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Pathways of adipose tissue androgen metabolism in women: depot differences and modulation by adipogenesis

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[1]*Molecular Endocrinology and Oncology Research Center, Laval University Medical Research Center;* [2]*Department of Nutrition,* [3]*Department of Surgery, Laval University;* [4]*Gynecology Unit, Laval University Medical Research Center, Québec City, Québec, Canada*

Blouin K, Nadeau M, Mailloux J, Daris M, Lebel S, Luu-The V, Tchernof A. Pathways of adipose tissue androgen metabolism in women: depot differences and modulation by adipogenesis. *Am J Physiol Endocrinol Metab* 296: E244–E255, 2009. First published November 4, 2008; doi:10.1152/ajpendo.00039.2008.—The objective was to examine pathways of androgen metabolism in abdominal adipose tissue in women. Abdominal subcutaneous (SC) and omental (OM) adipose tissue samples were surgically obtained in women. Total RNA was isolated from whole adipose tissue samples and from primary preadipocyte cultures before and after induction of differentiation. Expression levels of several steroid-converting enzyme transcripts were examined by real-time RT-PCR. Androgen conversion rates were also measured. We found higher expression levels in SC compared with OM adipose tissue for type 1 3β-hydroxysteroid dehydrogenase (3β-HSD-1; $P < 0.05$), for aldo-keto reductase 1C3 (AKR1C3; $P < 0.0001$), for AKR1C2 ($P < 0.0001$), and for the androgen receptor ($P < 0.0001$). 17β-HSD-2 mRNA levels were lower in SC adipose tissue ($P < 0.05$). Induction of adipocyte differentiation led to significantly increased expression levels in SC cultures for AKR1C3 (4.7-fold, $P < 0.01$), 11-*cis*-retinol dehydrogenase (6.9-fold, $P < 0.02$), AKR1C2 (5.6-fold, $P < 0.004$), P-450 aromatase (5.7-fold, $P < 0.02$), steroid sulfatase (3.1-fold, $P < 0.02$), estrogen receptor-β (11.8-fold, $P < 0.01$), and the androgen receptor (4.0-fold, $P < 0.0005$). Generally similar but nonsignificant trends were obtained in OM cultures. DHT inactivation rates increased with differentiation, this effect being mediated by dexamethasone alone, through a glucocorticoid receptor-dependent mechanism. In conclusion, higher mRNA levels of enzymes synthesizing and inactivating androgens are found in differentiated adipocytes, consistent with higher androgen-processing rates in these cells. Glucocorticoid-induced androgen inactivation may locally modulate the exposure of adipose cells to active androgens.

aldo-keto reductases; short-chain dehydrogenases; adipocyte differentiation; omental visceral fat; estrogen

A SURVEY OF THE LITERATURE on adipose tissue steroid conversions indicated that several isoforms of steroidogenic and steroid-inactivating enzymes can be detected in adipose tissue (3). Specifically, as many as 15 steroidogenic and steroid-inactivating enzyme mRNAs/activities have been detected to date in human adipose tissue, including aromatase, 3β-hydroxysteroid dehydrogenase (HSD) type 1, 3α-HSD type 3 [aldo-keto reductase 1C2 (AKR1C2)], 11β-HSD type 1 and type 2, 17β-HSD types 2, 3, and 5 (AKR1C3), 20α-HSD (AKR1C1), 7α-hydroxylase, 17α-hydroxylase, 5α-reductase, steroid sulfatase, and UDP-glucuronosyltransferase 2B15 (3–7, 11, 38). The capacity of peripheral tissues to synthesize and inactivate androgens/estrogens has been termed intracrinology (21, 22). Thus, in addition to its widely recognized endocrine chracteristics (20), adipose tissue also functions as an intracrine organ with respect to steroid hormones.

One of the steroidogenic enzymes expressed in adipose tissue, 11β-HSD-1, has attracted much scientific attention in recent years. Expression and activity of 11β-HSD-1 was demonstrated in fat cells from breast, omental, and subcutaneous adipose tissue (8, 42). Studies have found that the predominant activity in adipose tissue was the reduction of inactive cortisone to active cortisol (8, 19). Using cell cultures and transgenic mouse model studies by Bujalska et al. (9) and Masuzaki et al. (24) led to the demonstration that increased local cortisol production by 11β-HSD-1 may be one of the causal factors in the etiology of visceral obesity. These studies on local cortisol metabolism in fat have dramatically emphasized the importance of steroidogenic enzyme expression in the etiology of visceral obesity and related metabolic complications (9, 24). The impact of other steroidogenic enzymes that could modulate in a similar manner the exposure of abdominal adipocytes to active steroids, namely androgens and estrogens, remains to be established. In addition, regional adipose tissue depot differences in steroid-converting enzymes have not been examined for most of the enzymes identified in adipose tissue.

In the present study, we quantitatively examined the expression of a large number of steroidogenic and steroid-inactivating enzymes detected in fat biopsies of subcutaneous (SC) and omental (OM) adipose tissue obtained from a sample of women. To test the hypothesis that potential depot differences in the expression of steroid-converting enzymes may be the result of differential preadipocyte/mature adipocyte content in SC vs. OM adipose tissue, we also investigated the effects of adipocyte differentiation on the expression of steroid-converting enzymes and androgen metabolism.

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. *Study 1* included 14 women aged 41.5–58.8 yr [body mass index (BMI) 26.2 ± 2.6 kg/m², range 22.5–30.1 kg/m²] undergoing abdominal gynecological surgery. These women elected for total (*n* =

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13) or subtotal ($n = 1$) abdominal hysterectomies, some with salpingo-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 ($n = 1$), dysmenorhea ($n = 1$), severe premenstrual syndrome ($n = 1$), and menometrorrhagia ($n = 1$). *Study 2* included 12 women aged 41.1–49.0 yr (BMI 25.2 ± 2.7 kg/m^2, range 19.1–37.6 kg/m^2) also undergoing abdominal gynecological surgery. These women elected for total ($n = 11$) or subtotal ($n = 1$) abdominal hysterectomies, some with salpingo-oophorectomy of one ovary ($n = 3$) or both ($n = 2$). Reasons for surgery included one or more of the following: menorrhagia ($n = 5$), myoma/fibroids ($n = 10$), cervix cyst ($n = 1$), pelvic pain ($n = 1$), endometriosis ($n = 3$), postmenstrual bleeding ($n = 1$), dysmenorhea ($n = 2$), uterine poly ($n = 1$), and menometrorrhagia ($n = 1$). Preadipocytes were also isolated from fat samples obtained in women undergoing biliopancreatic diversion for the treatment of obesity and were used only in the experiments on dexamethasone alone (BMI ranging from 37 to 74 kg/m^2). These studies were approved by the medical ethics committees of Laval University and Laval University Medical Center as well as Laval Hospital for bariatric patients. All subjects provided written informed consent prior to their inclusion in the studies.

Adipose Tissue Sampling

Paired OM (epiploic) and SC 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 preadipocyte isolation, and the remaining tissue was immediately frozen at −80°C for subsequent analyses.

Preadipocyte Isolation and Primary Cultures

Fresh 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. Preadipocytes were isolated using a modification of the method previously described by Van Harmelen et al. (40). Briefly, the residual KRH buffer of the adipocyte isolation was centrifuged and the pellet washed in DMEM-F-12 supplemented with 10% fetal bovine serum, 2.5 µg/ml amphotericin B, and 50 µg/ml gentamicin. Cells were treated with erythrocyte lysis buffer (154 mM NH$_4$Cl, 10 mM K$_2$HPO$_4$, and 0.1 mM EDTA, pH 7.5), and DMEM-F-12 was added. Preadipocytes were then subsequently filtered through 140 and 30 µm of nylon mesh to remove endothelial cells, placed in culture plates, and cultured at 37°C under a 5% CO$_2$ atmosphere. Medium was changed every 2–3 days.

Induction of Adipocyte Differentiation

Cells were seeded in DMEM-F-12 at a density of 4×10^4 cells/cm^2 to obtain full confluence within 48 h. Cells were differentiated and maintained for 19–21 days according to a standard protocol (Zen-Bio, Research Triangle Park, NC). Differentiation medium consisted of DMEM-F-12 supplemented with a peroxisome proliferator-activated receptor-γ (PPARγ) agonist, insulin, dexamethasone, and 3-isobutyl-1-methylxanthine. Nondifferentiated preadipocytes were also grown to confluence and used for comparison. Cells were cultured in six-well plates in duplicates for mRNA quantification, and 96-well plates were grown in parallel to quantify adipogenesis and steroid conversions into metabolites.

Glycerol-3-Phosphate Dehydrogenase Activity Measurements

Glycerol-3-phosphate dehydrogenase (G3PDH) activity was used as a marker of adipocyte differentiation and measured according to Sottile and Seuwen (34), with some modifications. Differentiated cells

Table 1. *Oligonucleotides used in real-time RT-PCR quantification*

Gene	Oligonucleotide Sequence (5'→3' Sense/Antisense)	
	Study 1	Study 2
AKR1C2	CGGTCAAATTGGCAATAGAAGCC / CAACTCTGGTCGATGGGAATTGCT	CCGTCAAATTGGCAATAGAAGCC / CAACTCTGGTCGATGGGAATTGCT
AKR1C3	CAACCAGGTAGAATGTCATCCGTAT / ACCCATCGTTTGTCTCGTTGA	CAACCAGGTAGAATGTCATCCGTAT / ACCCATCGTTTGTCTCGTTGA
3β-HSD-1	AATCTGAAAAACCGGCGCACCC / CATTGTTGTTCAGGGCCTCGTTTA	CTTCAACCGCCACATAGTCACATT / AGGAGGGTGGAGCTTGATGACAT
17β-HSD-2	GCGCCTCCTGGTGCTCCAAATG / CGGCCATGCATTGTTTGTAGTCAGTCA	GCGCCTCCTGGTGCTCCAAATG / CGGCCATGCATTGTTTGTAGTCAGTCA
17β-HSD-3	GCCATGGAATTGGGAAAGCGTACT / CTCCCTGTAGTCCGCTCGATCTCTG	AAATGTGATAACCAAGACTGCTGAT / CCTGACCTTGGTGTTGAGCTT
RoDH		TTCGTGGGCCTGTACTACCCTTCTGC / CAGGTTCCCAAAGCCCGAGTCACA
RDH5		CTGATCTGTGACCCGGACCTAA / GGGGCAGAAATAAATCAAAGTCCTT
5α-Reductase-1	TGGCGATTATGTTCTGTACCTGTA / AACCACAAGCCAAAACCTATTAGA	TGGCGATTATGTTCTGTACCTGTA / AACCACAAGCCAAAACCTATTAGA
5α-Reductase-2	CGGTTTACGTTGGGTGTCTTTCTTATT / TGGCTCCAGAAACATACGTAAACAAG	
P450 aromatase	ACCCTTCTGCGTTCGTCGTCATGCTG / GGATTTTAACCACGATAGCACTTTTC	CGACAGGCTGGTACCGCCATGCTC / AAGAGGCAATAATAAAGGAAATCCAGAC
UGT2B15	GCTGTGGAAAGGTGCTAGTGTG / GCATTGACAAGAGTAGAAGCCGAAGA	
Steroid sulfatase	AGCCCTAATCCTGACCCTTTTCTTGG / CCGCCTCCACCGTTAGCCTCT	AGCCCTAATCCTGACCCTTTTCTTGG / CCGCCTCCACCGTTAGCCTCT
		TGCAAAATCTAACCCCTTAAGGAAGTG / CTCCCAGTACCCACAGTCCATCTC
ERα	AGCGCTAATCCTGACCCTTTTCTTGG / CCGCCTCCACCGTTAGCCTCT	CGCCGTGACCGATGCTTTGG / GCCCTTGTTACTCGCGATGCCTGAC
ERβ		ATTACAGCAAACCCCTATTCCATG / TTGCAGACAGTGTATCAGTGAAGGAAT
AR	AGCAGCAGGGTGAGGATGGTTC / GGCTCTGGGACGCAACCTCTC	TGGGCAGGAATTTGACGAAG / CGTCCCTTGGCTTATGCTCTCT
PPARγ2		TCGCCATTCAGAAGATCAGAGTAAA / ATTCAGAGACTTGTCATGGCATTTC
FABP4		ATTGAAGGTCGCTATGCCACAG / AACGACTCCTTGGGTATTTGCTTAA
LPL		GATGTCGCCCATGCTGCCCACCAACTCTG / GCAGGGCATTGAGGTTGGGAG
ATP synthase O subunit		
G6PDH	GATGTCCCCCTGTCCCACCAACTCTG / GCAGGGCATTGAGGTTGGGAG	

AKR1C2 and -3, aldo-keto reductase 1C2 and -3; HSD, hydroxysteroid dehydrogenase; RoDH, 3(α→β)-hydroxysteroid dehydrogenase; RDH5, 11-*cis*-retinol dehydrogenase; ERα and -β, estrogen receptor-α and -β; AR, androgen receptor; PPARγ2, peroxisome proliferator-activated receptor-γ2; FABP4, fatty acid-binding protein 4; LPL, lipoprotein lipase; UGT2B15, UDP-glucuronosyl transferase 2B15; G6PDH, glucose-6-phosphate dehydrogenase.

from three separate wells of 96-well plates were washed with PBS. Cold homogenization solution (100 μl/well; 20 mM Tris, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 7.3) was added to harvest cells, and samples were kept at −80°C until analysis. After thawing, samples were assayed for G3PDH activity in 96-well plates. To 100 μl of the sample, 90 μl of reaction mix (100 mM triethanolamine, 2.5 mM EDTA, 0.1 mM β-mercaptoethanol, 353 μM NADH, pH 7.7) was added and incubated for 10 min at 37°C. The assay was initiated by the addition of dihydroxyacetone phosphate (10 μl/well of an 8-mM stock solution), and a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA) was used to measure optical density at 340 nm at repeated intervals for 5 min. Purified G3PDH enzyme was used to generate a standard curve and calculate G3PDH activity in milliunits of purified enzyme. Proteins were quantified in duplicate by the BCA method in two separate wells and used to normalize for the amount of biological material. G3PDH activity was expressed as milliunits per microgram protein.

Lipid Accumulation

Oil red O staining followed by spectrophotometric analysis was performed to measure lipid accumulation, as described previously (30). Cells from three different wells of a 96-well plate were washed with PBS and fixed with formalin for 1 h. An oil red O solution in isopropanol was added to the wells and incubated for 2 h. After washing three times with dH$_2$O, oil red O retained by lipid droplets was eluted with isopropanol containing 4% Igepal CA-630. Optical density was measured at 490 nm.
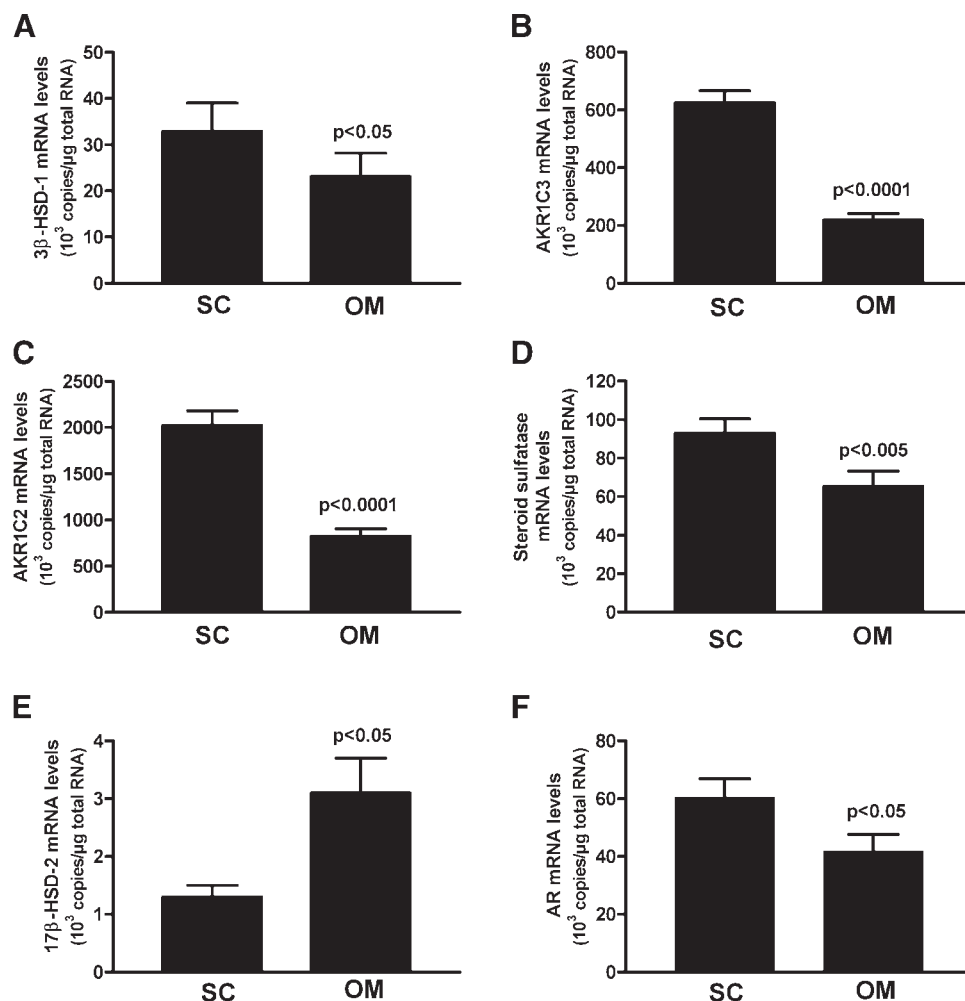
Real-Time PCR Measurements of Enzyme mRNA Abundance

Study 1. Total RNA was isolated from whole tissue samples using Trizol (Invitrogen Life Technologies). 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 Life Technologies), 300 ng of oligo dT$_{18}$, 500 μM dNTP, 10 mM DTT, and 34 units of porcine RNase inhibitor (Amersham Biosciences) in a final volume of 50 μl.

Study 2. Total RNA was isolated from primary differentiated and nondifferentiated cultures using Rneasy Lipid Tissue Mini Kit (Qiagen), following the manufacturer's recommendations. RNA quality was assessed with a bioanalyzer (Agilent Technologies), and RNA from two separate culture wells was pooled for real-time RT-PCR quantifications performed in duplicate. First-strand cDNA synthesis was accomplished using 0.5–5 μg of the isolated RNA in a reaction containing 200 units of Superscript III Rnase H-reverse transcriptase (Invitrogen Life Technologies), 300 ng of oligo dT$_{18}$, 500 μM dNTP, 5 mM DTT, and 40 units of Protector RNase inhibitor (Roche Diagnostics) in a final volume of 50 μl.

For both studies, resulting cDNA was 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 steroid-converting enzymes and nuclear receptors. The sets of primers used are listed in Table 1. The FastStart DNA Master Plus SYBR Green I kit (Roche Diagnostics) was used in a final

Fig. 1. Expression levels of enzymes involved in local androgen synthesis (*A* and *B*) and inactivation (*C* and *E*) of the steroid sulfatase (*D*) and androgen receptor (AR; *F*) in subcutaneous (SC) and omental (OM) adipose tissue of the 14 women in *study 1*. Means ± SE are shown. HSD, hydroxysteroid dehydrogenase; AKR1C2 and -3, aldo-keto reductase 1C2 and -3.

reaction volume of 20 µl containing 3 mM $MgCl_2$, 20 ng of each primer, and 20–200 ng of the cDNA template. The PCR was carried out according to the following conditions: 50 cycles of 95°C/10 s, 59–66°C/5 s, and 72°C/11 s and reading at 75°C/3 s, and temperature transition was 3°C/s for all reactions. PCR results were normalized according to subunit O of ATP synthase and glucose-6-phosphate dehydrogenase expression levels. 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, 10^2, 10^3, 10^4, 10^5, and 10^6 copies. The crossing points (Cp) to calculate the amount of copies in initial cDNA specimens were determined using the double derivative method (23). For each sample, the Cp value was divided by that of the housekeeping gene. To further minimize interassay 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 were expressed in normalized number of copies per microgram total RNA.

3α/β-Ketosteroid Reductase Activity

The conversion of DHT into the inactive metabolites 5α-androstan-3α,17β-diol (3α-diol) and 5α-androstan-3β,17β-diol (3β-diol) was measured in primary cultures before and after differentiation and in dexamethasone-treated preadipocytes with or without RU-486. Similar experiments were also performed using testosterone and androstenedione (D4-dione) as radioactive substrates. Cells were cultured in 96-well culture plates. Culture medium was changed for fresh medium containing the radiolabeled steroid (0.32 µM [14C]DHT, American Radiolabeled Chemicals; 0.47 µM [14C]testosterone and 0.42 µM [14C] D4-dione, PerkinElmer Life Sciences), and cells were incubated for 24 h. Steroids from culture medium were extracted twice with 1 volume ether, as described previously (16). The organic phases were pooled and evaporated to dryness. The steroids were solubilized in 50 µl of dichloromethane (reference standards were diluted in ethanol) and applied to Silica Gel 60 TLC plates (Merck, Darmstadt, Germany)
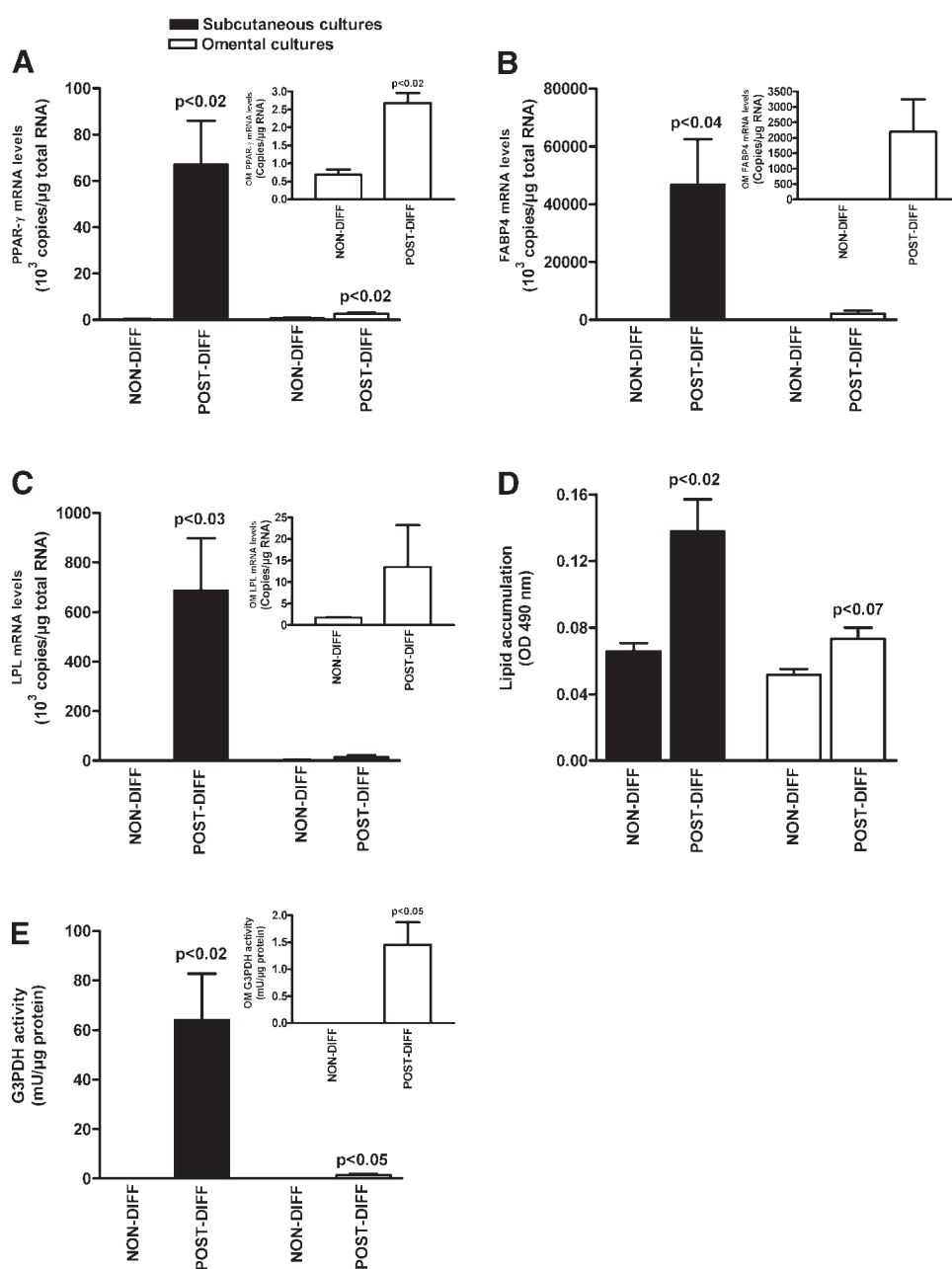

Fig. 2. Markers of adipocyte differentiation measured in SC ($n = 6$) and OM ($n = 4$) cultures before (non-diff) and after adipocyte differentiation (post-diff). Expression levels of peroxisome proliferator-activated receptor-γ (PPARγ; *A*), fatty acid-binding protein 4 (FABP4; *B*), lipoprotein lipase (LPL; *C*), lipid accumulation as measured by oil red O staining (*D*), and glycerol-3-phosphate dehydrogenase (G3PDH) activity (*E*). Black bars, SC cultures; open bars, OM cultures. Means ± SE are shown.

using 10-μl calibrated micropipets. The separation was done by migration in ether-ethyl acetate (1:1) to separate DHT, 3α-diol, and 3β-diol. The migration was performed in toluene-acetone (4:1) for other steroids. The radioactivity was detected using a Storm 860 PhosphorImager (Amersham Biosciences), and quantification was performed using the ImageQuant software version 5.1 (Amersham Biosciences). Total proteins were used in the calculation of activity values.

Statistical Analyses

Paired *t*-tests were used to compare expression levels between SC and OM adipose tissue and before vs. after adipocyte differentiation. The unpaired *t*-test was used to compare SC vs. OM in nondifferentiated preadipocyte cultures. Log$_{10}$ transformation was used to normalize nonnormal variables. Statistical analyses were performed with the JMP software (SAS Institute, Cary, NC).

RESULTS

Study 1: Expression Levels in Whole SC vs. OM Adipose Tissue

Comparative expression levels of steroid-converting enzymes and the androgen receptor in whole adipose tissue samples are shown in Fig. 1. AKR1C2 and AKR1C3 were expressed at remarkably high levels and showed important depot differences. There was no significant depot difference in the expression of 17β-HSD-3 (not shown). Androgen-synthesizing enzyme expression including 3β-HSD-1 and AKR1C3 was significantly higher in SC adipose tissue (Fig. 1, *A* and *B*). Messenger RNA abundance of AKR1C2 and of the steroid sulfatase was also significantly higher in SC compared with OM fat (Fig. 1, *C* and *D*). 17β-HSD-2 mRNA levels were significantly higher in OM adipose tissue. The expression levels of *P*-450 aromatase, Sult 2B1b, and 5α-reductase-1 and

-2, as well as UGT2B15, also tended to be higher in the SC compartment (not shown).

Study 2: Expression Levels Before and After Adipocyte Differentiation

To test the hypothesis that the observed SC vs. OM differences in steroid-converting enzyme expression may be the result of differential cellular composition in each depot, we examined depot differences in the expression of steroid-converting enzymes in nondifferentiated preadipocytes and the effects of adipocyte differentiation on the expression of these enzymes. In nondifferentiated preadipocytes, significant differences were observed only for steroid sulfatase and estrogen receptor-β (ERβ), which were expressed at significantly higher levels in OM vs. SC preadipocytes (180.2 ± 38.4 vs. 115.2 ± 47.3 10^3 copies/μg RNA, $P < 0.05$ for steroid sulfatase, and 0.6 ± 0.4 vs. 0.1 ± 0.1 10^3 copies/μg RNA, $P < 0.02$ for ERβ). The expression of AKR1C2 and AKR1C3 also tended to be higher in OM preadipocytes (not shown).

Figure 2 shows measurements of adipogenesis, including early markers (PPARγ expression) and late markers [fatty acid-binding protein 4 (FABP4) and lipoprotein lipase (LPL) expression, lipid accumulation, and G3PDH activity] of adipocyte differentiation. As observed in other studies (36), SC preadipocytes had higher differentiation rates compared with OM cells. As expected, the expression of PPARγ, FABP4, and LPL was significantly induced in SC differentiated cultures. Lipid accumulation and G3PDH activity were also significantly increased following adipogenesis in the SC depot. PPARγ expression and G3PDH activity increased significantly after differentiation in OM cultures, but less so than in SC cultures. OM FABP4 and LPL expression as well as lipid
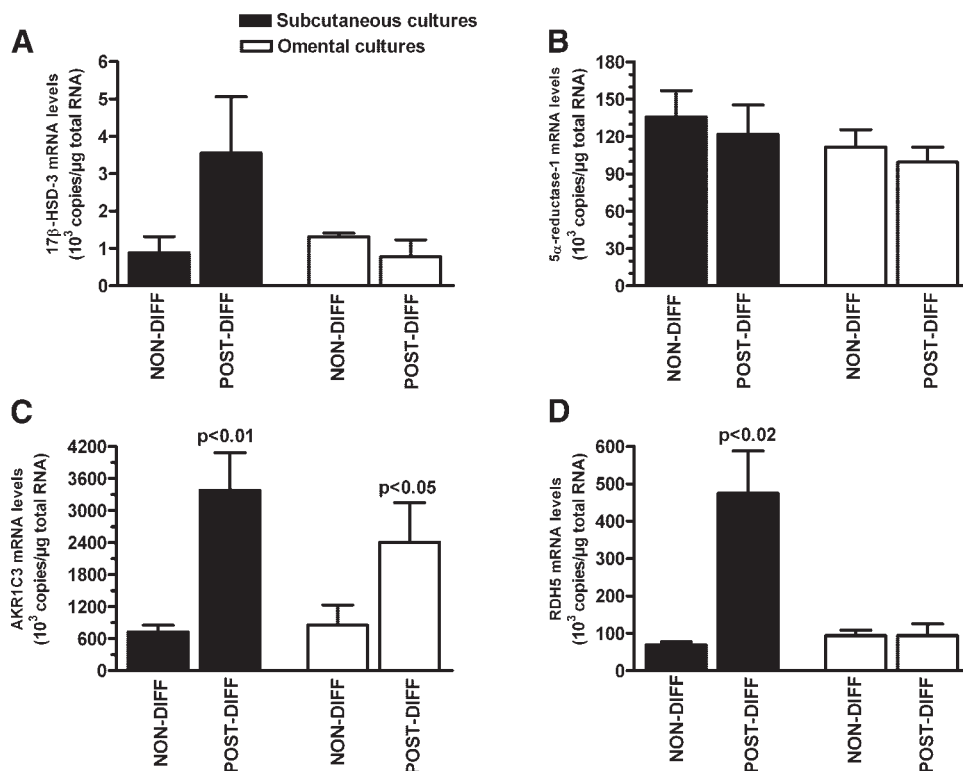

Fig. 3. Expression of steroidogenic enzymes involved in the local synthesis of active androgens in SC (*n* = 6) and OM (*n* = 4) cultures before and after adipocyte differentiation. Black bars, SC cultures; open bars, OM cultures. Means ± SE are shown.

A

B

Fig. 4. Expression of steroid-converting enzymes involved in androgen inactivation in SC ($n = 6$) and OM ($n = 4$) cultures before and after adipocyte differentiation. Black bars, SC cultures; open bars, OM cultures. Means $\pm$ SE are shown.
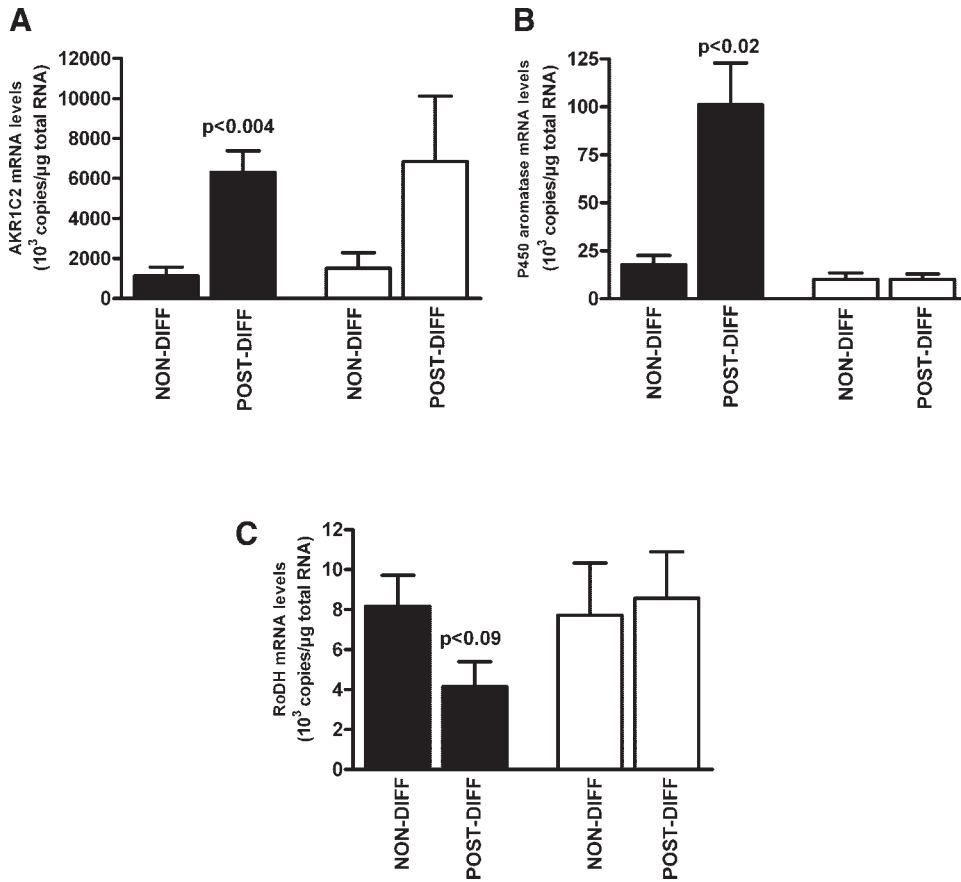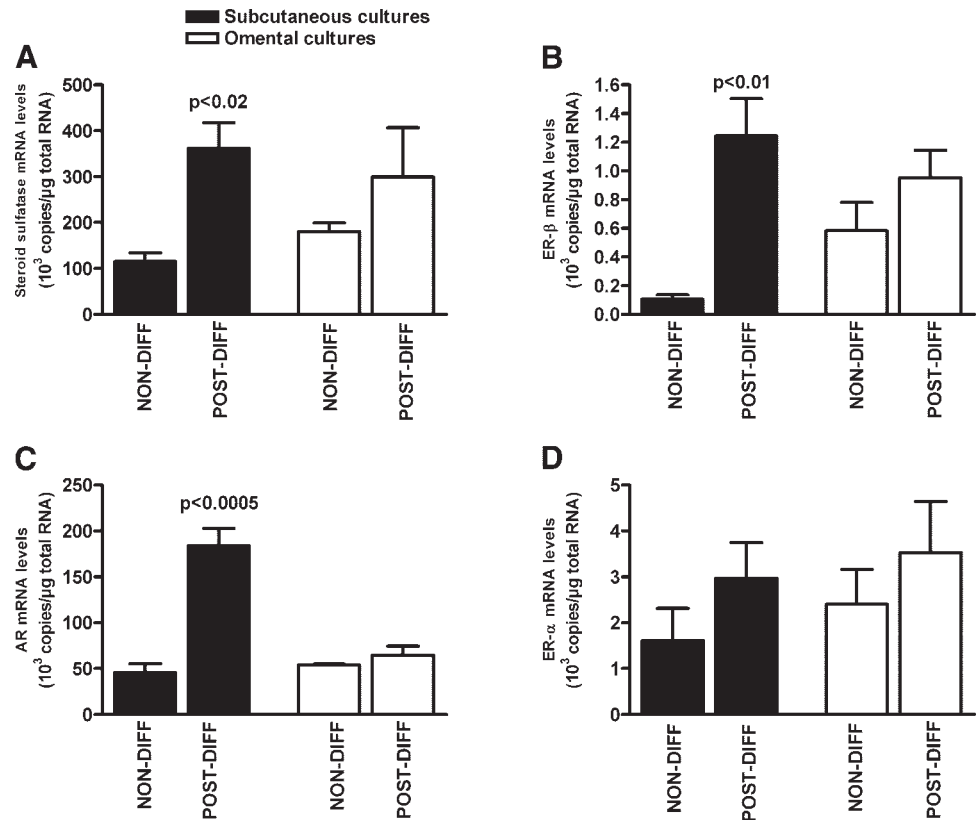
C

Fig. 5. Expression of steroid sulfatase and nuclear receptors involved in estrogen and androgen action in SC ($n = 6$) and OM ($n = 4$) cultures before and after adipocyte differentiation. Black bars, SC cultures; open bars, OM cultures. Means $\pm$ SE are shown. ERα and -β, estrogen receptor-α and -β, respectively.

A

B

C

D

accumulation tended to be increased after adipocyte differentiation, but the increase did not reach significance.

Figure 3 shows expression levels of enzymes involved in androgen synthesis in nondifferentiated preadipocytes and in differentiated cultures. The expression of 17β-HSD-3 tended to increase after differentiation in SC cells (Fig. 3A), whereas the mRNA abundance of 5α-reductase-1 tended to decrease with adipogenesis in both the SC and OM cultures (Fig. 3B). AKR1C3 mRNA expression strongly and significantly increased after adipocyte differentiation in the two fat depots (Fig. 3C). The expression of RDH5 (which synthesizes DHT from 3α-diol) also significantly increased after adipogenesis, but only in the SC cultures (Fig. 3D).

Figure 4 shows expression levels of enzymes involved in androgen inactivation in nondifferentiated preadipocytes and in differentiated cultures. The expression of AKR1C2 increased significantly following adipogenesis in the SC depot (Fig. 4A). A similar trend was observed in OM cells, but it did not reach statistical significance (Fig. 4A). P-450 aromatase mRNA levels also increased significantly after adipocyte differentiation in SC cultures only (Fig. 4B). Expression levels of $3(\alpha \rightarrow \beta)$-hydroxysteroid epimerase (RoDH) (which synthesizes 3β-diol from 3α-diol) expression levels tended to decrease following adipogenesis in the SC depot (Fig. 4C).

Expression levels of steroid sulfatase and nuclear receptors involved in estrogen and androgen action measured before and after adipocyte differentiation are shown in Fig. 5. The expression of steroid sulfatase, ERβ, and the androgen receptor was significantly increased after adipocyte differentiation in SC cultures (Fig. 5, A–C). A nonsignificant trend for increased mRNA levels after adipogenesis in OM cells was observed for steroid sulfatase, ERβ, and ERα (Fig. 5, A, B, and D, respectively).

DHT inactivation into 3α-diol and 3β-diol was measured before and after adipogenesis. Figure 6 shows that, consistent with the enzyme expression pattern, 3α-diol formation was strongly and significantly increased following adipocyte differentiation in both the SC and OM cultures (Fig. 6A). A slight, nonsignificant trend for decreased 3β-diol formation was observed in OM cells (Fig. 6B). There was no significant depot difference in the formation of 3α- and 3β-diol in nondifferentiated or differentiated cultures, but in nondifferentiated preadipocytes 3α-diol formation tended to be higher in the OM depot ($P < 0.08$). When cell cultures were incubated with radiolabeled testosterone, no significant DHT formation was observed (data not shown). The conversion of D4-dione into testosterone and DHT is shown in Fig. 7. Adipogenesis led to increased testosterone formation (statistically significant for SC cultures; Fig. 7C) and significantly decreased DHT synthesis (Fig. 7D). Cells also produced a small amounts of androstanedione from D4-dione (data not shown). Additional experiments indicated that dexamethasone alone was the adipogenic factor responsible for increasing DHT inactivation rates. The effects of dexamethasone on the conversion of DHT into the inactive metabolites 3α/β-diol in SC and OM preadipocytes are shown in Fig. 8. Dexamethasone at 100 nM and 1 μM significantly increased DHT inactivation in preadipocytes (Fig. 8A). The half-maximal effective concentration (EC$_{50}$) was 5.4 nM (Fig. 8B). The stimulatory effects of dexamethasone were completely blocked by the glucocorticoid receptor antagonist RU-486 (Fig. 8B).
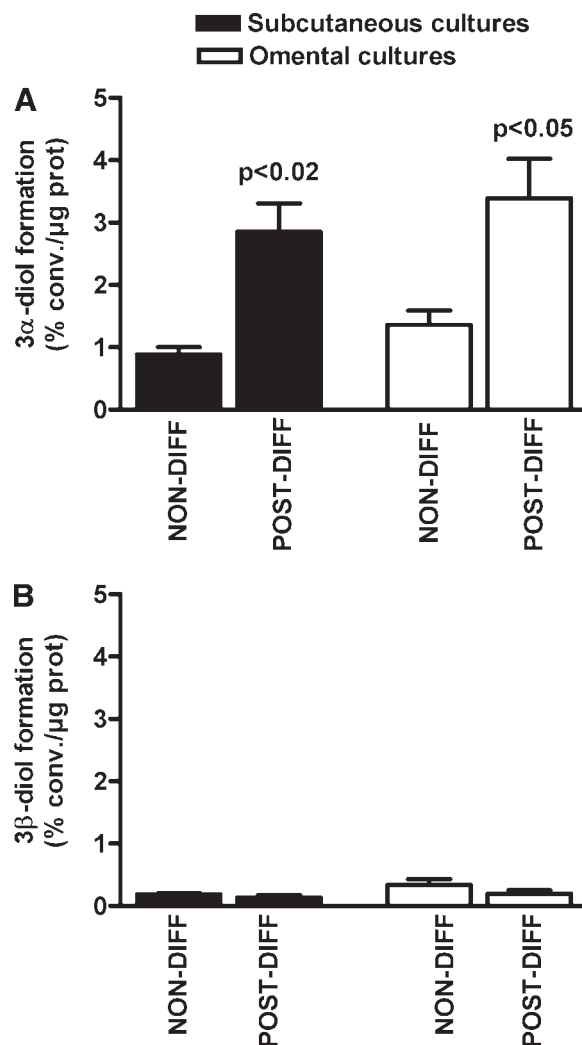

Fig. 6. Conversion of 5α-dihydrotestosterone (DHT) into the inactive metabolites 5α-androstan-3α,17β-diol (3α-diol) and 5α-androstan-3β,17β-diol (3β-diol) before and after adipocyte differentiation in SC ($n = 6$) and OM cultures ($n = 4$). A: 3α-diol formation; B: 3β-diol formation. Experiment performed in triplicate. Black bars, SC cultures; open bars, OM cultures. Means ± SE are shown.

Figure 9 shows pathways of androgen synthesis and inactivation in SC and OM adipose tissue. Large black arrows highlight enzymes for which expression levels were higher in an adipose tissue depot compared with the other. With the exception of 17β-HSD-2 that was more highly expressed in the OM depot, Fig. 9 emphasizes that expression levels of most enzymes involved in androgen synthesis and inactivation as well as the androgen receptor were higher in SC compared with OM adipose tissue.

DISCUSSION

The objectives of the present study were to investigate the expression of several steroidogenic and steroid-inactivating enzymes in abdominal SC and OM adipose tissue and preadipocytes obtained from women and to examine changes in the expression of these enzymes through adipocyte differentiation. In whole adipose tissue samples, the expression of aldo-keto reductases 1C (AKR1C2 and AKR1C3) was particularly high
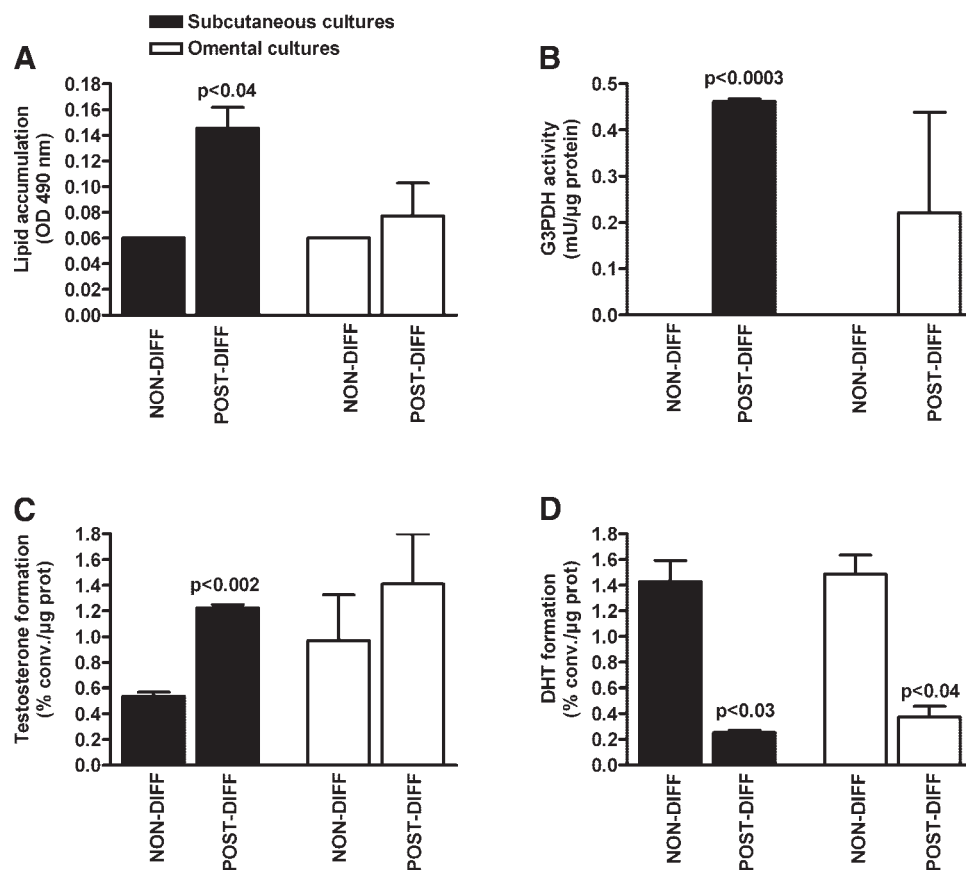
Fig. 7. Conversion of androstenedione into testosterone and DHT before and after adipocyte differentiation in SC ($n = 3$) and OM cultures ($n = 2$). *A*: lipid accumulation; *B*: G3PDH activity; *C*: testosterone formation; *D*: DHT production. Experiment performed in duplicate. Black bars, SC cultures; open bars, OM cultures. Means ± SE are shown.

compared with other steroid-metabolizing enzymes, with marked depot differences. We found higher expression levels in SC compared with OM fat for AKR1C2, AKR1C3, and 3β-HSD-1, for the steroid sulfatase, and for the androgen receptor. Expression levels of aromatase, 5α-reductase-1 and -2, 17β-HSD-3, Sult 2B1b, and UGT2B15 were not significantly different between SC and OM fat. The expression of 17β-HSD-2 was lower in SC adipose tissue. In agreement with our hypothesis, the majority of steroid-converting enzymes and steroid receptors were significantly increased (AKR1C3, RDH5, AKR1C2, *P*-450 aromatase, steroid sulfatase, ERβ, and the androgen receptor) or tended to increase (17β-HSD-3, ERα) following adipocyte differentiation. Our results suggest that specific expression patterns of steroid-related transcripts found in SC and OM adipose tissue actually reflect the relative proportion of mature adipocytes vs. preadipocytes in each adipose tissue depot.

We (4–7) and others (41) have previously emphasized the potential importance of AKR1C enzymes in the pathophysiology of visceral obesity. We have demonstrated that the expression (AKR1C1, -2, and -3) and activity (AKR1C1 and -2) of these enzymes were positively associated with visceral fat accumulation and OM adipocyte size in women (4–7). Wake et al. (41) also found a positive association between waist-to-hip ratio and the expression of AKR1C2 and AKR1C3 in men and women. Moreover, DHT inactivation was significantly higher in the omentum of women with visceral obesity (5, 6). Corbould et al. (10) demonstrated previously that the ratio of 17β-HSD-3 to *P*-450 aromatase mRNA was positively associated with BMI and waist circumference in OM adipose tissue,

whereas the reverse was found for SC fat, suggesting that OM fat may be more androgenic. Quinkler et al. (29) also observed that the expression of AKR1C3 decreased significantly after weight loss in SC fat from buttocks in women. Thus, data from several studies accumulate and increasingly suggest that sex hormone-converting enzymes, particularly AKR1C enzymes, may be involved in the modulation of body fat distribution.

In the present study, we demonstrated that the expression of AKR1C2 and AKR1C3 increased significantly (between 2.8- and 6.1-fold stimulation) after adipocyte differentiation in SC cultures. In microarray experiments, Tchkonia et al. (37) observed similar increases in the expression of AKR1C1, -2, and -3 after adipogenesis in clones derived from SC, mesenteric, and OM adipose tissue. In the present study, despite a lower differentiation rate in OM cells (Fig. 2), the expression of AKR1C2 showed a strong trend to increase with adipocyte differentiation, and AKR1C3 mRNA levels were even significantly increased. This trend was also observed for the steroid sulfatase and estrogen receptors. This suggests that some factors present in the differentiation medium may stimulate the expression of these enzymes independently of adipogenesis. We hereby demonstrate that dexamethasone is probably the effector responsible for the increase in the expression of AKR1C enzymes. We showed that DHT inactivation in preadipocytes was significantly increased by dexamethasone alone, in a dose-dependent manner, and that this effect was likely mediated by the glucocorticoid receptor. These results suggest a cross-talk between local glucocorticoid generation and local androgen metabolism in adipose tissue. The steroid sulfatase was studied recently by Valle et al. (38). In that study,
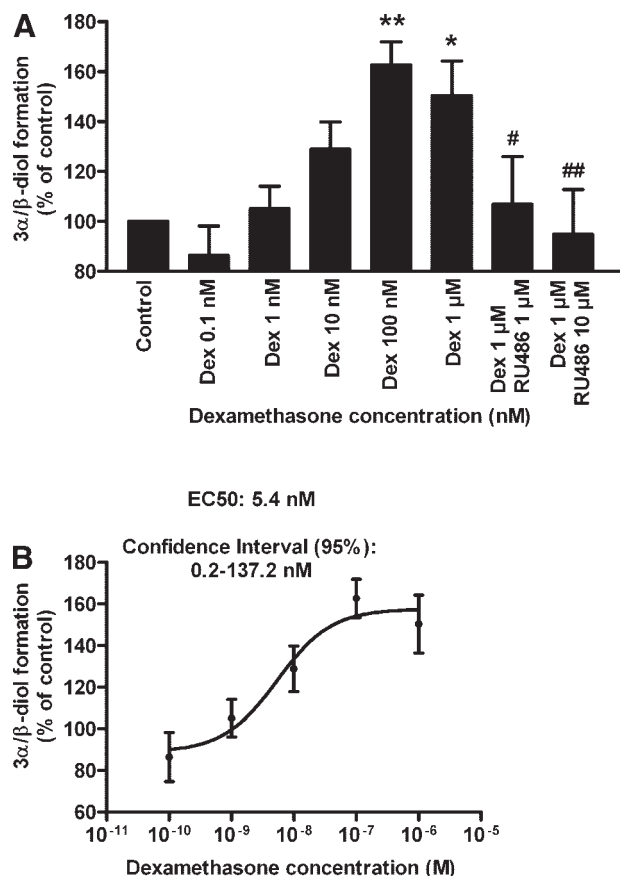
Fig. 8. *A*: the effects of dexamethasone (Dex) on the conversion of DHT into the inactive metabolites $3\alpha/\beta$-diol in SC ($n = 3$) and OM ($n = 3$ for 1 μM and $n = 4$ for other concentrations) preadipocytes were blocked by RU-486. *B*: half-maximal effective concentration (EC$_{50}$) of Dex. Means $\pm$ SE are shown. Different from the control by a posteriori mean contrast; *$P < 0.05$, **$P < 0.005$. Different from 1 μM Dex by a posteriori mean contrast; #$P < 0.05$, ##$P < 0.005$.

mRNA of this enzyme was present in SC adipose tissue from men and women, and no significant difference was observed in the conversion rate of dehydroepiandrosterone (DHEA)-S to DHEA between preadipocytes and mature adipocytes (38).

We observed that the expression of enzymes involved in opposite reactions was also increased following adipogenesis. This raises the question of what the net effect is on local androgen metabolism. For example, RDH5 catalyzes the back reaction of AKR1C2, i.e., the production of DHT using 3α-diol as substrate. The expression of both AKR1C2 and RDH5 was significantly increased after adipocyte differentiation. AKR1C2 mRNA levels increased 10-fold, whereas RHD5 expression levels increased fivefold. In agreement, the observed net effect on local androgen metabolism was that DHT inactivation significantly increased after adipogenesis. It should be kept in mind, however, that differences in the conversion of DHT into $3\alpha/\beta$-diol before and after differentiation may have been underestimated because radiolabeled DHT concentrations used were below the K_m. Quinkler et al. (29) previously performed extensive studies on AKR1C3. In that study, the quantitative expression of AKR1C3 was increased after adipocyte differentiation in both SC and OM cultures (29). The conversion of D4-dione to testosterone (17β-HSD activity) significantly in-

creased after adipogenesis in both depots, but in OM cells, the back reaction increased to a greater extent (29). In the present study, we confirmed that testosterone synthesis was significantly increased after adipocyte differentiation, at least in SC cultures. However, we did not detect the back reaction when cells were incubated with testosterone. In addition, we observed significant DHT synthesis after incubation in the presence of D4-dione. It is possible that depot differences in conversion rates for several other reactions may exist. Another concern is that AKR1C2 and AKR1C3 possess different ketosteroid reductase activities, namely 3α-HSD (DHT inactivation into 3α-diol), 20α-HSD (progesterone inactivation), 17β-HSD (testosterone synthesis from D4-dione), and possibly 3β-HSD (DHT inactivation into 3β-diol), but in different proportions (35, 43). This emphasizes the importance to study enzyme activities in addition to mRNA expression. Caution must be observed in the present study because enzyme activity was studied only for AKR1C2, AKR1C3, and 5α-reductase. Increased mRNA expression does not necessarily mean increased activity, and this prevents us from concluding for other enzymes on the basis of mRNA only.

Androgens inhibit adipocyte differentiation in murine cell lines (32, 33) and in human primary cultures (16a). At least one of the postulated limiting steps in the androgen pathway was increased at the mRNA level after adipocyte differentiation, namely *P*-450 aromatase. This is consistent with increased androgen processing after adipogenesis. No 5α-reduction of testosterone into DHT was observed. This finding was rather surprising because mRNA expression of 5α-reductase-1 was detected in both adipose tissue and primary cultures. Adipogenesis led to increased conversion of D4-dione into testosterone and to decreased DHT formation. We speculate that the significant increase in testosterone formation after adipocyte differentiation is likely due to higher AKR1C3 and 17β-HSD-3 mRNA expression. This increase would in turn reduce DHT formation by decreasing substrate availability for the following reaction sequence: D4-dione→androstanedione→DHT. These findings also suggest that marked changes in steroid-converting enzymes through adipogenesis may modulate the DHT/testosterone ratio in various cell types. The importance of the adipogenesis-induced increase in androgen receptor expression needs to be further examined in this light. The androgen receptor has been examined previously in human adipose tissue (14, 17, 28). Studies have demonstrated that androgen binding was significantly greater in visceral (OM and mesenteric) compared with SC preadipocytes (14, 17). In the present study, no significant depot difference was observed regarding the expression of the androgen receptor in nondifferentiated preadipocytes. In whole adipose tissue samples, the expression of the androgen receptor was significantly higher in SC vs. OM fat. Moreover, androgen receptor expression levels significantly increased after adipocyte differentiation in SC cultures. Dieudonné et al. (14) also observed that androgen receptor mRNA levels increased significantly after adipogenesis, whereas the protein was rather significantly decreased. Thus, it is not possible to conclude on the basis of androgen receptor mRNA data in the present study.

In whole adipose tissue samples, expression data suggest that SC adipose tissue is a more active steroidogenic tissue than OM fat, with indirect evidence for higher rates of both androgen synthesis from adrenal precursors and androgen inactiva-
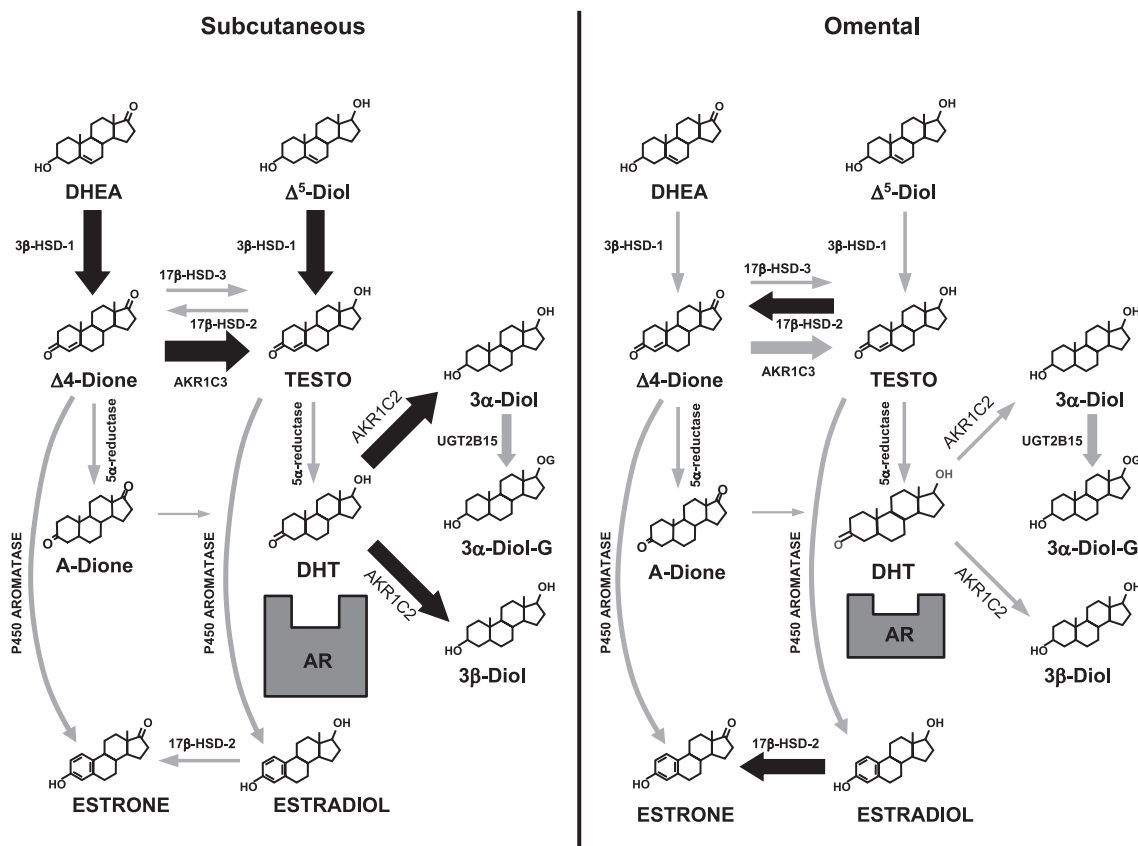
Fig. 9. Pathways of androgen synthesis and inactivation in SC and OM adipose tissue. Large black arrows highlight enzymes for which mRNA levels were higher in an adipose tissue depot compared with the other. DHEA, dehydroepiandrosterone; Δ5-Diol, androst-5-ene-3β,17β-diol; Δ4-Dione, androstenedione; A-Dione, androstanedione; TESTO, testosterone; G, glucuronide; UGT, UDP-glucuronosyltransferase.

tion (Fig. 9). Accordingly, we demonstrated previously that DHT and testosterone levels were higher in OM vs. SC adipose tissue (2). Van Schothorst et al. (39) also observed that weight gain through a high-fat diet in mice was associated with a downregulation in visceral fat of the expression of several enzymes (including 3β-HSD-1) involved in androgen synthesis. In our study, some regional differences found in whole adipose tissue were not maintained in differentiated cultures. This is not surprising, because primary cultures of differentiated adipocytes never reach differentiation stages comparable with whole tissue-derived mature cells and, in this sense, do not fully reflect the in vivo situation. Rather, they provide information on the impact of adipogenesis.

Estradiol increases preadipocyte proliferation in SC and OM fat in both men and women (1). In SC mature adipocytes, Palin et al. (26) have shown that 10^{-7} M estradiol significantly decreased LPL and increased HSL protein, whereas lower concentrations had the opposite effects. In the present study, we observed that P-450 aromatase expression levels increased significantly with adipogenesis in SC cultures. However, previous reports have shown that P-450 aromatase expression and activity were significantly lower in mature adipocytes compared with preadipocytes (15, 25). Methodological considerations such as RNA isolation in the presence of large amount of lipids from mature adipocytes may have been responsible for such discrepancies. In agreement with Dieudonné et al. (13), the expression of ERα in adipose cells was higher than

that of ERβ, possibly explaining why it was impossible in other studies to detect ERβ in preadipocytes (13, 18, 28). With the exception of one study (27), several groups observed results similar to the present study, i.e., higher expression levels in differentiated cultures vs. preadipocytes or higher expression levels in mature adipocytes vs. preadipocytes (12, 13, 28).

In conclusion, the expression of several steroid-converting enzymes and sex hormone nuclear receptors was significantly higher in SC vs. OM adipose tissue. This was generally reflected by mRNA levels of these enzymes increasing with adipocyte differentiation. This change in the expression profile with adipogenesis suggests that lipid-storing adipocytes have higher androgen-processing rates. Dexamethasone stimulated DHT inactivation in preadipocytes, suggesting the existence of a potential cross-talk between local glucocorticoid and androgen metabolism in adipose cells.

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