Racial differences in lipid metabolism in women with abdominal obesity

SUSAN B. RACETTE, JEFFREY F. HOROWITZ, BETTINA MITTENDORFER, AND SAMUEL KLEIN
Department of Internal Medicine, Washington University
School of Medicine, St. Louis, Missouri 63110

Received 28 October 1999; accepted in final form 10 March 2000

The mechanism(s) responsible for the differences in metabolic abnormalities between black and white women with abdominal obesity is not known. It has been hypothesized that increased fatty acid release from visceral/infra-abdominal (4) and subcutaneous fat (18) is responsible for many of the metabolic complications associated with abdominal obesity because of fatty acid effects on hepatic glucose production, insulin-mediated glucose uptake, and very-low-density lipoprotein (VLDL) production (13). Therefore, it is possible that racial differences in lipolytic sensitivity to insulin or epinephrine, the major plasma hormones that regulate fatty acid release from adipose tissue, contribute to the metabolic differences observed between black and white obese women. Although it has been shown that the antilipolytic effect of insulin in vitro is greater in abdominal subcutaneous adipocytes obtained from black than from white women with abdominal obesity (9), the same research group recently found that the suppressive effect of insulin on fatty acid rate of appearance (Ra) in plasma in vivo was similar in black and white obese women (2). These results make it unlikely that differences in insulin-mediated fatty acid metabolism are responsible for the metabolic differences between groups. However, the effect of race on the sensitivity of fatty acid kinetics to epinephrine has not been investigated.

The purpose of the present study was to evaluate whole body fatty acid kinetics in response to a physiological range of plasma epinephrine concentrations in black and white women with abdominal obesity, matched for age, BMI, percent body fat, fat-free mass (FFM), and waist-to-hip circumference ratio. The pancreatic hormonal clamp technique (19) was used to minimize differences in plasma insulin concentration between groups and prevent the confounding influence of epinephrine-stimulated insulin secretion (14).

METHODS

Subjects. Eighteen premenopausal women (9 black and 9 white) with abdominal obesity (BMI 32–40 kg/m², >40% body wt as fat, waist-to-hip circumference ratio ≥0.85),...
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>White Women</th>
<th>Black Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36 ± 2</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>99 ± 2</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>37 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>49 ± 1</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>kg</td>
<td>48 ± 2</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>51 ± 2</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>112 ± 2</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Waist-to-hip circumference ratio</td>
<td>0.89 ± 0.02</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>Abdominal adipose tissue, cm²</td>
<td>422 ± 31</td>
<td>410 ± 47</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td>170 ± 14</td>
<td>68 ± 9*</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *Significantly different from corresponding value in white women, P < 0.001.

matched on age, BMI, percent body fat, FFM, and waist-to-hip circumference ratio, participated in the study (Table 1). All subjects completed a comprehensive medical examination, including history and physical examination, electrocardiogram, standard blood and urine tests, and an oral glucose tolerance test. All subjects had normal glucose tolerance, and none had evidence of illness. The subjects were not engaged in regular physical activity. The study was approved by the Human Studies Committee and the General Clinical Research Center Scientific Advisory Committee of Washington University School of Medicine, and written informed consent was obtained from all volunteers before their participation.

Body composition. Total body fat mass (FM) and FFM were determined by dual-energy X-ray absorptiometry (QDR 1000/w, Hologic, Waltham, MA). Abdominal (subcutaneous and intra-abdominal) adipose tissue was quantified by magnetic resonance imaging (Siemens, Iselin, NJ) (1). A single-slice image at the L2-L3 interspace was analyzed for subcutaneous and intra-abdominal adipose tissue content.

Isotope and hormone infusion study. Subjects were admitted to the General Clinical Research Center on the evening before the isotope and hormone infusion study. At 1900 on the day of admission, subjects received a standard meal (55% carbohydrate, 30% fat, and 15% protein) containing 12 kcal/kg adjusted body weight. Adjusted body weight was calculated as follows: ideal body weight + 0.25(actual body weight – ideal body weight), where ideal body weight was based on the medium frame of the Metropolitan Life Insurance Company table (24). A snack comprised of two cans of Ensure (Ross Laboratories, Columbus, OH), containing a total of 500 kcal, 80 g of carbohydrate, 17.6 g of protein, and 12.2 g of fat, was served at 2230 and consumed within 30 min.

On the next morning, after subjects had fasted overnight, three catheters were inserted: one was placed into an antecubital vein to infuse hormones and glucose, the second into the contralateral antecubital vein to infuse palmitate tracer, and the third into a radial artery for blood sampling. Catheters were kept patent with infusions of 0.9% saline solution.

At 0800 (time 0), after withdrawal of a blood sample to measure fasting lipid concentrations and background isotopic enrichment, a 7-h isotope and hormone infusion study (Fig. 1) was started. [2,2-2H2]palmitate (98% atom percent excess) or [1-13C]palmitate (99% atom percent excess; Cambridge Isotope Laboratories, Andover, MA) bound to human albumin (Centeon LLC, Kankakee, IL) was infused at a constant rate (0.04 μmol · kg body wt⁻¹ · min⁻¹) for 1 h by means of a calibrated syringe pump (Harvard Apparatus, South Natick, MA) to assess basal plasma fatty acid kinetics. At 1 h, a pancreatic hormonal clamp (19) was initiated: somatostatin (0.17 μg · kg FFM⁻¹ · min⁻¹; BACHEM Feinchemikalien, Bubendorf, Switzerland), insulin (0.08 mU · kg FFM⁻¹ · min⁻¹; Novo Nordisk Pharmaceuticals, Princeton, NJ), and recombinant human growth hormone (0.00375 μg · kg FFM⁻¹ · min⁻¹; Genentech, San Francisco, CA) were infused continuously for 6 h (from 1 h to 7 h). The rate of insulin infusion during the pancreatic clamp was chosen to eliminate the confounding influence of basal hyperinsulinemia associated with abdominal obesity on the lipolytic response to epinephrine. Plasma glucose concentrations were monitored (Glucose AutoAnalyzer, Beckman Instruments, Fullerton, CA) every 10 min between 1 and 3.5 h, and 20% dextrose was infused as necessary to maintain euglycemia during the baseline period of the hormonal clamp. All subjects achieved euglycemia without dextrose infusion by 2.5 h. Therefore, dextrose was not infused during the last hour of the baseline period of the hormonal clamp or during the remainder of the infusion study. At 2.5 h, the palmitate tracer infusion was restarted and continued throughout the study to assess fatty acid kinetics during the baseline period of the pancreatic hormonal clamp and each stage of epinephrine infusion. At 3.5 h, a four-stage epinephrine infusion protocol was started. Epinephrine (Lederle Laboratories, Chicago, IL), containing ascorbic acid (0.5 mg/ml) to prevent degradation, was infused at 0.00125, 0.005, 0.0125, and 0.025 μg · kg FFM⁻¹ · min⁻¹. Each epinephrine infusion lasted 30 min, separated by a 30-min interval between stages without epinephrine infusion to reestablish basal epinephrine concentrations and basal fatty acid kinetics.

Fig. 1. Schematic diagram of isotope infusion study protocol. Epi 1, Epi 2, Epi 3, and Epi 4, epinephrine infusion at 0.00125, 0.005, 0.0125, and 0.025 μg · kg fat-free mass⁻¹ · min⁻¹, respectively.
Blood sampling. Blood samples were obtained before the beginning of the isotope infusion, every 5 min during the last 15 min of the basal period (4 samples between 45 min and 1 h) and the pancreatic hormonal clamp baseline period (4 samples between 3.25 and 3.5 h), and every 5 min throughout each 30-min epinephrine infusion (6 samples) to determine palmitate kinetics and substrate and hormone concentrations. Samples were collected in prechilled tubes containing EDTA to determine isotope enrichment and fatty acid concentration, reduced glutathione and EGTA to determine plasma catecholamine concentration, and EDTA with Trasylol to determine plasma insulin, C-peptide, and glucagon concentrations. After centrifugation, plasma was collected and stored at −70°C for subsequent analysis.

Analyses. Plasma catecholamine concentrations were determined radioenzymatically (31). Plasma insulin (26) and C-peptide (20) concentrations were measured by RIA. Plasma total cholesterol, high-density-lipoprotein (HDL) cholesterol, and triglycerides were determined enzymatically (Roche/Hitachi 917 Analyzer, Roche Diagnostics, Indianapolis, IN) with commercially available kits; low-density-lipoprotein (LDL) cholesterol was calculated as the difference between total and HDL cholesterol. Plasma free fatty acid concentrations were quantified by gas chromatography with heptadecanoic acid as an internal standard (35).

Plasma palmitate tracer-to-tracee ratio was determined by gas chromatography-mass spectrometry with an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with a capillary column (27). Briefly, plasma proteins were precipitated with acetone, and plasma lipids were extracted with hexane. Fatty acids were converted to their methyl esters with iodomethane and isolated by using solid-phase extraction cartridges. Samples were dried in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY), reconstituted in heptane, and transferred to auto sampler vials for gas chromatography-mass spectrometry analysis. Ions at mass-to-charge ratios 270.2, 271.2 (1-13C)palmitate, and 272.2 (12,2-

Calculations. Palmitate Rα in the basal state and during the baseline period of the pancreatic hormonal clamp were calculated using Steele’s equation for steady-state conditions (32). During steady-state conditions, palmitate Rα is equal to palmitate rate of disappearance (Rd). Plasma palmitate tracer-to-tracee ratio and concentration data obtained during each 30-min epinephrine infusion were smoothed by spline fitting (34), and the non-steady-state equation of Steele (32) was used to calculate palmitate kinetics during each epinephrine stage. The effective volume of distribution of palmitate was estimated to be 40 ml/kg.

Statistical analysis. Student’s t-test for independent samples was used to test the significance of differences in palmitate kinetics between black and white women. The responses of palmitate Rα to epinephrine were evaluated by a two-way (subject group × epinephrine stage) ANOVA for repeated measures. Significant F ratios from ANOVA were followed by Tukey’s post hoc analyses to assess the significance of differences in palmitate Rα between the black and white groups and between epinephrine stages. An α = 0.05 was considered to be statistically significant. Values are means ± SE.

RESULTS

Plasma hormone concentrations. Mean plasma epinephrine concentrations were similar in the black and white women during basal conditions and the baseline period of the pancreatic hormonal clamp (Fig. 2A). Plasma epinephrine concentrations increased progressively during epinephrine infusion at 0.00125, 0.005, 0.0125, and 0.025 μg · kg FFM−1 · min−1 (P < 0.05) and were the same in both groups. Plasma norepinephrine concentrations were similar in black and white women (average 1.04 ± 0.05 and 0.96 ± 0.05 nmol/l, respectively) throughout the study and were not affected by epinephrine infusion. Mean basal plasma insulin (Fig. 2B) and C-peptide (Fig. 2C) concentrations were similar in black and white women during basal conditions and the baseline period of the pancreatic hormonal clamp. Plasma glucose concentrations were similar in black and white women during basal conditions (70 ± 8 and 83 ± 13 mg/dl, respectively, P = 0.57) and identical throughout the epinephrine infusions (52 ± 4 and 52 ± 5 mg/dl, respectively).

Plasma lipids. Basal plasma triglyceride, total cholesterol, and LDL cholesterol concentrations were lower in black than in white women (Table 2). There
was a trend for higher plasma HDL cholesterol concentrations in black than in white women, but this difference was not statistically significant (Table 2). Basal plasma fatty acid concentrations tended to be lower in black than in white women (0.41 ± 0.05 vs. 0.50 ± 0.04 mmol/l), but the difference between groups was not statistically significant (P = 0.09). Plasma fatty acid concentrations increased significantly (P < 0.05) during the baseline period of the pancreatic hormonal clamp in black and white women (to 1.02 ± 0.08 and 1.11 ± 0.06 mmol/l, respectively, P = 0.29 between groups) but did not increase further with epinephrine infusion and were similar in black and white women (1.07 ± 0.09 and 1.21 ± 0.08 mmol/l, respectively, P = 0.13) during the epinephrine infusions.

Fatty acid kinetics. Basal palmitate Ra (and Rd), expressed relative to FM or FFM, was ~25% lower in our black than our white women (P < 0.05; Fig. 3). Palmitate Ra increased during the baseline period of the hormonal clamp in both groups. Although there was a trend toward an increase in palmitate Ra during each stage of epinephrine infusion, the increase was not significantly different from the baseline period during any of the four stages because of the large variability in Ra values (Fig. 4). The differences in palmitate Ra between black and white women persisted across all stages of epinephrine infusion. However, the relative and absolute increases in palmitate Ra from basal values were the same in the two groups (Fig. 5).

**DISCUSSION**

Although the prevalence of obesity is greater in black than in white women, black women have a lower risk of developing some of the metabolic complications associated with obesity when both groups are matched on BMI, body fat, and waist circumference or waist-to-hip circumference ratio (3, 8, 10). We hypothesized that the differences in metabolic abnormalities between groups may be related to racial differences in the regulation of fatty acid metabolism. Therefore, we evaluated basal fatty acid Ra and the sensitivity of fatty acid kinetics to epinephrine, the major plasma hormone that stimulates lipolysis, in black and white women with abdominal obesity. Values are means ± SE. The difference between black and white was significant (P < 0.05).

**Table 2. Basal plasma lipid concentrations**

<table>
<thead>
<tr>
<th></th>
<th>White Women</th>
<th>Black Women</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total triglycerides</td>
<td>115 ± 11</td>
<td>83 ± 12</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>190 ± 10</td>
<td>168 ± 5</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>124 ± 12</td>
<td>103 ± 5</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>42 ± 3</td>
<td>50 ± 4</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE in mg/dl. LDL, low-density lipoprotein; HDL, high-density lipoprotein. P value represents comparison between black and white women.
abdominal obesity compared with lean white women (17). The results from the present study demonstrate that obese black women are just as insensitive to the lipolytic effects of epinephrine as obese white women. The precise mechanism responsible for the blunted response is not known but may be related to a decrease in subcutaneous abdominal adipocyte \( \beta_2 \)-adrenergic receptor density observed in women with abdominal obesity (28). In fact, the blunted lipolytic response to epinephrine in abdominally obese white women in vivo is specific to abdominal, rather than femoral, subcutaneous adipose tissue (17). Although we assume that the lower whole body lipolytic sensitivity in our black women also was caused by a decreased response to epinephrine in upper-body fat, we did not evaluate regional lipolytic activity in our study subjects. It is possible that differences in body fat distribution contributed to the differences in basal fatty acid \( R_a \). Therefore, results from the present study, in conjunction with those reported by Albu et al., demonstrate that the sensitivity of fatty acid kinetics to inhibition by insulin and stimulation by epinephrine is the same in black and white obese women. It is possible that racial differences in body fat distribution contributed to the differences in basal fatty acid \( R_a \) between our black and white women. Increased fatty acid release from visceral/intra-abdominal (4) and subcutaneous (18) fat may be responsible for many of the metabolic complications associated with abdominal obesity, such as insulin resistance, hyperglycemia, and dyslipidemia (13). Excessive release of fatty acids from adipose tissue can impair metabolic sensitivity and may contribute to the pathogenesis of obesity-related complications.
We thank Renata Braudy and the nursing and dietary staff of the General Clinical Research Center for assistance in performing the experimental protocols and Dr. Guohong Zhao and Weiqing Feng for technical assistance.

This study was supported by National Institutes of Health Grants DK-37948, RR-00036 (General Clinical Research Center), AG-13629 (Claude D. Pepper Older Americans Independence Center), RR-00564 (Mass Spectrometry Resource), and DK-56341 (Clinical Nutrition Research Unit).

REFERENCES


