Estrogen Controls Lipolysis by Up-Regulating α2A-Adrenergic Receptors Directly in Human Adipose Tissue through the Estrogen Receptor α. Implications for the Female Fat Distribution

STEEN B. PEDERSEN, KURT KRISTENSEN, PERNILLE A. HERMANN, JOHN A. KATZENELLENBOGEN, AND BJØRN RICHELSEN

Department of Endocrinology and Metabolism (S.B.P., K.K., P.A.H., B.R.), Aarhus Amtssygehus, Aarhus University Hospital; Faculty of Health Sciences (B.R.), Aarhus University, DK-8000 Aarhus C., Denmark; and Department of Chemistry (J.A.K.), University of Illinois, Urbana, Illinois 61801

Estrogen seems to promote and maintain the typical female type of fat distribution that is characterized by accumulation of adipose tissue, especially in the sc fat depot, with only modest accumulation of adipose tissue intraabdominally. However, it is completely unknown how estrogen controls the fat accumulation.

We studied the effects of estradiol in vivo and in vitro on human adipose tissue metabolism and found that estradiol directly increases the number of antilipolytic α2A-adrenergic receptors in sc adipocytes. The increased number of α2A-adrenergic receptors caused an attenuated lipolytic response of epinephrine in sc adipocytes; in contrast, no effect of estrogen on α2A-adrenergic receptor mRNA expression was observed in adipocytes from the intraabdominal fat depot.

These findings show that estrogen lowers the lipolytic response in sc fat depot by increasing the number of antilipolytic α2A-adrenergic receptors, whereas estrogen seems not to affect lipolysis in adipocytes from the intraabdominal fat depot. Using estrogen receptor subtype-specific ligands, we found that this effect of estrogen was caused through the estrogen receptor subtype α.

These findings demonstrate that estrogen attenuates the lipolytic response through up-regulation of the number of antilipolytic α2A-adrenergic receptors only in sc and not in visceral fat depots. Thus, our findings offer an explanation how estrogen maintains the typical female sc fat distribution because estrogen seems to inhibit lipolysis only in sc depots and thereby shifts the assimilation of fat from intraabdominal depots to sc depots. (J Clin Endocrinol Metab 89: 1869–1878, 2004)

A DIPOSE TISSUE DISTRIBUTION has a large impact on the risks associated with obesity because sc fat accumulation is much less harmful than intraabdominal accumulation of adipose tissue (1, 2). Factors controlling human adipose tissue accumulation/metabolism are largely unknown; however, low estrogen levels (menopause) are somehow associated with loss of sc fat and gain of visceral fat (3). Furthermore, estrogen treatment of male-to-female transsexuals can increase the amount of sc adipose tissue; thus, estrogen changes the male fat distribution into a female type of fat accumulation (4).

The pathways by which estrogens affect the adipose tissue metabolism are not understood. Some studies indicate that estradiol might influence the lipolytic capability of the adipose tissue. In a study on male-to-female transsexuals, estradiol treatment inhibited basal lipolysis (5). Moreover, a study in postmenopausal women receiving hormone replacement therapy (HRT) showed inhibition of the epinephrine-stimulated lipolysis in human sc adipose tissue in the HRT group (6).

In humans, lipolysis is controlled through the balanced control of lipolytic β-adrenergic receptors and α2A-adrenergic receptor-mediated antilipolysis (7). In human visceral adipocytes, epinephrine stimulates lipolysis (high β-to-α2-adrenergic ratio), whereas it inhibits lipolysis in sc adipocytes (high α2-to-β-adrenergic ratio) (8). In addition, adipocytes from premenopausal women possess a higher α2-adrenergic receptor density than those from men (9).

Estradiol might also affect lipoprotein lipase (LPL) activity. LPL is important for the uptake of fat into the adipocytes, and it has been shown that the promoter region of the LPL gene contains a unique estrogen response element that is responsible for the inhibitory effect of estradiol on the LPL mRNA expression in 3T3-preadipocytes (10). A recent study in humans found reduced LPL activity in adipose tissue beneath a 17β-estradiol patch applied to the skin (11). Thus, estradiol might also affect uptake of fat in human adipose tissue.

We (12) have previously reported on the effects of HRT on s-leptin levels and body composition in a cohort of Danish perimenopausal women who were included in the Danish Osteoporosis Prevention Study. We reported that the women treated with estrogen had a significantly lower accumulation of adipose tissue on the truncus compared with control
women (12). Other studies have described similar findings of HRT (13), and studies using computerized tomography scan have been able to show that estrogen seems to maintain the sc localization of adipose tissue because women lacking estrogen experience a shift of their fat distribution from a predominantly sc fat depot toward a larger accumulation of adipose tissue intraabdominally (3).

For years it was thought that human adipose tissue did not contain estrogen receptors (ERs), and therefore any direct effect of estrogen on adipose tissue metabolism would be impossible (14). Recently, however, many groups including ourselves have been able to demonstrate that human adipose tissue actually expresses both ER mRNA as well as ER protein and has the capacity for specific estradiol binding (15–19). Furthermore, human adipose tissue contains both the classical ER-α as well as the recently discovered ER-β (15,16).

The present study was undertaken to determine the pathways in adipose tissue metabolism by which estrogen maintains the sc adipose tissue depot. Thus, adipose tissue biopsies were obtained in a subgroup of the Danish Osteoporosis Prevention Study women in which we (12) have reported previously on the effects on body composition. As the accumulation of adipose tissue is a balance between lipid mobilization and lipid assimilation, we studied the effects of estradiol on these two pathways. In the present paper, we were able to demonstrate that estradiol, through interaction with ER-α, increases the amount of α2-adrenergic receptors in human adipocytes, indicating that the status of human adipose tissue must be regarded as a true estrogen-sensitive organ and that this effect of estradiol might have implications for the female fat distribution.

Subjects and Methods

Subcutaneous abdominal adipose tissue was obtained by liposuction from six healthy women randomized to the group not retrieving treatment [age, 55.8 ± 1.4 yr; and body mass index (BMI), 25.5 ± 2.5 kg/m²] and six healthy women receiving HRT (age, 53.7 ± 1.3 yr; and BMI, 24.8 ± 1.0 kg/m²) using Trisekvens (Novo Nordisk, Malmö, Sweden), 12 tablets containing 2 mg estradiol, 10 tablets containing 2 mg estradiol, and 1 mg norethisteronacetate followed by six tablets containing 1 mg estradiol. The study was performed during the first estradiol period, and all women had received the treatment for 3 yr.

Adipose tissue (sc abdominal) for in vitro investigations was obtained from healthy women by liposuction during cosmetic surgery. None of the women received any medication.

Finally, paired sc abdominal and intraabdominal adipose tissue samples were obtained from five obese women (BMI, 48.2 ± 5.4 kg/m²) who underwent surgery for severe obesity (gastric banding). All adipose tissue samples were obtained after informed consent, and the study was approved by the local ethical committee in Aarhus County, Denmark.

Adipose tissue culture

Adipose tissue fragments (500 mg in each tube) were placed in 5 ml serum-free Medium 199 without Phenol red (Sigma-Aldrich, Copenhagen, Denmark) [supplemented with 25 mM HEPES, 5% BSA, and 1 mM insulin (Novo Nordisk, Bagsvaerd, Denmark)] in 50-ml plastic tubes. The cultures were placed in a humidified incubator and maintained at 37 °C for 24 h with the indicated hormones. Then, 100 mg of the adipose tissue was immediately frozen in liquid nitrogen and kept at −80 °C for RNA isolation and LPL activity measurement. The remaining adipose tissue fragments were used for isolation of mature adipocytes.

Isolation of human adipocytes

Adipocytes were isolated by collagenase digestion of the adipose tissue fragments in 10 mmol/liter HEPES buffer for 45 min at 37 °C as previously described (20). After the isolation procedure, adipocytes were washed three times in buffer containing 5% BSA and once in buffer containing 2.5% human serum albumin.

Lipolysis

Isolated adipocytes were incubated in 250 μl of 10 mm HEPES buffer containing 2.5% human serum albumin, 5 mM glucose, and electrolytes as previously described (21). Lipolysis was stimulated with isoproterenol (10 μM), epinephrine (100 μM), dibutyryl-cAMP (dbcAMP, 2 mM), and yohimbine (50 μM) for 120 min at 37 °C in triplicate. Glycerol was measured by a luciferase assay in a luminometer.

LPL activity measurements

LPL activity was determined essentially as previously described (22). Briefly, 200 μg adipocyte tissue was homogenized in a buffer containing 0.25 M sucrose, 1.0 mM K2EDTA (pH 7.4) at 4°C, and the homogenate was centrifuged 20 min at 12,000 × g at 4°C. The LPL activity in the postmitochondrial supernatant was determined by estimating the specific hydrolysis of [14C]triolein after 60 min of incubation. Free fatty acids were extracted from the incubation mixture as previously described (22) and measured by liquid scintillation counting.

Hormone-sensitive lipase (HSL) activity measurements

HSL was assayed as neutral cholesteryl esterase. Briefly, substrate for the assay was prepared [cholesteryl[14C]oleate, 0.043 mmol phosphatidylcholine, and 0.011 mmol cholesterol oleate in 100 mM potassium phosphate buffer (pH 7.0)] containing 5 mM sodium taurocholate. Adipose tissue was homogenized, the homogenate was centrifuged for 45 min at 20,000 × g at 4°C, the infranatant under the fat cake was carefully removed, and aliquots of 100 μl were assayed in triplicate for HSL activity by adding 10 μl of substrate at 37°C for 60 min.

α2-Adrenergic binding to adipocyte membranes

Adipocyte membranes were produced from isolated adipocytes essentially as previously described (23). The membranes were incubated with [3H]yohimbine, an α2-adrenoceptor antagonist, with an increasing amount of unlabelled yohimbine for 90 min; collected on glass fiber filters; washed three times on a Millipore vacuum device (Millipore Corp., Billerica, MA); dried; and counted by liquid scintillation.

Isolation of RNA

Adipose tissue samples (200 mg) were homogenized in TRIzol reagent (Life Technologies, Inc., Roskilde, Denmark), and total RNA was extracted following the manufacturer’s protocol. RNA was quantitated by measuring absorbency at 260 and 280 nm and the ratio was 1.8 or higher. The integrity of the RNA was checked by visual inspection of the two ribosomal RNAs on an ethidium bromide-stained agarose gel.

Real-time RT-PCR for mRNA analysis

Reverse transcription was performed using random hexamer primers at 23°C for 10 min and 42°C for 60 min and was terminated by increasing the temperature to 95°C for 10 min, as described by the manufacturer (GeneAmp RNA PCR Kit from Perkin-Elmer Cetus, Norwalk, CT). Then, PCR-mastermix containing the specific primers and Taq DNA polymerase (HotStar Taq, Qiagen, Inc., Valencia, CA) were added. The primers listed in Table 1 were designed using the Oligo Primer Analysis Software version 6.64 (Molecular Biology Insights, Inc., Cascade, CO).

Real-time quantitation of target gene (X0)-to-β-actin (R0) mRNA was performed with a SYBR-Green real-time PCR assay (Qiagen, Inc.) using an iCycler from Bio-Rad (Bio-Rad Laboratories, Hercules, CA) as previously described (16,24). The X0 and R0 mRNA were amplified in separate tubes. The increase in fluorescence was measured in real time during the extension step. The threshold cycle (Ct) was calculated, and
the relative gene expression was calculated essentially as described in
the User Bulletin no. 2, 1997, from Perkin-Elmer. Briefly, the X0-to-R0
ratio in each sample before amplification was calculated as X0/R0 = k ×
1/(2ΔCt), where ΔCt is the difference between Ct-target and Ct-reference,
and k is a constant, set to 1. All samples were amplified in duplicate. A
similar set-up was used for negative controls, except that the reverse
transcriptase was omitted, and no PCR products were detected under
these conditions.

Statistical analysis

Data are given as mean ±SEM. The statistical package SPSS version
11 (SPSS, Chicago, Illinois) was used and one-way ANOVA followed by
Duncan’s post hoc analysis was used to detect statistically significant
differences when more than two groups were compared; a P value < 0.05
was considered significant. When only two groups were compared,
Student’s t test was used.

Results

Effects of estradiol treatment in vivo (HSL activity and
adrenergic receptor mRNA)

Because estrogen seems to protect postmenopausal
women from losing subcutaneous adipose tissue, we investigated the
lipolytic capacity in abdominal subcutaneous adipose tissue from
placebo-treated and estradiol-treated women. There was a
tendency (although nonsignificant) toward a lower lipolytic
capacity in sc adipose tissue from estradiol-treated
women evaluated by measuring the HSL activity (P = 0.09)
data not shown). We then studied the mRNA expression
of adrenergic receptors in the estradiol- and placebo-
treated women (Fig. 1A). The estradiol-treated women had
significantly higher expression of adrenergic receptor
α-subtype 2A compared with the control group: the α2A-
subtype is the only α2-subtype important for adipose tissue
(25).

The gene expression of β-adrenergic receptors in adipose
tissue was unaltered by estradiol treatment of the women
(similar 1- and 2-receptor mRNA expression in the two
groups) (Fig. 1A). The estradiol-treated women had
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tissue was unaltered by estradiol treatment of the women
(similar 1- and 2-receptor mRNA expression in the two
groups) (Fig. 1A). The expression of the β3-adrenergic re-
ceptor was very low and was not different between the two
groups (data not shown).

Effects of estradiol on adrenergic receptors in vitro

Next we sought to determine whether the estradiol-
mediated increase in α2A-adrenergic receptor observed in
vitro could also be found using human adipose tissue in
culture. As shown in Fig. 1B, stimulation of the human tissue
fragments with estradiol (100 nM for 24 h) increased the
α2-adrenergic receptor mRNA expression significantly by
66 ± 17% (P < 0.05) (Fig. 1B).

The expression of lipolytic β-adrenergic receptors was also
unchanged after estradiol stimulation in vitro (Fig. 1B). The
expression of the β3-adrenoceptor subtype was very low,
and estradiol did not influence the expression (data not
shown).

To investigate whether the increased α2A-adrenergic re-
ceptor mRNA expression was followed by an increase at the
protein level, we determined the α2-adrenergic receptor by
ligand binding. Adipocyte membranes from estradiol-stim-
ulated (100 nM for 24 h) adipose tissue fragments had sig-
nificantly higher binding capacity (2.7-fold higher, P <
0.001), whereas the affinity was unaltered (Fig. 2).

Determination of the ER subtype involved

Human adipose tissue contains both the classical ER (19),
which now is named ER-α, as well as the newly discovered
ER-β (15, 16). Therefore, we investigated which of the re-
ceptor subtypes were involved in the effects of estradiol on
α2-adrenergic receptor number. We compared two non-
steroid ER-α agonists and compared the results with that of
17β-estradiol (which stimulates both subtypes) (Fig. 3). The
first compound used was 4,4'-di(4-propyl-[1H]pyrazole-1,3,5-triyl)
triphenol (PPT), an agonist with 410-fold selectivity for
ER-α over ER-β (26). PPT (10 nM) had the same stimu-
latory effect on yohimbine binding as estradiol (10 nM)
(Fig. 3). Finally, we used R.R-5,11-diethyl-5,6,11,12-tetrahy-
drocrystene-2,8-diol (THC): this compound is an ER-
agonist and a potent ER-β antagonist (27). Thus, any observed
effect on yohimbine binding would therefore go through
ER-α because THC would block effects mediated through
ER-β. As shown in Fig. 3, THC (10 nM) had the same stimu-
latory effect on yohimbine binding as PPT (10 nM) and
17β-estradiol (10 nM). Thus, the effect of estradiol on yohim-
bine binding in human adipocytes seems to be mediated
through the ER-α.

### TABLE 1. Primers

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<th>Gene</th>
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<td>TCGCAACCAAGCTTTGGCCGAGATG</td>
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<tr>
<td>α2B-Adrenergic receptor</td>
<td>TTACATCTTGCGCAAGGCTCTG</td>
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<td>α2C-Adrenergic receptor</td>
<td>GCCCTCTGCTGTTAGCTGCAAC</td>
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<tr>
<td>β1-Adrenergic receptor</td>
<td>GTGGTGGCTCTCTCTCCTGCA</td>
<td>161</td>
</tr>
<tr>
<td>β2-Adrenergic receptor</td>
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</tr>
<tr>
<td></td>
<td>GCGTAGCCCAGCCAGTTGAAGA</td>
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</tr>
<tr>
<td>β3-Adrenergic receptor</td>
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</tr>
<tr>
<td>LPL</td>
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<tr>
<td>β-Actin</td>
<td>ACAGGGTCTCCACACACTTGAGCCAGT</td>
<td>658</td>
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</table>
Estradiol effects on adipose tissue lipolysis (in vitro)

To determine whether these changes in the number of α2A-adrenergic receptors had any functional consequences, we investigated the lipolytic effect of various compounds that affect lipolysis through different pathways. Human sc adipose tissue fragments were incubated for 24 h with or without estradiol (100 nM). The expression pattern of adrenergic receptors α2A, β1, and β2 is presented (six controls and six estradiol-treated women). These studies revealed that the lipolytic response in control and estradiol-treated adipocytes were similar for isoproterenol (a pure β1-adrenergic agonist) and for dbcAMP (a stable cAMP analog) (Fig. 4). However, using epinephrine (an α- and β-adrenergic agonist) to stimulate lipolysis revealed a significant attenuation in the lipolytic response in estradiol-treated adipocytes compared with control adipocytes (Fig. 4).

If, however, yohimbine (an α2-adrenergic receptor blocker) was given together with epinephrine, the lipolytic response was similar in the two groups and equaled the lipolytic response observed using isoproterenol (Fig. 4). These findings demonstrate that the increased number of α2-adrenergic receptors also mediated increased antilipolytic effects in estradiol-stimulated human adipocytes.

Estradiol effects on α2A-adrenergic receptors in sc and visceral adipose tissue

To investigate whether estradiol might affect human adipose tissue depots differently, we investigated paired sc and visceral adipose tissue fragments in culture. As shown in Fig. 5, estradiol (100 nM for 24 h) was able to increase the α2A-

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Fig. 1. A, Adrenergic receptor mRNA expression (in vivo) in adipose tissue from women treated with or without estradiol for 3 yr. The expression pattern of adrenergic receptors α2A, β1, and β2 is presented (six controls and six estradiol-treated women). B, Human adipose tissue fragments incubated for 24 h with or without estradiol (100 nM). The expression pattern of adrenergic receptors α2A, β1, and β2 is presented. Mean ± SEM; *, significant difference between control and estradiol stimulation (P < 0.05; n = 6).
adrenergic receptor mRNA 1.7-fold \( (P < 0.001) \) in adipose tissue from the sc abdominal fat depot. In contrast, estradiol had no effect on \( \alpha_2A \)-adrenergic receptor mRNA expression in parallel incubations using adipose tissue fragments from the intraabdominal depot (Fig. 5).

**Estradiol effects on LPL**

**In vivo.** The HRT group had significantly lower adipose tissue LPL activity than the control group (52.8% of the control group; \( P < 0.05 \)) (Fig. 6A). However, the LPL mRNA expression was similar in adipose tissue from the two groups (Fig. 6B).

**In vitro.** To investigate whether the observed lower LPL activity in adipose tissue from estradiol-treated women was caused by estradiol acting directly at the adipocytes, human adipose tissue was cultured for up to 72 h. These studies revealed that the LPL activity in cultured human adipose tissue fragments rapidly fell to very low levels [as previously described also by Ottosson et al. (28)], and no effects of estradiol on LPL activity were observed under these conditions (data not shown). Therefore, we used dexamethasone to increase the LPL activity, and as shown in Fig. 6C, dexamethasone stimulation for at least 24 h increased LPL activity significantly above control incubations; after 72 h, the LPL...
activity was increased 2- to 3-fold above basal (Fig. 6C). However, addition of 100 nm estradiol concomitantly with dexamethasone did not influence LPL activity (Fig. 6C), demonstrating that estradiol had no direct effect on LPL activity. Furthermore, estradiol did not influence LPL mRNA expression (data not shown).

Discussion

In the present study, we have demonstrated, using both in vivo as well as in vitro data, that estradiol directly affects human adipocyte metabolism. In sc adipocytes, estradiol increases the number of antilipolytic α2A-adrenergic receptors, which is followed by an attenuated lipolytic response to epinephrine. However, the lipolytic machinery in the adipocytes is intact because the lipolytic response for other compounds is untouched by estradiol.

Previously, it was known from rats that lack of estrogen (ovariectomy) was causing obesity, which could be prevented by estradiol substitution, and several possible mechanisms have been suggested: estrogen lowers the food intake.
(29, 30); lowers adipose tissue LPL activity (31, 32); increases spontaneous physical activity (33); increases adipose tissue lipolysis (34); and somehow increases energy expenditure (30, 35).

However, in humans, the pathways involved in the estrogen-mediated control of adipose tissue metabolism are largely unknown. Studies indicate that the typical female fat pattern changes into a male type of adipose tissue accumulation during or after menopause, but this redistribution can largely be prevented by HRT (12, 13). Furthermore, estradiol treatment of transsexual males increases the amount of sc adipose tissue (4), indicating that estradiol is able to regulate human adipose tissue accumulation and especially seems to favor sc accumulation of adipose tissue.

Very few studies have previously investigated changes in human adipose tissue lipolysis after in vivo estrogen treatment, and two of them reported that lipolysis was reduced in estrogen-treated subjects (5, 6). In one study, basal lipolysis was diminished (5) (estradiol-treated male-to-female transsexuals), and in the other study, the epinephrine-stimulated lipolysis was attenuated with no change in basal lipolysis (6) (postmenopausal women receiving HRT). The latter findings are well in line with our present study, demonstrating a higher number of antilipolytic a2-adrenergic receptors in postmenopausal women receiving HRT.

Only a single study has previously investigated the balance between lipolytic and antlipolytic adrenergic receptors because they investigated lipolysis in pre- and postmeno-
pausal women using compounds that affect the lipolytic cascade at different steps. They were, however, unable to detect any significant differences in the lipolytic response in the two groups of women (36). Several differences between the latter study and ours might account for this discrepancy: 1) the premenopausal women in the study by Mauriege et al. (36) were investigated early in the follicular phase of their menstrual cycle (at a time point at which the plasma estradiol is at the lowest level), and, from our in vitro study, the estradiol effects seem to be rapid in onset (less than 24 h); 2) although they did not reach a significant difference in α2-adrenergic receptor-mediated antilipolysis, their data seem to support the notion that epinephrine and the pure α2-adrenergic receptor agonist UK14304 inhibited lipolysis more in premenopausal than in postmenopausal women.

Subcutaneous adipocytes from women have higher α2-adrenergic receptor density than adipocytes from men. In addition, the lipolytic response to epinephrine/norepinephrine in sc adipocytes from women is lower than that of men (9). Thus, our findings that estradiol increases α2A-adrenergic receptors in sc adipocyte tissue fragments therefore offer an explanation for these gender differences in sc adipose tissue metabolism.

In visceral adipocytes, epinephrine possesses a potent lipolytic activity (8). Thus, the finding that estradiol has no effect on α2A-adrenergic receptors in intraabdominal adipocytes but increases the number of α2A-adrenergic receptors in sc adipocytes might explain how estradiol can maintain the female fat distribution. It seems that estradiol controls the fat distribution by changing the lipolytic response in the two fat depots differently, thereby favoring fat accumulation in the sc depot at the expense of the visceral depot. Support for the notion that the ratio of lipolytic β- and antilipolytic α2-adrenergic receptors determines the tendency for accumulation of fat in the adipocytes is provided by studies using transgenic mice that overexpressed human α2A-adrenergic receptor in their adipose tissue. These transgenic mice had a tendency for developing obesity and had a reduced lipolytic capacity in the fat depot, demonstrating the highest expression of α2A-adrenergic receptors in relation to β-adrenergic receptors (37, 38).

Regarding the other possible pathway regulated by estrogen, namely the uptake of fat, it is known that estrogen lowers adipose tissue LPL activity in rodents (31, 32). In humans, a strong negative correlation between adipose tissue LPL activity and plasma estradiol levels, indicating an inhibitory effect of estradiol on adipose tissue LPL activity, has been reported (39). Moreover, it was recently demonstrated that adipose tissue LPL activity directly beneath an estradiol patch was lower than in adipose tissue beneath a placebo patch, whereas LPL mRNA levels were unchanged by estradiol (11). These in vivo findings were confirmed in the present study, demonstrating that estradiol-treated postmenopausal women had lower adipose tissue LPL activity (but a similar adipose tissue LPL mRNA level).

Furthermore, our culture experiments indicate that the estradiol effect on LPL activity observed in vitro might be indirect because no effect of estradiol on LPL activity could be found in vitro. Thus, the lower LPL activity in estradiol-treated women might be caused by estrogen-mediated changes in metabolites/other hormones or activity in the sympathetic nervous system (40).

However, in our culture, LPL activity fell rapidly to very low levels during incubation; thus, the model might not be optimal for studying the direct effects of estradiol on LPL activity. On the other hand, using a similar protocol, Ottoson et al. (28) were able to demonstrate that LPL activity was inhibited by GH.

Very recently, Palin et al. (41) studied the effects of estradiol on isolated human adipocytes incubated for approximately 48 h. They demonstrated that estradiol at high concentrations (100 nm) actually increased the amount of HSL protein and increased basal lipolysis; unfortunately, no assessment of epinephrine-stimulated lipolysis was performed. The observed effect of estrogen was attempted to be blocked by the use of an ER antagonist (ICI 182.780). However, ICI together with estrogen actually increased lipolysis and HSL levels even further. Moreover, they demonstrated a paradoxical effect of estradiol on LPL expression in the isolated adipocytes. Low concentrations of estrogen inhibited LPL, whereas higher concentrations increased the amount of LPL (41). The reason for these divergent results is not entirely clear, but it may be the use of whole adipose tissue fragments in the present study as compared with isolated adipocytes in the study of Palin et al. (41). Isolated human adipocytes are very fragile, and incubation of these cells for more than few hours is very difficult, whereas adipose tissue fragments are much more robust and can be kept in culture for days without many problems.

For years, it was thought that estrogen only affected adipose tissue metabolism indirectly by changes in metabolites/other hormones (42) because human adipose tissue was believed not to possess ERs (43, 44). However, recently, several groups including our own independently described the existence of ERs in human adipose tissue (15–19, 45), indicating that estrogen might be able to change adipose tissue metabolism directly. Our present results clearly show that the increase in α2-adrenergic receptor number is caused by estradiol acting directly at the adipocytes because estradiol both in vivo as well as in vitro increased the α2A-adrenergic receptor number. Furthermore, analysis of the α2-adrenergic receptor subtype 2A gene (GenBank accession no. AY032736) using a web-based promoter scanner (Alibaba2 version 2.1) revealed several sites where estradiol might regulate gene expression. Within the first 1700 bp in front of the starting ATG, several c-Jun, AP-1, and AP-2 binding sites were detected that are known to be typically estrogen-regulated transcription factors (46, 47). In addition, there are several Sp-1 binding sites that also can be regulated by estrogen (47). ER binds to the consensus estrogen response element palindrome GGTCAnnnTGACC; however, ERs also bind to imperfect estrogen response elements, and the promoter scanner suggested a possible ER binding site at location −1777 bp (tcTCAgcggTGACC). Thus, there are several possible localizations within the promoter where estradiol might regulate α2-adrenergic receptor transcription.

Using non-steroid selective ER agonists, we were able to study whether the stimulatory effect of estradiol on α2-
adrenergic receptor was mediated through ER-α or ER-β. Our results clearly show that stimulation of the ER-α was related to the estrogen-induced up-regulation of α2A-adrenergic receptor in sc adipose tissue.

In conclusion, we have demonstrated that estradiol increases the expression of α2-adrenergic receptors in human adipocytes through activation of the ER-α. The increase in anti-lipolytic α2-adrenergic receptors was followed by an attenuation of the epinephrine-induced lipolysis. Estradiol increased sc adipocyte α2A-adrenergic receptor content but had no effect on visceral adipose tissue α2A-adrenergic receptor content. These effects indicate that estradiol is probably maintaining the typical sc female pattern of fat distribution by inhibiting lipolysis in the sc fat depot and thus favoring fat accumulation in this depot at the expense of the visceral depot.

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Address all correspondence and requests for reprints to: Steen B. Pedersen, M.D., Ph.D., Department of Endocrinology and Metabolism, Aarhus Amtssygues, Aarhus University Hospital, DK-8000 Aarhus C., Denmark. E-mail: sbp@dadmnet.dk.

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