Systemic resistance to the antilipolytic effect of insulin in black and white women with visceral obesity

JEANINE B. ALBU, MICHAEL CURI, MEREDITH SHUR, LAURA MURPHY, DWIGHT E. MATTHEWS, AND F. XAVIER PI-SUNYER

Systemic resistance to the antilipolytic effect of insulin in black and white women with visceral obesity. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E551–E560, 1999.—This study was designed to determine the role of visceral adipose tissue (VAT) accumulation in systemic fat metabolism and to compare this in black and white women who differ in their manifestations of upper body obesity. Systemic glycerol and free fatty acid (FFA) turnover rates (rates of appearance, $R_A$) were measured in the basal state and during a pancreatic euglycemic clamp in nondiabetic, premenopausal, obese black and white women with a wide range of VAT accumulation. The slopes of the regression equations predicting basal and insulin-suppressed $R_A$Glycerol and $R_A$FFA from VAT area, age, and fat mass or fat-free mass did not significantly differ between black and white women. VAT area was the best predictor of the % suppressed $R_A$Glycerol and $R_A$FFA during the pancreatic clamp (partial $r = 0.76$, $P < 0.0001$ and partial $r = 0.60$, $P < 0.05$, respectively). Basal $R_A$Glycerol, but not $R_A$FFA, was lower in black than in white women ($P < 0.05$). During the clamp, black women showed greater insulin suppression of $R_A$Glycerol than of $R_A$FFA ($P < 0.0001$) and greater insulin suppression of $R_A$Glycerol ($P < 0.05$) but similar suppression of $R_A$FFA compared with white women. These differences were independent of age, fat mass, or fat-free mass and were partly explained by a lower VAT in black women. Thus, in both races, VAT accumulation was associated with systemic resistance to the antilipolytic effect of insulin and, in obese black women, systemic lipolysis measured as glycerol turnover rate was more responsive to insulin suppression than were systemic FFA turnover rates. VAT and relative visceral-to-subcutaneous AT accumulation was associated with the unfavorable metabolic traits independently of the overall degree of fatness (3). Whether VAT accumulation, in obesity, is associated with increased systemic FFA flux and/or systemic resistance to the antilipolytic effect of insulin is not known. In the one study of systemic FFA flux in 17 lean and obese women in which direct measurements of VAT were done (12), there was no significant effect of VAT accumulation on basal systemic FFA flux or on systemic FFA flux during a hyperinsulinemic euglycemic clamp. High doses of insulin were used in this study, which may have completely suppressed lipolysis, masking the potential relationship with VAT. Moreover, glycerol turnover rates were not measured, and the racial origin of the subjects was not specified in this study (12).

The manifestations of UBO are less pronounced in women of African-American origin (black) compared with age-matched, similarly obese Caucasian (non-Hispanic white) women (3, 29). Black women with UBO, compared with white women with UBO, have a smaller proportion of visceral than subcutaneous abdominal AT (3, 33). Also, abdominal subcutaneous adipocytes of black women with UBO are less resistant to the antilipolytic effect of insulin, measured in vitro, than abdominal subcutaneous adipocytes of age-matched, similarly obese, white women with UBO (16). Systemic lipolysis, measured in vivo, has not been previously compared in black vs. white obese women. Moreover, whether the potential relationship between VAT accumulation and systemic lipolysis is similar in both races is not known.

We measured systemic lipolysis, as both glycerol and FFA turnover rates, in the basal state, and the systemic antilipolytic effect of insulin during a pancreatic euglycemic clamp, choosing an insulin infusion rate that would produce only a partial suppression of lipolysis, to see differences among individuals. We studied nondiabetic, obese, premenopausal and age-matched black and white women with a wide range of both VAT area and sensitivity to insulin's effect on systemic glucose utilization ($S_I$), measured by the minimal model of Bergman et al. (5). VAT area was measured at midwaist by magnetic resonance imaging (MRI) in 27 women and by computed tomography (CT) scan in 2 women. We determined the relationships of the regional AT and body composition measurements with systemic glycerol and FFA turnover rates and with the systemic antilipolytic effect of insulin during the pancreatic clamp. Furthermore, we compared these relationships between races.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Subjects. Potential subjects were Caucasian (non-Hispanic white) or African-American (black), obese (body mass index [BMI] 27–45 kg/m²), premenopausal women over the age of 25 yr. Subjects were included in the study if all four grandparents were of either Caucasian or African ancestry. All subjects were in good health, nonparous, and of weight stable (±2 kg) for ≥6 mo before, and, at the time of the study, reported regular menstrual cycles. Absence of diabetes according to the National Diabetes Data Group Criteria (36) was based on a screening oral glucose tolerance test (OGTT). The Institutional Review Board of the Health Sciences Institute at St. Luke’s-Roosevelt Hospital Center approved the study. The subjects provided their written consent before participation.

Study protocol. To compare relationships between VAT and lipolysis, obese women of both races with a similarly wide range of visceral adiposity were recruited, as described in an earlier publication (3). The women accepted in the study had anthropometric, regional adipose tissue and SI measurements performed as outpatients within 1–3 mo of the lipolysis protocol. Sixteen black and thirteen white women of similar age and BMI but with a wider range of VAT and SI were recruited for the lipolysis measurements. The subjects were admitted to the Clinical Research Center (CRC) and fed a weight-maintaining diet. The amount of calories was further adjusted to prevent any fluctuation in weight. Body composition measurements were performed the day before the lipolysis measurements. Lipolysis was measured in the basal state after a 12-h overnight fast and during the pancreatic euglycemic clamp protocol (see Pancreatic Euglycemic Clamp Protocol) on day 4 or 5 in the CRC.

Anthropometric and visceral fat measurements. Minimum waist circumference (minimum circumference between the lower rib margin and the iliac crest, usually the midpoint or midwaist) and maximum hip circumference (below the iliac crest, with subject viewed from the front) were measured while the subjects were standing with heels together.

The amounts of visceral and subcutaneous adipose tissue were measured by MRI (GE System Signa Advantage 5.3 Scanner, GE Medical Systems, Milwaukee, WI), as described previously (3). AT areas [VAT and abdominal subcutaneous AT (SAT)] were determined from images obtained at midwaist (the midpoint between the last rib and the iliac crest at the L2–L3 level). The measurement of VAT volume by a similar technique has been recently validated in human cadavers (1); other measurements were performed the day before the lipolysis protocols at the same abdominal level as determined by bony anatomic landmarks. Images were read on the GE System Independent Physicians Console (GE Medical Systems) and on a Picker SystemVoxel Q software system (Picker International, Highland Hills, OH). In the same individual, CT measurements of abdominal AT areas were reported to give results both higher (by 4.4 and 12.8% for SAT and VAT, respectively) or lower (by 8 and 22.5% for SAT and VAT, respectively) than MRI measurements (41, 42). Thus we performed statistical analysis of the data both including and excluding the two individuals measured by CT scans.

Frequently sampled intravenous glucose tolerance test. S_i was measured according to the tolbutamide-modified frequently sampled intravenous glucose tolerance test, or FSIGT, of Bergman et al. (5). All subjects had this measurement within 10 days of the onset of their menstrual cycle. Plasma glucose and insulin were measured on frequently obtained samples, and S_i was calculated from these values with the nonlinear mathematical model of glucose disappearance (MINMOD program, copyright R. N. Bergman, 1986).

Body composition by dual-energy X-ray absorptiometry. Dual-energy X-ray absorptiometry (DEXA) was used to resolve body mass into bone mineral and soft tissue components (Lunar DP-4, software version 3.6, Lunar Radiation Corporation, Madison, WI). The coefficients of variation (CVs) for DEXA in our laboratory are 1.3% for bone mineral and 2.0% for total body fat (25). This model is a direct measurement of percent body fat (%Fat) by DEXA. Total body fat mass (FM) and fat-free mass (FFM) were then calculated from the weight recorded on the day of the measurement.

Lipolysis (glycerol and FFA turnover rates) measurements in the basal state. Glycerol and palmitate turnover rates (rate of appearance, R_a) were measured using stable isotopically labeled tracers. The tracers were prepared in sterile, pyrogen-free solutions of [1-13C]palmitic acid (99%, 13C, Masstrace, Woburn, MA), bound to fatty acid-free human albumin (courtesy of Miles Laboratories, West Haven, CT), and [3,2H_2]glycerol (99% deuterium, Masstrace) dissolved in normal saline (45).

At −120 min, a primed/continuous infusion of [3,2H_2]glycerol (1.6 µmol/kg and 0.11 µmol·kg^{-1}·min^{-1}, respectively) and, at −90 min, a continuous infusion of [1-13C]palmitate (0.07 µmol·kg^{-1}·min^{-1}·normal rate in the albumin mixture) were started (31, 46). Blood samples were collected in EDTA-containing vacutainers at 10-min intervals between −30 and 0 min of the 2-h isotope infusion via a butterfly needle inserted into the contralateral dorsal hand vein. The blood was drawn while the hand was heated (50–55°C) for sampling of arterialized blood. The line was kept patent with a slow infusion of 0.45% normal saline. The blood samples were immediately placed in a 4°C ice bath to prevent in vitro hydrolysis of endogenous triglycerides. Plasmas were promptly separated by centrifugation and stored at −20°C until analysis.

Pancreatic euglycemic clamp protocol. We used a pancreatic euglycemic clamp to equalize the insulin levels of our obese subjects, who had a wide range of S_i and, therefore, of fasting insulin levels (26). Because physiological concentrations of insulin profoundly suppressed FFA turnover rates in normal subjects and in subjects with insulin-dependent diabetes (7, 27), we chose the insulin infusion rates to produce only a partial suppression of lipolysis to see differences among individuals. A two-step euglycemic clamp was performed in each subject (Fig. 1) by use of the technique of DeFronzo et al. [13C] Palmitate

| [13C] Palmitate
| 120' | 90' | 90' |

| Somatostatin: 0.14 µg / kg FFM/min |
| Growth Hormone: 5 µg / kg FFM / min |

Baseline Low Insulin High Insulin

Indirect Calorimetry

Fig. 1. Protocol for pancreatic euglycemic clamp. FFM, fat-free mass.
Somatostatin (Bachem, Torrance, CA) was infused (0.14 μg·kg FFM⁻¹·min⁻¹) (28) starting at time 0 and throughout the duration of the clamp (180 min) to suppress endogenous production of insulin. Tracer infusions were started in the basal state (at −120 and −90 min, as described above), and insulin infusion was started at time 0 as a primed/continuous infusion (Humulins, Eli Lilly, Indianapolis, IN), first at a lower dose (prime: 3.9 and 2.75 μU·m⁻²·min⁻¹ for 2 and 8 min, respectively; continuous: 2 μU·m⁻²·min⁻¹) and then, at 90 min, at a higher dose (prime: 15.5 and 11 μU·m⁻²·min⁻¹ for 2 and 8 min, respectively, continuous: 8 μU·m⁻²·min⁻¹), until 180 min. The plasma glucose concentration was determined every 5 min from 0 to 180 min, and a variable rate of 20% glucose was infused to maintain plasma glucose constant at the basal level. Growth hormone (GH, courtesy of Eli Lilly), which is also suppressed by somatostatin, was added in physiological replacement doses via a continuous infusion of 5 ng·kg FFM⁻¹·min⁻¹ (26) from time 0 to 180 min. Glucagon was not replaced in this protocol because J. ensen et al. (27), in their studies of palmitate flux using somatostatin, showed that the addition of glucagon doubled basal glucose levels and caused breakthrough insulin release, causing a decrease in palmitate flux. The elimination of glucagon repletion caused glucose concentration to rise only slightly, and no change in palmitate flux occurred at steady state. This approach has been validated in a subsequent study by J. ensen (26).

The albumin-bound [1-¹³C]palmitate and the [¹³C]glycerol were infused throughout the basal period and the 90-min, low- and high-insulin clamp periods. Blood for measurement of plasma glycerol and palmitate enrichments and for hormone and metabolite determinations was collected every 10 min during the last 30 min of the basal period and of each of the 90-min clamp periods.

Determination of [¹³C]palmitate and [¹³C]glycerol enrichment in the plasma. For measurement of plasma [¹³C]palmitate enrichment, a 0.5-ml aliquot of plasma was added to a screw-cap tube containing 4 ml of “Dole’s Reagent” (40:10:1, 2-propanol-heptane-sulfuric acid); the tube was shaken and placed in a freezer to cool for 10 min before being centrifuged. The supernatant was transferred into a screw-cap tube containing 2 ml of water and 2 ml of heptane. The tubes were shaken for 5 min, and the clear top layer was carefully removed and placed into a screw-cap reaction vial. The liquid was evaporated to dryness under a steam of nitrogen gas, 50 μl of N-(t-butyldimethylsilyl)-N-methyl trifluoroacetamide, and MTBSTFA-pyridine mixture (5:1) were added, and the vials were capped and heated for 10 min at 70°C to form t-butyldimethylsilyl (TBDMS) ester of palmitate. Injections of TBDMS-palmitate samples were made into a gas chromatograph–mass spectrometer (GC-MS; model 5970, Hewlett-Packard, Palo Alto, CA) operated by electron impact ionization. The [M-57]⁺ ions at mass-to-charge ratio (m/z) = 313, 314, and 327 were corresponding to unlabeled, [¹³C]palmitate, and heptadecanoate internal standard, respectively (heptadecanoate was added to the plasma before extraction). The mole ratio of [¹³C]-labeled to unlabeled palmitate was calculated from the 314/313 integrated peak areas by use of a standard curve of known [¹³C]palmitate enrichments for calibration.

The measurement of plasma [¹³C]glycerol enrichment was performed as described (18). Briefly, heptafluorobutyrl anhydride and ethyl acetate (50:50) were added to form the t-heptafluorobutryl (HFB) glycerol derivative. Injections of HFB-glycerol were made into a GC-MS instrument (model 5988A, Hewlett-Packard) operated in negative chemical ionization mode. The [M-HF⁺]⁺ ions at m/z = 680 and 685 were monitored for unlabeled glycerol and [¹³C]glycerol, respectively. For the palmitate samples, the [¹³C]glycerol enrichments were calculated using the integrated areas under the 685/680 peaks and standards of known [¹³C]glycerol enrichment (18).

Hormone and metabolite assays. Plasma glucose was measured with a Beckman glucose analyzer (Beckman, Fullerton, CA; inter assay CV <4%). Plasma insulin was measured by the charcoal extraction method (CV 12%) (24), which does not distinguish insulin from proinsulin levels. Glucose and insulin areas under the 2-h time curve (AUC) during the OGTT were calculated by the trapezoidal method. Blood samples were taken from subjects after an overnight fast for the measurement of total and high-density lipoprotein (HDL) cholesterol (assay kits 225 and 215–13, Diagnostic Chemicals, Monroe, CT) and triglyceride levels (assay kit 210). The CVs were 2% for HDL cholesterol and 3% for triglycerides. Plasma GH was measured using a time-resolved fluorescence immunoassay (DELFI A system from Wallace, Gaithersburg, MD). The sensitivity of this assay is 0.05 ng/ml. Blood for glucagon level determination was collected in Trasylol-containing vacutainers, and glucagon levels were measured via a double-antibody radioimmunoassay kit (Diagnostic Products, Los Angeles, CA; CV 6.7%). Glycerol concentration was measured in plasma by the microfluorometric technique (CV 3%) (9) in the Hormone and Metabolite Laboratory of the New York Obesity Research Center. Plasma FFA concentrations were measured by the colorimetric assay (Biochemical Diagnostics, Brentwood, NY). The percentage of FFA as palmitate was measured on a gas chromatograph with a polar-packed column and a flame ionization detector in the plasma FFA fraction after extraction of lipids by the Dole method (15) and separation of the FFA by thin-layer chromatography (Adipose Tissue Core Laboratory of the New York Obesity Research Center).

Calculations. The rates of appearance of glycerol (RaGlycerol) and palmitate were calculated by the steady-state dilution equation for stable isotopic tracers (31, 46)

\[ R_a (\mu mol/min) = F \times (I_E/I_E_p - 1) \]

where F is the isotope infusion rate, IE is the isotopic enrichment of the infused (measured by GC-MS as described above), and IEp is the isotopic enrichment of plasma at isotopic equilibrium. During changes in insulin levels, new steady states were achieved and the Ra values were calculated by the steady-state equations for each of the clamp periods (26). The rate of appearance of FFA (RaFFA) was calculated as the Ra of palmitate multiplied by the percentage of palmitate from total plasma FFA, measured as described above.

Postabsorptive glycerol is assumed to originate exclusively in the AT (46) and is not reesterified by adipocytes, which lack α-glycerol kinase (10). Thus RaGlycerol in the plasma is a valid estimator of intracellular adipocyte lipolysis. RaGlycerol multiplied by 3 is assumed to reflect the rates of whole body lipolysis. In contrast, after triglyceride breakdown, the FFAs may undergo intra-adipocyte reesterification without being released in the circulation (primary re-esterification) (10). The contribution of this process (if any) to the systemic RaFFA has recently been challenged (17, 32, 38). Because the exact contribution of this process to the systemic RaFFA is presently unknown, we used RaGlycerol and RaFFA as independent indexes of systemic lipolysis.

Statistical analyses. Between-race comparisons of body composition, regional AT measurements, and metabolic measurements were performed by ANOVA.
Between-clamp period comparisons of the hormones, metabolites, and turnover rates were done by ANOVA with repeated measures. The mean value of the last three measurements of the basal and of each clamp period was used in all analyses.

Between-race comparisons of the hormones, metabolites, and turnover rates at each clamp period were done by ANOVA. To ensure that between-race differences of turnover rates, at each clamp period, were not due to between-race differences in hormonal levels, analysis of covariance, with the hormone levels (insulin, GH, and glucagon) and glucose infusion rate (GIR) as covariates, was also done after testing for parallelism of regression lines. Between-race comparisons of the change in the hormone, metabolite, and turnover rates during the clamp (as the difference between the absolute values at each clamp period) were done using contrast factor analysis.

Pearson's product-moment correlation coefficients were computed to determine the associations of the turnover rates (RaGlycerol and RaFFA) with measurements of regional adiposity (WHR, VAT, and SAT) and FM and FFM.

Between-clamp period comparisons of the hormones measured were significant after control for indexes of body composition (FM and FFM) and age. We have previously found that premenopausal black and white women recruited to have a greater degree of visceral AT (higher VAT) were older and fatter than those recruited to have a lesser degree of visceral AT (lower VAT) (3). Thus the degree of fatness and the age of subjects were considered potentially confounding variables when the relationship of VAT with rates of lipolysis was examined.

The analysis was done separately for basal RaGlycerol, insulin-suppressed RaGlycerol, basal RaFFA, and insulin-suppressed RaFFA as the dependent variable. For each one of these dependent variables, no more than three independent variables were used at a time in the regression equations, for example, age, VAT, and FM. The analysis was then repeated using other regional AT measurements (WHR and SAT) instead of VAT or also using FFM instead of FM. For each set of variables, the slopes and intercepts of the regression lines were compared between the groups of black and white women. When no significant differences were observed, partial correlation coefficients were computed, and results of combined groups were reported.

All values are means ± SE unless noted otherwise. The analyses were done using CSS:Statistica (Statsoft, Tulsa, OK) and SAS software (SAS Institute, Cary, NC).

### RESULTS

**Subject characteristics.** The subjects in both groups were obese and displayed a wide range of visceral adiposity and Si (BMI 29–41 and 27–41, VAT area 32–239 cm² and 25–258 cm², VAT/SAT 0.1–0.5 and 0.07–0.50, and Sì 0.3–8.4 and 0.6–8.6, for black and white women, respectively). Overall, the groups did not differ in age, body weight, body composition, regional AT distribution, Si, or 2-h insulin AUC during the OGTT (Table 1). However, the black women had significantly lower fasting and glucose AUC values during the OGTT (P < 0.001), slightly but not significantly lower fasting triglyceride values, and significantly higher HDL cholesterol levels (P < 0.01) than the white women. These differences are similar to those we found in larger samples of black and white women (3).

### Table 1. Subject characteristics and metabolic measurements

<table>
<thead>
<tr>
<th></th>
<th>Black Women (n=16)</th>
<th>White Women (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36.4 ± 1.7</td>
<td>34.5 ± 1.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.1 ± 1.0</td>
<td>34.7 ± 1.3</td>
</tr>
<tr>
<td>Percent fat</td>
<td>45.4 ± 1.0</td>
<td>46.9 ± 0.9</td>
</tr>
<tr>
<td>FM, kg</td>
<td>43.9 ± 2.0</td>
<td>44.4 ± 2.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.02</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>115.6 ± 12.6</td>
<td>133.4 ± 16.5</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>411.9 ± 28.5</td>
<td>422.6 ± 28.2</td>
</tr>
<tr>
<td>Si, µU·mL⁻¹·10⁻⁴·min⁻¹</td>
<td>2.68 ± 0.54</td>
<td>2.40 ± 0.65</td>
</tr>
<tr>
<td>Glucose AUC, µM/2 h</td>
<td>23.56 ± 0.85</td>
<td>29.24 ± 1.3†</td>
</tr>
<tr>
<td>Insulin AUC, µM/2 h</td>
<td>2.21 ± 247</td>
<td>2.595 ± 426</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>0.89 ± 0.09</td>
<td>1.19 ± 0.13</td>
</tr>
<tr>
<td>HDL cholesterol, mM</td>
<td>1.51 ± 0.09</td>
<td>1.18 ± 0.06‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; FM, fat mass; WHR, waist-to-hip circumference ratio; VAT and SAT, visceral and abdominal subcutaneous adipose tissue, respectively; Si, insulin sensitivity index; AUC, area under the time curve; HDL, high-density lipoprotein. *P < 0.01; †P < 0.001 by ANOVA.

The women who underwent the pancreatic clamp had similar body composition, regional AT, and metabolic characteristics to those of the overall sample [black vs. white: P = not significant (NS) for age: BMI, 34.9 ± 1.2 vs. 32.7 ± 1.4; %Fat, 45.2 ± 1 vs. 45.5 ± 1; VAT area, 100.0 ± 14.3 vs. 121.7 ± 18.7 cm²; Si, 3 ± 0.8 vs. 2.9 ± 0.9].

**Basal lipolytic rates.** Basal lipolysis measurements, RaGlycerol, and RaFFA, were performed in all 29 subjects. Basal RaGlycerol was lower in the black women (217.4 ± 11.8 vs. 262.5 ± 16.9 µmol/min, P < 0.05, by ANOVA). The basal RaFFA was not significantly different between the black and white groups (518.4 ± 39.8 vs. 569.7 ± 36.9 µmol/min, P = NS, by ANOVA).

Correlation coefficients of basal RaGlycerol and RaFFA with regional AT and body composition measurements are shown in Table 2. Basal RaGlycerol and RaFFA did not significantly correlate with VAT area in either group (Fig. 2, A and B). Basal RaGlycerol was significantly correlated with SAT area in black women only (r = 0.66, P < 0.01), whereas basal RaFFA was significantly correlated with WHR and SAT area in white women.

### Table 2. Correlation coefficients of basal glycerol and FFA turnover rates with regional adipose tissue and body composition variables for black and white women

<table>
<thead>
<tr>
<th></th>
<th>Black Women</th>
<th>White Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RaGlycerol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black women</td>
<td>+0.20</td>
<td>+0.32</td>
</tr>
<tr>
<td>White women</td>
<td>+0.23</td>
<td>+0.35</td>
</tr>
<tr>
<td>All women</td>
<td>+0.08</td>
<td>+0.37*</td>
</tr>
<tr>
<td><strong>RaFFA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black women</td>
<td>+0.07</td>
<td>-0.11</td>
</tr>
<tr>
<td>White women</td>
<td>+0.57*</td>
<td>+0.33</td>
</tr>
<tr>
<td>All women</td>
<td>+0.20</td>
<td>+0.11</td>
</tr>
</tbody>
</table>

Turnover rates (rates of appearance, Ra) of glycerol and free fatty acids (FFA) in 16 black and 13 white women are expressed in µmol/min. FFM, fat-free mass. *P < 0.05; †P < 0.01; §P < 0.001; ‡P < 0.0001.
SAT significantly predicted basal RaGlycerol or RaFFA, after adjustment for age and FM or FFM. Basal RaGlycerol remained lower in black women after adjustment for WHR, VAT, age, and turnover rates (see METHODS). Neither WHR, VAT, nor SAT significantly predicted basal RaGlycerol or RaFFA, after adjustment for age and FM or FFM.

The regression lines of equations computed for the prediction of basal RaGlycerol or RaFFA from the regional AT measurements, before and after adjustment for age and FM or FFM, had slopes that were not significantly different for the black compared with the white women. Basal RaGlycerol remained lower in black women after adjustment for WHR, SAT, age, and FM or FFM (P < 0.05) or VAT (P = 0.055, Fig. 2A), by analysis of covariance.

Multiple regression analysis was used to differentiate the independent effect of regional AT measurements from that of FM and FFM on the basal lipolytic turnover rates (see METHODS). Neither WHR, VAT, nor SAT significantly predicted basal RaGlycerol or RaFFA, after adjustment for age and FM or FFM.

The relationships of basal lipolytic rates with regional AT measurements, before and after adjustment for age and FM or FFM, were examined in the black and white groups by comparing the slopes and intercepts of the regression equations, as expressed during the high-insulin dose period (Table 3). Glucagon levels were lower at the low-insulin dose period compared with basal (P < 0.05); no further decrease was observed at the high-insulin dose period. GH levels were maintained throughout the clamp by exogenous infusion.

Plasma glucose was maintained at the basal level throughout the clamp by a variable glucose infusion (P = NS for comparison between the basal and the two clamp periods; Table 4). Fasting glucose levels were lower in black than in white women (P < 0.05). During the clamp, glucose levels (Table 4) and the exogenous GIR (average rate, 0.77 ± 0.1 vs. 1.08 ± 0.2 mg·kg⁻¹·min⁻¹, respectively) did not differ between the black and white groups (P = NS).

Plasma FFA and glycerol levels were significantly lower during the first 90 min of the clamp (low-insulin dose period) compared with basal (P < 0.05) and were higher during the second 90 min of the clamp (high-insulin dose period) compared with the low-insulin dose period (P < 0.05; Table 3). Glucagon levels were lower at the low-insulin dose period compared with basal (P < 0.05); no further decrease was observed at the high-insulin dose period. GH levels were maintained throughout the clamp by exogenous infusion.

Hormones and metabolites during the pancreatic euglycemic clamp. As designed, insulin levels were lower during the first 90 min of the clamp (low-insulin dose period) compared with basal (P < 0.05) and were higher during the second 90 min of the clamp (high-insulin dose period) compared with the low-insulin dose period (P < 0.05; Table 3). Glucagon levels were lower at the low-insulin dose period compared with basal (P < 0.05); no further decrease was observed at the high-insulin dose period. GH levels were maintained throughout the clamp by exogenous infusion.

Fig. 2. Relationships of basal lipolysis (rate of appearance (Ra) of glycerol (RaGlycerol) or FFA (RaFFA), in white (solid line) and black (dotted line) women. Subjects had VAT measurements by computed tomography (CT) scan. For both groups combined, r = 0.37, P < 0.05 and r = 0.11, P = nonsignificant (NS), for A and B, respectively. Intercepts of regression lines in A were different at P = 0.055. Ra values are means of last 3 measurements, taken during the last 30 min of baseline, at steady state.

Table 3. Hormone levels during clamp for black and white women

<table>
<thead>
<tr>
<th>Hormone (pM)</th>
<th>Baseline</th>
<th>Low Insulin</th>
<th>High Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>80.4 ± 8.4*</td>
<td>43.2 ± 3.0*</td>
<td>135.6 ± 10.2*</td>
</tr>
<tr>
<td>White</td>
<td>98.9 ± 20.6*</td>
<td>37.6 ± 6.5*</td>
<td>112.5 ± 10.4*</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>108.7 ± 14.8†</td>
<td>86.4 ± 11.6</td>
<td>82.9 ± 11.4</td>
</tr>
<tr>
<td>Black</td>
<td>136.2 ± 20.5†</td>
<td>120.7 ± 20.4</td>
<td>113.3 ± 18.6</td>
</tr>
<tr>
<td>White</td>
<td>0.70 ± 0.29</td>
<td>0.64 ± 0.03</td>
<td>0.63 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE of 10 black and 9 white women. *P < 0.05 for insulin at baseline vs. low- vs. high-insulin dose period, in both black and white groups. †P < 0.05 for glucagon at baseline vs. low- and high-insulin dose periods, in both black and white groups.

Table 4. Metabolite levels during clamp for black and white women

<table>
<thead>
<tr>
<th>Metabolite (µM)</th>
<th>Baseline</th>
<th>Low Insulin</th>
<th>High Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td>85.9 ± 4.6*</td>
<td>124.9 ± 9.9*</td>
<td>50.7 ± 5.0*</td>
</tr>
<tr>
<td>Glucose (µM)</td>
<td>702 ± 34†</td>
<td>979 ± 80†</td>
<td>456 ± 54†</td>
</tr>
<tr>
<td>Black</td>
<td>721 ± 41†</td>
<td>1049 ± 61†</td>
<td>554 ± 111†</td>
</tr>
<tr>
<td>White</td>
<td>4.9 ± 0.1‡</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolite (µM)</th>
<th>Baseline</th>
<th>Low Insulin</th>
<th>High Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td>80.5 ± 4.3*</td>
<td>109.8 ± 5.6*</td>
<td>64.9 ± 5.9*</td>
</tr>
<tr>
<td>Glucose (µM)</td>
<td>721 ± 41†</td>
<td>1049 ± 61†</td>
<td>554 ± 111†</td>
</tr>
<tr>
<td>Black</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of 10 black and 9 white women. *P < 0.05 for glucagon at baseline vs. low- vs. high-insulin dose period, in both black and white groups. †P < 0.05 for FFA at baseline vs. low- and high-insulin dose periods, in both black and white groups.
period of FFA and glycerol did not differ between the black and white groups.

Lipolytic rates during the clamp. Steady state of isotopic enrichment in the plasma was achieved for both tracers during the last 20 min of the basal and the two insulin infusion periods (Fig. 3, A and B). By experimental design, $\text{RaGlycerol}$ and $\text{RaFFA}$ increased at the low-insulin dose period ($P < 0.05$ compared with basal) and then were suppressed at the high-insulin dose period ($P < 0.05$ compared with basal and with the low-insulin dose period, by ANOVA with repeated measures).

$\text{RaGlycerol}$ ($\mu$mol/min) was not significantly different between the black and the white groups at basal and at the low-insulin and high-insulin dose periods ($P = 0.10$, $P = 0.8$, and $P = 0.14$, by ANOVA, respectively). However, the suppression of $\text{RaGlycerol}$ from the low- to the high-insulin dose period (expressed either as the absolute change, or as a % of the stimulated $\text{RaGlycerol}$) was greater in black than in white women ($P < 0.05$ by contrast factor analysis, Fig. 3A). This difference remained significant after adjustment for changes in insulin, GH, glucagon, glucose, and GIR during the clamp.

$\text{RaFFA}$ ($\mu$mol/min) was not significantly different between the black and the white groups at basal and at low-insulin and high-insulin dose periods ($P = 0.9$, $P = 0.6$, and $P = 0.9$, by ANOVA, respectively), nor were there any differences in the absolute or relative decrease in $\text{RaFFA}$ from the low- to the high-insulin dose period (Fig. 3B). Therefore, in black women, the same change in insulin levels produced a greater suppression of glycerol than of FFA turnover rates ($P < 0.0001$, by contrast factor analysis).

The relationships of $\text{RaGlycerol}$ and $\text{RaFFA}$ at the high-insulin dose period (as absolute rates in $\mu$mol/min) with regional AT measurements (WHR, SAT, or VAT areas) were examined in the black and white groups by comparing the slopes and intercepts of the regression equations before and after adjusting for age and FM or FFM (see METHODS). An interaction was found only for the univariate relationship of $\text{RaGlycerol}$ with WHR ($r = -0.10$, $P = 0.7$ and $r = 0.75$, $P < 0.05$ for black and white women, respectively, $P < 0.05$ for the difference in slopes). The slopes of the other regression equations, including all multivariate models, were not significantly different between the black and white groups. After adjustment for SAT, age, and FM or FFM, $\text{RaGlycerol}$ was lower ($P < 0.05$) but $\text{RaFFA}$ was similar, whereas after adjustment for VAT, the difference in $\text{RaGlycerol}$ diminished ($P = 0.2$), at the high-insulin dose period ($\mu$mol/min), in black compared with the white women (by analysis of covariance).

Correlation coefficients of $\text{RaGlycerol}$ and $\text{RaFFA}$, at high-insulin dose period ($\mu$mol/min), with regional AT and body composition measurements for the black and white groups combined, are shown in Table 5. $\text{RaGlycerol}$ was correlated with VAT area, FFM, and FM, whereas $\text{RaFFA}$ was correlated with VAT and SAT areas, FM, and FFM (Table 5). In multiple regression analyses (see METHODS), only VAT area, but not WHR or SAT, significantly predicted $\text{RaGlycerol}$ after adjust-

Table 5. Relationships of insulin-suppressed $\text{RaGlycerol}$ and $\text{RaFFA}$ with regional adipose tissue and body composition variables

Analyzes done for $\text{RaGlycerol}$ and $\text{RaFFA}$ at high-insulin dose period ($\mu$mol/min) in a group of 19 women, black and white combined. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; §$P < 0.0001$. 

$$
\begin{array}{|c|c|c|}
\hline
\text{Measure} & \text{Univariate Correlation} & \text{Partial Correlation (controlling for age and FM)} \\
\hline
\text{RaGlycerol} & & \\
\text{WHR} & +0.24 & +0.05 \\
\text{VAT, cm}^2 & +0.67‡ & +0.65‡ \\
\text{SAT, cm}^2 & +0.25 & -0.30 \\
\text{FM, kg} & +0.53* & -0.43 \\
\text{FFM, kg} & +0.65† & -0.43 \\
\hline
\text{RaFFA} & & \\
\text{WHR} & +0.31 & -0.12 \\
\text{VAT, cm}^2 & +0.49* & +0.32 \\
\text{SAT, cm}^2 & +0.55* & +0.01 \\
\text{FM, kg} & +0.71† & +0.40 \\
\text{FFM, kg} & +0.73§ & +0.40 \\
\hline
\end{array}
$$
ment for age and FM (Table 5) or FFM (partial r = 0.65, P < 0.01, not shown). The results were similar for RaFFA but fell short of statistical significance (Table 5).

We also quantified the systemic antilipolytic effect of insulin as the RaGlycerol or RaFFA at the high-insulin dose period expressed as a percentage of the value at the low-insulin dose period (%-suppressed rates; Table 6 and Fig. 4, A and B) and performed the same analyses as for the absolute values of the lipolytic rates (see METHODS). An interaction was found only for the univariate relationship of %-suppressed RaGlycerol with VAT area (r = 0.59, P = 0.07 and r = 0.89, P < 0.001, for black and white women, respectively; P < 0.05 and P = NS for the difference in slopes and intercepts, respectively; Fig. 4A). The slopes of the other regression equations, including all multivariate models, were not significantly different between the black and white groups. After adjustment for WHR, SAT, age, and FM (or FFM), the %-suppressed RaGlycerol was lower (i.e., greater insulin suppression of RaGlycerol, P < 0.05) and the %-suppressed RaFFA was similar in black compared with white women (by analysis of covariance).

For both groups combined, %-suppressed RaGlycerol and %-suppressed RaFFA were significantly correlated with VAT area but not with SAT area or with WHR (Table 6). In multiple regression analyses, the relationships between the rates of lipolysis and VAT remained significant after adjustment for age and FM (partial correlation coefficients shown in Table 6). Identical results were obtained after adjustment for age and FFM (not shown).

MRI vs. CT measurements of VAT. Because of the uncertainty related to the measurements of VAT by two different methods, all data analyses were performed without the two women who had VAT measurements by CT scan. The significance of all statistical analyses performed (see METHODS and RESULTS) were the same except for the following P values, which changed statistically: in Table 2, for the correlation of RaGlycerol and VAT, in all women, P = 0.052 instead of P < 0.05; in Table 6, for the univariate relationship of %-suppressed RaFFA and VAT, P = 0.056 instead of P < 0.05, and for the univariate relationship of %-suppressed RaFFA and FFM, P < 0.05 instead of P = 0.055. The two subjects measured by CT scan are identified in Figs. 2, A and B, and 4, A and B, by filled squares.

**DISCUSSION**

In this study we measured the effect of insulin on both FFA and glycerol turnover rates in an obese sample, homogenous with regard to age and gender but with a wide range of visceral obesity, measured as the VAT area at the umbilical level. Both black and white women were studied. We chose increased visceral AT, rather than a WHR over 0.85, as a surrogate for UBO because we have previously shown that 1) the WHR criteria did not distinguish between insulin-resistant and insulin-sensitive obese black women, 2) for the same WHR, black women had less VAT and more abdominal SAT than white women, and 3) increased VAT was similarly associated with insulin resistance (for glucose utilization) in both black and white women (3). VAT area was measured by MRI in most subjects; in two black women VAT area was measured by CT scans.

### Table 6. Relationships of insulin-suppressed RaGlycerol and RaFFA with regional adipose tissue and body composition variables

<table>
<thead>
<tr>
<th></th>
<th>Univariate Correlation</th>
<th>Partial Correlation (controlling for age and FM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RaGlycerol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>+0.17</td>
<td>−0.03</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>+0.74†</td>
<td>+0.76†</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>+0.04</td>
<td>−0.29</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>+0.24</td>
<td>+0.19</td>
</tr>
<tr>
<td><strong>RaFFA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>+0.17</td>
<td>+0.37</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>+0.48*</td>
<td>+0.60*</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>+0.41</td>
<td>+0.14</td>
</tr>
<tr>
<td>FM, kg</td>
<td>+0.45</td>
<td></td>
</tr>
<tr>
<td>FFM, kg</td>
<td>+0.45</td>
<td>+0.23</td>
</tr>
</tbody>
</table>

Analyses done for RaGlycerol and RaFFA at high-insulin dose as a % of values at low-insulin dose in a group of 19 women, black and white combined. *P < 0.05; †P < 0.001; ‡P < 0.0001.
Additional analyses performed without including the data from these two subjects did not significantly alter the overall results of the study. The women we studied here displayed a wide range of both visceral obesity and insulin sensitivity, as confirmed by measurement of $S_i$. We showed that the relationships of basal and insulin-suppressed lipolysis with regional AT measurements (VAT and SAT) did not significantly differ in the black and white groups. The antilipolytic effect of insulin was decreased in obese white and black women with increased VAT, and VAT was the best predictor of the effect of insulin on lipolysis, as measured by $R_g$Glycerol, independent of age, FM, or FFM. Although the black women exhibited the same systemic FFA turnover rates as the white women, they had lower basal and insulin-suppressed systemic glycerol turnover rates. These differences were partly explained by the small differences in VAT that were present in the black and white groups.

Previous studies reported conflicting results regarding the presence of resistance to the antilipolytic effect of insulin in obesity (11, 20, 21, 43). In these studies, the population samples were not homogenous in gender and age, and the subjects were not characterized by adipose tissue distribution. When UBO was characterized by the WHR criteria, the systemic FFA turnover rates were higher, and there was resistance to the antilipolytic effect of insulin in obese premenopausal white women with UBO compared with women with lower body obesity (LBO; WHR <0.75) (23, 26, 28, 34). In these studies, however, the amount of VAT was not directly measured, nor were direct measurements of glycerol turnover rates reported (23, 26, 28, 34). Colberg et al. (12) measured VAT and FFA turnover rates in obese premenopausal women and found no relationship between VAT and the effect of insulin on FFA turnover rates. These authors studied subjects who had lower VAT than our subjects, the racial origin of these subjects was not specified, and higher doses of insulin, but no somatostatin, were used during an euglycemic hyperinsulinemic clamp. These high doses of insulin may have completely suppressed lipolysis in their subjects, thereby masking the potential relationship with VAT. Moreover, the lipolysis was measured as FFA and not as glycerol turnover rates. Therefore, the association between VAT accumulation and decreased systemic antilipolytic effect of insulin may have gone unnoticed.

The finding of such an association may have important pathophysiological implications. First, the systemic resistance to the antilipolytic effect of insulin may reflect the presence of an enlarged, insulin-resistant visceral AT. In vitro, the antilipolytic sensitivity and responsiveness to insulin were decreased in omental compared with abdominal subcutaneous AT fragments from nonobese subjects (8), and the maximal responsiveness of lipolysis to insulin was decreased in omental compared with abdominal subcutaneous adipocytes from morbidly obese women (35, 39). However, in studies of hepatic vein catheterization, splanchnic glycerol release was estimated to be only ~5% of whole body glycerol in nonobese individuals (32), whereas splanchnic palmitate release contributed 15% to the whole body palmitate rate of appearance in lean and in obese women with UBO or LBO (34). It is thus possible that the differences in the systemic, insulin-suppressed $R_g$Glycerol that we found between the women with high and low VAT were due to differences in the glycerol released from AT depots other than the visceral AT.

Alternatively, an enlarged visceral AT may reflect resistance to the antilipolytic effect of insulin in the subcutaneous AT. A direct causal relationship between VAT and insulin resistance in SAT is not readily explained at the present time. VAT may only be a marker for insulin resistance in SAT. For example, when the subcutaneous AT becomes insulin resistant, fat accumulates in the visceral depot. This would not, however, be supported by the in vitro studies showing that VAT is more lipolytic and at least as insulin resistant as SAT (8, 35, 39). The differences between omental and subcutaneous adipocytes, however, have not been systematically assessed in women with various degrees of VAT accumulation.

It is possible that the pancreatic clamp design we used may have enhanced the contribution of splanchnic lipolysis to the systemic $R_g$Glycerol or $R_g$FFA. Lower insulin and higher GH levels than the usual concentrations may stimulate lipolysis. Thus the more lipolytic, insulin-resistant AT depots, within and between individuals, would contribute most to the systemic rates of lipolysis during the pancreatic clamp. In our subjects, basal systemic lipolysis measured as $R_g$FFA or $R_g$Glycerol was not correlated with VAT. Similar to previous reports (3, 6, 14), basal (fasting) insulin levels were significantly correlated with VAT, whereas basal (fasting) GH did not correlate with VAT in our subjects (not shown). The increased insulin levels in the basal state may have suppressed systemic lipolysis and FFA turnover rates, thereby masking the association with VAT. Thus the association of VAT with systemic lipolysis may have been apparent only during the pancreatic clamp, when the insulin levels were controlled exogenously.

Previously it was shown that the elevated basal FFA turnover rates in the white women with UBO originate from upper body AT other than visceral. White women with UBO, with a WHR >0.85 and with increased VAT and upper body SAT, were found to be resistant to the systemic antilipolytic effect of insulin (23, 26, 34). In accordance with this finding, we also found that basal FFA flux was correlated with WHR and abdominal SAT area. We did not find a relationship between abdominal SAT area and the systemic resistance to the antilipolytic effect of insulin. Preferential enlargement of upper rather than lower body SAT may be associated with resistance to the systemic antilipolytic effect of insulin. In this present study we could not account for this, because SAT measurements were done at only one area, the level of the umbilicus. In vivo, dose-response studies of the antilipolytic effect of insulin, in women with preferential enlargement of visceral and/or upper
body subcutaneous depots measured in their entirety, should be done to clarify these issues.

None of the previous in vivo studies of systemic lipolysis examined differences between black and white women. Black women in the United States have a much greater prevalence of obesity and type II diabetes mellitus than white women (37). In the Insulin Resistance Atherosclerosis Study (IRAS), African-American nondiabetic and diabetic men and women were more insulin resistant (by rates of systemic glucose utilization, $S_I$) than individuals in a similar, non-Hispanic Caucasian cohort (22). However, obese black women had lower triglyceride and higher HDL cholesterol levels than white women despite similar or even greater insulin resistance ($S_I$) (3). Moreover, black populations in the United States had more favorable lipid profiles in previous large epidemiological studies (4, 19). In the present study, we showed that basal and insulin-suppressed systemic rates of lipolysis, measured as $R_g$Glycerol, were lower in black compared with white women, whereas basal and insulin-suppressed FFA turnover rates did not differ in the black and white groups. These findings may have important pathophysiological implications. First, the lower rates of glycerol production were partly explained by differences in the size of VAT between the black and white groups. Additionally, systemic rates of lipolysis, either in SAT or VAT, may be lower in black than in white women despite a similar degree of insulin resistance as measured by $S_I$. Second, if systemic glycerol turnover rates were lower but systemic FFA turnover rates were similar in black as in white women, one has to postulate a decreased effect of insulin on intracellular “primary” AT reesterification (11, 17) or a rapid intravascular triacylglycerol hydrolysis, which may have increased the FFA availability and diluted the label in the black women. Therefore, in addition to differences in regional AT distribution, differences between black and white women may exist in the effect of insulin on lipolysis at the AT level (hormone-sensitive lipase), on lipolysis outside of SAT (lipoprotein lipase), or on FFA availability (primary FFA reesterification rates). We cannot compare our results with other studies because studies of systemic glycerol metabolism in obese black and white women have not been published to date.

Finally, the black women in this study were different from the white women in that they had lower glucose levels during OGTT and higher HDL cholesterol levels despite similar $S_I$. Also, although the difference in VAT area of the two groups was not statistically significant by ANOVA, after adjustment for FM and FFM, the black women had lower VAT than the white women. Whether obese women with these characteristics might always exhibit a differential effect of insulin on systemic $R_g$Glycerol vs. $R_g$FFA remains to be proven. Studies in larger numbers of women are needed to confirm whether or not there are indeed differences in lipolysis and FFA reesterification rates between black and white women.

In conclusion, characterization of visceral adipose tissue distribution in obesity is an important marker for an impaired systemic antilipolytic action of insulin and increased systemic FFA flux in nondiabetic, premenopausal obese women. Accurate measurements of the visceral and subcutaneous AT compartments in obese women could help predict insulin resistance of both carbohydrate and lipid metabolism.

We thank our volunteers, Yim Dam, Kangping Chen, Craig Mooney, and Chuck Gikler, for technical assistance, and Drs. Susan Fried and Jill Johnson for review of the manuscript. This study was supported in part by the National Institutes of Health Grants RO1-DK-40414, DK-26687, RR-00645–25, and K08-DK-02155 and by a grant from the Weight Watchers Foundation. Portions of this work have been presented previously at Biomedicine 96, Washington DC, 1996, and at Experimental Biology 97, New Orleans, LA, 1997.

Address for correspondence and reprint requests: J. B. Albu, Obesity Research Center, St. Luke’s/Roosevelt Hospital Center, 1111 Amsterdam Ave., New York, NY 10025 (E-mail: jba1@columbia.edu).

Received 5 August 1998; accepted in final form 20 May 1999.

REFERENCES


