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POSTPRANDIAL TRIGLYCERIDES AND ADIPOSE TISSUE STORAGE OF DIETARY FATTY ACIDS: IMPACT OF MENOPAUSE AND ESTRADIOL

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Abstract

Objective—Postprandial lipemia worsens after menopause, but the mechanism remains unknown. We hypothesized menopause-related postprandial lipemia would be: 1) associated with reduced storage of dietary fatty acids (FA) as triglyceride (TG) in subcutaneous adipose tissue (SAT); and 2) improved by short-term estradiol (E_2).

Design and Methods—We studied 23 pre- (mean±SD; 42±4yr) and 22 postmenopausal (55±4yr) women with similar total adiposity. A subset of postmenopausal women (n=12) were studied following 2 weeks of E_2 (0.15mg) and matching placebo in a random, cross-over design.

A liquid meal containing ^{14}C-oleic acid traced appearance of dietary FA in: serum (postprandial TG), breath (oxidation), and abdominal and femoral SAT (TG storage).

Results—Compared to premenopausal, healthy lean postmenopausal women had increased postprandial glucose and insulin and trend for higher TG, but similar dietary FA oxidation and storage. Adipocytes were larger in post- compared to premenopausal women, particularly in femoral SAT. Short-term E_2 reduced postprandial TG and insulin, but had no effect on oxidation or storage of dietary FA. E_2 increased the proportion of small adipocytes in femoral (but not abdominal) SAT.

Conclusions—Short-term E_2 attenuated menopause-related increases in postprandial TG and increased femoral adipocyte hyperplasia, but not through increased net storage of dietary FA.

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Introduction

Subcutaneous adipose tissue (SAT) buffers the flux of triglycerides (TG) after a meal[1]. Impaired uptake of dietary fatty acids (FA) into SAT may contribute to cardiometabolic risk through increases in postprandial TG and ectopic (e.g., visceral, hepatic, intramuscular) TG accumulation[1]. Women prior to menopause have more SAT and greater TG clearance (less postprandial lipemia) compared to men[2, 3]. After menopause postprandial TG clearance is reportedly reduced compared to premenopausal women, independent of differences in body mass index (BMI)[4]. These observations suggested sex hormones might play a role in postprandial TG clearance. Indeed, 6wks of estradiol (E_2) treatment appeared to improve TG clearance in postmenopausal women[5], but the mechanism is unknown. Potential mechanisms for increasing TG clearance include: delayed absorption of dietary FA, increased oxidation of dietary FA, reduced secretion of very low-density lipoprotein (VLDL)-TG, and increased storage of dietary FA as TG. Compared to men, premenopausal women appeared to store more dietary FA in SAT and this was associated with less TG storage elsewhere (visceral fat, liver, skeletal muscle)[6]. Consistent with this, postprandial lipemia was associated with greater visceral adiposity in premenopausal women[7]. Dietary fat oxidation may also be greater in women compared to men[8], although this is not a consistent finding[3]. It is not known whether there are menopause-related reductions in the oxidation or storage of dietary FA, but if so this might contribute to the observed increases in postprandial lipemia and ectopic (depots other than SAT) fat accumulation after menopause. We hypothesized that, compared to premenopausal women, postmenopausal women would have reduced postprandial TG clearance and a smaller proportion of meal-derived FA being oxidized and stored as TG in SAT. We postulated the reduced uptake of dietary FA by SAT would be associated with increased storage elsewhere (i.e., not accounted for in SAT). We further hypothesized that treating postmenopausal women with E_2 would improve storage of meal-derived FA in SAT TG and mitigate menopause-related differences in postprandial lipemia and ectopic storage.

Methods

Subjects

We studied 23 premenopausal (35–50yr) and 22 postmenopausal (48–60yr) women of similar total adiposity. All women were healthy, non-obese (BMI<30 kg/m^2), non-smokers, and sedentary to moderately physically active (exercise ≤150min/wk). Premenopausal women were normally menstruating (25–35 day cycles) and postmenopausal women were amenorrheic (≥1yr) or had undergone bilateral oophorectomy (FSH>30 IU/L). None of the women were currently (≤3mos) using estrogen-based hormones and none used lipid- or glucose-lowering medications. Women were excluded if they were not weight stable (±2kg over past 2mo) or had any metabolic or cardiovascular disease. Prior to enrollment, each woman provided informed consent. The protocol was approved by the Colorado Multiple Institutional Review Board.

Body composition assessment

Total and regional (trunk, leg) fat mass (FM) and fat-free mass (FFM) were measured by DXA (Hologic Discovery W, software version 11.2) as previously described[9]. CT scans of the abdomen (L2–L3 and L4–L5) and mid-thigh measured abdominal and femoral subcutaneous fat areas (SFA, cm^2) as previously described[10]. Visceral fat area (VFA, cm^2) and intermuscular fat area (IMFA) were calculated as the difference between total and subcutaneous fat areas. Visceral and subcutaneous FM (kg) were estimated as previously described[11]. The proportions of visceral and subcutaneous FM were multiplied by trunk FM and intermuscular and subcutaneous FM were multiplied by leg FM to estimate the proportions in the upper and lower body, respectively.

Physical activity assessment

The Yale Physical Activity Survey[12] was administered to assess time spent in a range of activities. Total time (hours/week) for each activity was multiplied by the intensity code (kcals/hour) provided by the survey and the sum of all activities (adjusted for season) used to estimate the energy expenditure (EE) of daily activities (kcal/day).

Test Meal visit (Figure 1)

Subjects consumed a 3-day standardized diet [Kcal=(23.9*FFM+372)*1.5][13] prepared by the Clinical Translational Research Center (CTRC) metabolic kitchen. Following an overnight fast, volunteers were admitted to the CTRC. Premenopausal women were studied during the follicular phase of their menstrual cycle (day 1–10). Resting EE was measured, breath samples were collected (described below), and an antecubital IV catheter was placed. After baseline sample collection volunteers consumed a liquid test meal (Boost Plus®, heavy cream, whey protein, and sugar) providing one third of estimated daily energy requirements containing 50% carbohydrate, 34% fat (13%saturated/13.5%monunsaturated/ 7.5%polyunsaturated) and 16% protein and labeled with 40μCi of [1- ^{14}C]oleic acid (Moravek Biochemicals). The composition of the test meal matched the composition of the lead-in diet. Blood was sampled at T = 0, 30, 60, 90, 120, 180, 240, 300, and 360 min for the assessment of ^{14}C, TG, free fatty acids (FFA), glucose, and insulin. Urine was collected for N_2 excretion. Subjects consumed a standardized lunch and dinner consisting of one third each of their daily energy requirements and then remained fasted overnight on the CTRC.

Energy expenditure and substrate oxidation

Resting and postprandial EE was measured by indirect calorimetry (Parvo Medics TrueOne 2400)[14]. Breath CO_2 samples were collected by having subjects exhale through a one-way filter into a scintillation vial containing 0.5mL benzethonium hydroxide (to trap 0.5mM CO_2), 2mL absolute methanol and 0.5mL phenolphthalein[3]. EE was measured and breath samples collected at T = 0, 60, 120, 180, 240, 300, 360, 540, 840, and 1440 min. Total fat and carbohydrate oxidation were calculated from CO_2 production, O_2 consumption, and urinary N_2 excretion as previously described[15]. $^{14}CO_2$ production (dpm/min) was calculated by multiplying the VCO_2 production (mL/min) by the breath specific activity ($^{14}CO_2$, dpm/mmol) and 1mmol CO_2/22.4mL. The amount of ^{14}C-oleic acid oxidized was calculated from the 24hr area under the curve (AUC) for $^{14}CO_2$ divided by the ^{14}C ingested.

Separation of lipid fractions

Blood samples collected at T=0, 60, 120, 180, 240, and 360 min were added to chilled EDTA-containing tubes and immediately spun (3,000 rpm, 10 min, 4 °C). Fresh plasma (2mL) was placed into polycarbonate tubes with NaN_3 (10%, 2uL) and PMSF (10mmol/L, 20uL) added to stabilize the triglyceride rich lipoproteins (TRL). Within 24hrs plasma was separated into TRL sub-fractions using a sequential floatation, ultracentrifugation method (large Svedberg flotation [Sf] >400, and medium Sf 20–400)[16]. The large sub-fraction consists primarily of chylomicron (CM) particles with small amounts of larger VLDL particles. The medium sub-fraction consists primarily of VLDL lipoproteins and some smaller CM particles. For collection of the large TRL, plasma was centrifuged (100,000 rpm, 10 min, 15 °C; Beckman TLX100), allowed to sit (1hr), and the supranate removed. For collection of the medium TRL, the infranate was overlaid with 1.0ml of NaCl solution (1.006 g/ml density) and centrifuged (100,000rpm, 2.5hr, 15 °C), allowed to sit (1hr), and the supranate removed. ^{14}C radioactivity was immediately assessed in each sub-fraction at each time point and the remaining sample stored at −80 °C for measurement of TG concentrations.

Adipose tissue biopsies

24h after the test meal, biopsies were taken from the abdominal and femoral SAT depots using a mini-liposuction suction technique as previously described[17]. Previous studies have shown that peak rate of uptake of dietary TG occurs approximately 4–6 hours after a meal, whereas uptake is near maximal by 24hrs[18]. Thus biopsies were done at 24h to determine net uptake of meal FA. Adipocyte cellularity and lipoprotein lipase (LPL, described below) activity were determined on an aliquot of fresh SAT sample and the remaining tissue frozen for later analysis of ^{14}C specific activity (SA).

Adipocyte cellularity

Immediately after collection, SAT was digested in Krebs-Ringer phosphate buffer containing collagenase (3 mg/ml) in 37°C shaking water bath for 30min. Average adipocyte size (552±288 cells/sample) was determined as previously described[19, 20]. In brief, collagenase-released adipocytes were stained with methylene blue and imaged (Olympus BX60 microscope, Canon Power Shot G5 digital camera) and counted with Cell Counting Analysis Program (CCAP; Mayo Clinic, Rochester, MN).

Adipose tissue LPL activity

Immediately after collection, heparin-releasable LPL activity (nmol FFA/min/g) was measured in fresh abdominal and femoral SAT samples as previously described[21]. Briefly, LPL was eluted from SAT fragments (~40 mg) into Krebs-Ringer phosphate buffer (pH 7.4) containing heparin (15 μg/mL). Enzyme activity was measured as hydrolyzed [^{14}C]- or [^{3}H]-oleic acid after incubation with a synthetic substrate.

^{14}C in adipose tissue and breath

Radioactivity in SAT was determined by liquid scintillation counting (LS6000TA; Beckman Instruments). Lipid was extracted from the SAT samples[22] and radioactivity was counted

(20min/ea) to a counting error <5% as described previously[23]. Specific activity (SA; dpm/g) in the abdominal and femoral SAT samples was multiplied by trunk and leg FM to calculate upper body (UBSQ) and lower body (LBSQ) subcutaneous TG storage. $^{14}CO_2$ SA was measured in breath samples to determine fat oxidation. Ectopic TG storage was estimated from ^{14}C not accounted for in oxidation or SAT storage as previously described[24].

Estradiol treatment

The Test Meal visit was performed on two occasions in a subset of 12 postmenopausal women following 2 weeks of transdermal E_2 (0.15mg; 3 × 0.05mg patches) and matching placebo patches in a random, cross-over manner. An average of 8±1 weeks separated the two tests; long enough for wash-out of E_2 but short enough for weight stability.

Blood analyses

Blood samples were stored at −80°C and analyzed in batch by the CTRC Core Laboratory. Glucose, TG (Beckman Coulter, Inc.), and FFA (Wako Chemicals, Inc.) were determined enzymatically; insulin, leptin, and adiponectin by radioimmunoassay (EMD Millipore, Corp.); and estradiol and sex hormone binding globulin (SHBG) by chemiluminescence (Beckman Coulter, Inc.). Sensitivity and precision details can be found at http://cctsi.ucdenver.edu/Research-Resources/CTRCs/Pages/Assays.aspx.

Statistics

The integrated AUC for postprandial (0–6 hours) outcomes (e.g, TG, glucose, insulin, substrate oxidation) were calculated by the trapezoidal method. Incremental AUC (IAUC) was determined to account for basal (fasting) concentrations. Statistical power (79%, α=0.05) for this study (with n= 23/group) was estimated from a previous study which reported a mean group difference and SD in TG_{IAUC} of 1.5±1.8 mmol*h/L (133±159mg*h/dL) between pre- and postmenopausal women[4]. Menopause-related group differences and E_2-mediated changes in outcomes were evaluated by one-way and repeated measures analysis of variance, respectively. All statistical analyses were done in SPSS (version 21, IBM Corporation). Data are presented as mean±SD unless otherwise specified.

Results

Subject Characteristics (Table 1)

The postmenopausal women were on average 10 years older than the premenopausal women. The pre- and postmenopausal groups were recruited to have similar total adiposity, but this resulted in postmenopausal women having less FFM and leg FM than the premenopausal women ($p<0.05$). Trunk FM and visceral adiposity were not different between the groups. The sub-group of 12 postmenopausal women enrolled in the transdermal E_2 treatment arm of the study had more visceral adiposity, but similar FFM and leg FM compared to the rest of the postmenopausal cohort. There were no differences in total daily activity EE between pre- and postmenopausal women (1212±946 vs. 1229±588 kcal/day, respectively) or the E_2 sub-group (1276±673 kcal/d).

Menopause-related differences

Compared to premenopausal women, postmenopausal women had a trend for higher total postprandial TG (TG_{IAUC} 6405±1482 vs. 3885±685, p=0.10; Figure 2A), due to less suppression of VLDL-TG (Figure 2E, p<0.05) and a trend for reduced clearance of CM-TG (Figure 2C, p=0.09). ^{14}C in the TRL sub-fractions did not differ between groups (data not shown), suggesting no difference in dietary FA appearing in CM and VLDL fractions. The postprandial TG excursions were accompanied by greater (p≤0.01) postprandial glucose and insulin excursions in post- compared to premenopausal women (Figures 3A and 3C, respectively). Postmenopausal women had lower (p<0.01) total AUC for 24-hr EE and 6-hr postprandial total carbohydrate and fat oxidation (Figures 4A, 4C and 4E). However, the lower EE and substrate utilization in postmenopausal compared to premenopausal women was directly related to their lower lean mass, as these group differences were not significant after adjustment for FFM (data not shown). There were no differences between pre- and postmenopausal women in the oxidation or net storage (relative or absolute) of dietary FA into abdominal or femoral SAT or elsewhere (Table 2). However, there were significant menopause-related differences in mean adipocyte size (Table 2). These differences were due to more large adipocytes (101–140μm) in the femoral (p<0.05), and a trend in the abdominal (p=0.12), region. Postmenopausal women also tended to have fewer small (21–60 μm) abdominal (p=0.12) and medium (61–100μm) femoral (p=0.12) adipocytes compared to premenopausal women. LPL activity was not different between groups (Table 2).

Differences in regional SAT depots

There were consistent depot-specific differences in SAT among pre- and postmenopausal women. Irrespective of group, less absolute and relative (Table 2) dietary FA was stored in lower versus upper body SAT. There was a trend (p=0.11) for mean cell diameter to be larger in the femoral region compared with the abdominal region in all women. Cell size was inversely related to dietary FA uptake in the femoral (r=−487, p≤0.001), but not abdominal (r=−0.186, p=0.26), region. Higher leptin concentrations were associated with a greater proportion of large adipocytes in abdominal SAT (r=0.460, p<0.01) and a lesser proportion of medium adipocytes in the femoral region (r=−0.342, p<0.05). LPL activity was consistently higher in femoral than abdominal SAT (p<0.01; Table 2) in both groups. Abdominal and femoral LPL activity were associated with mean cell size in abdominal (r=0.591, p<0.01) and femoral (r=0.317, p=0.05) SAT, respectively.

Acute effects of estradiol

Compared with placebo, 2 weeks of E_2 increased serum leptin (Table 1), reduced postprandial TG and insulin responses (Figures 2B and 3D; p<0.05), but had no effect on postprandial EE or nutrient oxidation (Figure 4B,D,F). E_2 did not alter the proportion of dietary FA that were oxidized or stored in SAT versus other depots (Table 2). Compared with placebo, E_2 did not change LPL activity in abdominal or femoral SAT. However, acute E_2 increased the percentage of small adipocytes (p<0.05) and tended to reduce the percentage of medium (p=0.11) and large adipocytes (p=0.06) in femoral, but not abdominal, SAT.

Discussion

This study demonstrated that healthy lean postmenopausal women tended to have higher postprandial TG, and significantly higher glucose and insulin, compared to premenopausal women; treatment with short-term (2 wks) E_2 reduced postprandial TG and insulin. In contrast to our hypothesis, there was no effect of menopause or E_2 on the oxidation or storage of dietary FA. There were region-specific and menopause-related differences in adipocyte size such that adipocytes were larger in femoral SAT in post- compared to premenopausal women. Short-term administration of E_2 to postmenopausal women increased serum leptin and the proportion of small adipocytes in the femoral region. Among all women, smaller adipocytes were associated with greater uptake of dietary FA by femoral SAT.

Sex hormones and postprandial lipemia

Fasting plasma TG concentrations are a risk factor for cardiovascular disease (CVD), particularly in women compared to men[25]. Postprandial TG may be an even better indicator of CVD risk because it is an integrated measure[26] of CM-TG clearance and VLDL-TG appearance and indicative of TG delivery to ectopic depots. Previous studies demonstrated that prior to menopause women have a blunted postprandial TG when compared to men[2, 3], but this advantage may be lost after menopause[4]. One study also suggested that E_2 treatment reduced postprandial lipemia in postmenopausal women, possibly through an increase in CM-TG clearance[5]. These preliminary observations suggested a role for sex hormones in postprandial TG clearance which our data generally supported. We extended these observations by testing one mechanism for increasing postprandial TG clearance, increased storage of dietary FA in SAT.

Sex hormones and dietary fatty acids

Early studies in women and men suggested a link between SAT storage of dietary FA, visceral adiposity, and postprandial lipemia. Sex differences in the proportion of dietary FA going to SAT versus elsewhere (i.e., ectopic) were reported[6], although not consistently[3]. Among women, variability in SAT storage appeared to explain the association between postprandial lipemia and abdominal adiposity[7]. However, a more recent study disassociated TG clearance and SAT storage; postprandial TG was elevated in postmenopausal, compared with premenopausal, women despite their storing a greater proportion of dietary FA in SAT[27]. Consistent with this, our study did not support a causal association between storage of dietary FA and postprandial TG clearance. Postprandial TG excursions were greater in postmenopausal, compared to premenopausal, women but storage of dietary FA in SAT was not different. Moreover, the greater TG response was accompanied by less suppression of VLDL-TG secretion. The fact that short-term E_2 improved TG clearance without a change in TG-FA storage provided further evidence that the mechanism by which sex hormones reduce postprandial lipemia is not through an increase in dietary FA storage.

Sex hormones and fat oxidation

Reduced oxidation of dietary fat is another mechanism by which postprandial lipemia might worsen. Dietary fat oxidation has been shown to be greater in women compared to men[8],

although not consistently[3]. We did not observe a menopause-related difference in the proportion of dietary fat oxidized (^{14}C in CO_2) but total fat and carbohydrate oxidation during the 6 hour postprandial period were lower in postmenopausal women, consistent with previous observations[27]. The lower substrate oxidation was consistent with the overall reduction in resting EE and appropriate for their lower FFM. Treating postmenopausal women with E_2 for 2 weeks had no effect on EE or substrate oxidation (dietary or total). Thus, reductions in fat oxidation did not explain group differences in postprandial TG.

Sex hormones and adipose tissue cellularity

There are region-specific sex differences in adipocyte cellularity. Compared to men, women have larger adipocytes in the femoral regions but smaller or similarly sized adipocytes in the abdominal (subcutaneous and visceral) regions[28, 29]. These sex- and region-specific differences in adipocyte size lead to different responses to overfeeding; femoral adipocytes increasing in cell number (hyperplasia) and abdominal adipocytes increasing in size (hypertrophy)[30]. In the present study, adipocytes were larger in postmenopausal compared to premenopausal women, particularly in the femoral region which responded to short-term E_2 treatment with an increase in the proportion of small adipocytes.

Sex hormones and adipose tissue LPL

Adipose tissue LPL hydrolyzes circulating TG to facilitate storage of FA in adipocytes. LPL activity is generally correlated with cell size and varies by sex and fat depot[29]. In the present study, LPL was related to cell size, but fasting abdominal and femoral SAT LPL activity (per gram) was not different between groups or in response to short-term E_2. Adjusting for differences in cell size (by analysis of covariance) did not change the results (data not shown). Our data are consistent with earlier studies which found no differences in abdominal LPL activity between pre- and postmenopausal women[31] or in response to percutaneous E_2 treatment[32].

Regional adipose tissue differences

Femoral SAT is metabolically distinct from abdominal SAT. The half-life of TG (net turnover) appears to be 50% longer in femoral compared to abdominal SAT[33, 34]. This slower turnover of TG in femoral SAT is consistent with the concept that this depot may be a relatively more effective *sink* for holding onto TG[1]. That said, in the current study proportionally less dietary FA was incorporated into the femoral than abdominal TG stores over a 24-hour period, suggesting the longer half-life for TG in this depot is due to slower mobilization rather than greater assimilation. Given that fasting LPL activity was higher in femoral compared to abdominal SAT, enzyme availability did not explain the lower femoral uptake of dietary FA. In the present study, femoral adipocytes were larger and the depot more sensitive to acute E_2 (responding with hyperplasia), compared to the abdominal depot, but whether this impacted TG clearance remains unclear.

Study Limitations

It is important to point out limitations to our study. First, we studied normal-weight women matched for total adiposity. Matching for total adiposity allowed a fair comparison of total

and relative uptake of dietary FA into SAT, but the resulting group differences in FFM lead to net differences in EE and substrate oxidation. Second, our women were non-obese and normolipidemic and E_2 treatment was short-term (2 wks). This cohort and study design allowed us to evaluate the effect of menopause and E_2 independent of obesity or changes in adiposity. However, the healthy cohort likely explains why differences in postprandial TG between groups were smaller than previously reported. The TG lowering effect of E_2 may have been more apparent in postmenopausal women with hypertriglyceridemia and treated for a longer period of time. It is also possible that peak rate of TG storage at 6hr, rather than net storage at 24hr, would have better reflected postprandial TG clearance. However, it is unlikely these limitations explain the lack of an effect of menopause or E_2 on the trafficking of dietary FA.

Conclusions

This study demonstrated that menopause-related increases in postprandial TG and insulin are attenuated with acute E_2 administration. There were no effects of menopause or E_2 on oxidation or storage of dietary FA. In the femoral region adipocytes were larger in postmenopausal, compared to premenopausal, women and short-term E_2 attenuated these differences. Taken together these data suggest that the loss of endogenous sex hormones at menopause and the addition of exogenous E_2 impact postprandial clearance of TG through mechanisms independent of subcutaneous adipose tissue dietary FA uptake.

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What is already known about this subject

- Postprandial triglycerides are increased in postmenopausal compared to premenopausal women.
- Estradiol has been shown to reduce postprandial triglycerides in postmenopausal women.

What this study adds

- Menopause-related differences and estradiol-mediated effects on postprandial triglyceride clearance did not appear to be mediated through subcutaneous adipose tissue storage of dietary fat.
- Short-term estradiol in postmenopausal women did not appear to increase storage of dietary fat in subcutaneous adipose tissue triglyceride.
- Short-term estradiol in postmenopausal women increased serum leptin and promoted femoral adipocyte hyperplasia.

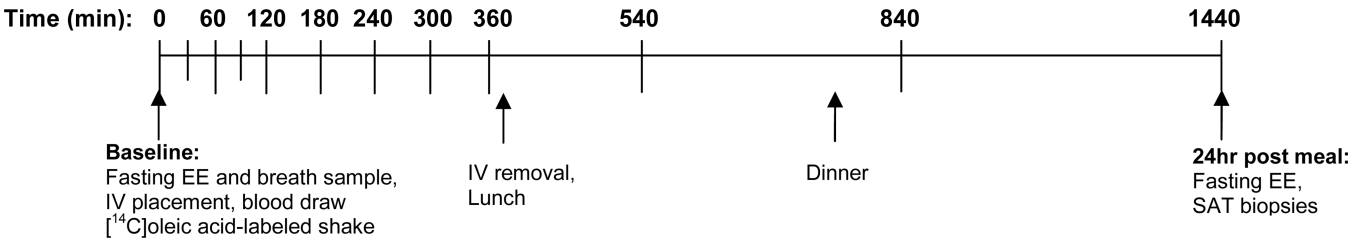

Figure 1.

Schematic timeline for 24-hour test meal visit. Fasting and postprandial blood samples (^{14}C-TG), breath samples ($^{14}CO_2$) and indirect calorimetry (energy expenditure) measures were collected at each time point.

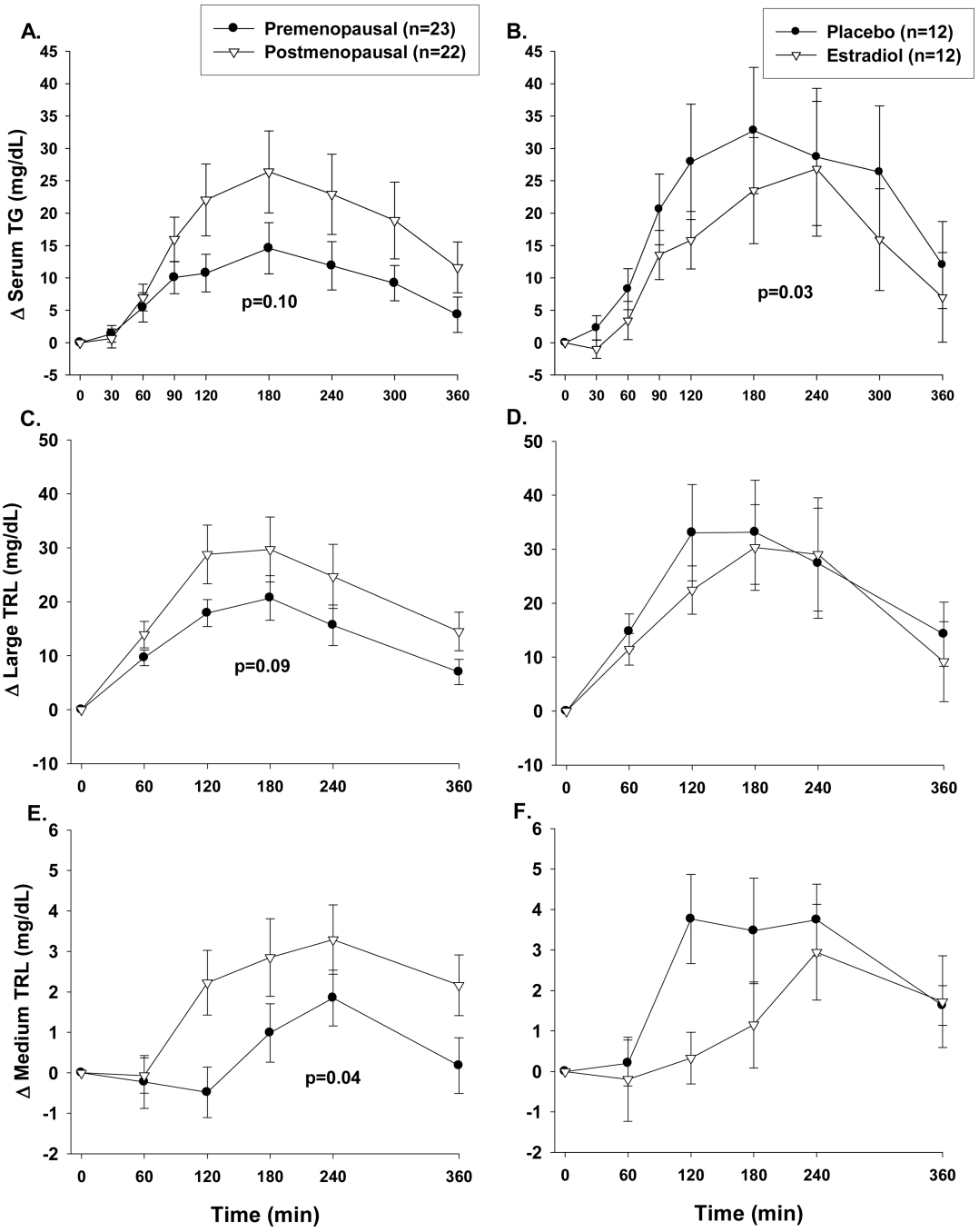

Figure 2.

Postprandial (6hr) responses in pre- (n=23) and postmenopausal (n=22) women or postmenopausal women (n=12) treated with and without 2 weeks of estradiol for: total serum triglycerides (panels A and B); large buoyant (Sf>400; primarily chylomicron) triglyceride rich lipoprotein (TRL) particles (panels C and D); and medium (Sf 20–400; primarily VLDL).) TRL particles (panels E and F). P-value for group difference in area under the curve.

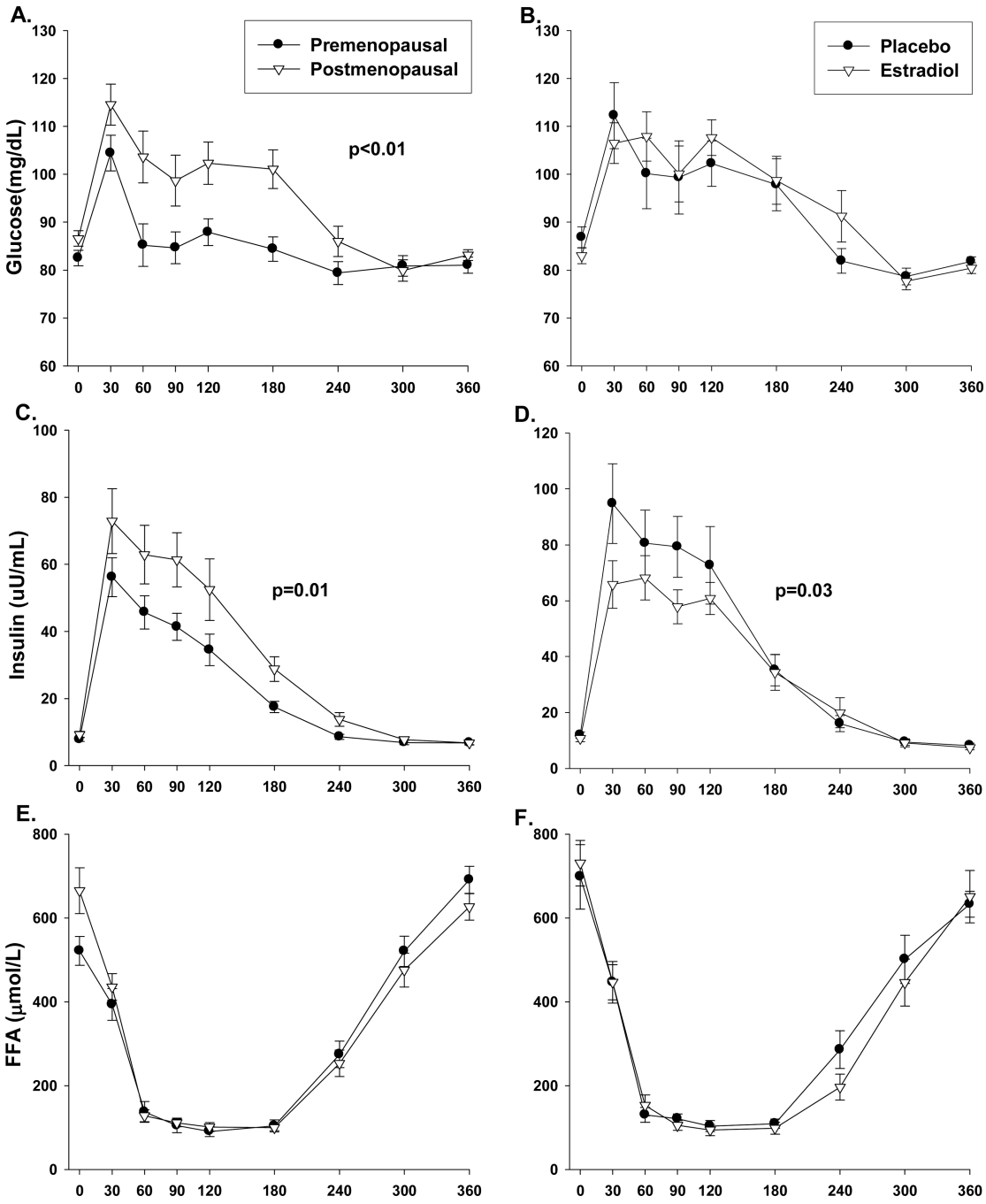

Figure 3.

Postprandial (6hr) responses in pre- (n=23) and postmenopausal (n=22) women or postmenopausal women (n=12) treated with and without 2 weeks of estradiol for: plasma glucose (panels A and B); insulin (panels C and D); and free fatty acids (panels E and F). P-value for group difference in area under the curve.

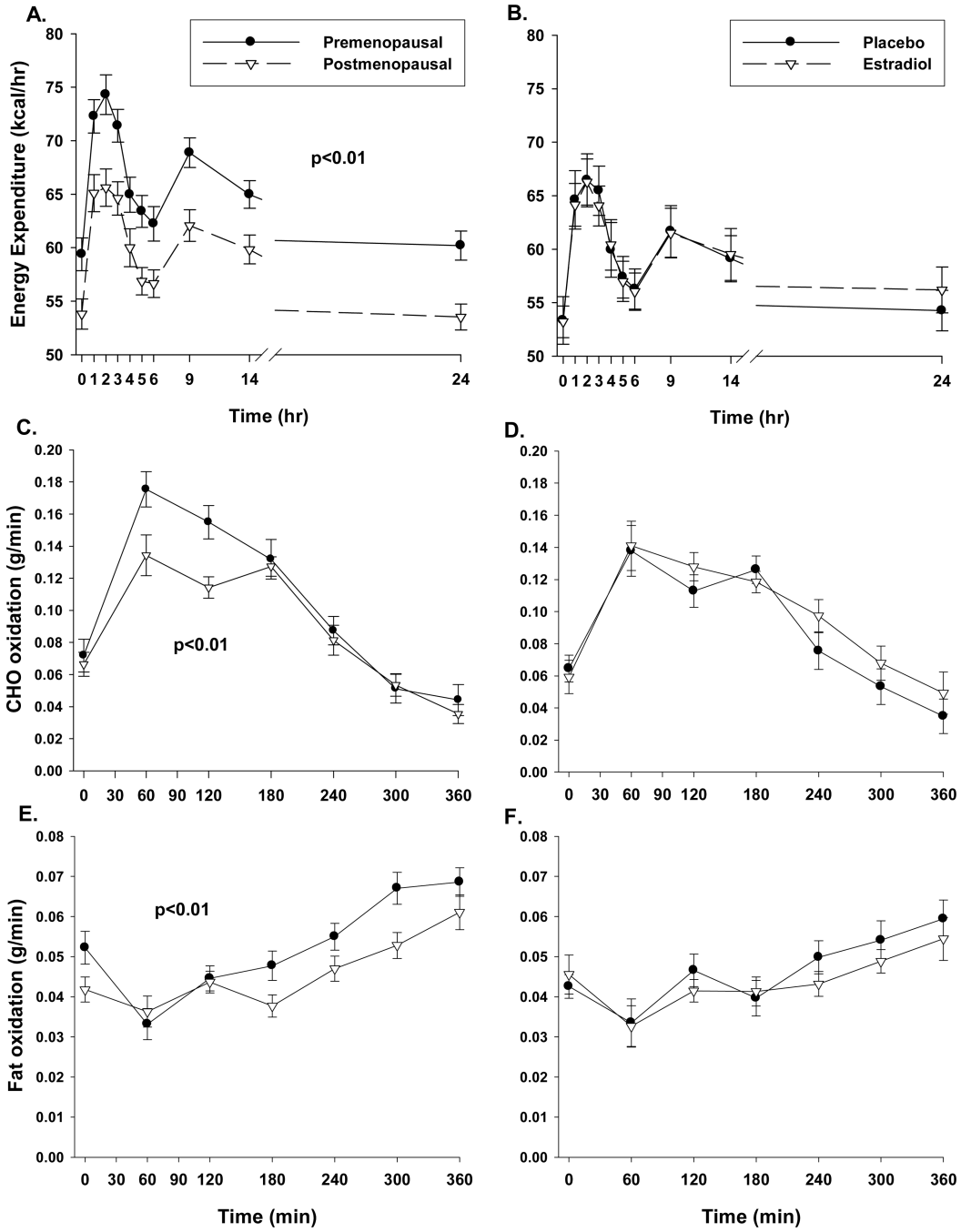

Figure 4.

Twenty four hour energy expenditure (panels A and B), postprandial fat oxidation (panels C and D) and carbohydrate (CHO) oxidation (panels E and F) in pre- (n=23) and postmenopausal (n=22) women or postmenopausal women (n=12) treated with and without 2 weeks of estradiol. P-value for group difference in area under the curve.

Table 1

Subject characteristics

	Premenopausal Women (n=23)	Postmenopausal Women (n=22)	Postmenopausal E_2 subgroup (n=12)
Age (yrs)	42 ± 4	55 ± 4*	56 ± 3
Years past menopause	n/a	7 ± 5	6 ± 5
Weight (kg)	67.7 ± 8.4	62.1 ± 8.2*	64.2 ± 9.9
BMI (kg/m^2)	24.2 ± 2.6	23.3 ± 2.4	24.1 ± 2.5
Fat mass (kg)	22.7 ± 5.3	21.7 ± 5.3	23.5 ± 5.5
%Fat	33.3 ± 4.8	34.5 ± 5.2	36.3 ± 4.3
Trunk fat mass (kg)	9.2 ± 2.6	9.7 ± 3.5	11.1 ± 3.6†
Leg fat mass (kg)	10.4 ± 2.5	8.8 ± 1.7*	9.0 ± 1.9
Fat-free mass (kg)	44.9 ± 4.6	40.5 ± 4.7*	40.7 ± 5.5
Abdominal SFA (cm^2)	228.6 ± 62.2	221.5 ± 70.5	247.2 ± 74.4
Femoral SFA (cm^2)	212.3 ± 58.3	173.1 ± 39.7*	173.5 ± 35.6
VFA (cm^2)	41.2 ± 24.7	57.6 ± 40.9	74.8 ± 40.2†
Femoral IMFA (cm^2)	16.6 ± 9.3	15.4 ± 5.8	16.0 ± 7.1
Fasting glucose (mg/dL)	83 ± 8	87 ± 8	83 ± 6
Fasting insulin (uU/mL)	8 ± 3	9 ± 4	11 ± 4
Estradiol (pg/mL)	91 ± 76	14 ± 6*	108 ± 65†
SHBG (nM/L)	64 ± 22	60 ± 25	66 ± 20†
Leptin (ng/mL)	13 ± 6	12 ± 7	17 ± 10†
Adiponectin (ug/mL)	15 ± 5	17 ± 9	15 ± 8

* $p<0.05$ vs premenopausal;

† $p<0.05$ vs postmenopausal untreated.

BMI, Body Mass Index; E_2, estradiol; IMFA, intermuscular fat area by CT; SFA, subcutaneous fat area by CT; SHBG, sex hormone binding globulin; VFA, visceral fat area by CT. Subgroup body composition data describe baseline (pre-E_2) characteristics; serum hormones and adipokines are the results of 2 weeks of transdermal E_2 treatment.

Table 2

Tissue cellularity, LPL activity and storage of dietary fatty acid

	Premenopausal Women	Postmenopausal Women	Postmenopausal Placebo	Postmenopausal E_2
Meal-derived fatty acid trafficking (relative)				
Oxidized (%)	25.7±7.3	23.6±7.4	22.1±9.2	23.3±4.2
Upper body SAT (%)	14.7±7.6	12.3±7.3	11.4±6.0	11.9±4.0
Lower body SAT (%)	10.4±5.8*	10.2±10.5*	5.7±3.4*	8.3±6.5*
Ectopic (%)	50.1±13.6	54.4±14.1	60.6±11.3	55.9±95
Meal-derived fatty acid uptake (absolute)				
Upper body SAT (mg meal TG/ g Tissue)	0.35±0.17	0.35±0.26	0.25±0.16	0.29±0.12
Lower body SAT (mg meal TG/ g Tissue)	0.27±0.14*	0.29±0.26*	0.16±0.10*	0.22±0.13*
Abdominal adipocyte size				
Mean diameter (μm)	45.0±12.2	55.1±19.4**	66.1±18.8	61.9±13.0
%small (21–60μm)	51.3±17.7	41.0±24.3+	26.5±18.6	32.9±17.6
%medium (61–100μm)	45.4±17.6	50.9±20.9	61.2±17.7	57.8±18.5
%large (101–140μm)	3.3±5.1	8.2±13.6+	12.3±17.1	9.3±11.2
Femoral adipocyte size				
Mean diameter (μm)	50.8±12.9	61.6±21.0**	76.1±16.5	56.9±18.7+
%small (21–60μm)	42.2±15.9	39.9±17.8	21.8±17.9	45.2±23.2†
%medium (61–100μm)	49.8±16.1	41.3±20.1+	49.0±16.3	41.9±19.6+
%large (101–140μm)	8.0±7.0	18.8±20.2**	29.3±22.8	12.9±11.9+
LPL activity				
Abdominal (nmol/g/min)	13.9±9.0	14.4±9.7	17.1±10.4	19.0±12.9
Femoral (nmol/g/min)	36.1±25.5*	38.4±31.2*	41.4±37.0*	34.0±24.1*

mean±SD;

* $p<0.05$ lower body different from upper body;

** $p<0.05$ postmenopausal different from premenopausal;

† $p\leq0.05$ E_2 treated different from placebo;

+ $p\leq0.12$ trend for group difference.

LPL, lipoprotein lipase; SAT, subcutaneous adipose tissue; TG, triglyceride.