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Effect of calorie restriction on spontaneous physical activity and body mass in mice divergently selected for basal metabolic rate (BMR)

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HIGHLIGHTS

- Selection of mice for basal metabolic rate affected spontaneous physical activity.
- Initial variation in physical activity is sustained during calorie restriction (CR).
- Variations in physical activity are not linked to body mass loss under CR.
- High physical activity is not always related to low metabolic thriftiness during CR.

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ABSTRACT

Spontaneous physical activity (SPA) represents an important component of daily energy expenditures in animals and humans. Intra-specific variation in SPA may be related to the susceptibility to metabolic disease or obesity. In particular, reduced SPA under conditions of limited food availability may conserve energy and prevent loss of body and fat mass ('thrifty genotype hypothesis'). However, both SPA and its changes during food restriction show wide inter-individual variations. We studied the effect of 30% caloric restriction (CR) on SPA in laboratory mice divergently selected for high (H-BMR) and low (L-BMR) basal metabolic rate. Selection increased SPA in the H-BMR line but did not change it in the L-BMR mice. This effect reflected changes in SPA intensity but not SPA duration. CR increased SPA intensity more strongly in the L-BMR line than in the H-BMR line and significantly modified the temporal variation of SPA. However, the initial between-line differences in SPA were not affected by CR. Loss of body mass during CR did not differ between both lines. Our results show that the H-BMR mice can maintain their genetically determined high SPA under conditions of reduced food intake without sacrificing their body mass. We hypothesize that this pattern may reflect the higher flexibility in the energy budget in the H-BMR line, as we showed previously that mice from this line reduced their BMR during CR. These energy savings may allow for the maintenance of elevated SPA in spite of reduced food intake. We conclude that the effect of CR on SPA is in large part determined by the initial level of BMR, whose variation may account for the lack of universal pattern of behavioural responses to CR.

1. Introduction

Different forms of spontaneous physical activity (SPA; defined also as non-exercise activity thermogenesis, NEAT) may significantly contribute to the daily energy expenditures in both people and animals [1–3]. Consequently, SPA can play an important role in maintaining the balance between the rate of energy acquisition and fat storage. In general, high SPA has beneficial effect on health and reduces the risk of obesity [2,4–8]. However, both humans and animals show large, heritable variations in SPA, which may significantly modulate the relationship between food intake and obesity [5,9,10]. Individuals may also vary with respect to their genetic makeup underlying the ability to accumulate fat stores in anticipation of food shortages (the concept of a 'thrifty genotype', see [11–13]). Although high SPA may improve fat deposition when food is plentiful (e.g., through more intense foraging), it may also elevate energy expenditures during periods of food scarcity. Thus, the SPA-induced energy expenditures under low food intake (calorie restriction, CR) may significantly contribute to the long-term beneficial effects of CR on obesity. Their efficacy, however, hinges on the relationship between CR and SPA, whose directionality as well as strength and timing varies between the studies (e.g. [14], reviewed in [15]).

Recently, several studies have highlighted the significance of the initial, between-subject variations of SPA in their response to CR or in CR-reduced body mass changes [16–19]. For example, the negative effect of 50% CR on SPA was more pronounced in rats selected for high intrinsic aerobic capacity and who manifested high initial SPA than in

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their low-capacity and less-active counterparts [19]. In contrast, changes in SPA incurred by 30% CR in outbred MF1 mice did not depend on their initial levels [16]. However, even though CR reduced SPA in the first study and elevated it in the second, in both studies, higher initial SPA and/or higher increases in SPA were related to greater body mass loss during CR (the same result was found in [17]). This pattern suggests that low initial SPA may represent an important component of the 'thrifty genotype', which conserves energy and body mass during CR.

Interpretation of CR-induced increases of SPA may not be straightforward because studies on CR usually involve comparisons between laboratory animals with restricted and unlimited access to food. Laboratory animals might have undergone adaptation to artificial conditions that could affect their responses to CR [20–22]. In particular, laboratory breeding is likely to incur inadvertent selection for increased food intake [23]. As a result, similar relative reductions of food intake may represent different stresses for laboratory and wild-derived animals. Moreover, laboratory animals reared under ad libitum feeding regimens are usually sedentary and easily become obese [24,25]. Thus, one may hypothesize that laboratory animals can elevate their SPA during CR simply because their routine activity (i.e., observed under ad libitum conditions) is much lower than that achieved when food is scarce. Therefore, we believe that studies on how CR affects laboratory animals with different pre-CR levels of SPA are essential to validate their relevance as models for understanding the potential beneficial effects of this treatment on both the extension of the lifespan and obesity in humans.

Here, we tested the effect of 30% CR on SPA in two lines of Swiss-Webster laboratory mice, divergently selected for either high (H-BMR) or low (L-BMR) levels of basal metabolic rate (BMR). Divergent selection for BMR has led to significant, genetically-based differences in SPA and food intake, both of which are higher in the H-BMR line [26]. These two lines are therefore an excellent model to investigate the effect of CR on SPA. First, SPA of the L-BMR line did not differ from that observed in randomly bred lines of Swiss mice [26]. Thus, the L-BMR line represents a 'typical' SPA of laboratory mice and serves as a 'control' to the H-BMR mice with elevated SPA. Second, differences in SPA between the H-BMR and L-BMR lines arose as a result of selection for BMR without simultaneous changes in maximum aerobic capacity [26]. Therefore, these two lines show different genetic correlations between key physiological and behavioural parameters than animal subjects of other experiments (e.g., [19]), and thus offer an excellent opportunity to cross-test the universality of the CR-SPA relationship. Finally, we previously showed that CR reduces BMR significantly more in mice with a higher initial value of this trait (H-BMR) [27]. Thus, the effect of CR on SPA is also likely to be line-specific.

In earlier studies, we found that, under ad lib feeding conditions, the H-BMR mice spent a larger part of their energy budget on SPA than those from the L-BMR line [26], while both lines showed similar body mass reduction during CR [27]. As explained previously, higher initial SPA may be expected to result in higher CR-mediated loss of body mass. Therefore, because H-BMR mice do not show higher body mass loss than their less active counterparts when food is restricted, we hypothesized that mice of this line should be more likely to reduce their SPA during CR than the L-BMR line.

2. Materials and methods

2.1. Animals and housing

Subjects in our experiment were Swiss-Webster mice (*Mus musculus* Linnaeus 1758) artificially selected for high and low body mass-corrected BMR. The selection experiment is described in detail elsewhere [26,28,29]. Briefly, the BMR of 12–16-week-old mice was measured for 3 h in an open-circuit respirometry system at an ambient temperature of 32 °C. Males and females characterized by the highest and lowest mass-corrected BMR were chosen as progenitors of the H-BMR and L-BMR selection lines, respectively. A similar procedure was repeated in subsequent offspring generations, yielding significant differentiation of the lines with respect to BMR, without simultaneous changes in body mass. Although both lines came from an unreplicated selection experiment, between-line differences in BMR and several other traits (including SPA; [26]) were large enough to claim that they represented a genuine change in frequencies of alleles directly related to BMR rather than genetic drift [26,28,29]. The conditions applied during the present experiment (ambient temperature of 23 °C and 12:12 light-dark cycle) were the same as those used throughout the course of the selection experiment.

2.2. Experimental design

The present experiment was carried out on mice from generation 34 of the selection experiment. After BMR measurements were completed, we measured the food intake of all mice selected for our experiment (see [27] for detailed description of the methods used) and then randomly assigned half of the mice within each of the selected lines to one of two feeding regimens: (i) unlimited access to food (ad libitum regimen, AL), or (ii) feeding every two days with a precise amount of food equal to 70% of their individually measured, two-day ad libitum food consumption (CR, analogous to method used in our previous experiment; [27]). This design created 4 experimental groups: (i) H-BMR mice fed AL, with an average two-day food consumption (mean ± s.e.m.) 11.94 ± 0.18 g, 27 individuals; (ii) H-BMR mice fed CR, offered 8.07 ± 0.16 g of food every two days (29 individuals); (iii) L-BMR mice fed AL, with an average two-day food consumption of 10.11 ± 0.12 g (29 individuals); (iv) L-BMR mice fed CR, offered 7.04 ± 0.10 g of food every two days (29 individuals). These groups were called H-BMR-AL, H-BMR-CR, L-BMR-AL, and L-BMR-CR. All mice were housed individually and had unlimited access to water. Because of logistical constraints, mice were assigned randomly to 4 replicates (blocks), each containing 27–30 mice (6–8 from each of four experimental groups). All experimental treatments were applied in these blocks with two-day intervals to provide sufficient time to quantify SPA.

All experimental procedures were approved by the Local Ethical Committee in Białystok (permission 52/2009).

2.3. Measurement of spontaneous physical activity (SPA)

SPA was measured during two-day measurement periods (hereafter called MPs). They were carried out during days 7–8 (MP 1), 15–16 (MP 2), and 29–30 (MP 3) after the implementation of CR. SPA was measured using passive infrared sensors (TL-xpress; Crow Electronics Engineering Inc., Fort Lee, NJ, USA) installed over each cage and monitored every 1 s by a computer (PCL-711 analog-digital interface; Advantech, Cincinnati, OH, USA). All disruptive activities related to human presence in a chamber (feeding CR mice, switching sensors between blocks of mice, body weight measurements) occurred between 08:00 and 10:00 am and these data were excluded from our dataset (these activities occurred only during the first day of MP, but we excluded these data from both days for consistency).

We calculated three variables quantifying different aspects of SPA (analogously to a recent study by Copes et al. [30]): (a) total SPA, i.e., the logical sum of signals calculated over 3-s periods (the same variable was used in an earlier study on the same mouse lines; [26]); (b) duration of SPA, calculated as the number of 1-min intervals during which any SPA was registered; (c) the average amount of activity per minute when any home-cage activity was occurring (subsequently referred to as SPA intensity), calculated as total SPA divided by the duration of SPA. Thus, total SPA represented all energy spent on SPA; that is, the product of the duration of the active phase (quantified by SPA duration) and the intensity of movements during the active phase (quantified by SPA intensity; both SPA duration and SPA intensity were shown to be significant predictors of intra-specific variation of food intake in Swiss mice; [30]). We calculated the total SPA and its