Impaired Capacity to Lose Visceral Adipose Tissue During Weight Reduction in Obese Postmenopausal Women With the Trp64Arg β3-Adrenoceptor Gene Variant

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Controversy exists regarding the association between the Trp64Arg variant of the β3-adrenoceptor gene and visceral obesity. The cross-sectional nature of most studies, the modest effect of the variant, and sex or ethnic differences between groups have contributed to discrepancies among investigations. To overcome these confounding factors, we examined the effect of the Trp64Arg variant on total and visceral adipose tissue loss, insulin sensitivity, and cardiovascular disease risk factors in response to weight reduction in obese older women. A total of 24 women (age 57 ± 4 years), including 1 Trp64Arg homozygote, 10 Trp64Arg heterozygotes, and 13 normal homozygotes, were admitted to a weight reduction program of 13 ± 3 months, with weight and nutritional intake stabilization established before testing. Total and regional adiposity were measured with dual-energy X-ray absorptiometry and computed tomography, insulin sensitivity was measured by the hyperinsulinemic-euglycemic clamp technique, and a blood lipid profile was obtained. No baseline differences were noted in adiposity measurements, glucose disposal, and lipid profiles among carriers and noncarriers of the variant allele. In response to weight loss, carriers and noncarriers of the Trp64Arg allele had similar reductions in body weight (–16.4 ± 5.0 vs. –14.1 ± 6.2 kg, NS) and body fat (–10.0 ± 5.2 vs. –11.5 ± 3.9 kg, NS). However, loss of visceral adipose tissue was 43% lower in carriers of the Trp64Arg allele compared with noncarriers (–46 ± 27 vs. –81 ± 51 cm², P = 0.05). Furthermore, there was less improvement in the total cholesterol–to–HDL cholesterol ratio (–0.18 ± 0.54 vs. –0.72 ± 0.56, P = 0.04) in carriers compared with noncarriers of the allele. Although glucose disposal improved in both groups, there was no difference in the magnitude of improvement between carriers and noncarriers of the variant allele. In conclusion, older obese women carrying the Trp64Arg β3-adrenoceptor gene variant have an impaired capacity to lose visceral adipose tissue in response to prolonged caloric restriction. Despite these genetic differences in loss of intra-abdominal adipose tissue, improvement in glucose disposal was similar between groups. Diabetes 49:1709–1713, 2000

The variant of the β3-adrenergic receptor gene coding for the replacement of tryptophan by arginine at codon 64 (Trp64Arg) has been associated with increased visceral adipose tissue accumulation and related comorbidities in several studies (1–6). These results, however, are unclear because other investigators have found no effect of the polymorphism on this phenotype (7–11). Although the phenotypic expression of the Trp64Arg variant remains unclear at present, Lönnqvist et al. (12) demonstrated that β3-adrenoceptor-mediated lipolysis was partly responsible for the increased free fatty acid release from the intra-abdominal adipose tissue depot in humans. More recently, several studies have investigated the possibility of an impaired β3-adrenoceptor-mediated lipolysis in omental fat from subjects with the Trp64Arg variant (13–15). Experiments performed with a selective β3-adrenoceptor agonist (14,16) suggest that the Trp64Arg variant is associated with impaired lipolysis in adipose tissue from the intra-abdominal cavity.

Discrepant results found among previous studies of the Trp64Arg variant of the β3-adrenoceptor gene and phenotypes of the insulin resistance syndrome may have been due to random sampling variations or cohort differences in ethnicity, sex, age, or degree of obesity. Discordant results may also be due to the cross-sectional nature of most investigations. Intervention studies provide a more rigorous approach to understanding the effects of this genetic variant, especially in obese populations. We have recently suggested that the obese state may mask moderate effects of this variant (11). Thus, we undertook a study in which obese women underwent weight reduction in an attempt to clarify possible effects of the β3-adrenoceptor gene variant on the ability to respond to weight loss. We tested the hypothesis that obese postmenopausal women with the Trp64Arg variant have an impaired capacity to lose intra-abdominal adipose tissue in response to a weight...
before the start and during the second hour of the infusion for determination of FGD. Blood samples were also taken during the last hour of the clamp. Blood samples were taken before and during the second hour of the infusion for determination of plasma [\(^{6,6}\text{H}_2\)]glucose enrichment. At 0900, the insulin infusion was begun and continued for an additional 2 h. Insulin was infused at a rate of 240 pmol - m\(^{-2}\) - min\(^{-1}\) to attain postprandial peripheral insulin levels and suppress endogenous glucose production (EGP). Blood glucose was monitored every 5 min during the insulin infusion, and euglycemia was maintained throughout the clamp by infusing 20\% dextrose at a variable rate. The duration of the insulin infusion was such that the rate of infused glucose reached a constant value by the second hour of the clamp. Blood samples were also taken during the last hour of the clamp for determination of [\(^{6,6}\text{H}_2\)]glucose enrichment. To maintain a constant enrichment of [\(^{6,6}\text{H}_2\)]glucose tracer in blood during the clamp, [\(^{6,6}\text{H}_2\)]glucose was added to the 20\%dextrose before the start of the study to produce an enrichment of 1 mol percent excess [\(^{2}\text{H}_2\)]glucose in the dextrose. Aliquots of heparinized plasma were stored at -60°C for later analysis. For plasma [\(^{6,6}\text{H}_2\)]glucose enrichment measurements, plasma was deproteinized with acetone, and the supernatants were decanted and evaporated to dryness under nitrogen. After adding 2\% butyliboron dihydroxide (Sigma, St. Louis, MO) in pyridine, the samples were allowed to sit for 24 h at room temperature. Acetic anhydride was added just before measurement to complete formation of the butyliboronate glucose derivatization formation. The butyliboronate glucose derivatives were measured by gas chromatography-mass spectrometry using electron impact ionization (model 5971; Hewlett-Packard, Palo Alto, CA). The [M-57] ions at m/z = 297 and 299 were monitored for unlabeled glucose and [\(^{6,6}\text{H}_2\)]glucose, respectively. The peak area ratios of 299/297 were determined by selected ion monitoring. A standard curve of known [\(^{6,6}\text{H}_2\)]glucose enrichment was run with each set of samples. From these ratios, the background-corrected and standard curve-corrected glucose enrichments were calculated in molar percent excess.

The purpose of the [\(^{6,6}\text{H}_2\)]glucose infusion was to provide measurement of basal FGD and glucose appearance and a measure of EGP during the clamp. The rate of EGP was calculated from the mean [\(^{6,6}\text{H}_2\)]glucose enrichment in plasma during the basal state as EGP = \((1/\text{IE}_{i})\left(\text{IE}_{i} - 1\right)\), where \(\text{IE}_{i}\) is the rate of [\(^{6,6}\text{H}_2\)]glucose infusion (milligrams per minute), E is the enrichment of the tracer enrichment in mole percent excess, and \(\text{IE}_{i}\) is the enrichment of [\(^{6,6}\text{H}_2\)]glucose in plasma during the basal state. During the hyperinsulinemic-euglycemic clamp, total glucose disposal (TGD) was also calculated from the plasma [\(^{3}\text{H}_2\)]glucose enrichment taken from blood samples during the last 30 min of the insulin infusion as TGD = \((1/\text{E}_{i} + \text{ME}_{i})\text{IE}_{i} - 1\), where \(\text{E}_{i}\) is the rate of exogenous dextrose infusion (milligrams per minute) and \(\text{ME}_{i}\) is the enrichment of [\(^{6,6}\text{H}_2\)]glucose in the infused dextrose (mole percent excess). The EGP during the clamp (\(\text{EGP}_{i}\)) was taken as the difference between TGD measured using [\(^{3}\text{H}_2\)]glucose and the mean rate of dextrose infusion during the last 30 min of the insulin infusion (M) as \(\text{EGP}_{i} = \text{TGD} - \text{M}\).

**Statistical analyses.** Values in Tables 1 and 2 are expressed as means ± SD. Study subjects were separated according to their \(\beta\)-adrenoceptor genotype into carriers (Arg/Arg homozygote and Trp/Trp heterozygotes) and noncarriers of the Arg64 allele (Trp/Trp normal homozygotes). Response to the weight loss protocol was compared in carriers and noncarriers of the variant allele using general linear models analysis of variance. The variance of glucose production during the clamp was significantly different between carriers and noncarriers of the variant allele. Thus, a nonparametric test (Mann-Whitney U test) was used to compare mean EGP during the clamp. Weight loss-induced changes between carriers and noncarriers of the variant allele were compared by Student’s t test. Significance level was accepted at \(p < 0.05\).

**RESULTS**

Table 1 shows total and regional adiposity values of carriers and noncarriers of the variant allele before and after the weight loss program. Women were in the weight loss protocol for an average of 13.4 ± 2.8 months (including weight stabilization). The program did not induce significant changes in leisure time physical activity (295 ± 188 vs. 247 ± 170 kcal/day before and after weight loss, respectively; \(p = 0.36\)). Carriers and noncarriers had similar physical characteristics and adiposity measures at baseline. Reductions in body weight, BMI, total fat mass, and fat-free mass were not significantly different between carriers and noncarriers of the variant allele. However, carriers of the Trp64Arg variant had smaller reductions in intra-abdominal fat compared with normal homozygotes (-81 ± 51 vs. -46 ± 27 cm, respectively; \(p = 0.05\)), which corresponds to a 43\% lower reduction in carriers of the Trp64Arg allele compared with noncarriers (\(p = 0.05\)). When Box-Cox-transformed intra-abdominal adipose tissue areas
were compared, taking into account group differences in the variance, the P value for this comparison was 0.04. Reductions in abdominal subcutaneous adipose tissue areas were not significantly different between genotypes.

Weight loss–induced changes in blood lipid profile, glucose disposal, and EGP are shown in Table 2. Total, LDL, and HDL cholesterol levels and triglyceride concentrations were similar in carriers and noncarriers of the variant allele at baseline. In response to weight loss, the lipid profile only tended to improve to the same extent in both groups. However, the total cholesterol–to–HDL cholesterol ratio improved significantly more with the weight loss in normal homozygotes compared with Trp64Arg allele carriers (0.72 ± 0.56 vs. 0.18 ± 0.54, P < 0.05, in noncarriers vs. carriers, respectively). Glucose disposal expressed either as absolute values or per kilogram of fat-free mass improved similarly in carriers and noncarriers of the variant allele (Table 2). EGP either in the basal state or during the clamp was also similar in both groups because no significant effect of weight loss was found. The insulin levels achieved during the clamp were similar before and after the weight loss program. Moreover, the levels achieved before and after the weight loss were not different between genotype groups (Table 2).

TABLE 1
Changes in body weight, BMI, and adiposity variables in response to weight loss

<table>
<thead>
<tr>
<th></th>
<th>Trp64Arg carriers (n = 11)</th>
<th>Normal homozygotes (n = 13)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline Change</td>
<td>Baseline Change</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.5 ± 5.4</td>
<td>58.0 ± 3.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.5 ± 19.6</td>
<td>95.5 ± 11.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.1 ± 6.9</td>
<td>35.4 ± 3.8</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>44.0 ± 3.1</td>
<td>43.8 ± 2.8</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>44.3 ± 4.5</td>
<td>46.0 ± 4.5</td>
</tr>
<tr>
<td>Abdominal adipose tissue areas (cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td>176 ± 77</td>
<td>211 ± 64</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>500 ± 105</td>
<td>504 ± 64</td>
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</tbody>
</table>

*Significant weight loss effect, P < 0.05; †significant genotype effect, P = 0.05.

Despite the absence of statistically significant genotype differences in baseline body fat mass, subcutaneous adipose tissue areas, or intra-abdominal adipose tissue areas, or intra-abdominal adipose tissue areas, the slightly lower adiposity of the Trp64Arg group may have contributed to the lower quantity of intra-abdominal fat lost in this group. Statistical adjustment of weight loss–induced changes in intra-abdominal fat for baseline body weight, BMI, total body fat mass, and abdominal subcutaneous area had no effect on the difference between carriers and noncarriers of the variant allele (data not shown). The adjustment for baseline intra-abdominal fat area slightly reduced the magnitude of the genotype difference in intra-abdominal fat loss (P = 0.13).

DISCUSSION

In the present study, we tested the hypothesis that obese postmenopausal women who harbor the Trp64Arg variant have a lower visceral fat loss in response to a weight reduction program compared with normal homozygotes. We found a 43% smaller decrease in intra-abdominal fat for baseline body weight, BMI, total body fat mass, and abdominal subcutaneous area had no effect on the difference between carriers and noncarriers of the variant allele (data not shown). The adjustment for baseline intra-abdominal fat area slightly reduced the magnitude of the genotype difference in intra-abdominal fat loss (P = 0.13).

TABLE 2
Changes in blood lipids and glucose disposal in response to weight loss

<table>
<thead>
<tr>
<th></th>
<th>Trp64Arg carriers (n = 11)*</th>
<th>Normal homozygotes (n = 13)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Change</td>
<td>Baseline Change</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.70 ± 0.14</td>
<td>1.74 ± 0.20</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.63 ± 0.23</td>
<td>5.63 ± 0.23</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.57 ± 0.24</td>
<td>3.30 ± 0.25</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.28 ± 0.07</td>
<td>1.24 ± 0.09</td>
</tr>
<tr>
<td>Total cholesterol–to–HDL cholesterol</td>
<td>4.54 ± 0.33</td>
<td>4.91 ± 0.42</td>
</tr>
<tr>
<td>Glucose disposal*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/min</td>
<td>329 ± 123</td>
<td>403 ± 191</td>
</tr>
<tr>
<td>mg · min⁻¹ · kg⁻¹ fat-free mass</td>
<td>7.65 ± 3.03</td>
<td>8.89 ± 4.07</td>
</tr>
<tr>
<td>Clamp insulin levels (μU/ml)</td>
<td>101 ± 31</td>
<td>97 ± 22</td>
</tr>
<tr>
<td>Endogenous glucose (mg/min)</td>
<td>Basal 155 ± 17</td>
<td>153 ± 15</td>
</tr>
<tr>
<td></td>
<td>Clamp§ 42 ± 48</td>
<td>8 ± 8</td>
</tr>
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</table>

*Clamp data were obtained in eight Trp64Arg carriers and nine normal homozygotes; †significant weight loss effect, P < 0.05; ‡significant genotype effect, P < 0.05; §the Mann-Whitney U nonparametric test was used because of unequal variances for this variable.
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ratio compared with normal homozygotes. Contrary to our expectations, we found no effect of the variant allele on weight loss–induced changes in glucose disposal.

To our knowledge, this is the first study to demonstrate an effect of the β3-adrenoceptor Trp64Arg variant on changes in visceral fat in response to weight loss. This study was prompted by conflicting data from previous studies that examined the association of the β3-adrenoceptor gene variant with features of the insulin resistance syndrome. The majority of these studies were observational, with some supporting a significant effect of the variant on adiposity or energy expenditure, whereas others found no meaningful effect (1–11). Given the probable moderate effect of this variant on amounts of body fat, we have put forth the hypothesis that the obese state per se may mask the effects of the Trp64Arg variant on obesity-related phenotypes (11). To address this issue, we used an intervention–weight loss paradigm to examine whether obese postmenopausal women with the variant have an impaired capacity to reduce intra-abdominal fat. The present experimental design, the concomitant use of criterion methods (i.e., clamps and computed tomography imaging), and weight and dietary stabilization before metabolic testing support the validity of our findings.

The physiological rationale underlying our hypothesis is two-fold. First, the intra-abdominal compartment is the primary site of β3-adrenoceptor gene expression (12). Second, it has been demonstrated that β3-adrenoceptor–induced lipolysis is lower in subjects carrying the Trp64Arg variant (14,16). Thus, the impaired capacity of Trp64Arg carriers to lose intra-abdominal fat in response to a weight reduction intervention observed in the present study may be attributable to impaired β3-adrenoceptor–mediated lipolysis in intra-abdominal fat of these subjects.

In the present study, we found a 43% lower intra-abdominal fat loss (35 cm2 difference in the response to weight loss) in women with the Trp64Arg allele compared with normal homozygotes. Despite the fact that baseline intra-abdominal fat area was different between genotypes, statistical control for this variable tended to reduce the magnitude of the difference observed between Trp64Arg carriers and normal women (from a 35 cm2 difference to a 22 cm2 difference, P = 0.13). However, the effect of the Trp64Arg allele on intra-abdominal fat loss was observed despite a similar total body fat loss in both groups (Table 1). The absence of difference in the magnitude of total and abdominal subcutaneous body fat loss between carriers and noncarriers of the Trp64Arg allele is at variance with a previous study in which smaller decreases in total fat were found in Japanese obese women with the Trp64Arg allele undergoing weight loss (2,4). Moreover, in these two investigations, it was found that the Trp64Arg variant was associated with a smaller decrease in the waist-to-hip ratio in women carrying the Trp64Arg allele, although these investigators did not directly measure intra-abdominal fat and failed to control for total fat loss. Thus, it was unclear in these studies whether carriers of the Trp64Arg allele have a reduced capacity to selectively lose intra-abdominal fat in response to weight loss. Although not directly comparable to the present study, the effect of the combination of both the Trp64Arg variant and the A-to-G mutation of the uncoupling protein 1 on the response to a weight loss intervention was also examined (24). These investigators found that women with both variants had lower weight loss compared with normal homozygotes, also suggesting a possible effect of the β3-adrenoceptor gene variant on the ability to lose weight. Taken together, results of the present study and others (2,4) suggest that the impaired response to weight reduction treatment in postmenopausal obese women with the Trp64Arg variant of the β3-adrenoceptor gene may be specific to the intra-abdominal fat compartment and independent of the loss of total and abdominal subcutaneous body fat mass.

Associations between intra-abdominal fat accumulation and features of Syndrome X have previously been reported (25,26). Thus, we initially hypothesized that genotype differences in intra-abdominal fat loss noted in the present study would be associated with a reduced capacity to improve insulin sensitivity with weight loss. Despite substantial improvement in glucose disposal in both groups, there was no difference in weight loss–induced changes in the M value between carriers and noncarriers of the variant allele. It is possible that the change in intra-abdominal adipose tissue is not a strong predictor of improvements in glucose disposal in a population of obese postmenopausal women undergoing weight loss. Accordingly, recent data from Goodpaster et al. (27) found that changes in intra-abdominal fat with weight loss explained only a small portion of the changes in the M value (14% of variance explained, r = 0.37).

Improvements in blood lipids in response to weight loss were modest and, despite statistical trends, they were found to be nonsignificant (Table 2). Thus, despite substantial weight loss, women in this study were somewhat resistant to the beneficial effects of weight reduction on their lipid profile. The effect of the Trp64Arg genotype on improvement in the blood lipid profile also appeared to be minor because a significant effect was detected only on the cholesterol-to-HDL cholesterol ratio. However, it should be kept in mind that women were still overweight at the end of the study (average BMI 30 ± 4 kg/m2). Moreover, post–weight loss intra-abdominal adipose tissue area was also elevated (average 130 ± 52 cm2). Other studies have shown that an intra-abdominal adipose tissue area >130 cm2 is likely to be found in combination with several alterations in the metabolic profile (28). Thus, it is likely that the rather moderate improvements in lipid profile and our inability to detect genotype effects is attributable to the persistent elevated intra-abdominal fat areas, even after weight loss.

In summary, we found that older obese women carrying the Trp64Arg β3-adrenoceptor gene variant have an impaired capacity to lose visceral adipose tissue in response to prolonged caloric restriction. This phenotypic trait may be attributable to an impaired β3-adrenoceptor–mediated lipolysis in the intra-abdominal fat of these subjects. Despite these genetic differences in loss of intra-abdominal adipose tissue, improvement in glucose disposal was similar between groups.

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