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Original Research

Prevention of hyperphagia prevents ovariectomy-induced triacylglycerol accumulation in liver, but not plasma

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ABSTRACT

Menopause is associated with higher plasma and liver triacylglycerol (TAG) and increased risk for cardiovascular disease. Lowering TAG in menopause may be beneficial; however, the mechanism underlying menopause-induced TAG accumulation is not clear. Ovariectomy is a model for menopause and is associated with metabolic alterations and hyperphagia. This study investigated the role of hyperphagia in ovariectomy-induced increases in blood and tissue TAG, as well as differences in lipid metabolism enzymes and resting metabolic measures. It was hypothesized that prevention of hyperphagia would restore blood and tissue TAG, enzyme expression, and metabolic measures to eugonadal levels. Ovariectomized rats were fed ad libitum (OVX + AL) or pair-fed (OVX + PF) relative to sham-operated rats (SHAM) to prevent hyperphagia. OVX + AL had higher TAG concentrations in liver and plasma than SHAM (60% and 50%, respectively), and prevention of hyperphagia in OVX + PF normalized TAG concentrations to SHAM levels in liver, but not plasma. OVX + AL also had 141% higher hepatic stearoyl-CoA desaturase 1 which was almost completely normalized to SHAM levels by pair-feeding, suggesting normalization of hepatic lipid storage. In contrast, skeletal muscle carnitine palmitoyl transferase 1 was 40% lower in OVX + AL than SHAM and was intermediate in OVX + PF, suggesting lower muscle fatty acid oxidation that may underlie the higher plasma TAG in OVX. No differences were seen in energy expenditure, VO_2, or VCO_2. Overall, this study indicates that prevention of hyperphagia resulting from ovarian hormone withdrawal normalizes hepatic TAG to eugonadal levels but has no effect on ovariectomy-induced increases in plasma TAG.

Abbreviations: OVX + AL, ovariectomized rat with ad libitum access to food; OVX + PF, ovariectomized rat pair-fed to sham-operated rat; SHAM, sham-operated rat; VO_2, volume of oxygen consumed; VCO_2, volume of carbon dioxide produced; SCD1, stearoyl-CoA desaturase 1; CPT-1a, carnitine palmitoyl transferase 1a; TAG, triacylglycerol; RER, respiratory exchange ratio; PDHK1, pyruvate dehydrogenase Kinase 1; FAS, fatty acid synthase; FADS, fatty acid desaturase; MFP2, multifunctional protein 2; PPARα, peroxisome proliferator activated receptor α; SREBP1c, sterol response element binding protein 1c; ERα, estrogen receptor α.

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1. Introduction

Menopause typically occurs after 40 years of age in women [1] and is associated with increased risk of cardiovascular disease [2]. Menopause is associated with increased adiposity and dyslipidemia [3] that includes elevated triacylglycerol (TAG) concentrations in plasma [4] and liver [5]. As plasma TAG is strongly associated with cardiovascular disease in women [6,7] and liver TAG accumulation is associated with hepatic dysfunction [8], normalization of TAG levels in menopausal women is likely cardioprotective. An improved understanding of the mechanisms underlying the increase in serum and liver TAG in menopause may inform strategies to improve the health of menopausal women.

Ovariectomized animals are a useful tool to study biochemical changes in tissues that occur in menopause. Ovariectomy results in similar biochemical changes to menopause, such as decreased serum 17β-estradiol [9], increased adiposity [10,11], increased hepatic TAG storage [12], and increased plasma TAG [13,14]. The cause of the TAG accumulation in ovariectomy is not clear, although ovariectomy causes hyperphagia [15,16] and decreases oxidation of carbohydrates [17] and lipids [18,19]. Prevention of hyperphagia to eugonadal levels may represent an easily-implemented strategy to decrease TAG accumulation resulting from lack of ovarian endocrine output; however, the contribution of ovariectomy-induced hyperphagia to increased TAG in plasma and liver in ovariectomy is not known. TAG concentrations are regulated by diet and metabolism, and it is not known if ovariectomy-induced hyperphagia affects liver and muscle oxidative enzyme expression. The effects of ovariectomy-induced hyperphagia on energy expenditure, activity, and fuel substrate preference are also unknown.

We hypothesized that prevention of hyperphagia in ovariectomized rats would normalize tissue and serum TAG concentration to eugonadal levels, and that this will be associated with normalization of the expression of oxidative and lipid storage enzymes. In addition, we hypothesized that prevention of ovariectomy-hyperphagia will normalize resting metabolic measures such as energy expenditure, VO_2, VCO_2, and the respiratory exchange ratio (RER, an indicator of oxidative substrate preference between fatty acids and carbohydrates), to eugonadal levels. To investigate this hypothesis, ovariectomy-induced hyperphagia was prevented by pair-feeding ovariectomized rats (OVX-PF) relative to eugonadal controls, and TAG accumulation, liver and muscle lipid metabolizing enzymes, and resting metabolic measures was compared to ad libitum-fed ovariectomized rats (OVX-AL). This study distinguishes the effect hyperphagia in OVX animals, thereby providing some evidence of the effectiveness of caloric restriction in ovarian hormonal removal.

2. Methods and materials

2.1. Animals

Animal procedures were ethically cleared by the University of Waterloo Animal Care Committee in accordance with the Canadian Council on Animal Care. Female Sprague–Dawley rats were ovariectomized or sham-operated by Harlan technicians at 5 weeks of age and arrived at the University of Waterloo at 6 weeks of age. Animals were housed in the animal facility in the Applied Health Sciences Faculty with 12:12 h light:dark cycle with temperature set at 21 ± 1°C. Animals had ad libitum access to water throughout the study. Upon arrival, ovariectomized rats were randomly assigned to receive either ad libitum access to food (OVX + AL, n = 6) or to be pair-fed (OVX + PF, n = 6) relative to the sham-operated rats (SHAM, n = 6). Food intake intervention was carried out over 33 days at which point rats were euthanized, therefore euthanasia occurred postnatal day 75 and 40 days post-ovariectomy. The pair feeding period was selected as 33 days based on previous work suggesting food intake in OVX + AL rats stabilizes at approximately 4–5 weeks post-ovariectomy [20]. The food consumed by SHAM rats was measured every 24 hours, and this amount was then provided to the OVX + PF rats to ensure that caloric consumption was identical between SHAM and OVX + PF. Pair-fed animals were provided food at the beginning of the dark phase, as this feeding schedule has been shown to be required to normalize body weight to sham-operated rats [21]. Diet was Harlan Teklad 8640 22/5 fixed formula diet providing 29% energy as protein, 17% as fat, and 54% as carbohydrate (ingredient list in online supplementary material) that has been used previously in our laboratory [22,23]. Body weight was measured every 4 days and food intake was measured daily, except during metabolic measurements. Rats were euthanized following an overnight fast by exsanguination after anaesthesia by intraperitoneal sodium pentobarbital injection (65 mg/kg). Liver, perirenal adipose, and posterior hindlimb skeletal muscle (mixture of soleus, red gastrocnemius and white gastrocnemius) were collected, washed in saline (0.9% v/v), and weighed before being snap-frozen in liquid N_2. Uterus and fallopian tubes were collected, washed in saline (0.9% w/v) and weighed. Plasma was isolated from whole blood by centrifugation at 1500*g*. All samples were stored at –80°C until analysis, and tissues were pulverized under liquid N_2 prior to analysis as previous [22].

2.2. Resting metabolic measurements

Whole body resting metabolism of the rats (n = 4 per group) was determined using a CLAMS (Comprehensive Lab Animal Monitoring System; Oxymax series; Columbus Instruments, Columbus, OH, USA) on the 23rd day of the study following two 24-h acclimatization sessions as previously described [24]. Four rats per group were analyzed due to the availability of monitoring units. Measurements were performed every 26 minutes over a 24-hour period following a 2-hour acclimatization/equilibration period. VO_2, VCO_2, total cage activity, and energy expenditure for each individual animal were recorded using Oxymax/CLAMS Software (Columbus Instruments) and exported for statistical analysis.

2.3. Measurement of enzyme and transcription factor expression by immunoblot

Expression of enzymes and transcription factors was assessed by immunoblot as described previously [22]. Briefly, homogenized protein samples were separated on 7.5% to 12.5% polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Following blocking, membranes were incubated in primary