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Sex differences in diurnal rhythms of food intake in mice caused by gonadal hormones and complement of sex chromosomes

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ABSTRACT

We measured diurnal rhythms of food intake, as well as body weight and composition, while varying three major classes of sex-biasing factors: activational and organizational effects of gonadal hormones, and sex chromosome complement (SCC). Four Core Genotypes (FCG) mice, comprising XX and XY gonadal males and XX and XY gonadal females, were either gonad-intact or gonadectomized (GDX) as adults (2.5 months); food intake was measured second-by-second for 7 days starting 5 weeks later, and body weight and composition were measured for 22 weeks thereafter. Gonadal males weighed more than females. GDX increased body weight/fat of gonadal females, but increased body fat and reduced body weight of males. After GDX, XX mice had greater body weight and more fat than XY mice. In gonad-intact mice, males had greater total food intake and more meals than females during the dark phase, but females had more food intake and meals and larger meals than males during the light phase. GDX reduced overall food intake irrespective of gonad type or SCC, and eliminated differences in feeding between groups with different gonads. Diurnal phase of feeding was influenced by all three sex-biasing variables. Gonad-intact females had earlier onset and acrophase (peak) of feeding relative to males. GDX caused a phase-advance of feeding, especially in XX mice, leading to an earlier onset of feeding in GDX XX vs. XY mice, but earlier acrophase in GDX males relative to females. Gonadal hormones and SCC interact in the control of diurnal rhythms of food intake.

Introduction

Males and females have distinct patterns of metabolic regulation, including the control of food intake, which contributes to sex differences in the development and progression of metabolic disease. Until recently, sex differences in feeding in mammals were attributed exclusively to the effects of gonadal hormones, especially estrogens and androgens, which regulate food intake and energy metabolism by acting on the brain and diverse peripheral tissues (Asarian and Geary, 2006, 2013; Karastergiou et al., 2012; Petersen, 1978; Witte et al., 2010). We recently reported that the complement of sex chromosomes also causes sex differences in body weight, adiposity, and susceptibility to metabolic dysregulation caused by a high fat diet. Using Four Core Genotypes (FCG) mice in which sex chromosome complement (SCC, XX vs. XY) is varied in mice with testes (gonadal males) or ovaries (gonadal females), we found that the presence of two X chromosomes (vs. one) is associated with greater body weight and adiposity, in both gonadal males and gonadal females that had been gonadectomized (GDX) as adults (Chen et al., 2012). The increased body weight and adiposity of XX male and female mice was related to increased food intake specifically during the inactive period of the diurnal cycle, without alterations in activity or energy expenditure relative to XY mice.

Emerging evidence in humans and rodents suggests that disruption of the diurnal feeding pattern, especially increased caloric intake during the inactive phase, is associated with obesity and metabolic syndrome (Colles et al., 2007; Ma et al., 2003; Sierra-Johnson et al., 2008). For example, night-time eating in humans is associated with enhanced weight gain and obesity compared to similar caloric intake during normal meal times (Gallant et al., 2012). Mice that consume a greater proportion of

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their daily calories than normal during the inactive light phase have increased body weight, adiposity, hepatic steatosis, and hyperinsulinemia (Arble et al., 2009; Chaix et al., 2014; Hatori et al., 2012). Conversely, restricting feeding in mice to the night prevents obesity and metabolic disease (Chaix et al., 2014; Hatori et al., 2012). Mice with mutations of the *Clock* gene, a key component of the molecular circadian clock, have altered diurnal feeding rhythms, light phase hyperphagia, hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia (Turek et al., 2005).

Our previous finding of greater food intake during the light phase in gonadectomized (GDX) XX mice, relative to XY, raises the question whether the greater adiposity in XX mice results from a diurnal shift in feeding caused by the presence of a second X chromosome. Here, we examine diurnal rhythms of food intake of FCG mice in greater detail, in groups of mice that differ in three major classes of sex-biasing factors: SCC (XX vs. XY), reversible activational effects of gonadal hormones (gonad-intact vs. GDX mice), and permanent organizational effects of gonadal hormones (differences between gonadal males vs. females that persist after GDX). Thus, the design allows assessment of the relative impact of each type of sex-biasing factor, and the potential interaction of the three factors.

Materials and methods

Mice

We used Four Core Genotypes (FCG) mice on a C57BL/6J (B6) background (B6.Cg-Tg(Sry)2Ei Sry^{dl1Rlb}/ArnoJ, Jackson Laboratories stock 10905; backcross generation greater than 23), bred at UCLA (Arnold and Chen, 2009; De Vries et al., 2002). In FCG mice, the testis-determining gene *Sry* is deleted from the Y chromosome and inserted as a transgene on chromosome 3 (Itoh et al., 2015), so that gonadal sex is no longer determined by SCC. Here, "male" refers to a mouse with *Sry*, born with testes, and "female" refers to a mouse without *Sry*, born with ovaries. FCG mice include XX males and females, and XY males and females (called XXM, XXF, XYM, XYF, respectively). The model is a 2 × 2 comparison in which gonadal sex and SCC (XX vs. XY) are varied independently, so that the independent and interacting effects of these variables can be assessed. The Y chromosome of FCG mice derives from strain 129. FCG B6 mice were studied with gonads intact or GDX at 75 days of age, as indicated.

The experimental design is intended to assess the roles of three major sources of sex-biasing factors: activational and organizational effects of gonadal hormones, and SCC. We compared the four genotypes with their gonads and after removal of the gonads, to test the role of testicular and ovarian secretions in adulthood ("activational effects"). Also compared were GDX mice that lack gonadal hormones as adults but that had either ovaries or testes prior to GDX, which allows detection of long-lasting effects of testicular (in mice with *Sry*) or ovarian secretions (in mice without *Sry*) up to several months after GDX. These long-lasting "organizational effects" in this model are confounded with direct effects of *Sry* outside the gonad. Finally, the design tests if either activational or organizational effects of hormones affect XX and XY mice differently (sex chromosome effects).

Two cohorts of mice were studied, one for longitudinal measurement of body weight and composition (Fig. 1), and the second for detailed studies of patterns of feeding (Figs. 2–5). Males and females were housed as single-sex groups (except when in BioDAQ cages, see below) and maintained at 23 °C with 12:12 LD (6 am/6 pm). The standard chow diet Purina 5001 contains approximately 5% fat (PMI Nutrition International, St. Louis, MO and Lab Diet 5001, www.labdiet.com). For studies in BioDAQ chambers, rodent diet AIN-93M was used (Research Diets, Inc., New Brunswick, NJ) which contains 4.1% fat. AIN-93M is a solid balanced rodent diet that causes minimum spillage, required for accurate measurement of food intake in BioDAQ chambers. Animal studies were performed under approval of the UCLA and Veterans Affairs Institutional Animal Care and Use Committees.

Genotyping

DNA was extracted from tails using Chelex resin (Bio-Rad, Hercules, CA). The genotype of mice was determined by PCR to amplify *Sry* (which determines gonadal sex) and the Y-chromosome repetitive sequence Ymt. Gonadal sex was confirmed at the time of gonadectomy or at the end of the experiment. Myogenin was used as an amplification control. The primers were myogenin-F: TTACGTCCATCGTGGACAGCAT, myogenin-R: TGGGCTGGGTGTTAGTCTTAT; Ymt-F: CTGGAGCTCTACAG TGATGA, Ymt-R CAGTTACCAATCAACACATCAC; Sry-F: AGCCCTACAGCC ACATGATA, Sry-R: GTCTTGCCTGTATGTGATGG.

Measurement of body weight and body composition

Body composition was determined at 30 weeks of age with a Mouse Minispec apparatus (Bruker Woodlands, TX) with Echo Medical Systems (Houston, TX) software (Taicher et al., 2003). This apparatus uses NMR spectroscopy for fat and lean mass measurements with coefficients of variation of <3%. Correlation between NMR and gravimetric measurements is better than 0.99.

BioDAQ food intake monitoring system

Food intake behavior and meal patterns were continuously monitored, with minimal human interference, using the BioDAQ Biological Data Acquisition episodic food intake monitor for mice (Research Diets, Inc., New Brunswick, NJ). Three weeks after gonadectomy at age 75 days, mice were habituated for one week to the AIN-93N diet, and then were housed as individuals in cages containing the BioDAQ monitors (n = 4 mice of each genotype). While housed in BioDAQ cages, mice received AIN-93M diet through a feeding hopper, which weighed the food every second at 0.01 g resolution. Water was provided ad libitum from regular water bottles. Gonad-intact FCG mice (n = 4 per group) were tested at the same age.

Mice were deemed not to be feeding if the hopper weight was stable, and feeding if the hopper weight changed by more than 0.01 g. Episodic feeding bouts, the smallest measured unit of feeding, were recorded automatically when the change in food hopper weight was greater than 0.01 g. Bouts were separated by an inter-bout interval, the time period between bouts during which there was no feeding activity. Meals were defined as comprising a series of bouts within 300 s of each other when the sum of food intake over all bouts was equal to or greater than 0.02 g. Meal beginning in one period (e.g., the light phase) was recorded in that period even if it extended into another period (e.g., dark phase). Measurements of meal patterns included total food eaten, meal size (food eaten per meal), meal number, and inter-meal interval (the time period between meals during which there was no feeding activity) as previously described (Stengel et al., 2010). The data were analyzed with BioDAQ Monitoring software 2.2.02 and spreadsheet software.

Baseline food intake for GDX and gonad-intact mice

It usually takes 3–5 days for mice to settle into a stable pattern of food intake in the novel BioDAQ cage environment. We report on feeding patterns for 7 days beginning on the 6th day after introduction into the BioDAQ cages. Thus, feeding is reported here during the period about 5–6 weeks after GDX. Patterns were measured for mice that were placed in BioDAQ cages at 4 weeks after GDX at 75 days of age, and for gonad-intact mice that were also approximately 103 days old.