

Expression and activity of 20 α -hydroxysteroid dehydrogenase (AKR1C1) in abdominal subcutaneous and omental adipose tissue in women

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ABSTRACT

We have examined the expression and activity of 20 α -hydroxysteroid dehydrogenase (20 α -HSD) in abdominal adipose tissue in women. This recently-characterized enzyme from the aldoketoreductase 1C family (AKR1C1) is responsible for the conversion of progesterone into 20 α -hydroxyprogesterone. Abdominal subcutaneous (SC) and omental (OM) adipose tissue biopsies were obtained from a sample of 32 women aged 47.7 \pm 5.9 years (BMI: 27.6 \pm 5.0 kg/m²) undergoing abdominal hysterectomies. Body composition and body fat distribution measurements were performed before the surgery by dual energy x-ray absorptiometry and computed tomography respectively. The expression of 20 α -HSD was determined by real-time RT-PCR, and its activity was measured in whole tissue homogenates. Messenger RNA and activity of the enzyme were detected in both the SC and OM fat depots, the two measures being significantly higher in the SC compartment. Women characterized by a visceral adipose tissue area greater than or equal to 100 cm² had an increased 20 α -HSD conversion rate in their omental adipose tissue compared to women without visceral obesity (13.99 \pm 2.07 vs. 7.92 \pm 0.83 fmol/ μ g protein/24h, p <0.05). Accordingly, a positive correlation was found between omental adipose tissue 20 α -HSD activity and computed tomography-measured visceral adipose tissue area (r =0.36, p <0.05). Significant positive correlations were also found between omental 20 α -HSD activity and omental adipocyte diameter (r =0.49, p <0.05) and omental adipose tissue LPL activity (r =0.36, p =0.06). In conclusion, 20 α -HSD activity and mRNA were detected in SC and OM adipose tissue in women, and omental 20 α -hydroxylation of progesterone was highest in women with visceral obesity. Further studies are required to establish whether local conversion of progesterone may impact on the metabolism and function of adipocytes located within the abdominal cavity.

INTRODUCTION

Excessive accumulation of adipose tissue in the abdominal region (abdominal obesity) has been demonstrated to be a strong correlate of obesity-related metabolic alterations leading to type 2 diabetes and cardiovascular disease (1,2). More specifically, fat accumulation located within the abdominal cavity (visceral obesity) has been closely related to the cluster of metabolic abnormalities now defined as the metabolic syndrome, which includes insulin resistance, hyperinsulinemia, elevated triglyceride levels, low HDL-cholesterol concentrations and hypertension (1-3).

The well-known sex difference in body fat distribution suggests that depot-specific regulation of adipose tissue mass is achieved at least partly through hormonal regulation of many cellular processes in each depot. Sex steroids are thought to regulate adipose tissue deposition by acting on the proliferation and/or differentiation of preadipocytes as well as lipogenesis and/or lipolysis in mature adipocytes (4-6). The specific role of progesterone on fat accumulation is not yet fully understood. Some studies found that progesterone stimulated fat accumulation, LPL-activity, lipogenesis and steroid-mediated differentiation of preadipocytes from various sources (7-12). On the other hand, Björntorp (13) suggested that progesterone could be responsible for the female fat distribution pattern via an anti-glucocorticoid action in abdominal fat, an hypothesis that was supported by *in vivo* and *in vitro* experiments reporting an inhibition of glucocorticoid-induced fat cell differentiation, lipogenesis or body fat accumulation by progesterone (14,15).

In recent years, adipose tissue was increasingly perceived as a metabolically active organ displaying endocrine, paracrine and autocrine signals (16). Several steroidogenic enzymes and steroid-converting activities have also been detected in adipose tissue or adipose cells (17),

including activities and/or mRNAs of aromatase, 3 β -hydroxysteroid dehydrogenase (HSD), type 3 3 α -HSD, type 1 11 β -HSD, type 2, 3 and 5 17 β -HSD, 7 α -hydroxylase, 17 α -hydroxylase, 5 α -reductase, and UDP-glucuronosyltransferase 2B15 (18-26). In the present study, we report on the expression and activity of 20 α -hydroxysteroid dehydrogenase (20 α -HSD), a member from the aldoketoreductase 1C enzyme family (AKR1C1) involved in the conversion of progesterone to its 20 α -hydroxylated metabolite, in abdominal subcutaneous and omental adipose tissue in women.

SUBJECTS AND METHODS

Subjects. Women of this study were recruited through the elective surgery schedule of the Gynecology Unit of the Laval University Medical Research Center for studies on adipose tissue metabolism. Thirty two healthy women aged 40 to 60 years undergoing abdominal gynecological surgery were included in the present analysis. All women of the study elected for total (n=31) or subtotal (n=1) abdominal hysterectomies, some with salpingo-oophorectomy of one (n=5) or two (n=19) ovaries. Reasons for surgery included one or more of the following: menorrhagia (n=11), myoma (n=18), pelvic pain (n=11), benign cyst (n=1), endometriosis (n=7), cystitis (n=3) and hydrosalpinx (n=3). Medication included thyroid hormones (n=3), GnRH agonist and/or hormone therapy (n=9), lipid lowering (n=1), or anti-hypertensive therapy (n=8), non-steroidal anti-inflammatory drugs (n=4), anti-depressors (n=9), anti-vertigo (n=1), anti-seizure (n=1) and asthma (n=6) medication. Seven women were taking nutritional supplements (vitamins, iron, calcium or glucosamine). Other types of medication included digoxin, acetaminophen, aspirin, allergy medicine, antibiotics, mesalamine, and anti-spasmodic medicine. Approbations by the medical ethics committees of Laval University and Laval University Medical Research Center were obtained. All subjects provided written informed consent before their inclusion in the study. Adipose tissue samples and biological/clinical data of the present group of women were used for a previous study on other steroidogenic enzymes (27).

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, using a Hologic QDR-2000 densitometer and the enhanced array whole-body software V5.73A (Hologic Inc., Bedford, MA). Measurement of abdominal subcutaneous and visceral adipose tissue cross-sectional areas was

performed by computed tomography as previously described (28), using a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI) and the Light Speed QX/I 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 vertebrae 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 -190 to -30 Hounsfield Units. The coefficient of variation between two analyses from the same observer (n=10) were 0%, 0.50% and 2.14% for total, subcutaneous and visceral adipose tissue areas, respectively.

Plasma hormone measurements. Plasma concentrations of DHEA, androstenedione, dihydrotestosterone, estrone, and estradiol were determined in 26 subjects of the study using high performance gas chromatography and chemical ionization mass spectrometry. Intra- and inter-assay precision did not exceed 5.9% for these measurements. Dihydrotestosterone, estrone, and estradiol values were below quantification level for 4, 5 and 3 subjects respectively. DHEA-S and estrone-sulfate concentrations were determined in 25 subjects of the study using HPLC and mass spectrometry and a PE Sciex API 300 tandem mass spectrometer (Perkin-Elmer, Foster City, CA) equipped with a Turbo ionspray source. Intra- and interassay precision did not exceed 6.4% for these measurements. Progesterone levels were determined in 27 subjects with a radioimmunoassay from Diagnostic System Laboratories (Webster, TX).

Adipose tissue sampling. Paired omental and subcutaneous adipose tissue samples were collected during the surgical procedure and immediately carried to the laboratory in 0.9% saline preheated at 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 lipase activity. Tissue samples were digested with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer for 45 minutes at 37°C according to a modified version of the Rodbell method (29). Adipocyte suspensions were filtered through nylon mesh and washed 3 times with KRH buffer. For cell size measurements, mature adipocyte suspensions were visualized using a contrast microscope attached to a camera and computer interface. Pictures were taken and the Scion Image software was used to measure the size of 250 adipocytes.

Basal lipolysis was measured by incubating isolated cell suspensions for 2 hours at 37°C. Glycerol release in the medium was measured by bioluminescence using the NADH-linked bacterial luciferase assay (30,31), an EG&G Berthold MicroLumat Plus Bioluminometer (LB 96 V), and the 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 ($\mu\text{mol glycerol}/\mu\text{m}^2 \times 10^8 / 2\text{h}$).

Lipoprotein lipase activity was determined in 30-50 mg frozen adipose tissue samples by the method of Taskinen (32). Tissue eluates were obtained by incubating the sample in Krebs Ringer

Phosphate buffer and heparin at 28°C for 90 minutes. The eluates were then incubated with excess concentrations of unlabeled and ¹⁴C-labeled triolein in a TRIS-albumin buffer emulsified with ultrasound. The reaction was carried out at 37°C for 60 minutes with agitation. The resulting free fatty acids liberated from triolein by the LPL reaction were isolated by the Belfrage extraction procedure. Porcine plasma was used as a source of Apo-CII to stimulate LPL activity, and unpasteurized cow's milk as an internal LPL activity standard for inter-assay variations. Activity results were expressed in $\mu\text{mol oleate}/10^6\text{cells/h}$.

Reverse transcriptase-PCR reactions. Total RNA was isolated using Trizol (Invitrogen Life Technologies) following the manufacturer's recommendations. Reverse transcription was performed with the Ambion Retroscript kit (Ambion Inc. Austin, Tx) using random decamer primers. PCR amplification conditions were 94°C/30 sec, 66°C/30 sec (63°C for control) and 72°C/30 sec for 35 cycles. The primers used spanned the junction for a 273 bp intron: 5'-CCT-ATA-GTG-CTC-TGG-GAT-CCC-AC -3', and 5'-AGG-ACC-ACA-ACC-CCA-CGC-TGT-3' (20 α -HSD). Amplified products were separated on 2% agarose gels stained with ethidium bromide. Sequencing of PCR products was performed using BigDye Terminator v3.1 cycle sequencing (ABI Prism, Applied biosystem, Foster city, CA) and analysis on an ABI 3730 automated DNA sequencer (PE Applied Biosystems, Foster City, CA).

Real-time PCR. 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 dT18, 500 μM dNTP, 10 mM DTT 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 expression of 20α-HSD using the primers described in the section above. 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, 50 cycles of (95°C/10 sec, 58°C/5 sec, 72°C/8 sec) with a temperature transition of 3°C/sec. For all the samples tested, no amplification was detected in a control tube containing water. Amplification of 20α-HSD and the housekeeping gene, glucose-6-phosphate dehydrogenase (G6PDH) which was quantified using the following primers: 5'-CAG-CGC-CTC-AAC-AGC-CAC-AT-3' and 5'-AAG-GGC-TTC-TCC-ACG-ATG-ATG-C-3', generated melting curves with a single peak and negligible non-specific amplification products. A universal standard curve was generated with ATPase 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 using the double derivative method. For each sample, the Cp value of 20α-HSD was divided by that of the housekeeping gene. In order to 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 is expressed in normalized number of copies per µg total RNA.

Enzymatic activities. 20α-HSD activity was measured in whole tissue homogenates. Tissue samples were homogenized with a Polytron in 50 mM sodium phosphate buffer (pH 7.4), 20% glycerol, 1 mM EDTA and 1 mM NADPH (33). ³H-labeled progesterone was added and

reactions were performed at 37°C in a final volume of 1 ml for 24 hours. Steroids from tissue homogenates were extracted twice with 1 volume ether as described previously (27). The organic phases were pooled and evaporated to dryness. The steroids, including reference standards, were solubilized in 50 µl dichloromethane (the 20α-hydroxyprogesterone standard was solubilized in ethanol) and applied to Silica Gel 60 TLC plates (Merck, Darmstadt, Germany) using 10 µl calibrated micropipets. The separation was performed by migration in toluene-acetone (4:1). Unlabeled 20α-hydroxyprogesterone was used as a standard and was detected under ultraviolet light. Radioactivity was detected using a Storm 860 PhosphorImager (Amersham Pharmacia Biotech Inc), and quantification was done using the ImageQuant software version 5.1 (Amersham Pharmacia Biotech Inc). Proteins in each tissue were quantified by the method of Lowry and used to normalize activity values.

Statistical analyses.

Paired t-tests were used to compare expression levels in SC and OM adipose tissue and unpaired t-tests were used to compare mean 20α-HSD activities between subjects with either high or low visceral fat areas. Spearman rank correlation coefficients were computed to quantify associations between progesterone-converting activity and adiposity measures. Statistical tests were performed on log₁₀-transformed data when the variables were non-normally distributed as tested using the Shapiro-Wilk W test. The following variables were not normally distributed: omental 20α-HSD activity, omental LPL adipose tissue activity, visceral adipose tissue area. A level of $\alpha \leq 0.05$ was considered as statistically significant. Analyses were performed using the JMP statistical software (SAS Institute, Cary, NC).

RESULTS

Female SC and OM adipose tissue expressed mRNA for 20 α -HSD, as shown by RT-PCR analysis (Fig. 1A). Expected transcripts of 160 bp were detected in tissue samples of several patients (two are shown on the Figure). Sequencing confirmed that the PCR products were 20 α -HSD. The minor amplicon noted for some of the samples likely corresponded to genomic DNA contamination of the sample (addition of a 273 bp intron). Using quantitative real-time PCR analysis in a subset of subjects we found that 20 α -HSD expression was 2.7-fold higher in SC than in OM adipose tissue when normalizing expression levels for glucose-6-phosphate dehydrogenase expression (paired t-test difference $p < 0.001$, $n = 8$; Fig. 1B).

Figure 2 shows a thin layer chromatogram of steroid products obtained after incubating whole SC or OM adipose tissue homogenates with radiolabeled progesterone for 24 h. The main conversion product form of progesterone was 20 α -hydroxyprogesterone (20 α -OH-Prog) (Fig 2. samples 1 to 4). As shown, enzymatic activity of 20 α -HSD was detected in SC and OM adipose tissue from two patients (C and D). These results are representative of experiments performed with samples from other patients of the study sample ($n = 32$).

Characteristics of the study sample are shown in Table 1. Subjects were aged 47.7 years and had a mean weight of 71.2 kg. They covered a wide range of body mass index values (from 19.3 to 39.4 kg/m²). The mean fat mass was 30 kg. Computed tomography measures also covered wide ranges: from 100 to 736 cm² for SC adipose tissue area and from 34 to 229 cm² for visceral adipose tissue area. Omental and subcutaneous adipose tissue metabolism measurements were consistent with previously reported regional differences with respect to adipocyte size and LPL

activity (34), the former being higher in the subcutaneous depot ($p < 0.0001$, test performed on \log_{10} -transformed values) and the latter being higher in the omental depot ($p < 0.05$, test performed on \log_{10} -transformed values).

Subcutaneous and omental progesterone conversion rates were not significantly different when comparing lean and obese women based on BMI or fat mass values, with the exception of a non-significant trend for higher subcutaneous 20α -HSD activity in obese women (not shown). When stratifying subjects for visceral adipose tissue area using a cut off value of 100 cm^2 (35), women with visceral obesity were characterized by a 1.8 fold higher 20α -HSD activity compared with women with low visceral adipose tissue area (Fig. 3). Subcutaneous progesterone conversion rates were not different in these two groups. When comparing the omental-subcutaneous differences, 20α -HSD activity was higher in the subcutaneous adipose tissue compartment of women with low visceral adipose tissue areas, whereas no regional difference was observed in viscerally obese women.

Figure 4 shows correlations between omental 20α -hydroxyprogesterone formation in omental fat tissue homogenates and adiposity as well as adipose tissue metabolism. We found significant positive correlations between omental 20α -HSD activity and visceral adipose tissue area ($r = 0.36$, $p < 0.05$), visceral adipocyte diameter ($r = 0.49$, $p < 0.05$) and a positive trend with omental LPL activity ($r = 0.36$, $p = 0.06$). Subcutaneous 20α -HSD activity was not significantly associated with adiposity measures or adipose tissue metabolism, with the exception of a trend for a positive correlation with total fat percentage ($r = 0.32$, $p = 0.08$). Basal lipolysis in both adipose tissue

depots was not significantly associated with 20 α -HSD activity in either compartment ($r=-0.06$, $p=0.78$ in SC adipose tissue; $r=-0.13$, $p=0.51$ in OM adipose tissue).

In the present sample, nine women did not have menstrual bleeding, 4 of which had reached a natural menopause and 5 of which had received GnRH agonist therapy. No differences were noted in subcutaneous or omental adipose tissue 20 α -HSD activity when comparing these groups of women (not shown). In addition, subcutaneous or omental adipose tissue 20 α -HSD activity in the nine women who were on hormone/GnRH replacement therapy were not significantly different from activities measured in other subjects of the sample (not shown). Plasma levels of sex hormones including estradiol, estrone-sulfate, progesterone, DHEA, DHEA-sulfate and androstenedione were not significantly associated with subcutaneous or omental adipose tissue 20 α -HSD activity in the present study.

DISCUSSION

In the present study, we investigated the expression and activity of 20 α -HSD, a member of the AKR1C family (AKR1C1) in abdominal adipose tissue. This enzyme catalyzes the conversion of progesterone into 20 α -hydroxyprogesterone and was previously found in several tissues such as the liver, ovaries, testes, adrenals and placenta (33). We detected mRNA and activity of 20 α -HSD in both SC and OM adipose tissue in women. Quantitative real-time PCR analyses showed that expression was higher in SC than in OM fat. A positive association was found between visceral adipose tissue area and omental adipose tissue activity of this enzyme. Moreover, 20 α -HSD in this depot correlated positively with omental adipocyte diameter and LPL activity. No association was found between subcutaneous adipose tissue 20 α -HSD activity and abdominal obesity. This study is the first to describe 20 α -HSD expression and activity in subcutaneous and omental adipose tissue. Our results show that omental adipose tissue 20 α -hydroxyprogesterone formation is highest in women characterized by visceral obesity. Further studies are required to establish whether regional hydroxylation of progesterone may impact locally on the metabolism and function of adipocytes located within the abdominal cavity.

The enzyme 20 α -HSD shares structure identity and sequence homology with 20 α -HSDs from several other species. In rodent models, it is believed that 20 α -HSD induction and conversion of progesterone to 20 α -hydroxyprogesterone in the corpus luteum may be responsible for pregnancy termination by causing the marked decrease in progesterone occurring before the initiation of parturition (36). In addition, a recent study demonstrated that an aldose reductase with 20 α -HSD activity was presumably responsible for the production of prostaglandin F $_{2\alpha}$ in bovine endometrium, suggesting additional roles of this enzyme in labor and luteolysis (37). Other

physiological roles in animal models may also include the protection of mice thymocytes against the toxic effects of progesterone (38), and regulation of myelin formation in the brain (39). The role of 20 α -HSD in humans is still poorly defined (33). The present finding of significant progesterone conversion to its 20 α -hydroxylated form and detection of 20-HSD mRNA in abdominal fat suggests that this enzyme may regulate the action of progesterone in this tissue. As opposed to the placenta, however, other progesterone metabolites may also be generated in adipose tissue through previously documented expression of 17 α -hydroxylase and 5 α -reductase in human adipose tissue or adipose cells (17,21,24).

The effects of progesterone itself on adipose tissue and adipose cell regulation have been investigated in several studies. Experiments performed in the murine 3T3-L1 cell line have shown that progesterone stimulates adipocyte differentiation as assessed by triglyceride accumulation and glyceraldehyde-3-phosphate dehydrogenase activity (10), and this lipogenic effect has been attributed to stimulation of adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c (ADD1/SREBP1c) and of key lipogenic genes such as fatty acid synthase (7). Progesterone has also been shown to have lipogenic properties in other models such as primary preadipocytes obtained from male and female rats (7,40), and brown adipocytes from male mice (41). Although progesterone receptor knockout mice are not characterized by a modified adiposity phenotype (42), the presence of progesterone receptor isoforms A and B in adipose tissue may provide a direct route for the regulation of adipose tissue by this steroid in women (43).

On the other hand, Björntorp suggested that this hormone might be responsible for the female pattern of obesity by acting as a glucocorticoid antagonist in abdominal adipose tissue depots, thereby preventing abdominal fat accumulation (13). This hypothesis is supported by experiments demonstrating inhibition of glucocorticoid-induced fat cell differentiation, lipogenesis and body fat accumulation by progesterone in rodents (14,15). Hence, studies have shown that progesterone may exhibit both lipogenic and anti-lipogenic properties, depending on the model and experimental approach used. It may be speculated that the depot origin of the fat cells may be critical in the effects observed, and that the hormone acts in a depot-specific manner. In this regard, modulation of progesterone concentrations through the regulation of 20 α -HSD expression may serve as a tissue-specific regulator of local hormone levels and action in adipose tissue. Another possibility would be that part of the effects of progesterone in adipose tissue are mediated through a yet undefined 20 α -hydroxyprogesterone action in adipose cells.

20 α -HSD is highly homologous to other enzymes from the AKR1C enzyme family in humans. For example, type 3 3 α -HSD (AKR1C2) shares 98% amino acid identity with 20 α -HSD and the identity with type 5 17 β -HSD (AKR1C3) is 88%. However, despite being nearly identical in amino acid sequence, these enzymes have distinct substrate specificities, with AKR1C2 being mostly involved in androgen inactivation (reaction of DHT to 3 α -androstenediol) and AKR1C3 being involved mostly in androgen synthesis (reaction of androstenedione to testosterone). AKR1C2 also exerts a slight 20 α -HSD activity toward progesterone, while AKR1C3 also exerts 3 α and 20 α activities toward DHT and progesterone (33). We recently demonstrated in the present sample that mRNA and/or activity of these two other enzymes from the AKR1C family, namely type 3 3 α -HSD (AKR1C2) and type 5 17 β -HSD (AKR1C3) are also detectable in

abdominal adipose tissue and primary preadipocyte cultures (27). In addition, rates of androgen inactivation measured in omental adipose tissue (3α -HSD activity) were significantly and positively associated with visceral adiposity in this sample of women. By examining 3α -reduction of DHT and 20α -hydroxylation of progesterone activities measured in our adipose tissue samples, we found that 3α -HSD activity (androgen inactivation) was positively correlated with 20α -HSD activity (progesterone hydroxylation) in both the omental ($r=0.46$, $p<0.01$) and subcutaneous ($r=0.37$, $p<0.05$) fat compartments. The relevance of the correlations observed between both activities in their respective depot is unclear. The fact that type 3 3α -HSD and 20α -HSD are highly homologous and that their activities are both correlated positively with visceral adipose tissue accumulation may suggest similar regulation mechanisms for both enzymes.

Due to the necessity of a surgical procedure to access the omental adipose tissue depot for sampling, the present study uses a cross-sectional design. Thus, based on the significant positive correlations between omental adipose tissue 20α -HSD activity and abdominal adiposity or omental adipose tissue metabolism, one cannot reach conclusions on causality. The question as to whether elevated 20α -HSD in omental adipose tissue of viscerally obese women is a causal agent for abdominal obesity or whether it is merely a consequence or adaptation to the obese state cannot be addressed. Further studies using *in vitro* approaches are warranted to resolve this issue.

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Table 1. Physical and metabolic characteristics of the sample of 32 women.

Variables	Mean	±	SD
Age (yr)	47.7	±	5.9
Weight (kg)	71.2	±	14.9
BMI (kg/m ²)	27.6	±	5.0
Fat mass (kg)	30.0	±	10.5
Fat-free mass (kg)	37.9	±	5.7
Subcutaneous AT area (cm ²)	350	±	133
Visceral AT area (cm ²)	102	±	47
<i>Subcutaneous AT metabolism</i>			
Adipocyte diameter (μm)	101.5	±	15.0
LPL activity (μmol oleate/10 ⁶ cells/h) ^a	10.7	±	9.7
Basal lipolysis (μmol glycerol/10 ⁸ cells×μm ²) ^b	2.1	±	1.0
<i>Omental AT metabolism</i>			
Adipocyte diameter (μm)	84.9	±	21.8
LPL activity (μmol oleate/10 ⁶ cells/h) ^a	8.1	±	6.1
Basal lipolysis (μmol glycerol/10 ⁸ cells×μm ²) ^a	2.2	±	1.4

AT, Adipose tissue

^an=29^bn=30

FIGURE HEADINGS

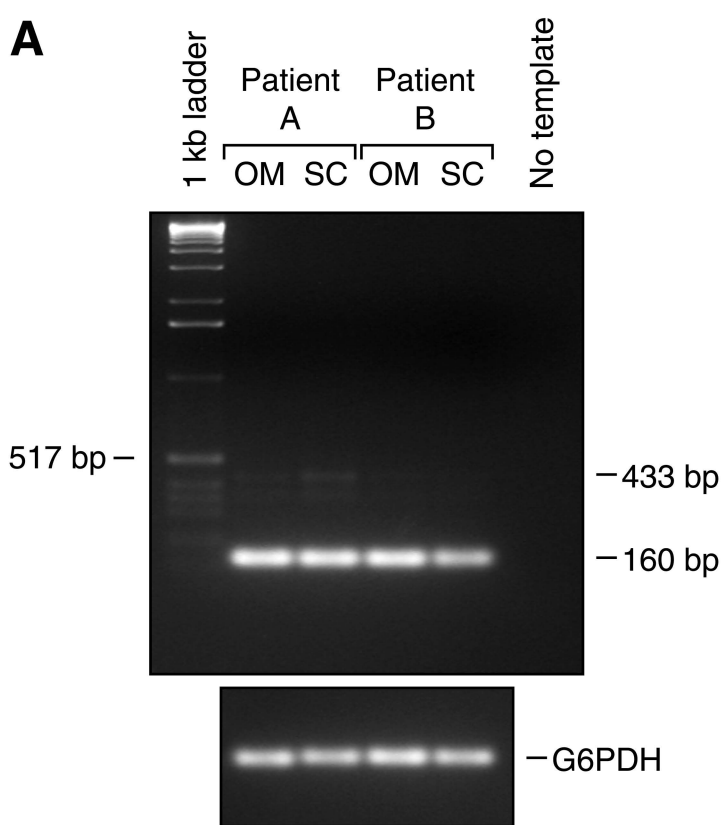
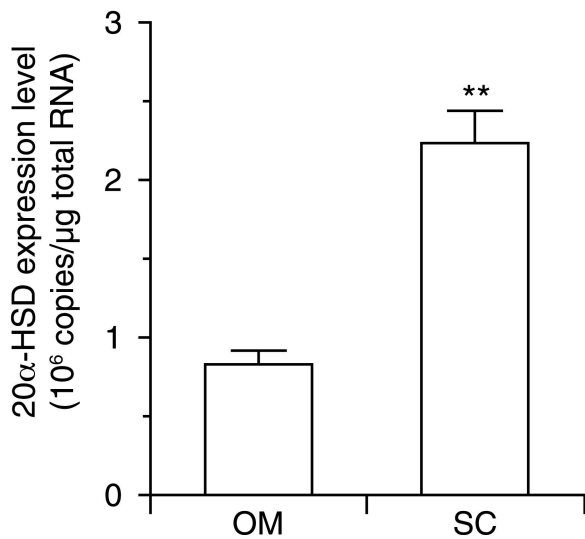
Figure 1. A. RT-PCR detection of 20α -HSD mRNA (160 bp) in subcutaneous (SC) and omental (OM) adipose tissue obtained in two women of the study (patients A and B; ages 43.8 and 48.3y, respectively). Results are representative of experiments performed in several patients. G6PDH: glucose-6-phosphate dehydrogenase. B. Real-time RT-PCR quantification of 20α -HSD in SC and OM adipose tissue samples obtained in eight women of the study. Age and adiposity characteristics of the subsample (n=8): age: 47.2 ± 4.5 years; BMI: 26.9 ± 3.3 kg/m²; fat mass 29.4 ± 7.2 kg; visceral adipose tissue area: 110.6 ± 60.2 cm² (mean \pm SD). **, p<0.001.

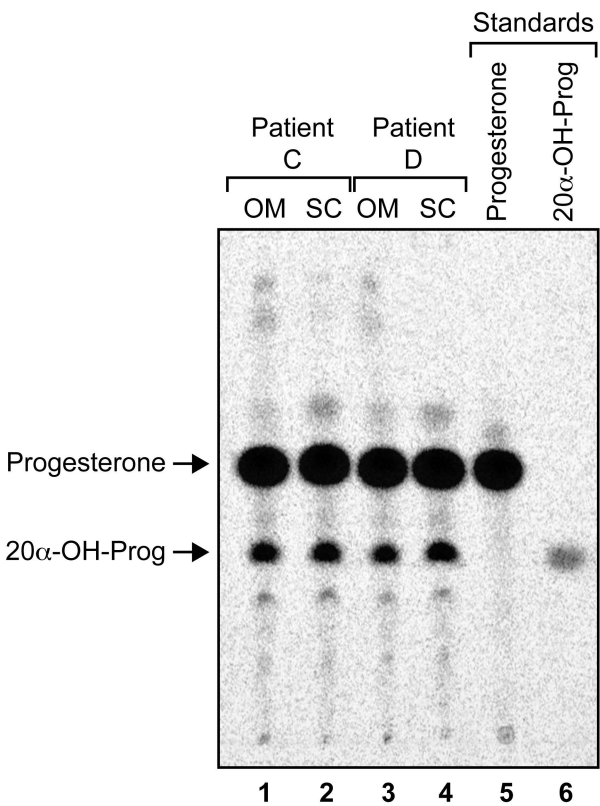
Figure 2. Thin layer chromatogram showing steroid products obtained when incubating SC or OM adipose tissue homogenates with radiolabeled ³H-progesterone. The chromatogram was photographed under ultraviolet light for the identification of the 20α -hydroxyprogesterone standard and this image was superimposed to the autoradiogram by image analysis. Results of patients C and D (ages 58.9 and 40.1y, respectively) were representative of experiments performed in several other homogenates.

Figure 3. Comparison of 20α -HSD activity measured in SC and OM homogenates in women with either low or high (< 100 cm² or \geq 100 cm²) visceral adipose tissue (AT) areas. For the comparison of omental 20α -HSD activity, the group with adipose tissue areas below 100 cm² included 17 subjects, and the group with adipose tissue areas greater than or equal to 100 cm² included 14 subjects. For the comparison of subcutaneous 20α -HSD activity, the group with adipose tissue areas below 100 cm² included 16 subjects, and the group with adipose tissue areas

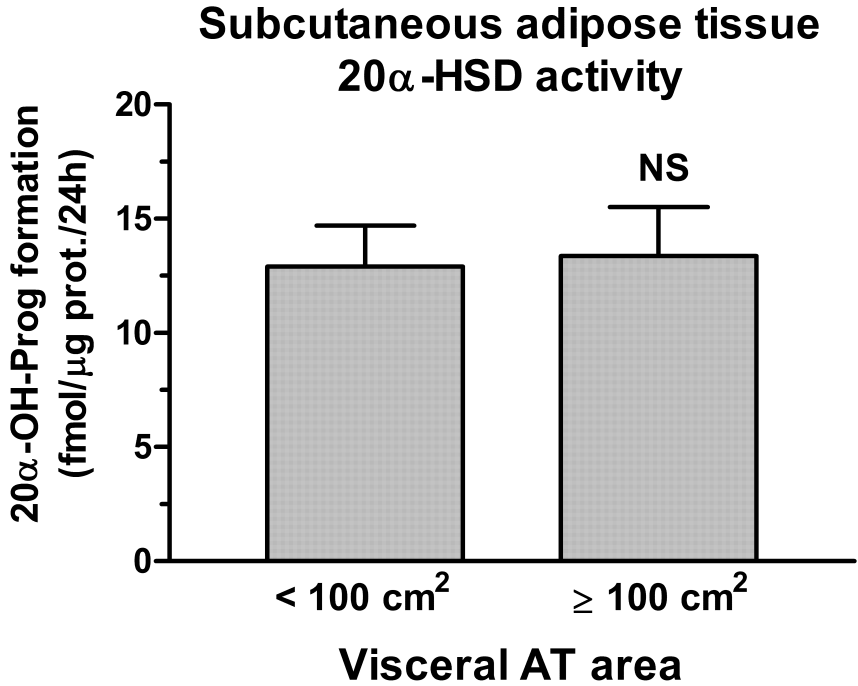
greater than or equal to 100 cm² included 13 subjects. Statistical test performed on log₁₀ transformed data for omental 20 α -HSD activity. NS, Non significant; *, p<0.05.

Figure 4. Correlations between 20 α -HSD activity measured in OM adipose tissue homogenates and A) visceral adipose tissue (AT) area (n=31), B) omental adipocyte diameter (n=32) and C) omental LPL activity (n=29). Log₁₀-transformed values are presented when variables were not normally distributed

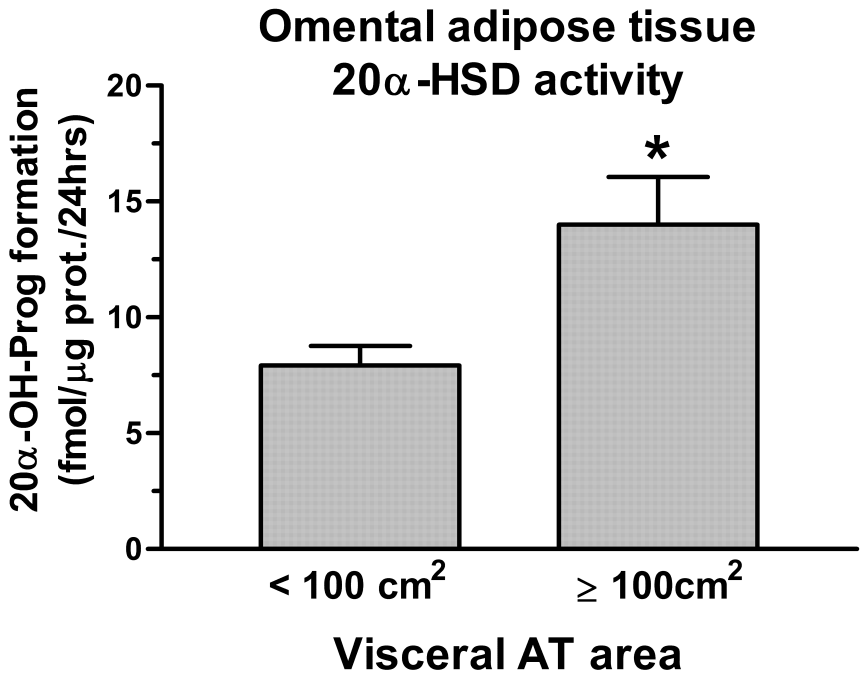
A**B**

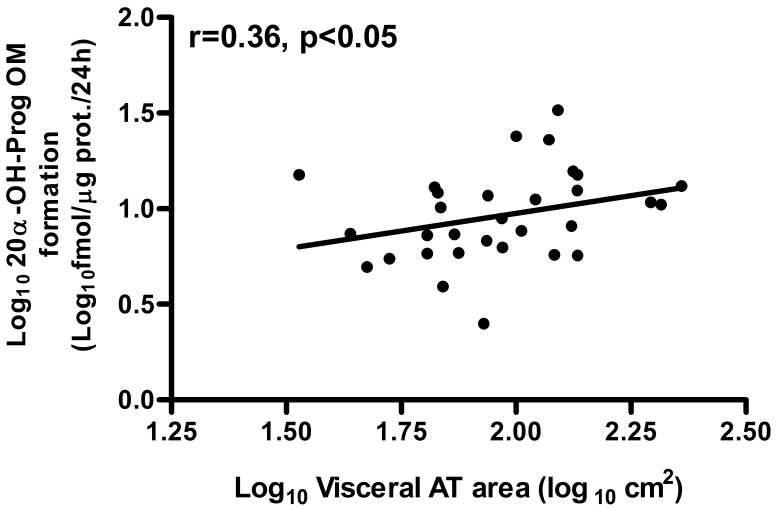
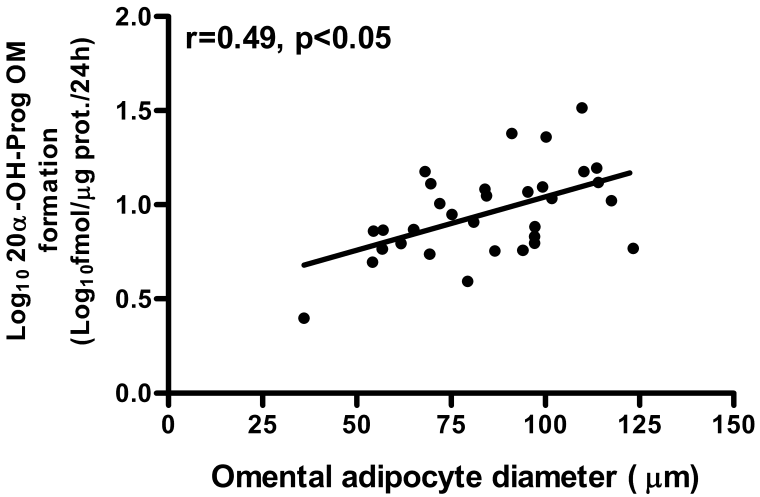


A



B



A**B****C**