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Differential estrogenic 17β-hydroxysteroid dehydrogenase activity and type 12 17β-hydroxysteroid dehydrogenase expression levels in preadipocytes and differentiated adipocytes

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ABSTRACT

Estradiol (E2) is produced locally in adipose tissue and could play an important role in fat distribution and accumulation, especially in women. It is well recognized that aromatase is expressed in adipose tissue; however the identity of its estrogenic 17β-hydroxysteroid dehydrogenase (17β-HSD) partner is not identified. To gain a better knowledge about the enzyme responsible for the conversion of estrone into estradiol, we determined the activity and expression levels of known estrogenic 17β-HSDs, namely types 1, 7 and 12 17β-HSD in preadipocytes before and after differentiation into mature adipocytes using an adipogenic media. Estrogenic 17β-HSD activity was assessed using [^{14}C]-labelled estrone, while mRNA expression levels of types 1, 7 and 12 17β-HSD were quantified using real-time PCR and protein expression levels of type 12 17β-HSD was determined using immunoblot analysis. The data indicate that there is a low conversion of E1 into E2 in preadipocytes; however this activity is increased ~5-fold ($p < 0.0001$) in differentiated adipocytes. The increased estrogenic 17β-HSD activity is consistent with the increase in protein expression levels of 17β-HSD12.

1. Introduction

There is increasing evidence showing that adipose tissue is a site of steroid metabolism [1], including the interconversion of estrone (E1) and estradiol (E2) [2]. The presence of both estrogen receptors (ERα and ERβ) in preadipocytes and mature adipocytes strongly suggests a role for active estrogen in these cells [3–8]. Accordingly, in postmenopausal women, in whom the production of E2 from ovarian source is absent, there is an increasing accumulation of adipose depot in the abdomen [9], similar to the situation found in men, while E2 treatment in postmenopausal women can reduce abdominal adiposity [10]. This E2-mediated reduction of adipose tissue mass is in agreement with data from studies using ERα [11,12] and aromatase [13] knock-out mice models, which have shown that these animals develop larger adipose tissue depots when compared with controls.

The role of E2 in the control of obesity and in fat distribution is however complex. On one hand, it has been shown that E2 plays a major role in the regulation of food intake and energy expenditure [14]; on the other hand, E2 clearly exerts important effects on the adipocyte metabolism itself. Indeed, it has previously been shown that there is a relationship between the lack of ovarian hormones in postmenopausal women and an increase in the omental/subcutaneous lipoprotein lipase activity ratio [15]. Other evidence supports that E2 down-regulates lipoprotein lipase expression and/or activity in adipocytes, and doing this, may influence the adipose tissue distribution [16–18]. In addition, E2 has been reported to affect preadipocyte proliferation [19].

The two specific steps in the biosynthesis of E2 are the aromatisation of 4-androstenedione into E1 followed by the transformation of E1 into E2 by estrogenic 17β-HSD enzymes. This last step indicates that estrogenic 17β-HSD enzymes are essential partners of aromatase in E2 biosynthesis. It is often described in the literature that E2 is produced by aromatisation of testosterone, suggesting that the step of 17-ketosteroid reduction of C19-steroid precedes the step of aromatisation. The recent cloning of separate 17β-HSD enzymes, specific for androgen (types 3 [20] and 5 [21,22] 17β-HSD) or estrogen (types 1 [23], 7 [24,25] and 12 [26,27] 17β-HSD), their tissue-specific distribution and the fact that the affinity of enzyme aromatase for 4-androstenedione (Km = 12 nM) is higher than for testosterone (Km = 41 nM) strongly suggest that the step of

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aromatisation precedes the step of 17-keto reduction of C18-steroid [28].

Since aromatase is already well recognized to be present in adipose tissue [29,30], in this report, we assess the role of estrogenic 17β-HSD enzymes in the local formation of E2 in subcutaneous and omental fat depots obtained from women undergoing abdominal hysterectomies, using preadipocytes and differentiated adipocytes in culture. The estrogenic 17β-HSD activity as well as expression levels of types 1, 7 and 12 17β-HSD were determined in preadipocytes and differentiated adipocytes coming from both fat depots.

2. Materials and methods

2.1. Subjects

Women of this study were recruited through the elective surgery schedule of the Gynecology Unit of the Laval University Medical Center (Table 1). The study included 26 women aged 33.4–49.5 years undergoing abdominal gynaecological surgery; not all women were pre-menopausal. Women of the study elected for a total ($n = 26$) or subtotal ($n = 1$) abdominal hysterectomies, some with salpingo-oophorectomy of one ($n = 3$) or two ($n = 2$) ovaries. Reasons for surgery included one or more of the following: menorrhagia/menometrorrhagia ($n = 12$), myoma/fibroids ($n = 17$), incapacitating dysmenorrhea ($n = 5$), pelvic pain ($n = 1$), endometriosis ($n = 7$), ovarian cyst ($n = 3$), uterine bleeding ($n = 2$), pelvic adhesion ($n = 1$). Medication included non-steroidal anti-inflammatory drugs ($n = 5$), antidepressants ($n = 3$), anti-fibrinolytic ($n = 3$) and asthma ($n = 3$), aspirin ($n = 3$), anti-hypertensive therapy ($n = 2$), hormone therapy ($n = 1$), lipid lowering ($n = 1$), anti-seizure ($n = 1$), anti-oedema ($n = 1$) and proton pump inhibitor ($n = 1$). This study was approved by the medical ethics committees of Laval University and Laval University Medical Center. All subjects provided written informed consent before their inclusion in the study.

2.2. Adipose tissue sampling

Paired subcutaneous and omental 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 frozen at −80 °C for subsequent analyses.

2.3. Preadipocyte isolation and primary cultures

Samples of whole subcutaneous and omental fat tissue were used for the isolation of preadipocytes, according to a modified version of the Rodbell method (1964). Adipose tissue biopsies were washed in PBS, then chopped and digested with collagenase type I in Krebs–Ringer–Henseleit buffer for 45 min at 37 °C. Adipocyte suspensions were filtered through nylon mesh and washed thrice with KRH buffer. For cell size measurements, mature adipocyte suspensions were visualized using a contrast microscope attached to a camera and a computer interface. Pictures of the cell suspensions were taken and the Scion Image software was used to measure the size (diameter) of 250 adipocytes for each tissue sample.

Preadipocytes were isolated using a modification of a method previously described [31]. Briefly, the residual KRH buffer of the adipocyte isolation was centrifuged and the pellet was washed in Dulbecco's modified Eagle's medium (DMEM)-F12 without phenol red, supplemented with 10% charcoal-stripped fetal bovine serum, 0.25 μg/ml amphotericin B and 50 μg/ml gentamicin. These cells were treated with erythrocyte lysis buffer (154 mM NH4Cl, 10 mM KH2PO4, and 0.1 mM EDTA pH 7.5). DMEM-F12 was added and preadipocytes were subsequently filtered through 140 and 30 μm nylon mesh to remove endothelial cells, placed in culture plates and cultured at 37 °C under a 5% CO_2 atmosphere. Subcutaneous preadipocytes were maintained in DMEM-F12 containing 10% fetal calf serum, while omental preadipocytes were cultured in serum-free DMEM-F12, supplemented with insulin, panthotenate, transferrin, T3, hydrocortisone, fibroblast growth factor and biotin, in order to eliminate any residual endothelial cells. Medium was changed every 2–3 days.

Since our study is aimed at comparing 17β-HSD reductase activity and expression levels of 17β-HSD12 in primary cell cultures before and after *in vitro* differentiation, no additional cell line was tested for these parameters.

2.4. Differentiation of preadipocytes

To avoid the effect of the status and treatment of women involved in the study, we use *in vitro* differentiation of preadipocytes into mature adipocytes using adipogenic media. Briefly, preadipocytes were plated near confluence in 12-well culture plates and then subjected to differentiation stimuli 24–48 h later. To achieve this, cells were exposed to a commercially available adipogenic media containing glucocorticoids, insulin and 3-isobutyl-1-methylxanthine (IBMX) (Zen Bio, Inc., Research Triangle Park, NC), for 16–20 days. For each differentiated well, one control well was maintained in basal media, throughout this period. These cell preparations were used for enzymatic assays, RNA extraction and immunoblot analysis. Pictures of differentiated adipocytes and preadipocytes were taken with an inverted and phase-contrast microscope (Diaphot, Nikon, Canada) in order to evaluate differentiation level. Typical morphology of undifferentiated preadipocytes and differentiated adipocytes is illustrated in Fig. 1.

2.5. Determination of enzymatic activity

Estrogenic 17β-HSD activity was determined in preadipocytes and differentiated adipocytes primary cultures, as previously described [25]. To have a better comparison between the two types of cells, preadipocytes were equally split in 2 wells; one well was submitted to differentiation while the other well was kept undifferentiated in basal media. To avoid proliferation, cells were plated at near confluence. At the end of the differentiation process, 50 nM of [^{14}C]-labelled-E1 was added to both undifferentiated and differentiated cells and was incubated for 48 h at 37 °C in a final volume of 2 ml, to determine the estrogenic 17β-HSD activity.

After incubation, steroids were extracted from culture media using ether and the organic solvent was evaporated under air stream. Steroids were dissolved in 50 μl dichloromethane and applied to Silica Gel 60 thin layer chromatography (TLC) plates (Merck). Commercial [^{14}C]-E1 and E2 were used as standards. The separation of steroid metabolites was performed using a toluene–acetone (4:1) migration solvent system. Radioactivity was detected and quantified using a Storm 860 PhosphorImager System (Molecular Dynamics Inc., Sunnyvale, CA).

Table 1
Characteristics of women of the study ($n = 27$).

Variable	Mean ± S.D.	Range
Age (years)	43.7 ± 3.9	33.4–49.5
Weight (kg)	62.0 ± 13.1	42.4–104.1
BMI (kg/m^2)	23.6 ± 4.4	17.6–36.0
Waist circumference (cm)	86.7 ± 12.2	69–118
Subcutaneous adipocyte diameter (μm)	99.4 ± 16	76.9–128.1
Omental adipocyte diameter (μm)	79.8 ± 15.4	58.6–113.2

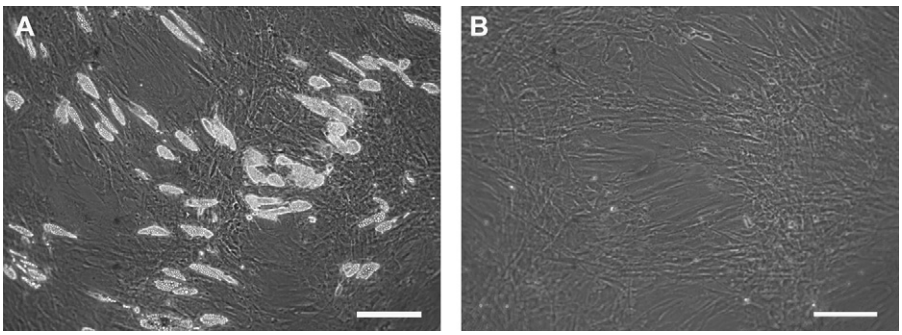

Fig. 1. Photographs of cultured preadipocytes and differentiated adipocytes. Preadipocytes (B) were cultured in basal media, while differentiated adipocytes (A) were obtained from exposition of preadipocytes to an adipogenic media for 16–20 days; both cultures are from the same patient and depot. Clear lipid droplets have been observed in differentiated adipocytes. Those cultures were used for enzymatic activity determination, mRNA quantitation and immunoblot analysis. Bar indicates 200 μm.

2.6. mRNA quantitation using real-time PCR

Total RNA was isolated using RNeasy Lipid tissue kit (Qiagen, Missisauga, Ontario), from preadipocytes and differentiated adipocytes cultures. Assessment of the quality of extracted total RNA was performed using the Bioanalyzer apparatus (Agilent technologies, Santa Clara, CA). First strand cDNA synthesis was accomplished using 1–5 μg total RNA in a reaction containing 200 units of Superscript III RNase H-reverse transcriptase (Invitrogen, Burlington, Ontario), 300 ng oligo$(dT)_{18}$, 500 μM dNTPs, 10 mM dithiothreitol, and 34 units porcine RNase inhibitor (Amersham Pharmacia Biotech Inc.) in a final volume of 50 μl incubated at 50 °C for 2 h. The resulting products were then treated with 1 μg RNase A for 30 min at 37 °C and purified with Qiaquick PCR purification kits (Qiagen, Missisauga, Ontario). A LightCycler PCR (Hoffman-La Roche Inc., Nutley, NJ) was used to measure quantitative expression using sets of primers shown in Table 2. The FastStart DNA Master SYBR green kit (Roche Diagnostics) was used in a final reaction volume of 20 μl containing 4 mM $MgCl_2$, 20 ng of each primer and 20 ng of the cDNA template. The PCR was carried out according to the following conditions: 95 °C/10 min, 40 cycles (95 °C/10 s, 62 °C/5 s, 72 °C/11 s, 81 °C/3 s), and temperature transition was 3 °C/s for all reactions. PCR results were normalized according to ATP5o expression levels. A specific standard curve was generated for each gene, 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 with the double-derivative method [32]. For each sample, the Cp value of the gene quantified was divided by that of the housekeeping gene. To further minimize inter-assay variability, this Cp ratio was then multiplied by the average Cp generated for housekeeping gene amplifications of all the samples examined in the present experiment.

2.7. Immunoblot analysis

Protein expression levels of type 12 17β-HSD in preadipocytes and differentiated adipocytes were determined by immunoblot analysis, as previously described [33], using an antibody raised against purified type 12 17β-HSD [34], and antiserum raised against type 1 17β-HSD [35]. An antibody raised against the transferrin receptor was used as a loading control (Zymed, San Francisco, USA). Briefly, cells were disrupted by sonication and electrophorized on 12% SDS–polyacrilamide gel. Separated peptides were transferred to a nitrocellulose filter using Bio-Rad power supply for 1 h at 100 V. Blots were treated using 5% fat-free milk in phosphate saline buffer (PBS) containing 0.2% NP-40 (Sigma–Aldrich Canada, Oakville, Ontario) for 1 h. Primary antibodies were added at a dilution of 1:500 and the blots were further incubated at 4° for 18 h. After incubation, immunoblots were washed thoroughly in PBS-NP-40 buffer, secondary antibodies conjugated with horseradish peroxidase (Amhersham Biosciences, Pistacaway, NJ) were added at a dilution 1:10000 and incubated for 1 h at room temperature. Blots were washed again in PBS-NP-40 buffer and revealed using enhanced chemiluminescent kit (PerkinElmer Life Science, Waltham, MA).

Table 2
Specific primers used for real-time PCR analysis.

Gene	Primers
HSD17b1	5′-TTCTTTGTCCCCTGGGTCTGTGT and 5′-ATGGGGGTCTCACTGTGTTGCT
HSD17b7	5′-TCCACCAAAAGCCTGAATCTCTC and 5′-GGGCTCACTATGTTTCTCAGGC
HSD17b12	5′-GGCTGGTCTTGAAATCGGCAT and 5′-TGCCACTGCCAGATGAAATGTT
ATP5o	5′-ATTGAAGGTCGCTATGCCACAG and 5′-AACGACTCCTTGGGTATTGCTTAA

2.8. Statistical analysis

Data are expressed as mean ± SEM. The analysis was performed using the JMP statistical software (SAS Institute, Cary, NC, USA). Paired *t*-test procedure was used to compare enzymatic activities and mRNA abundance in undifferentiated and differentiated adipocytes.

3. Results

3.1. Estrogenic 17β-HSD activity in preadipocytes and differentiated adipocytes

To determine whether there is a difference in the ability to produce active estrogens in preadipocytes and differentiated adipocytes, and whether this ability is related to subcutaneous or omental depot, we prepared primary preadipocytes and differentiated adipocytes from 12 subcutaneous and 9 omental adipose tissue biopsies and determined their estrogenic 17β-HSD activity converting E1 into E2, as described in Section 2. As illustrated in Fig. 2, preadipocytes from both subcutaneous and omental depots showed a low E1 conversion rate. Upon differentiation, adipocytes were found to exhibit a much higher (∼5-fold) 17β-HSD activity converting E1 into E2, in cells from both depots ($p < 0.0001$).

3.2. Determination of types 1, 7 and 12 17β-HSD mRNA expression levels using real-time PCR

To identify the enzyme that is responsible for the estrogenic 17β-HSD activity described above, we performed the quantifica-

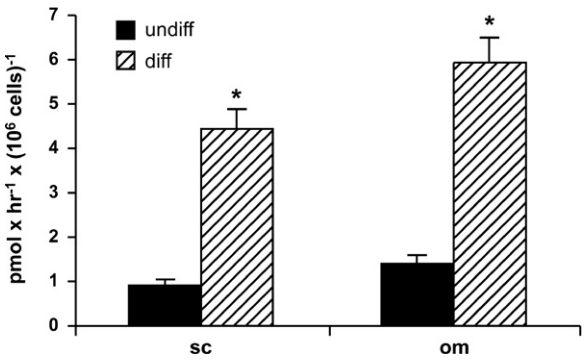

Fig. 2. Determination of estrogenic 17β-HSD activity in preadipocytes and differentiated adipocytes. Graphs showing the conversion of E1 into E2 in paired cultures of preadipocytes and differentiated adipocytes, obtained from subcutaneous (sc) and omental (om) adipose tissues biopsies. Experimental procedures were as described in Section 2. * indicates $p < 0.0001$ for differentiated adipocytes vs. preadipocytes.

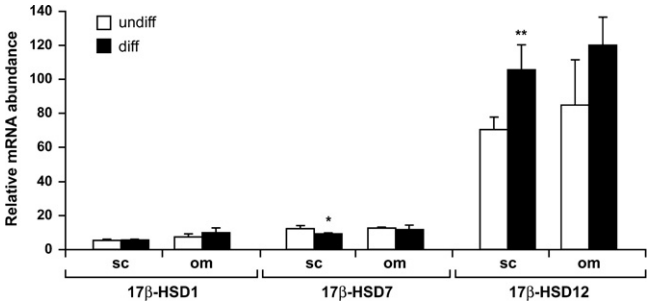

Fig. 3. Quantification of mRNA expression levels of estrogenic 17β-HSD enzymes. mRNA expression levels of type 1 17β-HSD (17β-HSD1), type 7 17β-HSD (17β-HSD7) and type 12 17β-HSD (17β-HSD12) in paired cultures of preadipocytes and differentiated adipocytes from subcutaneous (sc) and omental (om) fat depots were quantified using real-time PCR as described in Section 2. Expression levels of 17β-HSD enzymes are expressed as relative values using the housekeeping gene ATP5o expression levels as control. * indicates $p < 0.05$ and ** indicates $p < 0.01$ for differentiated adipocytes vs. preadipocytes.

tion of the mRNA expression levels of types 1, 7 and 12 17β-HSD enzymes, using real-time PCR, in 11 and 6 paired undifferentiated and differentiated adipocytes of subcutaneous and omental depots, respectively. As shown in Fig. 3, the mRNA expression levels of type 12 17β-HSD are much higher than those of types 1 and 7 17β-HSDs. Type 12 17β-HSD expression levels are also increased in differentiated adipocytes as compared to preadipocytes. In contrast, the mRNA expression levels of type 1 17β-HSD remain unchanged while those of type 7 17β-HSD appear to be decreased throughout the differentiation process.

3.3. Determination of type 12 17β-HSD protein level using immunoblot analysis

The increase in type 12 17β-HSD mRNA expression levels (~1.5-fold) found in differentiated adipocytes vs preadipocytes appears of lower amplitude than that of the estrogenic 17β-HSD activity (~5-fold) (Fig. 2). To determine whether this could be due to the difference of transcription and translation of 17β-HSD12, we performed immunoblot analysis using rabbit antibody raised against type 12 17β-HSD. As illustrated in Fig. 4, type 12 17β-HSD protein levels in differentiated adipocytes are much higher than those in preadipocytes. In contrast with the mRNA results, the data of type 12 17β-HSD protein levels appear to be in good agreement with the estrogenic 17β-HSD activity. In addition, immunoblot analysis using antibody raised against type 1 17β-HSD [35] did not permit to detect type 1 17β-HSD in these cells (data not shown).

4. Discussion

In the present study, we show that estrogenic 17β-HSD activity is increased following the differentiation of preadipocytes into adipocytes. This increased activity corresponds with an increase in protein (Fig. 4) expression of type 12 17β-HSD, and a high mRNA expression levels of type 12 17β-HSD as compared to the very low mRNA expression levels of two others estrogenic 17β-HSD enzymes, namely types 1 and 7 17β-HSD (Fig. 3). The results suggest that type 12 17β-HSD is probably involved in the local conversion of E1 into E2 in differentiated adipocytes. Indeed, type 12 17β-HSD catalyzes selectively the transformation of E1 into E2 in humans [27] and in primates [36] while it catalyses the transformation of both androgens and estrogens in the mouse [37] and in *C. elegans* [38].

In addition of its estrogenic 17β-HSD activity, it has been suggested that type 12 17β-HSD is involved in the elongation of fatty acids [26]. Whether the main physiological activity of type 12 17β-HSD is to perform estradiol biosynthesis or fatty acid elongation, or both, is still in investigation. Some recent data obtained in breast cancer cell lines are arguing against an estradiol-producing role for enzyme 17β-HSD12, mainly because they have found that type 1 17β-HSD is responsible for this activity in those cells [39]. Nevertheless, accumulated data obtained from our group suggest that type 12 17β-HSD might be an important 17β-HSD involved for intracrine estradiol biosynthesis. Indeed, since type 12 17β-HSD has been identified as a duplicate gene of type 3 17β-HSD, the enzyme responsible for male pseudohermaphrodism in boys having a deficiency of testicular 17β-HSD activity, the former is most likely also involved in steroidogenesis [27,40]. In agreement with their role in the formation of active sex steroids in human, type 3 17β-HSD

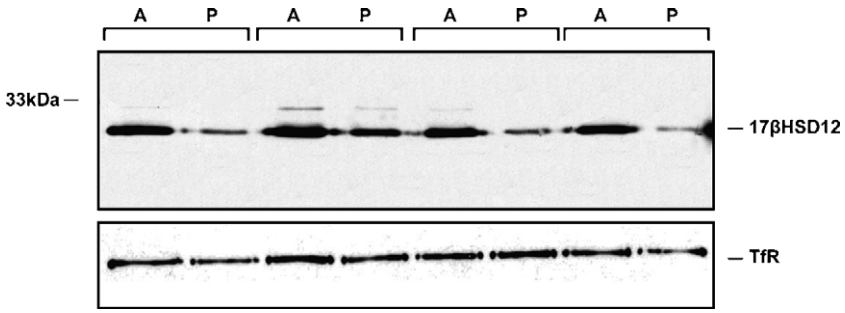

Fig. 4. Determination of type 12 17β-HSD protein level using immunoblot analysis. Western blots of 4 paired differentiated adipocytes (A) and undifferentiated preadipocytes (P), using a rabbit antibody raised against human type 12 17β-HSD; transferrin receptor antibody was used as a loading control. The experimental procedure was as described in Section 2.

is highly expressed in the testis, while type 12 17β-HSD is ubiquitously expressed, with higher expression levels in the mammary gland than types 1 and 7 17β-HSD [27]. The ability of type 12 17β-HSD to convert E1 into E2 is also observed in the mouse [37] and in *C. elegans* [38]. It is noteworthy that although let-767, the *C. elegans* ortholog of type 12 17β-HSD, is able to catalyze the transformation of E1 into E2, this organism does not possess estrogen receptors [41,42], thus suggesting that estrogen is not an active steroid in this organism. However, we [38] and others [43] have shown that let-767 is necessary for the transformation of cholesterol derivative into active compound crucial for *C. elegans* development. On the other hand, recent data have clearly shown that let-767 is required for the production of branched chain and long chain fatty acids [44]. Altogether, these data support that let-767 and most probably type 12 17β-HSD are multi-substrate enzymes able to catalyze the elongation of fatty acid [26] as well as the transformation of sex steroids and cholesterol [27,36,45]; the main activity of these enzymes in a typical cell type and tissue will depend on substrate availability.

Human and animal preadipocytes and adipocytes express both ERα and β, suggesting that local production of E2 could be involved in the modulation of ERα and β activity. It has been reported that E2 stimulates the proliferation of preadipocytes, as in mouse 3T3-L1 cell line [46] and in rat and human primary preadipocytes [47]. In contrast, the effects of E2 on adipocyte differentiation remain somehow controversial. Studies in rats have suggested that E2 stimulates the differentiation of preadipocytes into adipocytes [47]. However, Lea-Currie et al. have shown that E2 can inhibit the differentiation rate in mouse 3T3-L1 preadipocytes [46]. In addition of preadipocytes, similar results have been obtained with other stem-like cell lines: estradiol treatment of mouse preosteoblastic KS483 cells has been shown to enhance their differentiation into osteoblasts while it inhibits their differentiation into adipocytes [48], while mouse stromal osteoblast ST2 cell line treated with E2 have also revealed an inhibition of adipogenesis [49]. Similarly, using human bone marrow stromal cells, Heim et al. have shown that treatment with E2 inhibits adipogenic gene expression in differentiating cells; indeed, E2 appeared to downregulate several genes that display different expression profiles, PPARγ2 and C/EBPα being highly expressed early in the differentiation process (day 3) while LPL and adipsin being maximally expressed at the end of the differentiation (day 21) [50]. In this context, our finding that type 12 17β-HSD is maximally expressed at the end of the differentiation in human adipocytes could represent a mechanism that allows a time-dependent and cell type-specific regulation of estrogen production. This way, estradiol generation and its inhibitory effects regarding adipogenesis could be avoided during the differentiation process, while in adipocytes a high estradiol production would become possible, in order to regulate expression levels of important genes such as LPL and adipsin [50], as well as other crucial genes such as the β3-adrenergic receptor, which has already been shown to be stimulated by estradiol in adipocytes [51].

As mentioned above, the estrogenic 17β-HSD activity is increased ~5-fold in differentiated adipocytes. This increase of type 12 17β-HSD activity appears to correspond with the increase of type 12 17β-HSD protein expression levels Fig. 4. However, we cannot rule out the existence of an additional enzyme that could contribute to the conversion of E1 into E2 in differentiated adipocytes.

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References

[1] C. Belanger, V. Luu-The, P. Dupont, A. Tchernof, Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity, Horm. Metab. Res. 34 (11–12) (2002) 737–745.
[2] G. Bleau, K.D. Roberts, A. Chapdelaine, The in vitro and in vivo uptake and metabolism of steroids in human adipose tissue, J. Clin. Endocrinol. Metab. 39 (2) (1974) 236–246.
[3] T.M. Price, S.N. O'Brien, Determination of estrogen receptor messenger ribonucleic acid (mRNA) and cytochrome P450 aromatase mRNA levels in adipocytes and adipose stromal cells by competitive polymerase chain reaction amplification, J. Clin. Endocrinol. Metab. 77 (4) (1993) 1041–1045.
[4] M.N. Dieudonne, M.C. Leneveu, Y. Giudicelli, R. Pecquery, Evidence for functional estrogen receptors alpha and beta in human adipose cells: regional specificities and regulation by estrogens, Am. J. Physiol. Cell. Physiol. 286 (3) (2004) C655–661.
[5] S.B. Pedersen, P.S. Hansen, S. Lund, P.H. Andersen, A. Odgaard, B. Richelsen, Identification of oestrogen receptors and oestrogen receptor mRNA in human adipose tissue, Eur. J. Clin. Invest. 26 (4) (1996) 262–269.
[6] S.B. Pedersen, J.M. Bruun, F. Hube, K. Kristensen, H. Hauner, B. Richelsen, Demonstration of estrogen receptor subtypes alpha and beta in human adipose tissue: influences of adipose cell differentiation and fat depot localization, Mol. Cell. Endocrinol. 182 (1) (2001) 27–37.
[7] J.M. Joyner, L.J. Hutley, D.P. Cameron, Estrogen receptors in human preadipocytes, Endocrine 15 (2) (2001) 225–230.
[8] A. Anwar, P.G. McTernan, L.A. Anderson, J. Askaa, C.G. Moody, A.H. Barnett, M.C. Eggo, S. Kumar, Site-specific regulation of oestrogen receptor-alpha and -beta by oestradiol in human adipose tissue, Diabetes Obes. Metab. 3 (5) (2001) 338–349.
[9] Q. Wang, C. Hassager, P. Ravn, S. Wang, C. Christiansen, Total and regional body-composition changes in early postmenopausal women: age-related or menopause-related? Am. J. Clin. Nutr. 60 (6) (1994) 843–848.
[10] R.D. Hansen, C. Raja, R.J. Baber, D. Lieberman, B.J. Allen, Effects of 20-mg oestradiol implant therapy on bone mineral density, fat distribution and muscle mass in postmenopausal women, Acta. Diabetol. 1 (40 (Suppl)) (2003) S191–195.
[11] J.F. Couse, S.W. Curtis, T.F. Washburn, E.M. Eddy, D.W. Schomberg, K.S. Korach, Disruption of the mouse oestrogen receptor gene: resulting phenotypes and experimental findings, Biochem. Soc. Trans. 23 (4) (1995) 929–935.
[12] P.A. Heine, J.A. Taylor, G.A. Iwamoto, D.B. Lubahn, P.S. Cooke, Increased adipose tissue in male and female estrogen receptor-alpha knockout mice, Proc. Natl. Acad. Sci. U.S.A. 97 (23) (2000) 12729–12734.
[13] M.E. Jones, A.W. Thorburn, K.L. Britt, K.N. Hewitt, M.L. Misso, N.G. Wreford, J. Proietto, O.K. Oz, B.J. Leury, K.M. Robertson, S. Yao, E.R. Simpson, Aromatase-deficient (ArKO) mice accumulate excess adipose tissue, J. Steroid. Biochem. Mol. Biol. 79 (1–5) (2001) 3–9.
[14] G.N. Wade, J.M. Gray, T.J. Bartness, Gonadal influences on adiposity, Int. J. Obes. 1 (9 (Suppl)) (1985) 83–92.
[15] A. Tchernof, A. Desmeules, C. Richard, P. Laberge, M. Daris, J. Mailloux, C. Rheaume, P. Dupont, Ovarian hormone status and abdominal visceral adipose tissue metabolism, J. Clin. Endocrinol. Metab. 89 (7) (2004) 3425–3430.
[16] H. Homma, H. Kurachi, Y. Nishio, T. Takeda, T. Yamamoto, K. Adachi, K. Morishige, M. Ohmichi, Y. Matsuzawa, Y. Murata, Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter, J. Biol. Chem. 275 (15) (2000) 11404–11411.
[17] S.L. Palin, P.G. McTernan, L.A. Anderson, D.W. Sturdee, A.H. Barnett, S. Kumar, 17-Beta-estradiol and anti-estrogen ICI:compound 182,780 regulate expression of lipoprotein lipase and hormone-sensitive lipase in isolated subcutaneous abdominal adipocytes, Metabolism 52 (4) (2003) 383–388.
[18] T.M. Price, S.N. O'Brien, B.H. Welter, R. George, J. Anandjiwala, M. Kilgore, Estrogen regulation of adipose tissue lipoprotein lipase—possible mechanism of body fat distribution, Am. J. Obstet. Gynecol. 178 (1 Pt 1) (1998) 101–107.
[19] L.A. Anderson, P.G. McTernan, A.H. Barnett, S. Kumar, The effects of androgens and estrogens on preadipocyte proliferation in human adipose tissue: influence of gender and site, J. Clin. Endocrinol. Metab. 86 (10) (2001) 5045–5051.
[20] W.M. Geissler, D.L. Davis, L. Wu, K.D. Bradshaw, S. Patel, B.B. Mendonca, K.O. Elliston, J.D. Wilson, D.W. Russell, S. Andersson, Male pseudohermaphroditism caused by mutations of testicular 17 beta-hydroxysteroid dehydrogenase 3, Nat. Genet. 7 (1) (1994) 34–39.
[21] K.N. Qin, M.I. New, K.C. Cheng, Molecular cloning of multiple cDNAs encoding human enzymes structurally related to 3 alpha-hydroxysteroid dehydrogenase, J. Steroid. Biochem. Mol. Biol. 46 (6) (1993) 673–679.
[22] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu-The, Characteristics of a highly labile human type 5 17beta-hydroxysteroid dehydrogenase, Endocrinology 140 (2) (1999) 568–574.
[23] H. Peltoketo, V. Isomaa, O. Maentausta, R. Vihko, Complete amino acid sequence of human placental 17 beta-hydroxysteroid dehydrogenase deduced from cDNA, FEBS Lett. 239 (1) (1988) 73–77.
[24] A. Krazeisen, R. Breitling, K. Imai, S. Fritz, G. Moller, J. Adamski, Determination of cDNA, gene structure and chromosomal localization of the novel human 17beta-hydroxysteroid dehydrogenase type 7(1), FEBS Lett. 460 (2) (1999) 373–379.
[25] H. Liu, A. Robert, V. Luu-The, Cloning and characterization of human form 2 type 7 17beta-hydroxysteroid dehydrogenase, a primarily 3beta-keto reductase and estrogen activating and androgen inactivating enzyme, J. Steroid. Biochem. Mol. Biol. 94 (1–3) (2005) 173–179.
[26] Y.A. Moon, J.D. Horton, Identification of two mammalian reductases involved in the two-carbon fatty acyl elongation cascade, J. Biol. Chem. 278 (9) (2003) 7335–7343.

[27] V. Luu-The, P. Tremblay, F. Labrie, Characterization of type 12 17beta-hydroxysteroid dehydrogenase, an isoform of type 3 17beta-hydroxysteroid dehydrogenase responsible for estradiol formation in women, Mol. Endocrinol. 20 (2) (2006) 437–443.
[28] N. Yoshida, Y. Osawa, Purification of human placental aromatase cytochrome P-450 with monoclonal antibody and its characterization, Biochemistry 30 (12) (1991) 3003–3010.
[29] P.G. McTernan, A. Anwar, M.C. Eggo, A.H. Barnett, P.M. Stewart, S. Kumar, Gender differences in the regulation of P450 aromatase expression and activity in human adipose tissue, Int. J. Obes. Relat. Metab. Disord. 24 (7) (2000) 875–881.
[30] S.E. Bulun, E.R. Simpson, Competitive reverse transcription-polymerase chain reaction analysis indicates that levels of aromatase cytochrome P450 transcripts in adipose tissue of buttocks, thighs, and abdomen of women increase with advancing age, J. Clin. Endocrinol. Metab. 78 (2) (1994) 428–432.
[31] H. Hauner, P. Schmid, E.F. Pfeiffer, Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells, J. Clin. Endocrinol. Metab. 64 (4) (1987) 832–835.
[32] V. Luu-The, N. Paquet, E. Calvo, J. Cumps, Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction, Biotechniques 38 (2) (2005) 287–293.
[33] V. Luu-The, Y. Sugimoto, L. Puy, Y. Labrie, I. Lopez Solache, M. Singh, F. Labrie, Characterization, expression, and immunohistochemical localization of 5 alpha-reductase in human skin, J. Invest. Dermatol. 102 (2) (1994) 221–226.
[34] D. Song, G. Liu, V. Luu-The, D. Zhao, L. Wang, H. Zhang, G. Xueling, S. Li, L. Desy, F. Labrie, G. Pelletier, Expression of aromatase and 17beta-hydroxysteroid dehydrogenase types 1, 7 and 12 in breast cancer. An immunocytochemical study, J. Steroid. Biochem. Mol. Biol. 101 (2–3) (2006) 136–144.
[35] V. Luu The, C. Labrie, H.F. Zhao, J. Couet, Y. Lachance, J. Simard, G. Leblanc, J. Cote, D. Berube, R. Gagne, et al., Characterization of cDNAs for human estradiol 17 beta-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 5′-termini in human placenta, Mol. Endocrinol. 3 (8) (1989) 1301–1309.
[36] H. Liu, S. Zheng, V. Bellemare, G. Pelletier, F. Labrie, V. Luu-The, Expression and localization of estrogenic type 12 17beta-hydroxysteroid dehydrogenase in the cynomolgus monkey, BMC Biochem. 8 (2007) 2.
[37] P.G. Blanchard, V. Luu-The, Differential androgen and estrogen substrates specificity in the mouse and primates type 12 17beta-hydroxysteroid dehydrogenase, J. Endocrinol. 194 (2) (2007) 449–455.
[38] S. Desnoyers, P.G. Blanchard, J.F. St-Laurent, S.N. Gagnon, D.L. Baillie, V. Luu-The, *Caenorhabditis elegans* LET-767 is able to metabolize androgens and estrogens and likely shares common ancestor with human types 3 and 12 17{beta}-hydroxysteroid dehydrogenases, J. Endocrinol. 195 (2) (2007) 271–279.
[39] J.M. Day, P.A. Foster, H.J. Tutill, M.F. Parsons, S.P. Newman, S.K. Chander, G.M. Allan, H.R. Lawrence, N. Vicker, B.V. Potter, M.J. Reed, A. Purohit, 17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer, Int. J. Cancer 122 (9) (2008) 1931–1940.
[40] R. Mindnich, D. Deluca, J. Adamski, Identification and characterization of 17 beta-hydroxysteroid dehydrogenases in the zebrafish, Danio rerio, Mol. Cell. Endocrinol. 215 (1–2) (2004) 19–30.
[41] M.E. Baker, Evolution of adrenal and sex steroid action in vertebrates: a ligand-based mechanism for complexity, Bioessays 25 (4) (2003) 396–400.
[42] S. Bertrand, F.G. Brunet, H. Escriva, G. Parmentier, V. Laudet, M. Robinson-Rechavi, Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems, Mol. Biol. Evol. 21 (10) (2004) 1923–1937.
[43] L.M. Kuervers, C.L. Jones, N.J. O'Neil, D.L. Baillie, The sterol modifying enzyme LET-767 is essential for growth, reproduction and development in *Caenorhabditis elegans*, Mol. Genet. Genomics 270 (2) (2003) 121–131.
[44] E.V. Entchev, D. Schwudke, V. Zagoriy, V. Matyash, A. Bogdanova, B. Habermann, L. Zhu, A. Shevchenko, T.V. Kurzchalia, LET-767 is required for the production of branched chain and long chain fatty acids in *Caenorhabditis elegans*, J. Biol. Chem. 283 (25) (2008) 17550–17560.
[45] P.-G. Blanchard, V. Luu-The, Differential androgen and estrogen substrate specificity in the mouse and primates type 12 17β-hydroxysteroid dehydrogenase, J. Endocrinol. 194 (2) (2007) 449–455.
[46] Y.R. Lea-Currie, D. Monroe, M.K. McIntosh, Dehydroepiandrosterone and related steroids alter 3T3-L1 preadipocyte proliferation and differentiation, Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 123 (1) (1999) 17–25.
[47] M.N. Dieudonne, R. Pecquery, M.C. Leneveu, Y. Giudicelli, Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptor gamma2, Endocrinology 141 (2) (2000) 649–656.
[48] Z.C. Dang, R.L. van Bezooijen, M. Karperien, S.E. Papapoulos, C.W. Lowik, Exposure of KS483 cells to estrogen enhances osteogenesis and inhibits adipogenesis, J. Bone Miner. Res. 17 (3) (2002) 394–405.
[49] R. Okazaki, D. Inoue, M. Shibata, M. Saika, S. Kido, H. Ooka, H. Tomiyama, Y. Sakamoto, T. Matsumoto, Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) alpha or beta, Endocrinology 143 (6) (2002) 2349–2356.
[50] M. Heim, O. Frank, G. Kampmann, N. Sochocky, T. Pennimpede, P. Fuchs, W. Hunziker, P. Weber, I. Martin, I. Bendik, The phytoestrogen genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells, Endocrinology 145 (2) (2004) 848–859.
[51] M. Monjo, E. Pujol, P. Roca, alpha2- to beta3-Adrenoceptor switch in 3T3-L1 preadipocytes and adipocytes: modulation by testosterone, 17beta-estradiol, and progesterone, Am. J. Physiol. Endocrinol. Metab. 289 (1) (2005) E145–E150.