Depot differences in steroid receptor expression in adipose tissue: possible role of the local steroid milieu

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Depot differences in steroid receptor expression in adipose tissue: possible role of the local steroid milieu. Am J Physiol Endocrinol Metab 288: E200–E207, 2005. First published September 14, 2004; doi:10.1152/ajpendo.00270.2004.—Sex hormones play an important role in adipose tissue metabolism by activating specific receptors that alter several steps of the lipolytic and lipogenic signal cascade in depot- and sex-dependent manners. However, studies focusing on steroid receptor status in adipose tissue are scarce. In the present study, we analyzed steroid content [testosterone (T), 17β-estradiol (17β-E2), and progesterone (P4)] and steroid receptor mRNA levels in different rat adipose tissue depots. As expected, T levels were higher in males than in females (P = 0.031), whereas the reverse trend was observed for P4 (P < 0.001). It is noteworthy that 17β-E2 adipose tissue levels were higher in inguinal than in the rest of adipose tissues for both sexes, where no sex differences in 17β-E2 tissue levels were noted (P = 0.010 for retroperitoneal, P = 0.005 for gonadal, P = 0.018 for mesenteric). Regarding steroid receptor levels, androgen receptor (AR) and estrogen receptor (ER) densities were more clearly dependent on adipose depot location than on sex, with visceral depots showing overall higher mRNA densities than their subcutaneous counterparts. Besides, expression of ERα predominated over ERβ expression, and progesterone receptor (PR-B form and PR-A+B form) mRNAs were identical regardless of anatomic depot and sex. In vitro studies in 3T3-L1 cells showed that 17β-E2 increased ERα expression (P = 0.001) and AR expression (P = 0.001), indicating that estrogen can alter estrogenic and androgenic signaling in adipose tissue. The results highlighted in this study demonstrate important depot-dependent differences in the sensitivity of adipose tissues to sex hormones between visceral and subcutaneous depots that could be related to metabolic situations observed in response to sex hormones.

steroid receptors; testosterone; 17β-estradiol; progesterone

VISCERAL AND SUBCUTANEOUS adipose tissues display different metabolic properties, manifested by differences in the expression level of genes involved in fat cell metabolism, and in the secretion of adipose factors that could be involved in some pathologies in both rodents and humans (2, 3, 51, 59). Moreover, the existence of sex differences in adipose tissue metabolism affecting distribution pattern is well known. In this respect, we have previously investigated in rats the overall effect of sex and regional locations of the adipose tissue on some of the mechanisms affecting the lipolytic capacity, such as adrenergic receptor balance (45, 48) and several cascade steps at the postreceptor level, such as adenyl cyclase, protein kinase A and hormone-sensitive lipase (45). It has been suggested that these sex-dependent differences are due to variations in the hormonal environment, as sex hormones play an important role in the adipose tissue metabolism (32, 48).

Previous studies in rodents and humans have demonstrated that sex hormones are involved in the direct modulation of adipose tissue metabolism at multiple levels, acting at different steps of the lipolytic and lipogenic pathways (18, 23); coactivating or modulating the expression of adipogetic transcription factors (23); and altering the adipocyte proliferation (1), the glucose metabolism (15), and the expression of several adipocyte hormones (26, 35, 50, 56).

In addition to serving as a steroidal reservoir, adipose tissue is one of the most important extragonadal sources of steroids, due to the specific expression of steriodogenic enzymes, such as aromatase (4), suggesting a potential impact on the local adipose tissue metabolism that would be independent of the sex hormone plasma milieu (53). Steroid hormones exert their effects through specific receptors that belong to the superfamily of nuclear hormone receptors, which are widely expressed in several tissues including adipose tissue (7, 10, 20, 21, 31, 36). The steroid receptor action is tripartite, involving a receptor with its corresponding ligands and coregulator proteins that forms a complex that interacts with basal transcription factors that ultimately regulate transcription of target genes (5).

In this study, sex hormone content and steroid receptor mRNA were determined in adipose tissues from different regions in male and female rats to elucidate their different sensitivities to hormonal action. In addition, an in vitro model was used to further address the role of steroid hormones modulating steroid hormone receptor expression.

METHODS

Animals

One hundred ten-day-old male and female Wistar rats were used. Males and females were housed separately three per cage in the same room at 22°C, with a 12:12-h light-dark cycle, with free access to drinking water and standard chow pellets (Panlab, Barcelona, Spain). All animals were killed by decapitation at the start of the light cycle. Animal studies were in agreement with the Universitat de les Illes Balears Bioethics Committee guidelines for animal care.

Experimental Setting 1

Sex hormone content and receptor mRNA were analyzed in male and female white adipose tissue from different anatomic locations to assess possible depot and sex differences. Inguinal (subcutaneous depot), gonadal, and mesenteric (visceral depot) and retroperitoneal
tissues (nonsubcutaneous nonvisceral) were dissected and stored at -80°C until use.

**Experimental Setting 2**

To investigate whether the sex hormone content could be involved in the regulation of the steroid receptor mRNA expression in adipocytes, we designed an in vitro model using the murine 3T3-L1 preadipocyte cell line. Differentiated adipocytes were treated with a 10⁻⁷ M dose of testosterone (T), 17β-estradiol (17β-E₂), or progesterone (P₄).

**Steroid Assays**

Determination of T, 17β-E₂, and P₄ levels from adipose tissue samples was adapted from methods previously described (58). Briefly, 600 mg of frozen tissue were homogenized in 1 ml of phosphate buffer, pH 7.4. The homogenate was extracted twice with 5 ml of ethanol-acetone (1:1, vol/vol). The extract was concentrated under N₂ at 37°C, and the residue was dissolved in phosphate buffer containing 2% BSA. Levels of T, 17β-E₂, and P₄ in tissue extracts and plasma were analyzed using a commercially available immunoenzymatic determination kit.

**Cell Culture and Treatments**

Murine 3T3-L1 preadipocytes (American Type Culture Collection) were routinely cultured at 37°C in a humidified atmosphere with 8% CO₂. The cells were maintained in growth medium with the following constituents: DMEM supplemented with 10% newborn calf serum, 4 mM glutamine, and antibiotics (50 IU penicillin/ml and 50 μg streptomycin/ml).

Cell differentiation was initiated 2 days after confluence by incubation for 2 days in a differentiation medium containing 10% fetal calf serum, 4 mM glutamine, and antibiotics (50 μg penicillin/ml and 50 μg streptomycin/ml) and additionally 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, and 5 μM insulin. This was followed by a 2-day incubation in a differentiation medium containing 5 μM insulin and in the absence of insulin thereafter for 3 days, when cells presented a differentiated morphology with important lipid accumulation. Once differentiated, the cells were placed in serum-free medium consisting of DMEM-F12 (1:1) and supplemented with 0.5% BSA, 4 mM glutamine, and antibiotics (50 μg penicillin/ml and 50 μg streptomycin/ml) for hormone treatments.

The treatments were carried out on day 10 postconfluence. Sex hormones (T, 17β-E₂, and P₄) were dissolved in ethanol and added to the corresponding flasks to a final concentration of 10⁻⁷ M for 24 h.

**Total RNA Extraction**

Total RNA was isolated from rat adipose tissues and murine 3T3-L1 adipocytes using TriPure reagent as described earlier (47). The integrity of RNA was verified by optical density (OD) absorption ratio OD₂₆₀/OD₂₃₀ between 1.7 and 1.8.

**Reverse Transcription**

For reverse transcription (RT) of estrogen receptor-α (ERα), estrogen receptor β₁ (ERβ₁), androgen receptor (AR), and progesterone receptor (PR-B for the specific isoform B and PR-A+B for both isoforms), 1 μg of total RNA was reverse transcribed to cDNA at 42°C for 15 min with 25 U of MuLV reverse transcriptase in a 10-μl volume of RT reaction mixture containing 10 mM Tris·HCl (pH 9.0 at 25°C), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl₂, 2.5 μM random hexamers, 10 U of RNase inhibitor, and 500 μM of each dNTP in a DNA Gene Amp 9700 thermal cycler (Applied Biosystems, Barcelona, Spain).

**Real-Time RT-PCR**

The primers used were designed with specific primer analysis software Primer3 (Whitehead Institute for Biomedical Research) and Oligo Analyzer 3.0 (Integrated DNA Technologies), and the specificity of the sequences was analyzed by Fasta in the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/) (Table 1).

Real-time PCR was performed using a LightCycler rapid thermal cycler system (Roche Diagnostics, Barcelona, Spain). Reactions were performed in a 10-μl volume containing 3 μl of cDNA sample (diluted 1:10) with 0.5 μM primers and 3 mM MgCl₂ as well as dNTPs, Taq DNA polymerase, and reaction buffer provided in the LightCycler FastStart DNA Master SYBR Green I mix. All real-time conditions are summarized in Table 1. Product specificity was confirmed in initial experiments by agarose gel electrophoresis and routinely by melting curve analysis. PCR efficiencies were calculated from the slopes generated in the LightCycler Software 3.3.

For the mathematical analysis, crossing point (CP) values were used for each transcript. CP is defined as the point at which fluorescence rises appreciably above the background fluorescence. “Fit Point Method” was performed in the LightCycler software 3.3 (Roche Diagnostics, Barcelona, Spain).

<p>| Table 1. Oligonucleotide primer sequences and amplification conditions |
|-----------------|------------------|-----------------|-----------------|------------------|</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5'-3')</th>
<th>Forward and Reverse</th>
<th>Denaturation Temp. °C, s</th>
<th>Annealing Temp. °C, s</th>
<th>Elongation Temp. °C, s</th>
<th>Amplific Melting Temp. °C</th>
<th>PCR Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rAR</td>
<td>5'-ccc atc gac tat tac ttc cc-3'</td>
<td>95 (3)</td>
<td>60 (10)</td>
<td>72 (18)</td>
<td>84</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>rERα</td>
<td>5'-gtc cga tgg ccc gaa gta at-3'</td>
<td>95 (5)</td>
<td>60 (8)</td>
<td>72 (12)</td>
<td>83</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>rERβ₁</td>
<td>5'-ggc tgg cca aaa tcc ctc-3'</td>
<td>95 (3)</td>
<td>60 (8)</td>
<td>72 (12)</td>
<td>87</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>rPR-B</td>
<td>5'-gtg tgg tga tgg ctc tgg ctc-3'</td>
<td>95 (3)</td>
<td>60 (8)</td>
<td>72 (12)</td>
<td>84</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>rPR-A+B</td>
<td>5'-ggc ttc ctc ttc ctc ctc-3'</td>
<td>95 (5)</td>
<td>60 (8)</td>
<td>72 (15)</td>
<td>83</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>mAR</td>
<td>5'-gtg tgg tgg ccc ccc ccc ccc-3'</td>
<td>95 (10)</td>
<td>60 (8)</td>
<td>72 (12)</td>
<td>83</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>mERα</td>
<td>5'-gaa ccc ccc ccc ccc ccc ccc-3'</td>
<td>95 (10)</td>
<td>60 (8)</td>
<td>72 (12)</td>
<td>82</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>mPR-A+B</td>
<td>5'-cag cag ccc ccc ccc ccc ccc-3'</td>
<td>95 (5)</td>
<td>60 (8)</td>
<td>72 (15)</td>
<td>86</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

Temperature and time PCR conditions and amplification efficiencies are given (°C, s). r, rat primers; m, mouse primers; AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor.
Table 2. Precision and variability test of PCR amplifications

<table>
<thead>
<tr>
<th></th>
<th>rAR</th>
<th>rERα</th>
<th>rERβ</th>
<th>rPR-B</th>
<th>rPR-A+B</th>
<th>mAR</th>
<th>mERα</th>
<th>mPR-A+B</th>
</tr>
</thead>
</table>
| Intra-
run | 0.63 | 0.51 | 0.80 | 1.22  | 0.61    | 0.99 | 0.27 | 0.45    |
| Inter-
run   | 0.55 | 0.86 | 1.90 | 1.54  | 3.09    | 1.41 | 0.51 | 1.12    |

Intra-assay (test precision) and interassay variation (test variability) of LightCycler real-time RT-PCR. The coefficient of variation expressed as percentage (%) was calculated from 2 replicate determinations.

Diagnostics), at which CP was measured at constant fluorescence level. It should be noted that lower CP means higher expression rate and that a difference of 1 cycle number means theoretically a twofold difference in mRNA levels, although this is dependent on the amplification rate (between 1.7 and 2.2 in our study). Statistical data analysis was performed using the Relative Expression Software Tool (REST) for groupwise comparison and statistical analysis of relative expression results in real-time PCR, as previously described (43). Coefficient of variation based on the variation of CP from CP mean value expressed as percentage was given as test variability (Table 2). Test reproducibility for all investigated transcripts was low in intertest experiments and even lower in intratest experiments.

Drugs and Chemicals

T, 17β-E2, and P4 were supplied by Sigma (Madrid, Spain). Tripure reagent and LightCycler FastStart DNA Master SYBR Green I mix were provided by Roche Diagnostics (Madrid, Spain). The reaction reagents for the retrotranscriptase were supplied by Applied Biosystems (Barcelona, Spain), and the sex hormone immunoenzymatic determination kit was supplied by DIAIMETRA (Milan, Italy). Cell culture reagents were supplied by Sigma and Invitrogen (Barcelona, Spain), and routine chemicals were from Merck (Barcelona, Spain) and Panreac (Barcelona, Spain).

Statistical Analysis

Adipose tissue weight and sex steroid concentration data are presented as group mean values ± SE. Differences between groups were assessed by Student’s t-test in adipose tissue weight data and sex hormone plasma levels, and, in the case of sex hormone tissue levels, two-way analysis of variance (ANOVA) was used, followed by a post hoc least significant difference comparison when a depot effect (D) was shown. The analysis was performed with SPSS 10.0 for Windows. The level of probability was set at P < 0.05 as statistically significant for both ANOVA and Student’s t-test.

The statistical PCR data analysis was performed using REST. The statistical model used was the Pair Wise Fixed Reallocation Randomisation Test (43). Differences in expression between groups were assessed using the means for statistical significance by randomization tests, a proper model to avoid the normal distribution assumption of the data. The level of probability was set at P < 0.05 as statistically significant.

RESULTS

Adipose Tissue Weight

As seen in Table 3, adult male rats showed larger adipose depots than females in all different locations (P < 0.001 for inguinal, P = 0.001 for retroperitoneal and gonadal, P = 0.003 for mesenteric). When relative values with respect to body weight are considered, male rats showed higher values only for the inguinal depot (P = 0.014), and no sex differences were observed in gonadal, mesenteric, and retroperitoneal depots.

Steroid Concentrations in Plasma and Adipose Tissues

Plasma T levels were significantly higher in male than in female rats (P = 0.031). The 17β-E2 and P4 plasma concentrations were higher in female than in male rats, although these differences were of statistical significance only for P4 (P < 0.001).

The same sex profile was depicted when sex hormone contents of the adipose tissue were analyzed, as T levels were higher in males (sex effect P < 0.001), no differences between sexes were observed in 17β-E2 levels, and P4 levels were higher in females (sex effect P < 0.001; Table 4). Moreover, some depot-specific differences were shown, and T adipose tissue levels were lower in the inguinal depot than in the retroperitoneal (P = 0.030) and gonadal depots, although the latter did not reach statistical significance (P = 0.097). Moreover, the inguinal depot showed higher 17β-E2 levels than the rest of the adipose depots. No depot differences in P4 levels of tissue were observed (Table 4).

Regional and Sex Differences in Steroid Receptor mRNA Expression

AR. As shown in Table 5, male inguinal and retroperitoneal depots showed a lower AR expression (shown as CP, an inverse of expression data) than male gonadal and mesenteric depots (inguinal vs. gonadal, P = 0.035; retroperitoneal vs. gonadal, P = 0.003; retroperitoneal vs. mesenteric, P = 0.004). In contrast, in female rats, no tissue differences were found. Although AR mRNA levels were higher in almost all cases in males than in females, sex differences were not statistically significant for any of the adipose depots, probably due to the low mRNA levels and the high variability of AR shown by females.

ER. Two main estrogen receptors are involved in the estrogenic action, ERα and ERβ (34), and it is well established that both receptors have several splice variants whose presence can modify tissue-estrogenic responsiveness. Thus we decided first of all to find out which was the major form of both receptors

Table 3. Adipose tissue weight of different locations

<table>
<thead>
<tr>
<th>Adipose Tissue Weight, g tissue</th>
<th>Adipose Tissue, mg tissue/g animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3.13 ± 0.14</td>
</tr>
<tr>
<td>Female</td>
<td>1.65 ± 0.12*P &lt; 0.001</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.03 ± 0.15</td>
</tr>
<tr>
<td>Female</td>
<td>1.10 ± 0.18*P = 0.001</td>
</tr>
<tr>
<td>Gonadal</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3.01 ± 0.19</td>
</tr>
<tr>
<td>Female</td>
<td>2.09 ± 0.17*P = 0.001</td>
</tr>
<tr>
<td>Mesenteric</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.64 ± 0.16</td>
</tr>
<tr>
<td>Female</td>
<td>1.84 ± 0.17*P = 0.003</td>
</tr>
</tbody>
</table>

Data represent means ± SE of 12 animals per group. *Males vs. females; Student’s t-test significance was P < 0.05.
in adipose tissues by use of the primers and protocols previously described by classical RT-PCR (38, 44) before going further in the study of mRNA steroid receptors in the adipose depots. The presence of ERα spliced variants, previously described for other tissues (38), was almost negligible compared with the full-length form present in all of the adipose tissue analyzed, only ERβ3 and ERβ4 being detected and identified (Fig. 1A). In fact, ERβ isoforms β1 and β2 were not detected in adipose tissue with classical PCR, denoting lower expression levels of these receptors, although in this present experiment ERβ1, the most estradiol-sensitive isoform, but not ERβ2, was detected by real-time PCR (Fig 1B).

Both ERα full-length and ERβ1 mRNA analyses were undertaken using real-time PCR. Thus the male inguinal depot showed lower ERα mRNA levels compared with retroperitoneal (P = 0.005), gonadal (P = 0.001), and mesenteric depots (P = 0.002); the same tendency was observed in females but was statistically significant only for the gonadal depot (P = 0.001; Table 5). Moreover, in both sexes, the gonadal depot showed higher ERα mRNA levels than the retroperitoneal one (P = 0.040 for males, P = 0.037 for females). In addition, retroperitoneal, gonadal, and mesenteric adipose tissue differed in both sexes in ERα mRNA levels, showing only in the retroperitoneal adipose tissue of males a statistically significant threefold higher mRNA levels than females (P = 0.040).

For ERβ1 mRNA levels, only females showed differences between depots (Table 5), as inguinal adipose tissue had lower mRNA levels than mesenteric and retroperitoneal adipose

Table 5. Effect of sex and tissue location on steroid receptor mRNA expression in white adipose tissues

<table>
<thead>
<tr>
<th>Sex Steroid Receptor, CP ± SE</th>
<th>AR</th>
<th>ERα</th>
<th>ERβ1</th>
<th>PR-B</th>
<th>PR-A + B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27.4 ± 1.3 (1.0)</td>
<td>28.0 ± 0.4 (1.0)</td>
<td>38.7 ± 2.7 (1.0)</td>
<td>29.0 ± 0.8 (1.0)</td>
<td>31.7 ± 3.7 (1.0)</td>
</tr>
<tr>
<td>Female</td>
<td>28.3 ± 1.2 (0.5)</td>
<td>29.0 ± 0.8 (2.1)</td>
<td>41.2 ± 1.2 (0.2)</td>
<td>28.5 ± 0.4 (1.5)</td>
<td>32.0 ± 0.4 (0.8)</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28.8 ± 0.9 (0.4)</td>
<td>25.8 ± 0.4 (5.1)</td>
<td>36.9 ± 0.0 (3.5)</td>
<td>27.8 ± 0.5 (2.6)</td>
<td>33.0 ± 4.1 (0.4)</td>
</tr>
<tr>
<td>Female</td>
<td>28.8 ± 2.9 (0.4)</td>
<td>27.5 ± 0.9 (1.4)</td>
<td>38.1 ± 0.1 (1.5)</td>
<td>28.1 ± 0.7 (2.0)</td>
<td>33.2 ± 2.1 (0.4)</td>
</tr>
<tr>
<td>Gonadal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24.9 ± 1.5 (7.2)</td>
<td>24.6 ± 1.0 (12.4)</td>
<td>37.8 ± 2.2 (1.9)</td>
<td>28.8 ± 0.6 (1.2)</td>
<td>33.3 ± 1.3 (0.3)</td>
</tr>
<tr>
<td>Female</td>
<td>27.2 ± 2.2 (1.2)</td>
<td>25.5 ± 0.7 (6.4)</td>
<td>40.3 ± 0.5 (0.3)</td>
<td>28.3 ± 0.5 (1.7)</td>
<td>33.4 ± 1.3 (0.3)</td>
</tr>
<tr>
<td>Mesenteric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25.6 ± 0.8 (4.1)</td>
<td>25.5 ± 0.3 (6.4)</td>
<td>37.8 ± 1.4 (1.9)</td>
<td>28.7 ± 1.0 (1.3)</td>
<td>32.2 ± 1.0 (0.7)</td>
</tr>
<tr>
<td>Female</td>
<td>27.0 ± 1.4 (1.4)</td>
<td>26.2 ± 0.7 (3.8)</td>
<td>40.3 ± 0.5 (0.3)</td>
<td>28.3 ± 0.5 (1.7)</td>
<td>33.4 ± 1.3 (0.3)</td>
</tr>
</tbody>
</table>

Data represent crossing point (CP) means ± SE of 5–6 animals per group. Fold changes (in parentheses) were established using inguinal male as 1. Differences in expression between groups were assessed by Pair Wise Fixed Reallocation Randomisation Test. The level of probability was set at P < 0.05 as statistically significant: *males vs. females; †retroperitoneal, gonadal or mesenteric vs. inguinal; ‡retroperitoneal vs. gonadal or mesenteric.
To date, several studies demonstrate a role of the adipose tissue as reservoir and sex hormone extragonadal biosynthesis site (4, 9, 55). However, the differential role of the different depots in steroid metabolism remains unclear.

Adipose tissue sex hormone levels were markedly high, which is in agreement with the function of adipose tissue acting as a sex hormone reservoir. Nevertheless, it is noteworthy that no differences in 17β-E2 tissue levels were noted between females and males, which could suggest a local estrogen synthesis system independent of gonadal action (4, 54). In both sexes, inguinal adipose tissue, which is characterized by scarce vascularization (12), had a 17β-E2-to-T ratio two- to threefold higher than in other adipose tissues (P < 0.001, data not shown). To this extent, McTernan and coworkers (28, 29) have recently demonstrated that human subcutaneous preadipocytes showed a higher aromatase activity compared with visceral ones. Thus, the higher 17β-E2-to-T ratio shown by the inguinal depot could be related to the higher proliferative capacity of this tissue, especially when it is taken into account that estrogens can increase preadipocyte proliferation rate (1), whereas androgens can inhibit both proliferation and differentiation (11).

We have also observed variations in the mRNAs of steroid receptors that are adipose tissue location and sex dependent, which could be key in the understanding of adipose tissue steroid responsiveness.

The higher AR mRNA content observed in the gonadal and mesenteric adipose tissue (visceral depots), in contrast to inguinal (subcutaneous) and retroperitoneal tissues (nonvisceral nonsubcutaneous), is in agreement with the higher AR contents found in other studies (10, 21). These differences in the AR expression along with the differences found in T content suggest a higher responsiveness of visceral depots to androgens than the subcutaneous ones, which has also been previously reported (8) and could explain the depot-related differences in the androgen-modulated adipokines (26, 35, 50). This androgenic state in visceral depots could also be responsible for the higher lipolytic activity of this depot compared with the subcutaneous ones previously described (45, 57), as some studies have demonstrated that androgens increase the lipolytic capacity and specifically decrease lipoprotein lipase (LPL) in visceral depots (8, 46). These data are in agreement with the higher lipid accumulation shown by an AR knockout (ARKO) model compared with wild type (49).

T did not stimulate mRNA expression of its own receptor at the concentration tested. However, AR mRNA was slightly upregulated by 17β-E2 in this study, as reported as well in mouse uterus (42), and downregulated by P4. This could

Table 6. Effect of sex hormone treatment on ERα, AR, and PR-A+B mRNA levels in 3T3-L1 adipocyte cell line

<table>
<thead>
<tr>
<th>Sex Steroid Receptor</th>
<th>AR</th>
<th>ERα</th>
<th>PR-A+B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.0±0.1 (1.0)</td>
<td>31.1±0.3 (1.0)</td>
<td>27.0±0.5 (1.0)</td>
</tr>
<tr>
<td>Testosterone, 10−7 M</td>
<td>27.2±0.2 (0.9)</td>
<td>30.2±0.4 (1.8)</td>
<td>26.2±0.2 (1.5)</td>
</tr>
<tr>
<td>Estradiol, 10−7 M</td>
<td>26.6±0.1 (1.3)†</td>
<td>29.3±0.3 (3.2)†</td>
<td>26.3±0.3 (1.4)</td>
</tr>
<tr>
<td>Progesterone, 10−7 M</td>
<td>27.3±0.1 (0.8)*</td>
<td>30.8±0.3 (1.2)</td>
<td>27.0±0.1 (1.0)</td>
</tr>
</tbody>
</table>

Data represent CP means ± SE of 6 samples per group. Fold changes (in parentheses) were established using control group as 1. Differences in expression between groups were assessed by a Pair Wise Fixed Reallocation Randomisation Test. The level of probability was set as P < 0.05 as statistically significant: *P = 0.04, †P = 0.001, treatment vs. control.

Fig. 1. A: presence or absence of mRNA of estrogen receptor (ERα) full-length and δ3- and δ4-forms in several tissues. B: ERβ1 and ERβ2 in ovary and adipose tissue by classical RT-PCR.
explain why the tendency to lower AR mRNA levels observed in female depots showed higher P₄ levels and no differences vs. males in the 17β-E₂ levels.

Studies carried out in rodent models show that the effect of estrogens is location and hormonal status dependent (39). The differences in the ERα expression reported here could suggest a higher estrogen sensitivity in visceral than in subcutaneous depots, which could explain the higher estrogen-stimulated lipolysis in visceral adipose cells that is absent in subcutaneous ones (14) and also the higher adipose deposition present only in the visceral location in the absence of ER as occurred in the ERα knockout (αERKO) mice (17). These data, in conjunction with the findings of higher LPL by the aromatase knockout model (30), support the idea that both the local estrogen synthesis and ER mRNA expression reported in this study could be key factors in the adipose tissue metabolism and highlight an important depot-dependent estrogen-ER signaling.

ERα mRNA expression is also under the hormonal milieu control. We have observed in vitro that 17β-E₂ increases ERα mRNA levels. However, ERα mRNA expression stimulation by 17β-E₂ cannot itself explain why inguinal adipose tissue, with higher 17β-E₂ levels, has the lowest ERα mRNA expression and subsequently points to multiple hormonal effects (6, 13, 22), although specific culture conditions affecting ERα mRNA expression cannot be discarded (40).

In vitro T treatment also triggered a trend to increase ERα levels. These effects are in agreement with the ER upregulation reported in T-treated hamsters (20). Nevertheless, ERα mRNA expression was not stimulated in vitro by P₄, which would be in agreement with the slightly higher ERα mRNA levels in male depots, at least in the retroperitoneal depot.

The exact function of ERβ in adipose tissue remains unclear. Our study reported some sex and location differences in ERβ1 mRNA levels, although its expression was very low compared with that of ERα, suggesting that ERβ has a scant role in adipose tissue metabolism, and this is in accord with αERKO and βERKO studies (33, 37). Recently, it has been hypothesized that ERβ would act as modulator of ERα action (25), and therefore ERβ signaling could modify the tissue- and sex-specific response to estrogens predominantly exerted by ERα.

The presence of PR in adipose tissue is a matter of controversy. Several studies have suggested an absence of PR in male rat adipose depots, whereas in female adipocytes it has been proposed that PR is present due to estrogen stimulation (16, 41). However, in our study, the presence of PR mRNA, even in male adipose depots, has been clearly demonstrated. Nevertheless, no depot and sex differences were observed either for the specific B isoform or for the A+B combination. To this extent, our in vitro model failed to show any modulation of A+B mRNA expression under treatment with sex hormones, including 17β-E₂, which has been previously shown to upregulate PR in sheep adipose tissue (27).

P₄ seems to have a marked effect on adipose tissue metabolism (24, 52). Studies carried out by our group (Monjo M, unpublished results) have demonstrated that P₄ stimulates both the proliferation rate in preadipocytes and the β₃-adrenergic receptor mRNA expression in mature adipocytes. Thus the higher levels of P₄ reached in female adipose tissues could be responsible, at least in part, for the higher lipolytic capacity of females (45). From our data, it could be hypothesized that P₄ adipose tissue responsiveness is principally controlled by the differences in the P₄ content (higher in females than in males) instead of their nuclear receptor isoforms (A or B form), of which no differences in mRNA expression were observed between males and females. All in all, posttranscriptional regulation of steroid receptors by sex hormones that could alter the steroid receptor pool in the cell cannot be discarded.

In summary, our study demonstrates important depot- and sex-dependent differences in the sensitivity of adipose tissues to the sex hormones, with visceral depots having an overall higher androgen and estrogen receptor expression than subcutaneous ones. Furthermore, important depot-dependent differences in the sex hormone contents, especially 17β-estradiol, between subcutaneous and visceral depots have also been presented. These results could be key to understanding the sometimes contradictory effects of sex steroids on adipose tissue metabolism, which are dependent on factors such as hormone status, sex, and depot location.

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