Glucocorticoid-induced androgen inactivation by aldo-keto reductase 1C2 promotes adipogenesis in human preadipocytes

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1Endocrinology and Genomics, Laval University Medical Research Center; 2Department of Food Science and Nutrition; 3Department of Surgery, Laval University; and 4Gynecology Unit, Laval University Medical Research Center, Quebec City, Quebec, Canada

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Veilleux A, Côté JA, Blouin K, Nadeau M, Pelletier M, Marceau P, Laberge PY, Van Luu-The, Tchernof A. Glucocorticoid-induced androgen inactivation by aldo-keto reductase 1C2 promotes adipogenesis in human preadipocytes. Am J Physiol Endocrinol Metab 302: E941–E949, 2012. First published January 24, 2012; doi:10.1152/ajpendo.00069.2011.—Adipogenesis and lipid storage in human adipose tissue are inhibited by androgens such as DHT. Inactivation of DHT to 3α-diol is stimulated by glucocorticoids in human preadipocytes. We sought to characterize glucocorticoid-induced androgen inactivation in human preadipocytes and to establish its role in the antiadipogenic action of DHT. Subcutaneous and omental primary preadipocyte cultures were stimulated by glucocorticoids in human preadipocytes. We sought to characterize glucocorticoid-induced androgen inactivation in human preadipocytes and to establish its role in the antiadipogenic action of DHT. Subcutaneous and omental primary preadipocyte cultures were established from fat samples obtained in subjects undergoing abdominal surgeries. Inactivation of DHT to 3α/β-diol for 24 h was measured in dexamethasone- or vehicle-treated cells. Specific downregulation of aldo-keto reductase 1C (AKR1C) enzymes in human preadipocytes was achieved using RNA interference. In whole adipose tissue sample, cortisol production was positively correlated with androgen inactivation in both subcutaneous and omental adipose tissue ($P < 0.05$). Maximal dexamethasone (1 μM) stimulation of DHT inactivation was higher in omental compared with subcutaneous fat from men as well as subcutaneous and omental fat from women ($P < 0.05$). A significant positive correlation was observed between BMI and maximal dexamethasone-induced DHT inactivation rates in subcutaneous and omental adipose tissue of men and women ($r = 0.24, n = 26, P < 0.01$). siRNA-induced downregulation of AKR1C2, but not AKR1C1 or AKR1C3, significantly reduced basal and glucocorticoid-induced androgen inactivation rates ($P < 0.05$). The inhibitory action of DHT on preadipocyte differentiation was potentiated following AKR1C2 but not AKR1C1 or AKR1C3 downregulation. Specifically, lipid accumulation, G3PDH activity, and FABP4 mRNA expression in differentiated preadipocytes exposed to DHT were reduced further upon AKR1C2 siRNA transfection. We conclude that glucocorticoid-induced androgen inactivation is mediated by AKR1C2 and is particularly effective in omental preadipocytes of obese men. The interplay between glucocorticoids and AKR1C2-dependent androgen inactivation may locally modulate adipogenesis and lipid accumulation in a depot-specific manner.

3α-hydroxysteroid dehydrogenase; 5α-dihydrotestosterone; dexamethasone

The propensity to accumulate lipids in the visceral rather than in the subcutaneous adipose tissue compartment is associated with metabolic disorders. Little is known about the factors leading to preferential visceral fat accumulation when facing excess energy intake, but steroid hormones such as glucocorticoids and androgens appear to play a key role.

Active glucocorticoids such as cortisol are strong modulators of adipose tissue metabolism because they can alter body fat distribution (16, 24, 25) by impeding cellular proliferation and promoting differentiation of preadipocytes to mature adipocytes (10, 12). Stimulatory effects of glucocorticoids on preadipocyte differentiation are believed to involve the induction of lipoprotein lipase and glycerol-3-phosphate dehydrogenase (G3PDH) expression (10, 12, 17). Local reduction of cortisol to cortisol by type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1) may also regulate adipose tissue exposure to active glucocorticoids and promote lipid accumulation (9).

Androgens have been shown to reduce fat accumulation through inhibition of lipogenesis and stimulation of lipolysis (8). Active androgens such as 5α-dihydrotestosterone (DHT) inhibit adipogenesis in murine preadipocyte cell lines as well as in omental and subcutaneous human preadipocytes (5, 11). Although the exact mechanism is not established in human preadipocytes, in 3T3-L1 cells this effect is believed to involve induction of the β-catenin/Wnt signaling pathway as well as sequestration of peroxisome proliferator-activated receptor-γ (PPARγ) coactivator ARA70 by the activated androgen receptor (13, 21).

Fat tissue exposure to circulating androgens may be reduced by DHT inactivation to 5α-androstane-3α,17β-diol (3α-diol), a reaction generally attributed to enzymes of the aldo-keto reductase 1C (AKR1C) family (8). In this regard, DHT metabolism and AKR1C isofrom expression in adipose tissue were related to adiposity in several studies (1, 3, 4, 6, 7, 26).

We demonstrated previously that induction of preadipocyte differentiation led to significantly increased 3α-hydroxysteroid dehydrogenase (3α-HSD) activity as well as increased expression levels of AKR1C1, AKR1C2, and AKR1C3 (4). We also reported that dexamethasone, a potent glucocorticoid, induced a dose-dependent stimulation of androgen inactivation that was independent of the adipogenic process (4). The stimulation of DHT inactivation by glucocorticoids may add up to the distinct dynamics of androgens and glucocorticoids in the modulation of adipogenesis and fat accumulation.

The enzyme isofrom with the most pronounced in vitro 3α-HSD activity is AKR1C2 (27). Therefore, it has been suggested that this enzyme plays a key role in androgen inactivation. However, this postulate remains to be formally demonstrated in human preadipocytes. Moreover, there are no data on the biological factors that could influence glucocorticoid-induced androgen inactivation in these cells. Thus, the aim of the present study was to characterize the impact of sex,
fat depot, and adiposity on glucocorticoid-induced androgen inactivation rates in human preadipocytes. The contribution of AKR1C2 to this reaction and its impact on adipogenesis was also tested.

MATERIALS AND METHODS

Subject recruitment. The study included lean to obese women as well as men recruited through the elective surgery schedule of the Laval University Medical Center. Women were undergoing abdominal gynecological surgery for total or subtotal abdominal hysterectomies. Reasons for surgery in male patients included umbilical hernia, endocholecystectomy, giant parastomal hernia, and sigmoid resection. Morbidly obese women and men undergoing biliopancreatic diversion were recruited at the Laval University Cardiology and Pulmonology Institute. Body mass index (BMI) was measured on the morning of the surgery. Written informed consent was obtained from all patients. The project was approved by ethics committees of the University Medical Center.

Adipose tissue sampling and preadipocyte isolation. During the surgical procedure, subcutaneous and omental adipose tissue samples were collected at the site of surgical incision (lower abdomen) and at the distal portion of the greater omentum, respectively. Preadipocytes were isolated using a modification of the method of Van Harmelen et al. (23). A portion of each adipose tissue sample was digested for 45 min at 37°C in presence of type I collagenase. Digestion was performed in Krebs-Ringer-Henseleit buffer (25 mmol/l HEPES, 125 mmol/l NaCl, 3.7 mmol/l KCl, 5 mmol/l CaCl2, 2.5 mmol/l MgCl2, and 1 mmol/l K2HPO4, pH 7.4) supplemented with 5 mmol/l glucose, 0.1 μmol/l adenosine, 0.1 mg/ml ascorbic acid, and 4% electronegative grade BSA according to a modified version of the Rodbell method (20). Following digestion the cell suspension was filtered through nylon mesh, and adipocytes were washed three times with Krebs-Ringer-Henseleit buffer. Residual buffer, which contained the stromal-vascular fraction, was centrifuged, and the pellet was washed in DMEM-F-12. Preadipocytes were then filtered through 140 μm of nylon mesh and placed in culture plates at 37°C under 5% CO2 atmosphere. Cells were maintained in DMEM-F-12 supplemented with 5% fetal bovine serum, 1 ng/ml fibroblast growth factor, 10 μg/ml endothelial growth factor, 6 μM insulin, 2.5 μg/ml amphotericin B, and 50 μg/ml gentamicin. Medium was changed every 2–3 days. Subcutaneous preadipocyte cultures (n = 3) were also obtained from ZenBio. These cultures were plated and differentiated according to the manufacturer’s protocol (ZenBio).

Conversion rates of cortisone and DHT in whole adipose tissue samples. Frozen adipose tissue samples (50 mg) were homogenized in 500 μl of 100 mM sodium phosphate buffer, pH 7.4, containing 20% glycerol and 1 mM EDTA. Homogenates were centrifuged to remove lipids and the insoluble fraction. Cortisone conversion activity was assessed in 175 μl of homogenate with 50 μl of NADPH-regenerating system buffer [3 mM NADP+, 25 mM MgCl2, 50 mM glucose 6-phosphate (G6P), and 10 U/ml G6PD] and [14C]cortisone. DHT inactivation was assessed in 25 μl of homogenate with [14C]DHT. Conversion of [14C]cortisone to [14C]cortisol and [14C]DHT to [14C]3αβ-diol was followed for 24 h at 37°C with shaking. Steroids were extracted twice with diethyl ether. Glucocorticoids were resolved on thin-layer chromatography (TLC) with chloroform/ethanol (9:1, v/v). Androgens were resolved on thin layer chromatography (TLC) with toluene-acetone (4:1, vol/vol). TLC plates were dried, exposed, and visualized by storage phosphor autoradiography. Protein concentrations of the samples were determined using the BCA protocol (Pierce Biotechnology). Activities were assessed in duplicate and expressed as cortisol or 3αβ-diol produced per milligram of protein per hour (pmol·mg−1·h−1).

Conversion rates of DHT and progesterone in primary preadipocyte cultures. Preadipocyte cultures were incubated with dexamethasone for 48 h. During the last 24 h of incubation, 5,000 counts/min of [14C] DHT or progesterone was added to culture medium. Steroids were extracted using diethylther. Samples were resolved on TLC with toluene-acetone (4:1) and visualized as described previously (4). Using this method, the 3α- and 3β-metabolites of DHT were not separated. Thus, androgen inactivation rates are expressed as 3αβ-diol formation. However, we have shown previously that the 3α-diol metabolite is largely predominant and is the only one being modulated during adipogenesis (4).

RNA interference in human primary preadipocytes. Scramble (control) or AKR1C-specific siRNAs were transfected in preadipocytes using the calcium phosphate transfection method (15). Cells were transfected with control or target siRNA (100 nM) on days 1 and 3 following seeding at a confluence of 80% in 96-well plates. Scramble and AKR1C2-specific Dieter substrate siRNAs were designed, synthesized, and HPLC-purified by Integrated DNA Technologies as follows: scramble (sense: rUrtC-}

<table>
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<th>Genes</th>
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<tr>
<td>AKR1C1</td>
<td>−317</td>
<td>(+)</td>
<td>5′-GTGCAAGATTTGTTCT-3′</td>
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<tr>
<td>AKR1C2</td>
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<tr>
<td>AKR1C3</td>
<td>−326</td>
<td>(+)</td>
<td>5′-GTGCAAGATTTGTTCT-3′</td>
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GRE, glucocorticoid response element; AKR1C, aldo-keto reductase 1C. Consensus GRE: GTGCAAGATTTGTTCT.
GeneTools (Biotools). Results were expressed as relative units compared with Atp5o housekeeping gene expression (24).

Preadipocyte differentiation. Preadipocytes were seeded at a confluence of 80% in 96-well plates and transfected twice with siRNA (days 1 and 3). On day 4, differentiation of fully confluent preadipocyte cultures was induced using standardized differentiation medium and protocols (ZenBio). The medium consisted of DMEM-F-12 supplemented with a PPARγ agonist, insulin, dexamethasone, and 3-isobutyl-1-methylxanthine. DHT or vehicle was added to the differentiation medium prior to preadipocyte differentiation induction. Differentiation efficiency was assessed 21 days following induction.

**G3PDH activity measurements.** G3PDH activity was measured as described previously (5). Briefly, differentiated preadipocytes were homogenized (20 nM Tris, 1 mM EDTA, and 1 mM β-mercaptoethanol, pH 7.3), and G3PDH activity was assessed in reaction buffer (100 mM triethanolamine, 2.5 mM EDTA, 0.1 mM β-mercaptoethanol, 353 μM NADH, and 0.4 mM dihydroxyacetone phosphate, pH 7.7) for 10 min at 37°C. Optical density at 340 nm was measured at repeated intervals for 5 min, and milliunits of G3PDH activity was calculated using a standard curve of purified G3PDH enzyme. Proteins were quantified in duplicate by the BCA method. G3PDH activity was expressed as the percentage of control wells.

**Lipid accumulation.** Oil Red O staining, followed by contrast phase microscopy and spectrophotometric analysis, was performed to measure lipid accumulation, as described previously (5). Briefly, cells were washed with PBS and fixed with formalin for 1 h. Cells were then incubated for 2 h with a 4.9 mM Oil Red O solution and washed three times with ddH2O. Optical density at 490 nm was measured following cell disruption, with isopropanol containing 4% Igepal.

**Cytotoxicity and apoptosis assays.** Preadipocytes were transfected with target siRNA before cytotoxicity and apoptosis indices were measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay, based on the procedure described by Mosmann (25). Apoptosis was measured using the Apo-ONE Homogeneous Dead Cell Assay kit (Promega). Results were expressed as the percentage of dead cells compared with the control.

**RT-qPCR mRNA measurements.** Efficiency of gene downregulation was assessed by quantitative RT-PCR. Total RNA was isolated using the RNeasy extraction kit and on-column DNase (Qiagen). Complementary DNA amplification was performed in duplicate using the LightCycler 480 (Roche Diagnostics) and the SYBR Green I Master (Applied Biosystems) according to the following conditions: 95°C for 10 s, 59–62°C for 10 s, 72°C for 12 s, and then 72°C for 5 s (reading) repeated 50 times. Primer sequences for AKR1C1 (5′-CCG-AGC-AAG-TGG-CAC-CC-CC-GT), AKR1C2 (5′-CGC-TAA-TGG-CGA-ATG-TGG-ATG-GGA-ATT-GCT), and AKR1C3 (5′-CCA-CACA-AGT-TGG-ATG-TGG-CCT-GCT-TGA) were designed using PrimerExpress software (Applied Biosystems). Real-time PCR was performed using the LightCycler 480 II (Roche) with the SYBR Green I Master mix (Roche) and the Primers Express software (Applied Biosystems). The expression of AKR1C1, AKR1C2, and AKR1C3 was normalized to the expression of the housekeeping gene G3PDH (26). Gene expression levels were calculated using the comparative Ct method (27) and expressed as fold changes compared with control cultures. Results were expressed as the percentage of control.

**Fig. 2.** Response of 5α-dihydrotestosterone (DHT) inactivation to dexamethasone in human preadipocytes. 3αβ-Diol formation rates in preadipocyte cultures following 24-h exposure to 100 nM (A) and 1 μM dexamethasone (B). Preadipocyte cultures were derived from subcutaneous (n = 14; BMI 32.0 ± 13.8 kg/m², age 44.3 ± 5.6 yr) and omental (n = 10; BMI 36.0 ± 8.4 kg/m², age 45.8 ± 6.5 yr) adipose tissue in women as well as sc (n = 3; BMI 43.6 ± 7.8 kg/m², age 47.3 ± 4.6 yr) and OM (n = 3; BMI 45.6 ± 19.2 kg/m², age 54.0 ± 16.9 yr) adipose tissue in men. Data are expressed as the percentage over control (means ± SE) of ≥3 experiments in duplicate. #P ≤ 0.05 compared with sc preadipocytes within the same sex. *P ≤ 0.05 compared with women within the same adipose tissue depot.

**Fig. 3.** Response of DHT inactivation to dexamethasone in human preadipocytes according to BMI of women and men. 3αβ-Diol formation in preadipocyte cultures following 24-h exposure to 100 nM (A) and 1 μM dexamethasone (B) from women (sc, n = 11; OM, n = 9; BMI 35.0 ± 13.5 kg/m², age 46.5 ± 6.9 yr) and men (sc, n = 3; OM, n = 3; BMI 42.3 ± 11.2 kg/m², age 48.8 ± 3.3 yr). Data are expressed as the percentage over control (mean values of duplicate). Spearman correlation coefficients and P values are shown on the graphs.

rCrArG-rGrGrG-rArGrA-rArArG-rGrArA-rGrGrA; antisense: rCrUrC-rGrCrG-rUrUrU-rGrUrC-rGrCeU-rGrUG-U-A), AKR1C1 (sense: rCrArG-rCrArA-rArUrU-rGrGrC-rArArU-rUrGrA-rArGrC-rUrGG-C; antisense: rGrCrC-rArGrC-rUrUc-rArUrA-rUrGrG-rCrGrG-rCrGrA-rUrUrU-rGrGrU-rGrGrC-rGrGrC), AKR1C2 (sense: rUrGrC-rGrArC-rUrUc-rArGrU-rArArG-rCrUrA-rCrArG-rCrUA-A; antisense: rUr-rUrArG-rGrUrA-rGrUrA-rUrGrA-rArGrC-rGrArC-rCrArG), AKR1C3 (sense: rCrGrG-rGrArU-rArArA-rUrUrG-rCrUrA-rArGrU-rArGrA-rGrGrA; antisense: rUrGrC-rCrArA-rArArU-rCrUrA-rGrCrG-rGrUrG).

**RT-qPCR mRNA measurements.** Efficiency of gene downregulation was assessed by quantitative RT-PCR. Total RNA was isolated using the RNeasy extraction kit and on-column DNase (Qiagen). Complementary DNA amplification was performed in duplicate using the LightCycler 480 (Roche Diagnostics) and the SYBR Green I Master (Applied Biosystems) as follows: 95°C for 10 s, 59–62°C for 10 s, 72°C for 12 s, and then 72°C for 5 s (reading) repeated 50 times. Primer sequences for AKR1C1 (5′-CCT-ATA-GTG-CTC-TGG-GAT-CCC-AC-3′/5′-AGG-ACC-ACA-ACC-CCA-GGC-TGT), AKR1C2 (5′-CCG-TCA-AAT-TGG-CAA-TAG-AAG-CC-3′/5′-CCTG-ATC-AGA-TGC-AGA-TGC-GT), and AKR1C3 (5′-CCA-CAC-AGA-GTG-ATC-TGG-CTT-CAT-GTC-5′/5′-ACC-CAT-CTG-GCT-TAT-GCT-TGA) and fatty acid-binding protein 4 (FABP4; 5′-TGG-GCC-AGG-AAT-TTG-AAG-AG-AC-3′/5′-CGT-CCC-TTG-GCT-TAT-GCT-CCT-T) were designed using GeneTools (Biotools). Results were expressed as relative units compared with Atp5o housekeeping gene expression (24).
measured as described previously (5). Cytotoxicity of siRNA treatment was assessed by measuring adenylate kinase release in the medium using the ToxiLight nondestructive cytotoxicity bioassay kit (Lonza). Cell apoptosis was assessed using Caspase-Glo 3/7 assay kit (Promega, WI), which measured the activities of caspases 3–7. The absence of cytotoxic and apoptotic effects of the DHT treatment has been demonstrated previously (5).

Statistical analyses. Differences in mRNA expression or androgen inactivation rates between patient groups were tested using ANOVA followed by a Tukey post hoc test, whereas a posteriori mean contrasts were used to compare Oil Red O, G3PDH, and FABP4 mRNA measurements. Spearman correlation coefficients were computed to quantify associations between hormone conversion rates and BMI. Statistical analyses were performed using SAS (SAS Institute).

RESULTS

Cortisol production and androgen inactivation rates were measured in whole adipose tissue samples of women (Fig. 1). The rates of cortisol production by 11β-HSD1 were positively associated with the rate of DHT inactivation in both subcutaneous (r = 0.73, n = 43, P = 0.0001) and omental adipose tissue (r = 0.47, n = 43, P = 0.002). Hence, adipose tissue samples with a high glucocorticoid activation rate also showed an increased capacity to inactivate androgens. The potential interrelation between cortisol production and androgen inactivation is further supported by the finding of a glucocorticoid response element (GRE) in the promoter region of AKR1C enzymes (Table 1). We found a perfect consensus GRE in the promoter region of the AKR1C1 and AKR1C2 genes. An imperfect GRE sequence was observed in the promoter region of AKR1C2.

Inactivation of DHT to 3α/β-diol in the presence of 100 nM or 1 μM dexamethasone was measured in several cultures of subcutaneous and omental preadipocytes from women and men (Fig. 2). The increase in DHT inactivation was significant at both dexamethasone concentrations. No difference was observed in dexamethasone-induced 3α/β-diol formation between subcutaneous and omental preadipocyte cultures from women. However, stimulation of DHT inactivation by dexamethasone was more robust in preadipocytes derived from omental adipose tissue of men compared with their subcutaneous preadipocytes. Dexamethasone also had a greater impact on DHT inactivation rates in omental preadipocytes of men compared with preadipocytes from both fat depots in women. Figure 3 shows the association of dexamethasone-induced DHT inactivation rates with BMI in women and men. We observed a positive correlation between BMI of the adipose tissue donors and the response of preadipocytes to 100 nM (r = 0.60, n = 27, P < 0.001) and 1 μM dexamethasone (r = 0.43, n = 26, P < 0.05) (Fig. 3). Hence, compared with preadipocytes derived from adipose tissue of lean subjects, preadipocytes from obese subjects showed a higher sensitivity of 3α/β-diol formation to dexamethasone stimulation. Analyses were also performed separately in each adipose tissue depot. Whereas BMI was significantly associated with the response to 100 nM dexamethasone in subcutaneous preadipocytes (r = 0.68, n = 15, P < 0.01), a trend for an association was observed in omental preadipocytes (r = 0.49, n = 12, P = 0.1). At a dose of 1 μM, only the response of subcutaneous preadipocytes remained associated with BMI (r = 0.49, n = 14, P < 0.05). A significant correlation was also observed between BMI of women and DHT inactivation rates in the presence of 100 nM dexamethasone (r = 0.52, n = 20, P < 0.05). The number of preadipocyte cultures derived from adipose tissue in men was too low to perform an independent analysis.
To assess the involvement of AKR1C enzymes in dexamethasone-induced DHT inactivation, we have specifically downregulated AKR1C1, AKR1C2, or AKR1C3 isoform expression using siRNA. Transfection of human preadipocytes with siRNA designed against AKR1C1, AKR1C2, and AKR1C3 significantly reduced target gene expression by 82 ± 6, 84 ± 5, and 79 ± 17%, respectively (P < 0.01; Fig. 4A). Downregulation of these enzymes by RNA interference was highly specific since no siRNA cross-reactivity was observed between AKR1C enzymes. Progesterone and DHT inactivation rates were measured in human preadipocytes following siRNA-mediated downregulation of AKR1C enzymes. Although 20α-reductase activity in preadipocytes was reduced by AKR1C1 siRNA, this activity was not altered in AKR1C2- or AKR1C3-depleted preadipocytes (P < 0.05; Fig. 4B). The capacity of preadipocytes to inactivate DHT was reduced by one-half after downregulation of AKR1C2 expression (P < 0.05; Fig. 4C). Transfection of siRNA targeting AKR1C1 and AKR1C3 did not significantly alter 3α-reductase activity. More importantly, dexamethasone-induced DHT inactivation was blunted by downregulation of AKR1C2 expression (Fig. 4D). Similarly to preadipocytes treated with the scrambled siRNA, preadipocytes with targeted downregulation of AKR1C1 and AKR1C3 siRNA presented a significant and dose-dependent 3α-reductase response to dexamethasone.

Preadipocyte differentiation experiments were performed to test the impact of 3α-reductase activity on the sensitivity of cells to the antiadipogenic effects of DHT (Figs. 5 and 6). Following downregulation of AKR1C isoform expression, adipogenesis was induced in the presence of vehicle or several DHT concentrations. As reported previously, a dose-response inhibition of lipid accumulation by DHT was observed in differentiated human preadipocytes (5). In cells treated with the scrambled siRNA, antiadipogenic effects were already observed at 10 nM DHT using microscopy (Fig. 5) and spectrophotometric quantification (Fig. 6A, left) of Oil Red O staining. A trend toward reduced G3PDH activity was also observed in preadipocytes exposed to 10 nM DHT compared with vehicle-treated cells (Fig. 6B, left). Antiadipogenic effects of DHT remained similar in preadipocytes treated with AKR1C1 and AKR1C3 siRNA. Further-reduced Oil Red O lipid accumulation by 10 nM DHT was observed in preadipocytes with targeted downregulation of AKR1C2 compared with cells treated with the scrambled siRNA (Fig. 5). Consistent with these observations, spectrophotometric quantification of Oil Red O staining as well as G3PDH activity were reduced significantly in 10 nM DHT-treated preadipocytes transfected with the AKR1C2 siRNA compared with cells transfected with the scrambled siRNA (Fig. 6, A and B, left). Interestingly, whereas adipogenesis remained unaffected by 1 nM DHT in cells treated with the scrambled siRNA, the same concentration of DHT decreased lipid accumulation and G3PDH significantly in cells treated with AKR1C2 but not with AKR1C1 or AKR1C3 siRNA (Fig. 6, A and B, right). These results were further supported by reduced FABP4 mRNA expression in differentiated preadipocytes transfected with the AKR1C2 siRNA and incubated with 10 nM DHT (Fig. 6C). A trend toward decreased mRNA expression of the lipoprotein lipase (P = 0.1) and PPARγ (P = 0.09) was also observed in cells treated with the AKR1C2 siRNA and 10 nM DHT (data not shown). Finally, antiadipogenic actions of AKR1C2 siRNA downregulation and DHT were not induced by cytotoxicity or apoptosis compared with the scramble siRNA (data not shown).

**DISCUSSION**

The aim of the present study was to characterize glucocorticoid-induced androgen inactivation in human preadipocytes. We also aimed at testing the involvement of AKR1C2 in this reaction and the impact of this enzyme in modulating sensitivity of these cells to antiadipogenic effects of DHT. The results of our study support the notion that cortisol production and DHT inactivation are tightly regulated in adipose tissue. Preadipocytes derived from omental adipose tissue of men have higher dexamethasone-induced DHT inactivation compared with subcutaneous preadipocytes of men or cells from both fat depots of women. Moreover, higher stimulation of DHT inactivation was found in subcutaneous and omental preadipocyte cultures derived from the more obese individuals. Downregulation of AKR1C2 significantly decreased basal as
Fig. 6. Quantification of preadipocyte differentiation efficiency in the presence of DHT following siRNA-mediated AKR1C isoform downregulation. Differentiation efficiency of siRNA-treated preadipocytes was assessed using spectrophotometric quantification of Oil Red O staining (n = 3; BMI 29.3 ± 6.0 kg/m², age 49.8 ± 2.2 yr; A) and measurements of glycerol-3-phosphate dehydrogenase (G3PDH) activity (n = 7; BMI 28.8 ± 4.5 kg/m², age 49.2 ± 5.5 yr; B). Differentiation was induced with the adipogenic hormonal cocktail in the presence of 10 (left) or 1 nM DHT (right). Data are expressed as %Oil Red O staining or G3PDH activity measured in preadipocytes treated with vehicle and the scrambled siRNA (means ± SE). C: fatty acid-binding protein 4 (FABP4) mRNA expression in differentiated preadipocytes (n = 5; BMI 33.8 ± 18.9 kg/m², age 42.5 ± 20.4 yr). *P ≤ 0.05 compared with preadipocytes exposed to the same DHT concentration and treated with the scrambled siRNA. #P ≤ 0.05 compared with preadipocytes treated with vehicle and the scrambled siRNA.

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well as dexamethasone-induced 3α-reductase activity in human preadipocytes. Finally, we demonstrated that the antiadipogenic effects of DHT were increased following downregulation of AKR1C2 but not of AKR1C1 and AKR1C3 isoform expression. Even low DHT concentration (1 nM), which failed to inhibit adipogenesis in control cells, reduced lipid accumulation and G3PDH activity significantly in cells where AKR1C2 expression was downregulated. This study demonstrates for the first time that AKR1C2 is involved in basal and glucocorticoid-induced androgen inactivation and that this reaction modulates the antiadipogenic effects of DHT in human preadipocytes. Because regional and sex differences in dexamethasone-induced androgen inactivation were observed, our results suggest that modulation of AKR1C2-dependent androgen inactivation by glucocorticoids may represent a relevant hormonal mechanism involved in the regulation of body composition and fat distribution.

The possible involvement of AKR1C enzymes in adipose tissue hormone metabolism and body fat distribution was underlined by several studies (1, 3, 4, 6, 7, 26). Moreover,
higher DHT and progesterone inactivation rates were found in omental adipose tissue of viscerally obese women (1, 3, 6).

Zhang et al. (27) have performed experiments in intact cells stably expressing each AKR1C isoform to establish their relative contribution to 20α-HSD, 3α-HSD, and 17β-HSD activities (Fig. 7). They observed that AKR1C1 mainly inactivates progesterone into 20α-hydroxyprogesterone through its 20α-HSD activity but also converts 4-androsten-3,17-dione (D4-dione) to testosterone through its 17β-HSD activity (∼3–4% of its 20α-HSD activity) and inactivates DHT to 3α-diol through its 3α-HSD activity (∼8% of its 20α-HSD activity). AKR1C2 mostly inactivates DHT by its 3α-HSD activity. This isoform also possesses low levels of 17β-HSD activity (∼2–3% of its 3α-HSD activity) and 20α-HSD activity (∼10% of its 3α-HSD activity). Conversely, AKR1C3 has a strong 20α-HSD activity but also shows significant 17β-HSD (∼35% of its 20α-HSD activity) and low 3α-HSD activity (∼7–8% of its 20α-HSD activity). In the present study, we confirm that progesterone and DHT inactivation in human preadipocytes are at least partly mediated by the AKR1C isoforms. Reduced AKR1C1 expression was associated with a significantly lower 20α-progesterone formation. On the other hand, 3α-diol formation was significantly inhibited following siRNA-mediated downregulation of AKR1C2 but not AKR1C1 or AKR1C3 expression. Therefore, these results are in accord with previous in vitro experiments (27).

This study also points to the involvement of AKR1C2 in glucocorticoid-induced androgen inactivation in human preadipocytes. Our previous study showed that expression of AKR1C enzymes is strongly increased by induction of preadipocyte differentiation (4). This effect is likely mediated by glucocorticoids in a dose-dependent manner and independent of the adipogenic process (4). This induction was observed at both the activity and mRNA level (Ref. 4 and Tchernof A, unpublished results). Here, we demonstrate that siRNA-mediated downregulation of AKR1C2 but not AKR1C1 or AKR1C3 expression. Therefore, these results are in accord with previous in vitro experiments (27).

Regarding AKR1C3, other investigators have postulated that its moderate androgen-generating action (testosterone synthesis from 4-androsten-3,17-dione) or even its prostaglandin synthase activity could be relevant for obesity (18, 19, 26). We observed very low levels of 17β-oxygenate activity in preadipocytes despite strong AKR1C3 mRNA expression (6, 7). Moreover, recent evidence clearly points toward androgen inactivation as an important determinant of androgen action in adipose tissue (reviewed in Ref. 8). The present study supports the hypothesis that androgen inactivation by the AKR1C2 isoform is more relevant than androgen synthesis in the regulation of adipose tissue function in humans.

Individual impacts of glucocorticoids and androgens on adipocyte differentiation are rather well established (5, 9, 11). Our results now support the presence of an additional regulatory mechanism of adipogenesis by these hormones. Higher 11β-HSD1 reductase activity and local cortisol concentrations were observed in omental compared with subcutaneous adipose tissue of viscerally obese subjects (24, 25). In this study, we show that DHT inactivation rates were more strongly induced by glucocorticoids in omental compared with subcutaneous adipose tissue of obese subjects. Higher local glucocorticoid activation as well as higher sensitivity of androgen inactivation to glucocorticoids in omental adipose tissue of obese individuals support the existence of a differential regulation of hormonal exposure in subcutaneous vs. omental adipose tissue. In this context, generation of active glucocorticoids may create a permissive hormonal environment that promotes adipogenesis and lipid storage in a specific adipose tissue depot not only through direct glucocorticoid action but also possibly through DHT inactivation.

This study supports the role of AKR1C2-dependent androgen inactivation in the regulation of DHT antiadipogenic action, but some limitations need to be acknowledged. We did not assess the capacity of the glucocorticoid receptor to directly bind and promote transcription of the AKR1C2 gene. However, a functional GRE has been found in rat 3α-HSD (i.e., AKR1C9) (14). Binding of the activated glucocorticoid receptor to this element increases transcription of the AKR1C9 gene (14). Although we observed a perfect GRE sequence in the

![Fig. 7. Relative activities of AKR1C1, -2, and -3 toward various steroid substrates. Schematic enzyme reactions shown here are based on percentages from Zhang et al. (27) from their 6th bar graph illustration. Assessment of relative activities was performed in human embryonic kidney-293 cells stably expressing each isoform; 100% activity corresponds to 1.37, 1.70, and 0.38 nmol·h⁻¹·10⁶ cells for AKR1C1, -2, and -3 respectively.](image-url)
promoter of AKR1C2, the ability of glucocorticoids to bind this GRE and stimulate AKR1C2 expression remains to be confirmed. The androgenic environment of adipose tissue also includes testosterone, which is not inactivated by 3α-HSD activity. Therefore, AKR1C2 may regulate adipose tissue exposure to only a portion of the androgens available. The contribution of other inactivation pathways for androgens, such as glucuronidation by UGT2B15, was not tested in this study (22). Finally, we observed some residual 3α-HSD and 20α-HSD activities following target gene downregulation. This may be the result of an incomplete AKR1C1 and AKR1C2 knockdown, which is a normal feature of RNA interference. Moreover, we cannot exclude that part of 3α-HSD and 20α-HSD activities in human preadipocytes may possibly be mediated by other enzyme isoforms (27).

In conclusion, these results show that AKR1C2, but not AKR1C1 or AKR1C3, drives basal and glucocorticoid-induced androgen inactivation to 3α-diol in human preadipocytes. Moreover, we demonstrated that glucocorticoid action on AKR1C2-dependent androgen inactivation regulates DHT antiadipogenic action. Glucocorticoid-induced androgen inactivation suggests that, besides the direct adipogenic activity of active glucocorticoids, the latter hormone may also regulate availability and concomitant antiadipogenic effects of DHT. Because we observed regional-, sex-, and adiposity-related differences in glucocorticoid-induced androgen inactivation, the interplay between glucocorticoids and androgens may be involved in regional fat deposition. Modulation of AKR1C2 by active glucocorticoids may locally modify exposure of adipose cells to androgens and possibly represents a therapeutic target for fat tissue remodeling in obesity.

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DISCLOSURES
The authors have no conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

REFERENCES


