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Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice

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HIGHLIGHTS

- Male mice were housed in standard cages or with wheels for 3 weeks after weaning.
- All mice then experienced a sedentary phase for two months.
- Early-life wheel access increased adult voluntary exercise but not cage activity.
- The effect on plasma leptin concentrations depended on genetic background.
- Results are relevant for the importance of physical education for children.

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ABSTRACT

The purpose of this study was to evaluate the effects of early-life exercise on adult physical activity (wheel running, home-cage activity), body mass, food consumption, and circulating leptin levels in males from four replicate lines of mice selectively bred for high voluntary wheel running (High Runner or HR) and their four non-selected control (C) lines. Half of the mice were given wheel access shortly after weaning for three consecutive weeks. Wheel access was then removed for 52 days, followed by two weeks of adult wheel access for all mice. A blood sample taken prior to adult wheel testing was analyzed for circulating leptin concentration. Early-life wheel access significantly increased adult voluntary exercise on wheels during the first week of the second period of wheel access, for both HR and C mice, and HR ran more than C mice. During this same time period, activity in the home cages was not affected by early-age wheel access, and did not differ statistically between HR and C mice. Throughout the study, all mice with early wheel access had lower body masses than their sedentary counterparts, and HR mice had lower body masses than C mice. With wheel access, HR mice also ate significantly more than C mice. Early-life wheel access increased plasma leptin levels (adjusted statistically for fat-pad mass as a covariate) in C mice, but decreased them in HR mice. At sacrifice, early-life exercise had no statistically significant effects on visceral fat pad, heart (ventricle), liver or spleen masses (all adjusted statistically for variation in body mass). Results support the hypothesis that early-age exercise in mice can have at least transitory positive effects on adult levels of voluntary exercise, in addition to reducing body mass, and may be relevant for the public policy debates concerning the importance of physical education for children.

1. Introduction

Human obesity and its negative health consequences are caused by interactions among diet, level of physical activity, environmental factors, sex, genetic predisposition, and socio-cultural factors [1–9]. Like obesity itself, levels of physical activity and diet/caloric intake are products of both genes and numerous environmental effects acting across ontogenetic development. Some human studies have identified early-life risk factors for a sedentary lifestyle (e.g., [10,7]) and parental characteristics that are somewhat predictive of adolescent physical activity [11]. Overall, however, early-life environmental determinants of adult physical activity levels are poorly understood [12–20].

Given that exercise is a fundamental tool in metabolic health and the control of body weight, an essential line of questioning pertains to understanding its regulation and programming ([21,22]). The dramatic increase of the prevalence of obesity [23] heightens the need for new insights into mechanisms that govern energy balance and voluntary activity levels. This is especially critical because recovery from long-term

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obesity is particularly difficult due to an elevated defended body weight. Consistent with this concept, both juvenile obesity and diabetes tend to persist into adulthood, rendering such preventative measures as early exercise exposure a potentially useful option with long-term, beneficial effects [24]. Accumulating evidence suggests that early-life experiences can alter adult levels of voluntary exercise (VE) and/or spontaneous physical activity (SPA). For example, in a prospective birth-cohort study using accelerometers, parents' physical activity during pregnancy and early in the child's life showed a modest positive association with the child's physical activity at 11–12 years of age [11]. Less direct evidence comes from studies of individuals exposed to famine. Those exposed to the Dutch famine during gestation have increased adiposity and more atherogenic lipid profiles in later life that may be related to decreased physical activity [25,7]. Individuals exposed to the Chinese famine during fetal life or infancy have an increased risk of metabolic syndrome in adulthood [20]. However, as these sorts of studies are not experimental (no interventions applied), it is difficult to identify causal relationships.

Animal models are widely used for studies of early-life effects because they allow manipulations that would be neither feasible nor ethical in humans [26]. A few rodent studies demonstrate that juvenile exercise can affect adult activity levels. In male rats, 3 weeks of wheel access begun at 36 days of age reduced weight gain over the next 10 weeks [27]. Three weeks of post-weaning exercise in leptin-resistant rats bred to develop diet-induced obesity caused a sustained resistance to obesity on high-fat diet, in part due to increased central leptin sensitivity [28]. In rats genetically prone to early-onset, hyperphagia-induced obesity, post-weaning voluntary exercise for ~3 weeks caused long-term moderation of adiposity in males but not females [29].

In the present study we examined the effects of early-life wheel access on adult physical activity in a unique, genetically defined animal model, four selectively bred High Runner (HR) mouse lines and their four non-selected Control (C) lines [30–32]. The HR and C lines differ markedly in both voluntary wheel-running behavior [32] and baseline activity in the home cage when wheels are absent [33,34], which also allows tests for genotype-by-environment interactions. Previous studies of a subset of these lines have identified quantitative trait loci (QTL) that influence voluntary wheel running and body composition (e.g. [35–39]), but the importance of early-life environmental factors is unknown. Furthermore, using lines of mice with a wide range of running distances allows for assessment of possible threshold effects, or plateaus in benefit. Additionally, use of multiple genetic strains to some extent better mimics human ethnic and racial diversity in proneness to physical activity, obesity, metabolic syndrome, and related diseases (e.g., see [40–42]).

Based on associations observed in humans (e.g., [40]) several differences between HR and C mice suggest that they are likely to respond differently to early-life factors. For example, as compared with C, HR mice have higher $\dot{V}O_2$max and endurance during forced exercise, an altered brain reward system, elevated baseline circulating corticosterone levels (for possible relevance, see [43]), and increased depressive-like behaviors when wheel-deprived [44–47,33,48,49]. Moreover, previous studies document significant genotype-by-environment interactions in adult HR vs. C mice challenged with Western diet and housed with versus without wheels ([50–52,48]).

2. Materials & procedures

2.1. Experimental animals

Mice were from an artificial selection experiment that breeds for high voluntary wheel running activity [32]. Briefly, the base population was 224 outbred, genetically variable Hsd:ICR house mice. After two generations of random mating in our animal facility, 10 pairs of mice were used to create each of eight closed lines, four of which were randomly designated and bred for high running (HR) on wheels and the other four were the control (C) lines bred without regard to wheel running. During the normal selection experiment process, approximately 6–8 week old mice are individually housed in standard cages attached to a Wahman-type activity wheel (1.12 m circumference, 35.7 cm diameter, 10 cm wide running surface). Wheels are interfaced to a computer, which records revolutions in 1-minute intervals continuously for 6 days of wheel testing. Breeders for the next generation are chosen based on their wheel running on days 5 and 6. Within-family selection is applied. For the HR lines, the highest-running male and female within each family are chosen as breeders, whereas a random male and female are chosen from each family within the C lines (disallowing sib mating in all lines). Room temperatures are maintained at approximately 22 °C. Lights were on at 0700 with a 12:12 photoperiod. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604) are available ad libitum. Pregnant dams are given a breeder diet (Harlan Teklad Mouse Breeder Diet [S-2335] 7004) through weaning.

2.2. Early-life wheel access

Males from generation 59 were weaned at 21 days of age and housed individually in standard cages (total N = 98). Half of the experimental mice were allowed wheel access when they were approximately 24 ± 1 days old for a total of 21 days. The other subset of mice remained with their wheel access blocked and were designated as the young sedentary group. All mice had their home-cage activity (HCA, also referred to as spontaneous physical activity) monitored (see below). In addition, all mice had their food consumption and body mass monitored weekly throughout the experimental period.

2.3. Adult wheel testing

Wheels were removed after 3 weeks and all individuals remained in standard home cages for an additional 7^+ weeks (52 days). Following this sedentary phase, all mice were then granted wheel access for 2^+ weeks (16 days), with continued monitoring of home-cage activity, food consumption, and body mass.

2.4. Home-cage activity

Home-cage activity (HCA) was monitored using passive infrared motion-detector sensors [34] similar to [53] that detect movement within the standard housing cages attached to the wheels. Sensors were interfaced to a Macintosh personal computer that had custom Activity Recording Software developed by Dr. Mark A. Chappell. The software measured activity summed over every 1-minute interval for 23 h. The computer records 3 times per second and reads if there is movement (1) or no movement (0). Recordings are then averaged over 1-minute intervals and given values with arbitrary units. Total activity values in each 1-minute interval were summed to get total HCA for the entire daily period. The number of 1-minute intervals that show *any* HCA was also computed and tallied to indicate the duration (minutes) of HCA during the entire daily period. Dividing daily activity by the number of 1-minute intervals with any activity then gives an indication of the average intensity of activity when active. We also determined the single minute with the highest amount of HCA. All of these HCA measures had direct parallels from the wheel-data analyses [34]. Data for both HCA and wheel running were downloaded daily between 1200 and 1300 h.

2.5. Blood sampling, leptin assay, dissections

Prior to the second wheel-testing period, mice were anesthetized with isoflurane and bled through the retroorbital sinus [54]. Blood samples were spun at 13,000 RPM for 12 min and collected plasma was stored at −20 °C. Plasma leptin was measured using a Millipore Enzyme-linked Immunosorbent Assay (ELISA) kit (Mouse Leptin Assay