Impaired insulin action in subcutaneous adipocytes from women with visceral obesity

JULIA A. JOHNSON, SUSAN K. FRIED, F. XAVIER PI-SUNYER, AND JEANINE B. ALBU
Obesity Research Center, St. Luke’s-Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University, New York, New York 10025; and Department of Nutritional Sciences, Rutgers University, New Brunswick, New Jersey 08901

Received 20 December 1999; accepted in final form 24 August 2000

Impaired insulin action in subcutaneous adipocytes from women with visceral obesity. Am J Physiol Endocrinol Metab 280: E40–E49, 2001.—Visceral obesity is associated with resistance to the antilipolytic effect of insulin in vivo. We investigated whether subcutaneous abdominal and gluteal adipocytes from visceroally obese women exhibit insulin resistance in vitro. Subjects were obese black and white premenopausal non-diabetic women matched for visceral adipose tissue (VAT), total adiposity, and age. Independently of race and adipocyte size, increased VAT was associated with decreased sensitivity to insulin’s antilipolytic effect in subcutaneous abdominal and gluteal adipocytes. Absolute lipolytic rates at physiologically relevant concentrations of insulin or the adenosine receptor agonist N6-(phenylisopropyl)adenosine were higher in subjects with the highest vs. lowest VAT area. Independently of cell size, abdominal adipocytes were less sensitive to the antilipolytic effect of insulin than gluteal adipocytes, which may partly explain increased nonesterified fatty acid fluxes in upper vs. lower body obese women. Moreover, increased VAT was associated with decreased responsiveness, but not decreased sensitivity, to insulin’s stimulatory effect on glucose transport in abdominal adipocytes. These data suggest that insulin resistance of subcutaneous abdominal and, to a lesser extent, gluteal adipocytes may contribute to increased systemic lipolysis in both black and white visceroally obese women.

Abdominal and gluteal adipocytes; lipolysis; glucose transport; adipose tissue

INCREASED VISCERAL ADIPOSE TISSUE (VAT) has been associated with the insulin-resistance syndrome in upper body (abdominal) obesity (14). However, abdominal obesity is associated not only with excess VAT but also with increased upper body subcutaneous adipose tissue (SAT). A stronger relationship of in vivo insulin resistance to subcutaneous truncal fat than to intraperitoneal fat was shown in moderately obese men (3) and recently in a multiethnic group of lean and obese men and women (20). The relative contributions of visceral and subcutaneous abdominal adipose tissue metabolism to insulin resistance and its associated metabolic disorders are still uncertain (18, 47).

The mechanisms linking visceral obesity to decreased whole body insulin-stimulated glucose utilization are incompletely understood, but the increased availability of nonesterified fatty acids (NEFA) has been strongly implicated (9). Systemic NEFA flux has been shown to be increased in upper body obesity, as defined by an increased waist-to-hip ratio (WHR > 0.85; Ref. 24). Albu et al. (4) have also recently demonstrated that increased VAT, independently of total body fat, is associated with resistance to the systemic antilipolytic effect of insulin measured in vivo in obese women. Although visceral fat is known to exhibit a high lipolytic capacity in vitro (11, 36, 44), this depot has been shown to account for only 15% of systemic NEFA flux in women with upper body obesity (32). Therefore, the majority of NEFA released in these women was thought to derive from upper body SAT (21, 32).

A number of lines of evidence indicate that adipocytes from abdominal SAT are more lipolytically active in subjects with upper body or visceral obesity. Adipocytes from abdominal SAT of visceral obese women were found to exhibit elevated basal and β-adrenergically stimulated lipolysis (34, 39). Also, a recent study (33) found that adipocytes from abdominal SAT of visceroally obese men were more sensitive to β-adrenergic agonists and that this was linked to higher fasting insulin levels.

Additionally, insulin action in adipocytes from abdominal SAT has been shown to vary by race, as well as by WHR (17, 28). Adipocytes from abdominal SAT exhibited insulin resistance in premenopausal Caucasian (non-Hispanic white) women with a WHR > 0.85 but not in similarly obese African-American (black) women with a WHR > 0.85 (17). Obese black women, however, have less VAT for the same WHR compared with equally obese white women (5, 13, 30). Whether racial variation in VAT accumulation explains racial differences in insulin action in adipocytes from upper body SAT is not clear.

In the current study, we tested whether increased VAT is associated with impaired insulin action in adi-
pocytes from SAT and whether this relationship differs by race. A related aim was to determine whether insulin action differs in adipocytes from upper vs. lower body SAT in these women. We studied insulin action in subcutaneous abdominal and gluteal adipocytes from nondiabetic black and white premenopausal women who had a wide range of VAT area measured at midwaist and a wide range of insulin sensitivity (S_I) measured in vivo by the minimal model of Bergman et al. (8).

METHODS

Subjects. Potential subjects were 20- to 49-yr-old white (non-Hispanic Caucasian) or black (African-American), obese (body mass index (BMI) = 27–45 kg/m^2), premenopausal women. The extent of racial admixture was assessed from self-report; subjects were included in the study if all four grandparents were of either Caucasian or African ancestry. Obese women of both races with a similarly wide range of visceral adiposity were recruited, as described in an earlier publication (5). All subjects were in good health, nonparous, taking no prescription medications, weight stable (± 2 kg) for at least the 6 mo before the study, and regularly menstruating at the time of the study. Absence of diabetes according to the National Diabetes Data Group criteria (38) was based on a screening oral glucose tolerance test (OGTT). The study was approved by the Institutional Review Board of the Health Sciences Institute at St. Luke’s-Roosevelt Hospital Center. All subjects provided written consent before participation in the study.

Anthropometric measurements. Fasting weight and height were measured with subjects wearing only their undergarments. 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.

Body composition. Total body fat mass, fat-free mass (FFM), and percent body fat were determined by hydrodensitometry in the fasted state. The coefficient of variation for percent body fat by hydrodensitometry at our center is 2%. Different densities of FFM were used for black (1.106 g/cm^3) and white (1.100 g/cm^3) women in calculating percent body fat with the Siri equation. Our center quantified the density of FFM using a four-compartment model of body composition (19, 52). FFM density was found to be slightly greater in black than in white women, primarily due to heavier bone mass (41).

Visceral fat measurements. Areas of VAT and SAT were measured by magnetic resonance imaging (MRI; G.E. System Signa Advantage 5.3 Scanner, G.E. Medical Systems, Milwaukee, WI) as described (5). Adipose tissue areas (VAT and 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 validated in human cadavers (1). VAT area measured on a transverse body scan has been shown to correlate highly with total VAT volume (45, 50), especially at the L2-L3 level (2).

Three subjects who declined MRI measurements because of the discomfort associated with confinement were measured by computed tomography scans of the same body regions. Images were read on the G.E. System Independent Physicians Console (G.E. Medical Systems) and on a Picker System/Voxel Q software system (Picker International, Highland Heights, OH).

OGTT. OGTT was performed after a 12-h overnight fast. Plasma glucose (Beckman glucose analyzer, Fullerton, CA; coefficient of variation <4%) and insulin were measured in blood samples taken at 30-min intervals for 2 h after glucose ingestion (75 g dextrose, Baxter Healthcare, Valencia, CA). Plasma insulin was measured by radioimmunoassay using the charcoal extraction technique (coefficient of variation <12%; Ref. 22). This method does not distinguish insulin from proinsulin levels. The integral glucose and insulin areas under the curve (AUC) of the 2-h OGTT were estimated by the trapezoid method.

Frequently sampled intravenous glucose tolerance test. Sensitivity to insulin’s effect on systemic glucose utilization (S_I) was measured according to the tolbutamide-modified frequently sampled intravenous glucose tolerance test of Bergman et al. (8). The frequently sampled intravenous glucose tolerance test was performed after a 12-h overnight fast. All subjects had this measurement within 10 days of the determination 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).

SAT biopsy. Twenty-two white and 22 black women agreed to have needle aspirations of SAT; these were performed at 9 AM after a 12-h fast. The biopsies were performed without regard to phase of the menstrual cycle. Previous studies did not find significant differences between the follicular and luteal phases in insulin action on glucose transport and lipolysis in adipocytes from premenopausal women (31). The skin was anesthetized (2% lidocaine; Elkins-Sinn, Cherry Hill, NJ), and adipose tissue was obtained using a blunt-ended needle designed for liposuction (3-mm Spiriroti cannulas; Unitech Instruments, Fountain Valley, CA) from subcutaneous abdominal and gluteal sites, with no longer than 10 min elapsing between the abdominal and gluteal aspirations (17).

Adipocyte isolation. Adipocytes were isolated as described previously (17). Aspirated adipose tissue was washed with PBS (37°C, pH 7.4) and digested with collagenase (1 mg/ml, lot no. 47K121, Worthington Biochemical, Freehold, NJ) in Krebs-Henseleit HEPES (25 mM) buffer, pH 7.4, containing bicarbonate (10 mM), 5% albumin (CRG-7, lot no. H54707, Intergen, Purchase, NY) (KHHB-A buffer), and 0.5 mM glucose. After 45–60 min of collagenase digestion in a shaking water bath (60 cycles/min, 37°C), isolated adipocytes were filtered through a 250-μm nylon mesh (Tetko, Briarcliff Manor, NY) and washed four times by flotation with collagenase-free KHHB-A buffer at 37°C.

Determination of adipocyte size. Diameters of at least 200 cells from each adipocyte suspension were measured directly using a microscope with ocular micrometer (15). A frequency distribution plot of cell diameters was used to determine the mean fat cell diameter and standard deviation about the mean; from these, the mean fat cell volume and surface area were then determined using standard equations (19, 52). Mean fat cell weight (μg lipid/cell) was calculated from cell volume, assuming that the density of lipid is equal to that of triolein (0.915 g/l). The lipid content of an aliquot of cell suspension was determined by extraction (16). Fat cell density in suspension was calculated by dividing the lipid content of the cell suspension by the mean fat cell weight.

In vitro lipolysis measurements. Rates of lipolysis in isolated adipocytes were estimated by glycerol appearance as described (17). Adipocytes (10,000–20,000 cells/ml) were in-
cubated with gentle agitation (37°C, 60 cycles/min) for 2 h in KHBH-A buffer containing glucose (5 mM) and adenosine deaminase (ADA; 2 μg/ml). After incubation, cell-free medium was obtained by centrifugation of the cell layer through silicon oil. Glycerol was assayed in neutralized perchloric acid extracts of the incubation medium (12). Data were expressed as net glycerol release per 10^9 cells during 2-h incubation; glycerol present at the start of incubation was subtracted from all samples. Baseline incubations (ADA alone) were performed in quadruplicate. Incubations to determine the antilipolytic effects of insulin (25, 50, 100, and 400 pM) and of the nonhydrolyzable adenosine receptor agonist N^6-(phenylisopropyl)adenosine (PIA: 10, 20, 50, and 100 nM) were performed in triplicate.

**Determination of glucose transport.** Rates of basal and insulin-stimulated glucose uptake were measured in isolated subcutaneous abdominal adipocytes according to the method of Kashiwagi et al. (26). This assay reflects rates of cellular glucose transport measured directly with 3-O-methylglucose (26). Isolated adipocytes suspended in KHBH-A (20,000-40,000 cells/ml) were preincubated in triplicate with insulin (0, 25, 50, 100, 400, and 16,000 pM) in a shaking water bath (15 min, 37°C). A trace amount (300 nM) of d-[U-14C]glucose (0.1 μCi/ml) was then added, and the incubation was continued for 1 h at 37°C. The incubation was terminated by centrifuging the fat cell suspension through silicon oil in polyethylene microcentrifuge tubes. The tubes were cut through the oil layer containing the cell pellet and added to scintillation vials. Radioactivity associated with the fat cell layer was determined in a Packard Tri-Carb 1600TR liquid scintillation analyzer (Packard Instrument, Meriden, CT). Data were expressed as glucose clearance rate in femtoliters per cell per second.

**In vitro calculations.** The antilipolytic responses of insulin and PIA to ADA-stimulated lipolysis were assessed by dose-response curves. Sensitivity (dose giving half-maximal response [ED_{50}]) was defined as the concentration of insulin (or PIA) at which 50% suppression of lipolysis was observed: this was determined from log-logit plots of glycerol release rate vs. insulin (or PIA) concentration (6). In these calculations, the difference between lipolysis at baseline and at 400 pM insulin (or 100 nM PIA) was set at 100%. The maximum response was calculated as the absolute difference between the lowest rate of lipolysis observed under antilipolytic suppression and the ADA-stimulated lipolysis (when no insulin or PIA was present). This difference was also expressed as a percentage of the ADA-stimulated lipolysis without antilipolytic agent (% of baseline). A high ED_{50} denotes decreased antilipolytic sensitivity. The sensitivity and maximum response of glucose transport to insulin were assessed in a similar manner (26).

**Statistical analyses.** Data are reported as means ± SD (see text and Tables 1–3) or, for illustrative reasons, as means ± SE (see Figs. 1–6). One-way ANOVA was used to compare subject data (anthropometric, body composition, and visceral fat measurements) and in vitro data, both between races and between groups with high and low VAT areas. Lipolysis and glucose transport rates were log transformed if the data were not normally distributed.

Pearson’s product moment correlation coefficients were computed to measure associations among the variables. Where appropriate, multiple regression analysis was employed to control for the effect of total adiposity on the associations between in vitro insulin action and regional adipose tissue measurements. Prediction equations for the two racial groups were tested for parallelism, with the regression lines considered significantly different at the conventional α = 0.05 level. One-way ANOVA was also used to compare sensitivity to the antilipolytic effect of insulin (ED_{50}) between adipose tissue depots (abdominal vs. gluteal). The prediction equations of the relationships between ED_{50} and the respective depot’s mean cell size were computed, and regression lines were compared between depots. Statistical analyses were performed using CSS:Statistica for Windows, Release 5.1 (StatSoft, Tulsa, OK).

**RESULTS**

**Subjects.** The subject characteristics are shown in Table 1. The 22 black and 22 white women did not differ by age, BMI, body fat mass and percentage, VAT and SAT areas, insulin AUC during OGTT, or S1, WHR was higher (P < 0.05), and glucose AUC during OGTT was lower (P < 0.01) in the black than in the white group, as previously reported (5). Both groups displayed a wide range of visceral obesity (VAT range = 33–239 and 25–258 cm²) and S1 (range = 0.1–8.4 and 5.0–8.6 10^{-10} min^{-1} · μU·ml^{-1}), for black and white women, respectively). The mean subcutaneous cell size (wt) varied widely within black and white groups (abdominal ranges 0.36–0.97 and 0.32–1.24 μg, gluteal ranges 0.44–1.09 and 0.39–1.02 μg, respectively) but did not differ between the two races (P = nonsignificant for abdominal or gluteal adipocytes; Table 1). The mean subcutaneous abdominal cell size was smaller than the mean gluteal cell size in black women (P < 0.05) but did not differ by depot in white women (P = 0.6). The race by depot interaction for mean cell size was significant (P < 0.01).

**Lipoplosis: effects of race and visceral obesity.** Insulin inhibition of lipolysis was measured in adipocytes from abdominal SAT of 22 black and 20 white women and in gluteal adipocytes from a subset of 17 black and 14 white women. Measurements in both adipose tissue sites of the same subject were done in a subset of 16 black and 12 white women. The absolute rates of lipolysis and sensitivity to the antilipolytic effect of insulin, in either subcutaneous abdominal or gluteal adipocytes, did not differ by race (Fig. 1, A and B, respec-

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Black Women</th>
<th>White Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Age</td>
<td>37 ± 7</td>
<td>35 ± 6</td>
</tr>
<tr>
<td>BMI</td>
<td>35 ± 4</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>42 ± 8</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>44 ± 4</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.07</td>
<td>0.81 ± 0.06*</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>121 ± 53</td>
<td>137 ± 57</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>410 ± 105</td>
<td>408 ± 123</td>
</tr>
<tr>
<td>Mean abdominal cell size, μg</td>
<td>0.65 ± 0.16</td>
<td>0.69 ± 0.23</td>
</tr>
<tr>
<td>Mean gluteal cell size, μg</td>
<td>0.78 ± 0.19</td>
<td>0.68 ± 0.22</td>
</tr>
<tr>
<td>Glucose AUC, mU/m2 h</td>
<td>24 ± 4</td>
<td>29 ± 5*</td>
</tr>
<tr>
<td>Insulin AUC, pM/m2 h</td>
<td>2,399 ± 1,329</td>
<td>2,578 ± 1,637</td>
</tr>
<tr>
<td>S1, 10^{-10} min^{-1} · μU·ml^{-1}</td>
<td>2.4 ± 2.0</td>
<td>2.5 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; AUC, area under the curve during oral glucose tolerance test (OGTT); S1, insulin sensitivity index. *P < 0.05 for black vs. white women.
tively). When lipolysis data were expressed per cell surface area to adjust for depot and racial differences in mean cell size, similar results were obtained (not shown).

In the entire group of women, subcutaneous abdominal adipocytes were significantly less sensitive (higher ED$_{50}$) to the antilipolytic effect of insulin compared with gluteal adipocytes (Fig. 2; ED$_{50}$ = 47.7 ± 21.3 vs. 33.1 ± 13.7 pM, respectively, $P < 0.01$). No significant race by depot interaction was found for ED$_{50}$ ($P = 0.9$).

To determine whether decreased sensitivity of adipocytes to insulin (higher ED$_{50}$) was associated with variation in either mean fat cell size, total body fat, or visceral fat distribution, the correlations between lipolysis indexes (ADA-stimulated lipolysis, ED$_{50}$, and maximum response) and indexes of total or visceral obesity (adipocyte size, VAT, VAT-to-SAT ratio, and total fat mass) were calculated (Table 2). Because the slopes and intercepts of these relationships did not differ by race, data for all women are shown.

Sensitivity to the antilipolytic effect of insulin was decreased (higher ED$_{50}$), in both adipose tissue sites, in women with increasing visceral obesity. The ED$_{50}$ in subcutaneous abdominal adipocytes was positively correlated with VAT area ($P < 0.01$; Table 2 and Fig. 3A) and with the VAT-to-SAT ratio ($P < 0.01$). These correlations were present in gluteal adipocytes, albeit weaker (Table 2). The ED$_{50}$ values did not vary as a function of mean cell sizes or total body fat mass. However, the rates of basal (ADA-stimulated) lipolysis and the maximum antilipolytic response to insulin were greater with an increase in the respective subcutaneous cell sizes (Table 2). Neither total nor visceral adiposity was significantly correlated with the maximum response to insulin, expressed either as the absolute decrement in lipolysis from baseline (Table 2) or as a percentage of baseline lipolysis (not shown).

To assess the impact of visceral adiposity on subcutaneous abdominal adipocyte lipolysis at varying concentrations of insulin, absolute rates of lipolysis were compared for women in the highest (200 ± 34 cm$^2$, $n =$ 28) and lowest (63 ± 22 cm$^2$, $n =$ 20) visceral obesity groups. The ED$_{50}$s were greater with an increase in VAT area and with the VAT-to-SAT ratio ($P < 0.01$). These correlations were present in subcutaneous adipocytes, albeit weaker (Table 2), but not in gluteal adipocytes (Table 2). The ED$_{50}$ values did not vary as a function of mean cell sizes or total body fat mass.

![Fig. 1.](http://ajpendo.physiology.org/)

**Table 2. Simple correlations of mean cell size and regional and total adiposity measurements with antilipolytic effect of insulin in subcutaneous abdominal and gluteal adipocytes**

<table>
<thead>
<tr>
<th></th>
<th>Mean Cell Size</th>
<th>VAT-to-SAT Ratio</th>
<th>Fat Mass, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcutaneous abdominal (n = 42)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADA-stimulated lipolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cell, nmol·10$^{-5}$ cells·2 h$^{-1}$</td>
<td>0.51†</td>
<td>0.22</td>
<td>0.08</td>
</tr>
<tr>
<td>Per cell surface area, nmol·10$^{-9}$ cells·µm$^{-3}$·2 h$^{-1}$</td>
<td>0.09</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin maximum response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cell, nmol·10$^{-5}$ cells·2 h$^{-1}$</td>
<td>0.32*</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin sensitivity (ED$_{50}$), pM</td>
<td>0.10</td>
<td>0.46†</td>
<td>0.45†</td>
</tr>
<tr>
<td><strong>Gluteal (n = 31)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADA-stimulated lipolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cell, nmol·10$^{-5}$ cells·2 h$^{-1}$</td>
<td>0.41*</td>
<td>0.20</td>
<td>0.27</td>
</tr>
<tr>
<td>Per cell surface area, nmol·10$^{-9}$ cells·µm$^{-3}$·2 h$^{-1}$</td>
<td>0.09</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin maximum response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cell, nmol·10$^{-5}$ cells·2 h$^{-1}$</td>
<td>0.40*</td>
<td>0.27</td>
<td>0.29</td>
</tr>
<tr>
<td>Insulin sensitivity (ED$_{50}$), pM</td>
<td>0.28</td>
<td>0.38†</td>
<td>0.32†</td>
</tr>
</tbody>
</table>

ED$_{50}$, dose giving half-maximal response. ADA, adenosine deaminase. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.0001$.

![Fig. 2.](http://ajpendo.physiology.org/)
Fig. 3. A: relationship between sensitivity (ED$_{50}$) to antilipolytic effect of insulin in subcutaneous abdominal adipocytes and visceral adipose tissue (VAT) area in black (●; n = 22) and white (○; n = 20) obese women. There were no significant differences between races for coefficients and intercepts of regression lines. B: inhibitory effect of insulin on ADA-stimulated lipolysis in subcutaneous abdominal adipocytes from obese women in lowest (●; n = 11) and highest (○; n = 11) quartiles of VAT area. By ANOVA: P = 0.28 for baseline; P = 0.07 for 25 pM, P = 0.04 for 50 pM, P = 0.005 for 100 pM, P = 0.07 for 400 pM insulin concentration; P = 0.057 for insulin sensitivity (ED$_{50}$). *Significant differences between high and low VAT groups (P < 0.05).

SAT would thus be expected to be highest when visceral obesity is accompanied by subcutaneous fat cell hypertrophy due to higher rates of basal lipolysis (Table 2) and lower sensitivity to the antilipolytic effect of insulin (Fig. 3A).

The antilipolytic effect of PIA in abdominal SAT was also attenuated in viscerally obese women. Dose-response curves for the antilipolytic effect of PIA in subcutaneous abdominal adipocytes from women in the highest and lowest quartiles of VAT area are shown in Fig. 4. Analyses were performed similarly to those for insulin inhibition of lipolysis, as described in the previous paragraphs. Rates of lipolysis at 10 nM PIA were higher for the women in the highest VAT group compared with those in the lowest VAT group (P < 0.03). This difference was not significant when lipolysis rates were expressed per cell surface area or after adjustment for mean cell size by analysis of covariance. Sensitivity to the antilipolytic effect of PIA (ED$_{50}$) in abdominal SAT was positively correlated with VAT

![Fig. 4](http://ajpendo.physiology.org/). Inhibitory effect of N$^6$-(phenylisopropyl)adenosine (PIA) on ADA-stimulated lipolysis in subcutaneous abdominal adipocytes from obese women in lowest (●; n = 11) and highest (○; n = 11) quartiles of VAT area. By ANOVA: P = 0.14 for baseline; P = 0.03 for 10 nM, P = 0.07 for 20 nM, P = 0.3 for 50 nM, P = 0.25 for 100 nM insulin concentration; P = 0.3 for PIA sensitivity (ED$_{50}$). *Significant differences between high and low VAT groups (P < 0.05).
Glucose transport: effects of race and visceral obesity. Insulin-stimulated rates of glucose transport were measured in subcutaneous abdominal adipocytes only, in 22 black and a subset of 20 white women. The anthropometric and regional fat distribution characteristics of these women were similar to those of the larger group. Black and white groups did not differ with regard to absolute rates of insulin-stimulated glucose transport nor to the maximum response and sensitivity of glucose transport to insulin (Fig. 5). Similar results were obtained when glucose transport data were expressed per cell surface area (not shown). There were no significant interactions by race in the relationship of VAT to insulin’s effect on glucose transport in vitro; therefore, we combined racial groups for further analysis.

For both groups combined, the maximum response to insulin’s stimulatory effect on glucose transport was inversely related to VAT area \((n = 39, r = -0.31, P = 0.05, \text{Fig. 6A})\). However, sensitivity of glucose transport to insulin \((\text{ED}_{50})\) was not significantly related to VAT \((P = 0.8)\). The relationship between maximum response and VAT area was independent of mean cell size and was stronger when data were expressed per cell surface area \((r = -0.35, P = 0.027)\) or when data per cell were adjusted for cell size by analysis of covariance \((\text{partial } r = -0.36, P = 0.032)\). Insulin-stimulated rates of glucose transport (per cell) for the women in the highest VAT area quartiles were higher than those for the women in the lowest VAT area quartiles \((\text{Fig. 6B})\), but these differences fell short of statistical significance.

Correlations of in vivo and in vitro measurements. Because visceral obesity per se is associated with hyperinsulinemia and with resistance to insulin’s ability to stimulate glucose disposal in vivo, we examined the relationships of insulin levels (fasting and during OGTT) and \(S_I\) to measurements of insulin sensitivity in vitro (Table 3). In both subcutaneous abdominal and gluteal adipocytes, reduced sensitivity to the antilipo-
lytic effect of insulin (elevated ED$_{50}$) was strongly related to elevated fasting insulin levels and insulin AUC and to decreased $S_1$ (all $P < 0.05$). Additionally, the maximum response to insulin’s stimulatory effect on glucose transport in abdominal adipocytes was highly correlated with $S_1$ ($P = 0.004$) and was inversely related to fasting insulin and insulin AUC ($P < 0.03$).

**DISCUSSION**

We demonstrate for the first time in this study that subcutaneous adipocytes from black and white women with visceral obesity are less sensitive to the antilipolytic effects of insulin and the adenosine agonist PIA. Resistance to the antilipolytic effect of insulin in vitro was correlated with increased VAT accumulation, independently of the size of subcutaneous adipocytes and/or the level of total body fat mass. Thus the alterations in insulin action on subcutaneous adipocytes appear to be a specific feature of visceral obesity in premenopausal women and are not wholly attributable to increased adiposity per se.

Additionally, when measured at physiologically relevant concentrations of insulin or adenosine, absolute lipolytic rates were increased in subcutaneous fat cells from women with visceral obesity. These elevated rates of lipolysis were proportional to increased adipocyte size. In two studies (34, 39), viscerally obese women exhibited elevated rates of basal and $\beta$-adrenergic receptor-mediated lipolysis in subcutaneous adipocytes; however, these studies disagreed on whether these associations were independent of adipocyte size. Our data indicate that increased rates of lipolysis in the SAT of obese women may be independently influenced by adipocyte size, as well as by the presence of increased VAT.

These data further suggest that the increases in baseline systemic NEFA flux previously reported in upper body obese women (24) are at least partly attributable to 1) decreased sensitivity to the antilipolytic effect of insulin, independent of cell size; and to 2) increased lipolytic rates associated with subcutaneous fat cell hypertrophy. These in vitro data are also consistent with previous observations that NEFA flux after a meal is less well suppressed in upper body obesity (21, 46).

Albu et al. (4) have recently reported that women with greater degrees of visceral obesity are more resistant to the in vivo antilipolytic effect of insulin. Our in vitro data suggest that, at least partly, this systemic antilipolytic resistance may originate in SAT. The mechanisms responsible for this association are not known at the present time. Assessment of intracellular mechanisms for the insulin action abnormalities in SAT could have been helpful in better understanding the association with enlarged VAT. Future studies should examine whether insulin receptor binding capacity, autophosphorylation, or signal transduction (51) are impaired in subcutaneous adipocytes from viscerally obese individuals.

In animal experiments, it has been shown that the presence or absence of an enlarged specific fat depot may influence adipose tissue metabolism in another fat depot, as well as the systemic insulin levels. Sprague-Dawley rats that had epididymal and perinephric fat pads surgically removed were shown to have decreased plasma insulin levels relative to sham-operated rats and decreased expression of the cytokines tumor necrosis factor-$\alpha$ and leptin in other SAT (7). However, circulating NEFA levels were unchanged by this experimental manipulation (7). Expression of tumor necrosis factor-$\alpha$ and leptin has been shown to be regulated by circulating lipids and insulin (40, 42).

From these results we might hypothesize that, in viscerally obese humans, chronically elevated insulin levels (5) may directly lead to insulin insensitivity in subcutaneous adipocytes. Indeed, statistically, the relationship between VAT and diminished sensitivity to the antilipolytic effect of insulin in SAT in this study can be entirely explained by elevated insulin levels (fasting or during OGTT), independent of total body fatness. Hyperinsulinemia has also been statistically associated with increased sensitivity to $\beta$-adrenergic agonists in subcutaneous abdominal adipocytes from viscerally obese men (33).

In a previous report, Dowling et al. (17) assessed the impact of race and upper body obesity on insulin’s antilipolytic action in subcutaneous adipocytes. Upper body obese white women, characterized by an increased WHR, exhibited resistance to the antilipolytic effect of insulin in abdominal and gluteal adipocytes. However, black women with upper body obesity by the WHR criteria did not exhibit alterations in insulin action, when lower body obese black women of similar adiposity were used as controls. VAT areas were not determined in that study. In the current study, we found no racial differences in the relationship of VAT to absolute rates of lipolysis or glucose transport or to insulin responsiveness or sensitivity, in subcutaneous adipocytes. Thus the results of our previous study (17) could be explained if obese black women have less VAT for the same WHR compared with equally obese white women, as we and others have previously reported (5, 13).

Furthermore, we have demonstrated in this study that subcutaneous abdominal adipocytes are more resistant to the antilipolytic effect of insulin than gluteal adipocytes, independent of cell size. This in vitro finding may explain why, in vivo, the abdominal adipose tissue makes a greater contribution to postmeal systemic NEFA flux than does the gluteal adipose tissue, at least in lean and upper body obese women (21, 23). Viscerally obese subjects have increased abdominal SAT deposition relative to gluteal fat deposition. This could further account for an overall decreased systemic antilipolytic effect of insulin. It was not possible to detect this in the current study, because abdominal SAT was not measured in its entirety. However, using whole body MRI measurements, Kovara et al. (27) have recently reported that VAT is in fact associated with upper body rather than lower body SAT accumulation.
There has been considerable disagreement in previous in vitro studies regarding regional differences in sensitivity to insulin’s antilipolytic effect. Some investigators (43, 48) have found greater sensitivity to the antilipolytic effect of insulin in subcutaneous abdominal adipocytes and were unable to demonstrate any measurable insulin antilipolytic effect in lower body subcutaneous adipocytes. The lack of insulin effect in these studies may have been due to the use of norepinephrine to stimulate lipolysis, which resulted in significantly higher lipolytic rates in subcutaneous abdominal adipocytes due to regional differences in the distribution of α- and β-adrenergic receptors (35). Generally, when in vitro studies have been performed without catecholamine stimulation, subcutaneous upper and lower body (gluteal and femoral) fat cells appear to exhibit similar sensitivities to insulin’s antilipolytic effect (10, 17, 37).

Previously, Dowling et al. (17) reported that subcutaneous abdominal adipocytes were more resistant to the antilipolytic effect of insulin than gluteal adipocytes but only in lower body obese black women. In the present study, the decreased antilipolytic effect of insulin in abdominal compared with gluteal SAT was present in both black and white women, when analyses were done separately by race. We may have selected subjects, in both races, who had a broader abdominal vs. gluteal SAT distribution than in our previous study, where upper and lower body obese groups were selected by extremes of WHR.

Subcutaneous abdominal adipocytes from the viscerally obese women in this study also exhibited a decreased suppression of lipolysis at 10 nM PIA, an adenosine A₁-receptor agonist. In the previous study by Dowling et al. (17), upper body obese white women exhibited reduced adenosine sensitivity in vitro compared with similarly obese white women with lower body obesity. The interstitial concentration of adenosine in human adipose tissue normally ranges from 25 to 300 nM, with an average of ~130 nM (29). Because PIA exhibits a sixfold higher binding affinity relative to adenosine (49), these data suggest that altered adenosine sensitivity may further contribute to the elevated baseline lipolytic rates in viscerally obese subjects under physiological conditions. Decreased sensitivity to adenosine may be related to increased adenosine content in SAT and subsequent downregulation of adenosine receptor number (25). However, it is not known whether the SAT of women with visceral obesity has an increased adenosine content.

Additionally, subcutaneous abdominal adipocytes from viscerally obese women were less responsive to the stimulatory effect of insulin on glucose transport. However, sensitivity (ED₅₀) was not affected. The maximum response to insulin’s ability to stimulate glucose transport in subcutaneous abdominal adipocytes was decreased in women with increased VAT, particularly after adjusting for cell size. These results were similar to previous findings by Dowling et al. (17) in white women with various degrees of fat distribution assessed by WHR. It is unclear why the sensitivity to insulin’s antilipolytic effect was diminished in subcutaneous adipocytes from viscerally obese women, while the sensitivity to insulin’s ability to stimulate glucose transport was not. This pathway specificity of alterations in SI with visceral obesity suggests differences in the coupling of insulin receptor activation to signaling pathways regulating glucose transport and lipolysis or possibly divergence in these pathways downstream of insulin receptor autophosphorylation (51).

In summary, excess visceral adiposity is associated with insulin resistance of subcutaneous fat cells from both abdominal and gluteal depots. Subcutaneous adipocytes from viscerally obese women are less sensitive to the antilipolytic effect of insulin and less responsive to the stimulatory effect of insulin on glucose transport. In addition, adipocytes from upper body SAT are more resistant to the antilipolytic effect of insulin than are adipocytes from lower body SAT, providing a mechanism for the lesser effect of lower vs. upper body obesity on systemic NEFA flux. Moreover, visceral adiposity has similar effects in black and white women. These findings provide a cellular basis for explaining the increased systemic lipolysis seen in upper body and, to a greater extent, visceral obesity. These data support the hypothesis that increased nonesterified fatty acids derived from subcutaneous adipocytes may contribute to the relationship of visceral obesity with metabolic complications, including insulin resistance and dyslipidemia.

We thank the women who agreed to participate in this study, and we thank Yim Dam and Kangping Chen for technical assistance. This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1-DK-40414, DK-26687, and K08-DK-02155, and the National Center for Research Resources Grant RR-00645–25.

Portions of this work were presented at the annual meeting of the Federation of American Societies for Experimental Biology (Experimental Biology 96), Washington, DC, 1996, and at the 16th International Diabetes Federation Congress, Helsinki, Finland, 1998.

REFERENCES


