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Temporal relationships between food acquisition and voluntary exercise in mice

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ABSTRACT

Patterns of operant food acquisition in a closed economy and bouts of either voluntary wheel running (WR) or spontaneous locomotor activity in a standard condition (SC) with no wheel were examined in young adult male and female C57BL/6 mice across a range of nose poke prices (FUP) per food pellet. Both sexes showed vigorous WR or locomotor activity. At each FUP, WR groups had higher food intake than SC groups. Despite substantially higher mean body weight of males compared with females, intakes and activity did not differ by sex in the SC groups and males lost weight more rapidly as FUP increased. In contrast, WR males ran ~33% further per day than females, increased their food intake (above that of SC counterparts) more than females, and lost less body weight than SC males. By parsing the night in four 3 h epochs it was found that food intake declined progressively through the night in both WR and SC mice and that the hyperphagia of WR relative to SC groups was most evident early in the night, coincident with highest activity. No large or systematic sex differences were revealed in these temporal analyses. Analysis of data at 60 s resolution showed that pellet acquisition occurred in many small or short bouts, the timing of which was either intercalated or concurrent with either locomotor activity or WR. The results show that increased eating due to WR occurs concurrently with maximum running, and with no evidence of delayed compensation.

1. Introduction

The long term effectiveness of exercise as a weight loss strategy in humans depends on several factors including whether increased energy expenditure due to exercise affects food intake (Blundell et al., 2015; Schubert et al., 2013). In a recent meta-analysis, it was concluded that exercise has a minimal effect on food intake in the next few hours (Schubert et al., 2013). But out of 28 studies reported, 17 showed an increase and 6 a decrease in intake. Average increase in food intake was greatest in studies that used the highest intensity and expenditure mode of exercise (viz., running). Other studies have emphasized a high degree of individual variability in the effect of exercise on food intake (Blundell et al., 2015; Whybrow et al., 2008).

Previously sedentary men but not women lost weight during an identical long term exercise program (Donnelly et al., 2003). It was suggested that sex differences in hormonal responses to exercise may predispose women to a differential stimulation of appetite (Hagobian et al., 2009). However, a study examining energy compensation following moderate or high intensity exercise periods among lean subjects found that daily food intake increased by ~30% of the change in expenditure in both men and women (Whybrow et al., 2008). In contrast,

a study in which overnight-fasted healthy young men and women cycled for ~80 min (to achieve 30% increase in daily energy expenditure) found that food intake at a buffet meal 40 min later was 1.8 MJ higher in men but was not different in women, both compared with a no exercise session on another day (Hagobian et al., 2013). These studies do not provide a consistent account of sex difference in the effect of exercise on food intake, but are difficult to compare because they differ in many variables including initial weight or fitness, age, time of day, intensity of exercise, and duration of the measurement of intake.

These variables can be better controlled in research using laboratory animals, and one aim of the present study is to examine whether mice (*Mus musculus*) show sex differences in energy intake in relation to the expenditure of exercise. Voluntary wheel running (WR) is a common non-invasive way of increasing energy expenditure and many studies have reported the effect of WR on body weight in mice. Relatively few such studies have also reported food intake and most have used only one sex (males: Atalayer and Rowland, 2011; Goodrick 1978; Pendergast et al., 2014; females: Copes et al., 2015). An exception is a report by Kelly et al. (2011) in which individual food intake was recorded during 6 days wheel access. Food intake was positively correlated with distance run and, while males and females ran comparable

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distances, the slope of the regression of food intake *vs.* distance was ∼25% higher in males than females. This result suggests that females show less appetite stimulation than males in response to a given level of WR. However, neither this nor any other study of which we are aware reported the time course over which intake within a day is matched to or compensates for increased energy expenditure due to WR. If that time course is short (*e.g.,* 1 h or less), then WR and increased intake should overlap in time. Conversely, if that time course is long (e.g., several hours), then WR would be accompanied by normal or reduced food intake followed by hyperphagia during the non-running periods of the nycthemeron.

Previous work in our laboratory has studied food acquisition using an operant behavior closed economy protocol in which discrete food items are available at costs (nose poke responses per 20 mg pellet of food) that are increased every few days. One study (Atalayer and Rowland, 2011) examined exercise and we found that WR male mice (CD-1 strain) showed a 25% increase in daily food intake relative to SC (the sedentary group in that report). Intakes of SC mice showed typical demand elasticity, decreasing as price increased, whereas those of WR mice did not show appreciable elasticity. Thus, WR mice not only ate more than SC but were willing to work harder to maintain that intake. In another study using only SC, male C57BL/6 (B6) mice ate more than females at low cost but resembled females at high costs with the consequence that males showed greater elasticity of food demand than females (Rowland et al., 2015). This effect may be mediated by estrogen receptor α because females carrying its genomic knockout showed more food elasticity than wild type females (Minervini et al., 2015). We have not previously studied WR in female mice but, from the foregoing, we might reasonably hypothesize that WR females will show more hyperphagia than male WR, an hypothesis that is in apparent contradiction of the result obtained by Kelly et al. (2011), discussed above.

SC mice often eat slowly over prolonged periods, without defined meals, especially during the first half of the night (Goulding et al., 2008; Rowland, 2012; Rowland et al., 2015). Spontaneous circumnavigation of the home cage will bring SC animals into repeated proximity of food, as is evident in records from Goulding et al. (2008), or contact with the manipulandum that delivers food. It is possible that this proximity may promote close temporal intercalation of locomotion in the SC and feeding. In contrast, WR mice must terminate a bout of running and traverse the chamber to the location of the nose poke manipulandum and feeder. We thus hypothesize that eating patterns of WR mice may be more discrete or defined than in SC (no wheels) mice.

2. Materials and methods

2.1. Subjects

Adult male and female (N = 16 each) B6 mice were purchased from Envigo (Indianapolis, IN) at 3 months of age. The experiment was performed in two identical replications containing equal numbers of males and females in each cohort and condition, one year apart. Results from the two cohorts were similar and were combined, as planned, for analysis and presentation. Pre-experimentally, mice were housed individually in conventional polycarbonate shoebox cages with free access to Harlan #7912 pelleted chow and autoclaved water. Contact bedding (Sani-Chips, Harlan) was changed weekly. Ambient temperature was 23–24 °C, and relative humidity was 40–70%. The University of Florida Animal Care and Use Committee approved all procedures in this protocol with the stipulation that mice were removed from study on the day that body weight loss first exceeded 15% from that at the start of the experiment.

2.2. Operant behavior chambers

Experiments were conducted in 16 individual operant conditioning chambers (Med Associates, St. Albans, VT) each enclosed in a ventilated, sound-attenuating cubicle. A 7 W light in each cubicle provided illumination (Zeitgeber time ZT 0–12) from midnight until noon. The chambers measured 14 × 14 × 12 cm inside and were made of Plexiglas and with aluminum front and rear panels and steel rod floor (0.5 cm spacing). A paper-lined pan was placed 4.5 cm below the floor. Half of the cages had running wheels (55.8 cm circumference, 4–5 g resistance) mounted outside of the rear wall, accessible through a retracted wall panel. The remaining chambers (the SC) had no wheel but, to obtain a crude quantitative estimate of locomotor activity, one infrared photobeam was mounted 2 cm above the floor in the middle of one Plexiglas wall and a detector on the opposite wall; mice often patrol the perimeters of such enclosures, so each complete lap (∼0.5 m) in principle registered two beam breaks. A nose poke response device with a recess measuring 1 cm in diameter was located on the chamber front or intelligence panel, 3 cm from the right wall and 2 cm above the floor. A 0.75 cm diameter cue light positioned 5 cm above the nose poke recess was illuminated whenever food was available. A food trough was horizontally centered in the front panel, 1.5 cm to the left of the recess and 1 cm above the floor. Food was dropped into the trough from a 20 mg pellet dispenser fitted with a quiet stepping motor. Water was available freely from a sipper spout. A computer running Med-Associates software (St. Albans, VT) controlled experimental events and every 60 s recorded nose poke responses, pellet deliveries, and beam breaks or quarter wheel revolutions (1792 full revolutions = 1 km).

2.3. Procedure

Equal numbers of male and female mice were assigned at random to WR or SC groups. Males and females weighed means of 25.2 and 18.5 g, respectively, at the start of the study. Mice were tested in 23 h daily sessions during which a programmed number of nose poke responses (the fixed unit price or FUP) delivered a food pellet. After 2 days of initial task training at 2 responses per pellet, FUP was increased every 4 days in the sequence 5, 10, 25, 50, 100. Daily sessions started at noon (ZT12, the start of the dark cycle) and food could be earned at any time in the session. The wheel was always accessible for the WR mice. Each day at 1100 h, mice were removed to a holding cage with no food while the chambers were cleaned and serviced. Daily sessions started immediately after this service period.

2.4. Data analyses

The rod floor to the chambers precludes hoarding of pellets beyond any that may accumulate in the food trough. However, food was never found in the trough at the daily service so we have assumed this is true throughout each session and equate time of food acquisition with consumption. This is consistent with previous analyses of response runs which suggest that each pellet is eaten as soon as it is delivered (Rowland et al., 2014). Daily food intakes were corrected for spilled pellets, retrieved from the pan under the cage during the daily service. As we reported before (Minervini et al., 2014), mice spilled substantial amounts at low FUP (< 10) but the timing of this food wastage cannot be reliably determined from the record of pellet acquisition. Thus, intake and pattern data were analyzed only for FUP 10 and above, when spillage was < 5% of daily intake. Further, because not all animals completed FUP 100, these data at the highest cost were not included in the principal analyses and are presented separately. Data were analyzed by 3-way ANOVAs (main factors sex, activity condition, and FUP) with Holm-Sidak post hoc contrasts (P < 0.05).

Data were collected with 60 s resolution; intakes and/or activity were then collapsed across contiguous time bins to achieve various temporal integrations, up to an entire daily session. As we have reported previously (Rowland et al., 2015), group mean intakes were stable after the first day at each new FUP. To provide a temporal analysis of this stable behavior, detailed analyses of pellets and activity