CLINICAL STUDY

Estradiol acutely inhibits whole body lipid oxidation and attenuates lipolysis in subcutaneous adipose tissue: a randomized, placebo-controlled study in postmenopausal women

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Abstract

Context: Estradiol (E₂) promotes and maintains the female phenotype characterized by subcutaneous fat accumulation. There is evidence to suggest that this effect is due to increased anti-lipolytic α 2A-adrenergic receptors, but whether this requires long-term exposure to E₂ or is an immediate effect is not clear.

Objective: To study acute effects of a single dose (4 mg) of 17 β -E₂ on regional and systemic lipolysis.

Methods: Sixteen postmenopausal women (age, 59 $\pm$ 5 years; weight, 67 $\pm$ 10 kg; and BMI, 24.8 $\pm$ 2.9) were studied in a crossover design: i) placebo and ii) 4 mg E₂. Basal and adrenaline-stimulated regional lipolysis was assessed by microdialysis and substrate oxidation rates by indirect calorimetry. Tissue biopsies were obtained to assess lipoprotein lipase activity and mRNA expression of adrenergic, estrogen, cytokine, and vascular reactivity receptors.

Results: Acute E₂ stimulation significantly attenuated catecholamine-stimulated lipolysis in femoral subcutaneous adipose tissue (interstitial glycerol concentration (micromole/liter) ANOVA time vs treatment interaction, $P=0.01$) and lipolysis in general in abdominal adipose tissue (ANOVA treatment alone, $P<0.05$). E₂ also reduced basal lipid oxidation ((mg/kg per min) placebo, 0.58 $\pm$ 0.06 vs E₂, 0.45 $\pm$ 0.03; $P=0.03$) and induced a significantly higher expression of anti-lipolytic α 2A-adrenergic receptor mRNA ($P=0.02$) in skeletal muscle tissue as well as an upregulation of eNOS (NOS3) mRNA ($P=0.02$).

Conclusion: E₂ acutely attenuates the lipolytic response to catecholamines in subcutaneous adipose tissue, shifts muscular adrenergic receptor mRNA toward anti-lipolytic α 2A-receptors, decreases whole body lipid oxidation, and enhances expression of markers of vascular reactivity.

European Journal of Endocrinology 167 543–551

Introduction

Estradiol (E₂) is the female sex steroid with effects on primary and secondary female sex organs. E₂ also has direct antioxidant effects (1), changes vascular reactivity (2, 3), interacts with vascular smooth muscle (4, 5), and reduces the level of circulating cholesterol (6) and free fatty acids (FFAs) (7). Some of these effects are acute, taking place within minutes or hours (8), whereas the beneficial effects on lipid metabolism may be related to more prolonged effects of E₂ on muscle and fat distribution. Thus, the reduced muscle power observed after menopause (9) is readily counteracted by female hormone therapy (HT) containing estrogens (10), which also prevents abdominal fat accumulation

(11). Abdominal or visceral fat accumulation is associated with insulin resistance and dyslipidemia, notably elevated triglycerides (TGs), and high LDL-cholesterol (12).

Exactly how E₂ modulates fat partitioning and redistribution is still not completely clear. Studies in transsexuals treated with E₂ for a full year have demonstrated that lipolytic activity is reduced in both abdominal and femoral adipocytes during basal conditions (13). Likewise, 2 months of E₂ treatment significantly reduces whole body lipolysis in postmenopausal women assessed by whole body palmitate turnover (14). This effect may be due to an increased number of anti-lipolytic α -adrenergic receptors and a decreased number of lipolytic β -adrenergic receptors

(15), corroborated by the observation that nor-epinephrine-stimulated lipolysis in the abdominal region is attenuated when oral E_2 is administered (16). A number of long-term replacement studies thus suggest that E_2 to some extent modulates body composition via a gradual shift in catecholamine receptor composition favoring anti-lipolysis.

However, E_2 may act more acutely as demonstrated by Van Pelt *et al.* (17), who administered E_2 i.v. and observed significantly diminished adipose tissue regional lipolysis assessed by microdialysis. This suggests that E_2 not only acts via nuclear but also via membrane-bound receptors, which has indeed been demonstrated (18). However, to our knowledge, no group has hitherto studied whether this acute attenuation of regional tissue lipolysis is translated into decreased whole body lipid oxidation. In addition, no attempts to link regional microdialysis data to mRNA expression of lipid flux controlling catecholamine and estrogen receptors (ERs) have been done. The purpose of this study was therefore to study the effects of a single dose (4 mg) of E_2 on regional adipose tissue lipolysis assessed by microdialysis and whole body lipid turnover assessed by indirect calorimetry. As an addition to previous studies, we aimed to link any such findings to changes in catecholamine receptor mRNA expression. It was our primary hypothesis that a single dose of E_2 would result in significantly attenuated lipolysis, oxidation, and lipolytic catecholamine receptor mRNA expression.

Materials and methods

Subjects

Sixteen healthy postmenopausal women (age, 59 ± 5 years; weight, 67 ± 10 kg; and BMI, 24.8 ± 2.9) not on hormone replacement therapy were recruited through advertizing. All had normal routine biochemistry and had been postmenopausal at least 1 year preceding the study. Subjects with FSH levels exceeding the detection limits of our assay were excluded. All subjects received oral and written information concerning the study before giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee (#2002-0242). Study subjects were recruited over two periods with eight subjects recruited through 2005–2006 and another eight subjects recruited in 2008. Data on very LDL-TG (VLDL-TG) kinetics from the last eight subjects have previously been published (19).

Experimental protocol

The study was carried out as a single-blinded, placebo-controlled, randomized trial (Fig. 1). The participants

Figure 1 Study protocol. Participants were studied for a total of 6.5 h under basal conditions (180 min), conditions of regionally stimulated lipolysis (120 min), and systemically stimulated lipolysis (90 min). Biopsies were performed at peak estradiol concentrations ($t=210$ min).

were instructed not to perform any physical exercise or ingest alcohol 1 day before the start of the study.

E_2 (4 mg) or placebo was administered orally at $t=-120$ after an overnight fast (~ 10 h). Two intravenous catheters (Venflon; Viggo, Helsingborg, Sweden) were inserted in the antecubital vein of the right arm and in a dorsal vein of the right hand. The latter was placed in a heated box at 65°C , allowing for arterialized blood samples to be drawn. At $t=-90$, microdialysis catheters were placed.

From $t=0$ min and onward, blood samples were drawn every 30 min and analyzed for plasma glucose (PG), FFAs, cortisol, GH, insulin, C-peptide, and catecholamines. Sampling from the microdialysis catheters (interstitial fluid) commenced at $t=0$ min, allowing for 60 min of equilibration to minimize the influence of local edema and hemorrhage. The sampling was performed every 30 min and continued until $t=330$ min. From $t=120-240$ min, adrenaline was added to the microdialysis perfusion fluid at a final concentration of $10\ \mu\text{M/l}$ to enhance lipolysis locally (20).

From $t=240-330$ min, adrenaline was infused i.v. at a rate of $0.15\ \text{nmol/kg per min}$ (21). We aimed at limiting the increase in heart rate to no more than 50% during this study period, which was accomplished in all subjects.

Indirect calorimetry (Deltatrac Metabolic Monitor, Datex, Helsinki, Finland) with a ventilated hood at $40\ \text{l/min}$ was performed, during the basal period ($t=-30-0$ min) and during the last 30 min of the systemically stimulated lipolysis ($t=300-330$ min). Energy expenditure (EE), respiratory exchange ratio, and 24-h excretion of urea in urine were measured, and glucose, protein, and lipid oxidation were calculated (22).

Tissue biopsies

At $t=210$, a muscle biopsy was obtained from the vastus lateralis muscle with a Bergström biopsy needle under local anesthesia (1% lidocaine), a small incision having been made through the skin and muscle sheath 15–20 cm above the knee. A total amount of ~ 200 mg muscle was aspirated, cleaned for blood (within 15 s), and snap-frozen in liquid nitrogen. Muscle biopsies were stored at -80°C until analyzed. Immediately following the muscle biopsy, a subcutaneous fat biopsy from the periumbilical region was obtained by liposuction and snap-frozen in liquid nitrogen.

Microdialysis

After application of a local analgesic (Lidocaine), microdialysis catheters (CMA-60; CMA, Stockholm, Sweden) were inserted in subcutaneous adipose tissue in the periumbilical and femoral region. The microdialysis catheters have a molecular cutoff of 20 kDa and a membrane length of 30 mm and were perfused at a flow rate of 1 $\mu\text{l}/\text{min}$ using CMA-107 perfusion pumps (CMA). We attempted to assess the relative recovery of interstitial glycerol by the internal reference method with [^3H]glycerol (23). However, as adrenaline added to the perfusate introduced a dramatic variability in individual recovery rates (data not shown), interstitial glycerol concentrations were not corrected for relative recovery and may thus have been slightly underestimated. Perfusate and dialysate were counted using a Wallac 1450 liquid scintillation counter applying the Optiphase supermix scintillation fluid. Changes in interstitial glycerol concentration can be taken as an index of lipolysis (24, 25, 26).

Adipose tissue blood flow

Subcutaneous adipose tissue blood flow (ATBF) was assessed in the abdominal and femoral region by the local ^{133}Xe washout method (27). In short ~ 1 Mbq ^{133}Xe was injected into the area of interest and based on the rate constant (k) of the washout curve, ATBF was calculated as $\text{ATBF} = k \times \lambda \times 100$ (ml/100 g per min), where λ is the tissue to blood partition coefficient for ^{133}Xe at equilibrium; counts were collected every second and a straight line was fitted through the experimental points in a semilogarithmic diagram as a function of time. λ was calculated as $\lambda = 0.22 \times \text{SFT} + 2.99$, where SFT is the skin fold thickness of the abdominal adipose tissue (28).

Lipoprotein lipase activity measurements

Adipose tissue lipoprotein lipase (LPL) activity was determined essentially as described previously (29). Briefly, 200 mg adipose tissue was homogenized in a buffer containing 0.25 M sucrose, 1.0 mM K_2EDTA

(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 [^{14}C]triolein after 60 min of incubation. FFAs were extracted from the incubation mixture as described previously (29) and measured by liquid scintillation counting.

Muscle LPL activity was measured in ~ 30 mg of the muscle biopsy. This was cut (on ice) into small pieces (3–5 mg) and placed in incubation tubes. The tissue was incubated in a heparin-containing (200 IU L21) medium with a serum-activated triolein emulsion with ^{14}C -triolein as tracer and albumin as acceptor of released fatty acids. The mixture was incubated for 30 min on an ice bath and then for a further 120 min at 37°C in a shaking water bath. LPL activity was measured on the same day to eliminate the influence of day-to-day variation.

Isolation of RNA

Adipose tissue samples (200 mg) were homogenized in TRIzol reagent (Life Technologies, Inc.), and total RNA was extracted following the manufacturer's protocol. RNA was quantitated by measuring absorbency at 260 and 280 nm, and purity was assured by a ratio of 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, USA). Then, PCR mastermix containing the specific primers and *Taq* DNA polymerase (HotStar *Taq*; Qiagen, Inc.) were added. 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) as described previously (30). 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 (C_t) 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 $\text{X0/R0} = k \times 1/(2^{\Delta C_t})$, where ΔC_t is the difference between C_t -target and C_t -reference and k is a constant, set to 1. All samples were amplified in duplicate. A similar setup was used for negative controls, except that the reverse transcriptase was omitted, and no PCR products were detected under these conditions. The following targets were measured: $\alpha 2\text{A}$ -receptor,

$\beta 1$ -receptor, $\beta 3$ -receptor, $ER\alpha$, $ER\beta$, uncoupling protein 2 (UCP2), UCP3, adiponectin, hormone-sensitive lipase (HSL), LPL, interleukin 6, vascular cell adhesion molecule 1, intercellular adhesion molecule, monocyte chemoattractant protein, and endothelial derived nitric oxide synthase (eNOS).

Assays

PG was analyzed in duplicate using the glucose oxidase method (Beckman Coulter, Palo Alto, CA, USA). Measurements were performed immediately during the study. Insulin was measured with an immunoassay (DAKO, Glostrup, Denmark). Serum FFA was determined by a colorimetric method employing a commercial kit (Wako Pure Chemical Industries, Neuss, Germany). Serum 17β -E₂ was analyzed using a commercial ultrasensitive assay (Spectria Estradiol Sensitive RIA, Orion Diagnostica Espoo, Finland), with detection limits of 0.005 nmol/l. All samples from an individual patient were analyzed in the same assay. Adrenaline and noradrenaline were measured by HPLC (31). Glycerol in the microdialysis dialysate was measured in duplicate by an automated spectrophotometric kinetic enzymatic analyzer (CMA-600).

Statistical analysis

Results from blood samples (hormones and metabolites) are expressed as time-averaged values during the basal (0–240 min) and stimulated period ($t=240$ –330 min). To investigate the impact of E₂ on regional lipolysis, we used a mixed model with fixed effect terms treatment (placebo or E₂) and time and the interaction of time and treatment as the term of interest (time $\times$ treatment). Subjects were included as random effects to account for within-subject correlation. All other between-treatment differences were assessed by Student's *t*-test (parametric data) or Wilcoxon signed rank test (nonparametric data). *P* values <0.05 were considered significant. Statistical analysis was performed using SPSS version 11 (SPSS Inc., IBM, Somers, NY, USA). Parametric data are presented as mean $\pm$ S.E.M. and nonparametric data as medians (range).

Results

Hemodynamics

Basal heart rate (pulse_{BASAL} (beats/min), 61 ± 2 (placebo) vs 61 ± 2 (E₂); $P=0.57$), systolic blood pressure (systolic_{BASAL} (mmHg): placebo, 127 ± 4 vs E₂, 127 ± 3 ; $P=0.93$), and diastolic blood pressure (diastolic (mmHg): placebo, 79 ± 3 vs E₂, 79 ± 2 ; $P=0.77$) was unaffected by E₂. Likewise, the expected increase in heart rate and decrease in blood pressure induced by the systemic adrenaline infusion was similar regardless of treatment: (pulse_{SYSTEMIC} (beats/min):

placebo, 73 ± 3 vs E₂, 73 ± 2 ; $P=0.78$), (systolic_{SYSTEMIC} (mmHg): placebo, 117 ± 4 vs E₂, 117 ± 4 ; $P=0.95$), and (diastolic_{SYSTEMIC} (mmHg): placebo, 68 ± 2 vs E₂, 68 ± 2 ; $P=0.84$).

Circulating hormones

Participants had low to undetectable E₂ concentrations (0.08 ± 0.01 nmol/l) on the placebo study day (Fig. 2), while acute E₂ resulted in an approximate fourfold rise in E₂ concentrations with peak values occurring at $t=300$ min (0.35 ± 0.06 nmol/l). Adrenaline concentrations were low through basal and regionally stimulated lipolysis (adrenalin (pg/ml): placebo, 32.5 ± 3.4 vs E₂, 35.6 ± 4.6 ; $P=0.54$) and rose sharply to a plateau value (adrenalin (pg/ml): placebo, 308.6 ± 34.8 vs E, 300.9 ± 36.0 ; $P=0.83$) after initiation of the adrenaline infusion. Noradrenaline concentrations were comparable on both study days and only increased modestly during the adrenaline infusion. Insulin levels tended to decrease through basal and regionally stimulated lipolysis on both study days (insulin (pmol/l): placebo, 22.5 ± 2.3 vs E₂, 23.6 ± 2.6 ; $P=0.55$) with a similar increase in response to elevated glucose levels observed during the adrenaline infusion (insulin (pmol/l): placebo, 28.5 ± 3.1 vs E₂, 28.6 ± 2.8 ; $P=0.97$).

Metabolites

FFA levels were similar on both study days through the basal and regionally stimulated period (FFA (μ mol/l):

Figure 2 Circulating hormones and metabolites. Filled circle, placebo; open triangle, estradiol. Error bars are $\pm$ S.E.M., $n=16$.

placebo, 583 ± 33 vs E_2 , 575 ± 34 ; $P=0.86$; Fig. 2). Adrenaline infusion doubled FFA levels within 30 min to $\sim 1.200 \mu\text{mol/l}$ with a subsequent gradual decrease to $\sim 900 \mu\text{mol/l}$ at $t=330$ min. Blood glucose concentrations tended to decrease slightly through the basal and regionally stimulated period and rose sharply after initiation of the adrenaline infusion (Fig. 2). No significant differences in glucose levels between treatment days were observed.

Regional lipolysis

Three distinct patterns of adrenaline-induced lipolysis were observed (Fig. 3): i) in muscle, basal interstitial glycerol concentrations, and thus lipolysis, were unaffected by E_2 and the regional and systemic adrenaline stimulation elicited similar increases in interstitial glycerol concentrations (micromole/liter; repeated measurements ANOVA (RM-ANOVA; time vs treatment), $P=\text{NS}$). ii) In femoral adipose tissue, a similar lack of effect was observed in the basal period, whereas the lipolytic response to adrenaline-stimulated lipolysis was significantly attenuated when subjects were treated with E_2 (RM-ANOVA (time vs treatment), $P=0.01$). iii) In abdominal adipose tissue, by contrast, interstitial glycerol concentrations were lower after E_2 treatment already during the basal period and remained significantly subdued during local adrenaline and systemic stimulated lipolysis (RM-ANOVA (treatment), $P<0.05$). This anti-lipolytic effect was not enhanced by adding epinephrine to the perfusate (RM-ANOVA during the regional period (time vs treatment), $P=0.34$).

Substrate oxidation (indirect calorimetry)

During basal conditions, E_2 treatment significantly decreased whole body lipid oxidation rates (LIPOX_{basal} (mg/kg per min): placebo, 0.58 ± 0.06 vs E_2 , 0.45 ± 0.03 ; $P=0.03$; Fig. 4). As expected, glucose oxidation was increased, albeit not significantly (GOX_{basal} (mg/kg per min): placebo, 1.31 ± 0.11 vs E_2 , 1.48 ± 0.10 ; $P=0.10$). The adrenaline infusion elicited an immediate $\sim 20\%$ increase in EE regardless of treatment (data not shown), which could be entirely attributed to increased lipid oxidation. However, during systemic adrenaline stimulation, no difference was observed in neither lipid (LIPOX_{systemic} (mg/kg per min): placebo, 0.87 ± 0.09 vs E_2 , 0.87 ± 0.08 ; $P=0.98$) nor glucose (GOX_{systemic} (mg/kg per min): placebo, 1.06 ± 0.19 vs E_2 , 1.13 ± 0.14 ; $P=0.60$) oxidation (Fig. 5).

Basal total EE tended to decrease during E_2 treatment (EE_{basal} (kcal/24 h): placebo, 1297 ± 51 vs E_2 , 1237 ± 40 ; $P=0.06$), whereas this was not observed during adrenaline-stimulated conditions (EE_{systemic} (kcal/24 h): placebo, 1472 ± 56 vs E_2 , 1504 ± 57 ; $P=0.31$).

Figure 3 Regional lipolysis assessed by microdialysis. From $t=120$ – 240 adrenaline was added to the perfusate in order to enhance lipolysis and from $t=240$ – 330 adrenaline was infused i.v. The impact of estradiol on regional lipolysis was analyzed by repeated measurements ANOVA with time $\times$ treatment or treatment as the parameter of interest. Error bars are $\pm$ S.E.M., $n=16$. Filled circle, placebo; open triangle, estradiol.

LPL activity and mRNA expression

E_2 treatment resulted in a trend toward reduced LPL activity in skeletal muscle ($\sim 26\%$, $P=0.09$) whereas this was not the case in adipose tissue. No convincing trends were observed in LPL mRNA levels in muscle tissue or fat.

Catecholamine receptor gene expression

E_2 resulted in significantly increased anti-lipolytic α_2 -adrenergic receptor mRNA in muscle (Fig. 5B) and also tended to increase lipolytic β_1 -adrenergic receptor mRNA in that tissue (D). However, we were unable to detect any measurable effect of E_2 on the same receptor mRNA in adipose tissue (Fig. 5A and C).

Figure 4 Substrate oxidation rates. (A) lipid oxidation. E_2 administration significantly decreased basal lipid oxidation. (B) Glucose oxidation. No difference was observed between placebo and E_2 . All error bars are $\pm$ S.E.M., $n=15$ (one subject suffered from claustrophobia and could not cooperate). 'Basal' refers to $t=-60$ to 120 min whereas 'systemic' refers to systemic stimulation with adrenaline from $t=240$ to 330 min.

Markers of vascular reactivity (ATBF and eNOS mRNA)

During basal conditions, ATBF in both the femoral (ATBF_{BASAL} (ml/100 g per min): placebo, 1.01 ± 0.18 vs E_2 , 1.04 ± 0.13 ; $P=0.90$) and the abdominal (ATBF_{BASAL} (ml/100 g per min): placebo, 0.93 ± 0.11 vs E_2 , 0.84 ± 0.13 ; $P=0.58$) region was comparable. However, during systemic adrenaline infusion, E_2 treatment resulted in significantly diminished ATBF in the abdominal region (ATBF_{SYSTEMIC} (ml/100 g per min): placebo, 1.37 ± 0.15 vs E_2 , 0.98 ± 0.16 ; $P=0.01$), whereas no such effect was observed in the femoral region (ATBF_{SYSTEMIC} (ml/100 g per min): placebo, 1.50 ± 0.24 vs E_2 , 1.64 ± 0.17 ; $P=0.44$; Fig. 6).

In muscle, E_2 significantly increased eNOS (NOS3) mRNA expression ($P=0.02$). No significant effect was observed in adipose tissue.

Discussion

The principal results from this study show that a single dose of E_2 in postmenopausal women resulted in significant and immediate metabolic responses: i) the lipolytic response to local catecholamine stimulation in subcutaneous femoral adipose tissue was attenuated; ii) overall lipolysis was attenuated in subcutaneous abdominal adipose tissue; iii) an immediate shift in muscle adrenergic receptor expression toward anti-lipolytic α_2A -receptors occurred; iv) whole body fuel combustion shifted from lipid oxidation toward glucose consumption and a trend toward diminished LPL activity and thus extraction of lipids from circulating lipoproteins was observed; and v) finally, E_2 altered markers of vascular reactivity by stimulating eNOS mRNA expression in muscle tissue and decreasing adrenaline-stimulated ATBF in the abdominal region.

Menopause causes an extensive array of modulations in the typical female fat distribution, most notably a shift in fat depots from the lower body and subcutaneous depots to the upper body and visceral depots (32). Female sex HT abolishes this potentially deleterious effect of menopause and the accompanying decrease in estrogen levels (11), but whether this effect of E_2 on lipid metabolism is indirect or direct is not obvious. Adipocytes express both α - and β -ERs (33) and long-term E_2 treatment results in a shift in adrenergic receptor composition increasing the number of anti-lipolytic α_2A -receptors (15). Thus, the effect of E_2 on lipid metabolism and hence on body composition

Figure 5 Anti-lipolytic α_2A - and β_1 -receptor mRNA expression. Median values are represented by vertical lines as appropriate, $n=14$ (two subjects did not wish to have muscle biopsies taken). All units are arbitrary units (AU). Adipose tissue was obtained from the periumbilical region.

Figure 6 Markers of vascular reactivity. (A) Abdominal adipose tissue blood flow (ATBF) during systemic adrenaline infusion was significantly suppressed after estradiol treatment compared with placebo conditions. (B) Femoral ATBF did not show a similar trend as no differences between treatment days were observed. Error bars are $\pm$ S.E.M., $n=16$. (C and D) No difference was observed in eNOS mRNA expression in adipose tissue, whereas estradiol clearly upregulated eNOS mRNA in muscle, $n=14$. Data are presented as point plots with median values as vertical lines.

appears to be direct and mediated via ERs. These observations have led to renewed interest in adipocyte receptor subgroups, and recently, Garcia Dos Santos *et al.* (18) demonstrated the existence of a membrane-bound ER α . In that study, the authors demonstrated that physiological concentrations of E₂ rapidly lead to activated p42/p44 mitogen-activated protein kinase and that this effect could be blocked by an estrogen antagonist. Furthermore, infusion of conjugated estrogens increases insulin action within hours and decreases plasma glycerol concentrations during a hyperinsulinemic euglycemic clamp (34), rendering it plausible that a range of signaling cascades is activated acutely by E₂. It can therefore be speculated that adrenergic receptors as a result of E₂ administration are translocated to the cell surface of adipocytes within hours of stimulation. Cross talk between activated membrane-bound ERs and adrenergic receptors could therefore potentially facilitate a more rapid effect of E₂ than the classical pathway through nuclear receptors. It is therefore a major finding of this study that adrenaline-stimulated lipolysis was indeed markedly attenuated in subcutaneous adipose tissue when studied only 4 h after administration of a single dose of E₂. Although we failed to detect a significant upregulation of adipose tissue anti-lipolytic adrenergic receptor mRNA, this may therefore reflect the abovementioned rapid nongenomic effects of E₂ and that a longer observation period would have led to increases in the mRNA level.

Adipocyte function is tightly regulated by a range of hormones and enzyme systems. Hyperinsulinemia favors TG accumulation through inhibition of the HSL, whereas catecholamine stimulation through adrenergic β 1- and β 2-receptors stimulates lipolysis via increased HSL activity. Counteracting the lipolytic effects of adrenergic β -receptors, stimulation of the α 2A-receptor initiates a cascade blocking adenylate cyclase and ultimately resulting in inhibition of the HSL mimicking the effects of insulin (35). The ratio of lipolytic β -receptors to anti-lipolytic α -receptors is shifted by exposure to E₂ depending on the location of the fat depot: in visceral fat, E₂ has no detectable effect on this ratio, whereas a change toward anti-lipolytic α -receptors is observed in subcutaneous fat depots (15). This could suggest that the decline in estrogen observed after menopause promotes lipolysis and contributes to reduced subcutaneous adipose tissue. Data presented here demonstrate that in skeletal muscle a shift toward anti-lipolytic α 2A-receptors takes place on mRNA level within hours after E₂ exposure and in adipose tissue the same trend was observed.

In line with the findings of most (14, 36, 37, 38, 39), but not all (40), previous studies, whole body lipid oxidation was significantly decreased in our volunteers during E₂ treatment. However, whereas previous studies have been designed to study more long-term effects of estrogens with typical treatment periods lasting 1–12 months, this is to our knowledge the first study in humans reporting immediate effects of estrogen on lipid oxidation. Interestingly, animal studies have indicated that E₂ administered for brief periods results in significantly reduced hepatic carnitine palmitoyltransferase 1 (CPT1 (CPT1A)) mRNA expression (41) and thus reduces lipids transported into mitochondria for oxidation. Our results suggest that hepatic and/or peripheral lipid oxidation is effectively reduced within hours after E₂ treatment, an effect that must be largely independent of gene up or down regulation.

Somewhat to our surprise, E₂ reduced the known stimulatory effect of systemic adrenaline infusion on ATBF (42). However, as E₂ convincingly reduces lipid oxidation and subcutaneous lipolysis, it is intuitively appealing that it should also reduce ATBF to regional fat depots. E₂ treatment was also associated with a significant increase in eNOS mRNA expression in muscle, an effect that has been demonstrated before in animal and cell studies (43, 44). This may serve to further divert blood from adipose tissue toward muscle.

The data presented herein demonstrate that E₂ has immediate and therefore most likely nongenomic effects on lipid metabolism. In summary, E₂: i) attenuates the lipolytic response to adrenaline in subcutaneous femoral adipose tissue and overall lipolysis in subcutaneous abdominal adipose tissue; ii) inhibits basal whole body lipid oxidation; iii) upregulates anti-lipolytic α 2A-receptor mRNA in muscle; and iv) reduces the stimulatory effect of adrenaline on ATBF.

Declaration of interest

L Gormsen, B E Hjerrild, S B Pedersen, and S Nielsen have no disclosures. C H Gravholt has received lecture fees from Novo Nordisk. J S Christiansen has served as a consultant and received lecture fees from Novo Nordisk, Pfizer, and Ipsen.

Funding

The study was supported by grants from the Danish Health Research Council, grant number 22-01-0395, and the Novo Nordisk Foundation.

Author contribution statement

All studies were performed at The Medical Research Laboratories, Aarhus University Hospital, Denmark. L C Gormsen designed the experiments, performed metabolic studies, collected data, interpreted data, and wrote the manuscript. C Høst performed metabolic studies, collected data, interpreted data, and revised the manuscript. B E Hjerrild performed metabolic studies and collected data. S B Pedersen collected and interpreted data and revised the manuscript. J S Christiansen interpreted data and revised the manuscript. S Nielsen collected data, interpreted data, and revised the manuscript. C H Gravholt conceived the experiment, designed the study, collected and interpreted data, and revised the manuscript.

Acknowledgements

The authors thank Annette Mengel for excellent technical assistance. Roche Diagnostics kindly donated the microdialysis catheters.

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Received 14 May 2012

Revised version received 17 July 2012

Accepted 6 August 2012