Diet-induced hyperphagia in the rat is influenced by sex and exercise

Lisa A. Eckel and Shelley R. Moore

Program in Neuroscience and Department of Psychology, Florida State University, Tallahassee, Florida 32306-1270

Submitted 24 June 2004; accepted in final form 4 August 2004

Eckel, Lisa A., and Shelley R. Moore. Diet-induced hyperphagia in the rat is influenced by sex and exercise. Am J Physiol Regul Integr Comp Physiol 287: R1080–R1085, 2004. First published August 5, 2004; doi:10.1152/ajpregu.00424.2004.—Caloric intake is increased in rats fed a diet containing greater fat or sugar than that found in laboratory chow. Because such diet-induced hyperphagia has been studied primarily in sedentary male rats, our goal here was to investigate the effects of sex and exercise on caloric intake of a diet (chow supplemented with sweet milk) chosen for its ability to stimulate hyperphagia. Rats were housed individually in cages that provided access to running wheels, and daily caloric intake of chow alone and then chow plus sweet milk was monitored during sedentary and active conditions. In sedentary rats, chow intake was greater in males compared with females. Wheel running produced similar decreases in chow intake in both sexes. Availability of the chow plus milk diet increased caloric intake compared with that observed in chow-fed rats. This diet-induced hyperphagia was significantly greater in sedentary females (35.7 ± 3.1% increase) relative to sedentary males (9.1 ± 2.2% increase). In addition, 35% of sedentary females consuming the chow plus milk diet developed estrous cycle disruptions. Wheel running decreased intake of the chow plus milk diet in both sexes. In active males, diet-induced hyperphagia was abolished; caloric intake was reduced to that observed during chow feeding. In active female rats, diet-induced hyperphagia was attenuated but not abolished; caloric intake of the chow plus milk diet remained greater than that observed during chow feeding. We conclude that female rats are more vulnerable than male rats to this form of diet-induced hyperphagia.

METHODS

Animals and housing. Eight female and eight male Long-Evans rats (Charles River Breeding Laboratories, Raleigh, NC), weighing between 175 and 200 g at study onset, were housed individually in custom-designed, stainless-steel cages equipped with feeding niches and connected to Wahmann running wheels (35 cm in diameter). The room was maintained at 20 ± 2°C with a 12:12-h light-dark cycle.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(dark onset at 1300). Animal usage and all procedures were in compliance with the Florida State University Institutional Animal Care and Use Committee and the guidelines of the American Physiological Society (2).

Diets. Rats were given free access to ground rat chow (Purina 5001; 3.30 kcal/g) alone and then in combination with sweet milk (Eagle Brand sweetened condensed milk, diluted 1:2 with water; 1.35 kcal/g). While the overall carbohydrate content between sweet milk and chow was similar (59.0% and 54.3%, respectively), the sugar content (monosaccharides and disaccharides) was tenfold greater in the sweet milk relative to chow (59.0% and 5.9%, respectively). The sweet milk was chosen because it produces a reliable increase in daily caloric intake relative to that observed in chow-fed rats (13). Chow was presented in spill-resistant food cups located in feeding niches attached to the cages. The sweet milk was presented in spill-resistant bottles clipped to the sides of the cages. Food intake was monitored daily by weighing (±0.1 g) the food cups and bottles at 0900. Due to differences in caloric density between chow and the sweet milk, feeding data were converted to kilocalories to obtain a measure of total daily caloric intake when both diets were available. Throughout the experiment, water was freely available.

Running wheel activity. The running wheels could either revolve freely (active condition) or be in a locked position that prevented running (sedentary condition). Running wheel data were collected remotely by a computerized system (ESP 500; R. Henderson; Florida State University) using dipole magnets (DiLog Instruments; Tallahassee, FL) that continuously monitored the occurrence of wheel revolutions (±0.5 revolutions). During the active condition, wheel running for the previous 24 h was recorded daily at 0900.

Estrous cycle. Vaginal cytology samples were collected daily at 0900. Stage of the estrous cycle was then determined by examining the appearance and abundance of cells within each sample, as described previously (11, 12). Using this strategy, proestrus included the light period peak in estradiol secretion, and estrus included the subsequent dark period when female rats ovulate and display increased sexual receptivity (i.e., behavioral estrus). At study onset, all rats displayed regular, 4-day estrous cycles.

Procedure. Rats were adapted to the novel housing/feeding conditions for 7 days. After adaptation, caloric intake of chow was monitored for 8 days with wheels in the locked position and then for 8 days with wheels in the unlocked position. To induce hyperphagia, chow was then supplemented with free access to the sweet milk. Caloric intake of the chow plus milk diet was monitored for 8 days with wheels in the locked position and then for 8 days with wheels in the unlocked position. This design yielded four test periods: chow/no wheel, chow/wheel, chow plus milk/no wheel, and chow plus milk/wheel. Throughout the experiment, rats were weighed daily (±0.1 g) at 0900. Because male rats display steeper growth curves and accrue greater muscle mass than female rats (25), feeding data were expressed as kilocalories per 100 g body weight to control for sex differences in energy requirement. In female rats, data collection, which commenced when rats were in diestrus 1, was synchronized by phase of the 4-day estrous cycle to control for estrous-related changes in food intake and wheel running (12). Accordingly, 8-day test periods in male rats corresponded to two estrous cycles in female rats. A second reason for monitoring estrous cycle phase was to determine the impact of diet-induced hyperphagia on estrous cyclicity.

Statistical analyses. Preliminary analyses of daily caloric intake and running wheel activity in male rats revealed minimal day-to-day variability within each experimental test period. Similar analyses in female rats also revealed minimal day-to-day variability in these behavioral measures with the exception of an estrous-related decrease in caloric intake and increase in wheel running that was expressed during both the chow only and the chow plus milk feeding conditions. Because data collection was synchronized by cycle phase in female rats, the net effect of any estrous-related behavioral change was held constant during each experimental test period. Because of the lack of significant day-to-day variation in our behavioral measures, feeding and wheel running data were averaged across each experimental test period. The effect of sex, wheel availability, and diet on mean daily caloric intake was analyzed using a three-factor, mixed-design ANOVA with sex as the between-subject variable and wheel availability and diet as within-subject variables. Diet-induced hyperphagia was characterized by the percent increase in mean daily caloric intake when rats were switched from the chow diet to the chow plus milk diet during both sedentary and active conditions. The effect of sex and wheel availability on diet-induced hyperphagia was analyzed using a two-factor, mixed-design ANOVA with sex as the between-subject variable and wheel availability as the within-subject variable. The effect of sex and diet on mean daily running wheel activity was analyzed using an independent t-tests during sedentary and active conditions. Tukey’s honestly significant difference test was used to investigate differences between means following significant main or interactive ANOVA effects.

RESULTS

Caloric intake. Mean daily caloric intake was influenced by an interactive effect of sex, diet, and wheel availability [F(1,14) = 6.45, P = 0.02] (Fig. 1). In chow-fed rats (Fig. 1A), caloric intake was greater in males compared with females, regardless of wheel availability (P < 0.01). In both sexes, wheel access decreased caloric intake of chow (P < 0.05). As expected, switching rats from chow to the chow plus milk diet increased caloric intake in male and female rats (P < 0.01) (Fig. 1B). Caloric intake of chow plus milk was, however, greater in females, compared with males, regardless of wheel availability (P < 0.01). In both sexes, wheel access decreased caloric intake of chow plus milk (P < 0.01).

When chow and sweet milk were both available, male and female rats displayed a clear preference for the sweet milk (Table 1). Caloric intake of chow was greater in male rats compared with female rats [F(1,14) = 5.72, P = 0.03], and the

Fig. 1. Effect of exercise and diet on mean (±SE) daily caloric intake in male and female rats. A: in chow-fed rats, caloric intake was greater in males relative to females, regardless of wheel availability. Wheel access decreased caloric intake of chow in both sexes. B: availability of sweet milk, in addition to chow, increased caloric intake in male and female rats. In chow plus milk-fed rats, caloric intake was greater in females, relative to males. Wheel access decreased caloric intake of chow plus milk in both sexes. a Males greater than females, P < 0.01. b Wheel condition less than no-wheel condition, P < 0.05. c Females greater than males, P < 0.01.
availability of wheels decreased caloric intake of chow in both sexes \([F(1,14) = 48.27, P = 0.000007]\). Caloric intake of the sweet milk was greater in female rats compared with male rats \([F(1,14) = 23.44, P = 0.0003]\), and the availability of wheels decreased caloric intake of the milk diet in both sexes \([F(1,14) = 14.92, P = 0.002]\).

Diet-induced hyperphagia, defined as the percent increase in mean daily caloric intake when rats were switched from chow to the chow plus milk diet, is illustrated in Fig. 2. Regardless of wheel availability, diet-induced hyperphagia was greater in female rats compared with male rats \([F(1,14) = 29.95, P = 0.000008]\). Wheel access decreased diet-induced hyperphagia in both sexes \([F(1,14) = 16.51, P = 0.001]\). In male rats, wheel running abolished diet-induced hyperphagia; caloric intake of the chow plus milk diet was decreased to that observed when only the chow diet was available \([33.7 \pm 1.1 \text{ vs. } 33.0 \pm 0.8 \text{ kcal/100 g body wt, respectively, not significant (NS)}]\). In female rats, wheel running attenuated but did not abolish diet-induced hyperphagia; active female rats displayed greater caloric intake (i.e., hyperphagia) when the chow plus milk diet was available compared with when only the chow diet was available \([36.0 \pm 1.3 \text{ vs. } 30.9 \pm 0.9 \text{ kcal/100 g body wt, respectively, } P = 0.01]\).

**DISCUSSION**

Diet-induced hyperphagia, particularly when combined with a sedentary lifestyle, appears to be a leading cause of obesity in our society (20, 22). Thus understanding how physical activity impacts one’s appetite and diet choices is paramount to identifying those individuals at risk for developing obesity. Because the prevalence of obesity is greater in women compared with men (31), it is important that such research be conducted in both sexes. In light of these issues, our goal here was to examine the effects of sex and exercise on daily caloric intake in a rat model of diet-induced hyperphagia. Initially, the effect of wheel running on daily caloric intake of a chow diet was
assessed in male and female rats. To promote diet-induced hyperphagia, chow was then supplemented with sweet milk and the effect of voluntary wheel running on daily caloric intake of the sweetened chow plus milk diet was determined. There were three principal findings. First, daily caloric intake of the chow diet was greater in male rats compared with female rats. Second, diet-induced hyperphagia was more pronounced in female rats. Third, wheel running decreased diet-induced hyperphagia in both male and female rats.

**Caloric intake, activity, and estrous cyclicity in rats consuming laboratory chow.** Under our sedentary condition, when access to running wheels was not permitted, daily caloric intake of chow was greater in male rats compared with female rats. This finding is consistent with previous studies in which caloric intake was compared in sedentary male and female rats fed similar chow diets (6, 25). In these studies, however, feeding data were collected during periods when sex differences in daily weight gain were prominent, and caloric intake was not expressed in relation to body weight. Thus the accelerated growth curves of male rats relative to female rats may have contributed to a differential energy requirement that could account for previous reports of sex differences in caloric intake. While plausible, our current findings do not support this hypothesis because daily caloric intake, expressed in proportion to body weight, was greater in male rats compared with female rats. Because a sex difference in body weight cannot explain the relative hyperphagia observed in male rats, some other factor(s) must underlie the sex difference in caloric intake observed here and in previous studies involving sedentary, chow-fed rats.

Two lines of evidence suggest that sex differences in caloric intake are related to sex differences in gonadal hormone secretion. First, peripheral administration of testosterone increases caloric intake in chow-fed male rats (17). Because testosterone does not appear to interact with neural circuits involved in the control of food intake (30), its orexigenic properties are believed to arise from its ability to stimulate the development of lean muscle mass (35). Although not quantified in the present study, it is likely that our male rats had greater lean muscle mass and, therefore, greater energy requirement than our similarly aged female rats. This could have contributed to the sex difference in caloric intake observed here. Second, it is well established that estradiol exerts both phasic and tonic inhibitory effects on food intake in female rats (10). The phasic inhibition is expressed during the estrous phase of the ovarian reproductive cycle (12). Elevated plasma estradiol concentration precedes the decrease in caloric intake that occurs during estrus relative to diestrous and proestrous phases (14). The tonic inhibition is revealed by ovariectomy; bilateral removal of the ovaries increases caloric intake (16, 24). Such hyperphagia has been attributed to the loss of estradiol, rather than progesterone, because estradiol treatment alone restores daily food intake to that observed in gonadally intact rats (16). The greater concentration of plasma estradiol in female rats likely contributed to the sex difference in caloric intake observed here.

Although the majority of research investigating physical activity level in rodents has been conducted in males (38), the few studies involving both males and females suggest that daily physical activity, like food intake, is sexually dimorphic. Specifically, females are more active than males when given access to an open field (39), the elevated plus maze (26), and running wheels (7, 23). The present findings are consistent with this previous research. Under our active condition, when access to running wheels was permitted, daily wheel running was significantly greater in chow-fed female rats compared with chow-fed male rats. Like sex differences in caloric intake, this sex difference appears to be mediated at least in part by estradiol. In rats, wheel running is reduced by ovariectomy (18), estradiol treatment alone after ovariectomy reinstates normal levels of wheel running (18), and wheel running is increased during estrus relative to nonestrous phases (12).

In the present study, wheel running suppressed chow intake in male and female rats. Our findings in males are consistent with previous studies that report an exercise-related decline in chow intake in male rats either given access to running wheels (1, 21) or forced to run on a treadmill (29). In contrast, our findings in females do not support previous reports that wheel running has either no effect (12) or a slight stimulatory effect (3, 32) on chow intake in female rats. These discrepant findings may be related to differences in energy requirement at the time of testing. In previous studies, rats were heavier and their body weight was relatively stable. Here, data were collected in younger rats during a period of accelerated growth. Thus it is possible that only older, weight-stable female rats are able to compensate for the increased energy expenditure associated with wheel running by increasing their daily caloric intake.

When the chow diet was available, all rats displayed regular 4-day estrous cycles during both the sedentary and the active condition. While forced wheel running has been shown to suppress the pulsatile secretion of luteinizing hormone and delay the onset of puberty in young rats (28), this and previous studies (9, 12) suggest that voluntary wheel running does not disrupt the estrous cycle of adult rats. In support of this, voluntary wheel running has been shown to facilitate estrous cyclicity and reproductive function in hamsters housed in a short-day, nonbreeding environment (5).

**Caloric intake, activity, and estrous cyclicity in rats consuming chow plus milk.** As expected, an increase in daily caloric intake was observed in male and female rats when their chow diet was supplemented with sweet milk. This finding is consistent with previous studies in which caloric intake was monitored in rats fed high-carbohydrate diets consisting of chow plus various concentrations of sweetened solutions (21, 36). To investigate what aspect of a high-carbohydrate diet promotes overeating, Seilafi and colleagues (37) examined the relative contributions of preabsorptive and postabsorptive actions of carbohydrate intake in a rat model of diet-induced hyperphagia. They found that intragastric infusions of a Poly-cose solution did not stimulate overeating or weight gain in rats unless the infusions were paired with the taste of a sweet solution (37). Thus taste appears to be more important than the postingestive actions of carbohydrates in driving overeating in rats exposed to a sweetened diet. Our data are consistent with this observation. While chow and sweet milk were similar in overall carbohydrate content (~60%), diet-induced hyperphagia was driven entirely by overconsumption of sweet milk (Table 1).

Under our sedentary condition, the expression of diet-induced hyperphagia was sexually dimorphic; female rats displayed greater caloric intake of the chow plus milk diet than male rats. To the best of our knowledge, only one other study...
has examined whether sex influences the development of diet-induced hyperphagia in rats fed a sweetened diet. In this study (21), male and female rats were given access to chow supplemented with a 32% sucrose solution. While this diet stimulated hyperphagia in both male and female rats, the authors did not report any sex difference in the magnitude of overeating. Interpretation of these findings is limited, however, by several factors. There was no direct statistical comparison between males and females; males received less exposure to the palatable diet than females; and males were older, heavier, and displayed greater caloric intake than females at the start of the study.

The greater expression of diet-induced hyperphagia in female rats may have been driven by a sex difference in taste preference. Several studies indicate that female rats display a stronger preference than male rats for both nutritive (e.g., sucrose) and nonnutritive (e.g., saccharin) sweet solutions during intake tests ranging from 30 min to 24 h (21, 36). Alternatively, the greater expression of diet-induced hyperphagia observed here in female rats may be related to greater behavioral responsivity to a diet choice relative to male rats. In a previous study, female rats increased their daily caloric intake more than male rats when given free access to both a Polycose solution and a sucrose solution (36).

Access to running wheels decreased diet-induced hyperphagia in male and female rats. In active male rats, daily caloric intake of the chow plus milk diet was reduced to the level observed in chow-fed male rats. In previous studies involving male rats, diet-induced hyperphagia, after exposure to high-fat, high-carbohydrate, or cafeteria diets, was abolished by voluntary wheel running (21) and by forced exercise on a treadmill (33). Additionally, chronic exercise in running wheels was recently shown to lower the defended level of weight gain and body adiposity of diet-induced obese male rats, despite a change in hypothalamic neuropeptide expression that is normally associated with increased caloric intake (27). Thus exercising male rats do not appear to make the required behavioral or metabolic adjustments to compensate for negative energy balance or lowered energy stores.

In contrast to our findings in male rats, wheel running attenuated but failed to eliminate diet-induced hyperphagia in female rats. One mechanism that could explain why wheel running did not abolish overconsumption of the chow plus milk diet in female rats, similar to that observed in male rats, involves hypoactivity. Consumption of the more palatable chow plus milk diet was associated with a decrease in daily wheel running in female rats, but not male rats, relative to that observed when only the chow diet was available (Fig. 3). Because wheel running suppressed appetite in both male and female chow-fed rats, the relative decline in wheel running in chow plus milk-fed female rats may have enabled greater caloric intake of the palatable diet. It is also possible that less time spent in the running wheels allowed female rats to spend proportionally more time engaged in ingestive behavior. Additional studies investigating the temporal relationship between wheel running and ingestive behavior in male and female rats fed a sweetened diet will be required to examine this hypothesis directly.

When the chow plus milk diet was available, estrous cycle disruptions were observed in three of the eight rats during the sedentary condition. The prolonged presence of leukocytes and epithelial cells in daily vaginal cytology samples indicate that rats with estrous cycle disruptions experienced a prolonged diestrous phase. In a previous study, similar disruptions in estrous cyclicity were reported in sedentary female rats fed a cafeteria diet (19). In this study, rats displayed hyperphagia and an increase in brown adipose tissue (BAT) thermogenesis. Returning rats to a chow diet restored estrous cyclicity on normalization of food intake and BAT thermogenesis. Thus the increase in BAT thermogenesis may provide a signal that results in disruption of estrous cyclicity in overfed rats (19). Interestingly, the availability of running wheels restored regular estrous cyclicity to two of the three rats during the active condition. The increased energy expenditure associated with wheel running may mediate this effect. In a previous study, voluntary wheel running facilitated estrous cyclicity in hamsters housed in a short-day, nonbreeding environment (5).

In summary, the goal of our study was to determine the effects of sex and exercise on the development of diet-induced hyperphagia in male and female rats fed a palatable, sweetened diet. Our results indicate that diet-induced hyperphagia is greater in female rats and that exercise decreases diet-induced hyperphagia in both male and female rats. Although wheel running eliminated diet-induced hyperphagia in male rats, female rats continued to overeat the chow plus milk diet relative to the chow alone. We conclude, therefore, that female rats are more vulnerable than male rats to diet-induced hyperphagia stimulated by the availability of a palatable, sweetened diet. Our findings have obvious clinical importance, given that hyperphagia and lack of exercise are two primary factors that contribute to weight gain and obesity, a phenomenon that has reached epidemic proportions worldwide (34, 42). Research investigating the mechanism underlying the greater hyperphagia observed here in female rats relative to male rats is critical given a recent report that the incidence of obesity is greater in women than in men (31). Further research of the hormonal basis for the sex differences observed here is necessary to determine why females are more likely to overeat when given a palatable, sweetened diet. Finally, it will be crucial to expose male and female rats to sweetened diets for longer periods to determine whether the sex difference in this form of diet-induced hyperphagia translates into a sex difference in weight gain and, ultimately, obesity.

GRANTS
This work was supported by National Institute of Mental Health Grant MH-63787 (L. A. Eckel).

REFERENCES