Expression and activity of steroid aldoketoreductases 1C in omental adipose tissue are positive correlates of adiposity in women

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Sex steroid hormones have been the focus of several studies on fat distribution and related comorbidities, but most investigations have been limited to measures of hormone concentrations in the plasma (34). The association between circulating sex hormones and adipose tissue distribution is well established (34). However, local androgen/estrogen synthesis and inactivation (adipose tissue intracrinology) have received little attention in the context of abdominal obesity. Recent evidence has been published supporting the importance of steroid conversions in adipose tissue. The generation of active cortisol through expression of type 1 11β-hydroxysteroid dehydrogenase (11β-HSD-1) in abdominal adipose tissue, which has been shown to increase exposure of omental adipocytes to cortisol (7), appears to be of particular interest. Studies by Bujalska et al. (8) and Masuzaki et al. (23) using cell cultures and transgenic models recently led to the suggestion that increased expression of 11β-HSD-1 in abdominal adipose tissue may represent a common molecular etiology for visceral obesity and the metabolic syndrome. In addition, Corbould et al. (9) indicated that the type 3 17β-HSD to-aromatase expression ratio in omental adipose tissue was positively correlated with abdominal obesity in women, suggesting that androstenedione conversion to testosterone is increased in female abdominal obesity. These studies provide support to the potential importance of steroid conversions in adipose tissue.

Whether the expression of other adipose tissue steroidogenic enzymes that could decrease or increase exposure of omental adipocytes to active steroids is related to abdominal fat distribution remains to be determined.

Three human HSDs that are also members of the aldoketoreductase (AKR) superfamily have recently been characterized (12, 13, 38), namely, type 3 3α-HSD, type 5 17β-HSD, and 20α-HSD. These enzymes are mainly involved in the inactivation of dihydrotestosterone to 5α-androstane-3α,17β-diol (3α-diol) and in the conversion of progesterone to 20α-hydroxyprogesterone (38). Type 5 17β-HSD also partly catalyzes the reaction of androstenedione to testosterone (38). Thus AKR1C enzymes function primarily as metabolizing enzymes, with reducing activities toward the 20α- and 3α-positions of progesterone and dihydrotestosterone (DHT), respectively (38). In recent studies (6), we found a positive correlation between CT-measured visceral adipose tissue area and omental adipose tissue conversion of DHT to inactive 3α-diol (3α-HSD activity), as well as progesterone 20α-reduction (20α-HSD activity) (5, 6). To further document the relationship between...
steroid AKRs and abdominal obesity, we tested the hypothesis that progesterone and androgen 20α- and 3α-reduction activities, as well as mRNA abundance of AKR1C enzymes involved in these reactions, are increased in adipose tissue of women with abdominal-visceral obesity. We examined the activity and mRNA abundance of AKR1C enzymes in abdominal subcutaneous and omental adipose tissue biopsies obtained from age- and body fat mass-matched women with or without visceral obesity.

SUBJECTS AND METHODS

Subjects. Women in this study were recruited through the elective surgery schedule of the Gynecology Unit of the Laval University Medical Center. The study included 14 healthy women, aged 41.5–58.8 yr, undergoing abdominal gynecological surgery. For the present study, seven women with elevated visceral adipose tissue areas were selected from a larger pool of patients (n = 83) and were matched for age and total body fat mass with seven women from the same pool who were characterized by low visceral adipose tissue areas. These women elected for total (n = 13) or subtotal (n = 1) abdominal hysterectomies, some with salpingoo-oophorectomy of one (n = 4) or two (n = 7) ovaries. Reasons for surgery included one or more of the following: menorrhagia (n = 6), myoma/fibroids (n = 6), pelvic pain (n = 3), ovarian cysts (n = 1), endometriosis (n = 3), uterine cervix elongation (n = 1), uterine polyp and postmenstrual bleeding (n = 1), dysmenorrhea (n = 1), severe premenstrual syndrome (n = 1), and menometrorrhagia (n = 1). This study was approved by the medical ethics committees of Laval University and Laval University Medical Center. All subjects provided written informed consent before their inclusion in the study.

Body fatness and body fat distribution measurements. These tests were performed on the morning of or a few days before the surgery. Measures of total body fat mass, fat percentage, and fat-free mass were determined by dual-energy X-ray absorptiometry by use of a Hologic QDR-2000 densitometer and the enhanced-array whole body software (v5.73A; Hologic, Bedford, MA). Measurement of abdominal subcutaneous and visceral adipose tissue cross-sectional areas was performed by computed tomography (CT) as previously described (10), using a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI) and the Light Speed QXi 1.0 production software. Subjects were examined in the supine position, with arms stretched above the head. The scan was performed at the L4–L5 vertebral level, using a scout image of the body to establish the precise scanning position. The quantification of visceral adipose tissue area was done by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body with the computer interface of the scanner. Adipose tissue was highlighted and computed using an attenuation range of 100 to 190 Hounsfield units. The coefficients of variation between two analyses (10, same observer) were 0.50 and 2.14% for subcutaneous and visceral adipose tissue areas, respectively.

Plasma hormone measurements. Plasma concentrations of dehydroepiandrosterone (DHEA), androstenedione, DHT, estrone, and estradiol were determined using high-performance gas chromatography and chemical ionization mass spectrometry. Intra- and interassay coefficients of variation did not exceed 5.9% for these measurements. DHEA-sulfate (DHEA-S) and androsterone-glucuronide concentrations were determined by use of HPLC and mass spectrometry with a PE Sciex API 300 tandem mass spectrometer (Perkin-Elmer, Foster City, CA) equipped with a Turbo ion spray source. Intra- and interassay coefficients of variation did not exceed 6.4% for these measurements. Progesterone, testosterone, and sex hormone-binding globulin (SHBG) levels were determined with radioimmunoasays from Diagnostic System Laboratories (Webster, TX). The free androgen index was calculated as testosterone/SHBG × 100.

Adipose tissue sampling. Paired omental (epiploic) and subcutaneous adipose tissue samples were collected during the surgical procedure and immediately carried to the laboratory in 0.9% saline preheated to 37°C. A portion of the biopsy was used for adipocyte isolation, and the remaining tissue was immediately frozen at −80°C for subsequent analyses.

Adipocyte isolation, lipolysis, and lipoprotein lipase activity. Tissue samples were digested with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer for 45 min at 37°C, according to a modified version of the Rodbell method (31). Adipocyte suspensions were filtered through nylon mesh and washed three times with KRH buffer. For cell size measurements, mature adipocyte suspensions were visualized with a contrast microscope attached to a camera and computer interface. Pictures of cell suspensions were taken, and Scion Image software was used to measure the size (diameter) of 250 adipocytes for each tissue sample. Average sizes of each cell population were used in analyses.

Lipolysis experiments were performed by incubation of isolated cell suspensions for 2 h at 37°C. Glycerol release in the medium was measured by bioluminescence, using the NADH-linked bacterial luciferase assay (15, 17), an EG&G Berthold Microlumat plus bioluminometer (LB 96 V), and WinGlow software. The average coefficient of variation for duplicate glycerol release measurements was 14.1%. Lipid weight of the cell suspension was measured by performing Dole’s extraction, and lipolysis results were expressed as a function of adipocyte surface area [nmol glycerol/(μm² × 10⁸)·2 h⁻¹].

Lipoprotein lipase activity was determined in 30- to 50-mg frozen adipose tissue samples by the method of Taskinen et al. (33). Tissue eluates were incubated by incubating the samples in Krebs-Ringer-phosphate buffer and heparin at 28°C for 90 min. The eluates were then incubated with excess concentrations of unlabeled and [¹¹C]triollein in a Tris-albumin buffer emulsified with ultrason for the reaction was carried out at 37°C for 60 min with agitation. The resulting free fatty acids liberated from triollein by the lipoprotein lipase (LPL) reaction were isolated by the Belfrage extraction procedure. Porcine plasma was used as a source of apolipoprotein (apo) C-II to stimulate LPL activity, and unpasteurized cow’s milk was used as an internal LPL activity standard for interassay variations. Activity results were expressed in nanomoles of oleate per hour per 10⁶ cells.

Real-time PCR measurements of enzyme mRNA abundance. Total RNA was isolated from whole tissue samples using TRIzol (Invitrogen Life Technologies), following the manufacturer’s recommendations. First-strand cDNA synthesis was accomplished using 5 μg of the isolated RNA in a reaction containing 200 units of Superscript II RNase H-Reverse Transcriptase (Invitrogen), 300 ng of oligo(dT)₁₈, 500 μM dNTP, 10 mM dTTP, and 34 units of porcine RNase inhibitor (Amersham Pharmacia) in a final volume of 50 μL. The resulting products were then treated with 1 μg of RNase A for 30 min at 37°C and purified thereafter with Qiaquick PCR purification kits (Qiagen). For quantitative PCR analyses, a Light-Cycler PCR (Roche Diagnostics) was used to measure the mRNA abundance of AKR1C enzymes. The following sets of primers were used: 5′-CGG-TAG-AAG-CC-3′, 5′-CAA-CTG-TGG-ATG-GGA-ATT-GCT-3′, 5′-GAC-AGG-ACC-ACC-CCA-CGC-GTG-3′, respectively, for type 3 3α-HSD, type 5 17β-HSD, and 20a-HSD cDNAs. The FastStart DNA Master SYBR Green kit (Roche Diagnostics) was used in a final reaction volume of 20 μL containing 3 mM MgCl₂, 20 ng of each primer, and 20 ng of the cDNA template. The PCR was carried out according to the following conditions: 95°C/10 min and 50 cycles of 95°C/10 s, 58°C/5 s, and 72°C/8 s, with a temperature transition of 3°C/s. Glucose-6-phosphate dehydrogenase (G6PDH) was used as the housekeeping gene and was measured using

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the following primers: 5′-CAG-CCG-CTC-AAC-AGC-CAC-AT-3′ and 5′-AAG-GGC-TTC-TCC-ACG-ATG-ATG-C-3′. A universal standard curve was generated with ATPasase from an amplification with perfect efficiency (i.e., efficiency coefficient E = 2.00), using cDNA amounts of 0, 10², 10³, 10⁴, 10⁵, and 10⁶ copies. The crossing points (Cp) to calculate the amount of copies in initial cDNA specimens were determined with the double-derivative method. For each sample, the Cp value of 20α-HSD was divided by that of the housekeeping gene. To further minimize inter assay variability, this Cp ratio was then multiplied by the average Cp generated for housekeeping gene amplifications of all samples examined in the present experiment. PCR data are expressed as normalized numbers of copies per microgram of total RNA.

AKR1C enzyme activity measurements. The activities of 3α-HSD and 20α-HSD were measured in whole tissue homogenates. For these measurements, frozen tissues were homogenized with a Polytron in 50 mM sodium phosphate buffer (pH 7.4), 20% glycerol, 1 mM EDTA, and NADPH at 1 mM for 3α-HSD activity and at 0.4 mM for 20α-HSD activity (12, 38). [3H]progesterone and [14C]DHT were added in separate aliquots, and reactions were performed at 37°C in a final volume of 1 ml for 24 h. Steroids from tissue homogenates were extracted twice with 1 vol of ether. The organic phases from both extractions were pooled and evaporated to dryness. The steroids were solubilized in 50 ml of dichloromethane (reference standards were diluted in ethanol) and applied to Silica Gel 60 TLC plates (Merck, Darmstadt, Germany), using 10-μl calibrated micropipets. Unlabeled 20α-progesterone was used as a standard and was detected under ultraviolet light. The separation was performed by migration in toluene-acetone (4:1), the radioactivity was detected using a Storm PhosphorImager (Amersham Pharmacia Biotech), and quantification was done using ImageQuant software (v5.1, Amersham Pharmacia Biotech). Proteins in each tissue homogenate were quantified by the method of Lowry and used in the calculation of activity values. Type 5 17β-HSD was previously shown to be extremely labile, with an almost complete loss of activity in tissue or cell homogenates compared with intact cells (6, 13), which prevented us from measuring this activity in tissue homogenates.

Statistical analyses. Adipose tissue AKR1C mRNA abundance and activities were compared between groups by Student’s t-tests. These tests were performed on log10-transformed values for variables that were not normally distributed (age, subcutaneous adipose tissue area, visceral adipose tissue area, and omental adipose tissue area; 3α-HSD, and 20α-HSD activities). Homogeneity of variances between groups was tested with the Levene test, accepting unequal variances at P ≤ 0.05. Variances were unequal for subcutaneous adipose tissue adipocyte diameter and omental adipose tissue 20α-HSD activity. The Welch analysis of variance procedure was used to compare means for these variables. Spearman rank correlation coefficients were computed to assess the magnitude of the associations between adipose tissue AKR1C enzyme mRNA abundance or activity and other variables. Statistical analyses were performed with JMP software (SAS Institute, Cary, NC).

RESULTS

Characteristics of the study sample. Characteristics of the women studied are shown in Table 1. By design, the two groups of women were characterized by similar age and total body fat mass values. However, computed tomography-measured visceral adipose tissue area was significantly different between groups (P < 0.0001). On the other hand, abdominal subcutaneous adipose tissue areas were similar in both groups. Consistent with their higher visceral fat area, visually obese women were characterized by a greater omental adipocyte diameter and adipose tissue LPL activity compared with women with low visceral adipose tissue areas. Omental adipose tissue basal lipolysis and measures of subcutaneous adipose tissue metabolism were not significantly different between groups. In addition, no significant group difference was found for any of the circulating sex steroid hormones examined (not shown).

Group differences: AKR1C enzyme mRNA abundance and activity. AKR1C enzyme mRNA abundance and activities measured in abdominal subcutaneous and omental adipose tissue of age- and body fat mass-matched women with or without visceral obesity are shown in Table 2. Omental adipose tissue mRNA abundance of 3α-HSD-3 and 20α-HSD was significantly higher in women with visceral obesity. Omental adipose tissue mRNA abundance of 17β-HSD-5 also tended to be higher in women with visceral obesity (P = 0.07). On the other hand, subcutaneous adipose tissue mRNA abundance of all three enzymes was not significantly different between groups. 3α-HSD and 20α-HSD activities measured in both omental and subcutaneous adipose tissue homogenates were significantly higher in women with visceral obesity.

Correlations between AKR1C mRNA abundance and adiposity as well as metabolic variables. Concordant with group differences in omental adipose tissue AKR1C enzyme mRNA abundance, significant positive correlations were found between omental adipose tissue 20α-HSD and 3α-HSD-3 mRNA abundance and total body fat mass, visceral adipose tissue area, omental adipocyte diameter, and omental LPL activity (Fig. 1). Subcutaneous enzyme mRNA abundance was not significantly related to any adiposity measure or adipocyte metabolic parameter in any fat depot (not shown). Circulating levels of unesterified DHEA were negatively correlated with omental and subcutaneous adipose tissue mRNA abundance of 17β-HSD-5 (r = −0.56, P < 0.05, and r = −0.60, P < 0.04,

Table 1. Physical characteristics and adipose tissue metabolism measurements

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Low VAT Area (n = 7)</th>
<th>High VAT Area (n = 7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr†</td>
<td>45.6 ± 4.1</td>
<td>48.7 ± 5.8</td>
<td>0.27</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 ± 2.5</td>
<td>27.0 ± 2.7</td>
<td>0.31</td>
</tr>
<tr>
<td>Total body fat mass, kg</td>
<td>27.1 ± 6.0</td>
<td>29.3 ± 5.8</td>
<td>0.51</td>
</tr>
<tr>
<td>Adipose tissue area, cm²†</td>
<td>11 105</td>
<td>12 05 106</td>
<td>0.0001</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>58 6 104</td>
<td>12 8 106</td>
<td>0.91</td>
</tr>
<tr>
<td>Visceral</td>
<td>65 9 104</td>
<td>149 39 104</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7, except *n = 6. Physical characteristics and adipose tissue metabolism measurements of women in the study with low or high visceral adipose tissue (VAT) area, matched for age and total body fat mass. LPL, lipoprotein lipase; BMI, body mass index. †Statistical tests performed on log10-transformed values.
Table 2. Omental and subcutaneous adipose tissue mRNA abundance and activities of AKR1C enzymes

<table>
<thead>
<tr>
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<th>Low VAT Area (n = 7)</th>
<th>High VAT Area (n = 7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Omental adipose tissue</strong></td>
<td></td>
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<tr>
<td>20α-HSD mRNA</td>
<td>0.681 ± 0.070</td>
<td>0.941 ± 0.079</td>
<td>0.03</td>
</tr>
<tr>
<td>3α-HSD-3 mRNA</td>
<td>0.645 ± 0.065</td>
<td>1.000 ± 0.119</td>
<td>0.03</td>
</tr>
<tr>
<td>17β-HSD-5 mRNA</td>
<td>0.258 ± 0.034</td>
<td>0.178 ± 0.022</td>
<td>0.07</td>
</tr>
<tr>
<td>20α-HSD activity†</td>
<td>17.0 ± 6.8</td>
<td>30.2 ± 14.8*</td>
<td>0.08</td>
</tr>
<tr>
<td>3α-HSD activity†</td>
<td>50.8 ± 18.5</td>
<td>101.2 ± 51.0</td>
<td>0.02</td>
</tr>
<tr>
<td>17β-HSD activity</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

| **Subcutaneous adipose tissue** |                      |                       |         |
| 20α-HSD mRNA          | 2.290 ± 0.288        | 2.238 ± 0.109         | 0.87    |
| 3α-HSD-3 mRNA         | 1.991 ± 0.262        | 2.059 ± 0.194         | 0.86    |
| 17β-HSD-5 mRNA        | 0.586 ± 0.063        | 0.663 ± 0.591         | 0.39    |
| 20α-HSD activity      | 20.0 ± 11.5*         | 53.7 ± 29.8*          | 0.04    |
| 3α-HSD activity       | 55.2 ± 35.7*         | 152.4 ± 63.3*         | 0.0008  |
| 17β-HSD activity      | ND                   | ND                    |         |

Values are means ± SE; n = 7, except *n = 6. Omental and subcutaneous adipose tissue mRNA abundance and activities of aldoketoreductase (AKR) 1C enzymes in age- and body fat mass-matched women with low or high VAT area. Enzyme mRNA abundance is expressed as 10⁶ copies/µg total RNA (see SUBJECTS AND METHODS); enzyme activities are expressed as fmol/µg protein⁻¹·24 h⁻¹. HSD, hydroxysteroid dehydrogenase; ND, not done. †Statistical tests performed on log₁₀-transformed values.

Fig. 1. Correlations between omental (Om) adipose tissue (AT) 20α-hydroxysteroid dehydrogenase (20α-HSD) and 3α-HSD-3 mRNA abundance and total body fat mass (n = 14), visceral adipose tissue area (n = 14), omental adipocyte size (n = 13), and omental adipose tissue lipoprotein lipase (LPL) activity (n = 13). Spearman rank correlation coefficients are shown.

Correlations between AKR1C activities and adiposity as well as metabolic variables. Similar to 20α-HSD and 3α-HSD-3 mRNA levels, omental activities of 20α-HSD and 3α-HSD were positively related to visceral adipose tissue area (r = 0.56 and r = 0.61, respectively, P < 0.05), omental adipocyte size (r = 0.83 and r = 0.82, respectively, P < 0.001; Fig. 2), and omental adipose tissue LPL activity (r = 0.69 and r = 0.70, respectively, P < 0.01). Subcutaneous adipose tissue activities of these enzymes were correlated positively with total body fat mass (r = 0.59 and r = 0.55, respectively, P < 0.06), visceral adipose tissue area (r = 0.60 and r = 0.73, respectively, P < 0.05), and omental adipocyte size (r = 0.82 and r = 0.76, respectively, P < 0.01). However, the correlations between subcutaneous enzyme activities and subcutaneous...
adipose tissue area or LPL activity in any depot were nonsignificant. Subcutaneous 20α-HSD and 3α-HSD activities were positively associated with subcutaneous adipocyte size \( (r = 0.72 \text{ and } r = 0.60, \text{ respectively, } P < 0.06) \). Circulating levels of free DHEA were negatively associated with omental adipose tissue 20α-HSD \( (r = -0.58, P < 0.05) \) and 3α-HSD \( (r = -0.58, P < 0.04) \) activities. Enzyme activities were not associated with circulating levels of any other steroid examined.

Subcutaneous adipose tissue 20α-HSD and 3α-HSD-3 mRNA abundance was not related to subcutaneous activities of the enzymes. Activity and mRNA abundance of 3α-HSD-3 were significantly associated only in omental adipose tissue \( (r = 0.55, P < 0.05) \). On the other hand, activity or mRNA of a given enzyme was strongly related to that of the other in a given depot. Omental adipose tissue 20α-HSD and 3α-HSD-3 mRNA levels as well as 20α-HSD and 3α-HSD activities were positively intercorrelated \( (r = 0.75 \text{ and } r = 0.86, \text{ respectively, } P < 0.003) \). Subcutaneous adipose tissue 20α-HSD and 3α-HSD-3 mRNA levels as well as 20α-HSD and 3α-HSD activities were also positively intercorrelated \( (r = 0.86 \text{ and } r = 0.94, \text{ respectively, } P < 0.0001) \).

**DISCUSSION**

The aim of the present study was to investigate the relationship between steroid AKR1C mRNA abundance and activity in abdominal adipose tissue compartments and visceral obesity in women. Although the association between increased circulating total and free androgen levels and abdominal fat accumulation in women is well documented \( (34) \), sex steroid metabolism at the local level within adipose tissue has received little attention in the context of visceral obesity. On the basis of our \( (5, 6) \) previous demonstration of the presence of AKR1C in abdominal adipose tissue, we tested the hypothesis that progesterone and androgen 20α- and 3α-reduction activities as well as mRNA abundance of AKR1C enzymes involved in these reactions are increased in adipose tissue of women with abdominal-visceral obesity. We found that women with elevated visceral adipose tissue accumulations have increased omental adipose tissue mRNA abundance and activities of two AKR1C enzymes involved in the reduction of DHT and progesterone compared with women with low visceral adipose tissue accumulations. Further studies are required to elucidate whether increased inactivation of progesterone and DHT in abdominal adipose tissue impacts locally on fat metabolism in abdominally obese women.

Several steroidogenic enzymes have been detected to date in adipose tissue \( (\text{reviewed in Ref. } 3) \). As mentioned, we \( (5, 6) \) have previously demonstrated that all three AKR1C enzymes examined in the present study were detected in abdominal adipose tissue compartments of women. We \( (5, 6) \) also had reported positive correlations between omental adipose tissue activities of both 3α-HSD and 20α-HSD and visceral adipose tissue area and omental adipocyte size. However, from these studies, it was not possible to determine whether expression levels of these enzymes were also modulated in visceral obesity and, most importantly, whether visceral obesity per se rather than increased total body fat mass was associated with increased omental adipose tissue androgen and progesterone 3α- and 20α-reduction activities. The present study was designed to address this question specifically. Women of our sample were chosen on the basis of low or high visceral adipose tissue areas but were matched for age and total body fat mass. Subcutaneous adipose tissue area measured by computed tomography was also similar between groups. The main adiposity difference between groups was, therefore, visceral adipose tissue area, which was 2.3-fold higher in one group than the other. Our finding that the mRNA abundance and activity of both 20α-HSD and 3α-HSD \( (\text{type } 3) \) are increased in abdominal adipose tissue of viscerally obese women suggests a link with visceral adipose tissue accumulation that is independent of the higher total body fat mass accumulations also observed frequently in viscerally obese women.

AKR1C enzymes are highly homologous. 3α-HSD-3 shares 98% amino acid identity with 20α-HSD, and its identity with type 5 17β-HSD is 88% \( (14, 25, 28) \). However, despite being nearly identical in amino acid sequence, these enzymes have distinct substrate specificities, with 3α-HSD-3 being involved mostly in androgen inactivation \( (\text{reaction of DHT to } 5α\text{-androstane-3α,17β-diol}) \) and 20α-HSD as well as type 5 17β-HSD being involved mostly in the formation of 20α-hydroxyprogesterone from progesterone. There is, however, a slight substrate overlap, with 3α-HSD-3 also exerting a low 20α-HSD activity toward progesterone. Type 5 17β-HSD also partly catalyzes the reaction of androstenedione to testosterone \( (38) \). The use of specific sets of primers for amplification and of distinct steroid substrates for activity measures in tissue homogenates suggests that the close relationship between mRNA abundance and activity of 3α-HSD and 20α-HSD in a given fat depot is not related to failure of distinguishing each enzyme methodologically. On the other hand, the fact that
3α-HSD-3 and 20α-HSD are highly homologous and that their activities and mRNA abundance are both correlated positively with visceral adipose tissue accumulation may suggest similar regulation mechanisms for both enzymes. At the present time, it cannot be excluded that the increased 3α-HSD expression (and activity) observed in omental adipose tissue of abdominally obese women is related to the slight ability of this enzyme to reduce progesterone in addition to DHT. Human AKR1C enzymes apparently have evolved to function either as 3α- or 20α-HSDs (28). Further studies are required to establish which activity is physiologically relevant to abdominal-visceral obesity in women.

Potentially important impacts on body fat distribution and adipocyte physiology have been postulated for both androgens and progesterone. Androgens have been shown to exert a lipolytic action in adipocytes from rats (36). Accordingly, fatty acid turnover was increased in men treated with testosterone (21, 22). Testosterone treatment also inhibited the activity of adipose tissue LPL (21, 22). On the other hand, the specific role of progesterone on fat metabolism is not yet completely elucidated, as some studies found that progesterone stimulated fat accumulation, LPL activity, lipogenesis, and steroid-mediated differentiation of preadipocytes (20, 32, 35), and others reported that progesterone could be responsible for the female fat distribution pattern via an anti-glucocorticoid action in abdominal fat (26, 37). Whether increased metabolism of these steroids in adipose tissue of obese women, through enzymes examined here, relates to actually increased or reduced binding of the steroids to their receptors remains unclear (16). In the present study, we found that androgen and progesterone inactivation activities in omental adipose tissue were positively associated with adipocyte size and LPL activity, suggesting a possible relationship between sex steroid inactivation and adipocyte metabolism. No association was observed with lipolysis, although only basal lipolysis was examined. Other measures of lipolysis, including hormone-sensitive lipase activity and lipidic sensitivity to adrenergic agonists, have been shown to be modulated by sex hormones (2, 11). Their association with AKR1C enzyme expression and activities remains to be determined.

As discussed above, plausible hypotheses to explain the link between AKR1C enzymes and adiposity likely involve the effects of progesterone and/or androgens on adiposity regulation. However, they may also relate to the metabolism of these hormones per se. There is the possibility that progesterone and DHT act through their own receptors and that the balance between active and inactive hormone plays a role in the regulation of fat cell function and metabolism. There also is the possibility that the balance between progesterone and its metabolites determines progesterone’s anti-glucocorticoid (4) and anti-mineralocorticoid (29) actions on adipocytes. Studies on the kidney have shown that conversion of progesterone to 20α-hydroxyprogesterone prevents the anti-mineralocorticoid action of the parent hormone, which provides a role for 20α-HSD in the regulation of blood pressure (29). Similar mechanisms may be operative with respect to adipose tissue, since aldosterone has been shown to induce differentiation in brown adipocytes through binding to the mineralocorticoid receptor (27). Finally, progesterone (or androgen) effects may also be mediated by locally produced hydroxy metabolites through other pathways. Further studies are needed to elucidate the physiological mechanisms underlying our observations.

In the present study, no group differences in plasma levels of sex steroids were observed. In addition, circulating levels of androgens, estrogens, and progesterone were not consistently associated with either adiposity measures or AKR1C enzyme mRNA abundance and activity in adipose tissue. In this regard, it must be kept in mind that correlations between plasma sex hormone levels and visceral adiposity, although highly significant, are generally of low-to-moderate magnitude. The relatively small sample size of the present study may explain the lack of a consistent association pattern between circulating sex steroids and adiposity or AKR1C enzymes. On the other hand, plasma levels of the weak androgen DHEA appeared to be negatively associated with 17β-HSD-5 mRNA as well as 3α-HSD and 20α-HSD activity. Testosterone was positively associated with omental fat 20α-HSD mRNA abundance, whereas plasma DHT was negatively associated with 17β-HSD-5 mRNA abundance. The mechanisms underlying these unexpected associations are unclear. However, apparent discrepancies between adipose tissue steroidogenic enzymes and circulating steroid levels recently have been discussed by another group (30), who found low basal circulating cortisol levels in conjunction with Cushingoid features and elevated regeneration of cortisol in adipose tissue through the activity of 11β-HSD-1 in obese women. The observation of a concomitant increase in cortisol inactivation in the liver was proposed as an explanation for this apparent paradox (30). The documented presence of AKR1C enzymes in several other tissues, including the liver (12), and possible tissue-specific regulation of these enzymes may be related to our observation of an inconsistent association pattern between circulating sex hormones and AKR1C enzymes in adipose tissue.

The present study is limited by its cross-sectional design. Due to the restrictive nature of omental adipose tissue sampling, it is not possible to use longitudinal designs and determine whether increased AKR1C enzyme expression in women is causally related to accelerated abdominal fat accumulation. In addition, although omental AKR1C enzyme expression and activities were stronger correlates of adiposity and adipocyte metabolic parameters compared with subcutaneous fat enzyme measures, our finding of significant group differences in subcutaneous adipose tissue enzyme activities (with no concomitant differences in mRNA) may suggest that the regulation of abdominal adipose tissue AKR1C enzyme activities in visceral obesity is not necessarily restricted to the omental depot. Future studies using in vitro cell culture systems may help clarify these issues. We also have to keep in mind that the small sample size may explain the rather high correlation coefficients observed among variables. Caution must be used in comparing strength of the associations observed among the various measures of this study. Finally, peripheral gluteal or femoral adipose tissue was not examined in the present study; this could have provided further insights into the potential role of androgen and progesterone metabolism through AKR1C enzymes in female body fat distribution.

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