Ethnic differences in in vitro glyceride synthesis in subcutaneous and omental adipose tissue

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Bower, J. F., S. Vadlamudi, and H. A. Barakat. Ethnic differences in in vitro glyceride synthesis in subcutaneous and omental adipose tissue. Am J Physiol Endocrinol Metab 283: E988–E993, 2002. First published July 30, 2002; 10.1152/ajpendo.00225.2002.—Considerable evidence suggests that there are ethnic differences in lipid metabolism between African American and Caucasian women, which may result in increased synthesis of fat in adipose tissue. The purpose of this study was to measure the in vitro rates of \( ^{14} \text{C} \)glucose incorporation into the glyceride-glycerol backbone of triglycerides (TG) and diglycerides (DG) in abdominal subcutaneous (SAT) and omental adipose tissue (OAT). Morbidly obese African American women had faster rates of clearance of TG from circulation that is mediated by lipoprotein lipase (LPL) or a decreased synthesis of VLDL particles in due to an increased uptake of TG-rich lipoproteins (24, 30–34). These differences in plasma TG may be in part contribute to the increased weight gain and obesity seen in African American women (13, 14, 19–21).

The purpose of this study was to examine possible differences in the in vitro rates of newly synthesized glycerides in African American and Caucasian women. This was accomplished by measuring the rates of incorporation of \( ^{14} \text{C} \)glucose into TG and diglycerides (DG) in abdominal subcutaneous (SAT) and omental adipose tissue (OAT) from African American and Caucasian women. In addition, the rates of reesterification were evaluated in a subset of obese women to determine whether there were differences in the recycling of free fatty acids (FFA).

METHODS

Subjects. Four groups of women participated in this study: preobese [body mass index (BMI) 24.5–28.8] African Americans and Caucasians and morbidly obese (BMI > 35) African Americans and Caucasians. The participants were free of vascular disease, diabetes, cancer, or emotional distress and were not taking medications that might affect carbohydrate or lipid metabolism. The subjects were not taking hormone replacement therapy or birth control pills. The women who participated in this study were recruited consecutively over a period of 18 mo from the Department of Surgery at East Carolina School of Medicine and were divided into groups on the basis of BMI according to the guidelines of the World Health Organization; preobese ranged in BMI from 24.5 to 28.8, and the morbidly obese ranged in BMI from 35 to 70 kg/m\(^2\). African American women were included in this study only if their parents and grandparents were of African American descent. Body mass and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively, and BMI was calculated. Waist-to-hip ratio (WHR) was measured only in the preobese subjects due to inherent inaccuracies of waist and hip measurements in morbidly obese subjects. WHR was measured as the ratio of the minimal waist circumference to the maximal circumference of the buttocks. Forty-seven women were recruited for this study: 18 obese Caucasian, 15
obese African American, 7 preobese Caucasian, and 7 preobese African American. SAT and OAT were obtained from these volunteers during abdominal surgery for gastric bypass or total abdominal hysterectomy. Abdominal SAT was dissected from the epigastric region of the abdomen, and abdominal OAT was dissected from the greater omentum. Blood samples were collected 1 wk before surgery, and plasma was analyzed for glucose and insulin concentrations as previously described (24). Written consent was obtained from all of the subjects after they were informed of the nature of the study. The Institutional Review Board for human subject research approved the protocols used in this study.

Plasma analyses. Blood was collected from the subjects after a 12-h fast, and a preservative solution containing sodium azide (50 mg/ml) and aprotonin (1 TIU/ml) was added (2 µl/ml blood). Plasma was prepared by centrifugation, aliquoted, and stored at −80°C until analyzed. Samples were analyzed spectrophotometrically for glucose (18-UV; Sigma Chemical, St. Louis, MO) and by microparticle enzyme immunoassay for insulin (IMX, Abbott Labs, Abbott Park, IL).

Incubations. To measure possible differences in glyceride synthesis in the cell volume, we used the method described by Edens et al. (11). This method gives an accurate assessment of glyceride synthesis with the use of [14C]glucose as a substrate. It has been shown that glucose is incorporated solely into the glyceride-glycerol backbone and that only negligible amounts are incorporated into FFA. With the additional measurements of esterification and lipolysis, the rate of reesterification can be determined to assess the amount of recycling that occurs during synthesis. Human adipose tissue was obtained from surgery and transported to the laboratory immediately in RPMI 1640 (Invitrogen, Frederick, MD). The tissue was washed with phosphate-buffered saline (PBS), cleaned, minced, and preincubated in incubation medium [4% essentially fatty acid-free BSA (Sigma Chemical) and 4 mM glucose in Krebs-Ringer bicarbonate buffer] for 30 min at 37°C in a 95% O2-5% CO2 atmosphere. After incubation, 100-mg pieces were weighed and transferred to the assay tubes containing incubation medium, [preincubation buffer containing 0.5 mM oleic acid and radioactive substrates [14C]glucose (final specific activity 0.4 uCi/µmol) and [3H]oleate (final specific activity 20 µCi/mmol)]. Incubations occurred for 1 h at 37°C in a 95% O2-5% CO2 atmosphere. All assays were run in triplicate. After incubation, tissues were washed with PBS to remove unincorporated label, blotted dry, and transferred to a new tube for homogenization. Homogenates were extracted with a 2:1 chloroform-methanol mixture and centrifuged. The organic phase was transferred to a preweighed vial and allowed to evaporate and was then reweighed to determine lipid weight. A known amount of sample was loaded onto silica plates, and the lipids were separated by thin-layer chromatography using heptane-isopropyl ether-acetic acid (60:40:3). Bands corresponding to DG and TG were scraped and counted for radioactivity.

Cell size determination. Adipocytes were prepared according to the method of Rodbell (27), and adipocyte cell size was determined according to the method of Di Girolamo et al. (9). One gram of adipose tissue was digested, the average diameter of >150 cells was determined by ocular gradient, and total lipid was extracted from a 100-mg piece of adipose to determine the cell volume. The average cell diameter was converted to the mean fat cell volume by the equation (V = πD^2/6) and multiplied by the density of lipid, 0.915 g/ml, to give the mean fat cell TG content. The TG content of tissue was divided by the mean fat cell TG content to give the number of cells per gram tissue.

Lipolysis. Glycerol concentration in the medium was determined for the experiments that measured rates of reesterification. Glycerol was determined as described by Boobis and Maughan (5), in which medium from the incubation tubes was removed before termination of the reaction, and the glycerol concentration was determined by measuring the fluorescence at 420 nm.

Reesterification. The percentage of reesterification was calculated as previously described by Leibel and Hirsch (22). Reesterification is a measure of the incorporation of unlabeled fatty acids into newly synthesized glycerides during the incubation. The rate of reesterification of fatty acids is the difference between esterified fatty acids accounted for by [3H]oleate and the total moles of fatty acids that must have been esterified to give [14C]glyceride formation. First, the rate of unlabeled FFA must be calculated as: E(U) = [3·(nmol [14C]TG) - nmol [3H]TG + 2·(nmol [14C]DG) - nmol [3H]DG], where the [3H]oleate represents the [3H]oleate esterified to [14C]glucose backbone. Second, because the rate of glycerol release can be measured during the incubation, it is then possible to calculate the percentage of these FFA released by TG hydrolysis (L = 3·moles of glycerol released) that were reesterified to newly synthesized TG or DG. Therefore, the percentage of reesterification %RE = [E(U)/(3·L)]·100.

Statistics. To examine significant differences between the races within each group (obese African American vs. obese Caucasian), Student’s t-tests were performed, and P < 0.05 implied statistical significance. All analysis was performed on SPSS version 9.0 (SPSS, Chicago, IL).

RESULTS

Table 1 shows the characteristics of the subjects in this study. There were no significant differences in age, BMI, glucose, or insulin concentrations between the races in either the preobese or obese groups. WHR measurements in preobese subjects were also similar between the races. There were no significant differences in the average cell diameter of obese women in either SAT or OAT. Preobese African American women had a larger SAT cell diameter compared with that of preobese Caucasian women (P < 0.05), but there were no differences in OAT cell size.

The rates of incorporation of [14C]glucose into TG (Fig. 1A) and DG (Fig. 1B) of obese women are shown in Fig. 1. There were no significant differences in the rates of newly synthesized TG or DG in SAT between the races. Obese African American women had significantly higher rates of TG synthesis in OAT compared with obese Caucasian women (P = 0.003; Fig. 1A). There were no differences in newly synthesized DG in OAT of the obese women. Similar to previous reports (11, 12), DG accumulation accounted for 21–24% in the SAT depending on the group, whereas OAT had a larger DG accumulation, with 31–41% depending on the group (Fig. 1B).

The rates of incorporation of [14C]glucose into TG (Fig. 2A) and DG (Fig. 2B) of preobese women are shown in Fig. 2. There were no significant differences in the rates of newly synthesized TG or DG in SAT between the races. Preobese African American women had significantly higher rates of TG synthesis in OAT.
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Obese Women</th>
<th>Preobese Women</th>
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<tr>
<td></td>
<td>African American</td>
<td>Caucasian</td>
</tr>
<tr>
<td>$n$</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>41 ± 1.4</td>
<td>41 ± 1.9</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>45 ± 2.5</td>
<td>51 ± 2.3</td>
</tr>
<tr>
<td>WHR</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>92 ± 4.3</td>
<td>90 ± 3.5</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>12.2 ± 3.8</td>
<td>12.6 ± 2.8</td>
</tr>
<tr>
<td>Cell Diameter, (μm)</td>
<td>122 ± 3.2</td>
<td>125 ± 2.8</td>
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<td></td>
<td>110 ± 4.8</td>
<td>112 ± 2.7</td>
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Values are shown as means ± SE. BMI, body mass index; WHR, waist-to-hip ratio; ND, not determined. Subjects were fasted overnight, blood was drawn, and plasma was prepared for analysis. Student's $t$-tests were performed to determine significant differences ($^*P < 0.05$) for obese African American vs. Caucasian and preobese African American vs. Caucasian women.

compared with preobese Caucasian women ($P < 0.001$; Fig. 2A). There were no differences in newly synthesized DG in OAT of the preobese women. DG accumulation accounted for 21–29% in the SAT depending on the group, whereas OAT had a larger DG accumulation, with 30–53% depending on the group (Fig. 1B).

To determine whether the increase in the capacity of OAT was due to an increase in the recycling of FFA, we measured the rate of reesterification in a subset of the obese group (Table 2). Basal measurements of lipolysis and esterification were similar in both groups. There were no statistical differences found between the races in any of the parameters calculated for reesterification. The rates of reesterification were 34–37% in SAT and 26–30% in OAT, depending on the group. Therefore, it appears that the increased synthesis found in OAT of African American women was not due to significant differences in reesterification.

To better evaluate the rates of glyceride synthesis, we examined the relationship between cell size and the rates of incorporation of glucose into total lipid (TG + DG) in both adipose tissue depots. As expected, total lipid synthesis from glucose was significantly correlated with cell size in both depots and in both races (Fig. 3, A and B). There were no significant differences between the slopes of the regression lines in SAT ($P = 0.34$) between the races; however, the slopes of the regression lines were significantly different in OAT ($P < 0.001$) between African American and Caucasian women.

**DISCUSSION**

The novel findings of this study are that African American women have an increased capacity to syn-
thesize TG from glucose in OAT compared with Caucasian women regardless of the presence of obesity. This increase in synthesis is not due to differences in cell size or the rates of reesterification. Also, we confirmed previous reports that larger cells have higher rates of synthesis than smaller cells by showing that the rates of synthesis of glyceride-glycerol correlated with average cell diameter in both adipose tissue depots.

The rates of incorporation of glucose into TG and DG measured in this study are in agreement with previous reports (11, 22), and these results confirm previous reports that obesity is associated with an increase in cell size and a concomitant increase in glyceride synthesis (4, 16, 18, 28, 29), indicating that larger cells synthesize more fat than smaller cells whether they are from an obese or a lean subject. SAT tends to have higher rates of glyceride synthesis than OAT, which is probably due to a larger average cell size in SAT than in OAT, as seen in this study (average obese SAT 123.5 vs. OAT 98.0 μm and average preobese SAT 111.0 vs. OAT 81.7 μm) and in others (11, 25). In addition, SAT had a lower percentage of DG accumulation than OAT (24 vs. 39%, respectively), indicating that the synthesis of TG accounts for the majority of newly synthesized glycerides.

Preobese and obese African American women had a significant increase in the synthesis of glycerides in vitro in OAT compared with their Caucasian counterparts. The increased rate of TG synthesis in OAT tended to reduce the percentage of DG accumulation in OAT, but this decrease was not significantly different. These data suggest that African American women have a higher capacity to acquire the substrates needed for glyceride synthesis compared with Caucasian women, which may suggest that African American women tend to accumulate more visceral fat than Caucasian women. However, the result regarding visceral fat deposition in African American and Caucasian women seems to be inconclusive. Although several studies have reported that African American women have less visceral fat than Caucasian women, in most of these studies the African American women tended to have higher BMIs than Caucasian women. Furthermore, many of these studies reported less visceral adipose tissue relative to total adipose tissue or, after correction for total body fat mass, waist circumference or BMI (2, 7, 8, 17, 23). In addition, some studies even reported that African American women had an increase in SAT compared with Caucasians, which resulted in an overall increase in total adipose tissue (8, 17). Although the amount of visceral fat was not determined in our subjects, the BMI and WHR of the preobese subjects were not significantly different between the races, suggesting that there were no differences in visceral fat deposition between our groups. Because we found that both preobese and obese African American women have increased rates of synthesis in OAT, this may indicate that the OAT of African Amer-

### Table 2. Reesterification in obese women

<table>
<thead>
<tr>
<th>Subcutaneous</th>
<th>Omental</th>
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<tbody>
<tr>
<td>African American</td>
<td>Caucasian</td>
</tr>
<tr>
<td>L</td>
<td>444 ± 87</td>
</tr>
<tr>
<td>G</td>
<td>380 ± 56</td>
</tr>
<tr>
<td>E(3H)</td>
<td>542 ± 80</td>
</tr>
<tr>
<td>E(U)</td>
<td>494 ± 99</td>
</tr>
<tr>
<td>%RE</td>
<td>37 ± 13</td>
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Values are means ± SE expressed as nmol·10⁶ cells⁻¹·h⁻¹. Percent reesterification (%RE) was determined in subcutaneous and omental adipose tissue in a subset of obese African American and Caucasian women. All parameters were measured under basal conditions. Calculation of L, G, E(3H), E(U), and RE are described in METHODS, where L = rate of lipolysis, G = rate of [¹⁴C] glucose incorporation into glycerides, E(3H) = rate of [³H] oleate incorporation into glycerides, E(U) = rate of incorporation of unlabeled fatty acids into glycerides, and %RE = rate of reesterification. Student’s t-tests were performed to determine significant differences at P < 0.05 for obese African American vs. Caucasian women.
ican women is metabolically more active than the OAT of Caucasian women. However, the rates of lipolysis in our subsets of obese patients were not different, nor were there differences in the rates of reesterification. On the other hand, an important observation in this study is that, although African American women have an increased capacity to synthesize fat in SAT compared with Caucasian women, the correlation of cell size to total lipid synthesis in OAT was stronger in the Caucasian women. This suggests that African American women have a higher and less dramatic increase in synthesis as their fat cells become larger, whereas Caucasian women have a lower but more rapid increase in synthesis as their fat cells become larger. However, because these experiments were carried out under in vitro conditions, the preferential increase in synthesis in OAT in vitro needs to be explored further to determine whether these results play a significant role in vivo.

Surprisingly, there were no differences in the rates of synthesis in SAT between the races in either preobese or obese women. Preobese African American women did have a significantly larger average cell size in SAT; however, the rate of glyceride synthesis was not statistically different. The significance of an increase in SAT cell size in preobese African American women may be linked to the availability of substrates needed for glyceride synthesis. We (6) and others (15) have recently reported that lean African American women have a faster clearance of lipid from circulation after a fat load, due in part to an increase in postheparin LPL activity and expression in SAT. This suggests that, although the capacity to synthesize glycerides in vitro was similar between the races, in vivo, lean and preobese African American women may have an increased availability of substrate to SAT and, therefore, an increased capacity to synthesize fat.

Although the exact mechanisms underlying the preferential increase in glyceride synthesis in OAT of African American women need to be more thoroughly examined in future studies, it seems reasonable to speculate that these differences may be due to increased enzyme activity or increased availability of substrates. To date, there have been no measurements of the activities of the enzymes that are involved in the pathway of glyceride synthesis between the races. Therefore, examination of key enzymes that may influence the regulation of glyceride synthesis, such as phosphofructokinase, glycerol-3-phosphate dehydrogenase, diacylglycerol acyltransferase, and glycerol phosphate acyltransferase, is needed. In addition, the uptake of the substrates needed for TG synthesis, i.e., glucose and FFA, is regulated by distinct processes. Glucose transport into the cell is regulated by glucose transporters, GLUT1 at basal and GLUT4 upon insulin stimulation (26). Our experiments measured rates of glyceride synthesis under basal conditions. This may mean that increased glyceride synthesis would be due to an increase in basal glucose transport, mediated by GLUT1. This suggests that African American women have a higher transport than Caucasian women of glucose uptake that might be related to a higher expression of GLUT1. Therefore, direct measurements of glucose transport and utilization of glucose in adipocytes are needed. Fatty acid transporters have been shown to mediate FFA uptake into adipocytes (1, 3). There has been considerable work on the identification and characterization of FFA transporters, but further work is needed to better understand their role in glyceride synthesis. In vivo, the availability of FFA to the cell is mediated in part by LPL (10). Recent evidence has shown that lean and preobese African Americans have higher postheparin LPL activity than Caucasians (6, 8), suggesting that African Americans have access to an excess of FFA in circulation. The increase in LPL activity found in lean and preobese African Americans may indicate that they have an increased availability of substrate needed for glyceride synthesis, which may accelerate the accumulation of fat and, therefore, the onset of obesity. However, this increased availability of substrate due to LPL is attenuated in the obese African American women, indicating that there must also be an enhanced uptake of FFA in both preobese and obese African American women that helps maintain an increased rate of glyceride synthesis. Therefore, measurements of FFA uptake and incorporation into adipocytes in African Americans are needed.

Taken together, these data suggest that the mechanisms of TG synthesis may be different between African American and Caucasian women. However, it appears as though these differences are manifested primarily in OAT. This suggests that OAT may have an increased ability to acquire substrates from circulation. Because there are no significant differences in the rate of reesterification between the races, perhaps the uptake of glucose, which serves as the backbone of the lipid, may be a key component to the differences seen between these groups. Therefore, future studies should be directed toward examining the uptake of both glucose and FFA into adipocytes, as well as key enzyme activities, to better understand the ethnic differences in glyceride synthesis between African American and Caucasian women.

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