Estradiol effects on subcutaneous adipose tissue lipolysis in premenopausal women are adipose tissue depot specific and treatment dependent

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Gavin KM, Cooper EE, Raymer DK, Hickner RC. Estradiol effects on subcutaneous adipose tissue lipolysis in premenopausal women are adipose tissue depot specific and treatment dependent. Am J Physiol Endocrinol Metab 304: E1167–E1174, 2013. First published March 26, 2013; doi:10.1152/ajpendo.00023.2013.—Estrogen has direct effects within adipose tissue and has been implicated in regional adiposity; however, the influence of estrogen on in vivo lipolysis is unclear. The purpose of this study was to investigate the effect of local 17β-estradiol (E2) on subcutaneous adipose tissue (SAT) lipolysis in premenopausal women. In vivo lipolysis (dialysate glycerol) was measured in 17 women (age 27.4 ± 2.0 yr, BMI 29.7 ± 0.5 kg/m2) via microdialysis of abdominal (AB) and gluteal (GL) SAT. Glycerol was measured at baseline and during acute interventions to increase lipolysis including local perfusion of isoproterenol (ISO, β-adrenergic agonist, 1.0 μmol/l), phentolamine (PHEN, α-adrenergic antagonist, 0.1 mmol/l), and submaximal exercise (60% VO2peak, 30 min); all with and without coperfusion of E2 (500 nmol/l). E2 coperfusion blunted the lipolytic response to ISO in AB (E2 196 ± 31%, control 258 ± 26%, P = 0.003) but not in GL (E2 113 ± 14%, control 111 ± 12%, P = 0.43) adipose tissue. At rest, perfusion of PHEN with ISO did not change dialysate glycerol. Submaximal exercise during ISO + PHEN increased dialysate glycerol in the AB (56 ± 9%) and GL (62 ± 12%) regions. Probes perfused with E2 during exercise and ISO + PHEN had an increased lipolytic response in AB (90 ± 9%, P = 0.007) but a lower response in GL (35 ± 7%, P = 0.05) SAT compared with no-E2 conditions. E2 effects on lipolysis are region specific and may work through both adrenergic and adrenergic-independent mechanisms to potentiate and/or blunt SAT lipolysis in premenopausal women.

lipolysis; estrogen; exercise; regional adiposity; microdialysis

WOMEN HAVE THE PROPENSITY to accumulate more adipose tissue than men, particularly in the subcutaneous depot and gynoid region (19). Reduced cardiometabolic disease risk in premenopausal women has been attributed to this differential body fat distribution (18) and the benign (35) or even protective effect of lower body adiposity (28, 33, 34, 38). Alterations in the circulating hormonal milieu with menopause, such as the decline in circulating estrogens, have been implicated in age-related shifts toward a central body fat distribution and cardiometabolic risk (10, 24).

Although associative data supporting the role of premenopausal levels of circulating estradiol (70–1,500 pmol/l depen-

dent on menstrual cycle phase) in maintaining gynoid body fat distribution is available (22), mechanisms of sex steroid action within adipose tissue and how this may impact body fat distribution and lipid metabolism remain unclear. Adipose tissue serves not only as a sink for hormones from the circulation but also as an endocrine organ producing sex hormones that act in paracrine and autocrine fashions, more recently termed intracrinology (3). The ability of adipose tissue to regulate concentrations of sex steroids locally reveals what may be an important evolutionary mechanism to help determine or regulate regional adiposity. Although other locally produced and metabolized sex steroids such as progesterone and androgens may be involved, the discovery of estrogen receptor-α and -β (ERα, ERβ) (26, 29), as well as the G protein-coupled estrogen receptor (GPER) (14) in human adipose tissue, supports the important role of direct genomic and nongenomic actions of estrogens within this tissue. Furthermore, a role for estrogen in fat metabolism is evident in men with aromatase deficiency syndrome, who typically present with abdominal adiposity and a BMI in the overweight-to-obese range (15).

The balance or imbalance of triacylglycerol accumulation and free fatty acid release within a specific adipose depot is responsible for changes in local adiposity. A lower lipolytic rate in the gluteal depot of premenopausal women has been implicated in the increased adiposity demonstrated in this region (23, 40); however, the mechanisms underlying this regional difference in lipolysis are not completely understood. Importantly, acute intravenous administration of conjugated estrogens has been shown to decrease basal lipolysis in the abdominal and femoral subcutaneous adipose tissue of estrogen-deficient postmenopausal women as measured by the in situ technique of microdialysis (37). However, similar studies in premenopausal women examining acute modulation of lipolysis by 17β-estradiol (E2) are lacking, and this knowledge is of paramount importance in uncovering the mechanisms underlying the region-specific adiposity demonstrated by premenopausal women. In addition, this information may help in understanding the shift in body fat distribution demonstrated in women after menopause. Therefore, the purpose of this study was to investigate the effect of locally perfused E2 on basal and stimulated subcutaneous adipose tissue lipolytic rate in the abdominal and gluteal regions of overweight-to-obese premenopausal women.

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MATERIALS AND METHODS

Participants

Seventeen overweight-to-obese premenopausal women (9 Caucasian, 8 African American, 27.4 ± 2.0 yr, 81.7 ± 2.3 kg, BMI 29.7 ± 0.5 kg/m²) between 18 and 44 yr old were studied (Table 1). Participants were eumenorrheic (average cycle length 30 ± 1 days), not taking hormonal contraceptives (no use in ≥6 mo at study entry), weight stable (<3 kg weight change in previous 6 mo), and not regularly active (exercise <30 min/day, <2 days/wk). Exclusion criteria evaluated by medical history questionaire included trying to get pregnant, currently pregnant or lactating, smoking, self-reported history of diabetes, metabolic or cardiovascular diseases, and taking any medications known to alter lipid metabolism or blood flow. Participants were informed verbally and in writing of the purpose, risks, and benefits of the research and provided informed consent prior to enrollment in the study. This study was approved by the Medical Center Institutional Review Board at East Carolina University.

Experimental Procedures

Initial visit. Participants reported to the Human Performance Lab at East Carolina University to obtain written informed consent, collect baseline information, and record self-reported dates of menses for the previous 6–12 mo. Weight was measured on an electronic scale and recorded to the nearest 0.1 kg, and height was measured with a standard stadiometer to the nearest centimeter. Minimal waist and hip circumferences were measured according to previously published guidelines, and waist-to-hip ratio (WHR) was calculated (21). Fat-free mass (lean mass + bone mineral content), fat mass (total, android, and gynoid), and percent body fat were determined using dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, Madison, WI). Amylase and gynoid regions were determined according to manufacturer recommendations.

A VO2peak test of aerobic fitness was conducted on a Corival LODE cycle ergometer using a TrueOne 2400 metabolic cart (ParvoMedics, Sandy, UT) for indirect calorimetry measurements. Expiratory gases were monitored throughout the test, and heart rate (HR; polar hear rate monitor, Polar Electro, Lake Success, NY) and blood pressure (manual measurements every 2 min) were monitored during exercise and recovery. Resistance started at 25 W and increased 15–25 W every 2 min until volitional fatigue. The test was deemed successful according to the guidelines set forth by the American College of Sports Medicine (25).

Microdialysis visit. Participants reported to the East Carolina Diabetes and Obesity Institute at the East Carolina Heart Institute at 0800 after an overnight (&gt;10 h) fast during the follicular phase (day 5 ± 1 after start of menses) of the menstrual cycle for a single microdialysis visit. Follicular timing of the microdialysis visit was important to ensure 1) similar circulating E2 concentrations between participants and 2) low circulating E2 concentrations to minimize local effects of circulating E2 within the adipose tissue. Menstrual cycle phase was confirmed by blood draw the morning of the visit (Table 2). All participants rested in a semirecumbent position for the duration of the study with the exception of the cycling session.

An indwelling polyethylene catheter (iv) was inserted into an antecubital vein for blood sampling. Blood was collected in lithium heparin or EDTA tubes for plasma or in untreated vacutainers for serum. Plasma and serum were obtained by centrifugation, and aliquots were immediately stored at −80°C until later batch analysis. Resting energy expenditure (REE) measurements were made via indirect calorimetry (ParvoMedics TrueOne 2400) for 25 min during quiet rest (~120 min after probe insertion). The final 20 min of measurements were averaged for final REE.

The skin over a small area of the subcutaneous adipose tissue (SAT) region of interest was desensitized to pain with ethyl chloride spray, and four microdialysis probes (CMA 20 MD Elite Probe 10 mm, 20 kDa cutoff; CMA Microdialysis/Harvard Apparatus, Holliston, MA) were inserted unilaterally into the upper gluteal (GL) SAT ~3–10 cm right or left of the medial line of the buttock as well as unilaterally into the abdominal (AB) SAT ~3–6 cm lateral to the umbilicus for a total of eight probes, all tested simultaneously. Probes were placed at least 2 cm from one another to avoid interference from adjacent probes and at a depth of ~1 cm into the SAT. No sample was collected during the first hour post-probe insertion in order to allow for equilibration of the microdialysis system (4, 13).

Microdialysis was performed with continuous perfusion of a 0.9% saline solution containing 10 mmol/l ethanol (indirect indicator of local blood flow) (12, 13) (perfusate, control) at 2.0 μl/min (CMA107 microinfusion pumps; M Dialysis, N. Chelmsford, MA). After exchange with the SAT interstitial contents, the perfused solution was collected at the exit end of the probe (dialysate) and stored at −4°C for analysis of ethanol within 48 h and subsequently stored at −20°C for batch analysis of dialysate glycerol (in vivo indicator of lipolysis) and determination of interstitial E2. The experimental design for the microdialysis visit (Fig. 1) included five stages in which in vivo

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Body mass index, kg/m²</th>
<th>Waist circumference, cm</th>
<th>Waist-to-hip ratio</th>
<th>Body fat, %</th>
<th>Android fat mass, kg</th>
<th>Gynoid fat mass, kg</th>
<th>Total cholesterol, mmol/l</th>
<th>HDL-cholesterol, mmol/l</th>
<th>LDL-cholesterol, mmol/l</th>
<th>Triglycerides, mmol/l*</th>
<th>Fasting glucose, mmol/l</th>
<th>Fasting insulin, pmol/l</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>27.4 ± 2.0</td>
<td>81.7 ± 2.3</td>
<td>29.7 ± 0.5</td>
<td>89.3 ± 1.1</td>
<td>0.79 ± 0.01</td>
<td>3.1 ± 0.3</td>
<td>7.0 ± 0.3</td>
<td>3.9 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>0.73 (0.57–0.93)</td>
<td>4.8 ± 0.1</td>
<td>55.0 ± 5.5</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Skewed variable, reexponentiated to geometric mean and 95% confidence interval. HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2. Gynecologic history and baseline sex hormones

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Cycle length, days</th>
<th>Age at menarche, yr</th>
<th>Parity, yes/no</th>
<th>Time since HC use, yr</th>
<th>Estradiol, pmol/l*</th>
<th>Estrenone, pmol/l*</th>
<th>Progesterone, nmol/l*</th>
<th>Testosterone, nmol/l</th>
<th>FSH, IU/l</th>
<th>LH, IU/l</th>
<th>SHBG, nmol/l*</th>
<th>DHEA-S, μmol/l</th>
<th>Interstitial estradiol</th>
<th>Gluteral, pmol/l*†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>30.4 ± 0.7</td>
<td>11.7 ± 0.3</td>
<td>4/13</td>
<td>5.6 ± 2.5</td>
<td>226 (147–348)</td>
<td>0.36 (0.24–0.54)</td>
<td>1.08 ± 0.13</td>
<td>7.3 ± 0.9</td>
<td>5.6 ± 0.9</td>
<td>39.5 (32.6–47.8)</td>
<td>4.5 ± 0.5</td>
<td>415 (220–783)</td>
<td>380 (268–540)</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Skewed variable, reexponentiated to geometric mean and 95% confidence interval; †n = 13, HC, hormonal contraceptives; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; DHEA-S, dihydroepiandrosterone sulfate.
lipolysis was assessed under either basal or pharmacological and physiological interventions designed to increase lipolysis. The SAT was locally perfused with the following solutions throughout the visit, under both resting (stage 1–4) and submaximal aerobic exercise (stage 5) conditions: control 0.9% saline solution, 1.0 μmol/l isoproterenol [ISO, β-adrenergic agonist] and ISO + 0.1 μmol/l phenolamine (PHEN, α-adrenergic antagonist) and ISO + 0.1 mmol/l phenolamine (PHEN, α-AR antagonist) (Sigma Aldrich, St. Louis, MO). All conditions were studied with and without coperfusion of 500 nmol/l E2 (Sigma Aldrich) to investigate E2’s effect on basal and induced lipolysis. Dialysate samples were collected every 15–30 min.

Previous microdialysis studies have demonstrated that perfusion of ISO and PHEN at the concentrations utilized in the current protocol induce maximal alterations in lipolysis (1, 2). Furthermore, use of these pharmacological agents allow for specific AR targeted perturbations of lipolysis to gain a more in-depth understanding of which pharmacological agents introduce into the SAT by microdialysis remain under both resting (stage 1–4) and submaximal aerobic exercise (stage 5) conditions: control 0.9% saline solution, 1.0 μmol/l isoproterenol [ISO, β-adrenergic (AR) agonist] and ISO + 0.1 mmol/l phenolamine (PHEN, α-AR agonist) (Sigma Aldrich, St. Louis, MO). All conditions were studied with and without coperfusion of 500 nmol/l E2 (Sigma Aldrich) to investigate E2’s effect on basal and induced lipolysis. Dialysate samples were collected every 15–30 min.

Microdialysis samples and calculations. Dialysate glycerol (Glycdialysate; basal: interstitial E2 concentrations throughout the microdialysis visit [baseline, 226 (147–348) pmol/l; preexercise, 234 (147–373) pmol/l; postexercise, 269 (163–443) pmol/l; mean 95% CI].

Stage 5 involved 30 min of stationary cycling on a Monark Ergometric 828E cycle ergometer at 60% of each participant’s own previously determined V\textsubscript{O2peak}. Submaximal exercise was included as a physiological stimulator of lipolysis, as exercise at 60% V\textsubscript{O2peak} has been previously reported to elicit maximal lipolytic stimulation (9). HR was monitored throughout (Polar), and indirect calorimetry measurements (ParvoMedics) were taken from 0 to 7 and 20 to 25 min. Two 15-min dialysate samples were collected during exercise, with microdialysis probes removed after the exercise session.

Sample Analysis

Microdialysis samples and calculations. Dialysate glycerol (Glycdialysate; index of in vivo lipolysis) concentration was analyzed using a CMA/ 600 automated microdialysis analyzer (M Dialysis). A concentration of 10 mmol/l ethanol has been shown to effectively detect blood flow changes around the microdialysis probe in SAT (7). The ethanol outflow-to-inflow ratio (O:I) is inversely related to blood flow and was calculated: ethanol outflow:inflow = [ethanol\textsubscript{dialysate}/ethanol\textsubscript{perfusate}]. Dialysate and perfusate ethanol was measured in our laboratory using a previously described enzymatic, fluorometric assay (13). Dialysate E2 was measured using a Salivary Estradiol ELISA [SLV–4188; DRG Instruments, Marburg, Germany; Intra-assay coefficient of variation (CV) 2.2%].

In vitro microdialysis studies. In vitro studies previously conducted by our laboratory estimated the relative in vitro glycerol, ethanol, and E2 recoveries over the microdialysis membrane to be 59.5 ± 2.9, 89.4 ± 3.0, and 21.5 ± 4.3%, respectively, at the flow rate of 2 μl/min (CMA/Ellite 10-mm membrane probes used in both the in vivo and in vitro studies). Interstitial E2 was calculated: interstitial E2 = [dialysate E2]/[(1 – in vivo O:1)/(in vitro ethanol\textsubscript{relative recovery}/in vitro E2\textsubscript{relative recovery})].

The in vitro relative recoveries of E2 and ethanol and in vivo recovery of ethanol were used to estimate the concentration of E2 passing from the perfusate into the SAT in vivo. An estimated 5–20% of the E2 passed over the microdialysis membrane into the SAT (starting perfusate E2 = 500 nmol/l, estimated local SAT E2 concentration in the immediate area of the microdialysis membrane = 25–100 nmol/l). It is also important to remember that the exchange of E2 over the membrane, and therefore local exposure, is also dependent on local blood flow and dispersion of the perfusate into a volume of interstitial fluid surrounding the 10 mm long × 0.5 mm diameter probe. Although the perfused E2 concentration was above the known circulating physiological range, the SAT interstitial concentration of E2 was unclear. Therefore it was important that our perfusion solution was of a concentration sufficient to increase the local SAT E2 concentration.

Blood samples. Serum samples of glucose, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL)-cholesterol were determined using enzymatic/colorimetric methods, and low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald equation (8). Serum E2, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, testosterone, and insulin were determined by Electrochemiluminescence Immunoassay (UniCel DxC 600i Synchron® Access Clinical System; Beckman Coulter, Brea, CA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: [fasting glucose (mmol/l) × fasting insulin (μU/ml)]/22.5 (5). Plasma glycerol (collected in lithium heparin) was determined in our laboratory using the Sigma quantitative enzymatic free glycerol determination kit (F6428; Sigma-Aldrich, St. Louis, MO; intra- and interassay CVs respectively 2.4% and 7.1%). The University of Colorado Denver Clinical and Translational Research Center (CTRC) Core Laboratory conducted the following analyses: estrone by conventional radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX; intra- and interassay CVs respectively 8.7 and 11.7%), FFA by enzymatic colorimetric methods (Wako Diagnostics, Richmond, VA; 1.1 and 5.6%), dehydroepiandrosterone sulfate (DHEA-S) and sex hormone-binding globulin (SHBG) by electrochemiluminescence immunoassay (Beckman Coulter, DHEA-S 2.3 and 3.4%, SHBG 3.6 and 5.7%), and luteinizing hormone (LH), progesterone, testosterone, and insulin were determined by Electrochemiluminescence Immunoassay (UniCel DxC 600i Synchron® Access Clinical System; Beckman Coulter, Brea, CA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: [fasting glucose (mmol/l) × fasting insulin (μU/ml)]/22.5 (5). Plasma glycerol (collected in lithium heparin) was determined in our laboratory using the Sigma quantitative enzymatic free glycerol determination kit (F6428; Sigma-Aldrich, St. Louis, MO; intra- and interassay CVs respectively 2.4% and 7.1%). The University of Colorado Denver Clinical and Translational Research Center (CTRC) Core Laboratory conducted the following analyses: estrone by conventional radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX; intra- and interassay CVs respectively 8.7 and 11.7%), FFA by enzymatic colorimetric methods (Wako Diagnostics, Richmond, VA; 1.1 and 5.6%), dehydroepiandrosterone sulfate (DHEA-S) and sex hormone-binding globulin (SHBG) by electrochemiluminescence immunoassay (Beckman Coulter, DHEA-S 2.3 and 3.4%, SHBG 3.6 and 5.7%), exercise.

Statistics

Skewed distribution of some variables necessitated logarithmic transformation, in which case the reexponentiated geometric mean and 95% CI are presented. Paired t-tests were used to determine differences in metabolic characteristics between rest and exercise. Repeated-measures ANOVA was used to analyze changes in circulating factors at rest and during exercise. Three-way repeated-measure ANOVA (probe × time × region) was used to determine overall probe and regional differences in dialysate glycerol. Two-way repeated-measures ANOVA tests (probe × region) were used to determine regional differences in dialysate glycerol response to stimulated lipolysis with and without E2 coperfusion. When indicated by a significant
**F**-statistic, post hoc analyses to determine significant mean differences between the groups were conducted by *t*-tests with the Bonferroni correction. Identical analyses were conducted for ethanol O:I ratio. Analyses were completed using IBM SPSS statistics v. 19. Data are presented as means ± SE unless otherwise noted, and *α* was set at 0.05.

**RESULTS**

**Participant Characteristics**

Participant characteristics are presented in Tables 1 and 2. Two participants exhibited elevated circulating E2, estrone, and LH values, indicative of late follicular phase timing. Microdialysis results from these women were consistent with the remainder of the study sample; therefore their data were included in all analyses. Reduced sample sizes for microdialysis measurements resulted from microdialysis probe failures (n = 2), dialysate flow interruptions (n = 5), or dialysate glycerol outliers (>2 SD above the mean) under basal (control) conditions (n = 1).

AB and GL interstitial E2 concentration was determined from pooled control (basal) dialysate samples from the four probes in each region in a subset of women (n = 13; Table 2). Interstitial concentrations were ~400 pmol/l, which provided a clear indication that the local concentration of E2 perfused by microdialysis (500 nmol/l, with ~60 nmol/l passing over the membrane) was sufficient to increase local SAT E2 concentrations.

The mean REE for the group was 1,457 ± 45 kcal/day and resting respiratory exchange ratio (RER) 0.77 ± 0.01. The women exercised at 60 ± 2.1% of previously determined V̇O₂peak for 30 min, resulting in an increase in RER (0.88 ± 0.01) and HR (rest 76 ± 3, exercise 154 ± 4 beats/min) (both *P* < 0.001). Blood samples were taken at three time points: baseline, preexercise, and exercise (Table 3).

**Physiological and Pharmacological Modulation of Lipolysis**

Fifteen minutes of submaximal exercise increased Glyc<sub>dialysate</sub> more in the AB than in the GL region (Fig. 2A). Similarly, the increase in Glyc<sub>dialysate</sub> in response to ISO was lower in the GL than in the AB region (Fig. 2B). The addition of PHEN to the ISO perfusion did not change Glyc<sub>dialysate</sub> from ISO perfusion alone in either the AB or GL regions (Fig. 2B). Thirty minutes of submaximal exercise during ISO + PHEN perfusion ("maximal" stimulation/disinhibition) further increased Glyc<sub>dialysate</sub> from ISO + PHEN perfusion alone but to a similar extent in AB and GL SAT (Fig. 2B). There were no significant racial differences in the responses to pharmacological and/or exercise stimulation of lipolysis (data not shown).

**Effect of Local E<sub>2</sub> Perfusion**

**Basal.** Local perfusion of E2 for 180 min did not change basal Glyc<sub>dialysate</sub> compared with control probes over the same time period (Fig. 3A). There was a main effect for Glyc<sub>dialysate</sub> to decrease throughout the study day in all probes. Under basal resting conditions, ethanol O:I was dependent on the adipose tissue region as well as probe perfusion conditions (Fig. 3B); mean O:I was higher in the gluteal compared with abdominal region (AB 0.79 ± 0.01, GL 0.85 ± 0.01, *P* = 0.001), indicative of a lower blood flow in the gluteal region.

**Exercise.** Thirty minutes of submaximal exercise increased Glyc<sub>dialysate</sub> in all probes, but this response was significantly lower in the GL region (Fig. 4A). There was no difference in the response of the control and E2 probes in either region (Fig.
The lipolytic response to interventions with pharmacological agents was dependent on the specific perfusion conditions, SAT region, and coperfusion of E2 (Fig. 4B). Ethanol O:I changed differently in the probes over the study visit (Fig. 4D). The change between each of the individual conditions is analyzed independently below.

**Isoproterenol.** The magnitude of the Glyc_dialysate response to ISO perfusion (β-AR agonist) was dependent on E2 coperfusion as well as adipose tissue depot (probe × region, $P = 0.003$). AB Glyc_dialysate increased with ISO perfusion (258 ± 26%), but this response was attenuated in the E2-coperfused probe (196 ± 31%, $P = 0.003$). In the GL region, the increase in Glyc_dialysate with ISO perfusion did not differ between the control and E2 probes (control 111 ± 12%, E2 113 ± 14%, $P = 0.43$). O:I did not change differently between probes during ISO perfusion.

**ISO and PHEN.** The addition of PHEN (α-AR antagonist) to the ISO perfusion did not significantly change Glyc_dialysate from ISO alone regardless of E2 coperfusion or region (AB control $-2 ± 6\%$, E2 $-3 ± 3\%$, GL control $3 ± 5\%$, E2 $-2 ± 2\%$). The addition of PHEN to the perfusate did change the O:I ratio dependently on E2 perfusion. O:I in the control probes did not change (AB $-1 ± 2\%$, GL 2 ± 2%) but increased in the E2-perfused probes (AB 10 ± 3%, GL 6 ± 1%, effect of E2 $P = 0.009$).

**ISO, PHEN, and exercise ("maximal" conditions).** The change in Glyc_dialysate with the addition of submaximal aerobic exercise to ISO + PHEN perfusion was dependent on SAT region and E2 perfusion (probe × region, $P = 0.001$). In the AB region, 30 min of exercise along with ISO + PHEN perfusion resulted in a greater increase in Glyc_dialysate in the E2-coperfused probe compared with the control probe (control 56 ± 9%, E2 90 ± 15%, $P = 0.007$), whereas in the gluteal region the E2-perfused probe had a smaller percent increase in Glyc_dialysate than the control probe (control 62 ± 12%, E2 35 ± 7%, $P = 0.05$). Ethanol O:I did not change differently between the probes during maximal stimulation.

**DISCUSSION**

This is the first study to locally modulate the SAT sex steroid profile to determine how changes in E2 may directly affect lipolysis. The novel finding of this study is that increasing the local SAT E2 concentration does change local lipolytic rate as measured via the in situ technique of microdialysis, reinforcing a modulatory role for E2 in female adipose tissue metabolism. Importantly, our results indicate that the influence of E2 is dependent on the adipose tissue depot of interest as well as the specific regulatory mechanism targeted.

The importance of understanding estrogen action in adipose tissue is underscored by the fact that adipose tissue is an estrogen-producing organ, particularly in postmenopausal women, where adipose tissue is the major site of estrogen production (32). Indeed, SAT aromatase mRNA expression is higher in the buttocks and thighs than in the abdomen and increases (2- to 4-fold) with age in all regions (6). Acute alterations in local E2 concentrations in the current study allows for more detailed insight into the consequences of increasing local E2 production in women with age and how this may affect regional lipolysis and body fat distribution.

In agreement with previous research (23, 30, 40), we found lower body SAT to be less lipolytically responsive under two conditions: 1) β-AR agonist perfusion and 2) submaximal exercise (believed to be primarily catecholamine mediated). Lower lipolytic response in the gluteal region may be involved in the lack of, or possibly negative, relationship between lower body obesity and cardiometabolic disease risk (31, 36, 41). Sequestering circulating lipids and releasing fewer free fatty acids into the circulation may help to protect women with a propensity for storing adipose in the gluteal-femoral region from excess exposure to circulating lipids and the negative

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Fig. 3. Dialysate glycerol and ethanol outflow-inflow ratio (O:I) at rest with or without 17β-estradiol (E2) perfusion as measured by microdialysis in AB and GL SAT in overweight-to-obese premenopausal women. E2 perfusion started after the 30-min sample in E2 probes only. Analysis by 2-way repeated-measures ANOVA. A: dialysate glycerol. Main effect of time, $P < 0.001$. B: ethanol O:I. Time × probe, $P = 0.02$; time $P < 0.001$; probe $P < 0.05$. Open symbols and dashed lines (○, □) represent control probe (0.9% saline + 10 mmol/l ethanol); and closed symbols and solid line (●, ■) represent E2 probe (500 nmol/l E2 in 0.9% saline + 10 mmol/l ethanol). Circles represent AB: squares represent GL. AB $n = 14$, GL $n = 16$. *$P < 0.05$ vs. GL.
Fig. 4. Change in dialysate glycerol and ethanol O:1 with or without E2 perfusion as measured by microdialysis during physiological and pharmacological perturbations in AB and GL SAT in overweight-to-obese premenopausal women. Analysis by 3-way repeated-measures ANOVA. A: physiological modulation by cycle ergometry at 60% \( \dot{V}O_{2}\text{peak} \). Probe \( \times \) time \( \times \) region, \( P = 0.48 \); probe, \( P = 0.12 \); time, \( P = 0.001 \); region, \( P = 0.001 \). B: pharmacological modulation by perfusion of ISO (1.0 \( \mu \)mol/l), ISO + PHEN (0.1 mmol/l), and ISO + PHEN + 60% \( \dot{V}O_{2}\text{peak} \). Probe \( \times \) time \( \times \) region, \( P = 0.03 \); probe, \( P = 0.05 \); time, \( P < 0.001 \); region, \( P < 0.001 \). C: ethanol O:1 measured simultaneously to A. Time \( \times \) region \( \times \) probe, \( P = 0.12 \); probe, \( P = 0.38 \); time, \( P = 0.001 \); region, \( P = 0.04 \). D: ethanol O:1 measured simultaneously to B. Time \( \times \) region \( \times \) probe, \( P = 0.15 \); time \( \times \) probe, \( P < 0.001 \); time, \( P < 0.001 \); probe, \( P = 0.84 \); region, \( P = 0.14 \). Open symbols and dashed lines represent control perfused (0.9% saline + 10 mmol/l ethanol); closed symbols and solid line represent E2 perfusion (500 nmol/l E2 in 0.9% saline + 10 mmol/l ethanol). Circles represent AB; squares represent GL. Data are means \( \pm \) SE. A and C: AB \( n = 14 \), GL \( n = 16 \); B and D: AB and GL \( n = 15 \). *Main effect of region, \( P < 0.01 \).

Effects of E2 on Basal Lipolysis

We know of only one other study acutely modulating hormonal status in vivo to investigate changes in whole body and regional lipolysis. Van Pelt et al. found that an acute intravenous bolus of exogenous conjugated estrogens decreased basal lipolysis in femoral, and to a lesser extent abdominal, SAT in postmenopausal women, supporting a role for estrogens in regional adipose tissue E2 levels. Changes in local E2, at least at the concentrations used in the current study, do not appear to influence β-AR-stimulated lipolysis in the gluteal region.

Depot-Dependent Effects of E2

Of particular interest is our observation of a divergent response to E2 perfusion in the abdominal and gluteal SAT. In addition to the region-specific effect of E2 perfusion on ISO stimulation, we found that E2 blunts lipolysis in the gluteal region but potentiates lipolysis in the abdominal region during ‘maximally’ stimulated/disinhibited conditions. Because perfusion of ISO and PHEN at the concentrations used in the present study led to maximal changes in AR-mediated lipolysis in previous investigations (1, 2), we believe that increases in lipolysis with submaximal exercise during pharmacological perfusion here was due to non-adrenergically mediated mechanisms. However, it is possible that some, or all, of the increase in lipolysis could be a result of further catecholamine stimulation of the β-ARs if the concentration of ISO used here did not fully stimulate the β-ARs.
We hypothesize that the divergent regional effects of E2 on lipolysis during “maximal” stimulation/disinhibition occur through a yet to be determined adrenergic-independent mediator of lipolysis. Atrial natriuretic peptide, insulin, adenosine, cortisol, and nitric oxide are all well known non-adrenergic regulators of lipolysis (17) through which estrogen may influence local lipolytic rate. More studies are necessary to fully elucidate the effect of varying concentrations of E2 on non-adrenergically mediated lipolysis and the mechanisms behind this influence, particularly if estrogen acts directly on lipolysis through the adipose tissue ERs.

We recognize that the concentration of E2 perfused was above the circulating physiological range. Because of the uncertainty of interstitial E2 concentrations, it was important that the concentration be sufficient to increase local estrogen, particularly considering the limited net movement (~12%) of the perfused E2 over the dialysis membrane. It is possible that the dose of E2 may have altered lipolysis in a manner that was not physiological; however, because adipose tissue is an estrogen-producing organ as well as a sink for estrogen from the circulation (3), local adipose tissue estrogen concentrations are probably much higher than typically considered physiological according to circulating measurements. In preliminary correlational analysis, regional body composition measurements, hormone-associated changes in lipolysis, and measured interstitial E2 concentrations were not associated (data not shown). Nevertheless, we believe our ability to detect these expected relations was limited by our homogenous population and lack of a highly sensitive and reliable E2 assay platform, such as mass spectrometry, to measure E2 in our dialysate. It is important for future studies to establish accepted values for tissue-specific estrogen concentrations in order to fully appreciate the influence of E2 on adipose tissue metabolism.

Adipose Tissue Blood Flow

The use of the ethanol technique provides a qualitative assessment of changes in microvascular exchange, a key determinant in the concentration of substances recovered in microdialysate. We recognize that this technique does not provide a direct quantitative measurement of changes in local blood flow, and therefore our results should be interpreted with caution; however, our past experience (12, 13) indicates that it is a reliable qualitative indicator of acute changes in local blood flow. We therefore believe that the ethanol technique provided adequate monitoring to properly interpret the dialysate glycerol data in the context of changes in local blood flow. Although estrogen is known to have vasodilatory effects in large vessels and the coronary microvasculature in postmenopausal women (11, 27, 39), acute changes in circulating estrogen levels do not affect microvascular responses in normally cycling premenopausal women (16), in agreement with the finding that E2 perfusion per se did not change SAT blood flow considerably in the current study. The ethanol O:1 ratio did change over time throughout the microdialysis visit; however, there was not a specific effect of E2 perfusion to change the blood flow response differently between probes. The ISO + PHEN condition was the only exception, during which O:1 in the E2 probes (AB and GL) increased, indicating a decrease in local blood flow. However, as blood flow decreases, recovery over the microdialysis membrane also decreases; therefore, our ability to detect a possible E2-induced increase in lipolysis was likely reduced under these conditions. Finally, differences in O:1 responses between probes were not apparent under conditions in which E2 had a significant effect on lipolysis, making it unlikely that observed differences in lipolysis between E2 and control probes were simply a result of differences in local blood flow.

Conclusions

In conclusion, exposure to high concentrations of E2 leads to region- and pathway-dependent modulation of SAT lipolysis in premenopausal women. We found that E2 blunts β-adrenergic stimulation of lipolysis in the abdominal, but not gluteal, SAT. These findings implicate local adipose estrogen concentrations in modulating abdominal SAT mobilization, a mechanism that may be involved in increased abdominal adiposity in postmenopausal women. However, under conditions during which lipolysis is “maximally” stimulated, E2 had a potentiating effect on lipolysis in the abdominal SAT while also having a suppressive effect on lipolysis in the gluteal SAT, potentially a result of region-specific nonadrenergically mediated mechanisms. The divergent regional responses apparent under these conditions may be involved in the maintenance of the gynoid body fat distribution in premenopausal women even during times of increased lipid mobilizing signals. Our results shed light on why previous studies have demonstrated conflicting results in regard to pro- or antilipolytic effects of E2 in adipose tissue of women. When estrogen-mediated modulation of lipolysis is being investigated, particular attention must be paid with regard to the specific lipolytic regulatory pathways as well as the adipose tissue depot(s) under investigation. Future investigations to determine the physiological range of interstitial E2 concentrations in pre- and postmenopausal women and the adrenergic or nonadrenergic mechanisms through which this local estrogen works to modulate regional lipolysis are needed.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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