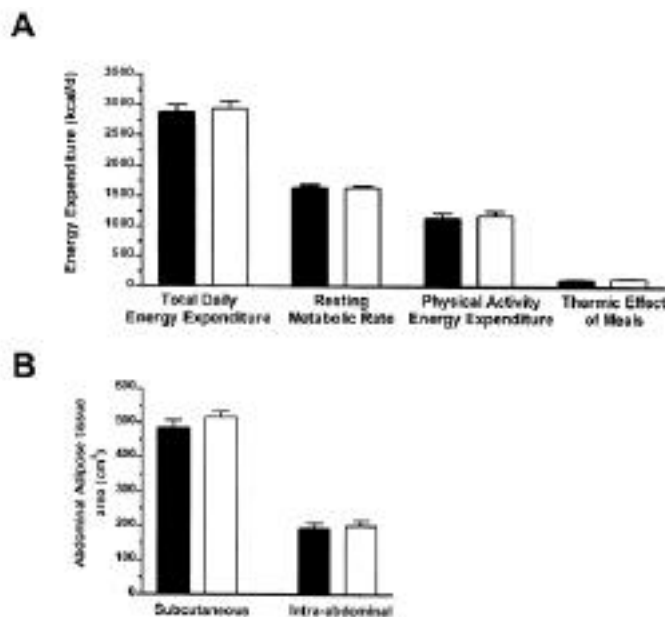


FIG. 1. TEE and its components (A) and abdominal adipose tissue areas (B) among postmenopausal obese women with or without the Trp64Arg variant of the  $\beta_3$ -adrenergic receptor gene. TEE and physical activity energy expenditure were measured in a subset of 18 Trp64Arg heterozygous (■) and 16 normal homozygous (□) subjects.

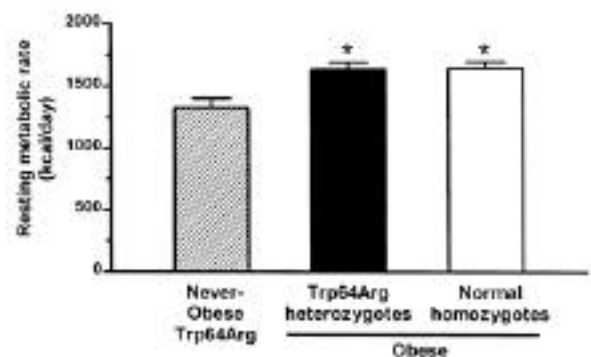


FIG. 2. RMR in never-obese Trp64Arg heterozygous women ( $n = 16$ ) compared with obese postmenopausal women with ( $n = 23$ ) or without ( $n = 19$ ) the Trp64Arg variant. \* $P < 0.05$ .

pared RMR (the largest component of daily energy expenditure) between never-obese heterozygous and obese heterozygous women. We found that the never-obese group had a 317 kcal/day lower RMR value, independent of body composition and age. This finding suggests that the modest effect of the gene is probably most apparent in individuals who have not yet expressed the obese state. These results must be interpreted with caution, however, given the small sample size, the cross-sectional nature of the comparison, and the fact that we did not have all the outcome variables for the never-obese group. Other studies in never-obese individuals with a greater number of phenotypes examined are clearly needed to confirm this finding. Thus, although further experimental evidence is needed, we raise the possibility that the obese state may be a possible confounding factor in experimental designs.

In summary, the results of the present study do not support the hypothesis that the Trp64Arg variant of the  $\beta_3$ -adrenoceptor gene is associated with differences in energy expenditure, fat metabolism, or abdominal-visceral obesity in postmenopausal obese older women. However, the obese state may be a confounding factor in the study of the effects of this genetic variant on energy expenditure. Further studies in the postobese state may be required to elucidate the effects of the Trp64Arg variant in postmenopausal Caucasian women.

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