

Estrogen Controls Lipolysis by Up-Regulating α 2A-Adrenergic Receptors Directly in Human Adipose Tissue through the Estrogen Receptor α . Implications for the Female Fat Distribution

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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 α 2A-adrenergic receptors in sc adipocytes. The increased number of α 2A-adrenergic receptors caused an attenuated lipolytic response of epinephrine in sc adipocytes; in contrast, no effect of estrogen on α 2A-adrenergic receptor mRNA expression was observed in adipocytes from the intraabdominal fat depot.

These findings show that estrogen lowers the lipolytic re-

sponse in sc fat depot by increasing the number of antilipolytic α 2A-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 α 2A-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)

ADIPOSE 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 epineph-

rine-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 α 2A-adrenergic receptor-mediated antilipolysis (7). In human visceral adipocytes, epinephrine stimulates lipolysis (high β - to α 2-adrenergic ratio), whereas it inhibits lipolysis in sc adipocytes (high α 2- to β -adrenergic ratio) (8). In addition, adipocytes from premenopausal women possess a higher α 2-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 17 β -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

Abbreviations: BMI, Body mass index; Ct, threshold cycle; dbcAMP, dibutryl cAMP; ER, estrogen receptor; HRT, hormone replacement therapy; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PPT, 4,4',4''-(4-propyl-[¹H]-pyrazole-1,3,5-triyl)trisphenol; THC, R,R-5,11-diethyl-5,6,11,12-tetrahydrochrysen-2,8,-diol.

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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 α 2-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 nM 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 μ l 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 μ M), epinephrine (100 μ M), dibutyl-cAMP (dbcAMP, 2 mM), and yohimbine (50 μ M) 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 mg adipose 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 \times g at 4 C. The LPL activity in the post-mitochondrial 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-[¹⁻¹⁴C]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 \times g at 4 C, the infranatant under the fat cake was carefully removed, and aliquots of 100 μ l were assayed in triplicate for HSL activity by adding 10 μ l of substrate at 37 C for 60 min.

α 2-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 α 2-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 $X0/R0 = k \times 1/(2^{\Delta Ct})$, 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 \pm SEM. The statistical package SPSS version 11 (SPSS, Chicago, IL) 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 sc adipose tissue, we investigated the lipolytic capacity in abdominal sc 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 α 2A-subtype is the only α 2-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 β 1- and β 2-receptor mRNA expression in the two groups) (Fig. 1A). The expression of the β 3-adrenergic receptor 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 α 2A-adrenergic receptor observed *in*

vivo 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 α 2-adrenergic receptor mRNA expression significantly by $66 \pm 17\%$ (*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 β 3-adrenoceptor subtype was very low, and estradiol did not influence the expression (data not shown).

To investigate whether the increased α 2A-adrenergic receptor mRNA expression was followed by an increase at the protein level, we determined the α 2-adrenergic receptor by ligand binding. Adipocyte membranes from estradiol-stimulated (100 nM for 24 h) adipose tissue fragments had significantly 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 receptor subtypes were involved in the effects of estradiol on α 2-adrenergic receptor number. We compared two non-steroid ER- α agonists and compared the results with that of 17 β -estradiol (which stimulates both subtypes) (Fig. 3). The first compound used was 4,4',4''-(4-propyl-[³H]pyrazole-1,3,5-triyl)trisphenol (PPT), an agonist with 410-fold selectivity for ER- α over ER- β (26). PPT (10 nM) had the same stimulatory effect on yohimbine binding as estradiol (10 nM) (Fig. 3). Finally, we used *R,R*-5,11-diethyl-5,6,11,12-tetrahydrochrysene-2,8,-diol (THC): this compound is an ER- α 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 stimulatory effect on yohimbine binding as PPT (10 nM) and 17 β -estradiol (10 nM). Thus, the effect of estradiol on yohimbine binding in human adipocytes seems to be mediated through the ER- α .

TABLE 1. Primers

Gene	Primer	Product length (bp)
α 2A-Adrenergic receptor	GGCCTGCTCATGCTGCTCACC TCGCACCAAGTCTTGCCGAAGT	200
α 2B-Adrenergic receptor	TTACCATCTTCGGCAACCGTCTG GCCTCCTGGTTGAGCTTGAC	436
α 2C-Adrenergic receptor	GTGGTGGGCTTCCTCATCGTCT TGATGGCACACAGATGCACGATC	268
β 1-Adrenergic receptor	GTGGCCCTACGCGAGCAGAAG GCGTAGCCAGCCAGTTGAAGA	161
β 2-Adrenergic receptor	ACAGCCATTGCCAAGTTCGAG ATGATCACCCGGGCCTTATTCTT	296
β 3-Adrenergic receptor	CCCAATACGCCAACCAGT CGGCCAGCGAAGTCACGAA	178
LPL	ATGGAGAGCAAAGCCCTGCTC CACCCAACCTCATACATTCC	273
β -Actin	ACGGGGTCAACCCACTGTGC CTAGAAGCATTTGCGGTGACGATG	658

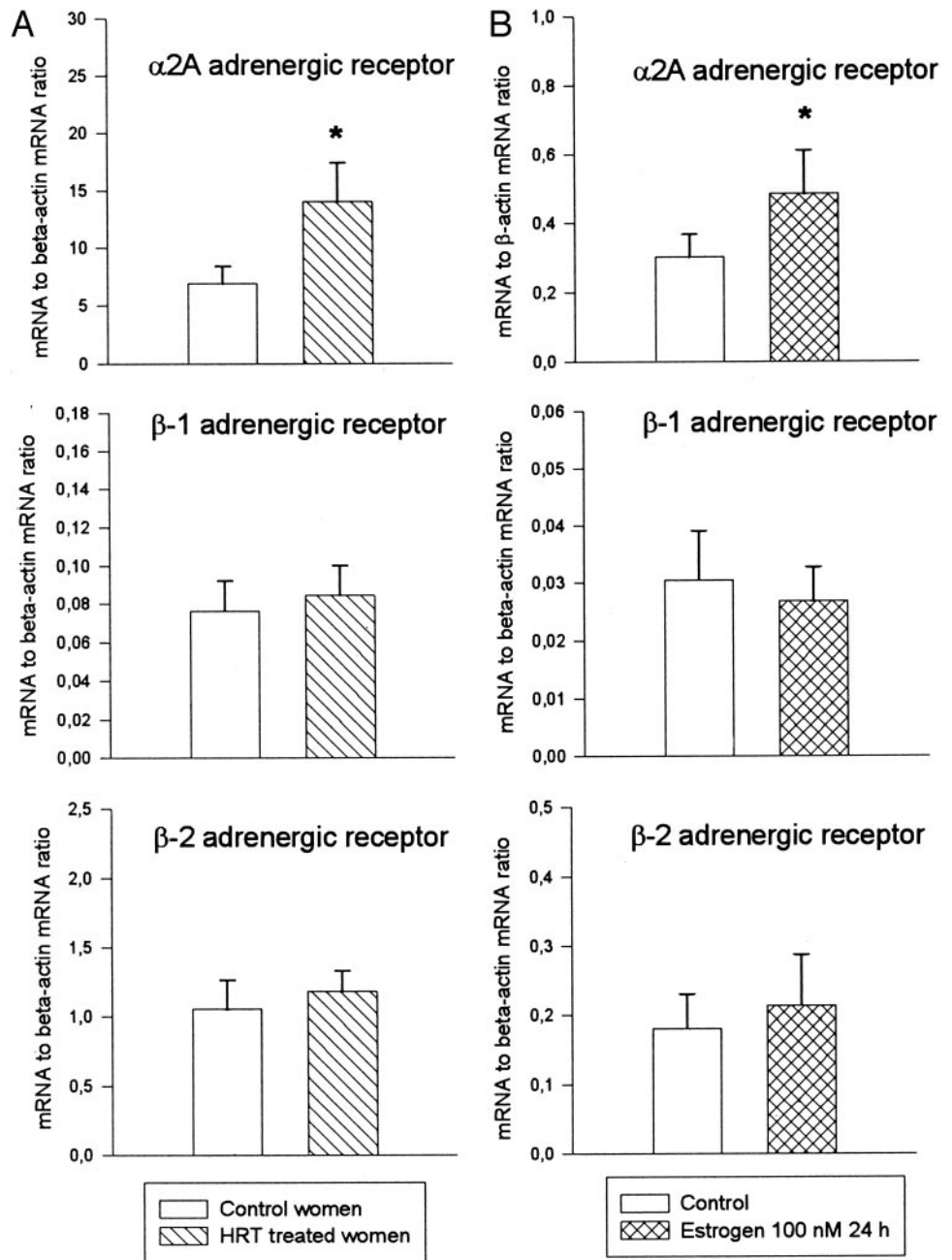


FIG. 1. A, Adrenergic receptor mRNA expression (*in vivo*) in adipose tissue from women treated with or without estradiol for 3 yr. The expression pattern of adrenergic receptors α 2A, β 1, and β 2 is presented (six controls and six estradiol-treated women). B, Human adipose tissue fragments incubated for 24 h with or without estradiol (100 nM). The expression pattern of adrenergic receptors α 2A, β 1, and β 2 is presented. Mean \pm SEM; *, significant difference between control and estradiol stimulation ($P < 0.05$; $n = 6$).

Estradiol effects on adipose tissue lipolysis (*in vitro*)

To determine whether these changes in the number of α 2A-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 without or with estradiol (100 nM), and then adipocytes were isolated. These studies revealed that the lipolytic response in control and estradiol-treated adipocytes were similar for isoproterenol (a pure β -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 α 2-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 α 2-adrenergic receptors also mediated increased antilipolytic effects in estradiol-stimulated human adipocytes.

Estradiol effects on α 2A-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 α 2A-

FIG. 2. Yohimbine (YOH) binding in isolated plasma membranes from sc human adipose tissue incubated with or without estradiol (100 nM) for 24 h. Labeled yohimbine was used to assess the number of α 2A-adrenergic receptors (three experiments, adipose tissue obtained from healthy women during liposuction). Conc, Concentration.

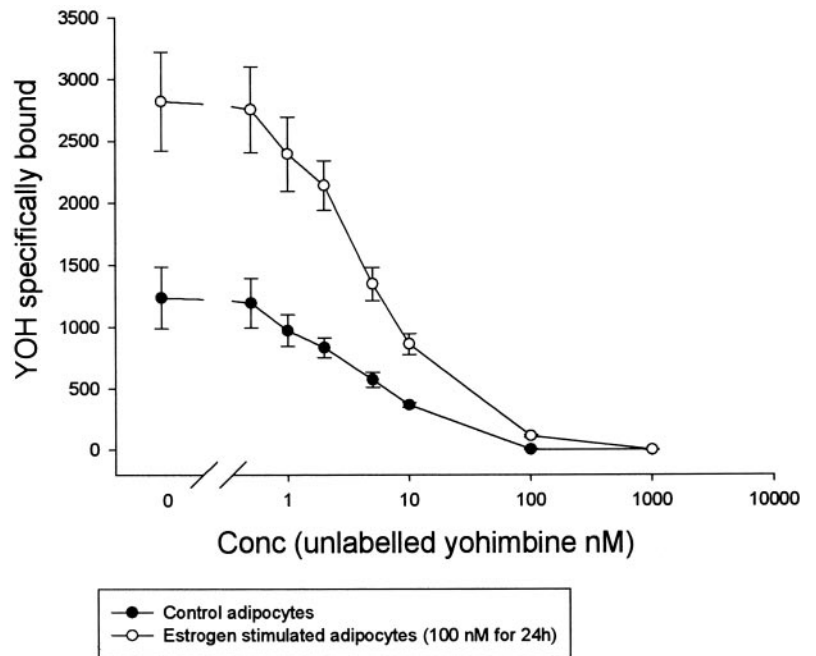
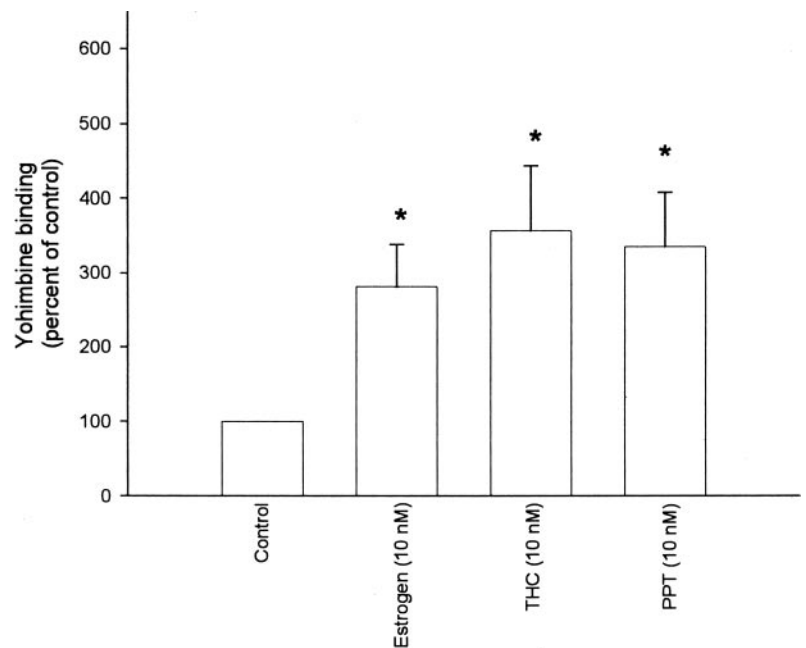


FIG. 3. Yohimbine binding to plasma membranes from isolated human adipocytes. Adipose tissue fragments were incubated with estradiol (10 nM), PPT (an ER- α agonist, 10 nM), THC (an ER- α agonist and ER- β antagonist, 10 nM) for 24 h. Then isolated plasma membranes were prepared. Mean \pm SEM; *, $P < 0.05$ compared with control (five experiments, adipose tissue obtained from healthy women during liposuction).



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 α 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 α 2A-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

FIG. 4. Characterization of the lipolytic response in human adipocytes stimulated with and without estradiol (100 nM) for 24 h. Lipolysis was stimulated with isoproterenol (Isop, 10 μ M), epinephrine (Epi, 100 μ M), epinephrine (100 μ M) together with yohimbine (50 μ M) (Epi+Yoh), and finally, dbcAMP (2 mM). Mean \pm SEM; *, denotes significant difference between control cells and estradiol-stimulated cells ($P < 0.05$; n = 3; adipose tissue obtained from healthy women during liposuction).

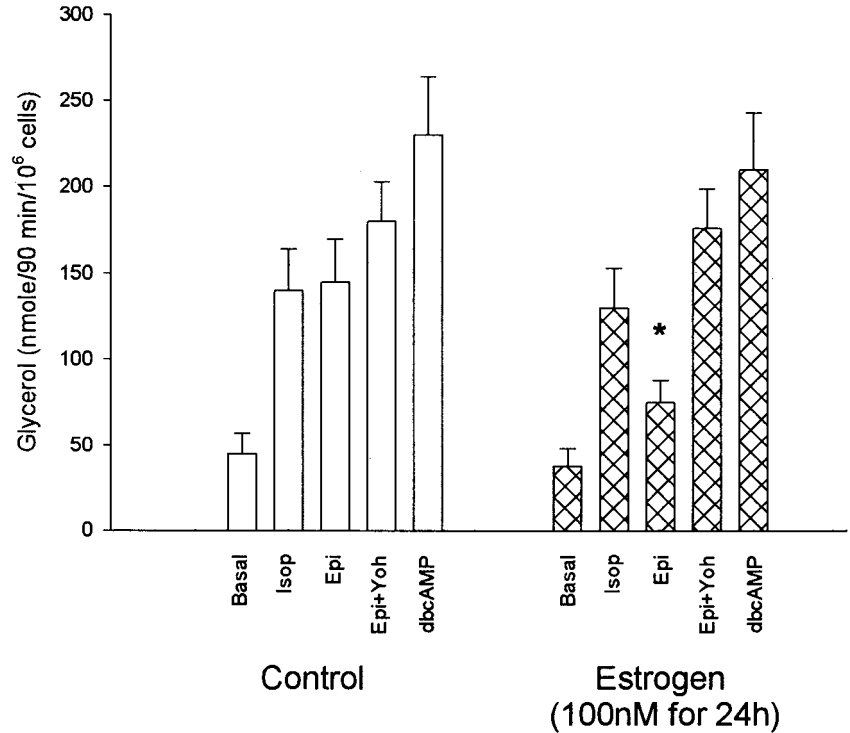
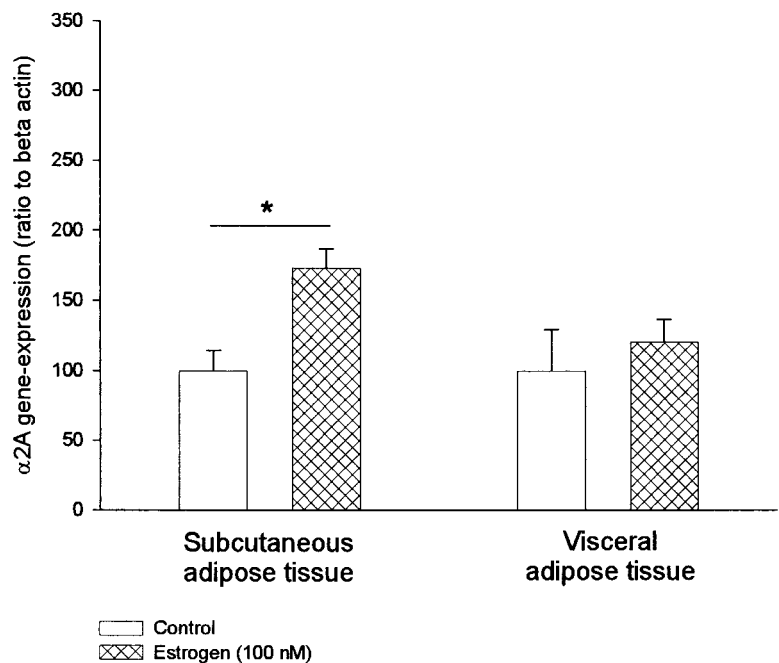


FIG. 5. Effects of estradiol on sc and visceral adipose tissue α 2A-adrenergic receptor mRNA expression. Paired sc and visceral adipose tissue was obtained from obese women during obesity surgery, incubated for 24 h with and without 100 nM estradiol. Mean \pm SEM of five experiments; *, $P < 0.001$.



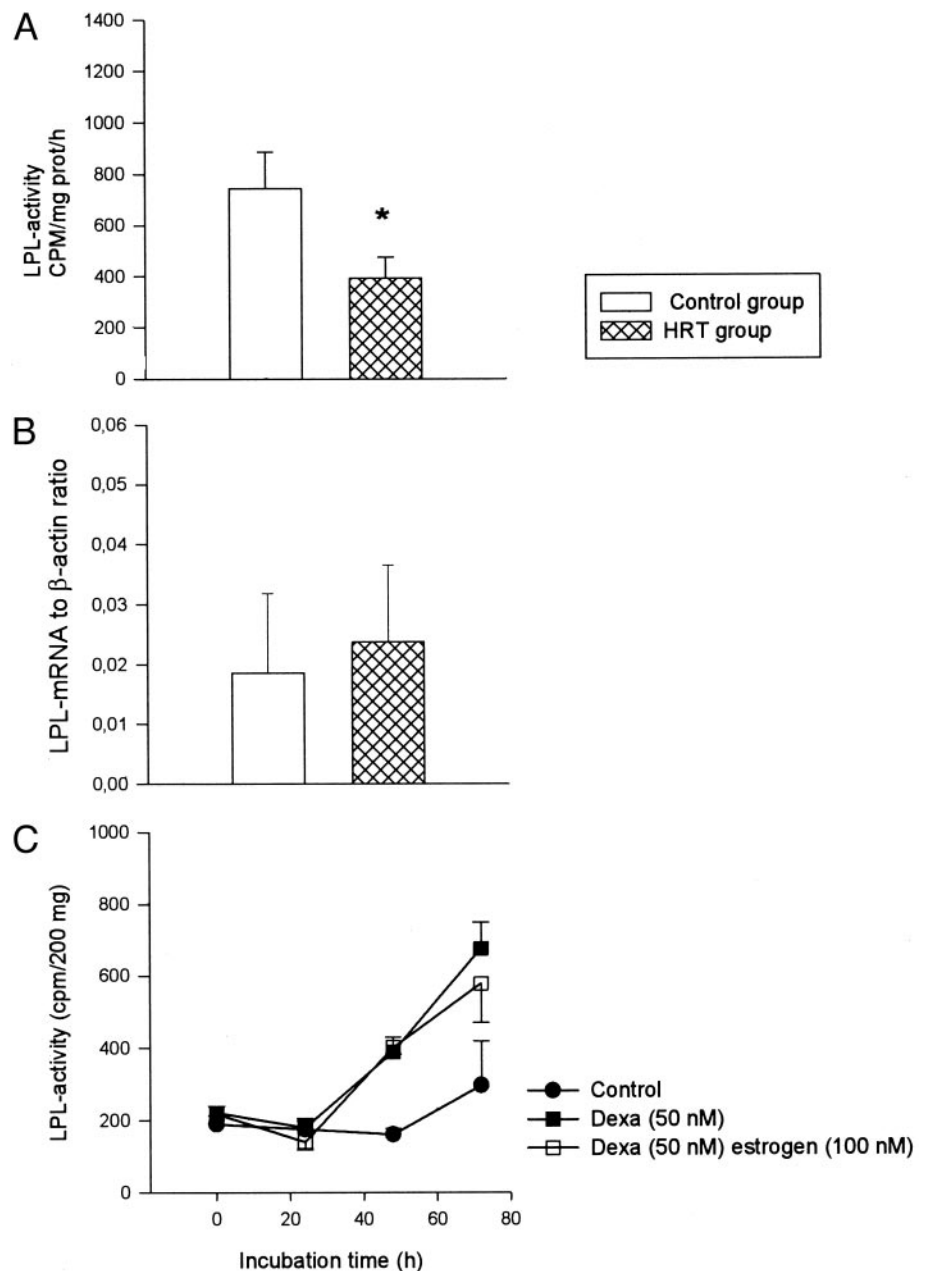


FIG. 6. A, LPL activity and B, LPL mRNA expression (*in vivo*) in abdominal sc adipose tissue from women treated with or without estradiol for 3 yr. Mean \pm SEM; *, $P < 0.05$ (six controls and six estradiol-treated women). C, LPL activity (*in vitro*) in human adipose tissue fragments cultured for up to 72 h. Closed circle, Control incubations; closed square, LPL activity in (50 nM) dexamethasone (Dexa)-stimulated adipose tissue. Open square, LPL activity in (50 nM) dexamethasone- and (100 nM) estradiol-stimulated adipose tissue fragments (three experiments, adipose tissue obtained from healthy women during liposuction).

(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 α 2-adrenergic receptors in postmenopausal women receiving HRT.

Only a single study has previously investigated the balance between lipolytic and antilipolytic 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 α 2-adrenergic receptor-mediated antilipolysis, their data seem to support the notion that epinephrine and the pure α 2-adrenergic receptor agonist UK14304 inhibited lipolysis more in premenopausal than in postmenopausal women.

Subcutaneous adipocytes from women have higher α 2-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 α 2A-adrenergic receptors in sc adipose 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 α 2A-adrenergic receptors in intraabdominal adipocytes but increases the number of α 2A-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 α 2-adrenergic receptors determine the tendency for accumulation of fat in the adipocytes is provided by studies using transgenic mice that overexpressed human α 2A-adrenergic receptor in their adipose tissue. These transgenic mice had a tendency for developing obesity and had a reduced epinephrine-mediated lipolytic capacity in the fat depot, demonstrating the highest expression of α 2A-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, Ottosson *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 162,780). 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 α 2-adrenergic receptor number is caused by estradiol acting directly at the adipocytes because estradiol both *in vivo* as well as *in vitro* increased the α 2A-adrenergic receptor number. Furthermore, analysis of the α 2-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 (tcTCAgccgTGACC). Thus, there are several possible localizations within the promoter where estradiol might regulate α 2-adrenergic receptor transcription.

Using non-steroid selective ER agonists, we were able to study whether the stimulatory effect of estradiol on α 2-

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 α 2A-adrenergic receptor in sc adipose tissue.

In conclusion, we have demonstrated that estradiol increases the expression of α 2-adrenergic receptors in human adipocytes through activation of the ER- α . The increase in anti-lipolytic α 2-adrenergic receptors was followed by an attenuation of the epinephrine-induced lipolysis. Estradiol increased sc adipocyte α 2A-adrenergic receptor content but had no effect on visceral adipose tissue α 2A-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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References

- Björntorp P 1988 The associations between obesity, adipose tissue distribution and disease. *Acta Med Scand Suppl* 723:121–134
- Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G 1984 Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br Med J (Clin Res Ed)* 288:1401–1404
- Toth MJ, Tchernof A, Sites CK, Poehlman ET 2000 Effect of menopausal status on body composition and abdominal fat distribution. *Int J Obes Relat Metab Disord* 24:226–231
- Elbers JM, Asscheman H, Seidell JC, Gooren LJ 1999 Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am J Physiol* 276(2 Pt 1):E317–E325
- Elbers JM, de Jong S, Teerlink T, Asscheman H, Seidell JC, Gooren LJ 1999 Changes in fat cell size and *in vitro* lipolytic activity of abdominal and gluteal adipocytes after a one-year cross-sex hormone administration in transsexuals. *Metabolism* 48:1371–1377
- Lindberg UB, Crona N, Silfverstolpe G, Björntorp P, Rebuffe-Scrive M 1990 Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. *Horm Metab Res* 22:345–351
- LaFontan M, Berlan M 1993 Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res* 34:1057–1091
- Richelsen B, Pedersen SB, Møller-Pedersen T, Bak JF 1991 Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E2. *Metabolism* 40:990–996
- Richelsen B 1986 Increased α 2- but similar β -adrenergic receptor activities in subcutaneous gluteal adipocytes from females compared with males. *Eur J Clin Invest* 16:302–309
- Homma H, Kurachi H, Nishio Y, Takeda T, Yamamoto T, Adachi K, Morishige K, Ohmichi M, Matsuzawa Y, Murata Y 2000 Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *J Biol Chem* 275:11404–11411
- Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J, Kilgore M 1998 Estrogen regulation of adipose tissue lipoprotein lipase—possible mechanism of body fat distribution. *Am J Obstet Gynecol* 178:101–107
- Kristensen K, Pedersen SB, Vestergaard P, Mosekilde L, Richelsen B 1999 Hormone replacement therapy affects body composition and leptin differently in obese and non-obese postmenopausal women. *J Endocrinol* 163:55–62
- Haarbo J, Marslew U, Gottfredsen A, Christiansen C 1991 Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. *Metabolism* 40:1323–1326
- Björntorp P, Ottosson M, Rebuffe-Scrive M, Xu X 1990 Regional obesity and steroid hormone interactions in human adipose tissue. In: Bray GA, Ricquier D, Spiegelman BM, eds. *Obesity: towards a molecular approach*. New York: Wiley-Liss, Inc.; 147–157
- Crandall DL, Busler DE, Novak TJ, Weber RV, Kral JG 1998 Identification of estrogen receptor β RNA in human breast and abdominal subcutaneous adipose tissue. *Biochem Biophys Res Commun* 248:523–526
- Pedersen SB, Bruun JM, Hube F, Kristensen K, Hauner H, Richelsen B 2001 Demonstration of estrogen receptor subtypes α and β in human adipose tissue: influences of adipose cell differentiation and fat depot localization. *Mol Cell Endocrinol* 182:27–37
- Price TM, O'Brien SN 1993 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* 77:1041–1045
- Mizutani T, Nishikawa Y, Adachi H, Enomoto T, Ikegami H, Kurachi H, Nomura T, Miyake A 1994 Identification of estrogen receptor in human adipose tissue and adipocytes. *J Clin Endocrinol Metab* 78:950–954
- Pedersen SB, Hansen PS, Lund S, Andersen PH, Odgaard A, Richelsen B 1996 Identification of oestrogen receptors and oestrogen receptor mRNA in human adipose tissue. *Eur J Clin Invest* 26:262–269
- Pedersen O, Højlund E, Lindskov HO 1982 Insulin binding and action on fat cells from young healthy females and males. *Am J Physiol* 243:E158–E167
- Richelsen B, Eriksen EF, Beck-Nielsen H, Pedersen O 1984 Prostaglandin E2 receptor binding and action in human fat cells. *J Clin Endocrinol Metab* 59:7–12
- Hietanen E, Greenwood MR 1977 A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. *J Lipid Res* 18:480–490
- Pedersen SB, Borglum JD, Schmitz O, Bak JF, Sørensen NS, Richelsen B 1993 Abdominal obesity is associated with insulin resistance and reduced glycogen synthetase activity in skeletal muscle. *Metabolism* 42:998–1005
- Buhl ES, Jessen N, Schmitz O, Pedersen SB, Pedersen O, Holman GD, Lund S 2001 Chronic treatment with 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. *Diabetes* 50:12–17
- Castan I, Devedjian JC, Valet P, Paris H, LaFontan M 1995 Human adipocytes express α 2-adrenergic receptor of the α 2A- subtype only: pharmacological and genetic evidence. *Fundam Clin Pharmacol* 9:569–575
- Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS, Katzenellenbogen JA 2000 Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor- α -selective agonists. *J Med Chem* 43:4934–4947
- Meyers MJ, Sun J, Carlson KE, Katzenellenbogen BS, Katzenellenbogen JA 1999 Estrogen receptor subtype-selective ligands: asymmetric synthesis and biological evaluation of *cis*- and *trans*-5,11-dialkyl-5,6,11,12-tetrahydrochrysenes. *J Med Chem* 42:2456–2468
- Ottosson M, Vikman-Adolfsson K, Enerback S, Elander A, Björntorp P, Eden S 1995 Growth hormone inhibits lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 80:936–941
- Gray JM, Wade GN 1981 Food intake, body weight, and adiposity in female rats: actions and interactions of progestins and antiestrogens. *Am J Physiol* 240:E474–E481
- Pedersen SB, Bruun JM, Kristensen K, Richelsen B 2001 Regulation of UCP1, UCP2, and UCP3 mRNA expression in brown adipose tissue, white adipose tissue, and skeletal muscle in rats by estrogen. *Biochem Biophys Res Commun* 288:191–197
- Pedersen SB, Borglum JD, Møller-Pedersen T, Richelsen B 1992 Effects of *in vivo* estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. *Mol Cell Endocrinol* 85:13–19
- Gray JM, Wade GN 1980 Cytoplasmic estrogen, but not progestin, binding sites in male rat adipose tissues. *Am J Physiol* 239:E237–E241
- Roy EJ, Wade GN 1975 Role of estrogens in androgen-induced spontaneous activity in male rats. *J Comp Physiol Psychol* 89:573–579
- Darimont C, Delansorne R, Paris J, Ailhaud G, Negrel R 1997 Influence of estrogenic status on the lipolytic activity of parametrial adipose tissue *in vivo*: an *in situ* microdialysis study. *Endocrinology* 138:1092–1096
- Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS 2000 Increased adipose tissue in male and female estrogen receptor- α knockout mice. *Proc Natl Acad Sci USA* 97:12729–12734
- Mauriege P, Imbeault P, Prud'homme D, Tremblay A, Nadeau A, Despres JP 2000 Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution. *J Clin Endocrinol Metab* 85:2446–2454
- Boucher J, Castan-Laurell I, Le Lay S, Grujic D, Sibrac D, Krief S, Lafontan M, Lowell BB, Dugail I, Saulnier-Blache JS, Valet P 2002 Human α 2A-adrenergic receptor gene expressed in transgenic mouse adipose tissue under the control of its regulatory elements. *J Mol Endocrinol* 29:251–264
- Valet P, Grujic D, Wade J, Ito M, Zingaretti MC, Soloveva V, Ross SR, Graves RA, Cinti S, Lafontan M, Lowell BB 2000 Expression of human α 2-adrenergic receptors in adipose tissue of β 3-adrenergic receptor-deficient mice promotes diet-induced obesity. *J Biol Chem* 275:34797–34802

39. Iverius PH, Brunzell JD 1988 Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. *J Clin Invest* 82:1106–1112
40. Lazzarini SJ, Wade GN 1991 Role of sympathetic nerves in effects of estradiol on rat white adipose tissue. *Am J Physiol* 260(1 Pt 2):R47–R51
41. Palin SL, McTernan PG, Anderson LA, Sturdee DW, Barnett AH, Kumar S 2003 17 β -Estradiol and anti-estrogen ICI:compound 182,780 regulate expression of lipoprotein lipase and hormone-sensitive lipase in isolated subcutaneous abdominal adipocytes. *Metabolism* 52:383–388
42. Björntorp P 1991 Adipose tissue distribution and function. *Int J Obes* 15:67–81
43. Brönnegård M, Ottosson M, Böös J, Marcus C, Björntorp P 1994 Lack of evidence for estrogen and progesterone receptors in human adipose tissue. *J Steroid Biochem Mol Biol* 51:275–281
44. Rebuffé-Scrive M, Brönnegård M, Nilsson A, Eldh J, Gustafsson J-A, Björntorp P 1990 Steroid hormone receptors in human adipose tissues. *J Clin Endocrinol Metab* 71:1215–1219
45. Pedersen SB, Fuglsig S, Sjogren P, Richelsen B 1996 Identification of steroid receptors in human adipose tissue. *Eur J Clin Invest* 26:1051–1056
46. Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS 1997 Differential ligand activation of estrogen receptors ER α and ER β at AP1 sites. *Science* 277:1508–1510
47. Garcia E, Lacasa D, Giudicelli Y 2000 Estradiol stimulation of *c-fos* and *c-jun* expressions and activator protein-1 deoxyribonucleic acid binding activity in rat white adipocyte. *Endocrinology* 141:2837–2846

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