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Progesterone metabolism in adipose cells

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ABSTRACT

The aim of the present study was to investigate pathways of progesterone metabolism in human adipose cells. Adipose tissue samples from the omental (OM) and subcutaneous (SC) fat compartments were surgically obtained in women. In isolated mature adipocytes, progesterone was converted to 20α-hydroxyprogesterone as the main metabolite, most likely through the activity of aldo-keto reductases 1C1, 2 and 3 (20α-HSD, 3α-HSD type 3 and 17β-HSD type 5, respectively). In cultured preadipocytes, progesterone was converted to several metabolites identified using bidimensional thin layer chromatography, with or without the dual inhibitor of 5α-reductase type 1 and 2 (17β-*N*,*N*-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)). Major metabolites identified in OM and SC preadipocytes which were incubated for 24 h with ^{14}C-labelled progesterone were 20α-hydroxyprogesterone, 5α-pregnane-3α/β-ol-20-one, 5α- and 5β-pregnanedione, 5α- and 5β-pregnane-20α-ol-3-one, 5α-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β-20α-diol. Induction of preadipocyte differentiation increased expression levels of AKR1C1 and modified the pattern of progesterone metabolism substantially, leaving 20α-hydroxyprogesterone as the main metabolite generated. On the other hand, progesterone itself showed no consistent effect on adipocyte differentiation. In conclusion, preadipocytes and lipid-storing, mature adipocytes efficiently generate progesterone metabolites in women, which is consistent with rather modest effects progesterone on abdominal fat cell differentiation.

1. Introduction

Findings of several studies (Anderson et al., 2001; Price et al., 1998; Roncari and Van, 1978) suggest that sex steroids may be regarded as critical modulators of adipose tissue deposition through the regulation of preadipocyte proliferation and/or differentiation as well as lipogenesis and/or lipolysis of mature adipocytes. A series of *in vivo* and *in vitro* observations (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981; Wiper-Bergeron et al., 2003) has shown that progesterone may stimulate fat accretion by increasing lipoprotein lipase (LPL) activity, lipid synthesis and steroid-mediated differentiation of preadipocytes. These results, however, are not unanimous, as Hamosh and Hamosh (1975) reported no effect of progesterone on LPL activity in rat adipose tissue. Bjorntorp (1997) suggested that progesterone could be involved in the presence of a female fat distribution pattern via an anti-glucocorticoid action in abdominal adipose tissue. This notion was partially supported by studies showing that progesterone inhibited glucocorticoid-induced fat cell differentiation, lipogenesis, or body fat accumulation (Pedersen et al., 2003; Xu et al., 1990).

Regarding progesterone metabolism, we have reported high adipose tissue levels of 20α-hydroxysteroid dehydrogenase (20α-HSD, AKR1C1), an enzyme involved in the inactivation of progesterone (Blanchette et al., 2005). Findings of our previous studies indicated that the 20α-reduction of progesterone was increased in omental (OM) adipose tissue of women characterized by abdominal obesity (Blanchette et al., 2005; Blouin et al., 2005). In addition to the 20α-hydroxy group, the progesterone molecule contains additional functional groups that may be modified by other steroid-converting enzymes also expressed in adipose tissue such as 5α-reductase and 3α-hydroxysteroid dehydrogenase type 3 (3α-HSD-3, AKR1C2) (Belanger et al., 2002).

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As a member of the aldo-keto reductase (AKR) superfamily, AKR1C1 is mainly involved in the conversion of progesterone into its biologically inactive metabolite 20α-hydroxyprogesterone (Penning, 1997), the reverse reaction being minor within a cellular context (Rizner et al., 2006). This enzyme has been detected in rat ovary, testes, adrenals, placenta, liver, thymus, T-dependent areas of other lymphoid organs, kindney and lung tissue of mice (Imamura et al., 2007), as well as human uterus (Mori and Wiest, 1979; Penning et al., 2000; Wilcox and Wiest, 1966; Wiest and Wilcox, 1961; Weinstein, 1977; Zhang et al., 2000). Findings from the group of Penning suggest that AKR1C1 appears to possess the plasticity which allows it to interconvert potent androgens, estrogens, and progestins into their cognate inactive metabolites, the most catalytically efficient conversion being that of progesterone to its inactive metabolite (20α-hydroxyprogesterone) (Penning et al., 2000). In mouse liver cytosol, both NADPH and NADH are involved in the activity of 20α-HSD as the cofactors, whereas NADPH is the only cofactor for the 20α-HSD effect in mouse kidney (Shimade et al., 2006). Additionally, 20α-HSD activity is found to be catalyzed by type 1 or type 2 17β-HSD in human placenta (Penning, 1997). In human renal cytosol (Quinkler et al., 2004), high conversion rates of progesterone to 20α-dihydro-progesterone were found. Wiebe (2006) reported that progesterone was directly converted to the 4-pregnenes, 3α-hydroxy-4-pregnen-20-one (3α-dihydroprogesterone; 3αHP) and 20α-hydroxyprogesterone through 3α-HSD and 20α-HSD activities, and through the irreversible catalysis of 5α-reductase, and then these metabolites were converted to 5α-pregnane, 5α-pregnane-3,20-dione (5α-dihydroprogesterone; 5αP). These previous reports indicated that 20α-HSD activity is also found in other non-reproductive tissues and appears to generate differences in the effect of progesterone.

The discrepancies in studies that have examined the impact of progesterone on adipose tissue function and the newly described possibility of pre-receptor progesterone inactivation through 20α-HSD and other enzymes in adipose tissue prompted us to re-examine progesterone action and metabolism in cultured adipose cells from women. The present study focuses on the comparison of progesterone metabolite formation in preadipocytes and lipid-storing adipocytes.

2. Subjects and methods

2.1. Subjects and adipose tissue sampling

Samples were obtained from 24 women aged 26–62 years (BMI $42.11 \pm 16.62\,kg/m^2$, range $21.4–74.31\,kg/m^2$) undergoing abdominal gynecological ($n = 9$) or bariatric ($n = 15$) surgery. According to their BMI value, 7 women had normal BMI (29.2%), 4 were obese (16.6%), and 13 were morbidly obese (54.2%). Medication and drug history included anti-diabetic therapy ($n = 7$); lipid-lowering ($n = 4$) or antihypertensive therapy ($n = 5$), diuretics ($n = 2$), anti-depressants ($n = 4$). All these treatments were used in obese ($n = 4$) and morbidly obese ($n = 11$) patients undergoing bariatric surgery. Approval of the medical ethics committees of Laval University, Laval University Medical Research Center and Laval Hospital was obtained. All subjects provided written informed consent before their inclusion in the study.

Adipose tissue samples were collected during the surgical procedure at the site of incision (subcutaneous adipose tissue, SC) and from the greater omentum (omental adipose tissue, OM). Samples were immediately carried to the laboratory in 0.9% saline preheated at 37 °C. A portion of the biopsy was used for adipocyte isolation and primary cultures, and the remaining tissue was immediately frozen at −80 °C for subsequent analyses.

2.2. Adipocyte isolation and primary preadipocyte cultures

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 (Rodbell, 1964). Adipocyte suspensions were filtered through nylon mesh and washed three times with KRH buffer. Mature adipocyte suspensions were incubated with ^{14}C-labelled progesterone at 37 °C with shaking for 24 h. Preadipocytes were isolated using a modified method previously described by Hauner (Hauner et al., 2001). Briefly, the residual buffer of the adipocyte isolation was centrifuged and the pellet was washed in DMEM-F12 supplemented with 10% fetal bovine serum, 2.5 μg/ml amphotericin B, 1.00 U/ml penicillin and 50 g/ml streptomycin. Cells were treated with erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA, pH 7.5) and washed again with DMEM-F12. Preadipocytes were seeded in 12-well culture plates and cultured at 37 °C under a 5% CO_2 atmosphere. Medium was changed every 2–3 days. For steroid converting activities before and after fat cell differentiation, preadipocytes were incubated 24 h with ^{14}C-labelled progesterone at baseline and 16–20 days after inducing differentiation using differentiation medium with insulin, dexamethasone and a PPARγ agonist (Zen-Bio, Research Triangle Park, NC). The concentration of radiolabelled progesterone was 0.44 μM per well which is close to the K_m value of human 20α-HSD in intact transfected cells, 0.6 μM (Zhang et al., 2000).

2.3. Glycerol-3-phosphate dehydrogenase (G3PDH) activity measurements and lipid accumulation

G3PDH activity was used as a marker of adipocyte differentiation and measured according to Sottile and Seuwen (2001) with some modifications. Differentiated cells 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 a 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 during 5 min. Purified G3PDH enzyme was used to generate a standard curve and calculate G3PDH activity in mU 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 mU/μg protein.

Oil red O staining followed by spectrophotometric analysis was performed to measure lipid accumulation as previously described (Ramirez-Zacarias et al., 1992). 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_2O, oil red O retained by lipid droplets was eluted with isopropanol containing 4% Igepal CA-630. Optical density was measured at 490 nm.

Based on previous studies suggesting that progesterone may have insulin-like (Lacasa et al., 2001) or anti-glucocorticoid-like action (Bjorntorp, 1997; Pedersen et al., 2003; Xu et al., 1990), cultured SC or OM preadipocytes were differentiated by incubation with various concentrations of progesterone (10^{-5} to 10^{-8} M) or without progesterone in differentiation media containing the following insulin and dexamethasone concentrations: (1) 100 nM insulin and 1 μM dexamethasone; (2) 25 nM insulin and 1 μM dexamethasone; (3) 100 nM insulin and 0.25 μM dexamethasone; (4) 25 nM insulin and 0.25 μM dexamethasone. Ethanol alone was added in untreated controls. A PPARγ agonist was included in all four experimental conditions. After differentiation, G3PDH enzymatic activity measurement and oil red O lipid staining were performed to assess the effect of progesterone on fat cell differentiation and lipid accumulation.

2.4. Enzymatic activities and metabolite formation

After incubation with ^{14}C-labelled progesterone for 24 h, steroids were extracted twice with one volume ether as described previously (Blouin et al., 2003). The organic phases were pooled and evaporated to dryness. Steroids were solubilized in 50 μl dichloromethane (reference standards were solubilized in 100% ethanol) and applied to Silica Gel 60 thin layer chromatogram (TLC) plates (Merk, Darmstadt, Germany). The separation was performed by migration in toluene–acetone (4:1). Unlabelled 20α-hydroxyprogesterone was used as a standard and was detected under ultraviolet light. Bidimensional thin layer chromatography was performed according to (Wiebe et al., 2000), and samples were applied to Silica Gel 60 TLC plates and separated by two successive migrations in chloroform–ether (10:3) and two migrations in hexane–ethyl acetate (5:2). The extracted sample was applied on the lower right hand corner of 20 cm × 20 cm silica gel 60 F254 aluminium sheet (Merck KGaA, Germany), and separated by two successive migrations in solvent 1 (chloroform:ether, 10:3, v/v) and then turned 90° to perform two successive migrations in solvent system 2 (hexane:ethyl acetate, 5:2, v/v). The migrations of cold steroid reference standards were also performed at the same time to identify metabolites. Plates were analyzed using a Storm 860 PhospholmagerTM (Amersham Pharmacia Biotech Inc.) and the proportion of each metabolite was quantified using the ImageQuant software version 5.1 (Amersham Pharmacia Biotech Inc.).

2.5. Real-time PCR measurements of enzyme mRNA abundance

Total RNA was isolated from primary differentiated and non-differentiated 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. 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 AKR1C1 and progesterone receptor (PR). The sets of primers were: 5′-CCT-ATA-GTG-CTC-TGG-GAT-CCC-AC-3′, and 5′-AGG-ACC-ACA-ACC-CCA-CGC-TGT-3′ (AKR1C1); and 5′-TGA-GCT-TAA-TGG-TGT-TTG-GTC-TAG-GA-3′, and 5′-TTC-TTT-CAT-CCG-CTG-TTC-ATT-TAG-TATT-3′ (progesterone receptor). The FastStart DNA Master Plus SYBRGreen I kit (Roche Diagnostics) was used in a final 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, 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 expression levels. This gene was found to display remarkably stable expression levels from embryonic life through adulthood in various tissues (Warrington et al., 2000). 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 (Luu-The et al., 2005). For each sample, the Cp value was divided by that of the housekeeping gene. In order to further minimize inter-assay variability, this Cp ratio was then multiplied by the average Cp generated for housekeeping gene amplifications of all samples examined in the present experiment. PCR data were expressed in normalized number of copies per μg total RNA.

2.6. Statistical analysis

Unpaired *t*-tests were used in comparisons of metabolite mean radioactivity intensities between cultured preadipocytes and adipocytes induced to differentiate, including: (1) 5α- pregnanedione, (2) unconverted progesterone, (3) 5α/β-pregnane-20α-ol-3-one and 5α-pregnane-3α/β-ol-20-one, (3) 20α-hydroxyprogesterone, (4) 5β-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol, (5) as well as total metabolites. Paired *t*-tests were performed to compare the difference in the relative intensity of the above-mentioned metabolites as well as unconverted progesterone, between cultured preadipocytes and differentiated adipocytes. Analyses were performed using the JMP statistical software (SAS Institute, Cary, NC).

3. Results

Fig. 1A shows a thin-layer chromatography of steroid products in mature adipocytes and preadipocytes from SC and OM samples

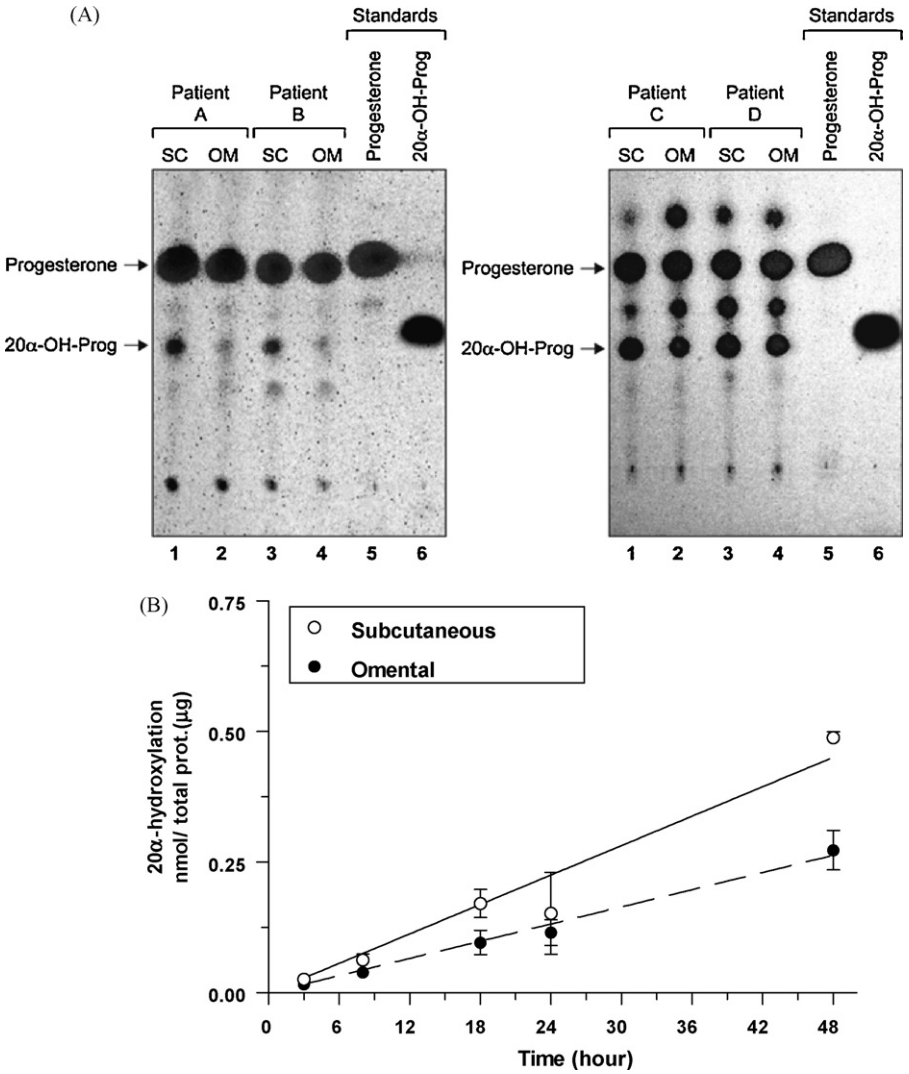

Fig. 1. (A) Thin layer chromatograms showing steroid products obtained when incubating SC or OM isolated mature adipocytes from obese women with radiolabelled ^{3}H-progesterone (left panel), and incubating SC or OM preadipocytes from obese women with radiolabelled ^{14}C-progesterone (right panel). The chromatograms were photographed under ultraviolet light for the identification of the 20α-hydroxyprogesterone standard (20α-OH-Prog) and this image was superimposed to the autoradiogram by image analysis. (B) 20α-reduced metabolite production in subcutaneous (SC) and omental (OM) preadipocyte primary cultures at different time points. Preadipocytes from two subcutaneous and three omental samples were treated with ^{14}C-labeled progesterone (0.44 μM) for the various times indicated. Data presented as mean ± S.E.M.

after incubation with radiolabelled progesterone for 24 h. In both fat depots, we found that 20α-hydroxyprogesterone was the predominant conversion product of progesterone. These results are representative of experiments performed with mature adipocytes and isolated preadipocytes from several other patients. In time course experiments, 20α-reduction of progesterone was linear over 48 h.

By using bidimensional thin layer chromatography, we performed a more detailed analysis of progesterone metabolites generated by intact primary SC and OM preadipocytes from women. Fig. 2 shows a bidimensional thin layer chromatography of steroid products obtained after incubating SC and OM preadipocytes for 24 h with radiolabelled progesterone. Metabolites generated included 20α-hydroxyprogesterone, 5α-pregnane-3α/β-ol-20-one, 5α- and 5β-pregnanedione, 5α- and 5β-pregnane-20α/β-ol-3-one, 5α-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β-20α-diol. Progesterone was mainly converted to 20α-hydroxyprogesterone, whereas 5β-pregnane-3α/β-20α-diol was also generated after incubating with the dual inhibitor of 5α-reductase type 1 and 2 (17β-*N*,*N*-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)) (Fig. 2B). These results are representative of several other preadipocyte cultures. The postulated pathways of progesterone inactivation in preadipocytes are shown in Fig. 3.

We found that the pattern of metabolites generated was altered during adipocyte differentiation (Fig. 4). A total of six adipose tissue samples were examined (three SC fat samples and three OM fat samples). At baseline, intact preadipocyte cultures generated a mixture of metabolites similar to that shown in Fig. 2. However, in lipid-storing adipocytes after differentiation, 20α-hydroxyprogesterone formation was proportionally increased. Table 1 shows the quantification of relative intensities of

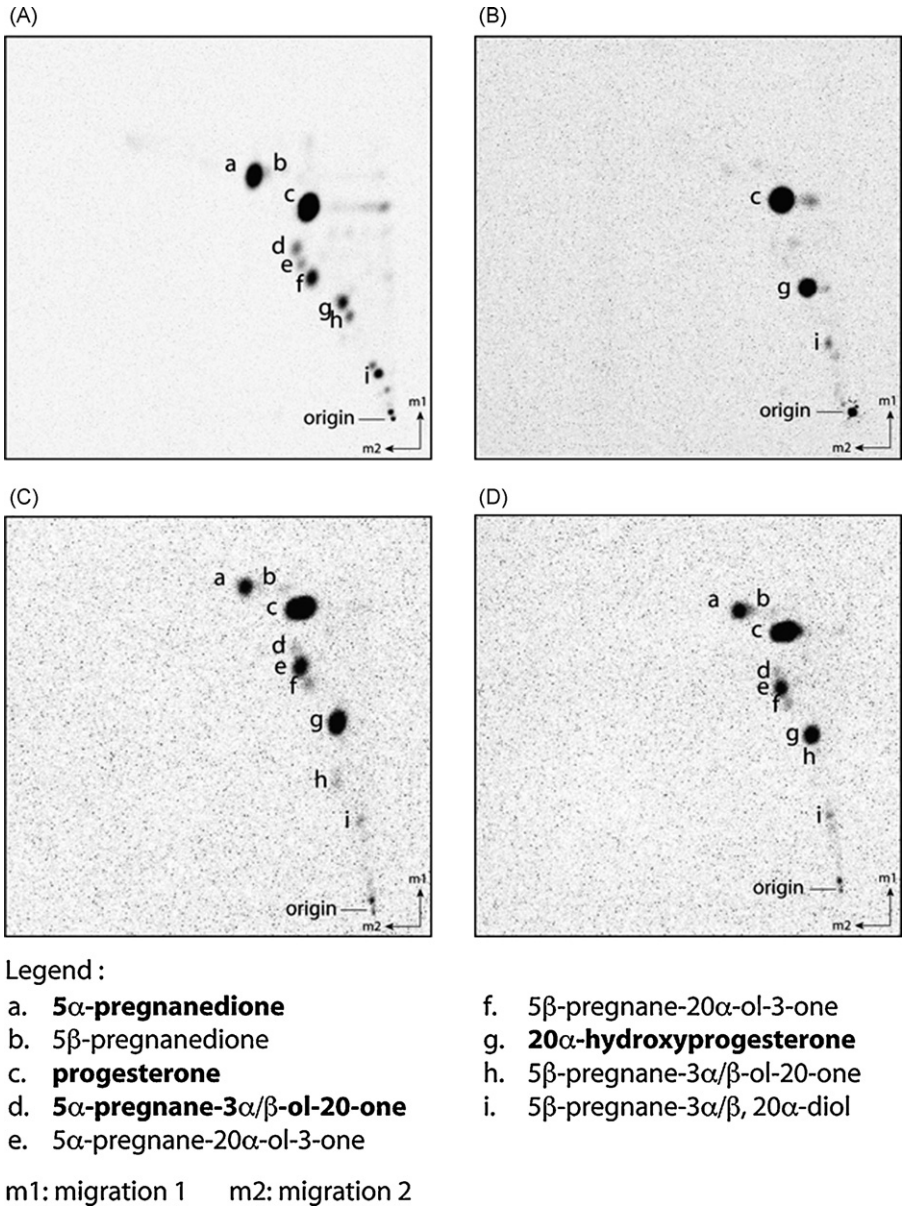

Fig. 2. Bidimensional thin layer chromatogram showing progesterone metabolites obtained when incubating SC preadipocytes with radiolabelled ^{14}C-progesterone (A), or with radiolabelled ^{14}C-progesterone and the dual inhibitor of 5α-reductase type 1 and 2 (17β-*N*,*N*-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)) (B), and when incubating SC (C) or OM (D) preadipocytes from obese subjects.

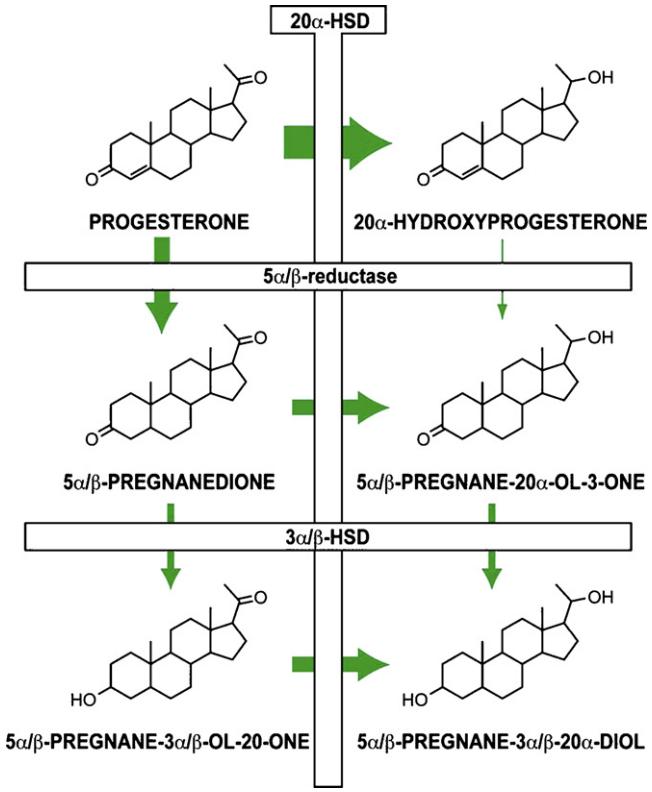

Fig. 3. Postulated pathways of progesterone metabolism in human preadipocytes (HSD: hydroxysteroid dehydrogenase).

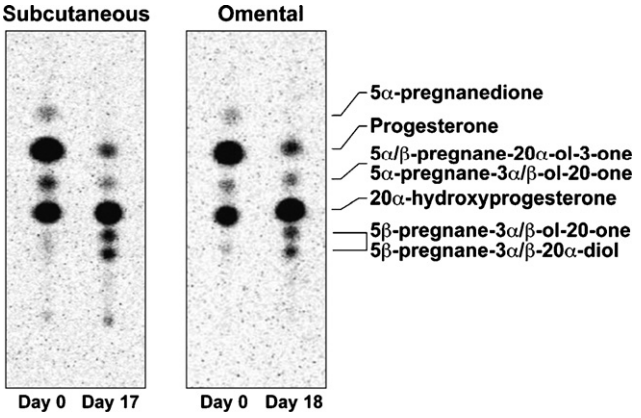

Fig. 4. Thin layer chromatograms showing steroid products obtained when incubating preadipocytes before and after hormone-induced fat cell differentiation.

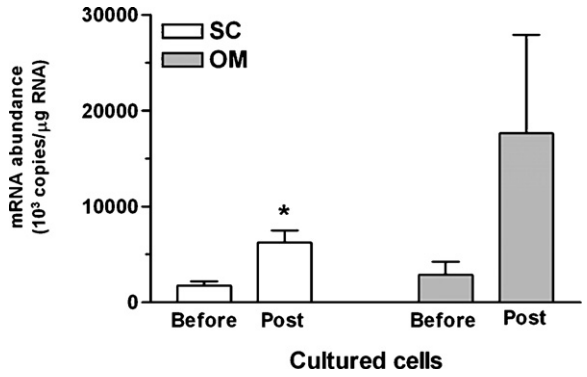

Fig. 5. Expression levels of AKR1C1 (20α-HSD) mRNA in preadipocytes (before differentiation) or post-differentiated adipose cells from subcutaneous (SC) ($n = 6$) and omental (OM) ($n = 4$) adipose tissue. Data presented as mean ± S.E.M. *$p < 0.05$, post-differentiated fat cells vs. preadipocytes (before differentiation) cultured fat cells. In SC samples, average G3PDH activity reached after differentiation was 64.03 ± 45.8 mU/μg protein, and average lipid staining increased by 216.1 ± 85.9% in differentiated adipocytes vs. preadipocytes. In OM samples, average G3PDH activity reached after differentiation was 1.45 ± 0.84 mU/μg protein, and average lipid staining increased by 143.1 ± 30.3% in differentiated adipocytes vs. preadipocytes.

several metabolites based on the radioactivity of the TLC. Proportions of 20α-hydroxyprogesterone, 5β-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol were significantly higher in differentiated adipocytes compared to preadipocytes. The proportion of total metabolite formation also increased in differentiated adipocytes. Unconverted progesterone, 5α/β-pregnane-20α-ol-3-one, 5α-pregnane-3α/β-ol-20-one, and 5α-pregnanedione were consistently lower in differentiated adipocytes compared to preadipocytes.

In a similar experiment, expression levels of AKR1C1 (20α-HSD) mRNA were obtained in preadipocytes (before differentiation) or post-differentiated adipose cells from SC ($n = 6$) and OM ($n = 4$) adipose tissue (Fig. 5). We found that the expression level of AKR1C1 mRNA in differentiated adipocytes was significantly higher than in preadipocytes in the SC depot. We also found that the expression level of AKR1C1 mRNA was increased following adipocyte differentiation in OM compartments, although the trend failed to reach significance. PR expression was examined in whole SC and OM adipose tissue samples of one patient. We found that expression levels were very low. For this reason, PR expression was not investigated in isolated cells.

Using differentiation media with various concentrations of insulin and dexamethasone, cultured SC or OM preadipocytes were incubated with (10^{-5} to 10^{-8}M) or without progesterone. No consistent effect of progesterone was observed on biochemical measurements of fat cell differentiation including G3PDH enzymatic activity and oil red O measurements (data not shown).

Table 1
Progesterone metabolites generated by preadipocytes and mature adipocytes from two OM fat samples and four SC fat samples incubated with radiolabelled progesterone for 24 h.

Metabolites	Preadipocytes ($n = 6$)	Differentiated adipocytes ($n = 6$)	Significance
5α-Pregnanedione	6.59 ± 4.3	2.24 ± 2.4	NS ($P = 0.1$)
Progesterone	68.3 ± 4.0	10.01 ± 4.6	$P < 0.0001$
5α/β-pregnane-20α-ol-3-one and 5α-pregnane-3α/β-ol-20-one	5.62 ± 2.7	3.93 ± 1.6	NS ($P = 0.14$)
20α-Hydroxyprogesterone	16.2 ± 9.3	72.72 ± 9.9	$P = 0.0001$
5β-Pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol	2.80 ± 1.7	9.2 ± 2.0	$P = 0.0007$
Total metabolites	31.2 ± 4.0	88.1 ± 5.5	$P < 0.0001$

Values are presented in percentage of total radioactivity. Samples from the OM and SC depots were pooled since no depot difference was found. Average G3PDH activity reached after differentiation was 0.19 ± 0.14 mU/μg protein, and average lipid staining increased by 195.7 ± 71.8% in differentiated adipocytes vs. preadipocytes. Mean ± S.D. are shown. NS: not significant.

4. Discussion

Although the effects of progesterone on adipose tissue and adipocytes from humans (Belanger et al., 2002; Bjorntorp, 1997; Blanchette et al., 2005; Blouin et al., 2005) and animals (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981) have been extensively studied, this is the first report to take into account and document pathways of progesterone inactivation within adipose tissue. In the present study, using SC and OM adipose tissue from women, we examined the local metabolic pathways of progesterone in isolated mature adipocytes and cultured adipocytes. Incubations with radiolabelled progesterone for 24 h were carried out to identify the various steroid products in isolated mature adipocytes, cultured preadipocytes and lipid-storing differentiated adipocytes. We found that preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. However, overall metabolite formation increased in differentiated adipocytes, with 20α-hydroxyprogesterone as the main metabolite. These findings further validate the detection of 20α-HSD, 3α/β-HSD and 5α-reductase activity in preadipocytes, and support the notion of a complex regulation of steroid action through locally expressed steroid-converting enzymes. On the other hand, progesterone had no consistent effect on fat cell differentiation in the present study, which is rather consistent with the observed patterns of progesterone inactivation.

In our previous work on abdominal adipose tissue obtained in women, we reported 20α-HSD mRNA and activity in SC and OM adipose tissue (Blanchette et al., 2005), and a positive correlation was observed between adiposity and activity of steroid aldo-keto reductases 1C, namely type 3 3α-HSD, type 5 17β-HSD and 20α-HSD (Blouin et al., 2005). Experimental data showed that progesterone may interact with glucocorticoid receptors (Pedersen et al., 1992), and that progesterone may generate anti-glucocorticoid effects in vitro (Schmidt et al., 1998; Xu et al., 1990). In ovariectomized (OVX) rats, increasing body lipid and protein content were restrained by estradiol replacement (Richard, 1986), whereas progesterone had no effect on energy intake and weight gain in this model (Richard, 1986; Wade and Gray, 1979). Conversely, progesterone increased adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression of rat preadipocytes in a dose-dependent manner (Lacasa et al., 2001), and it stimulated steroid-mediated differentiation of 3T3-L1 preadipocytes (Rondinone et al., 1992; Wiper-Bergeron et al., 2003). The receptor mediating these effects has not been clearly identified. Our preliminary experiments on PR expression in whole tissue are consistent with previous studies reporting low PR in adipose tissue, mature adipocytes or preadipocytes (Mayes and Watson, 2004). Low PR expression and significant 20α-HSD activity mediating the conversion of progesterone into inactive 20α-hydroxyprogesterone may contribute to the inconsistent effect of progesterone observed on adipocyte differentiation.

Through the reduction by aldo-keto reductase 1C (AKR1C) enzymes and 5α/5β reductase activity, progesterone was converted into a complex mixture of metabolites in preadipocytes. These results are consistent with previous studies in other cell types (Quinkler et al., 1999; Zhang et al., 2000). The finding of a slight 5β-reductase activity in adipose cells is novel. Our finding of increased progesterone metabolism in differentiated cells suggests that the differentiation of preadipocytes into lipid-storing mature adipocytes has effects on the pattern of progesterone metabolism. The modulation of progesterone inactivation rates or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes, particularly AKR1C1.

Hormonal steroids bind to specific receptors to exert their actions and the effect is determined by both the local concentration of the hormone and the receptor number (Vanderbilt et al., 1987; Webb et al., 1992). We have examined progesterone receptor (PR) expression in whole SC and OM adipose tissue samples and found that expression levels were very low. Wiebe reported that receptors of progesterone metabolites (such as 5α-pregnane-3,20-dione and 3α-hydroxy-4-pregnen-20-one) were observed on the cell membrane and involved in cell-signaling pathways related to protein kinase C, phospholipase C, or IP3-induced Ca^{2+} mobilization and Ca^{2+} channels. Their findings suggested a selective nongenomic mechanism (Wiebe, 1997, 2006). Progesterone may induce the acceleration of Xenopus oocyte maturation (Bayaa et al., 2000; Tian et al., 2000), stimulate acrosome reaction in sperm, modulate neurotransmitter and neural excitability (McEwen, 1991), and contribute to the activation of the Src/Ras/MAPK pathway in breast cancer cells (Migliaccio et al., 1998). Whether such rapid, membrane-initiated effects of progesterone (Leonhardt et al., 2003) are present in adipocytes remains unclear at this time.

Non-conjugated metabolites of progesterone such as allopregnanolone (5α-pregnane-3α-ol-20-one) and pregnanolone (5β-pregnane-3α-ol-20-one) were generated in preadipocytes. These steroids are considered as neurosteroids and potent positive modulators of gamma-aminobutyric acid type A (GABAA) receptors (Lambert et al., 2001; Majewska et al., 1986). GABA type A receptor is regarded as the target of important compounds, such as anxiolytic benzodiazepines (BZs), barbiturates, neurosteroids, and certain volatile anaesthetics (Davies, 2003; Lambert et al., 2003; Sieghart, 1995; Watanabe et al., 2002). Metabolites of progesterone as well as deoxycorticosterone (DOC) may actually represent endogenous ligands for this receptor (Darlison et al., 2005). Allopregnanolone effects on GABA receptors include mood alterations such as pubertal mood swings, prementstrual syndrome (PMS), postpartum blues, and perimenopause (Smith et al., 2007). Several studies on animals also reported that allopregnanolone inhibits learning and memory and increases appetite, as well as anxiety (Chen et al., 1996; Gulinello et al., 2001; Johansson et al., 2002). Several lines of investigation on GABAA receptor agonists including allopregnanolone reported that treatment with low dose of this compound generates loss of impulse control, negative mood, and aggression/irritability (Ferrari et al., 1997; Fish et al., 2001; Masia et al., 2000; Miczek et al., 2003). However, sedation, hypnosis, anxiolysis, and antiepileptic effects are induced when treating with high doses (Herzog, 1991; Sundstrom et al., 1998; Wieland et al., 1991). In the present study, we found that the production of progesterone metabolites was significantly increased in differentiated adipocytes, and a trend for increased allopregnanolone production was found in differentiated adipocytes. It remains to be clearly established whether adipose tissue-derived progesterone metabolites play a role in modulating central nervous system responses. At the local level, adipose tissue has been shown to have benzodiazepine binding sites, and locally produced allopregnanolone may be a ligand for these receptors (Gonzalez Solveyra et al., 1988).

It should be noted that medication used by the patients was not considered in the statistical analysis. Given the relative consistency of our observations from patient to patient, we suggest that a confounding effect of a specific drug is unlikely. Future studies could investigate whether progesterone inactivation is modulated by exogenous factors.

In conclusion, the differentiation of preadipocytes into mature adipocytes affects the pattern of progesterone metabolism. Preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. Metabolite formation increased in mature

adipocytes, and 20α-hydroxy-progesterone was the main metabolite. The efficient conversion of progesterone to inactive metabolites in adipose tissue and the low expression of progesterone receptor are concordant with the inconsistent effect of progesterone on fat cell differentiation in humans. The modulation of progesterone inactivation or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes and differential sensitivity to progesterone action.

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