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Reduced metabolic disease risk profile by voluntary wheel running accompanying juvenile Western diet in rats bred for high and low voluntary exercise

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HIGHLIGHTS

- We have developed a model of high (HVR) and low (LVR) voluntary running rats.
- We assessed genetic and lifestyle factors influencing metabolic risk in HVR and LVR.
- Body fat % is not different in HVR and LVR despite a 5–7 fold running difference.
- HVR consume more food than LVR independent of wheel running and Western diet.
- Running and Western diet differentially influence hypothalamic mRNAs in HVR and LVR.

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ABSTRACT

Metabolic disease risk is influenced by genetics and modifiable factors, such as physical activity and diet. Beginning at 6 weeks of age, rats selectively bred for high (HVR) versus low voluntary running distance (LVR) behaviors were housed in a complex design with or without voluntary running wheels being fed either a standard or Western (WD, 42% kcal from fat and added sucrose) diet for 8 weeks. Upon intervention completion, percent body fat, leptin, insulin, and mediobasal hypothalamic mRNAs related to appetite control were assessed. Wheel access led to differences in body weight, food intake, and serum leptin and insulin. Intriguingly, percent body fat, leptin, and insulin did not differ between HVR and LVR lines in response to the two levels of voluntary running, regardless of diet, after the 8 wk. experiment despite HVR eating more calories than LVR regardless of diet and voluntarily running 5–7 times further in wheels than LVR. In response to WD, we observed increases in Cart and Lepr mediobasal hypothalamic mRNA in HVR, but no differences in LVR. Npy mRNA was intrinsically greater in LVR than HVR, while wheel access led to greater Pomc and Cart mRNA in LVR versus HVR. These data suggest that despite greater consumption of WD, HVR animals respond similarly to WD as LVR as a result, in part, of their increased wheel running behavior. Furthermore, high physical activity in HVR may offset the deleterious effects of a WD on adiposity despite greater energy intake in this group.

1. Introduction

The prevalence of childhood obesity has increased in the past several decades [1] and ~70% of obese adolescents become obese adults [2,3], and are at risk for its associated co-morbidities [4], such as cardiovascular disease (CVD) and type II diabetes. One potential approach to counteract childhood obesity is to increase physical activity, which is associated with decreased risk factors of CVD such as insulin resistance, hypertension, and adiposity [5]. However, accelerometer data suggests that ~60% of children and ~90% of adolescents fail to achieve recommended levels of daily physical activity [6]. Furthermore, lifetime physical inactivity accelerates secondary aging and is associated with increased risk of chronic disease and reduced lifespan [7].

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We have developed a rat model selectively bred for high vs. low voluntary wheel running (HVR and LVR, respectively). After 9 generations (G), wheel running was 8.5 and 11.0-fold greater in HVR than LVR for males and females, respectively [8]. Furthermore, both HVR and LVR have diverged from outbred rats, with G9 HVR running 1.4-fold greater and G9 LVR running 6.9-fold less than the founding population [8]. While numerous changes in mesolimbic gene expression and function differences between HVR and LVR have been reported [8–11], no differences in body weight, body fat, food intake, or muscle phenotype were observed through G9 [8]. However, by G12 HVR rats paradoxically had lower body weight compared to LVR despite consuming the same amount of calories when sedentary and more when given voluntary running wheels [11].

While physical activity may help prevent obesity, diet and genetic factors may affect obesity development. Selective breeding for either resistance or sensitivity to diet-induced obesity (DIO) [12–14] suggest that polygenic patterns of inheritance may afford protection from obesity. Alternatively, selective breeding for rats with high intrinsic aerobic capacity provides protection from DIO [15] and from metabolic and cardiovascular complications [16]. Commenting on selected lines of mice for high distances of voluntary wheel running, Garland et al. (See [17–21]) state that high "activity-selected lines of mice…have revealed alterations in brain function that seem to indicate changes in motivation or propensity to exercise on wheels." Garland's group has reported that mice selected for high wheel running display co-selected phenotypes of increased cage activity [22], lower body fat [23], and varied responses to DIO [24] when compared to non-selected control lines. However, similar comparisons with LVR have not been made between rodents bred for HVR and LVR.

Given the high percentage of childhood obesity persistence into adulthood [2,3] and evidence for gene-by-environment interactions influencing childhood obesity development [25], we sought to determine if various genetic and environmental factors influenced the development of obesity in our HVR and LVR rats. In the present study, we addressed the following questions: 1) do either HVR or LVR display resistance to any of the negative effects (increased fat mass, insulin and/or leptin levels) of a Western diet (WD)?; and 2) if so, are these differences preferentially due to differential expression of mediobasal hypothalamic mRNAs related to body weight regulation or voluntary physical activity-related factors?

2. Materials and methods

2.1. Experimental animals

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. Female rats from the 11th–12th generations (G) of an artificial selection process for high and low voluntary wheel running behavior were used in this experiment. The selective breeding process used to generate HVR and LVR rats has been previously described [8,26]. In short, the founding population consisted of outbred Wistar rats (Charles River Raleigh, Raleigh, NC). Thirteen families were bred for HVR and LVR. In each generation rats are provided access to running wheels from 28 to 34 days of age. Within each HVR and LVR family, the highest (HVR) and lowest (LVR) running male and female are chosen as breeders based upon wheel running distance during nights 5 and 6 of the selection period.

In the present study, female G 11-12 HVR and LVR rats were weaned at 21 days of age and group housed until the beginning of the experiment. Female rats were employed for this study due to the fact that females typically run further than males [27,28]. Further rationale for usage of female rats is that their body mass plateaus, minimizing the effect of continued body mass growth in male rats, and our usage of female rats balances the predominance of male rodents in the literature. However, the voluntary wheel running rhythm of female rats varies with their 4-day estrous cycle (i.e., running distance peak every 4th night), with peak running occurring at proestrus [29]. Animals were fed Purina Formulab Diet 5008 during this time. Rats were maintained in a 12:12-h light/dark cycle at 21–22 °C, and food and water were provided ad libitum throughout the entire experiment.

2.2. Experimental design

At 5 weeks of age (35–37 days of age) (Pre-intervention week), HVR and LVR rats were single housed and randomly provided either access to voluntary running wheels (circumference = 1.08 m; Tecniplast 2154F0105, Tecniplast, Italy) or remained sedentary and were fed a normal diet (ND) (Formluab Diet 5008, Purina, 16% kilocalories (kcal) from fat). This randomization was done to ensure no initial within-line differences in wheel running would be present after further grouping by diet. At 6 weeks of age, rats within each line previously randomized into wheel running and sedentary treatments were further divided and provided either a ND or a Western diet (WD) (Harlan Teklad TD.88137 WD, 42% kcal from fat and the final weight including 341.46 g/kg from sucrose). These divisions resulted in groups composed of: a) those that were sedentary and maintained on a ND (HVR-NDsed, LVR-NDsed (n = 8 per group)); b) those that were sedentary but were fed a WD (HVR-WDsed (n = 8), LVR-WDsed (n = 8)), c) those that had wheel access and maintained a ND (HVR-NDrun (n = 8), LVR-NDrun (n = 8)), and d) those that had wheel access and fed a WD (HVR-WDrun (n = 8), LVR-WDrun (n = 8)). These treatments were continued for 8 weeks. Voluntary wheel running distance (km) and time (min) were recorded every 24-h using Sigma Sport BC 800 bicycle computers (Cherry Creek Cyclery, Foster Falls, VA). Body weight and food consumption were measured weekly. Food consumption was measured as the difference in hopper mass between successive weeks. Due to differences in the mass-specific energy content between the ND and WD, we converted food consumption from grams to energy intake using 3.3 kcal/g and 4.5 kcal/g for the ND and WD, respectively.

After the 8-week treatment (rats 98–101 days of age), rats were fasted for 8-h and sacrificed between 1700 and 1900, up to two hours prior to the dark cycle, with carbon dioxide asphyxiation. Rats were sacrificed at the proestrus stage of the estrous cycle, as determined by vaginal cytology, to minimize the effect of cycling sex hormones.

2.3. Body composition phenotyping

During the week of sacrifice, all rats were anesthetized with isoflurane inhalation and whole body composition was measured using a Hologic QDR-1000/w dual-energy X-ray absorptiometry machine calibrated for rats.

2.4. Serum leptin and insulin measurements

At the time of sacrifice, whole blood samples were removed via heart stick using a 21-gauge needle and syringe, placed in a serum separator tube, centrifuged at 1300 ×g for 10 min, and aliquoted into 1.7 ml microcentrifuge tubes and stored at −20 °C until further use. Serum (dilution 1/10) from fasted animals were assayed in duplicate for the concentration of leptin using a mouse/rat leptin quantitative ELISA assay (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Insulin levels were measured using a rat-specific insulin ELISA (ALPCO) according to the supplier's instructions. To avoid adding error in our molecular measurements, fasting serum measurements were used to prevent potential effects on hypothalamic neuropeptide expression and other neuroendocrine pathways that regulate energy homeostasis [30]. The intra-assay coefficients of variation (CV) for leptin and insulin assays were 2.9 and 5.9%, respectively, and the inter-assay CV for leptin and insulin assays were 2.5 and 4.8%, respectively.