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Original article

Overweight postmenopausal women with different plasma estradiol concentrations present with a similar pattern of energy expenditure and substrate oxidation rate before and after a fatty meal challenge

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SUMMARY

Menopause-related withdrawal of ovarian estrogens is associated with reduced energy metabolism and overall impairment of substrate oxidation. Estradiol's withdrawal after menopause is associated with a reduction in energy metabolism and impaired substrate oxidation, which contributes to weight gain and visceral fat accumulation. Here we aimed to investigate the association between plasma estradiol concentrations and energy expenditure (EE)/substrate oxidation in a group of overweight postmenopausal women before and after a fatty meal challenge. Women were divided into three groups according to their plasma estradiol concentrations (E2): group 1 – $E_2 \leq 39$, group 2 – $40 \leq E_2 \leq 59$, and group 3 – $E_2 \geq 60$ pg/mL. VO_2 and VCO_2 volumes were collected following indirect calorimetry 5 h following a single lipid overload meal (1100 kcal, 72% of fat). For comparisons between groups and within the same group, a linear regression model with mixed effects was applied ($P < 0.05$). Forty-four women aged 55 ± 0.7 years-old, 8 ± 1.1 years following menopause, with a BMI of 30.5 ± 0.5 kg/m^2, and 41.9 ± 0.7% of body fat were enrolled the study. Plasma E2 concentrations were: group 1 – 30.4 ± 1.9, group 2 – 46.9 ± 1.5, and group 3 – 91.3 ± 12.0 pg/mL ($P < 0.0001$). EE at baseline and in the resting state was 1320 ± 24.3 kcal/d, and increased to 1440 ± 27.0 kcal/d 30 min following ingestion of the fatty meal ($P < 0.0001$), and rose again to an average of 1475 ± 30.3 kcal/d at the completion of experiment ($P < 0.0001$). Carbohydrate oxidation (Chox) was 0.155 ± 0.01 g/min at resting, maintained as 0.133 ± 0.00 g/min 30 min after ingestion of the fatty meal, and was 0.123 ± 0.01 g/min at the end of the testing period. Lipid oxidation (Lipox) was 0.041 ± 0.003 g/min at resting, increasing to 0.054 ± 0.003 g/min at 30 min ($P = 0.01$), and reaching 0.063 ± 0.003 g/min at the end of the experiment ($P < 0.0001$). There was no difference between groups for EE, Chox or Lipox. Our data suggest that EE and substrate oxidation were modulated following a lipid-meal challenge equally in all groups and this did not differ with plasma E2 concentrations.

1. Background and aims

Climacterium is characterized by the transition between reproductive and non-reproductive phases of women's life, in which menopause occurs [7,41]. This period has gained more and more attention due to an increase in life expectancy for women. In fact, most women now spend more than one-third of their lives after menopause [6]. Importantly, menopause is associated with abdominal fat accumulation and comorbid conditions such as type II diabetes and cardiovascular diseases [10,38]. Some of these changes are associated with a sedentary lifestyle and excessive intake of calories from high saturated fat foods [35,36], but the contribution of changes in estradiol levels associated with menopause is not fully known.

In addition to its effects on reproduction, E2 plays an important role in non-reproductive tissues, such as the pancreas [26], skeletal muscle [2], adipose tissue [8], bone [37], blood circulation [3] and central nervous system [5,25]. Importantly, E2 are key regulators of energy metabolism [27,30]. In the central nervous system, the effects of E2 on energy balance are primarily mediated by estrogen receptor

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alpha (ERα). Through non-genomic effects of E2 on extranuclear ERα, a rapid increase in the anorexigenic pathway occurs, leading to a reduction of food intake and increase in energy expenditure (EE) [23]. Recent studies have also reported the importance of peripheral E2-regulation of EE through "beiging" of adipose tissues which improves glucose homeostasis and may increase EE [24,30]. In this context, some clinical and experimental studies demonstrate estrogen replacement therapy in postmenopausal women reduces weight gain and abdominal fat accumulation [4,19].

Plasma E2 concentration widely fluctuate during perimenopause. After menopause, E2 levels are low, no longer fluctuate and estrone becomes the predominant circulating estrogen [34]. Interestingly, E2 levels in postmenopausal women on E2 therapy also exhibit variability, mainly due to age, BMI, ethnicity and smoking status [13]. Here we sought to determine the association of E2 concentration and energy metabolism in a group of postmenopausal women using hormone replacement therapy or not. Additionally, due to the prevalence of saturated fat intake in this population as seen in our previous work [35,36], we investigated the metabolic response to a high fat meal in postmenopausal women with varying levels of E2.

2. Methods

2.1. Subjects

Postmenopausal women who were seen at Climacteric Outpatient Clinic (Hospital das Clínicas, Universidade de São Paulo – HCFMRP/USP), and Centro de Saúde Escola (CSE Cuiabá-FMRP/USP) were invited to enroll the study. Inclusion criteria included menopausal status achieved at least one year prior to enrollment and a BMI between 25.0 and 39.9 kg/m^2. Exclusion criteria were smoking, hypo/hyperthyroidism, and use of corticosteroids. The study protocol was approved by the Ethical Committee of HCFMRP/USP (10655/2008). All participants provided written informed consent.

2.2. Study design

Data collection consisted of: anthropometric measurements, body composition, plasma measurements of E2 and follicle stimulating hormone (FSH), as well as VO_2 and VCO_2. According to the plasma E2 concentration, participants were enrolled in one of the following groups: group 1 – E2 $\leq$ 39, group 2 – 40 $\leq$ E2 $\leq$ 59, or group 3 – E2 $\geq$ 60 pg/mL.

2.3. Nutritional parameters

BW and height were obtained in the morning following an overnight fast [20]. BMI was calculated as BW (kg)/[height (m)]^2. Participants were classified according to the World Health Organization [45] into the following categories: 25.0 > BMI < 29.9 kg/m^2 as overweight, and $\geq$30 kg/m^2 as obese. Body composition was assessed by dual energy X-ray absorptiometry (DEXA) (Hologic QDR 4500 W®, Bedford, USA), and a range of 22.1–23.9% of body fat was used as the reference range for ideal body adiposity [20].

2.4. Energy expenditure and substrate oxidation

Inspired O_2 volume (VO_2) expired CO_2 (VCO_2) were measured through indirect calorimetry (Sensor Calorimeter Medics Vmax 29®, Yorba, USA), which was validated for the Brazilian population [43]. The resting metabolic rate (RMR) was obtained at fasting and for 30 min or until a "steady state" has been reached (steady state is defined as no alterations higher than 5% during 5 consecutive min). Energy expenditure (EE) was measured after a high fat lipid meal challenge over a 5 h-period with measurements occurring at 30, 90, 150, 210 and 270 min, in accordance with Refs. [17,32]. EE was calculated as $(3.94 \times VO_2 + 1.106 \times VCO_2) \times 1440$ kcal/d [44]. Carbohydrate oxidation (Chox) was calculated using the formula $[(4.55 \times VCO_2) - (3.21 \times VO_2)] \times 1440$ g/d, and lipid oxidation (Lipox) was calculated as $(1.67 \times VO_2) - (1.67 \times VCO_2)] \times 1440$ g/d [14].

2.5. Lipid overload meal

A single lipid meal containing 1100 kcal was provided to the subjects and it contained 72% of the kcals as fat, 13% of the kcals as protein, and 15% of the kcals as carbohydrates, which meets the definition of a high fat-lipid challenge test meal in accordance to Luscombe-Marsh et al. [21] and Smith et al., 2000 [46]. The meal consisted of foods rich in lipids such as fatty cheese, whole milk, avocado, egg and butter. The protein content was determined using the Kjeldahl method [1], the lipids content was calculated using Soxlet [1], and the carbohydrate content determined according to food composition tables [40,42]. The total calories content was determined according to the same food composition tables.

2.6. Plasma biochemical analysis

Fasting plasma E2 and FSH were determined by chemioluminescence (Immulite® 2000 immunoassay system, Siemens Healthcare GmbH, Erlangen, Germany).

2.7. Statistical analysis

Data were presented as mean ± SEM. Non-parametrical Kruskal–Wallis was used to compare general (age, menopausal years, plasma E2 and FHS levels) and anthropometric features between groups. A linear regression with mixed model was used to compare different times of the EE and substrate oxidation within the same group and between groups. $P < 0.05$ was considered statistical significant. SAS® 9.2, R software and GraphPad were used.

3. Results

3.1. General and anthropometric features

150 women were initially contacted of which 44 of them met the criteria for enrollment into the study. Sixteen subjects were

Table 1
General and anthropometric features.

	Group 1	Group 2	Group 3	*P* value
Age (years)	57 ± 1.2	55 ± 1.1	54 ± 1.6	0.34
Postmenopause (years)	7 ± 1.3	8 ± 2.2	9 ± 2.1	0.65
Body weight (kg)	73.4 ± 2.3	75.2 ± 2.5	76.4 ± 3.2	0.85
Height (m)	1.56 ± 0.02^{a,b}	1.55 ± 0.01^{a}	1.60 ± 0.01^{b}	0.04
BMI (kg/m^2)	30.3 ± 1.0	31.2 ± 0.9	29.8 ± 1.1	0.66
WC (cm)	90.3 ± 2.4	94.7 ± 1.9	89.5 ± 3.4	0.34
HC (cm)	106.9 ± 2.0	108.6 ± 2.2	108.2 ± 2.7	0.84
WC/HC ratio	0.85 ± 0.02	0.87 ± 0.01	0.83 ± 0.03	0.22
Body fat mass DEXA (%)	41.3 ± 1.2	41.9 ± 1.1	43.0 ± 1.0	0.73
Body lean mass DEXA (%)	58.7 ± 1.2	58.2 ± 1.1	57.0 ± 1.0	0.73
E2 (pg/ml)	30.4 ± 1.9^{a}	46.9 ± 1.5^{b}	91.3 ± 12.0^{c}	<0.0001
FSH (μUI/ml)	53.5 ± 7.4	51.4 ± 7.5	36.8 ± 6.5	0.25

BMI = body mass index, WC = waist circumference, HC = hip circumference, DEXA = dual energy X-ray absorptiometry, E2 = estradiol, FSH = follicular stimulating hormone. Different letters in the same row indicate $P < 0.05$ regarding the respective variable.