Differences in transport of fatty acids and expression of fatty acid transporting proteins in adipose tissue of obese black and white women

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Bower, Joseph F., Julianne M. Davis, Enhui Hao, and Hisham A. Barakat. Differences in transport of fatty acids and expression of fatty acid transporting proteins in adipose tissue of obese black and white women. Am J Physiol Endocrinol Metab 290: E87–E91, 2006; doi:10.1152/ajpendo.00194.2005.—We have reported that the rate of de novo triglyceride (TG) synthesis by omental, but not subcutaneous, adipose tissue was higher in African-American women (AAW) than in Caucasian women (CAW). The purpose of this study was to explore the potential mechanisms underlying this increase. Toward that end, we determined the activities of key enzymes in the pathway of TG synthesis, the rates of uptake of fatty acids by adipocytes, mRNA and protein levels of the fatty acid-transporting proteins FAT/Cd36 and FATP4, and mRNA and protein levels of PPARγ in omental fat of AAW and CAW. The results showed 1) no difference in the activity of phosphofructokinase, glycerol-3-phosphate dehydrogenase, or diacylglycerol acyltransferase; 2) a higher rate of fatty acid uptake by adipocytes of the AAW; 3) an increase in the mRNA and protein levels of CD36 and FATP4 in the fat of the AAW; and 4) an increase in the mRNA and protein levels of PPARγ, which can stimulate the expression of CD36 and FATP. These results suggest that the increase in the transport of fatty acid, which is mediated by the overexpression of the transport proteins in the omental adipose tissue of the AAW, might contribute to the higher prevalence of obesity in AAW.

METHODS

Subjects

All subjects recruited for this study were women undergoing gastric bypass surgery. Blood samples were collected from these patients 1 wk before their surgery, and plasma was analyzed for glucose and insulin. Subcutaneous and omental adipose tissue was obtained from these volunteers during the surgery as we previously described (8). Subjects participated in this study if they 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. AAW were included in this study only if their parents and grandparents were all 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. Written consent was obtained from the participants after they were informed of the nature of the study. The Institutional Review Board of the University and Medical Center approved the protocols to be used.

Adipocyte Preparation, Cell Size Determination, and Tissue Fractionation

Adipose tissue was obtained from surgery, placed in RPMI and immediately delivered to our laboratory. Adipose tissue samples were washed and cleaned of connective tissue. Adipocytes were prepared from ~3 g of tissue for cell size determination, as previously described (8, 13, 14, 29), and for experiments involving substrate uptake (described below). Another 5 g were quick-frozen and stored for later RNA extraction, tissue fractionation, protein determination, and enzyme activity assays.

For tissue fractions, 1 g of adipose tissue was homogenized in 2.0 ml of homogenization buffer (0.05 M Tris·HCl, 0.1 M KCl, 5 mM MgSO4, 1 mM EDTA, pH 7.5). Homogenates were filtered through four layers of cheesecloth, an aliquot centrifuged for 20 min at 14,000 g, and the supernant fraction was retained. A fraction of this supernant was centrifuged further for 1 h at 100,000 g at 4°C to obtain the microsomal pellet. The microsomal pellet was resuspended in 400 μl

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of STE buffer (0.25 M sucrose, 10 mM Tris·HCl pH 7.4, 1.0 mM EDTA), and the protein concentration was determined.

**Enzyme Assays**

**Phosphofructokinase.** Phosphofructokinase activity was determined as previously described by Kemp (19). One hundred microliters of adipose tissue-soluble fraction (14,000 g supernatant) were added to the assay buffer, containing the auxiliary enzymes (36 U/ml aldolase, 36 U/ml triose phosphate isomerase, and glycerophosphate dehydrogenase) in a final volume of 3 ml, and the decrease in absorbance at 340 nm was followed for 5 min. Enzyme activity is expressed as units per milligram of protein where one unit equals the conversion of one micromole of fructose 6-phosphate to fructose bisphosphate per minute. The reaction was linear with protein concentration and time.

**Glycerol-3-phosphate dehydrogenase.** Glycerol-3-phosphate dehydrogenase activity was determined in adipose tissue-soluble fraction as described by Harding et al. (15). The decrease in absorbance at 340 nm was followed for 5 min. Activity was expressed as units per minute per milligram of protein where one unit equals the oxidation of one micromole of NADH. Reactions were linear with time and protein.

**Diacylglycerol acyltransferase.** Diacylglycerol acyltransferase activity was determined in the microsomal fraction of adipose tissue as described by Coleman (10). After incubation for 10 min at 25°C, the reaction was terminated by the addition of 5 ml of ice-cold 400 μM phloretin, and the mixture was quick-filtered through a 1.13-cm GF/A filter at a rate of 50 mmHg vacuum pressure. The cells were then washed twice with 1 ml of ice-cold PBS, and the filters were counted.

**Western Blot Analysis**

The mass of the various proteins was determined by Western blot analysis as we have previously described (5) except that detection was by ECL Plus (Amersham Pharmacia Biotech, Piscataway, NJ), and the blots were quantified using a phosphorimager (Molecular Dynamics, Piscataway, NJ). All primary and secondary antibodies were purchased from Santa Cruz Biotecnology (Santa Cruz, CA). A sample was randomly picked to serve as an internal control and was run on every gel. After quantification on the phosphorimager, this sample was assigned a value of 1.0, and ratios were calculated by comparisons with each sample.

**RNA isolation and RT-PCR**

Total RNA was extracted from the quick-frozen adipose tissue with TRIzol reagent (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions. Messenger RNA (mRNA) levels were determined using RT-PCR. For the RT reaction, 5 μg of purified total RNA were reverse transcribed using avian myeloblastosis virus RT (Roche Diagnostics), as recommended by the manufacturer. The set of specific primers for the different genes is shown Table 1.

For amplification, single-stranded cDNA products were denatured and subjected to amplification by the PCR (35 cycles). The reaction mixture contained 0.2 mM 2-deoxyribonucleotide 5-triphosphate, 2.5 U/100 μl Taq DNA polymerase (Roche Diagnostics), and 1× PCR buffer (1.5 mM MgCl2) with a Gene-Amp PCR system. Amplification of β-actin mRNA served as control. Each PCR cycle consisted of denaturing at 94°C for 5 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. Final extension of 72°C for 10 min was added after the 35th amplification cycle. The PCR products were separated on 2.5% agarose gels with ethidium bromide. The data were analyzed using the Fluorchem Digital Imaging System with AlphaEaseFC Software (Alpha Innoteck, San Leandro, CA). The relative expression level of mRNA of the various genes was normalized to β-actin.

**Table 2. Characteristics of the subjects**

<table>
<thead>
<tr>
<th></th>
<th>AAW</th>
<th>CAW</th>
<th>P Value</th>
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<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>42±1.2</td>
<td>42±1.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>45±1.8</td>
<td>49±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>90±2.6</td>
<td>90±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>12.1±2.2</td>
<td>12.4±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>78.3±0.6</td>
<td>123±14.1</td>
<td>&lt;0.05</td>
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<tr>
<td>Fatty acids, nM</td>
<td>546±49</td>
<td>758±43</td>
<td>&lt;0.05</td>
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</table>

Values are means ± SE. AAW, African-American women; CAW, Caucasian women; NS, not significant.

**Table 3. Enzyme activity in subcutaneous adipose tissue**

<table>
<thead>
<tr>
<th></th>
<th>AAW</th>
<th>CAW</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>PFK, U/min-1·mg protein-1</td>
<td>0.46±0.03</td>
<td>0.55±0.03</td>
<td>0.090</td>
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<tr>
<td>G3PDH, U/min-1·mg protein-1</td>
<td>1.16±0.12</td>
<td>1.36±0.14</td>
<td>0.283</td>
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<tr>
<td>DGAT, nmol/min-1·mg protein-1</td>
<td>11.3±1.67</td>
<td>11.3±1.50</td>
<td>0.860</td>
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</tbody>
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Values are means ± SE. PFK, phosphofructokinase; G3PDH, glycerol-3-phosphate dehydrogenase; DGAT, diacylglycerol acyltransferase. Enzyme assays and activity units are as described in METHODS.
CAW (Table 6). Because of the role that PPAR-omental adipose tissue of the AAW compared with those of the CAW, might be due to the small sample size. On the other hand, the difference did not reach statistical significance, which CD36 tended to be higher in the AAW than in the CAW, but tissue of CAW. In omental adipose tissue, mRNA levels of CD36 and FATP4 were not different in subcutaneous normalization to and mRNA levels of CD36 or FATP are reported here after dehydrogenase, and diacylglycerol acyltransferase in subcutaneous and omental fat are shown in Tables 3 and 4, respectively. No differences in the activities of any of these enzymes were observed between the AAW and CAW in either tissue. This suggests that the pathways for TG synthesis are intact in the adipose tissue of the AAW.

Because glyceride synthesis depends on the availability of fatty acids to the adipocytes, which in turn depends on the transport of these substrates inside the adipocytes, we sought to determine whether there were differences between the AAW and CAW in the expression of CD36 and FATP, the two major fatty acid-transporting proteins in adipose tissue. Using RT-PCR, we amplified the individual genes along with β-actin, and mRNA levels of CD36 or FATP are reported here after normalization to β-actin. As shown in Table 5, mRNA levels of CD36 and FATP4 were not different in subcutaneous adipose tissue of AAW from those in subcutaneous adipose tissue of CAW. In omental adipose tissue, mRNA levels of CD36 tended to be higher in the AAW than in the CAW, but the difference did not reach statistical significance, which might be due to the small sample size. On the other hand, FATP mRNA levels were significantly (P < 0.05) higher in the omental adipose tissue of the AAW compared with those of the CAW (Table 6). Because of the role that PPARγ has in the stimulation of the expression of the transport proteins, we also determined mRNA levels of PPARγ in the two fat depots. No differences in mRNA levels were observed in subcutaneous tissue, but mRNA levels of PPARγ were significantly higher in the omental fat of the AAW.

Because no significant differences in the subcutaneous fat were found in the above-mentioned parameters, we did not pursue other determinations in this tissue. Rather, we further characterized the differences in omental adipose tissue. Thus we measured the protein concentration of these transporters in membrane preparations of omental fat from the AAW and CAW by immunoblotting. Fig. 1 shows a representative blot of the two transporters, and Table 7 shows that the protein concentrations of both CD36 and FATP were higher (P < 0.05) in the AAW than in the CAW. Measurement of protein levels of PPARγ (Table 7) in adipose tissue of AAW and CAW showed a significant (P < 0.05) increase (~40% increase) in the adipose tissue of the AAW compared with the CAW.

In efforts to further ascertain the significance of the changes in the expression of the transport proteins in omental adipose tissue of AAW, we measured the rate of uptake of [1-14C]oleic acid by freshly isolated adipocytes. As shown in Fig. 2, the rate of [1-14C]oleic acid uptake was significantly (P < 0.05) higher in the adipose tissue of the AAW than in the CAW. From these results it appears that uptake of fatty acids by omental fat of the AAW is upregulated and could explain, at least in part, the higher rates of glyceride synthesis by this tissue that we observed earlier.

**DISCUSSION**

In a previous study, we examined differences in the capacity of adipose tissue of AAW and CAW to synthesize glycerides de novo and found no differences between the two groups in the rates of TG synthesis in subcutaneous adipose tissue. On the other hand, we found that the rate of TG synthesis in omental adipose tissue was higher in AAW than in the CAW. The results of the current study shed light on some of the underlying mechanisms behind the increase in the capacity of

<table>
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<th>Gene</th>
<th>AAW</th>
<th>CAW</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>1.00±0.03</td>
<td>0.98±0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>FATP4</td>
<td>0.94±0.03</td>
<td>0.87±0.18</td>
<td>0.59</td>
</tr>
<tr>
<td>PPARγ</td>
<td>0.35±0.03</td>
<td>0.25±0.06</td>
<td>0.14</td>
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</table>

Values are means ± SE.

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Fig. 1. Representative Western blot of fatty acid transporter proteins CD36 and FATP4 in omental adipose tissue of African-American women (AAW) and Caucasian women (CAW) showing the differences in the intensities of the bands of proteins from the two groups.
We have observed earlier (8) that omental adipose tissue to for substrate uptake, coupled with the increased capacity that fat to take up these substrates is higher. This increased capacity glucose are elevated in the circulation of obese AAW, synthesis would be assumed that, when and if the levels of fatty acids or raise the following question: How do these data apply to the in vitro experiments, where enzyme activities and other parameters were measured under optimal conditions. These findings showed no difference in the activities of the key enzymes that are needed for glyceride synthesis (Table 4). Rather the higher capacity of omental adipose tissue of AAW to synthesize fat is likely due, at least in part, to the higher rates of fatty acid uptake by adipocytes from of the AAW (Fig. 2). The lower free fatty acid concentrations in the plasma of the AAW (Table 3) might be partly due to the higher rate of uptake of the fatty acids by the adipose tissue of these women. This increased capacity to take up these substrates from the surroundings is congruent with the increased expression of the transporting proteins both at the mRNA and the protein levels (Tables 6 and 7, respectively). Furthermore, the increased expression of the FATPs appears to be linked with the increased expression of PPARγ (Tables 6 and 7), which has been reported to be involved in the regulation of the expression of these proteins. These findings were independent of cell size, because no differences in cell size between the two groups were observed (8). Thus it appears that the capacity of omental fat of AAW to synthesize fat is upregulated at the genetic level.

The findings in the current study of a higher rate of fatty acid uptake by omental adipose tissue of AAW were obtained from in vitro experiments, where enzyme activities and other parameters were measured under optimal conditions. These findings raise the following question: How do these data apply to the in vivo situation, and what would be their biological significance? When the data from the present study are considered, it would be assumed that, when and if the levels of fatty acids or glucose are elevated in the circulation of obese AAW, synthesis of TG would be increased because the capacity of omental fat to take up these substrates is higher. This increased capacity for substrate uptake, coupled with the increased capacity that we have observed earlier (8) of omental adipose tissue to synthesize fat de novo, raises the issue of the relevance to the in vivo situation. Some studies reported that obese AAW have less visceral fat than obese CAW with similar BMI and waist-to-hip ratio (4, 11, 22), whereas other studies showed that AAW are more obese than CAW and as such have more visceral fat (17). Our findings of the higher potential of omental adipose tissue of AAW to take up the necessary substrates, along with its increased potential for de novo TG synthesis, would favor the concept that the visceral fat compartment in the obese AAW would be larger than, or at least the same as, that in the CAW.

It should be pointed out that the current study examined the differences in fatty acid uptake in obese patients. Future studies should be aimed at determining whether the changes that were observed in the current study correlate with body weight. In addition, it is imperative that similar studies be done in lean AAW and CAW. If similar findings were observed in lean women, that might shed light on the potential causes of the enhanced obesity in AAW.

In summary, the results from this study indicate that the potential for synthesis and deposition of TG in omental, but not subcutaneous, adipose tissue is higher in obese AAW than in obese CAW. This might be due to a higher rate of fatty acid uptake as well as a higher expression, at both the mRNA and protein levels of the transporting proteins. This increased expression, in turn, might be mediated at the gene level because of the higher expression of PPARγ. These findings shed light on some of the potential causes of the enhanced obesity in AAW.

### Table 7. Protein levels in omental adipose tissue

<table>
<thead>
<tr>
<th>Protein</th>
<th>AAW</th>
<th>CAW</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>0.89±0.07</td>
<td>0.57±0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>FATP4</td>
<td>0.87±0.26</td>
<td>0.52±0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>PPARγ</td>
<td>0.73±0.09</td>
<td>0.44±0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Fatty acid transporters both at the mRNA and the protein levels (Tables 6 and 7) have been reported to be involved in the regulation of the expression of these proteins. Enhanced expression, in turn, might be mediated at the gene level because of the higher expression of PPARγ. These findings shed light on some of the potential causes of the enhanced obesity in AAW.

**REFERENCES**