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Influence of ovarian and non-ovarian estrogens on weight gain in response to disruption of sweet taste — calorie relations in female rats

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ABSTRACT

Regulation of energy balance in female rats is known to differ along a number of dimensions compared to male rats. Previous work from our lab has demonstrated that in female rats fed dietary supplements containing high-intensity sweeteners that may disrupt a predictive relation between sweet tastes and calories, excess weight gain is demonstrated only when females are also fed a diet high in fat and sugar, and is evidenced primarily in animals already prone to gain excess weight. In contrast, male rats show excess weight gain when fed saccharin-sweetened yogurt supplements when fed both standard chow diets and diets high in fat and sugar, and regardless of their proneness to excess weight gain. The goal of the present experiments was to determine whether ovarian, or other sources of estrogens, contributes to the resistance to excess weight gain in female rats fed standard chow diets along with dietary supplements sweetened with yogurt. Results of the first experiment indicated that when the ovaries were removed surgically in adult female rats, patterns of weight gain were similar in animals fed saccharin-sweetened compared to glucose-sweetened yogurt supplements. In the second experiment, when the ovaries were surgically removed in adult female rats, and local production of estrogens was suppressed with the aromatase inhibitor anastrozole, females fed the saccharin-sweetened yogurt consumed more energy and gained more weight than females fed the glucose-sweetened yogurt. However, when the ovaries were surgically removed prior to the onset of puberty (at 24–25 days of age), females given saccharin-sweetened yogurt along with vehicle gained excess weight. In contrast, weight gain was similar in those given saccharin-sweetened and glucose-sweetened yogurt along with anastrozole. The results suggest that behavioral differences between males and females in response to disruption of sweet→calorie relations may result from differences in patterns of local estrogen production. These differences may be established developmentally during the pubertal period in females.

Introduction

Overweight and obesity represent increasingly pressing health problems in the United States and throughout the world (e.g., Flegal et al., 2012; James, 2008; Ogden et al., 2012). Efforts to reduce the spread of obesity have focused on a variety of factors, including changing the food environment to reduce energy intake. One method promoted for reducing energy intake is to replace ingredients in the diet that provide energy, such as sugars and fats, with substitutes, such as the high intensity sweeteners saccharin, acesulfame potassium (AceK) and sucralose and the fat substitute olestra, which mimic the sensory properties of sugars and fats (American Dietetic Association, 2004). The rationale behind such substitution approaches is that these replacers will result in reduced energy intake. However, whether such reductions in energy intake actually occur remains an open question. For example, a number of epidemiological studies link consumption of beverages sweetened with high-intensity sweeteners to increased risk of overweight, obesity, diabetes, cardiovascular disease and metabolic syndrome (e.g., Dhingra et al., 2007; Duffey et al., 2012; Fowler et al., 2008; Gardener et al., 2012; Laska et al., 2012; Ludwig, 2009; Lutsey et al., 2008; Nettleton et al., 2009; Yang, 2010) while other studies do not appear to demonstrate such a link (e.g., Bellisle and Drewnowski, 2007; Schulze et al., 2004).

Previously we reported that male rats given high intensity sweeteners (i.e., saccharin, acesulfame potassium, and Stevia extracts) exhibit increased energy intake and body weight gain (for review, see Swithers et al., 2010). We have provided evidence that these deficits in regulating energy balance are based on the disruption of the learned signaling relationship between sweet tastes and caloric or energetic outcomes (Davidson et al., 2011). This relationship is formed early in life (Swithers et al., 2012) presumably as a result of normal experience with consuming sweet-tasting, caloric foods and fluids.

This learned relation between taste and calories may provide one mechanism that animals use to regulate energy intake. Based on the

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principles of Pavlovian conditioning, as a consequence of experience with sweet tastes in foods that provide energy in the form of calories, may come to show conditioned, anticipatory responses that prepare them to effectively utilize that energy (e.g., Woods and Ramsay, 2000). Within this framework, consuming high-intensity sweeteners that taste sweet but deliver few or no calories, should interfere with energy regulation by weakening this predictive relationship (e.g., Davidson and Swithers, 2004; Swithers and Davidson, 2008). This mechanism may contribute to some of the associations observed in humans between consumption of foods and beverages sweetened with high-intensity sweeteners and increased risk of negative health outcomes including overweight, obesity, and metabolic syndrome.

To date, most of the work examining the consequences of disrupting sweet taste-calorie associations on long-term food intake and body weight gain has focused on lean male rats, although data suggest that the most likely consumers of such "diet" foods and beverages are women and those already overweight or obese (e.g., Duffey et al., 2012; Fowler et al., 2008). Recent data from our lab suggest that negative effects of consuming high-intensity sweeteners on weight control may be greatest for these overweight or likely to become overweight. We found that female rats prone to excess weight gain and fed a diet high in fat and sugar, show increased body weight when given dietary supplements sweetened with saccharin compared to those given supplements sweetened with glucose (Swithers et al., submitted for publication). However, compared to male rats, female rats consuming a standard low-fat lab chow diet appear to be relatively resistant to the additional body weight gain related to consumption of high-intensity sweeteners; that is female rats given saccharin-sweetened diets gain similar amounts as female rats given glucose-sweetened diets (Swithers et al., submitted for publication). Further, the responses of female rats to high-intensity sweeteners when maintained on diets high in fat and sugar are modulated by their phenotype; females identified as prone to diet-induced obesity (DIO) prior to introduction of the sweeteners show excess weight gain compared to those given a caloric sweetener, while females identified as resistant to diet-induced obesity (DR) gain similar amounts of weight when given caloric and non-caloric sweeteners. In contrast, both DIO and DR males show excess weight gain when provided with high-intensity sweeteners compared to caloric sweeteners (Swithers et al., submitted for publication).

The mechanisms that might underlie differences in patterns of responding to high-intensity sweeteners, and the resistance to weight gain on a standard chow diet, in female compared to male rats are unknown. However, short-term data from rats tested during the prepubertal period suggest that prior to the onset of puberty (e.g. 15–25 days of age), responses to high-intensity sweeteners are similar in male and female rats fed a standard chow diet (e.g., Davidson and Swithers, 2004; Swithers et al., 2012), suggesting that changes occurring during puberty may alter responses of female rats to high-intensity sweeteners. One implication of this finding is that ovarian estrogens might play a role in differences in responses of female rats before puberty compared to following puberty. Alternatively, it is becoming increasingly recognized that physiological and neural function can be influenced not only by circulating estrogens derived from the ovaries, but also by estrogens synthesized locally from precursors such as cholesterol and testosterone through activity of the CYP450 enzyme aromatase. In the rat, tissues expressing aromatase that may be particularly critical for energy regulation include the liver, adipose tissue, hypothalamus, and even taste buds of the circumvallate papillae (Azcoitia et al., 2011; Belanger et al., 2002; Charlier et al., 2010; Cornil, 2009; Cornil and Charlier, 2010; Cornil et al., 2006; Sanghera et al., 1991; Shibuya et al., 2003; Simpson et al., 2000; Toyoshima et al., 2007). Thus, differences in responding in female rats could also reflect changes in local estrogen synthesis through aromatase activity that follow puberty.

The goal of the present studies was to examine whether ovarian or other estrogens contribute to the observed resistance to weight gain in adult female rats given high-intensity sweeteners. In Experiment 1, the ovaries of adult female rats were removed surgically and the effects of consuming dietary supplements sweetened with the high-intensity sweetener saccharin were compared to supplements sweetened with glucose in female rats. In Experiment 2, the goal was to examine whether blocking the activity of aromatase, thereby inhibiting local estrogen synthesis, affected responding to saccharin-versus glucose-sweetened yogurt diet supplements and whether the consequences of inhibiting local estrogen synthesis by surgical removal of the ovaries were different compared to after puberty. The aromatase inhibitor anastrozole was used because it is designed for oral delivery, and has been previously demonstrated to significantly suppress estrogen levels in OVX female rats at the dose employed (0.1 mg/kg; Dukes, 1997; Wang et al., 2009). In rats, anastrozole is typically delivered by gavage or provided in drinking water as the sole source of fluids, which can diminish the amount of fluid consumed and thereby impact food intake. Preliminary work demonstrated that when anastrozole or its vehicle (ethanol) was mixed into the dietary supplement we typically use, low-fat yogurt, animals reliably consumed the entire portion of yogurt, and therefore the entire dose of the drug. We took advantage of the willingness of animals to consume the drug voluntarily by dissolving it in the yogurt supplements, allowing us to avoid stress associated with repeated gavage or suppression of fluid intake associated with providing it in drinking water.

Materials and methods

Experiment 1

Subjects were 25 adult female Sprague–Dawley rats approximately 70 days of age (Harlan, Indianapolis) that received ad libitum access to a standard laboratory chow (Lab Diets 5012) for one week after arrival in the lab. Then, all rats were anesthetized with ketamine and xylazine prior to bilateral ovariectomy (OVX), which was performed as described previously (Swithers et al., 2008). Butorphanol (2.5 mg/kg s.c.) was used for post-operative analgesia. Animals were allowed to recover for 3 weeks prior to being assigned to Glucose or Saccharin groups matched on body weight (Means = 260.1 ± 4.04 and 260.8 ± 4.2 g, Glucose [n = 12] and Saccharin [n = 13] groups, respectively). Animals in both groups were given access to 30 g yogurt (Dannon low-fat yogurt ~0.6 kcal/g) daily 6 days per week for 4 weeks. Yogurt was provided unsweetened 3 days per week and was sweetened with 0.3% saccharin (Saccharin group) or 20% glucose (Glucose group) on the remaining 3 days per week. Chow and water alone were available on the 7th day of each week. Yogurt intake, food intake and body weight were measured daily. Weight gain was analyzed by a two-way, repeated measures ANOVA (Sweetener × Day) with sweetener as a between-subjects factor and day as a within-subjects factor. Total energy intake (chow plus yogurt) was analyzed with a separate one-way (Sweetener) ANOVA.

Experiment 2

Experiment 2 differed in several ways from Experiment 1 due to differences in the time at which the studies were conducted in the lab. Subjects in this experiment were 48 adult female Sprague–Dawley rats approximately 70 days of age (Harlan, Indianapolis) and weighing 175–200 g upon arrival and 34 adolescent female Sprague–Dawley rats, identified by litter of origin, received from Harlan (Indianapolis) at 21 days of age. In the adolescent group, 1 or 2 animals per litter were assigned to each of the experimental groups, and sample sizes were 8–9 per group due to the availability of animals identified by litter at the specified age. Sample sizes were 12 per group in the animals OVX as adults as in Experiment 1; litter