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Voluntary running decreases nonexercise activity in lean and diet-induced obese mice

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HIGHLIGHTS

- Voluntary exercise failed to decrease body weight in obese and lean mice.
- Voluntary exercise causes compensatory behaviors, including decreased SPA.
- SPA is a key component of daily energy expenditure.
- By decreasing SPA, voluntary exercise induced-weight reduction also decreases.

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ABSTRACT

Purpose: Determine whether voluntary wheel running triggers compensatory changes in nonexercise activity in lean and high-fat diet fed mice.
Methods: C57Bl/6 mice received a control (C) or a high-fat diet (H) and half of them had free access to a running wheel 5 days/week (CE and HE, respectively) for 10 weeks. Energy intake, nonexercise activity (global activity, distance covered and average speed of displacement in the home cage) and energy expenditure (EE) were evaluated at weeks 5 and 10 during the 2 days without the wheels.
Results: High-fat diet increased weight gain in H (110%) and HE (60%) groups compared to C and CE groups, respectively, with no effect of exercise. Wheel running increased energy intake (26% CE, 11% HE in week 5; 7% CE, 45% HE in week 10) and decreased distance covered (26% for both CE and HE in week 5; 35% CE and 13% HE in week 10) and average speed (35% CE and 13% HE in week 5; 45% CE and 18% HE in week 10) compared to the respective nonexercised groups. In week 10 there was an interaction between diet and exercise for global activity, which was reduced nearly 18% in CE, H, and HE groups compared to C. Access to a running wheel increased EE in week 5 (11% CE and 16% HE) but not in week 10, which is consistent with the period of highest running (number of turns: weeks 1–5 nearly 100% > weeks 6–10 for CE and HE groups). EE was reduced in H (19%) and HE (12%) groups compared to C and CE, in week 10.
Conclusion: Voluntary running causes a compensatory decrease in nonexercise activity and an increase in energy intake, both contributing to the lack of effect of exercise on body mass.

1. Introduction

Obesity is a complex disease with genetic, epigenetic, environmental and behavioral components. Between 1980 and 2013, the worldwide prevalence of overweight and obesity increased 27.5% for adults and 47.1% for children. In the same time span, the number of overweight or obese people rose from 857 million to 2.1 billion [30].

Exercise is an important tool in the fight against obesity as it can create a negative energy balance. However, studies in humans [28,33,34] and rodents [18,21,39] have failed to meet the expected body weight reduction after increasing daily energy expenditure through exercise. Additionally, significant variation in body weight in response to an exercise training protocol has been seen even in a setting of controlled energy intake [6].

Nonexercise activities, or spontaneous physical activity (SPA), are activities of day-to-day living other than exercise per se, including fidgeting, maintenance of posture and other body movements of daily

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life [24,42]. For rodents, SPA refers to all forms of activities, including ambulatory and non-ambulatory behavior [15]. SPA has been shown to protect against weight gain in response to overeating and it seems to have a fundamental role in energy homeostasis [24,41]. High levels of SPA have been shown to protect rats from fat mass gain [41] whereas a decline in SPA contributes to diet-induced obesity in mice [4,41]. In humans, changing SPA toward more sedentary behavior, e.g. sitting or other very low-energy demanding activities, may account for the failure of some exercise programs in reaching the predicted weight loss [28,34].

Morio et al. (1998) found that 14 weeks of progressive endurance training in elderly people did not change their total daily energy expenditure during the training period due to a compensatory decrease in free-living activities. Among the categories of activities they investigated, they found a reduction in time spent walking [28]. The longer the periods in sedentary behaviors, the shorter the time spent in higher intensity physical activities, contributing to an overall reduction in energy expenditure. Sedentary behavior is associated with overweight and obesity, and has other deleterious health effects such as metabolic alterations and premature death [22,32].

High-fat diet fed mice offer an adequate model to study obesity and type 2 diabetes mellitus [2,43], and wheel running has being used to analyze the effects of exercise on diet-induced obesity (DIO) [7,21]. However, wheel running is not always accompanied by changes in body weight or body composition [18,21,39]. The existence of an undefined distance threshold below which voluntary running does not protect from DIO [7], that there is a compensatory increase in energy intake [18,39], or the idea that wheel running just substitutes other forms of equivalent energy-consuming behaviors [18] have been proposed to explain the discrepancy in literature. Only recently a compensatory change in SPA in mice submitted to voluntary exercise has begun to be investigated [1,7,36], but to date, there have been no investigations in DIO mice. Therefore, our objective was to determine the effect of free access to running wheels on nonexercise activity and energy homeostasis in diet-induced obese mice.

2. Methods

2.1. Animals

The experiments were approved by the Institutional Ethics Committee on Animal Use (CEUA 5042110514). Eight-week-old male C57Bl/6 mice were obtained from the Center for Development of Animal Models for Medicine and Biology (CEDEME, Federal University of São Paulo). They were kept at the laboratory animal facility of the Department of Bioscience in a temperature-controlled room (22 °C) with a 12:12-h light-dark cycle (7:00–19:00 h) and had free access to a control diet with a caloric composition of 16.5% fat, 65.7% carbohydrate, and 17.7% protein, and a caloric density of 3.82 kcal/g. After a four-week adaptation period mice (12 weeks-old) were randomly assigned into one of two diet groups for 10 weeks: they were either kept on the same control diet (C group) or fed a high-fat diet (H group) with a caloric composition of 60.2% fat, 26% carbohydrate, and 13.8% protein, with a caloric density of 5.23 kcal/g. In both diets, the source of carbohydrate was maize starch, dextrinized maize starch and sucrose; protein was from casein and fat was from soybean oil. In the high-fat diet, there was the addition of lard. Half of the mice of both C and H groups stayed in individual cages with free access to a running wheel five days a week (CE and HE groups, respectively). Both diets were purchased from Rhoster (Rhoster Indústria e Comércio LTDA, São Paulo, Brazil). The study was performed in two independent sets of experiments with $n = 4–5$ mice/group in each (total $n = 9, 8, 10$ and 8 for C, CE, H and HE groups, respectively).

2.2. Voluntary exercise

Mice were housed individually in home cages equipped with a running wheel (Panlab-Harvard Apparatus, Barcelona, Spain) for 10 weeks, five days a week, and rested for 2 consecutive days every week. The wheel (diameter 34.5 cm; width 9 cm) was mounted outside the home cage to preserve animal life space. The total number of wheel rotations was registered daily on an external LE907 individual counter, and the mean value for each week was calculated. Wheel running, commonly used as a model of exercise, is not equivalent to spontaneous physical activity as it engages different neural and physiological mechanisms [31,37].

2.3. Body weight and caloric intake

Body weight was recorded once a week during the entire experiment. Caloric intake was measured in weeks 5 and 10 while spontaneous physical activity was being monitored (on days without access to a running wheel). Average daily consumption was determined by subtracting the weight of the remaining food removed after 48 h from the weight of food given, with care taken to account for spillage.

2.4. Indirect calorimetry

Indirect calorimetry was analyzed for 48 h in the beginning of the fifth (transition from 4th to 5th week) and tenth (transition from 9th to 10th week) weeks at room temperature (22 °C) using an indirect calorimetry system. In short, mice were placed individually in specifically designed calorimeter chambers (Oxylet system, Panlab-Harvard Apparatus, Barcelona, Spain) with free access to diet and water. Oxygen consumption and carbon dioxide production, as well as energy expenditure, were calculated using METABOLISM software (Panlab-Harvard Apparatus, Barcelona, Spain). During indirect calorimetry measurement, mice of the CE and HE groups had no access to a running wheel. Data for energy expenditure were analyzed as areas under the curves of energy expenditure in the full (24 h), light (uninterrupted light cycle, from 7:00 to 19:00) and dark cycles (mean of the two dark cycles) calculated from values of each mouse using the trapezoidal method [26].

2.5. Nonexercise or spontaneous physical activity (SPA)

SPA was measured by infrared beam sensors [40] using an IR Actimeter system composed of a 2-dimensional (X and Y axes) square frame (25 × 25 cm), each frame containing 16 × 16 infrared beams, 1.3 cm apart (Panlab-Harvard Apparatus, Barcelona, Spain). SPA was recorded individually in the end of the fifth and tenth weeks for 48 consecutive hours and it was determined using the ActiTrack v2.7 software (Panlab-Harvard Apparatus, Barcelona, Spain). The software allowed the determination of global activity, being the sum of stereotypes (the number of samples where the position of the subject is different from its position during the previous sample and equal to its position from the second sample, back in time) and locomotion (the number of samples where the position of the subject is different from its position during the previous sample and different to the position of the second sample, back in time), and also the determination of the distance travelled and average speed of displacement. As for the indirect calorimetry, SPA was analyzed on the days the mice in the CE and HE groups had no access to a running wheel and the areas under the curves of activity in the full (24 h), light (uninterrupted light cycle, from 7:00 to 19:00) and dark cycles (mean of the two dark cycles) were calculated from values of each mouse using the trapezoidal method [26].

2.6. Carcass lipid and protein content

Mice were euthanized by decapitation after CO_2 inhalation. Then, retroperitoneal and epididymal adipose tissues were excised and weighed. The carcasses were eviscerated and the remnants, including the retroperitoneal and epididymal fat depots, were weighed and stored at −80 °C. The lipid content was measured as described by Stansbie et al. (1976) and standardized using the method described by Oller Do