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Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity

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ABSTRACT

Though obesity is common, some people remain resistant to weight gain even in an obesogenic environment. The propensity to remain lean may be partly associated with high endurance capacity along with high spontaneous physical activity and the energy expenditure of activity, called non-exercise activity thermogenesis (NEAT). Previous studies have shown that high-capacity running rats (HCR) are lean compared to low-capacity runners (LCR), which are susceptible to cardiovascular disease and metabolic syndrome. Here, we examine the effect of diet on spontaneous activity and NEAT, as well as potential mechanisms underlying these traits, in rats selectively bred for high or low intrinsic aerobic endurance capacity. Compared to LCR, HCR were resistant to the sizeable increases in body mass and fat mass induced by a high-fat diet; HCR also had lower levels of circulating leptin. HCR were consistently more active than LCR, and had lower fuel economy of activity, regardless of diet. Nonetheless, both HCR and LCR showed a similar decrease in daily activity levels after high-fat feeding, as well as decreases in hypothalamic orexin-A content. The HCR were more sensitive to the NEAT-activating effects of intra-paraventricular orexin-A compared to LCR, especially after high-fat feeding. Lastly, levels of cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) in the skeletal muscle of HCR were consistently higher than LCR, and the high-fat diet decreased skeletal muscle PEPCK-C in both groups of rats. Differences in muscle PEPCK were not secondary to the differing amount of activity. This suggests the possibility that intrinsic differences in physical activity levels may originate at the level of the skeletal muscle, which could alter brain responsiveness to neuropeptides and other factors that regulate spontaneous daily activity and NEAT.

Introduction

Obesity continues to increase worldwide (Prentice, 2006; Wyatt et al., 2006), along with the health problems associated with it (Wyatt et al., 2006). Over the past several decades, physical activity levels in Western populations have steadily decreased at the same time as obesity has increased (Kruger et al., 2007a; Kruger et al., 2007b; Livingstone et al., 1991). This trend exists despite the plethora of treatments and programs available for body weight management, most of which focus on the roles of diet and exercise. The importance of everyday physical activity to total daily energy expenditure (TDEE) is increasingly being recognized. The energy spent while engaging in activities of daily living, called non-exercise activity thermogenesis (NEAT), can have a significant contribution to TDEE, energy balance, body weight, and health (Church et al., 2007; Dauncey, 1990; Johannsen et al., 2008; Levine et al., 1999; Levine et al., 2005), and differences in physical activity can contribute to obesity propensity and weight gain (Mustelin et al., 2009). There is a large amount of inter-individual variation in the amount of activity people engage in as well as their NEAT, and these correlate with leanness, health, and fitness (Hamilton et al., 2007; Levine et al., 1999; Levine et al., 2005). Moreover, changes in diet composition or caloric intake can alter NEAT (Bjursell et al., 2008; Novak et al., 2006a), and the ability to fend off weight gain in the face of increased caloric intake also varies between individuals (Levine et al., 1999).

Although social, cognitive, and environmental factors can surely impact physical activity levels, the innate, biological influences may be equally important. Studies examining physical activity levels in twins revealed a highly heritable component (Joosen et al., 2005; Kaprio et al., 1981). As in humans, there is a large amount of inter-individual variation in physical activity levels in rats, and lower levels of physical activity are associated with higher body weight and

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obesity (Dauncey, 1986; Dauncey and Brown, 1987; Novak et al., 2006a; Teske et al., 2006a; Tou and Wade, 2002). Previously, we have shown that rats selectively bred for diet-induced obesity (DIO) are less physically active than their diet-resistant (DR) counterparts after obesity induced by a high-fat diet (Novak et al., 2006a). Investigating energy balance in selectively-bred animals may yield insights into the neural and endocrine underpinnings of obesity and physical activity that are relevant to human physiology. Another interesting and highly relevant animal model that contrasts for metabolic disease risk has been developed by selectively breeding rats for high and low intrinsic aerobic capacity measured by distance run to fatigue on a treadmill test. The high-capacity runners (HCR) remain lean on a calorie-dense diet (Britton and Koch, 2001; Noland et al., 2007; Wisloff et al., 2005). The low-capacity runners (LCR), on the other hand, readily develop insulin resistance and symptoms of cardiovascular disease (Wisloff et al., 2005). Though these rats were not bred to be lean or obese *per se*, the HCR/LCR rats are a highly useful model of leanness vs. obesity (Wisloff et al., 2005). Recently, we have demonstrated that HCR are consistently more active than LCR, and that this is not secondary to body mass (Novak et al., 2009). HCR also engage in more voluntary activity on a running wheel (Burghardt et al., 2006; Waters et al., 2008), though this type of activity is much more variable between rats, specifically between HCR rats (Burghardt et al., 2006). Thus, high daily activity levels seem to be an inherent part of the lean, high-intrinsic-endurance phenotype (Novak et al., 2009). Moreover, the link between innate endurance capacity and high spontaneous activity appears to extend to humans (Novak et al., 2009).

Alterations in diet also affect NEAT (Bjursell et al., 2008; Levine et al., 1999; Rosenbaum et al., 2003). In fact, access to a high-fat Western diet can suppress activity levels within only a few days (Bjursell et al., 2008). Long-term, obesity-prone DIO rats show a decrease in their daily activity levels when on a high-fat diet, whereas their diet-resistant counterparts do not (Novak et al., 2006a). It is possible that the lean, highly active phenotype is also resistant to diet-induced suppression of physical activity levels. Using rats artificially selected for high and low intrinsic aerobic capacity, we first sought to determine whether individual differences in activity can be modulated by high-fat feeding by comparing spontaneous physical activity and energy expenditure before and after one month on a high-fat diet. Energy expenditure of activity was also measured directly while rats walked on a treadmill, allowing for the dissection of this component of energy expenditure.

Several central neuropeptides and peripheral circulating hormones affect physical activity and NEAT (Kotz et al., 2008; Novak et al., 2006a; Novak and Levine, 2007; Novak et al., 2006b; Teske et al., 2008). What is not known, though, is which of these factors might help explain the individual variations in activity levels noted in those with tendencies for obesity or leanness. Our next objective was to identify biological factors that could potentially underlie the high and low activity levels seen in association with obesity propensity. First, we started by examining peripheral and circulating factors that are both altered in obesity and associated with altered levels of physical activity, including adiponectin (Bjursell et al., 2008), leptin (Choi et al., 2008), and corticosterone (Cador et al., 1993; Veldhuis et al., 1982). Second, we tested the potential role of the enzyme cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) in skeletal muscle. In mice, genetically enhancing PEPCK-C in skeletal muscle results in a lean, active, high-endurance mouse (Hakimi et al., 2007; Hanson and Hakimi, 2008). We have previously demonstrated that the lean HCR have heightened skeletal muscle PEPCK-C levels and enzymatic activity (Novak et al., 2009), implicating this enzyme as an important factor in the lean, active, high-endurance phenotype.

Third, we investigated potential differences within the brain that may account for the differential levels of physical activity between HCR and LCR (Novak et al., 2009). Others and we have previously demonstrated a potent effect of orexin-A on physical activity, as well as differences in brain orexin associated with obesity and obesity propensity (Kotz et al., 2006; Novak et al., 2006a; Teske et al., 2006a). More recently, alterations in the *Snark* gene have been found to result in alterations in wheel running in mice (Ichinoseki-Sekine et al., 2009), making this gene an interesting potential candidate mechanism for intrinsic differences in spontaneous physical activity as well. To determine if any of these variables were likely to contribute to the intrinsic differences or diet-related changes in physical activity in these rats, we investigated the effects of phenotype (high and low intrinsic aerobic capacity) in conjunction with diet on physical activity, energy expenditure, and weight gain, as well as potential mediators including central, circulating, and peripheral factors.

Methods

The data described here were generated from four separate studies: (1) Physical activity and energy expenditure (24-hour and acute activity after orexin-A) were measured in male HCR and LCR before and after one month on a high-fat diet (rats approximately 7 months old). (2) To measure the energy expenditure during resting and activity more precisely, calorimetry was conducted while female rats rested and walked on a treadmill. (3) Body weight and food intake were measured in four groups: HCR and LCR on regular and high-fat diets (rats approximately 4 months old). (4) To determine whether the difference in physical activity levels affects skeletal muscle PEPCK-C levels, we compared female HCR and LCR in standard calorimetry acclimation housing with housing of reduced size. Females were specifically chosen for the final studies because female HCR and LCR do not have a large magnitude of difference in body weight and size between groups as males do. In addition, we previously confirmed that female HCR and LCR are nearly identical to males in their behavioral phenotype (Novak et al., 2009); for this reason, the use of female rats was justified in this study. The reproductive cycle of these female rats, however, was not monitored nor accounted for. All studies and procedures were approved by the Mayo Institutional Animal Care and Use Committee. Rats, selectively bred at the University of Michigan, were air shipped to the Mayo Clinic animal facility at 4–6 mo of age. After arrival at the facility, rats were housed on a 12:12 light:dark cycle, without forced treadmill running or access to running wheels, and given food and water *ad lib* unless otherwise noted.

Daily physical activity

Male rats (10 HCR and 10 LCR, generation 20) were individually housed on a 24-hour light:dark cycle and allowed *ad lib* access to standard chow (Lab Diet 5001) and tap water. Each rat underwent four phases of the study protocol: After implantation of a guide cannula aimed at the paraventricular nucleus of the hypothalamus (PVN), we measured (1) 2-hour orexin-A-induced physical activity and energy expenditure, and (2) 24-hour spontaneous daily activity; (3) after 29 days on a high-fat diet (Research Diets D12492, 60% calories from fat), we measured 24-hour spontaneous daily activity; and (4) 2-hour orexin-A-induced activity and energy expenditure was measured a second time. In this way, we could compare spontaneous daily activity with sensitivity to the NEAT-activating effects of orexin-A both before and after one month on the high-fat diet. The high-fat diet contained 5.24 kcal/g as determined by Atwater values, with 20% kcal from each protein and carbohydrate. The standard laboratory rodent diet contained 28% kcal from protein, 12% from fat, and 60% from carbohydrate, with 3.36 kcal/g as calculated from Atwater values.

Measurement of spontaneous physical activity and energy expenditure

Rats were acclimated to the calorimetry room and a simulated calorimetry chamber for at least 24 h prior to measurement. For each