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Time-restricted feeding improves insulin resistance and hepatic steatosis in a mouse model of postmenopausal obesity

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ABSTRACT

Background. Menopause is associated with significant hormonal changes that result in increased total body fat and abdominal fat, amplifying the risk for metabolic syndrome and diseases such as diabetes, cardiovascular disease and cancer in postmenopausal women. Intermittent fasting regimens hold significant health benefit promise for obese humans, however, regimens that include extreme daytime calorie restriction or daytime fasting are generally associated with hunger and irritability, hampering long-term compliance and adoption in the clinical setting. Time-restricted feeding (TRF), a regimen allowing eating only during a specific period in the normal circadian feeding cycle, without calorie restriction, may increase compliance and provide a more clinically viable method for reducing the detrimental metabolic consequences associated with obesity.

Methods. We tested TRF as an intervention in a mouse model of postmenopausal obesity. Metabolic parameters were measured using Clinical Laboratory Animal Monitoring System (CLAMS) and we carried out glucose tolerance tests. We also stained liver sections with oil red O to examine steatosis and measured gene expression related to gluconeogenesis.

Results. Preexisting metabolic disease was significantly attenuated during 7 weeks of TRF. Despite having access to the same high fat diet (HFD) as ad libitum fed (ALF) mice, TRF mice experienced rapid weight loss followed by a delayed improvement in insulin resistance and a reduced severity of hepatic steatosis by having access to the HFD for only 8 h during their normal nocturnal feeding period. The lower respiratory exchange ratio in the TRF group compared with the ALF group early in the dark phase suggested that fat was the predominant fuel source in the TRF group and correlated with gene expression analyses that suggested a switch from gluconeogenesis to ketogenesis. In addition, TRF mice were more physically active than ALF fed mice.

Conclusions. Our data support further analysis of TRF as a clinically viable form of intermittent fasting to improve metabolic health due to obesity.

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1. Introduction

Obesity is a strong risk factor for type 2 diabetes and several types of cancer including breast, colon, liver, and prostate cancer. Just prior to and after menopause, women experience increased adiposity (% fat mass) that contributes to the increased incidence of obesity and the metabolic syndrome with time after menopause [1–3]. Nonalcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which are manifestations of the metabolic syndrome and precursors to cirrhosis of the liver, also increase following menopause [4,5]. Furthermore, inflammation and insulin resistance that are associated with obesity are known risk factors for breast cancer especially in postmenopausal women [6]. Older women consume disproportionately more health care than other segments of the population and published data document statistically significant gender differences in chronic disease risk factors, the associations of risk factors with disease, and chronic disease rates [7–10]. Considering the increased risk that obesity poses for human disease, dietary obesity prevention and intervention strategies may be effective for mitigating obesity's harmful sequelae and reducing the medical and emotional burden on society. Indeed, dieting (restricting food intake to lose weight), caloric restriction (CR, energy restriction without incurring malnutrition) and intermittent fasting (IF, cycling between fasting and non-fasting periods) can reverse many of the detrimental effects of obesity [1,11–13]. One of the most common IF protocols is alternate day fasting (ADF) consisting of 24 h of ad libitum feeding followed by 24 h of fasting or severe CR (75–90% restriction of energy needs on 1–2 days per week) that reduces circulating cholesterol and triglycerides, and decreases blood pressure, fat mass and insulin resistance [14].

At the mechanistic level, CR reduces growth factor activation of Akt and mTORC1 and induces AMPK and the sirtuins, and CR mimetics such as metformin or everolimus are being tested in a number of cancer-related clinical trials [11,15]. ADF reduces oxidative stress and cancer incidence in rodents [16] but has not been shown to provide any advantages over CR for weight loss in humans [17]. Despite promising data in animals [18,19], these dietary interventions have not been adopted in the clinic, possibly reflecting difficulties patients face when trying to incorporate CR or ADF into their daily routine and/or problems with long-term compliance as these regimens are associated with hunger and irritability and the benefits can take weeks or months to materialize [20,21]. Indeed, a recent IF study reported that while compliance was strong during the study period, the majority of participants found the fasting days made daily living more difficult and only 18% would adhere to the regimen if prescribed by a physician [22]. A nutritional intervention that has the same benefits but is easier to maintain would greatly increase long-term compliance.

There is increasing evidence that time-restricted feeding (TRF), the practice of restricting the time of calorie intake, but not the amount of calorie intake, to an 8–12 h window that corresponds with daily circadian rhythms (e.g., eating during the night or "dark phase" for nocturnal mice), is an alternative approach for metabolic disease and cancer prevention that might be easier to implement in terms of compliance [13]. Food signals entrain peripheral clock rhythms and amplitudes. In fact, synchronizing feeding-fasting with normal circadian rhythms appears to improve oscillations in circadian clock gene expression, enhance energy metabolism, and reduce inflammation, while loss of circadian clock genes dysregulates metabolism and inflammatory responses [23,24]. For example, loss of the core clock component protein cryptochrome (Cry) leads to constitutive elevation of proinflammatory cytokines in a cell-autonomous manner in mice [25] and loss of the clock gene period circadian clock 2 (Per2) causes systemic and liver-specific perturbations in glucose metabolism and altered food intake behavior [26].

TRF during the dark phase fully protects male mice from obesity, hyperinsulinemia, hepatic steatosis, and inflammation, despite their consuming the equivalent amount of calories as ad libitum-fed (ALF) mice [23,27]. In female *Drosophila melanogaster*, TRF attenuates cardiac aging and prevents body weight gain compared with ALF, without reducing calorie intake [28]. We have found that restricting access to a pro-inflammatory, western-style high-fat diet, without restricting calorie intake, ameliorates hepatic steatosis and insulin resistance in ovariectomized (OVX) female mice, a postmenopausal model.

2. Materials and Methods

2.1. Animals and Diets

All animal experiments were carried out in accordance with the guidelines of the NIH and were approved by the University of California, San Diego, Institutional Animal Care and Use Committee. Mice were maintained in a facility with a 12 h light/12 h dark cycle with water and food ad libitum. Forty-five female C57BL/6 N mice (Charles River, Wilmington, MA) aged 7–8 weeks were ovariectomized (OVX) and body weights measured weekly thereafter. At 10 weeks of age 15 mice were continued on normal chow (NC, 12% kcal from fat; 3.02 kcal/g; Purina 5001, LabDiet, St. Louis, MO) and the remaining 30 mice introduced to a high fat diet (HFD, 60% kcal from fat; 5.24 kcal/g; D12492, Research Diets, New Brunswick, NJ). Both diets in pellet form were initially weighed and placed into standard wire food racks. Remaining pellets were weighed the next day to calculate the amount of food consumed before additional pellets were added and the new total amount of food weighed.

At 19 weeks of age when the HFD mice reached an average of 40 g (9 weeks of HFD feeding), they were divided into ALF and TRF groups of 15 mice each. The TRF group had access to the HFD for 8 h per day during the dark cycle from Zeitgeber time (ZT) 16 (10 pm) to ZT time 0 (6 am). ZT 0 is lights-on (6 am) and ZT 12 is lights-off (6 pm). The ambient room temperature for group housed mice was 22 ± 1 °C. At 6 am, the TRF group was moved to clean boxes for the fasting period to prevent foraging and coprophagia, after which time the mice were placed in their home boxes. Mice in all groups were handled daily at the same time to control for any handling stress and minimize experimental variation between groups [29]. Mice were euthanized 7 weeks after commencing the TRF intervention, the bilateral #3 and #4 mammary and parametrial fat pads were removed and weighed and the liver frozen for further analysis.