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2016

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UNIVERSITY OF CALIFORNIA
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Effects of Western Diet and Early-Life Exercise Opportunity on Voluntary Exercise and
Spontaneous Physical Activity in Mice Bred for Voluntary Wheel Running

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Wendy Acosta

March 2016

Dissertation Committee:

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The Dissertation of Wendy Acosta is approved:

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ACKNOWLEDGEMENTS

This dissertation is made possible with the help of many. I would not be here if it was not for the endless help of the following:

Contrary to most of my department's beliefs, I would like to thank my God who has made all this possible. Without Him, I would not be here. I would also like to thank my dissertation committee: Dr. Theodore Garland, Jr. (chair), Dr. Mark Chappell, Dr. Wendy Saltzman, and Dr. Richard Cardullo. Thank you for all of your patience and support throughout my lengthy stay. Ted, thank you for always pushing my limits and helping me reach this, once thought, unreachable goal.

Thank you to Maria Franco and Melissa Gomez. Comadre, you have been such a positive figure in my life. Thank you for being so strong, resilient and showing me, I can do it. Thank you for picking me up when I was down, helping me laugh through the good times and bad. I thank you for being an awesome role model. Melissa, I thank you for being there for me and knowing the answers to all of my questions. Thank you for sharing some laughs and all your compassion. Thank you to the entire Graduate Division for my temporary employment.

Thank you to all my fellow Garland lab members (past and present): Tom Meek, Liz Dlugosz, Heidi Schutz, Erik Kolb, Rob Hannon, Gerald Claghorn, Ralph Albuquerque, Layla Hiramatsu, Jennifer Singleton, Zoe Thompson, Marcell Cadney, Jarren Kay. We endured together! :) Thank you for your friendship and your help. Thank you to all of the undergraduate students who have helped us endlessly through many hours of mouse duty.

I do not know how to thank my mother and father for all that they have done. I do not know what I would do without you both. I love you both for all the sacrifices and all the love you have shown me. You have given me undeniable support in everything and have taught me how to try to be an excellent parent to my bundle of joy: my boy, Nathan. I thank you, baby boy, too for your existence, which adds so much more meaning to my life and gave me that extra motivation to get this done.

Thank you to my sisters: Yolie and Christina and to my brothers: David, Jesse, and Albert. Thank you for making me tough and encouraging me to keep going. Thank you to my nieces and nephews, who also cheered me on through my schooling. Last, but not least, thanks to the father of my son, Josh. Babe, thank you for pushing me forward and picking me up when I was ready to give up. Thank you for giving me my joy, my baby boy, Nathan Daniel Barkan.

Chapter 2 of this dissertation has been published in the *Physiology & Behavior* journal and is adopted with permission from Elsevier (full citation below):

Acosta, W., T. H. Meek, H. Schutz, E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2015.

Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice. *Physiol Behav* 149:279-286.

ABSTRACT OF THE DISSERTATION

Effects of Western Diet and Early-Life Exercise Opportunity on Voluntary Exercise and Spontaneous Physical Activity in Mice Bred for Voluntary Wheel Running

by

Wendy Acosta

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, March 2016
Dr. Theodore Garland, Jr., Chairperson

Elucidating genetic and environmental factors that affect physical activity, dietary choices, and their interactions is essential for attempts to ameliorate the obesity epidemic. Using mice selectively bred for high voluntary wheel-running behavior (four replicate HR lines) and their four non-selected control (C) lines, I investigated early-life effects on adult physical activity, preference for Western diet (WD), and whole-animal metabolic rate and fuel usage with WD.

Early-life access to wheels increased adult wheel running but had no statistical effect on spontaneous physical activity (SPA) of adult males in their attached home cages. The early-exercise effect on wheel running disappeared after one week, but body mass was reduced throughout the experiment. Early-life exercise reduced circulating leptin concentrations in HR lines, but increase them in C lines (genotype-by-environment interaction).

Both HR and C mice highly preferred WD, high in fat and sucrose, over standard chow. After 17 days of wheel acclimation, but not after six days, HR had a stronger preference for WD than did C mice, which further increased their wheel running and decreased their SPA. When a separate set of mice was switched from standard chow to WD, the effects on wheel running depended on both sex and linetype in a complicated fashion.

Fuel usage was studied indirectly by whole-animal respirometry. Female HR and C mice did not differ statistically in minimal resting metabolic rate, but the former had higher levels of maximal oxygen consumption during voluntary wheel running. Contrary to my hypothesis, I found no evidence for a reduced respiratory exchange ratio (which would indicate greater reliance on lipids) in HR mice at rest, during maximal voluntary exercise or measured over a 23-hour period, either on standard chow or with WD.

Overall, my research provides evidence for important, genetically based differences between the HR and C lines, sex differences, and sex-by-linetype interactions. These results encourage use of the HR mice as an anti-obesity model in further studies of the complex physiological and neurobiological mechanisms that interact with sex and genetic background in ways that may allow some individuals to resist the adverse effects of obesogenic environments.

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Dissertation Introduction

Broader Impacts: Obesity

Obesity is an increasingly prevalent disorder in industrialized societies. More than 68% of the adult U.S. population is overweight or obese, meaning they have a body mass index (BMI) greater than 25 ((Flegal et al. 2010a; U.S. National Library of Medicine 2012)). Obesity amongst children is also a growing epidemic, with almost 20% of 2-19 year olds in the U.S. being obese (Ford et al. 2008; Flegal et al. 2010b; Ogden CL 2010). Obese children have an 85% likelihood of becoming obese adults (Kiehl et al. 2001). All obese individuals have an increased risk of diabetes, hypertension, cholesterol, atherosclerosis, and additional cardiovascular diseases (Kiehl et al. 2001; Hill et al. 2012).

Role of Exercise

The two most commonly advanced reasons for the increase in obesity over recent decades are reductions in physical activity and consumption of high-calorie diets (McAllister et al. 2009). Thus, understanding relationships among activity, food consumption, energy balance, and the regulation of body weight are critical for the battle against obesity. Numerous studies identify certain environmental and socio-cultural factors, including physical inactivity and "Western" diets, as key contributors to increasing obesity and its comorbidities in adults, adolescents, and even children (Steinberger et al. 2009; Flegal et al. 2010b; Halpern et al. 2010). Conversely, a "good" diet (e.g., relatively low in fat and sugar) and higher levels of physical activity promote

physical fitness while lowering the risk for obesity, diabetes, and their comorbidities (Blair and Morris 2009); (Hills et al. 2011); (Ross and McGuire 2011).

Importantly, not only exercise *per se*, but also incidental or spontaneous physical activity (SPA) can have beneficial effects in humans and animal models (e.g., (Levine 2007); (Novak et al. 2009); (Ross and McGuire 2011). Spontaneous physical activity is defined as any activity not encompassed within voluntary exercise, such as fidgeting, grooming or walking to the bathroom, among many other actions (Garland T et al. 2011). As mentioned in (Copes et al. 2015), spontaneous physical activity can be measured inside rodent home cages using photobeams, force plates, passive infrared sensors, video recording or implanted transmitters. The amount of activity inside their home-cage, or SPA, may be negatively related to voluntary exercise if there were temporal or energy constraints (e.g., if the amount of time needed for sleep and other maintenance functions is relatively fixed, then more time spent exercising voluntarily may allow less time for moving around in the cage). Alternatively, if the overall level of physical activity is regulated (the activity-stat hypothesis of (Rowland 1998); see also (Eisenmann and Wickel 2009))), then increases in exercise should lead to reductions in SPA.

Many comparative and evolutionary physiologists have argued for the importance of locomotion, locomotor performance, and/or exercise in animal evolution (e.g., see review and references in (Ekkekakis 2005)). Locomotion and physical performance make survival and reproductive success possible through foraging, outrunning predators, and finding mates. Moreover, locomotion or physical exercise has been identified as one of the key factors promoting health and well being (Flegal et al. 2010a). Physical activity

is consistently associated with reduced all-cause mortality. Increasing physical fitness can reduce the risk of death by 12% over a period of 19 years increasing exercise capacity by one metabolic equivalent (Myers et al. 2002). Physical fitness and mortality are inversely associated in many epidemiological studies in healthy subjects; the higher the exercise capacity among older men, the lower the mortality (Kokkinos et al. 2010) (Flegal et al. 2010a), (Blair and Morris 2009).

Plenty of evidence suggests that voluntary exercise decreases body fat (Westerterp and Goran 1997; Goodpaster et al. 2003; McClelland 2004; Mustelin et al. 2009). Decreased body fat and increased exercise improve human blood lipid profiles (Gauthier et al. 2004) and lower blood pressure (Mundal et al. 1998). In addition, exercise improves cardiovascular physical fitness, often measured by the maximal rate of oxygen consumption during a graded treadmill test (Booth and Roberts 2008). In turn, higher levels of physical fitness have been shown to reduce all-cause mortality, regardless of status as overweight or obese (Blair and Morris 2009). Despite all the benefits of physical exercise, most Americans do not get enough exercise (Centers for Disease Control and Prevention National Center for Chronic Disease Prevention and Health Promotion 1996; U.S. Department of Health and Human Services 2002; Troiano et al. 2008).

Genetic, social, and environmental factors influence behaviors, physical fitness, and health outcomes. Of these, the most feasible to manipulate are environmental factors that can alter behavior so that physical fitness and health are benefited. Behavior that promotes physical activity is highly recommended to prevent/reduce incidences of

cardiovascular diseases, obesity, type 2 diabetes, stroke, pulmonary diseases (chronic obstructive pulmonary disease and asthma), and several cancers (colon, pancreatic, breast, and prostate) (Booth et al. 2002).

Obesity and resultant cardiovascular diseases affect both sexes and it is important to study both, but studies of females are uncommon for both humans and mice. In biomedical research, females are the main subjects only 37% of the time (Kim et al. 2010). Studies of both sexes are important because men and women show different symptoms and progressions for many common diseases, may require different dosages of medications, and also often respond differently to a given type of treatment. For example, males and females show different symptoms during heart attacks (Mosca L et al. 2007). The typical symptom described for a myocardial infarction is chest pain, and this is common in males, but females also may feel unusual fatigue, sleep disturbance, shortness of breath, indigestion, and anxiety in addition to the other general symptoms (McSweeney et al. 2003).

Benefits of physical activity are also demonstrated in adult rodent studies. In a study by (Simi et al. 1991), they demonstrated increased oxygen capacity with endurance training and high-fat diet. Other rodent studies established the positive training effects at increasing VO_2 max (Swallow et al. 1998b; Houle-Leroy et al. 2000; Helge 2002). In another example, Shima and colleagues demonstrated physical activity may have a protective effect against the development of type II diabetes in rats (Shima et al. 1993). Increased spontaneous physical activity was also positively associated with obesity resistance in rats (Teske et al. 2014).

Although many studies have examined effects of training in adult rodents and humans, few have investigated effects of early-life exercise or amounts of physical activity on adult activity, physical fitness, health or metabolic biomarkers. (Patterson et al. 2008) gave diet-induced-obesity (DIO) and diet-resistant (DR) rats three weeks of early-onset exercise and found that was sufficient to significantly decrease body mass throughout thirteen weeks and to significantly decrease fat pad masses. In a later study, Patterson et al. showed evidence of early-onset exercise delaying negative effects of obesity for up to ten weeks with no wheel access in high-fat-fed DIO rats (Patterson et al. 2009).

Role of Food Choice

Food consumption can be influenced by food preference, and most animals in their natural environment have some opportunity to choose among different types of food. Moreover, diet composition can have important effects on physiology, which can in turn affect an organism's ability to perform various types of behavior, such as migration.

One aspect of food choice involves varying lipid composition. Unsaturated fatty acids are more fluid (at a given temperature) and undergo peroxidation more easily than saturated fatty acids (Shaikh and Edidin 2006), which allows higher rates of flux into metabolizing cells, and may facilitate higher rates of fatty acid oxidation. A field study demonstrated that just prior to their long-distance migration from Canada to South America, sandpipers consumed a diet rich in amphipod crustaceans, which have greater fat content than other crustaceans (Weber 2009). Moreover, the chosen shrimp are high

in polyunsaturated fatty acids (PUFA), which modifies phospholipid membranes by increasing the fluidity and permeability (Maillet and Weber 2006), and certain polyunsaturated fatty acids have been shown to increase treadmill endurance-running performance in rats (Ayre and Hulbert 1997).

Diet composition has been shown to affect bird physiology. For example, captive white-throated sparrow fed a high-protein, low-carbohydrate insect diet or a high-carbohydrate, low-protein grain showed differences in plasma uric acid concentrations, but not glycerol or nonesterified fatty acid concentrations after an inactive overnight fast (Smith et al. 2007). In a follow-up study, wild-caught white-throated sparrows were fed one of four diets: low-protein, high-carbohydrate (LPHC); low-protein, high-fat (LPHF); high-protein, low carbohydrate (HPLC); or high-protein, low-fat (HPLF), and then body composition and lipid metabolites were measured (Smith and McWilliams 2009). Results indicated that levels of triglyceride, B-hydroxybutyrate, and nonesterified fatty acids varied depending on the diets. The greatest fat accumulation was in birds fed the LPHC. Additionally, high-fat diets increased dietary fat utilization and circulating concentration of B-hydroxybutyrate and nonesterified fatty acids (Smith and McWilliams 2009).

In another study, wild-caught yellow-rumped warblers were offered a choice between a diet high in saturated fat or unsaturated fat. The majority of individuals preferred diets containing mono-unsaturated fatty acids (one category of unsaturated fatty acids) rather than the diet high in saturated fats (McWilliams et al. 2002). Other research on wild-caught red-eyed vireos established preference for long-chain unsaturated fatty

acids over long-chain saturated fatty acids (Pierce et al. 2004). Results from a follow-up study demonstrated that red-eyed vireos do not have improved aerobic performance (mass-specific peak metabolic rate) with a highly unsaturated fatty acid diet as compared with a lower unsaturated fatty acid diet (Pierce et al. 2005). In summary, avian research suggests that diet preference may be determined by coming performance demands and that there seems to be a general tendency for preference of diets high in unsaturated fats as opposed to saturated fats.

Less directly related to physical activity per se, many mammals are known to alter diet prior to hibernation. For example, grizzly bears consumed soluble carbohydrates or low in fiber diets, depending upon seasonal availability and nutritional content, just prior to torpor or hibernating periods (Hamer and Herrero 1987). Similarly, lemurs were determined to undergo pre-hibernation-fattening by consuming greater quantities of highly sugary fruits over other plant species (Fietz and Ganzhorn 1999). Wild-caught chipmunks were fed one of two fatty diets 8 weeks prior to their hibernation period. One group was fed normal chow, high in saturated fats, and the other was normal chow high in unsaturated fats. After hibernation conditions were simulated, no matter the diet consumed, levels of unsaturated fatty acids increased in both dietary groups, suggesting that unsaturated lipids in tissues and membranes are important for successful hibernation (Geiser 1990). Although some non-human animal studies suggest they choose their food to affect their physiological performance, it is not always the case. For example, wild-caught squirrels did not consume more PUFA when it was offered to them prior to their pre-hibernation amounts (Frank et al. 1998).

Although various non-human animals have been shown to selectively choose their food in ways that affect their physiological performance abilities, they do not necessarily consume more of a certain diet under all conditions, such as when an altered performance capacity is not required. For example, wild-caught squirrels in a lab setting did not consume more polyunsaturated fatty acids (PUFAs) when it was offered to them prior to their pre-hibernation amounts (Frank et al. 1998).

In the context of exercise performance, many studies of humans and rodents have examined the effects of various macro- or micronutrients on performance abilities. A recent review analyzing dietary supplements in athletes confirms that they often purposely consume certain foods to benefit performance (Maughan et al. 2004). To promote tissue growth and repair, and to improve strength and power, some athletes consume more than the adequate amounts of amino acids (glutamine, leucine, lysine), metabolites of amino acids (hydroxymethylbutyrate), trace elements (boron, chromium, vanadium, zinc), protein (creatine), herbs (chrysin), bicarbonate, and caffeine. Of these, survey results suggest that creatine, caffeine, and bicarbonate improved performance through various pathways (Maughan et al. 2004).

In a different review, consumption of various herbs improved performance (endurance and strength), improved recovery, maintained health during intense periods of exercise, built muscle mass, and reduced body fat (Bucci 2000). For example, ginseng improved maximal oxygen uptake, increased alertness, and the time to exhaustion. However, abuse of this, as with other stimulants, has negative effects, such as sleeplessness, nervousness, hypertension, skin eruptions, and diarrhea (Bucci 2000).

Another commonly used herb is ephedra; it is commonly thought to enhance physical performance, but study results indicate otherwise. Ephedra is known to increase nervousness, anxiety, heart palpitations, headaches, nausea, hyperthermia, hypertension, cardiac arrhythmias, and even to cause occasional deaths (Bucci 2000). The Maughan and Bucci reviews show that people have tried to enhance their physical performance abilities by consuming dietary supplements; sometimes they work, but other times may result in dire consequences.

A rat study demonstrated that 12 weeks of high-fat feeding increased VO_{2max} by 15 percent in sedentary rats, whereas endurance training of the same duration increased it by 20 percent (Simi et al. 1991), and the effects were additive. In a different study, researchers determined how diet composition affects exercise endurance and body composition. Rats were trained and fed one of two different types of diets (high-fat or high-carbohydrate) for 8 weeks. Half of the rats had their diets switched for 3 days prior to endurance testing. Rats receiving the high-fat diet first, followed by the high-carbohydrate diet, fatigued later than the rats fed a high-carbohydrate or high-fat diet only (Lapachet et al. 1996). In another study of rats fed one of three diets (high in saturated fat, high in n-3 fatty acids, or high in n-6 fatty acids), those fed the high n-6 diet were shown to increase treadmill endurance-running performance compared to the other groups (Ayre and Hulbert 1997).

Mice of the inbred C57BL/6 strain were tested for food preference after a subset had early exposure to a high-fat diet immediately after weaning (Teegarden et al. 2009). Specifically, juvenile mice were fed a high-fat diet for one week, fed regular mouse chow

for approximately eight weeks, and then presented with 3 different diets: high fat, high protein, or high carbohydrate. There was an increased general preference for the high-fat diet for all mice, but the pre-exposed mice had a significantly greater preference. This study established that brief early exposure to high-fat diet can increase preference for this diet in adulthood.

Human food preferences are influenced by various personal, socioeconomic, cultural, and intrinsic biological factors (Shepherd 1998). At least in Western societies, people show a general preference for foods high in sugars and fats, and it is well established that the consumption of such diets has increased in Western countries in recent years (Kant 2000). However, increased consumption of these foods often does not occur in preparation for some costly activity, let alone hibernation. Instead, increased consumption of foods high in fats and sugars may occur because they are commonly available and/or rewarding, i.e., they trigger a hedonic response. Moreover, greater preference for "Western diets" among U.S. adults and children has resulted in the unfortunate consequence of weight gain (Bray and Popkin 1998; Drewnowski 2007), which in turn is associated with increased risk of developing coronary heart disease, type 2 diabetes, hypertension, metabolic syndrome, and some forms of cancer (Munnelly and Feehan 2002; U.S. Department of Health and Human Services 2002; Ford et al. 2004; Flegal et al. 2010a). Therefore, eating healthier foods is another highly recommended and flexible method of improving health.

Given that simply increasing physical activity and consuming fewer calories (while also reducing consumption of fats and sugars) would decrease the incidence of

obesity and associated diseases, one would think this problem would be easily fixed by education and public policy that encourages activity and the choice of healthy diets. However, major efforts in these areas have, so far, not been enough to stop the negative health trends, and much more knowledge is needed to understand behaviors behind these two larger phenomena.

The mechanisms behind maintenance of healthy weights as influenced by physical activity (both voluntary exercise and spontaneous activity) and food preferences are difficult to study in human subjects, and so rodent models are often examined (Garland et al., 2011). Laboratory strains of mice are particularly convenient to study because of the ease in handling, housing, breeding, behavioral testing, etc. Laboratory mice are also good models for addressing human health concerns because they exhibit much of the genetic, behavioral, and physiological variation that exists in human populations (Svenson et al. 2007). Laboratory mice are not only well-studied, but also share many of the diseases that humans suffer, including diabetes, atherosclerosis, heart disease, cancer, anemia, hypertension, obesity, and asthma (Peters et al. 2007).

Energy balance: fuel usage, metabolism during exercise

Fats (lipids) and sugars (carbohydrates) are the primary fuel sources for energy metabolism in mammals. Lipids are the most chemically reduced of all fuels and they can be stored without water. Consequently, fats (38KJ/gram) provide more energy per unit mass than carbohydrates (18KJ/gram) (Jeukendrup et al. 1998). Carbohydrates are essential when high ATP turnover rates are necessary and when oxygen availability is

compromised (Weber and Haman 2004). Prolonged high-fat diet consumption (3-49 days) increases whole body lipid utilization and fat oxidation (Askew 1984; Iossa et al. 2002; Achten and Jeukendrup 2004).

In an exercising land mammal, oxygen is consumed and, usually, both lipids and/or carbohydrates are oxidized to produce carbon dioxide, water, and ATP. Carbohydrates and fats produce different amounts of CO₂ and require different amounts of O₂ when they are oxidized. The ratio of VCO₂/VO₂ in expired air, or the respiratory exchange ratio (RER), will be 0.69-0.73 when only fat is oxidized (depending on the length of the fatty acid) or closer to 1.0 when glucose or carbohydrates are oxidized (Jeukendrup et al. 1998) .

During sustained exercise, locomotor muscles depend on intramuscular fuels (muscle glycogen and muscle triacylglycerol) and circulatory fuels brought to working muscles from remote storage sites, such as the liver (hepatic glucose) or fat depots (adipose tissue lipids). At low-intensity exercise, ATP production is primarily derived from fat oxidation (Askew 1984; Achten and Jeukendrup 2004; Weber and Haman 2004). As exercise intensity increases (65-80% VO₂max), carbohydrates become the primary fuel source. Highly trained athletes, such as long-distance runners, have increased fat oxidation during aerobic exercise (Weber 2009). During acute, high-intensity exercise, as in sprint-speed running, very aerobic mammals with high maximal oxygen capacity (VO₂max) rely relatively more on intramuscular fuels (muscle glycogen and muscle triacylglycerol) and relatively less on circulatory fuels than sedentary mammals (Weber and Haman 2004). In another study comparing dogs, horses and human athletes, Poole

and Erickson demonstrated exercise training effects alter cardiovascular and pulmonary structure and function (Poole and Erickson 2011).

Lipid metabolism

Lipid composition and the regulation of lipid metabolism can affect performance ability during exercise (McClelland 2004). However, some literature suggests fat intake can impede performance, whereas other studies show performance enhancement. There is evidence that fat intake decreases performance in cycling athletes as compared to a carbohydrate-rich diet (Burke and Hawley 2006). In another study, human subjects were fed a high-fat diet (HFD) for 5 days and bicycle tested. Afterward, the same group of people received a high-carbohydrate diet (HCD) for the same duration and they were again exercise tested. Blood samples were taken before and after exercise trials. Lower respiratory exchange ratio during $VO_2\text{max}$ and slightly higher $VO_2\text{max}$ were seen after the high-fat diet than after the high-carbohydrate diet (Jansson and Kaijser 1982); thus, literature on the influences of HFD on humans can be somewhat contradictory.

In rodents, some studies have shown that a high-fat diet (HFD) can stimulate physical activity. For example, mice selectively bred for high or low body fat ("fat" mice had 22% body fat, "lean" mice had 4% body fat) were fed either a HFD or a regular rodent diet for 42 days. The lean mice ran more than the fat mice throughout the 42 days, but fat mice on a high-fat diet significantly increased their wheel running as compared to fat mice on the regular diet (Simoncic et al. 2008). In a separate experiment presented in the same paper, they also demonstrated home-cage activity differences between the two strains on regular chow. Lean mice were more active than fat mice (Simoncic et al.

2008), but they did not report effects of a high-fat diet (Meek et al. 2010; Meek et al. 2012).

Lipid oxidation is increased with exercise training and high-fat feeding (Jansson and Kaijser 1982). High-fat diets and high plasma fatty acid levels result in improvements in B-oxidation capacity, as seen by increases in citrate synthase (CS), carnitine acyl-transferase, and B-hydroxy-acyl-CoA dehydrogenase (HOAD) activities in rats' deep vastus muscles (Cheng et al. 1997). Rats fed a high-fat diet for 12 weeks had increased endurance-exercise capacity by 15% in sedentary rats and 35% in rats that also received endurance training via treadmill exercise (Simi et al. 1991). Additionally, HFD and exercise both increased CS and HOAD activity (Simi et al. 1991).

Model of study

Mice for the experiments described in this dissertation were obtained from four replicate High Runner (HR) lines lines that have been selectively bred for high voluntary wheel running, along with their four non-selected Control (C) lines (Swallow et al. 1998a). Outbred Hsd:ICR house mice (*Mus domesticus*) (N = 224) were used as the founding population, purchased from Harlan-Sprague-Dawley (Indianapolis, Indiana, USA). After two generations of random mating, eight closed populations or lines were established. Four lines are selected for their high voluntary wheel running (HR lines) on days 5 & 6 of a 6-day wheel test, while the other four control (C) lines are bred without regard to wheel running. Briefly, approximately 600 mice are tested each generation (at 6-8 weeks of age) in standard housing cages that are attached to Wahman-type activity wheels for 6 days (1.12-m circumference, 35.7-cm diameter stainless-steel; Lafayette

Instruments, Lafayette, IN). An automated photocell system counts the number of revolution per minute for ~23.5 hours and uploads it to an attached MS-DOS computer system (software from San Diego Instruments; San Diego, CA). Complete selection experiment protocol can be found elsewhere (Swallow et al. 1998a). Unless indicated otherwise, mice are kept with *ad libitum* food (Harlan Teklad Laboratory Rodent Diet (8604) and water, a 12:12h L:D cycle, and a temperature of ~22°C.

Various studies of the HR mice demonstrate that aerobic exercise training via eight weeks of wheel access enhances capacity for fatty acid oxidation as shown by mitochondrial enzyme activities. Cytochrome-c oxidase, citrate synthase, and carnitine palmitoyl-transferase are increased in the HR mice relative to C mice (Houle-Leroy et al. 2000). HR mice with wheel access generally showed greater training responses than the C mice with wheel access (Houle-Leroy et al. 2000). Exercise in HR and C mice also causes decreases in body mass (Swallow et al. 1999) and body fat (Swallow et al. 2001), as well as increased whole-animal maximal oxygen consumption (Swallow et al. 1998b).

In a study of one HR line (Line #7) and one Control line (Line #2), mice were fed either a HFD or a high-carbohydrate diet (HCD) while housed without wheel access. All mice fed the HFD had greater resting metabolic rate, and daily energy expenditure than the HCD-fed mice (Vaanholt et al. 2008). However, female HR mice fed the HFD not only ate more than those on the HCD, but they also had greatly increased spontaneous home-cage activity (although not statistically significant, probably due to small sample sizes), elevated daily energy expenditure, and did not gain weight (Vaanholt et al. 2008). In contrast, male HR mice and both sexes of C mice had significant weight gain. Thus,

HR females showed a unique and very interesting effect in response to a high-fat diet, which led to my proposal for further investigation.

In a very recent study, male mice from all eight lines were either fed a Western diet (high fat and added sucrose) or a standard rodent diet for eight weeks and results indicated Western-fed HR mice increased their daily wheel running, resulting in levels that were up to 5.6-fold higher than C mice, whose wheel running was unaffected by WD (Meek TH et al. 2010). Findings were important, not only because HR mice were able to increase wheel running by up to 52% (during week four) while consuming a Western diet (Meek et al 2010), but also because these lines of mice had reached a plateau since approximately generation 17-25 (Careau et al. 2013). Furthermore, C mice were not affected by the Western diet; they maintained similar levels of running with either diet. It was suggested that HR mice might have a better ability to oxidize lipids in the diet and utilize that energy (Meek TH et al. 2010).

Dissertation Chapters

The first dissertation chapter explored the propensity to engage in adult physical activity when given early-life exercise opportunity. Like many other behavioral and physiological traits, it is possible that the propensity to engage in physical activity can be influenced by conditions that an organism experiences during its early ontogeny. Before my study, there was no information for either rodents or humans to directly support the idea that the introduction of physical activity during childhood or adolescence actually increased physical activity later in life. When behaviors or practices are habituated at young ages (i.e., habits are formed), animals are more likely to repeat those practices later

in life. We exposed a subset of HR and C mice to early-life wheel access and compared adult physical activity with a subset that did not have early-life wheel access. We also measured body mass, food consumption, home-cage activity, wheel running, plasma leptin levels and organ masses.

The second dissertation chapter explored food choice and its effects on HR and C mice. Food preferences can be influenced by many things, including: diet composition, hedonic responses, genetic predisposition, sex, and the need or desire to improve physical performance. Fat and sugar content in the diet can also have some bearing on both voluntary and spontaneous physical activity (measured here by wheel running and home-cage activity, respectively), aerobic performance, body fat, and enzyme levels in various tissues. Because a previous study showed enhanced wheel running while consuming a Western diet (high in fat and sucrose), rather than standard chow (Meek et al. 2010), we investigated whether HR and C mice would prefer to consume it, given a choice between Western diet and the standard chow. Furthermore, we were interested in determining if consumption of the Western diet increased wheel running in HR mice as demonstrated previously. We measured body mass, food consumption, home-cage activity, and wheel running.

Chapter three investigated fuel usage in HR and C mice as indicated by whole-animal respirometry. Aerobic metabolism was measured using indirect calorimetry where oxygen and the metabolic waste-product, carbon dioxide, were measured to estimate metabolic rates. Oxygen consumption and carbon dioxide production increase with greater energetic demand, as with increased running. We wanted to determine if HR

mice relied more on lipids to fuel their higher activity levels. We compared gas-exchange rates of HR and C mice while being fed a standard rodent chow (Harlan Teklad Laboratory Rodent Diet [W]-8604) or a "Western" rodent diet (Harlan Teklad Custom Research Western Diet TD.88137) (42% of total KJ came from fat and 43% of total KJ came from sucrose). We measured oxygen consumption, carbon dioxide production, body mass, food consumption, wheel running, and home-cage activity. The goals of these studies were to further investigate differences that have evolved along with the artificial selection for high-voluntary wheel running, which may help elucidate key features that allow them to be anti-obesogenic.

References

- Achten, J., and A. E. Jeukendrup. 2004. Optimizing fat oxidation through exercise and diet. *Nutrition* 20:716-727.
- Askew, E. W. 1984. Role of fat metabolism in exercise. *Clinics in sports medicine* 3:605-621.
- Ayre, K. J., and A. J. Hulbert. 1997. Dietary fatty acid profile affects endurance in rats. *Lipids* 32:1265-1270.
- Blair, S. N., and J. N. Morris. 2009. Healthy hearts-and the universal benefits of being physically active: physical activity and health. *Ann. Epidemiol.* 19:253-256.
- Booth, F. W., M. V. Chakravarthy, S. E. Gordon, and E. E. Spangenburg. 2002. Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. *Journal of Applied Physiology* 93:3-30.
- Booth, F. W., and C. K. Roberts. 2008. Linking performance and chronic disease risk: indices of physical performance are surrogates for health. *British Journal of Sports Medicine* 42:950-952.
- Bray, G. A., and B. M. Popkin. 1998. Dietary fat intake does affect obesity! *American Journal of Clinical Nutrition* 68:1157-1173.
- Bucci, L. R. 2000. Selected herbals and human exercise performance. *American Journal of Clinical Nutrition* 72:624S-636.
- Burke, L. M., and J. A. Hawley. 2006. Fat and carbohydrate for exercise. *Current Opinion in Clinical Nutrition & Metabolic Care* 9:476-481.
- Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2013. Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. *Evolution* 67:3102-3119.
- Centers for Disease Control and Prevention National Center for Chronic Disease Prevention and Health Promotion, D. o. N. a. P. A. 1996. A report of the surgeon general: physical activity and health at-a-glance.
- Cheng, B., O. Karamizrak, T. D. Noakes, S. C. Dennis, and E. V. Lambert. 1997. Time course of the effects of a high-fat diet and voluntary exercise on muscle enzyme activity in Long-Evans rats. *Physiol. Behav.* 61:701-705.

- Copes, L. E., H. Schutz, E. M. Dlugosz, W. Acosta, M. A. Chappell, and T. Garland Jr. 2015. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. *Physiol. Behav.* 149:86-94.
- Drewnowski, A. 2007. The real contribution of added sugars and fats to obesity. *Epidemiologic Reviews* 29:160-171.
- Eisenmann, J. C., and E. E. Wickel. 2009. The biological basis of physical activity in children: revisited. *Pediatric Exercise Science* 21:257-272.
- Ekkekakis, P., E. E. Hall, and S. J. Petruzzello. 2005. Variation and homogeneity in affective responses to physical activity of varying intensities: an alternative perspective on dose – response based on evolutionary considerations. *J. Sports Sci.* 23:477-500.
- Fietz, J., and J. U. Ganzhorn. 1999. Feeding ecology of the hibernating primate *Cheirogaleus medius*: how does it get so fat? *Oecologia* 121:157-164.
- Flegal, K. M., M. D. Carroll, C. L. Ogden, and L. R. Curtin. 2010a. Prevalence and trends in obesity among U.S. adults, 1999-2008. *Journal of the American Medical Association* 303:235-241.
- Flegal, K. M., C. L. Ogden, J. A. Yanovski, D. S. Freedman, J. A. Shepherd, B. I. Graubard, and L. G. Borrud. 2010b. High adiposity and high body mass index-for-age in US children and adolescents overall and by race-ethnic group. *American Journal of Clinical Nutrition* 91:1020-1026.
- Ford, E. S., W. H. Giles, and A. H. Mokdad. 2004. Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care* 27:2444-2449.
- Ford, E. S., C. Y. Li, G. X. Zhao, W. S. Pearson, and A. H. Mokdad. 2008. Prevalence of the metabolic syndrome among U.S. adolescents using the definition from the international diabetes federation. *Diabetes Care* 31:587-589.
- Frank, C. L., E. S. Dierenfeld, and K. B. Storey. 1998. The relationship between lipid peroxidation, hibernation, and food selection in mammals. *Am. Zool.* 38:341-349.
- Garland T, J., Schutz H, Chappell MA, Keeney BK, Meek TM, Copes LE, Acosta W, Drenowatz C, Maciel RC, van Dijk G, Kotz CM, and E. JC. 2011. The biological control of voluntary exercise, spontaneous physical activity, and daily energy expenditure in relation to obesity: human and rodent perspectives. *Journal of Experimental Biology* In press.

- Gauthier, M. S., K. Couturier, A. Charbonneau, and J. M. Lavoie. 2004. Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. *Int. J. Obes.* 28:1064-1071.
- Geiser, F. 1990. Influence of polyunsaturated and saturated dietary lipids on adipose tissue, brain and mitochondrial membrane fatty acid composition of a mammalian hibernator. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* 1046:159-166.
- Goodpaster, B. H., A. Katsiaras, and D. E. Kelley. 2003. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 52:2191-2197.
- Halpern, A., M. C. Mancini, M. E. C. Magalhaes, M. Fisberg, R. Radominski, M. C. Bertolami, A. Bertolami, M. E. de Melo, M. T. Zanella, M. S. Queiroz, and M. Nery. 2010. Metabolic syndrome, dyslipidemia, hypertension and type 2 diabetes in youth: from diagnosis to treatment. *Diabetology & Metabolic Syndrome* 2.
- Hamer, D., and S. Herrero. 1987. Grizzly bear food and habitat in the front ranges of Banff National Park, Alberta. *Bears: Their Biology and Management* 7:199-213.
- Helge, J. W. 2002. Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc.* 34:1499-1504.
- Hill, J. O., H. R. Wyatt, and J. C. Peters. 2012. Energy Balance and Obesity. *Circulation* 126:126-132.
- Hills, A. P., L. B. Andersen, and N. M. Byrne. 2011. Physical activity and obesity in children. *British Journal of Sports Medicine* 45:866-870.
- Houle-Leroy, P., T. Garland, J. G. Swallow, and H. Guderley. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. *Journal of Applied Physiology* 89:1608-1616.
- Iossa, S., M. P. Mollica, L. Lionetti, R. Crescenzo, M. Botta, and G. Liverini. 2002. Skeletal muscle oxidative capacity in rats fed high-fat diet. *Int. J. Obes.* 26:65-72.
- Jansson, E., and L. Kaijser. 1982. Effect of diet on the utilization of blood-borne and intramuscular substrates during exercise in man. *Acta Physiol. Scand.* 115:19-30.
- Jeukendrup, A. E., W. H. M. Saris, and A. J. M. Wagenmakers. 1998. Fat metabolism during exercise: a review. *Int J Sports Med* 19:371-379.

- Kant, A. K. 2000. Consumption of energy-dense, nutrient-poor foods by adult Americans: nutritional and health implications. The third National Health and Nutrition Examination Survey, 1988-1994. *American Journal of Clinical Nutrition* 72:929-936.
- Kiess, W., A. Reich, G. Muller, K. Meyer, A. Galler, J. Bennek, and J. Kratzsch. 2001. Clinical aspects of obesity in childhood and adolescence - diagnosis, treatment and prevention. *Int. J. Obes.* 25:S75-S79.
- Kim, A. M., C. M. Tingen, and T. K. Woodruff. 2010. Sex bias in trials and treatment must end. *Nature* 465:688-689.
- Kokkinos, P., J. Myers, C. Faselis, D. B. Panagiotakos, M. Doumas, A. Pittaras, A. Manolis, Kokkinos, J. Peter, P. Karasik, M. Greenberg, V. Papademetriou, and R. Fletcher. 2010. Exercise Capacity and Mortality in Older Men. *Circulation* 122:790-797.
- Lapachet, R. A. B., W. C. Miller, and D. A. Arnall. 1996. Body fat and exercise endurance in trained rats adapted to a high-fat and/or high-carbohydrate diet. *Journal of Applied Physiology* 80:1173-1179.
- Levine, J. A. 2007. Nonexercise activity thermogenesis--liberating the life-force. *Journal Internal Medicine* 262:273-287.
- Maillet, D., and J. M. Weber. 2006. Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. *Journal of Experimental Biology* 209:2686-2695.
- Maughan, R. J., D. S. King, and T. Lea. 2004. Dietary supplements. *J. Sports Sci.* 22:95-113.
- McAllister, E. J., N. V. Dhurandhar, S. W. Keith, L. J. Aronne, J. Barger, M. Baskin, R. M. Benca, J. Biggio, M. M. Boggiano, J. C. Eisenmann, M. Elobeid, K. R. Fontaine, P. Gluckman, E. C. Hanlon, P. Katzmarzyk, A. Pietrobelli, D. T. Redden, D. M. Ruden, C. Wang, R. A. Waterland, S. M. Wright, and D. B. Allison. 2009. Ten putative contributors to the obesity epidemic. *Critical Reviews in Food Science and Nutrition* 49:868-913.
- McClelland, G. B. 2004. Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 139:443-460.

- McSweeney, J. C., M. Cody, rsquo, P. Sullivan, K. Elberson, D. K. Moser, and B. J. Garvin. 2003. Women's Early Warning Symptoms of Acute Myocardial Infarction. *Circulation*:01.CIR.0000097116.0000029625.0000097117C.
- McWilliams, S. R., S. B. Kearney, and W. H. Karasov. 2002. Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. *Journal of Avian Biology* 33:167-174.
- Meek, T., J. Eisenmann, and T. Garland, Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *Int. J. Obes.* 34:960-969.
- Meek TH, Eisenmann JC, and G. T. Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *Int. J. Obes.*:1-10.
- Meek, T. H., E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2012. Effects of leptin treatment and Western diet on wheel running in selectively bred high runner mice. *Physiol Behav* 106:252-258.
- Mosca L, Banka CL, Benjamin EJ, Berra K, Bushnell C, Dolor RJ, Ganiats TG, Gomes AS, Gornik HL, Gracia C, Gulati M, Haan CK, Judelson DR, Keenan N, Kelepouris E, Michos ED, Newby LK, Oparil S, Ouyang P, Oz MC, Petitti D, Pinn VW, Redberg RF, Scott R, Sherif K, Smith SC Jr., Sopko G, Steinhorn RH, Stone NJ, Taubert KA, Todd BA, Urbina E, and Wenger NK. 2007. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. *Circulation* 115:1481-1501.
- Mundal, R., S. E. Kjeldsen, L. Sandvik, G. Erikssen, E. Thaulow, and J. Erikssen. 1998. Clustering of coronary risk factors with increasing blood pressure at rest and during exercise. *J. Hypertens.* 16:19-22.
- Munnely, P., and S. Feehan. 2002. An obesity clinic model. *Proc. Nutr. Soc.* 61:9-10.
- Mustelin, L., K. Silventoinen, K. Pietilainen, A. Rissanen, and J. Kaprio. 2009. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *Int. J. Obes.* 33:29-36.
- Myers, J., M. Prakash, V. Froelicher, D. Do, S. Partington, and J. E. Atwood. 2002. Exercise Capacity and Mortality among Men Referred for Exercise Testing. *N. Engl. J. Med.* 346:793-801.
- Novak, C. M., C. Escande, S. M. Gerber, E. N. Chini, M. Zhang, S. L. Britton, L. G. Koch, and J. A. Levine. 2009. Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. *PLoS One* 12:e5869.

- Ogden CL, F. K. 2010. Changes in terminology for childhood overweight and obesity. *Natl Health Stat Report* 25:1-5.
- Patterson, C. M., S. G. Bouret, A. A. Dunn-Meynell, and B. E. Levin. 2009. Three weeks of postweaning exercise in DIO rats produces prolonged increases in central leptin sensitivity and signaling. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 296:R537-R548.
- Patterson, C. M., A. A. Dunn-Meynell, and B. E. Levin. 2008. Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 294:R290-R301.
- Peters, L. L., R. F. Robledo, C. J. Bult, G. A. Churchill, B. J. Paigen, and K. L. Svenson. 2007. The mouse as a model for human biology: a resource guide for complex trait analysis. *Nat Rev Genet* 8:58-69.
- Pierce, B. J., S. R. McWilliams, T. P. O'Connor, A. R. Place, and C. G. Guglielmo. 2005. Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *Journal of Experimental Biology* 208:1277-1285.
- Pierce, B. J., S. R. McWilliams, A. R. Place, and M. A. Huguenin. 2004. Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyed Vireos (*Vireo olivaceus*). *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 138:503-514.
- Poole, D. C., and H. H. Erickson. 2011. Highly athletic terrestrial mammals: horses and dogs. *Compr Physiol* 1:1-37.
- Ross, R., and K. A. McGuire. 2011. Incidental physical activity is positively associated with cardiorespiratory fitness. *Medicine & Science in Sports & Exercise* 43:2189-2194.
- Rowland, T. W. 1998. The biological basis of physical activity. *Med. Sci. Sports Exerc.* 30:392-399.
- Shaikh, S. R., and M. Edidin. 2006. Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. *American Journal of Clinical Nutrition* 84:1277-1289.
- Shepherd, R., and Mela, D. 1998. Food choice: Factors influencing food choice. Pp. 843-850 *in* M. Sadler, Strain, J.J., Caballero, B., ed. *Encyclopedia of Human Nutrition*. Academic Press.

- Shima, K., K. Shi, T. Sano, T. Iwami, A. Mizuno, and Y. Noma. 1993. Is exercise training effective in preventing diabetes mellitus in the Otsuka-Long-Evans-Tokushima Fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus? *Metabolism* 42:971-977.
- Simi, B., B. Sempore, M. H. Mayet, and R. J. Favier. 1991. Additive effects of training and high-fat diet on energy-metabolism during exercise. *Journal of Applied Physiology* 71:197-203.
- Simoncic, M., S. Horvat, P. L. Stevenson, L. Bunger, M. C. Holmes, C. J. Kenyon, J. R. Speakman, and N. M. Morton. 2008. Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. *Behavior Genetics* 38:292-300.
- Smith, S. B., and S. R. McWilliams. 2009. Dietary macronutrients affect lipid metabolites and body composition of a migratory passerine, the White-Throated Sparrow (*Zonotrichia albicollis*). *Physiol. Biochem. Zool.* 82:258-269.
- Smith, S. B., S. R. McWilliams, and C. G. Guglielmo. 2007. Effect of diet composition on plasma metabolite profiles in a migratory songbird. *Condor* 109:48-58.
- Steinberger, J., S. R. Daniels, R. H. Eckel, L. Hayman, R. H. Lustig, B. McCrindle, and M. L. Mietus-Snyder. 2009. Progress and challenges in metabolic syndrome in children and adolescents a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 119:628-647.
- Svenson, K. L., R. Von Smith, P. A. Magnani, H. R. Suetin, B. Paigen, J. K. Naggert, R. Li, G. A. Churchill, and L. L. Peters. 2007. Multiple trait measurements in 43 inbred mouse strains capture the phenotypic diversity characteristic of human populations. Pp. 2369-2378.
- Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics* 28:227-237.
- Swallow, J. G., T. Garland, P. A. Carter, W. Z. Zhan, and G. C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *Journal of Applied Physiology* 84:69-76.
- Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland. 2001. Food consumption and body composition in mice selected for high wheel-running activity. *J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol.* 171:651-659.

- Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland, Jr. 1999. Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. *Journal of Experimental Biology* 202:2513-2520.
- Teegarden, S. L., A. N. Scott, and T. L. Bale. 2009. Early life exposure to a high fat diet promotes long-term changes in dietary preferences and central reward signaling. *Neuroscience* 162:924-932.
- Teske, J. A., C. J. Billington, and Kotz C.M. 2014. Mechanisms underlying obesity resistance associated with high spontaneous physical activity. *Neuroscience* 256:91-100.
- Troiano, R. P., D. Berrigan, K. W. Dodd, L. C. Masse, T. Tilert, and M. McDowell. 2008. Physical activity in the United States measured by accelerometer. *Medicine & Science in Sports & Exercise* 40:181-188.
- U.S. Department of Health and Human Services. 2002. Physical activity fundamental to preventing disease. U.S. Department of Health and Human Services Centers for Disease Control and Prevention National Center for Chronic Disease Prevention and Health Promotion, Division of Nutrition and Physical Activity:1-19
<http://health.gov/paguidelines/guidelines/default.aspx#toc>.
- U.S. National Library of Medicine. 2012. Overweight and Obesity Statistics. Pp. 1-4 *in* U. S. D. o. H. a. H. Services, ed. National Institutes of Health- The National Institute of Diabetes and Digestive and Kidney Diseases.
- Vaanholt, L. M., I. Jonas, M. Doornbos, K. A. Schubert, C. Nyakas, T. Garland, G. H. Visser, and G. van Dijk. 2008. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int. J. Obes.* 32:1566-1575.
- Weber, J.-M., and F. Haman. 2004. Oxidative fuel selection: adjusting mix and flux to stay alive. *International Congress Series* 1275:22-31.
- Weber, J. M. 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. *Journal of Experimental Biology* 212:593-597.
- Westerterp, K. R., and M. I. Goran. 1997. Relationship between physical activity related energy expenditure and body composition: a gender difference. *Int. J. Obes.* 21:184-188.

Chapter 1

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

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Running Title: Early exercise, adult activity & food consumption

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.

ABSTRACT

The purpose of this study was to evaluate the effects of early 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 eight weeks, 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. 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 to 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.

Keywords: Early-life factors; Exercise; Food consumption; Genotype-by-environment interaction; Selection experiment; Spontaneous physical activity; Wheel running

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 (Eisenmann 2004; Eisenmann 2006; Adam and Epel 2007); (Papas et al. 2007); (Coccorello et al. 2009); (McAllister et al. 2009); (Stein et al. 2009); (Patterson and Abizaid 2013); (Yang and Huffman 2013). 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., (Hallal et al. 2006); (Stein et al. 2009)) and parental characteristics that are somewhat predictive of adolescent physical activity (Mattocks et al. 2008). Overall, however, early-life environmental determinants of adult physical activity levels are poorly understood (Dishman et al. 1985); (Sallis and Hovell 1990); (Twisk 2001; Jung et al. 2006); (Andersen et al. 2009);(Eisenmann and Wickel 2009); (Koeneman et al. 2011) ; (Baker et al. 2015);(Li et al. 2013).

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 ((Warburton et al. 2006); (Hayes and Kriska 2008)). The dramatic increases of in the prevalence of obesity (Smyth and Heron 2006) 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 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 (Eisenmann 2003). 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 (Mattocks et al. 2008). 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 (Lussana et al. 2008); (Stein et al. 2009). Individuals exposed to the Chinese famine during fetal life or infancy have an increased risk of metabolic syndrome in adulthood (Li et al. 2013). 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 (Nathanielsz 2006). 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 (Patterson and Levin 2007). 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 (Patterson et al. 2009). 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 (Schroeder et al. 2010).

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 (Swallow et al. 1998b; Garland et al. 2011a; Garland et al. 2011b). The HR and C lines differ markedly in both voluntary wheel-running behavior (Swallow et al. 1998b) and baseline activity in the home cage when wheels are absent (Malisch et al. 2009) (Copes et al. 2015), 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. (Hannon et al. 2008); (Nehrenberg et al. 2009); (Kelly et al. 2011); (Kelly et al. 2012); (Kelly et al. 2014), 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 (Steinberger et al. 2009); (Belcher et al. 2010); (Lopez et al. 2013).

Based on associations observed in humans (e.g., (Steinberger et al. 2009) several differences between HR and C mice suggest they are likely to respond differently to early-life factors. For example, as compared with C, HR mice have higher VO₂max and endurance during forced exercise, an altered brain reward system, elevated baseline

circulating corticosterone levels (for possible relevance, see (Weaver 2009), and increased depressive-like behaviors when wheel-deprived (Rhodes et al. 2005); (Rezende et al. 2006); (Malisch et al. 2007); (Malisch et al. 2008); (Malisch et al. 2009); (Meek et al. 2009); (Keeney et al. 2012). 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 ((Vaanholt et al. 2008) (Meek et al. 2010) (Meek et al. 2012); (Meek et al. 2009)).

2. Materials & procedures

2.1. Experimental animals

Mice were from an artificial selection experiment that breeds for high voluntary wheel running activity (Swallow et al. 1998b). 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, mice approximately 6-8 week old 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 (Copes et al. 2015) similar to (Gebczynski and Konarzewski 2009)) 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 hours. 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 were 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 (Copes et al. 2015). Data for both HCA and wheel running were downloaded daily between 1200-1300 hours.

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 (Girard et al. 2007). Blood samples were spun at 13,000 RPM for 12 minutes and collected plasma was stored at -20°C. Plasma leptin was measured using a Millipore Enzyme-linked Immunosorbent Assay (ELISA) kit (Mouse Leptin Assay Catalog # EZML-82K). Plasma samples were diluted and measured in duplicate in 96-well plates. Absorbances were read at 450 nm in a SpectraMax Plus

microplate reader (Molecular Devices, Sunnyvale, CA, USA) and compared with a standard curve generated individually for each plate.

After the 2-week period of adult wheel testing, mice were euthanized via CO₂ asphyxiation and dissected. Body mass and body length measurements from the tip of the nose to the base of the tail were taken. Visceral fat pad (combined visceral perirenal, periovaric, parametrial, and perivescical fat masses (Cinti 2005) along with heart ventricles, liver, and spleen were dissected and weighed. All methods were approved by the Institutional Animal Use and Care Committee of the University of California, Riverside.

2.6. Statistical analyses

Sample sizes were chosen based on previous experimental studies of these lines of mice. Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects and REML estimation. Linetype (HR or C) and wheel access (if applicable) were fixed effects; line was nested within line type as a random effect. Effects of linetype, wheel access, and their interaction were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6. Covariates depended on the trait being analyzed and included age, body mass, wheel freeness (an inverse measure of rotational resistance), home cage sensor calibration (measure of sensor motion sensitivity), and total wheel running. Dependent variable and/or covariates were transformed as necessary to improve the homogeneity of the spread of the covariates,

linearity of relations with covariates, and normality of residuals. All P values are 2-tailed unless otherwise indicated.

3. Results

3.1. Body mass

Early-life wheel access tended to decrease body mass in both Control and High Runner lines of mice (**Fig. 1**), but the effect was never statistically significant (Online Supplemental Material Tables S1-S3). Mice from HR lines were significantly lighter than C from week 3 through the end of the experiment. No interaction between early-life wheel access and linetype was ever statistically significant. A repeated-measures analysis supported the foregoing results, with a highly significant effect of week ($F = 135.07$; d.f. = 12,72; $P < 0.0001$), a trend for wheel access to reduce body mass ($F = 3.99$; d.f. = 1,6; $P = 0.0926$), a trend for HR mice to be smaller than C mice ($F = 4.93$; d.f. = 1,6; $P = 0.0682$), a strong week-by-linetype interaction ($F = 4.11$; d.f. = 12,72; $P < 0.0001$), no week-by-early-life exercise interaction ($F = 0.77$; d.f. = 12,72; $P = 0.6745$), and no week-by-early-life exercise-by-linetype interaction ($F = 0.45$; d.f. = 13,72; $P = 0.9450$).

3.1. Food consumption

With body mass as a covariate, food consumption during week 3 of early-life wheel access (**Fig. 2A**) was significantly increased by wheel access in HR mice, but not in C (wheel access by linetype interaction $P = 0.0183$). Results were similar during week 2 (Supplemental Material Table S4). During the first week of adult wheel access (**Fig.**

2B), food consumption was higher in HR mice (linetype $P = 0.0310$), but was not affected by early-life wheel access ($P = 0.8793$; interaction $P = 0.2301$). Similarly, during the second week of adult wheel access, food consumption was higher in HR mice (linetype $P = 0.0470$), with neither an effect of early-life wheel access ($P=0.7845$) nor an interaction ($P=0.9992$) (Supplemental Material Table S6).

3.1. Wheel running

During week 3 of early-life wheel access ($N = 47$), HR mice ran approximately 6-fold more revolutions per day than C ($P = 0.0018$) (**Fig. 3A**). When tested as adults ($N = 89$), mice that experienced early-life exercise ran more than sedentary individuals ($P = 0.0168$), HR mice ran more than C ($P = 0.0021$), with no statistical interaction ($P = 0.2245$) (**Fig. 3B**). During the second week of adult wheel access (Table S7), HR mice continued to run more than C mice ($P < 0.0001$), but the effect of early-life wheel access had disappeared ($P = 0.9261$), with no linetype-by-early exercise interaction ($P = 0.5929$).

3.1. Home-cage activity

During the third week of early-life wheel access (**Fig. 4A**), all mice with wheels had reduced spontaneous physical activity in their home cages ($P < 0.0001$), but the reduction was greater in HR mice than in C (interaction $P = 0.0388$). During the first week of adult wheel access, HCA was not statistically affected by any factor (**Fig. 4B**: early exercise $P=0.8773$, linetype $P=0.5155$, interaction $P=0.9728$). Results were similar during the second week of adult wheel access (early exercise $P=0.3991$, linetype $P=0.5069$, interaction $P=0.5779$: Supplemental Material Table S7).

3.1. Plasma leptin concentrations

Plasma leptin levels (measured one week prior to adult wheel testing) were strongly positively correlated with fat pad mass in both C (**Fig. 5A**) and HR (**Fig. 5B**) mice. Adjusted for fat-pad mass as a covariate, leptin levels were increased in C mice that had early-life wheel access, but decreased in HR mice that had early exercise (**Fig. 5C**: linetype x wheel access interaction $P = 0.0206$).

3.1. Organ masses

As shown in the Supplemental Material (Table S8), early-life exercise did not have a statistically significant effect on fat pad mass, heart ventricle, liver or spleen masses (with either body mass or body length as a covariate), nor did we find any interaction effects. However, early-life exercise did tend to reduce fat pad mass in both C and HR mice ($P = 0.1228$, adjusted for body mass as a covariate). In addition, HR mice tended to have had larger hearts (adjusted for body mass) than C mice ($P = 0.0616$).

4. Discussion

We tested the hypothesis that early-life exposure to exercise (voluntary wheel running) would affect the propensity to exercise in adult mice. Consistent with our hypothesis, three weeks of wheel access beginning at weaning significantly increased adult wheel running in both genetically selected High Runner lines and in non-selected control lines (Fig. 3B), whereas simultaneously measured home-cage activity was unaffected (Fig. 4B).

Wheel access caused a statistically significant increase in mass-adjusted food consumption by juvenile HR but not C mice (Fig. 2A, Table 4). The explanation for a

lack of increase in C mice is unclear, but a few previous studies of other strains of mice have also reported no increase in food consumption with wheel access ((Basterfield et al. 2009; Jung et al. 2010)). In general, the effect of wheel access on food consumption will be fairly directly related to the amount of running in rodents, and so should be greater in strains that run more (e.g., see (Swallow et al. 2001)). Our observation for C mice is of interest, as it suggests modest levels of exercise can perhaps lower body mass without necessarily triggering homeostatic compensatory responses in food intake. If true, and if this relationship exists in humans, then it could prove of value for ultimately determining recommended daily exercise criteria.

In adult mice, no effect of early-life wheel access on mass-adjusted food consumption was detected (Fig. 2B, Supplemental Material Tables S5 and S6). Overall, mice with wheel access as juveniles tended to be smaller than no-wheel-access mice, but the effect was not statistically significant (Fig. 1 and Online Supplemental Material Tables S1-S3). Reductions in growth rate and body mass caused by chronic wheel access have been reported many times previously in rodents, including in the HR and C lines of mice ((Swallow et al. 1999); references therein).

Leptin is a hormone secreted by adipocytes, generally in proportion to fat mass. In the present study, as would be expected, visceral fat pad mass was a strong positive predictor of circulating leptin concentrations (Fig. 5). Leptin affects neuroendocrine regulation of body weight by inhibiting food intake and/or increasing energy expenditure. We found that early-life wheel access increased adult plasma leptin levels (adjusted statistically for fat-pad mass as a covariate) in C mice, but decreased them in HR, thus

demonstrating a genotype-by-environment interaction (Fig. 5). Previously, we found that long-term wheel exposure results in low body fat and leptin levels in HR mice, and that either Western diet (high in fat and with added sucrose) or exogenous leptin treatment increased wheel running in adult male HR mice ((Vaanholt et al. 2008; Meek et al. 2010; Meek et al. 2012). This work, together with the current observations, suggests leptin availability is one of the contributing factors regulating wheel running in HR mice. Moreover, leptin appears to play a role in the bidirectional control of activity. This assertion is supported by the fact that restoration of low leptin levels promotes even greater running distances in HR mice, indicating that insufficient leptin concentrations may limit wheel running by serving as a “stop” signal to reduce motivation for activity. Furthermore, elevated levels of leptin, whether through chronic inactivity (Fig. 5A) or experimental high fat diet feeding (Meek et al. 2010), appear to initiate marked homeostatic compensatory increases in volitional physical activity in the form of wheel running, presumably to prevent further weight gain. Collectively, these findings implicate leptin as a critical mediator of physical activity, and in coordinating energetic outputs, such as exercise, with energy intake.

The compensatory hyperphagia observed in HR mice when given access to wheels during the present study suggests that exercise modifies the responses of the central nervous system to adjust energetic needs. Consistent with this hypothesis, the adiposity signal leptin, which acts primarily in the CNS to exert its behavioral effects, remains suppressed through at least 7 week after juvenile wheel exposure in HR mice (Fig. 5), indicating a long-lasting improved sensitivity to the hormone. Interestingly, the

C mice did not exhibit this pattern, but instead developed elevated leptin levels 7 weeks after wheel exposure (Fig. 5). This change in leptin occurred despite no statistically significant effect on body mass or visceral fat pad mass (Fig. 1, Supplemental Material), indicating some disassociation between circulating leptin concentrations and body fat percentage. Further quantitative measures of adiposity, including whole-body levels, will be needed to test this possibility under conditions of altered exercise and/or diet. Another consideration when interpreting changes in circulating leptin concentrations is that although leptin treatment does not alter wheel running in wild type mice (Krawczewski Carhuatanta et al. 2011; Morton et al. 2011) (but does in our HR mice: (Meek et al. 2012), exercise alone does improve leptin sensitivity in rodents, even on a high fat diet. This improvement in leptin sensitivity was demonstrated by greater weight loss and reductions in food intake after an ICV leptin injection, as compared to sedentary mice, and this effect occurred independently of baseline adiposity (Krawczewski Carhuatanta et al. 2011). Thus, although we observed an increase in leptin 7 weeks after juvenile wheel exposure in our C mice, this response may be a harbinger of leptin resistance and future weight gain (none occurred during the present study) in response to the cessation of exercise, or possibly a long lasting physiological response to reverse the exercise induced hyperphagia after wheel access was revoked.

We did not observe a statistically significant effect of early-life wheel access on the trajectory of body-mass gain with age, but mice given early-exercise did tend to be lighter (Fig. 1). The diet used here, as in our routine selective breeding protocols (Teklad Laboratory Rodent Diet [W]-8604), is not considered obesogenic for mice, so it would be

of interest to repeat these studies with a high-fat or Western diet, which is obesogenic for these lines of mice ((Meek et al. 2010; Meek et al. 2012; Meek et al. 2014)). Another point worth mentioning is that "room temperature," as used in the present study and in most studies of laboratory house mice, generally imposes a mild cold stress ((Tschop et al. 2012): [at least when mice are housed individually and/or without nesting materials]). As this sort of cold exposure can protect against obesity in some mice (Feldmann et al. 2009), and housing under different thermal conditions results in alterations in energy expenditure and food intake, it would also be of interest to repeat the present study in mice housed within their thermal-neutral zone (Kaiyala et al. 2015).

Although mice with early-life wheel access ran more on wheels when retested as adults, the effect disappeared after approximately one week because both C (+33.5%) and HR (+58.6%) mice that did not have early-life wheel access increased their wheel running more than those that did have early-life exercise (C -4.0%, HR +27.9%)(see Supplemental Material Table S8). Although the positive effect of early-life exercise lasted for only one week, it is important to note that one week in the life of a mouse is equivalent to approximately nine months for humans ((Demetrius 2006);(Flurkey et al. 2007)). On the other hand, some early-life human intervention studies indicate that beneficial effects can last for at least one year (e.g., (Nemet et al. 2005)). In any case, our results potentially suggest that any positive effects of early-life exercise on adult exercise propensity will require reinforcement and maintenance if they are to be long-lasting.

In summary, we found that early-life access to voluntary exercise had lasting effects on the behavior and physiology of adult mice in ways that depended on genetic

background. Throughout the study, all mice with early exercise were lighter than their non-exercised counterparts, but the effect on plasma leptin concentrations and food consumption depended on genetic background. In all mice, early-life wheel access specifically increased adult wheel running and not cage activity. Use of the polygenic High Runner mouse lines and their controls, the former produced by selective breeding rather than manipulation of a single gene, allows exploration of wide variation in genetically based levels of voluntary exercise, aerobic capacity, body fat, food consumption (Copes et al. 2015), and leptin levels.

Conflict of Interest Statement

The authors declare no conflict of interest.

Acknowledgments

Supported by NSF grant IOS-1121273 to TG. We thank two anonymous reviewers for comments that improved the manuscript.

Supplementary information is available online.

Figure Legends

Figure 1. Early-life wheel access tended to decrease body mass in both Control and High Runner (HR) lines of mice, but the effect was never statistically significant for any measure (Online Supplemental Material Tables S1-S3). Mice from HR lines were lighter than C, and the difference reached statistical significance (2-tailed $P < 0.05$ at the time points indicated by *). No interaction between early-life wheel access and linetype was ever statistically significant. Values are least square means (LSM) + associated SE from SAS Procedure Mixed. Each of the four groups included 24-25 males.

Figure 2. Wheel access significantly increased food consumption during week 3 of early-life wheel access (A) in HR mice, but not in C (wheel access by linetype interaction $P = 0.0143$). During the first week of adult wheel access (B), food consumption was higher in HR mice (linetype $P = 0.0384$), but was not affected by early-life wheel access ($P = 0.7819$; interaction $P = 0.4779$). Mass-adjusted food consumption LSM \pm SE.

Figure 3. Wheel running during the third week of early-life wheel access (beginning just after weaning) and following two months of no wheel access for any group. During week 3, HR mice ran more than non-selected control lines, as reported in numerous previous studies. During week 12, HR mice again ran more than C, and for both linetypes those individual who had early-life wheel access ran significantly more than those that did not. Wheel revolutions per day LSM \pm SE.

Figure 4. During the third week of early-life wheel access, mice housed with wheels showed reduced activity in the home cages attached to the wheels. Relative to mice housed without wheels, mice from the HR lines showed a greater reduction in SPA than did C mice. During week 12, the amount of home-cage activity was somewhat reduced in all groups, and no differences among groups were apparent. Values are LSM \pm SE.

Figure 5. Early-life wheel access increased adult plasma leptin levels (adjusted statistically for fat-pad mass) in C mice (+29% on the backtransformed raw scale), but decreased them in HR (-20%) with juvenile exercise. A) leptin levels (square-root transformed) plotted versus visceral fat pad mass (\log_{10} -transformed) for mice from non-selected Control lines (without wheel access [open symbols], with early life wheel access [solid symbols]). B) leptin levels versus visceral fat pad mass for mice from selectively bred HR lines. C) Least squares means and standard errors from nested analysis of covariance in SAS Procedure Mixed, with \log_{10} t fat pad mass as a covariate. These transformations of leptin and fat pad mass were chosen because they best achieved homogeneity of the spread of the covariate, linearity of the relation with the covariate, and normality of residuals from the statistical model.

Figure 1.1 Body mass

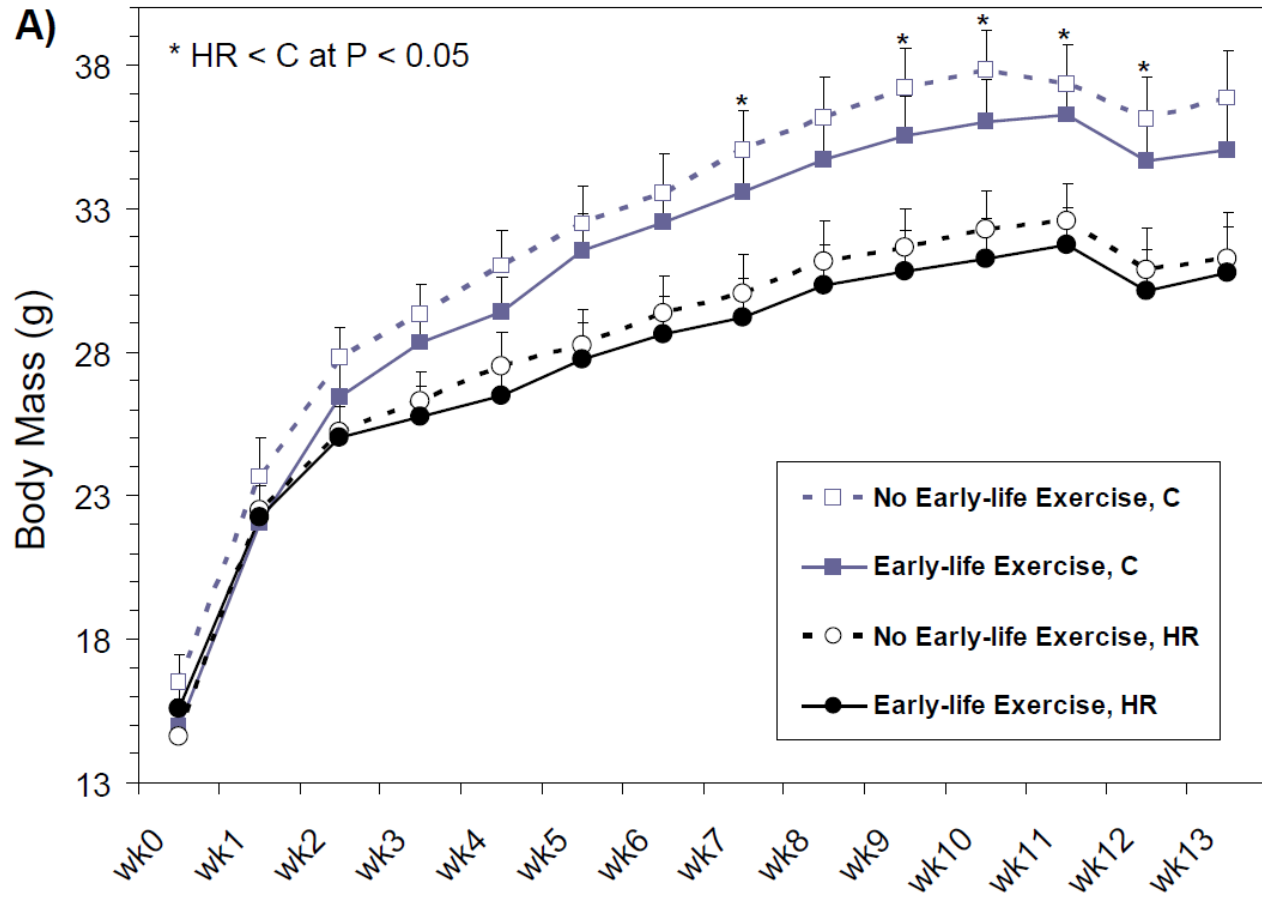


Figure 1.1 Body mass- continued

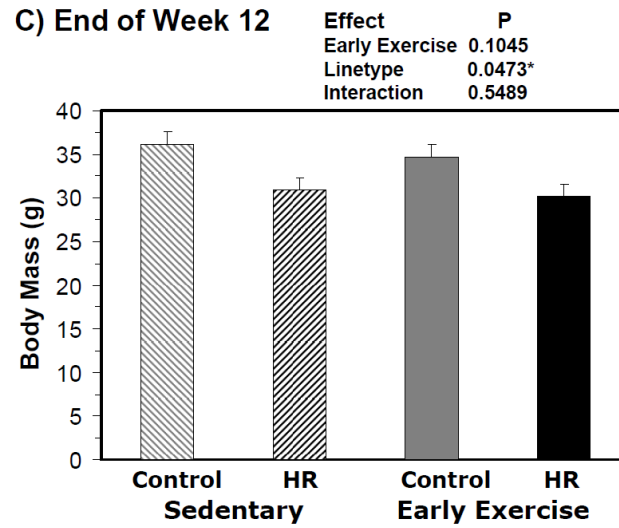
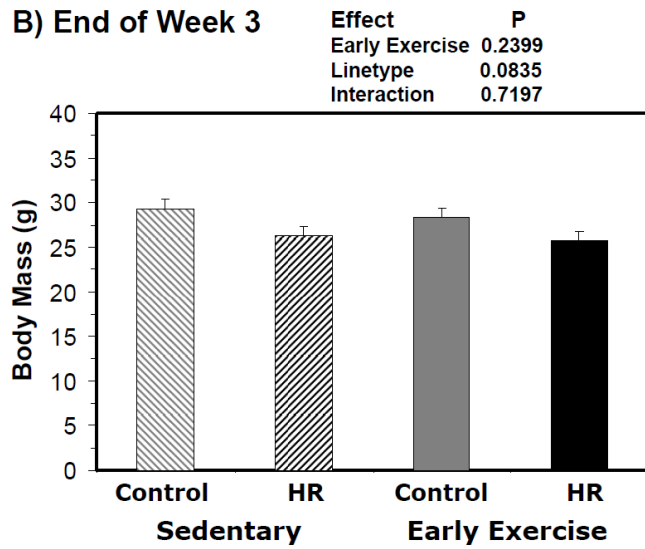


Figure 1.2 Food consumption

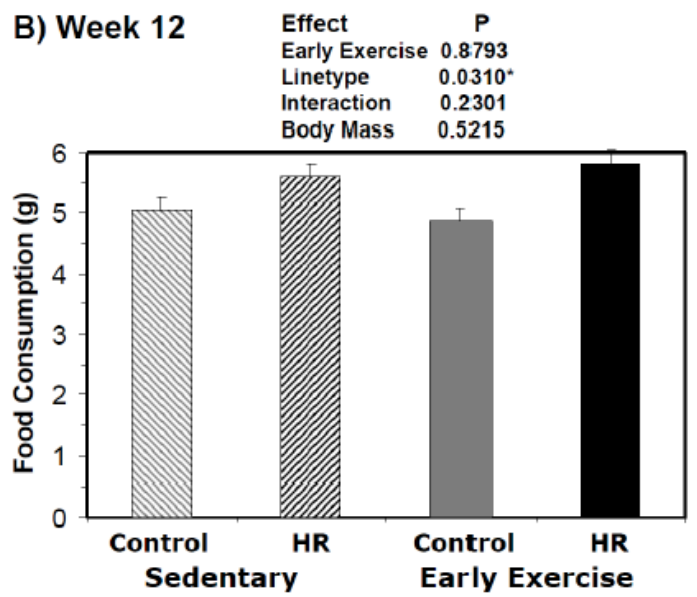
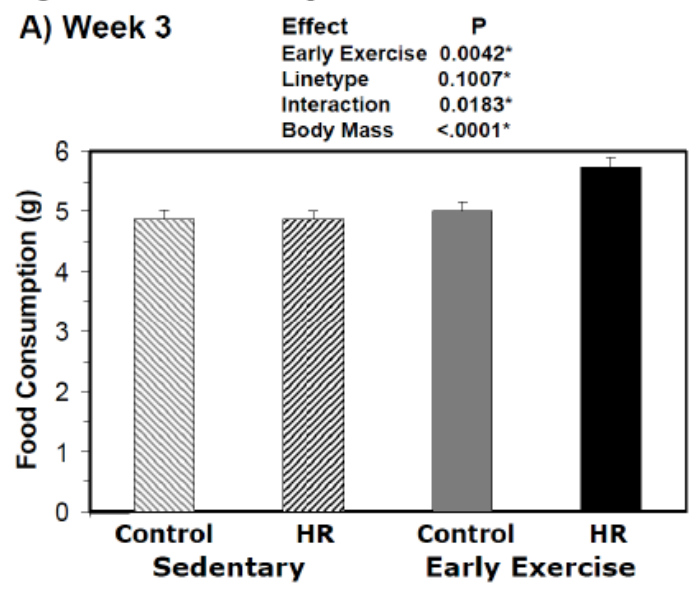


Figure 1.3 Wheel running

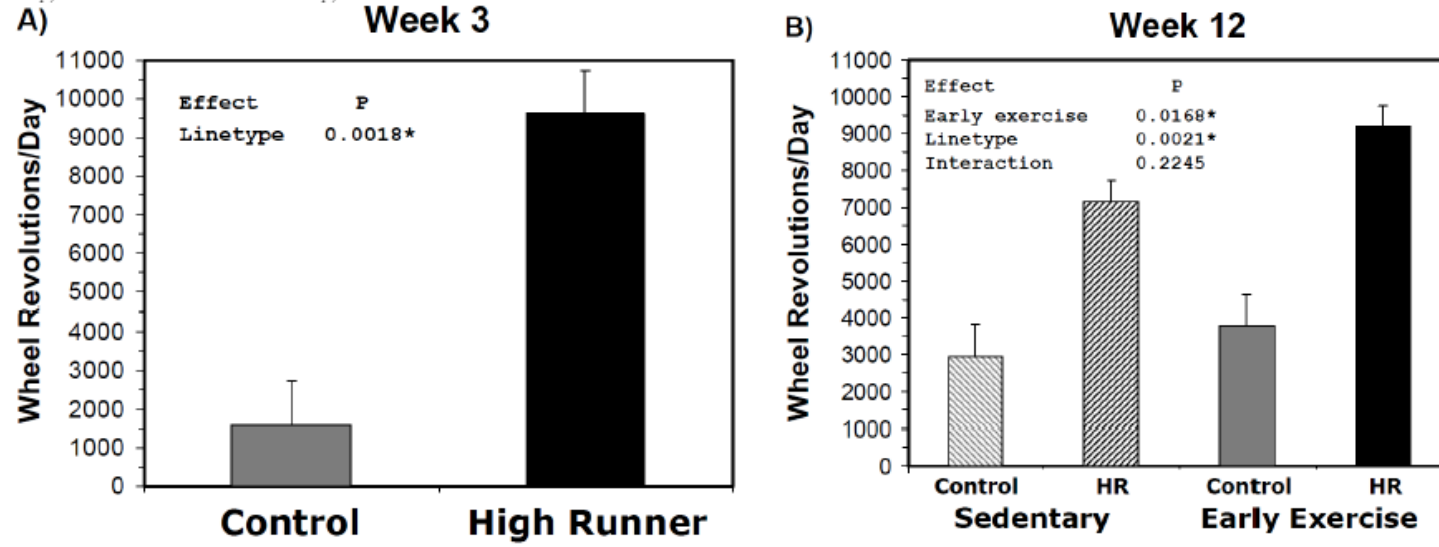


Figure 1.4 Total home-cage activity (HCA)

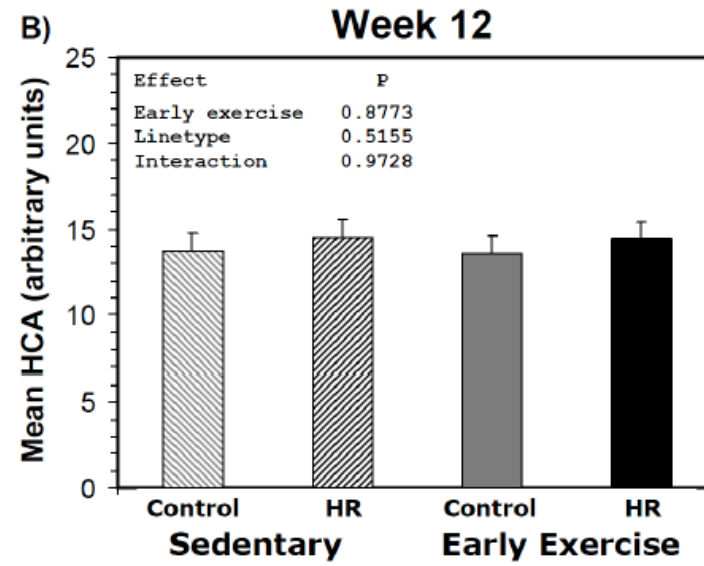
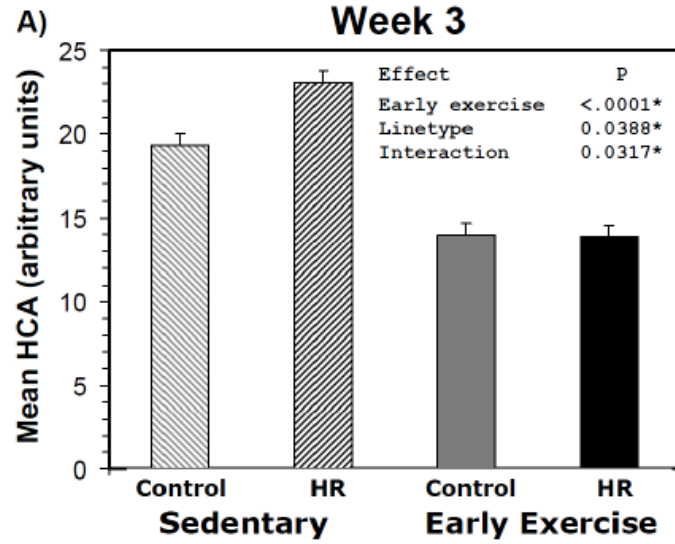
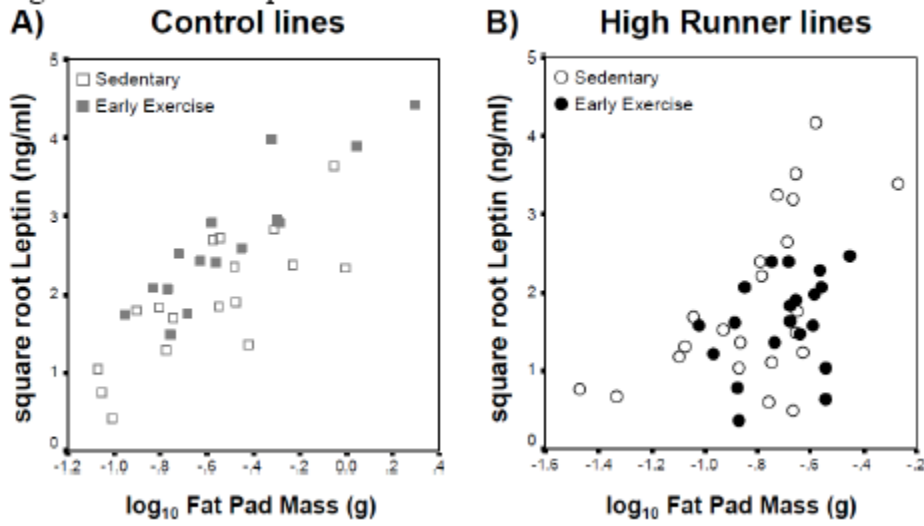
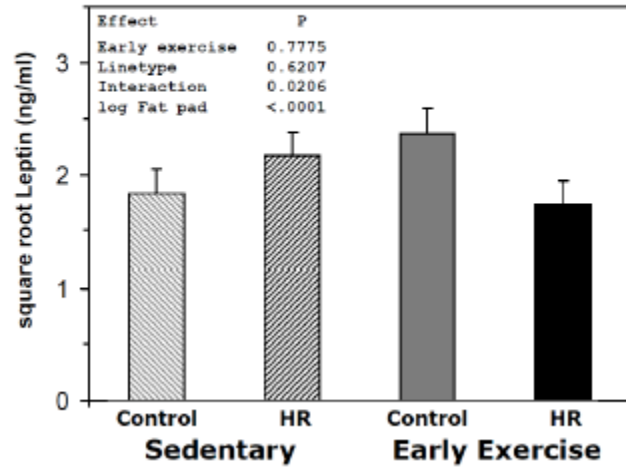


Figure 1.5 Plasma leptin concentrations



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C) Least Square Means + SEs



Online Supplemental Material

Table 1.1 Body mass (g) of all mice before, during, and after three-week period of early-life wheel access. Mice were weaned when 3 weeks old, then had access to wheels (or not) for three weeks. Values are LSM \pm SE.

Variable Name	EEwk0	SE	EEwk1	SE	EEwk2	SE	EEwk3	SE
No Wheels, C	16.507	\pm 0.981	23.654	\pm 1.348	27.775	\pm 1.100	29.320	\pm 1.050
No Wheels, HR	14.620	\pm 0.953	22.476	\pm 1.324	25.234	\pm 1.080	26.295	\pm 1.028
Wheels, C	14.956	\pm 0.981	22.025	\pm 1.348	26.429	\pm 1.100	28.347	\pm 1.050
Wheels, HR	15.567	\pm 0.953	22.238	\pm 1.324	25.003	\pm 1.080	25.758	\pm 1.035
Early Exercise P	0.6594		0.2075		0.2011		0.2399	
Linetype P	0.6148		0.7942		0.2175		0.0835	
Interaction P	0.1034		0.3329		0.3490		0.7197	
N	98		98		98		97	

Table 1.2 Body mass (g) of all mice during the sedentary period (no wheel access), with mice weighed at the start of each week. Values are LSM \pm SE.

Variable Name	Restwk1	SE	Restwk2	SE	Restwk3	SE	Restwk4	SE
No Wheels, C	31.020 \pm	1.204	32.472 \pm	1.283	33.549 \pm	1.344	35.052 \pm	1.355
No Wheels, HR	27.485 \pm	1.180	28.209 \pm	1.269	29.323 \pm	1.330	30.035 \pm	1.341
Wheels, C	29.413 \pm	1.204	31.534 \pm	1.287	32.496 \pm	1.347	33.587 \pm	1.360
Wheels, HR	26.500 \pm	1.188	27.723 \pm	1.273	28.610 \pm	1.334	29.196 \pm	1.345
Early Exercise P	0.0858		0.1963		0.1262		0.0703	
Linetype P	0.0853		0.0594		0.0681		0.0428	
Interaction P	0.6402		0.6601		0.7446		0.5723	
N	97		96		96		96	

Variable Name	Restwk5	SE	Restwk6	SE	Restwk7	SE	Restwk8	SE
No Wheels, C	36.173 \pm	1.418	37.203 \pm	1.390	37.789 \pm	1.421	37.346 \pm	1.348
No Wheels, HR	31.167 \pm	1.403	31.628 \pm	1.373	32.242 \pm	1.400	32.574 \pm	1.309
Wheels, C	34.678 \pm	1.422	35.517 \pm	1.393	36.033 \pm	1.418	36.238 \pm	1.330
Wheels, HR	30.333 \pm	1.407	30.822 \pm	1.378	31.235 \pm	1.401	31.725 \pm	1.310
Early Exercise P	0.0768		0.0667		0.0600		0.1705	
Linetype P	0.0510		0.0338		0.0347		0.0390	
Interaction P	0.5668		0.4600		0.5540		0.8430	
N	96		96		94		93	

Table 1.3. Body mass (g) of mice at end of adult weeks of wheel access, with mice weighed at the start of each week. Values are LSM \pm SE.

Variable Name	AEwk1	SE	AEwk2	SE
No Wheels, C	36.117 \pm	1.465	36.850 \pm	1.655
Wheels, C	34.663 \pm	1.451	35.035 \pm	1.645
No Wheels, HR	30.865 \pm	1.435	31.234 \pm	1.627
Wheels, HR	30.136 \pm	1.436	30.753 \pm	1.628
Early Exercise P	0.1045		0.1059	
Linetype P	0.0473		0.0690	
Interaction P	0.5489		0.3116	
N	93		93	

Table 1.4. Daily food consumption (g) measured over 7 days for all mice during the three-week period of early-life exercise (wheel access). Values are LSM \pm SE.

	FCWKD1	SE	FCWKD2	SE	FCWKD3	SE
No Wheels, C	5.2304	\pm 0.1523	5.4225	\pm 0.1106	4.8699	\pm 0.1505
No Wheels, HR	5.3407	\pm 0.1437	5.4700	\pm 0.1046	4.8568	\pm 0.1460
Wheels, C	5.0392	\pm 0.1517	5.5066	\pm 0.1105	5.0153	\pm 0.1540
Wheels, HR	5.5365	\pm 0.1458	6.1824	\pm 0.1093	5.7417	\pm 0.1499
Early Exercise P	0.9853		0.0064		0.0042	
Linetype P	0.1272		0.0258		0.1007	
Interaction P	0.1605		0.0176		0.0183	
Body Mass P	<.0001		<.0001		<.0001	
N	91		92		94	

Table 1.5. Daily food consumption (g) measured over 7 days for all mice throughout the sedentary period (no wheel access). (Food consumption was not measured during experimental week 4). Values are LSM \pm SE.

	FCWKD5	SE	FCWKD6	SE	FCWKD7	SE	FCWKD8	SE
No Wheels, C	5.2238	\pm 0.1485	5.3231	\pm 0.2336	5.4323	\pm 0.1955	5.5975	\pm 0.1434
No Wheels, HR	5.6133	\pm 0.1429	5.8967	\pm 0.2235	5.7523	\pm 0.1863	5.6406	\pm 0.1367
Wheels, C	5.1474	\pm 0.1456	5.0574	\pm 0.2324	5.2070	\pm 0.1900	5.0727	\pm 0.1449
Wheels, HR	5.6340	\pm 0.1453	5.9503	\pm 0.2306	5.8145	\pm 0.1924	5.7108	\pm 0.1416
Early Exercise P	0.7673		0.5912		0.5058		0.1341	
Linetype P	0.0628		0.0363		0.1171		0.0781	
Interaction P	0.6070		0.6312		0.2521		0.0628	
Body Mass P	<.0001		<.0001		<.0001		<.0001	
N	92		90		89		91	

	FCWKD9	SE	FCWKD10	SE	FCWKD11	SE
No Wheels, C	5.4170	\pm 0.1346	4.2953	\pm 0.1201	4.4559	\pm 0.1066
No Wheels, HR	5.4786	\pm 0.1285	4.4581	\pm 0.1133	4.4942	\pm 0.0995
Wheels, C	5.0598	\pm 0.1302	3.9807	\pm 0.1197	4.1145	\pm 0.1053
Wheels, HR	5.7430	\pm 0.1340	4.5266	\pm 0.1199	4.5928	\pm 0.1030
Early Exercise P	0.6038		0.2171		0.2171	
Linetype P	0.0769		0.0792		0.0792	
Interaction P	0.0095		0.0462		0.0462	
Body Mass P	<.0001		<.0001		<.0001	
N	92		90		90	

Table 1.6. Daily food consumption (g) measured over 7 days for all mice during the two-week period of adult wheel access. Values are LSM \pm SE.

	FCWKD12	SE	FCWKD13	SE
No Wheels, C	5.0438	\pm 0.2173	5.5814	\pm 0.3164
No Wheels, HR	5.6068	\pm 0.2015	6.5466	\pm 0.2956
Wheels, C	4.8715	\pm 0.2069	5.5191	\pm 0.3049
Wheels, HR	5.8259	\pm 0.2101	6.4847	\pm 0.3019
Early Exercise P	0.8793		0.7845	
Linetype P	0.0310		0.0470	
Interaction P	0.2301		0.9992	
Body Mass P	0.5215		0.7068	
N	90		90	

Table 1.7. Average daily wheel running and home-cage activity measured over 7-day periods during the three weeks of early-life wheel access and then again during the two-week period of adult wheel access. Values are LSM \pm SE.

Wheel Running (revolutions/day)

Experimental week	week1		week2		week3	
Variable name	EEwk1	SE	EEwk2	SE	EEwk3	SE
Age	~4 Weeks		~5 Weeks		~6 Weeks	
No Wheels, C						
No Wheels, HR						
Wheels, C	533 \pm 226		1,064 \pm 831		1,595 \pm 1,143	
Wheels, HR	2,241 \pm 201		6,722 \pm 787		9,640 \pm 1,103	
Early Exercise P						
Linetype P	0.0013		0.0026		0.0018	
Interaction P						
N	*43		47		47	

*different N because line 1 mice were born later than most others and so missed a few days of wheel access

Experimental week	week12		week13	
Variable name	AEwk1	SE	AEwk2	SE
Age	~15 Weeks		~16 Weeks	
No Wheels, C	2,943 \pm 883		3,931 \pm 659	
No Wheels, HR	7,142 \pm 569		11,326 \pm 638	
Wheels, C	3,783 \pm 848		3,632 \pm 615	
Wheels, HR	9,187 \pm 553		11,748 \pm 618	
Early Exercise P	0.0168		0.9261	
Linetype P	0.0021		<.0001	
Interaction P	0.2245		0.5929	
N	89		89	

Table 1.7. Average daily wheel running and home-cage activity measured- continued
Home-cage Activity (arbitrary units)

Experimental week	week1		week2		week3	
Variable name	EEwk1	SE	EEwk2	SE	EEwk3	SE
Age	~4 Weeks		~5 Weeks		~6 Weeks	
No Wheels, C	21.4	± 0.94	20.3	± 1.09	19.4	± 0.69
No Wheels, HR	22.3	± 0.87	24.7	± 1.05	23.1	± 0.67
Wheels, C	16.1	± 0.99	15.8	± 1.09	14.0	± 0.69
Wheels, HR	17.3	± 0.87	15.6	± 1.07	13.9	± 0.69
Early Exercise P	0.0012		0.0001		<.0001	
Linetype P	0.2997		0.1609		0.0388	
Interaction P	0.8612		0.0263		0.0317	
N	*88		96		97	

*different N because line 1 mice were born later than most others and so missed a few days of wheel access

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Experimental week	week12		week13	
Variable name	AEwk1	SE	AEwk2	SE
Age	~15 Weeks		~16 Weeks	
No Wheels, C	13.7	± 1.04	3.6	± 0.16
No Wheels, HR	14.5	± 1.01	3.7	± 0.15
Wheels, C	13.6	± 1.03	3.4	± 0.15
Wheels, HR	14.5	± 0.99	3.6	± 0.15
Early Exercise P	0.8773		0.3991	
Linetype P	0.5155		0.5069	
Interaction P	0.9728		0.5779	
N	89		89	

Table 1.8. Organ masses (g) and body length (mm) of all mice at the end of the two-week period of adult wheel access. Values are LSM \pm SE.

	log ₁₀ Visceral Fat Pad Mass		log ₁₀ Heart Ventricle Mass		log ₁₀ Liver Mass		log ₁₀ Spleen Mass		Body Length	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
No Wheels, C	-0.7150	± 1.204 ₀	-0.8056	± 0.018 ₃	0.2937	± 0.024 ₀	-1.0727	± 0.039 ₃	99.098 ₂	± 1.118 ₁
No Wheels, HR	-0.6421	± 1.204 ₀	-0.7593	± 0.017 ₂	0.2809	± 0.023 ₃	-1.0763	± 0.037 ₈	95.514 ₉	± 1.076 ₀
Wheels, C	-0.6458	± 1.180 ₄	-0.8159	± 0.017 ₅	0.2891	± 0.023 ₇	-1.0946	± 0.038 ₁	97.087 ₆	± 1.105 ₁
Wheels, HR	-0.5599	± 1.188 ₁	-0.7525	± 0.017 ₅	0.2975	± 0.023 ₄	-1.1005	± 0.038 ₂	95.011 ₁	± 1.089 ₆
Early Exercise P	0.1228		0.8521		0.6273		0.1625		0.0873	
Linetype P	0.4952		0.0616		0.9460		0.9315		0.0941	
Interaction P	0.8811		0.3732		0.3970		0.9391		0.2671	
log ₁₀ Body Mass P	<0.000 ₁		0.0002		<0.000 ₁		<0.000 ₁			
N	92		92		92		92		90	

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References

- Adam, T. C., and E. S. Epel. 2007. Stress, eating and the reward system. *Physiol Behav.* 91:449-458.
- Andersen, L. G., L. Angquist, M. Gamborg, L. Byberg, C. Bengtsson, D. Canoy, J. G. Eriksson, M. Eriksson, M. R. Jarvelin, L. Lissner, T. I. Nilsen, M. Osler, K. Overvad, F. Rasmussen, M. K. Salonen, L. Schack-Nielsen, T. H. Tammelin, T. P. Tuomainen, T. I. A. Sorensen, and J. L. Baker. 2009. Birth weight in relation to leisure time physical activity in adolescence and adulthood: meta-analysis of results from 13 Nordic cohorts. *Plos One* 4.
- Baker, M. S., Li G., Kohorst J.J., and Waterland R.A. 2015. Fetal growth restriction promotes physical inactivity and obesity in female mice. *Int J Obes* 39:98-104.
- Basterfield, L., L. K. Lumley, and J. C. Mathers. 2009. Wheel running in female C57BL/6J mice: impact of oestrus and dietary fat and effects on sleep and body mass. *Int J Obes (Lond)*. 33:212-218.
- Belcher, B. R., D. Berrigan, K. W. Dodd, B. A. Emken, C. P. Chou, and D. Spruijt-Metz. 2010. Physical activity in US youth: effect of race/ethnicity, age, gender, and weight status. *Med. Sci. Sports Exerc.* 42:2211-2221.
- Cinti, S. 2005. The adipose organ. *Prostaglandins Leukotrienes Essential Fatty Acids.* 73:9-15.
- Coccorello, R., F. R. D'Amato, and A. Moles. 2009. Chronic social stress, hedonism and vulnerability to obesity: Lessons from Rodents. *Neurosci. Biobehav. Rev.* 33:537-550.
- Copes, L. E., H. Schutz, E. M. Dlugosz, W. Acosta, M. A. Chappell, and T. Garland Jr. 2015. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. *Physiol. Behav.* 149:86-94.
- Demetrius, L. 2006. Aging in mouse and human systems: a comparative study. *Ann N Y Acad Sci.* 1067:66-82.
- Dishman, R. K., J. F. Sallis, and D. R. Orenstein. 1985. The determinants of physical activity and exercise. *Public Health Reports* 100:158-171.
- Eisenmann, J. C. 2003. Secular trends in variables associated with the metabolic syndrome of North American children and adolescents: A review and synthesis. *Am. J. Hum. Biol.* 15:786-794.

- Eisenmann, J. C. 2004. Physical activity and cardiovascular disease risk factors in children and adolescents: An overview. *Can. J. Cardiol.* 20:295-301.
- Eisenmann, J. C. 2006. Insight into the causes of the recent secular trend in pediatric obesity: Common sense does not always prevail for complex, multi-factorial phenotypes. *Prev. Med.* 42:329-335.
- Eisenmann, J. C., and E. E. Wickel. 2009. The biological basis of physical activity in children: revisited. *Pediatric Exercise Science* 21:257-272.
- Feldmann, H. M., V. Golozoubova, B. Cannon, and J. Nedergaard. 2009. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9:203-209.
- Flurkey, K., J. M. Curren, and D. E. Harrison. 2007. The Mouse in Aging Research. Pp. 637–672 *in* e. a. Fox JG, editors, ed. *The Mouse in Biomedical Research* 2nd ed. American College Laboratory Animal Medicine (Elsevier), Burlington, MA.
- Garland, T., Jr., S. A. Kelly, J. L. Malisch, E. M. Kolb, R. M. Hannon, B. K. Keeney, S. L. Van Cleave, and K. M. Middleton. 2011a. How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proceedings of the Royal Society B: Biological Sciences* 278:574-581.
- Garland, T., Jr., H. Schutz, M. A. Chappell, B. K. Keeney, T. H. Meek, L. E. Copes, W. Acosta, C. Drenowatz, R. C. Maciel, G. van Dijk, C. M. Kotz, and J. C. Eisenmann. 2011b. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *Journal of Experimental Biology* 214:206-229.
- Gebczynski, A. K., and M. Konarzewski. 2009. Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy. *Journal of Experimental Biology* 212:2872-2878.
- Girard, I., E. Rezende, and T. Garland, Jr. 2007. Leptin levels and body composition of mice selectively bred for high voluntary locomotor activity. *Physiol. Biochem. Zool.* 80:568-579.
- Hallal, P. C., J. C. K. Wells, F. F. Reichert, L. Anselmi, and C. G. Victora. 2006. Early determinants of physical activity in adolescence: prospective birth cohort study. *Br. Med. J.* 332:1002-1005.
- Hannon, R. M., S. A. Kelly, K. M. Middleton, E. M. Kolb, D. Pomp, and T. Garland. 2008. Phenotypic effects of the "mini-muscle" allele in a large HR x C57BL/6J mouse backcross. *Journal of Heredity* 99:349-354.

- Hayes, C., and A. Kriska. 2008. Role of physical activity in diabetes management and prevention. *J Am Diet Assoc.* 108:S19-23.
- Jung, A. P., T. S. Curtis, M. J. Turner, and J. T. Lightfoot. 2006. Influence of age of exposure to a running wheel on activity in inbred mice. *Med. Sci. Sports Exerc.* 38:51-56.
- Jung, A. P., T. S. Curtis, M. J. Turner, and J. T. Lightfoot. 2010. Physical activity and food consumption in high- and low-active inbred mouse strains. *Medicine & Science in Sports & Exercise* 42:1826-1833.
- Kaiyala, K. J., K. Ogimoto, J. T. Nelson, M. W. Schwartz, and G. J. Morton. 2015. Leptin signaling is required for adaptive changes in food intake, but not energy expenditure, in response to different thermal conditions. *PLoS One* 10:e0119391.
- Keeney, B., T. Meek, K. Middleton, L. Holness, and T. Garland, Jr. 2012. Sex differences in cannabinoid receptor-1 (CB1) pharmacology in mice selectively bred for high voluntary wheel-running behavior. *Pharmacol. Biochem. Behav.* 101:528-537.
- Kelly, S. A., D. L. Nehrenberg, K. Hua, T. Garland, Jr., and D. Pomp. 2011. Exercise, weight loss, and changes in body composition in mice: phenotypic relationships and genetic architecture. *Physiol Genomics* 43:199-212.
- Kelly, S. A., D. L. Nehrenberg, K. Hua, T. Garland, Jr., and D. Pomp. 2014. Quantitative genomics of voluntary exercise in mice: transcriptional analysis and mapping of expression QTL in muscle. *Physiol Genomics* 46:593-601.
- Kelly, S. A., D. L. Nehrenberg, K. Hua, T. Garland, and D. Pomp. 2012. Functional genomic architecture of predisposition to voluntary exercise in mice: expression QTL in the brain. *Genetics* 191:643-654.
- Koeneman, M. A., M. W. Verheijden, M. J. M. Chinapaw, and M. Hopman-Rock. 2011. Determinants of physical activity and exercise in healthy older adults: a systematic review. *Int J Behav Nutr Phys Act* 8:142.
- Krawczewski Carhuatanta, K., A., G. Demuro, M. H. Tschop, P. Pfluger, T., S. Benoit, C., and S. Obici. 2011. Voluntary exercise improves high-fat diet-induced leptin resistance independent of adiposity. *Endocrinology* 152:2655-2664.
- Li, G., J. J. Kohorst, W. Zhang, E. Laritsky, G. Kunde-Ramamoorthy, M. S. Baker, M. L. Fiorotto, and R. A. Waterland. 2013. Early postnatal nutrition determines adult physical activity and energy expenditure in female mice. *Diabetes* 62:2773-2783.

- Lopez, D. S., S. B. Peskoe, C. E. Joshi, A. Dobs, M. Feinleib, N. Kanarek, W. G. Nelson, E. Selvin, S. Rohrmann, and E. A. Platz. 2013. Racial/ethnic differences in serum sex steroid hormone concentrations in US adolescent males. *Cancer Causes Control* 24:817-826.
- Lussana, F., R. C. Painter, M. C. Ocke, H. R. Buller, P. M. Bossuyt, and T. J. Roseboom. 2008. Prenatal exposure to the Dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. *The American Journal of Clinical Nutrition* 88:1648-1652.
- Malisch, J., C. Breuner, E. Kolb, H. Wada, R. Hannon, M. Chappell, K. Middleton, and T. Garland, Jr. 2009. Behavioral despair and home-cage activity in mice with chronically elevated baseline corticosterone concentrations. *Behavior Genetics* 39:192-201.
- Malisch, J. L., C. W. Breuner, F. R. Gomes, M. A. Chappell, and T. Garland. 2008. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *General and Comparative Endocrinology* 156:210-217.
- Malisch, J. L., W. Saltzman, F. R. Gomes, E. L. Rezende, D. R. Jeske, and T. Garland. 2007. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol. Biochem. Zool.* 80:146-156.
- Mattocks, C., K. Deere, S. Leary, A. Ness, K. Tilling, S. N. Blair, and C. Riddoch. 2008. Early life determinants of physical activity in 11 to 12 year olds: cohort study. *Br. Med. J.* 336:26-29.
- McAllister, E. J., N. V. Dhurandhar, S. W. Keith, L. J. Aronne, J. Barger, M. Baskin, R. M. Benca, J. Biggio, M. M. Boggiano, J. C. Eisenmann, M. Elobeid, K. R. Fontaine, P. Gluckman, E. C. Hanlon, P. Katzmarzyk, A. Pietrobelli, D. T. Redden, D. M. Ruden, C. Wang, R. A. Waterland, S. M. Wright, and D. B. Allison. 2009. Ten putative contributors to the obesity epidemic. *Critical Reviews in Food Science and Nutrition* 49:868-913.
- Meek, T., J. Eisenmann, and T. Garland, Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *Int. J. Obes.* 34:960-969.
- Meek, T. H., E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2012. Effects of leptin treatment and Western diet on wheel running in selectively bred high runner mice. *Physiol Behav* 106:252-258.

- Meek, T. H., J. C. Eisenmann, B. K. Keeney, R. M. Hannon, E. M. Dlugosz, and T. Garland. 2014. Effects of early-life exposure to Western diet and wheel access on metabolic syndrome profiles in mice bred for high voluntary exercise. *Genes, Brain and Behavior* 13:322-332.
- Meek, T. H., B. P. Lonquich, R. M. Hannon, and T. Garland. 2009. Endurance capacity of mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology* 212:2908-2917.
- Morton, G. J., K. J. Kaiyala, J. D. Fisher, K. Ogimoto, M. W. Schwartz, and B. E. Wisse. 2011. Identification of a physiological role for leptin in the regulation of ambulatory activity and wheel running in mice. *Am J Physiol Endocrinol Metab* 300:9.
- Nathanielsz, P. W. 2006. Animal Models That Elucidate Basic Principles of the Developmental Origins of Adult Diseases. *ILAR Journal* 47:73-82.
- Nehrenberg, D. L., K. Hua, D. Estrada-Smith, T. Garland, and D. Pomp. 2009. Voluntary exercise and its effects on body composition depend on genetic selection history. *Obesity* 17:1402-1409.
- Nemet, D., S. Barkan, Y. Epstein, O. Friedland, G. Kowen, and A. Eliakim. 2005. Short- and long-term beneficial effects of a combined dietary-behavioral-physical activity intervention for the treatment of childhood obesity. *Pediatrics*. 115:e443-449.
- Papas, M. A., A. J. Alberg, R. Ewing, K. J. Helzlsouer, T. L. Gary, and A. C. Klassen. 2007. The built environment and obesity. *Epidemiologic Reviews* 29:129-143.
- Patterson, C. M., S. G. Bouret, A. A. Dunn-Meynell, and B. E. Levin. 2009. Three weeks of postweaning exercise in DIO rats produces prolonged increases in central leptin sensitivity and signaling. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 296:R537-R548.
- Patterson, C. M., and B. E. Levin. 2007. Role of exercise in the central regulation of energy homeostasis and in the prevention of obesity. *Neuroendocrinology* 87:65-70.
- Patterson, Z. R., and A. B. Abizaid. 2013. Stress induced obesity: Lessons from rodent models of stress. *Frontiers in Neuroscience* 7.
- Rezende, E. L., F. R. Gomes, J. L. Malisch, M. A. Chappell, and T. Garland. 2006. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. *Journal of Applied Physiology* 101:477-485.

- Rhodes, J. S., S. C. Gammie, and T. J. Garland. 2005. Neurobiology of mice selected for high voluntary wheel-running activity. *Integr. Comp. Biol.* 45:438-455.
- Sallis, J. F., and M. F. Hovell. 1990. Determinants of Exercise Behavior. *Exerc. Sport Sci. Rev.* 18:307-330.
- Schroeder, M., L. Shbiro, V. Gelber, and A. Weller. 2010. Post-weaning voluntary exercise exerts long-term moderation of adiposity in males but not in females in an animal model of early-onset obesity. *Hormones and Behavior* 57:496-505.
- Smyth, S., and A. Heron. 2006. Diabetes and obesity: the twin epidemics. *Nat Med.* 12:75-80.
- Stein, A. D., A. Rundle, N. Wada, R. A. Goldbohm, and L. H. Lumey. 2009. Associations of gestational exposure to famine with energy balance and macronutrient density of the diet at age 58 years differ according to the reference population used. *The Journal of Nutrition* 139:1555-1561.
- Steinberger, J., S. R. Daniels, R. H. Eckel, L. Hayman, R. H. Lustig, B. McCrindle, and M. L. Mietus-Snyder. 2009. Progress and challenges in metabolic syndrome in children and adolescents a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 119:628-647.
- Swallow, J. G., T. Garland, P. A. Carter, W. Z. Zhan, and G. C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *Journal of Applied Physiology* 84:69-76.
- Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland, Jr. 1999. Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. *Journal of Experimental Biology* 202:2513-2520.
- Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland, Jr. 2001. Food consumption and body composition in mice selected for high wheel-running activity. *J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol.* 171:651-659.
- Tschop, M. H., J. R. Speakman, J. R. S. Arch, J. Auwerx, J. C. Bruning, L. Chan, R. H. Eckel, R. V. Farese Jr, J. E. Galgani, C. Hambly, M. A. Herman, T. L. Horvath, B. B. Kahn, S. C. Kozma, E. Maratos-Flier, T. D. Muller, H. Munzberg, P. T. Pfluger, L. Plum, M. L. Reitman, K. Rahmouni, G. I. Shulman, G. Thomas, C. R.

- Kahn, and E. Ravussin. 2012. A guide to analysis of mouse energy metabolism. *Nat Meth* 9:57-63.
- Twisk, J. W. R. 2001. Physical activity guidelines for children and adolescents: A critical review. *Sports Med.* 31:617-627.
- Vaanholt, L. M., I. Jonas, M. Doornbos, K. A. Schubert, C. Nyakas, T. Garland, G. H. Visser, and G. van Dijk. 2008. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int. J. Obes.* 32:1566-1575.
- Warburton, D. E., C. W. Nicol, and S. S. Bredin. 2006. Health benefits of physical activity: the evidence. *Can Med Assoc J* 174:801-809.
- Weaver, I. C. G. 2009. Epigenetic effects of glucocorticoids. *Seminars in Fetal & Neonatal Medicine* 14:143-150.
- Yang, Z. Y., and S. L. Huffman. 2013. Nutrition in pregnancy and early childhood and associations with obesity in developing countries. *Matern. Child Nutr.* 9:105-119.

Chapter 2

Preference for Western diet is altered in High Runner mice and affects voluntary exercise and spontaneous physical activity in a genotype-dependent manner

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Running Title: Food preference, Western Diet, home cage activity, wheel running

HIGHLIGHTS

- Preference for Western diet (WD) was tested in genetically High Runner (HR) mice
- HR mice of both sexes preferred WD to a significantly greater extent than did Control mice
- WD had a larger stimulatory effect on wheel running in HR mice than in Control mice
- Home-cage activity declined when wheel running increased (including with WD)
- Substituting WD for chow revealed strong genotype-by-environment interactions

ABSTRACT

Do animals choose diets that positively affect their performance abilities? We addressed this question by examining preference for Western diet (WD), high in fat and sugar, versus standard rodent chow in adults of both sexes from 4 lines of mice selectively bred for high levels of voluntary wheel running (High Runner or HR lines) and 4 non-selected control (C) lines. We also assessed whether food preference or substitution affects physical activity (wheel running and/or spontaneous physical activity [SPA] in the attached home cages). In experiment 1 (generation 56), mice were given 6 days of wheel acclimation (as is used routinely to pick breeders in the selection experiment) prior to a 2-day food choice trial. In experiment 2 (generation 56), 17 days of wheel acclimation allowed mice to reach a stable level of daily running, followed by a 7-day food-choice trial. In experiment 3 (generation 58), mice had 6 days of wheel acclimation with standard chow, after which half were switched to WD for two days. In experiment 1, WD was highly preferred by all mice, with somewhat greater preference in male C mice. In experiment 2, wheel running increased and SPA decreased continuously for the first 14 days of adult wheel testing, followed by 3-day plateaus in both. During the subsequent 7-day food choice trial, HR mice of both sexes preferred WD significantly more than did C mice. Wheel running increased in all groups except males from C lines, with the increase being significantly greater in HR than C, while SPA declined further in all groups. In experiment 3, the effect of being switched to WD depended on both linetype and sex. On standard chow, only HR females showed a significant change in wheel running during nights 7+8, increasing by 10%. In contrast, on WD, C females (+28%), HR females

(+33%), and HR males (+10%) all significantly increased their daily wheel running distances. Our results show that dietary preferences have coadapted with performance abilities in lines of mice bred for high levels of voluntary exercise.

Keywords: Food choice, Genotype-by-environment interaction, Selection experiment, Sex differences, Spontaneous physical activity, Wheel running

1. Introduction

Many studies link physical inactivity and/or inappropriate diet to various health problems and chronic diseases in modern human populations (e.g., obesity, cardiovascular disease, hypertension, diabetes) (Booth et al. 2002; Roberts and Barnard 2005), although the exact causes remain controversial (e.g., (Westerterp and Speakman 2008; McAllister et al. 2009)). Nonetheless, it is well established that various types of exercise ((Carroll and Dudfield 2004; Booth and Roberts 2008; Bouassida et al. 2008)) as well as dietary manipulations ((Kant 2000; Drewnowski 2007; Centers for Disease Control and Prevention National Center for Chronic Disease Prevention and Health Promotion 2010; Westerterp 2010; Bauer et al. 2013)) can lead to improvements in body composition, metabolic status, and mental health.

Several lines of evidence indicate that diet and physical activity can influence each other, both acutely and chronically. Effects of physical activity on appetite have been studied extensively ((Mayer et al. 1954; King et al. 1997; King 1998; Westerterp 2010)). Generally, food or energy intake increases as physical activity increases (Mayer et al. 1954; Smith et al. 1982; Koteja et al. 1999b; Westerterp 2010). Moreover, in a few cases, physical activity has been shown to affect dietary choice. For example, Tour de France cyclists voluntarily consumed more carbohydrates as their primary fuel source during their training than when not training to maximize glycogen power output (Saris et al. 1989).

In laboratory rodents, diet choice and composition affect physical activity and whole-animal performance. For example, 3 months of high-fat diet increased maximal

aerobic capacity ($VO_2\text{max}$) by 15 percent in sedentary rats, whereas endurance training for 3 months increased $VO_2\text{max}$ by 20 percent (Simi et al. 1991), and the effects were additive. A different study of rats, compared effects of a high-fat versus a high-carbohydrate diet fed for 2 months on exercise endurance and body composition. Half of the rats had their diets switched for 3 days prior to endurance testing. Rats that received the high-fat diet first, followed by the high-carbohydrate diet, fatigued later than the rats fed a high-carbohydrate or high-fat diet only (Lapachet et al. 1996). In another study of rats fed one of three diets (high in saturated fat, high in n-3 fatty acids, or high in n-6 fatty acids), those fed the high n-6 diet increased treadmill endurance-running performance compared to the other groups (Ayre and Hulbert 1997). Increased consumption of polyunsaturated fatty acids (PUFAs) has also increased treadmill endurance-running performance in rats (Ayre and Hulbert 1997). A large literature also concerns effects of dietary supplements and carbohydrate loading on human athletic performance (e.g., (Mundal et al. 1998; Bucci 2000)).

Diet choice and composition can also affect physical activity and whole-animal performance in wild animals. For example, a field study demonstrated that just prior to their long-distance migration from Canada to South America, sandpipers consumed a diet rich in amphipod crustaceans, which have greater polyunsaturated fatty acids (PUFA) content than other crustaceans (Weber 2009). Unsaturated fatty acids have increased fluidity, accelerating peroxidation rates and influx into cells more than saturated fatty acids (Shaikh and Edidin 2006) (Maillet and Weber 2006). Enhanced fuel accessibility may be the reason sandpipers consume certain crustaceans prior to migration. Similarly,

migratory red-eyed vireos prefer long-chain unsaturated fatty acids over long-chain saturated fatty acids (Pierce et al. 2004). In a different experiment, vireos had improved aerobic performance (mass-specific peak metabolic rate during forced exercise) with a diet containing lower unsaturated fatty acids as compared with a diet containing more unsaturated fatty acid (Pierce et al. 2005). These and other studies (e.g., (Frank et al. 1998); (Weber 2009)) support the general hypothesis that dietary preferences should coadapt with other aspects of behavioral and physiological ecology in ways that facilitate organismal performance abilities ((Bauwens et al. 1995); (Angilletta Jr et al. 2006)).

The purpose of the present study was to examine preference for "Western diet" (high in fat and sugar) and the effects of Western diet on physical activity in a unique rodent model: four replicate lines of High Runner (HR) mice that have experienced long-term selective breeding for high levels of voluntary exercise on wheels, as compared with four non-selected control (C) lines (Swallow et al. 1998a; Swallow et al. 2009; Acosta et al. 2015). In addition to running voluntarily 2.5-3-fold more revolutions per day, HR mice have increased endurance capacity (Meek et al. 2009) and maximal aerobic capacity (Kolb et al. 2010), reduced body fat (Swallow et al. 2001; Girard et al. 2007), lower circulating leptin levels that are not explained solely by their lower body fat (Girard et al. 2007; Acosta et al. 2015), and alterations in the brain reward system (Rhodes et al. 2005; Belke and Garland 2007), and may experience withdrawal symptoms when wheel access is removed (Kolb et al. 2013). Moreover, previous studies found that Western diet can positively affect wheel running in male HR mice but not in C (Meek et al. 2010; Meek et al. 2014). This unusual response of HR mice could be related to constraints that their

hyper-lean phenotype places on sustained endurance exercise (given that they have inherently high motivation for voluntary exercise) and/or an effect on the reward they receive from running, alternatives that have yet to be discriminated.

Here, we expand on previous studies by including measures of dietary preference, home-cage activity, early-life testing effects on adults and testing both sexes. We hypothesized that HR mice would have elevated preference for Western diet compared to C mice, and that this differences might depend on context, e.g., whether they were fully acclimated to wheels when tested. In addition, we hypothesized that HR mice might respond differently from C mice, in terms of wheel running and/or home-cage activity, when given Western diet. Finally, we expected males and females might differ in food preferences and responses to Western diet in a manner that depends on genetic background (C vs, HR).

2. Materials and procedures

2.1 Experimental animals

Mice employed in these experiments were from generations 56 and 58 of a broader, ongoing artificial selection experiment that breeds for high voluntary wheel running activity (for more details on the selection process, see (Swallow et al. 1998a; Careau et al. 2013);(Careau et al. 2015). The base population consisted of 224 outbred, genetically variable Hsd:ICR house mice (*Mus musculus*). Ten pairs of mice were used to create eight closed lines, four of which were randomly designated and bred for high

running (HR) on wheels and the other four were non-selected control (C) lines, bred without regard to wheel running. In each generation, mice are weaned at 21 days of age and housed in same-sex groups of four until approximately 6-8 weeks of age, when they 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 that records revolutions in 1-minute intervals continuously for 6 days of wheel testing. In the HR lines, breeders for the next generation are chosen based on their wheel running for days 5 and 6. For the HR lines, the highest-running males and females within each family are chosen as breeders, whereas random males and females are chosen from within families in the C lines (sibling mating is disallowed in all lines). Room temperature was maintained at approximately 22°C. Lights were on at 0700 with a photoperiod of 12:12h. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604 or Harlan Teklad Custom Research Western Diet TD.88137) were available *ad libitum*.

2.2 Experiment 1 (N= 326) and experiment 2 (N=96): Preference for Western diet

Two sets of mice sampled from generation 56 were studied. Both groups were tested at approximately 6-8 weeks of age, similar to the standard selection procedures. In experiment 1, mice were given short-term wheel access prior to food choice trials (6 days, as is used routinely to pick breeders). In experiment 2, mice had 17 days of wheel access (long-term) prior to food choice trials. This extended period of wheel access allowed mice to reach a plateau in wheel running, as has been reported previously (e.g.,

see (Swallow et al. 2001)). Diet preference was measured by dividing the standard food hoppers into two subsections with a stainless steel plate. Each half was filled with a weighed amount of either standard chow or Western diet. Food hoppers were reweighed after seven additional days of wheel access, and the bedding (wood shavings) was checked for pieces of uneaten food that were weighed and accounted for in determination of food intake (Koteja et al. 2003). All mice were monitored for wheel running, food consumption, and body mass; home-cage activity was also monitored in experiment 2 (long-term) mice.

2.3 Experiment 3(N=313): Substitution of Western diet

Mice from generation 58 were subjected to food substitution. After having wheel access and standard chow for 6 days (the usual for the selection experiment protocol) half of the mice were switched to Western diet for two days. We used these last two days because the selection criterion in the ongoing experiment uses two days of wheel running data. Each food hopper was weighed as described above, before and after the two days of additional wheel running.

2.4 Measurement of wheel running and home-cage activity

Mice were individually housed in standard cages attached to a Wahman-type activity wheel (as described above). Wheel running was recorded for 23 h each day (1300-1200), with the final hour used for data collection and checks of wheel function and mouse health.

Spontaneous physical activity (SPA) in the attached home cages was monitored as previously described (Copes et al. 2015) using infrared motion-detector sensors (similar to (Gebczynski and Konarzewski 2009)) that detect movement within the standard housing cages. Briefly, sensors were interfaced to a Macintosh personal computer running custom recording software developed by Mark A. Chappell. The software measured activity summed over every 1-minute interval for 23 hours. Sensor status was recorded 3 times per second as either movement (1) or no movement (0). A mean value (0-1) with arbitrary units was computed for each minute. Total home-cage activity (referred to as spontaneous physical activity or SPA) was taken as the sum of all activity over 23 hours. The number of 1-minute intervals showing *any* SPA were also computed and tallied to indicate the duration (minutes) of SPA 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 SPA. The SPA measures were recorded simultaneously with wheel running. Further SPA measurement details are listed elsewhere (Copes et al. 2015). Data for both SPA and wheel running were downloaded daily between 1200 and 1300 hours. All methods were approved by and are in agreement with the regulations of the Institutional Animal Use and Care Committee of the University of California, Riverside.

2.5 Statistical analyses

Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects. Linetype (HR or C), diet type (if applicable), and sex were treated as fixed effects; line was nested within line type as a random effect. Effects of linetype, sex, and diet and their respective interactions were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6. Covariates depended on the trait being analyzed and included age, body mass, wheel freeness (an inverse measure of rotational resistance (Copes et al. 2015)), SPA sensor calibration (measure of sensor motion sensitivity (Copes et al. 2015)), and total amount of wheel running. Dependent variable and/or covariates were transformed as necessary to improve the homogeneity of the spread of the covariates, linearity of relations, and the normality of the residuals.

3. Results

3.1 Experiment 1 and Experiment 2: Preference for Western diet

3.11 Wheel running before food choice

Mice from the High Runner lines, as expected from numerous previous studies (e.g., (Koteja et al. 1999a; Meek et al. 2010; Acosta et al. 2015; Copes et al. 2015)), ran significantly more than those from Control lines in both short-term (experiment 1, Fig. 1A) and long-term (experiment 2, Fig. 1B) wheel-access groups. HR mice ran

significantly more than C mice before the food choice on days 5 + 6 of wheel access ($P=0.0006$), showing a nearly-significant sex effect, with females running more than males on days 5 and 6 ($P=0.0578$), and a non-significant sex-by-linetype interaction ($P=0.3886$) (Fig. 1A). For the long-term mice (experiment 2), HR ran significantly more than C mice before the food choice on days 14-17 of wheel access ($P<0.0001$), females ran more than males ($P=0.0050$), and a significant sex-by-linetype interaction ($P=0.0182$) was found, indicating a larger differential in females than males on both an absolute basis (9,298 versus 5,029 revolutions/day, respectively) and proportional basis (increase of 199.8% in females versus increase of 132.7% in males) (Fig. 1b).

3.12 Food choice

Most mice exhibited a preference for Western diet (86%) after 6 days of wheel access (Fig. 2A), and this pattern was accentuated after 17 days of wheel access, when only 3% of the individuals preferred standard chow (Fig. 2C). Beyond the overall tendency of mice to prefer Western diet, we measured differences among groups in the strength of this preference. After 6 days of wheel access, male mice from the Control lines tended to show stronger preferences than male HR mice (Fig. 2B: interaction $P=0.0612$). The pattern was different after 17 days of wheel acclimation, with High Runner mice of both sexes showing stronger preference for Western diet (Fig. 2D: linetype $P=0.0424$).

3.13 Wheel running during short-term food choice

As shown in Figure 3, all mice in experiment 1 increased wheel running during the food-choice trial on days 7 & 8. The difference in wheel running was positive for all groups, and was significant for Control Females ($P=0.0428$), HR Females ($P<0.0001$), and HR Males ($P=0.0011$), but not for Control Males ($P=0.3716$). Females and HR mice showed significantly larger increases than males and C mice (sex $P=0.0115$, linetype $P=0.0033$), respectively, with no significant interaction ($P=0.1992$).

3.14 Wheel running and home-cage activity during long-term food choice

As expected, HR mice ran also more than C mice during long-term wheel access (Fig. 4A). Food choice was tested after mice appeared to have reached a running plateau (days 14-17 in Fig. 4A). During the diet-choice trial (days 18-24), when HR mice of both sexes showed elevated preferences for Western diet (see above and Fig. 2D), wheel running increased in all mice as compared with days 14-17 (Fig. 4B: Control Female $P=0.0106$, Control Male $P=0.1032$, HR Female $P=0.0008$, HR Male $P=0.0012$). Analysis of these differences in wheel running indicated the increases were significantly greater in the HR mice (linetype $P=0.0200$), with no effect of sex ($P=0.2699$) and no interaction ($P=0.8006$).

Reciprocal to increased wheel running, SPA decreased throughout the experiment across all groups (Fig. 4C). After food choice was introduced, SPA tended to decrease further in all groups (Control Female $P=0.0686$, Control Male $P=0.0407$, HR Female

P=0.0246, HR Male P=0.1258), with no statistical difference between linetypes or sexes, nor an interaction (Fig. 4D).

3.2 Experiment 3: Substitution of Western diet

3.21 Effects of Western diet on wheel running

On days 5 & 6 of the 6-day wheel exposure (Fig. 5A) with standard chow (N=313), HR mice ran much more than C (P<0.0001), as expected, but with no sex effect (P=0.1469), or interaction (P=0.6629). The effects of administering Western diet to half of the mice on days 7 & 8 depended on sex and linetype in a complex manner. Statistically significant interactions occurred for sex*diet (P=0.0174) and sex*linetype (P=0.0061), and marginally for diet*linetype (P=0.0868) and sex*diet*linetype (P=0.1102). For females (Fig. 5B), mice from Control lines remaining on standard chow showed no statistical change in wheel running from days 5 & 6 to 7 & 8. In contrast, HR females on standard diet (+9.9%, P=0.0070), Control females on WD (+27.8%, P=0.0176), and especially HR females on WD (+32.9%, P<0.0001) all showed significant increases (based on comparisons of least squares means with the null hypothesis of no change; % changes calculated by comparison with least squares means from combined-sex analysis). Note that the figures depict absolute changes in revolutions run per day, and not the percentage changes, which are presented parenthetically. For males (Fig. 5C), the only group showing a statistically significant change was HR switched to Western diet, which increased approximately 10% in average revolutions/day (P=0.0138).

3.22 Effects of Western diet on home-cage activity

On days 5 & 6 of the 6-day wheel exposure, males were less active in their home cages than females ($P=0.0295$), but HR and C mice did not differ statistically ($P=0.8146$), nor was there an interaction ($P=0.5487$). On days 7 & 8, with Western diet, males were still less active than females ($P=0.0454$), HR and C did not differ ($P=0.9281$), and Western diet reduced activity ($P=0.0430$). None of the interaction terms were statistically significant (all $P > 0.29$). When the changes in home-cage activity were analyzed, all mice tended to become less active with Western diet ($P=0.0074$). None of the other main effects or interaction terms was statistically significant (all $P > 0.24$).

4. Discussion

We tested the hypothesis that mice from lines selectively bred for high levels of voluntary wheel running would have altered preferences for Western diet, as compared with mice from non-selected control lines, and that their response to Western diet in terms of physical activity might also differ from C mice. Elevated preferences for WD in HR mice could arise from a general alteration of the reward system (Roberts and Barnard 2005; Belke and Garland 2007) and/or a greater need for lipids associated with their higher levels of physical activity and energy expenditure (even when housed without wheels:(Copes et al. 2015); and references therein). As expected from many previous studies of rodents (Reed et al. 1997; Levine et al. 2003; Laugerette et al. 2005; Kasper et al. 2014), Western diet was highly preferred by most mice, regardless of sex or linetype

(Fig. 2A,C). Consistent with our hypothesis, HR mice of both sexes showed enhanced preferences after 17 days of wheel acclimation (Fig. 2D), although not after 6 days (Fig. 2B). Moreover, administration of WD after 6 days of wheel access (no choice given) had a complex interactive effect on wheel running that depended on both sex and linetype (Fig. 5).

In general, the regulation of lipid intake is a complex behavior controlled by both instantaneous orosensory stimuli (i.e., texture, odor, and taste) and delayed post-ingestive signals (Drewnowski 1997; Gilbertson 1998). For rodents, various studies attribute the palatability of high-fat foods to such factors as texture, flavor, taste, and post-ingestive effects (Levine et al. 2003; Laugerette et al. 2005; Manabe et al. 2010). Traits that might account for an elevated preference for high-fat foods (as observed in the HR mice) include altered numbers of fatty acid receptors on the tongue, which could increase palatability of fatty foods, possibly affect appetite for dietary fat, and potentially enhance the hedonic response.

Alternatively, the promotion of wheel running by HFD may serve as an indirect mechanism leading to the reinforcement of the HFD preference. In other words, the dietary change allowed the HR mice to engage in additional activity, which itself is known to be rewarding ((Sherwin 1998); (Belke and Garland 2007); (Novak et al. 2012)), and through this process they developed a stronger preference for HFD. Although this possibility has not been specifically tested, it is consistent with our data indicating that the elevated preference for Western diet in HR mice does not arise within the first week

(Fig. 2B), but appears to require more time (Fig. 2D), which is what would be expected for the gradual elevation in running to generate a learned (reinforced) response.

The Western diet used here contains not only more fat but also more sucrose than standard chow. Numerous studies show that rodents generally prefer sweet tastes, especially as compared with bitter-tasting foods (Reed et al. 1997; Lemon 2015). Consuming sugar-sweetened foods or beverages in humans, rodents, and other mammals leads to rewarding sensations, as indicated by the upregulation of opioid and dopamine receptors in the nucleus accumbens (Gosnell and Levine 2009). Hence, both sugar and high-fat diet (HFD) rewards may explain why both HR and C mice preferred Western diet. Future studies will be required to determine the proximate physiological and neurobiological bases of elevated fat and/or sucrose preferences in the HR lines of mice.

In addition to most mice consuming more of the Western diet, most mice also increased wheel running when they had access to Western diet (Figs. 3, 4, 5). As expected from numerous previous studies (Swallow et al. 1998a; Dlugosz et al. 2009; Meek et al. 2010; Acosta et al. 2015), HR mice continued to run more than C mice in all experiments when they had access to WD (Figs. 1, 3, 4, 5). Based on two previous studies that examined only males (Meek et al. 2010), we expected that HR mice would run even more than C mice with the WD, whereas this effect might not occur in C mice. Our results were only partially consistent with these expectations. After 6 days of wheel acclimation, access to WD for two days in a choice paradigm (Experiment 1) resulted in significantly increased wheel running for all mice except Control males, consistent with earlier reports. After 17 days of wheel acclimation (Experiment 2), by which time mice

had reached stable (plateaued) levels of running, access to WD for seven days increased wheel running (revolutions/day) in both HR and C mice of both sexes, but with a statistically greater effect in HR mice of both sexes (Fig. 4A,B). In experiment 3, mice had 6 days of wheel access (as used to choose breeders in the ongoing selection experiment) followed by replacement of standard chow with Western diet for half of the mice. This treatment demonstrated a complex interactive effect on wheel running that varied by sex and linetype (Fig. 5).

Meek and colleagues (Meek et al. 2010), who studied only males of the HR and C lines, suggested the ingestion of certain macronutrients is important for muscular activity and delaying fatigue during exercise, although not via polyunsaturated fatty acids (PUFAs), as they are not the major component of the WD used in our experiments. As Meek et al. further proposed, HR mice might run more with a WD because of the neuro-hormonal system that maintains energetic balance and regulates total body weight. In particular, the increased wheel running by HR mice on WD may be a compensatory mechanism to deal with the excess caloric intake making them run more as a consequence of eating more.

Alternatively, Western Diet may increase running because diets high in fat have many physiological effects that act to promote endurance performance. Consumption of high-fat diet may increase fatty acid metabolism, which would allow fat to fuel muscular activity (Turner et al. 2007; Templeman et al. 2012). More specifically, chronic consumption of HFD increases fatty acid oxidative capacity of skeletal muscle in rats and mice, as indicated by increased activity of β -hydroxyacyl CoA dehydrogenase (β HAD),

medium-chain acyl-CoA dehydrogenase (MCAD), carnitine palmitoyl-transferase (CPT-1), and citrate synthase (CS), as well as and elevated protein expression of PGC-1 α , UCP3 and mitochondrial respiratory chain subunits (Turner et al. 2007). A subset of these and other factors (CCO, CS, CPT, and HK) are indeed higher in the HR mice, as compared with C, when given chronic (8 weeks) wheel access on standard chow (Houle-Leroy et al. 2000). The effects of WD on wheel running observed in Experiment 3 (Fig. 5) occurred during days 7 and 8 of wheel access, and it is unknown if any of the biochemical and molecular changes described above could have occurred by that time.

Whatever the mechanism that underlies a stimulatory effect of WD on wheel running, our finding that the effects of HFD on physical activity depend on genetic background is consistent with studies of a different selection experiment, in which mice were bred to be lean or to fat (Simoncic et al. 2008). On regular diet, mice from the lean line had higher wheel running and greater home-cage activity (measured via vertical posture allocation) than those from the fat line. When mice were switched to a calorie-matched HFD, mice from the fat line significantly increased their wheel running, up to the level of lean-line mice, while mice from the lean line were unaffected (Simoncic et al. 2008).

Several previous studies have shown that home-cage activity of rodents tends to decline when wheel access is provided (Copes et al. 2015) and references therein). Here, we present novel evidence that home-cage activity decreases with access to Western diet, possibly as compensation for increases in wheel running, and that this effect is general for both sexes and both linetypes that we studied (Fig. 4). The decreased home-cage

activity may be directly related to the increase in wheel running through homeostatic compensatory mechanisms targeting energy balance or potentially the overall level of physical activity (i.e., the "activity-stat" hypothesis: (Rowland 1998; Eisenmann and Wickel 2009; Garland et al. 2011)). Alternatively, some sort of reward substitution could be occurring (Belke et al. 2006). However, we cannot rule out the possibility that WD has some sort of direct effect on SPA (e.g., see (Vaanholt et al. 2008)).

In conclusion, the present results add to a growing body of work that characterizes the behavioral and physiological profile of mice that have been selectively bred for high levels of voluntary exercise on wheels. More generally, they show that dietary preferences can evolve as a byproduct of such selective breeding, and that the effects of a Western diet can vary depending on sex and genetic background. Moreover, the dietary preference of HR mice has evolved in a way that facilitates the trait for which they have been bred, i.e., high voluntary wheel running, and hence this qualifies as an example of coadaptation (Angilletta Jr et al. 2006). Further studies will be required to elucidate the mechanistic basis of these differences in preference and behavioral response to Western diet, as well as the basis of sex differences (e.g., Fig. 5).

Conflict of Interest Statement

The authors declare no conflict of interest.

Acknowledgments

Supported by NSF grant IOS-1121273 to TG.

Figure Legends

Fig. 2.1 Mice from High Runner lines ran significantly more than those from non-selected Control lines. Values are least square means (LSM) + associated SEs from SAS Procedure Mixed. A) Values from days 5 and 6, prior to mice having a choice between standard chow and Western diet (Experiment 1, short-term mice). B) Values from days 14-17, prior to mice having a choice between standard chow and Western diet (Experiment 2, long-term mice).

Fig. 2.2 Food consumption when mice were offered a choice between standard chow and Western diet (same mice as in Fig. 1). A) After six days of wheel access (as is used routinely in the selection experiment for choosing breeders), most mice preferred Western diet, although 14% ate little of it. B) All mice consumed more Western Diet than standard chow, with a marginally significant interaction between sex and linetype ($P=0.0612$), indicating that males from the Control lines showed somewhat stronger preferences than did the other three groups. Values are least square means (LSM) + associated SEs from SAS Procedure Mixed. C) Preference for Western diet was even stronger after mice had 17 days of wheel access prior to the choice trial, when only 3% of the individuals ate little WD. D) After 17 days of wheel acclimation, all groups of mice again preferred WD, but the preference was stronger for mice from High Runner lines of both sexes ($P=0.0424$).

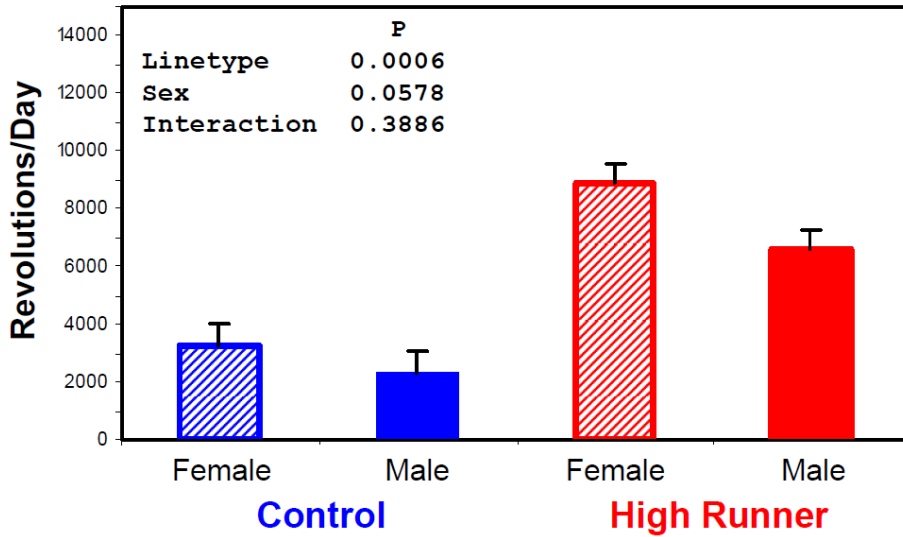
Fig. 2.3 In Experiment 1 (short-term mice), all groups except Control males ran significantly more on days 7 & 8 of wheel access, when they had a choice between Western diet and standard chow, as compared with days 5 & 6. Values are least square means (LSM) + associated SEs from SAS Procedure Mixed. The increase in running was significantly greater for HR mice and for females rather than C mice or males (linetype $P=0.0033$, sex $P=0.0115$), with no interaction ($P=0.1992$).

Fig. 2.4 A) In experiment 2 (long-term mice), both sexes of High Runner mice ran significantly more than C lines at all times. B) During the diet-choice trial (days 18-24), when HR mice of both sexes showed elevated preferences for Western diet (see Fig. 2D), wheel running increased in all mice as compared with days 14-17. Analysis of these differences in wheel running indicated the increases were significantly greater in the HR mice (linetype $P=0.0200$), with no effect of sex ($P=0.2699$) and no interaction ($P=0.8006$). C) As wheel running increased across the experiment, home-cage activity decreased for all four groups. After food choice was introduced, SPA tended to decrease further in all groups. D) During the food-choice trial, home-cage activity of all groups decreased (while wheel running increased: Fig. 4B), with no statistical difference between linetypes or sexes, nor an interaction.

Fig. 2.5 A) During days 5 & 6 prior to food-substitution in Experiment 3, HR mice ran significantly more than C, and females showed no statistically significant difference from males ($N = 313$). Age and a measure of wheel freeness were also included in the statistical model (results not shown). When half of the mice were switched to Western diet on days 7 & 8, the change in wheel running depended on linetype in both B) females and C) males. See text for further statistical results. Asterisks indicate that the change in wheel running within a given group differed significantly ($P<0.05$) from zero. All values are least squares means and standard errors from SAS Procedure Mixed.

Figure 2.1 Wheel running prior to food choice

A) Wheel Running on days 5 + 6 of **short-term Mice**



B) Wheel Running on days 14-17 of **long-term Mice**

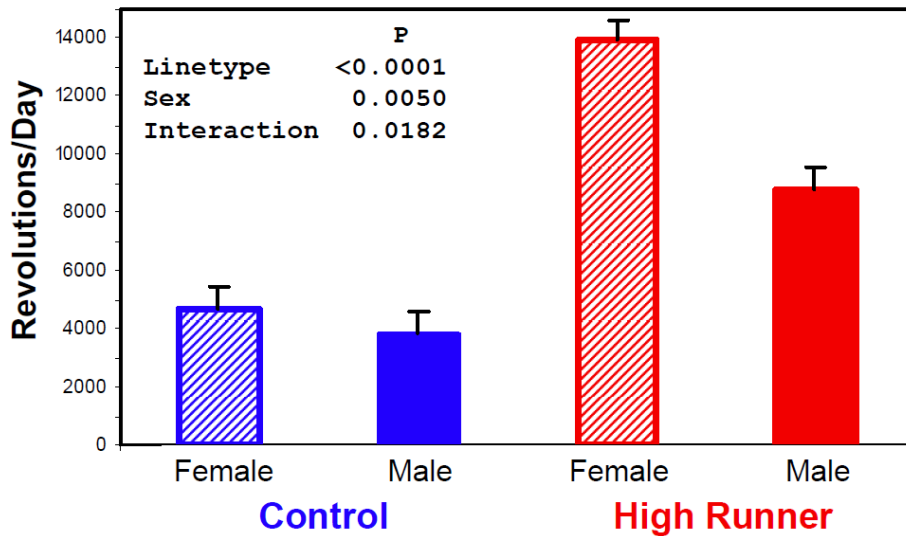
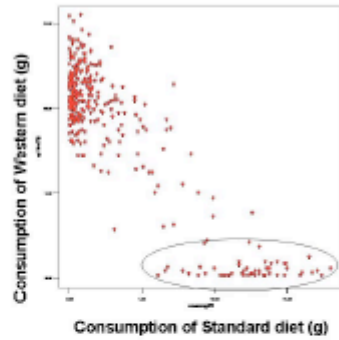


Figure 2.2 Food consumption and dietary choice

A)

Results: Food Consumption

Food Consumption with choice for **short-term mice**:



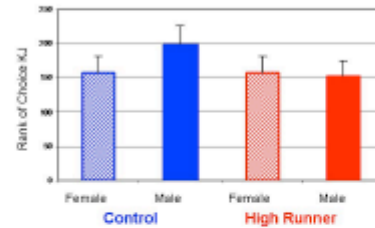
46 do not eat much Western diet = 14%

B)

Results: Dietary Choice

Rank of food choice (W(W+S)) on days 7+8 of **short-term mice**:

	P
Linetype 1	0.2413
Sex	0.3510
Interaction	0.0912



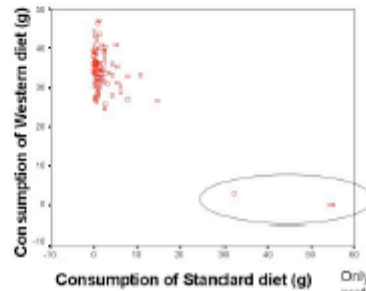
Adjusted Means ± Standard Errors

27

C)

Results: Food Consumption

Food Consumption with choice for **long-term mice**:



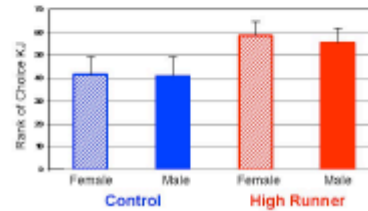
Only 3 do not prefer Western diet = 3%

D)

Results: Dietary Choice

Choice KJ = Western KJ/Western KJ + Standard KJ) on days 15-24 of **long-term mice**:

	P
Linetype 1	0.0424
Sex	0.8125
Interaction	0.8217



Adjusted Means ± Standard Errors

3

Figure 2.3 Difference in wheel running of short-term mice

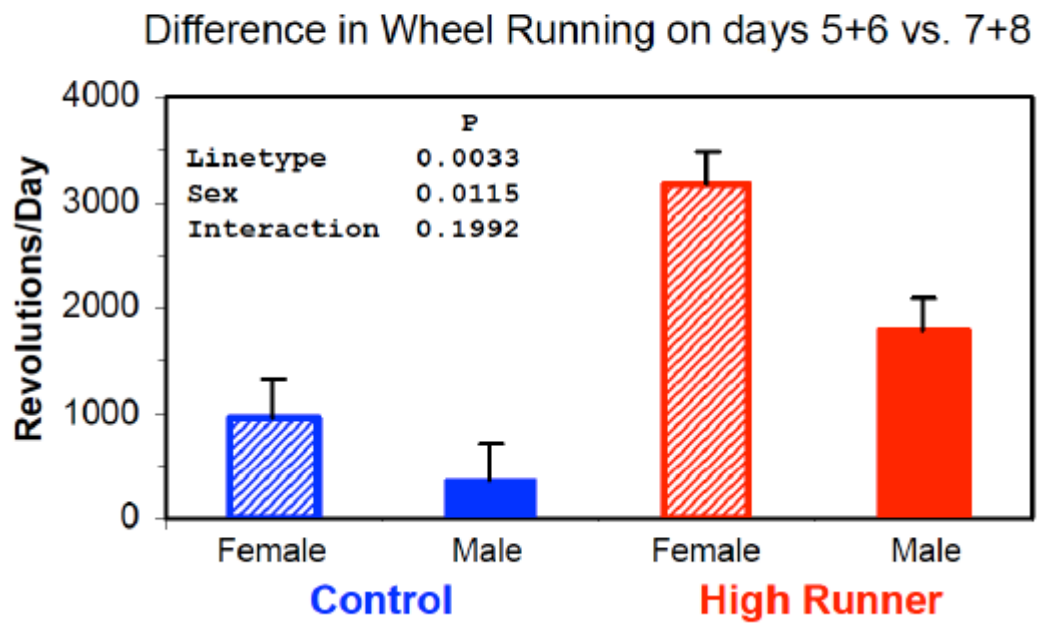
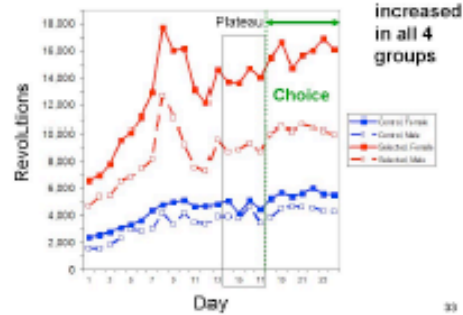


Figure 2.4 Wheel running and SPA of long-term mice

A)

Results: Wheel Running

Wheel running of long-term mice:



C:\data\spg\030305\p030305\wheelrunning\wheelrunning

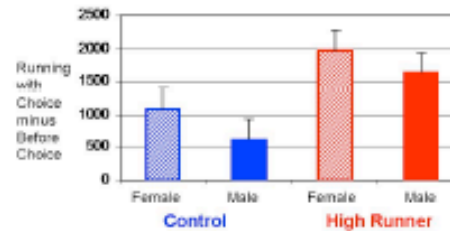
33

B)

Results: Wheel Running Response to Choice

Wheel running pre- (14-17) vs. post- (18-24) choice of long-term mice:

	P
LineType 2	0.0200
Sex	0.2659
Interaction	0.8006



Adjusted Means ± Standard Errors

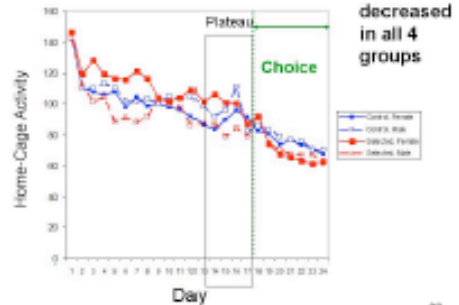
C:\data\spg\030305\p030305\wheelrunning\wheelrunning

34

C)

Results: Home Cage Activity

HCA of long-term mice:



C:\data\spg\030305\p030305\hca\hca

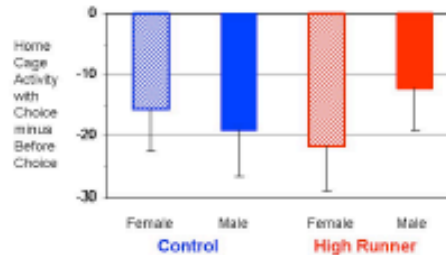
35

D)

Results: Home Cage Activity Response to Choice

HCA pre- (14-17) vs. post- (18-24) choice of long-term mice:

	P
LineType 2	0.9613
Sex	0.6304
Interaction	0.2198

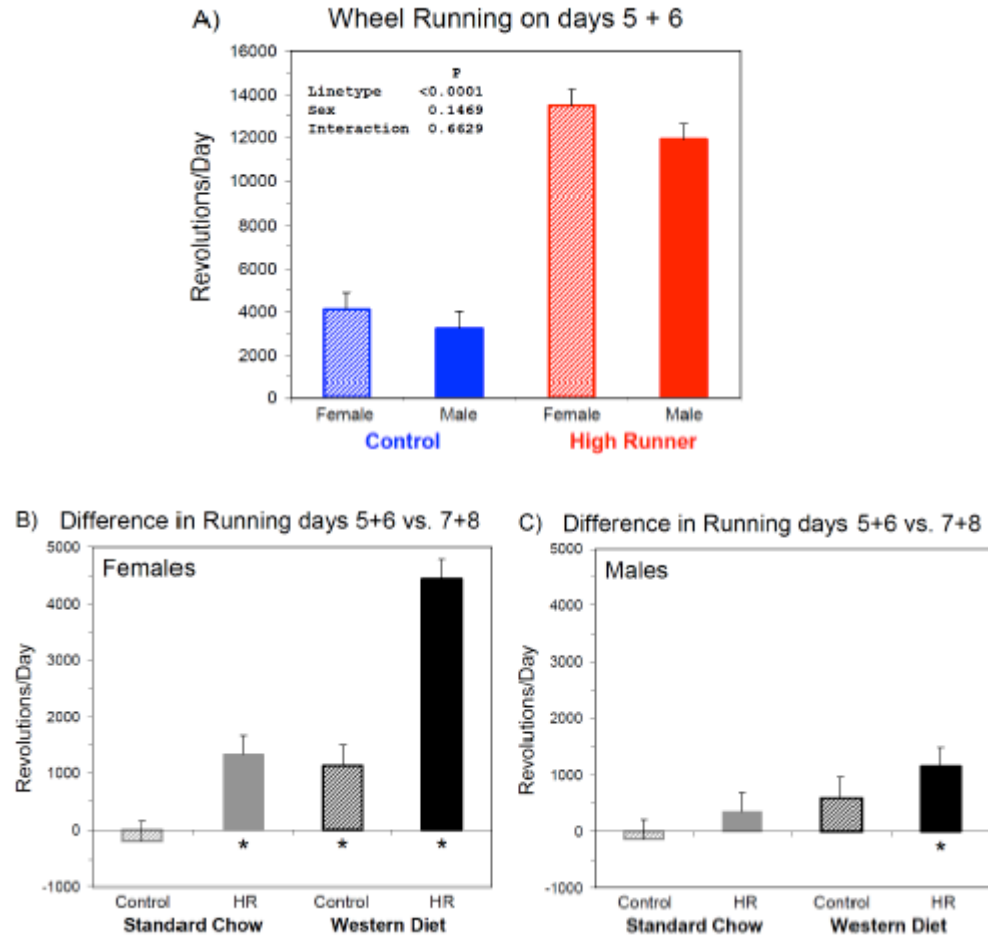


Adjusted Means ± Standard Errors

C:\data\spg\030305\p030305\hca\hca

37

Figure 2.5 Wheel running difference with and without Western diet



References

- Acosta, W., T. H. Meek, H. Schutz, E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2015. Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice. *Physiol Behav* 149:279-286.
- Angilletta Jr, M. J., A. F. Bennett, H. Guderley, C. A. Navas, F. Seebacher, and R. S. Wilson. 2006. Coadaptation: A Unifying Principle in Evolutionary Thermal Biology*. *Physiol. Biochem. Zool.* 79:282-294.
- Bauer, F., J. W. Beulens, A. D. van der, C. Wijmenga, D. E. Grobbee, A. M. Spijkerman, Y. T. van der Schouw, and N. C. Onland-Moret. 2013. Dietary patterns and the risk of type 2 diabetes in overweight and obese individuals. *Eur J Nutr* 52:1127-1134.
- Bauwens, D., T. Garland Jr, A. M. Castilla, and R. Van Damme. 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution*:848-863.
- Belke, T. W., and T. Garland, Jr., 2007. A brief opportunity to run does not function as a reinforcer for mice selected for high daily wheel-running rates. *Journal of the Experimental Analysis of Behavior* 88:199-213.
- Belke, T. W., W. D. Pierce, and I. D. Duncan. 2006. Reinforcement value and substitutability of sucrose and wheel running: Implications for activity anorexia. *Journal of the Experimental Analysis of Behavior* 86:131-158.
- Booth, F. W., M. V. Chakravarthy, S. E. Gordon, and E. E. Spangenburg. 2002. Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. *Journal of Applied Physiology* 93:3-30.
- Bucci, L. R. 2000. Selected herbals and human exercise performance. *American Journal of Clinical Nutrition* 72:624S-636.
- Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2013. Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. *Evolution* 67:3102-3119.
- Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2015. Evolution of the additive genetic variance-covariance matrix under continuous directional selection on a complex behavioural phenotype. *Proceedings of the Royal Society of London B: Biological Sciences* 282.

- Centers for Disease Control and Prevention National Center for Chronic Disease Prevention and Health Promotion, D. o. N. a. P. A. 2010. Adult obesity: Obesity rises among adults. Pp. 1-4 *in* D. o. N. National Center for Chronic Disease Prevention and Health Promotion, Physical Activitiy and Obesity, ed.
- Copes, L. E., H. Schutz, E. M. Dlugosz, W. Acosta, M. A. Chappell, and T. Garland Jr. 2015. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. *Physiol. Behav.* 149:86-94.
- Dlugosz, E. M., M. A. Chappell, D. G. McGillivray, D. A. Syme, and T. Garland, Jr. 2009. Locomotor trade-offs in mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology* 212:2612-2618.
- Drewnowski, A. 1997. Taste preferences and food intake. *Annual Review of Nutrition* 17:237-253.
- Drewnowski, A. 2007. The Real Contribution of Added Sugars and Fats to Obesity. *Epidemiologic Reviews* 29:160-171.
- Eisenmann, J. C., and E. E. Wickel. 2009. The biological basis of physical activity in children: revisited. *Pediatric Exercise Science* 21:257-272.
- Frank, C. L., E. S. Dierenfeld, and K. B. Storey. 1998. The relationship between lipid peroxidation, hibernation, and food selection in mammals. *Am. Zool.* 38:341-349.
- Garland, T., Jr., H. Schutz, M. A. Chappell, B. K. Keeney, T. H. Meek, L. E. Copes, W. Acosta, C. Drenowatz, R. C. Maciel, G. van Dijk, C. M. Kotz, and J. C. Eisenmann. 2011. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *Journal of Experimental Biology* 214:206-229.
- Gebczynski, A. K., and M. Konarzewski. 2009. Locomotor activity of mice divergently selected for basal metabolic rate: a test of hypotheses on the evolution of endothermy. *Journal of Evolutionary Biology* 22:1212-1220.
- Gilbertson, T. A. 1998. Gustatory mechanisms for the detection of fat. *Current Opinion in Neurobiology* 8:447-452.
- Girard, I., E. Rezende, and T. Garland, Jr. 2007. Leptin levels and body composition of mice selectively bred for high voluntary locomotor activity. *Physiol. Biochem. Zool.* 80:568-579.

- Gosnell, B. A., and A. S. Levine. 2009. Reward systems and food intake: role of opioids. *Int J Obes* 33:S54-S58.
- Houle-Leroy, P., T. Garland, J. G. Swallow, and H. Guderley. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. *Journal of Applied Physiology* 89:1608-1616.
- Kant, A. K. 2000. Consumption of energy-dense, nutrient-poor foods by adult Americans: nutritional and health implications. The third National Health and Nutrition Examination Survey, 1988-1994. *American Journal of Clinical Nutrition* 72:929-936.
- Kasper, J. M., S. B. Johnson, and J. D. Hommel. 2014. Fat Preference: A Novel Model of Eating Behavior in Rats. e51575.
- King, N. A. 1998. The relationship between physical activity and food intake. *Proc Nutr Soc.* 57:77-84.
- King, N. A., A. Tremblay, and J. E. Blundell. 1997. Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* 29:1076-1089.
- Kolb, E. M., S. A. Kelly, K. M. Middleton, L. S. Sermsakdi, M. A. Chappell, and T. Garland, Jr. 2010. Erythropoietin elevates VO_{2,max} but not voluntary wheel running in mice. *Journal of Experimental Biology* 213:510-519.
- Kolb, E. M., E. L. Rezende, L. Holness, A. Radtke, S. K. Lee, A. Obenaus, and T. Garland. 2013. Mice selectively bred for high voluntary wheel running have larger midbrains: support for the mosaic model of brain evolution. *The Journal of Experimental Biology* 216:515-523.
- Koteja, P., P. A. Carter, J. G. Swallow, and T. Garland, Jr. 2003. Food wasting by house mice: variation among individuals, families, and genetic lines. *Physiology Behavior* 80:375-383.
- Koteja, P., T. Garland, Jr., J. K. Sax, J. G. Swallow, and P. A. Carter. 1999a. Behaviour of house mice artificially selected for high levels of voluntary wheel running. *Animal Behaviour* 58:1307-1318.
- Koteja, P., J. G. Swallow, P. A. Carter, and T. Garland, Jr. 1999b. Energy cost of wheel running in house mice: implications for coadaptation of locomotion and energy budgets. *Physiological & Biochemical Zoology* 72:238-249.

- Laugerette, F., P. Passilly-Degrace, B. Patris, I. Niot, M. Febbraio, J.-P. Montmayeur, and P. Besnard. 2005. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *The Journal of Clinical Investigation* 115:3177-3184.
- Lemon, C. 2015. Perceptual and Neural Responses to Sweet Taste in Humans and Rodents. *Chemosensory Perception* 8:46-52.
- Levine, A. S., C. M. Kotz, and B. A. Gosnell. 2003. Sugars and Fats: The Neurobiology of Preference. *The Journal of Nutrition* 133:831S-834S.
- Maillet, D., and J. M. Weber. 2006. Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. *Journal of Experimental Biology* 209:2686-2695.
- Manabe, Y., S. Matsumura, and T. Fushiki. 2010. Preference for High-Fat Food in Animals *in F. i. Neuroscience*, ed. Fat Detection: Taste, Texture, and Post Ingestive Effects. CRC Press, Boca Raton, FL.
- Mayer, J., N. B. Marshall, J. J. Vitale, J. H. Christensen, M. B. Mashayekhi, and F. J. Stare. 1954. Exercise, Food Intake and Body Weight in Normal Rats and Genetically Obese Adult Mice. *American Journal of Physiology -- Legacy Content* 177:544-548.
- Meek, T., J. Eisenmann, and T. Garland, Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *Int. J. Obes.* 34:960-969.
- Meek, T. H., J. C. Eisenmann, B. K. Keeney, R. M. Hannon, E. M. Dlugosz, and T. Garland. 2014. Effects of early-life exposure to Western diet and wheel access on metabolic syndrome profiles in mice bred for high voluntary exercise. *Genes, Brain and Behavior* 13:322-332.
- Meek, T. H., B. P. Lonquich, R. M. Hannon, and T. Garland. 2009. Endurance capacity of mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology* 212:2908-2917.
- Mundal, R., S. E. Kjeldsen, L. Sandvik, G. Erikssen, E. Thaulow, and J. Erikssen. 1998. Clustering of coronary risk factors with increasing blood pressure at rest and during exercise. *J. Hypertens.* 16:19-22.
- Novak, C. M., P. R. Burghardt, and J. A. Levine. 2012. The use of a running wheel to measure activity in rodents: Relationship to energy balance, general activity, and reward. *Neuroscience and Biobehavioral Reviews* 36:1001-1014.

- Pierce, B. J., S. R. McWilliams, T. P. O'Connor, A. R. Place, and C. G. Guglielmo. 2005. Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *Journal of Experimental Biology* 208:1277-1285.
- Pierce, B. J., S. R. McWilliams, A. R. Place, and M. A. Huguenin. 2004. Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyed Vireos (*Vireo olivaceus*). *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 138:503-514.
- Reed, D. R., A. A. Bachmanov, G. K. Beauchamp, M. G. Tordoff, and R. A. Price. 1997. Heritable Variation in Food Preferences and Their Contribution to Obesity. *Behavior genetics* 27:373-387.
- Rhodes, J. S., S. C. Gammie, and T. J. Garland. 2005. Neurobiology of mice selected for high voluntary wheel-running activity. *Integr. Comp. Biol.* 45:438-455.
- Roberts, C. K., and R. J. Barnard. 2005. Effects of exercise and diet on chronic disease. *Journal of Applied Physiology* 98:3-30.
- Rowland, T. W. 1998. The biological basis of physical activity. *Med. Sci. Sports Exerc.* 30:392-399.
- Saris, W. H. M., M. A. van Erp-Baart, F. Brouns, K. R. Westerterp, and F. Ten Hoor. 1989. Study on food intake and energy expenditure during extreme sustained exercise: the Tour de France. *Int J Sports Med* 10:S26-S31.
- Shaikh, S. R., and M. Edidin. 2006. Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. *American Journal of Clinical Nutrition* 84:1277-1289.
- Sherwin, C. M. 1998. Voluntary wheel running: a review and novel interpretation. *Animal Behavior* 56:11-27.
- Simonic, M., S. Horvat, P. L. Stevenson, L. Bungler, M. C. Holmes, C. J. Kenyon, J. R. Speakman, and N. M. Morton. 2008. Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. *Behavior Genetics* 38:292-300.
- Smith, M. P., J. Mendez, M. Druckenmiller, and P. M. Kris-Etherton. 1982. Exercise intensity, dietary intake, and high-density lipoprotein cholesterol in young female competitive swimmers. *Am J Clin Nutr.* 36:251-255.

- Swallow, J., J. Hayes, P. Koteja, and T. Garland, Jr. 2009. Selection experiments and experimental evolution of performance and physiology. *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments*:301-351.
- Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics* 28:227-237.
- Swallow, J. G., P. Koteja, P. A. Carter, and T. Garland, Jr. 2001. Food consumption and body composition in mice selected for high wheel-running activity. *J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol.* 171:651-659.
- Templeman, N. M., H. Schutz, T. Garland, Jr., and G. B. McClelland. 2012. Do mice bred selectively for high locomotor activity have a greater reliance on lipids to power submaximal aerobic exercise? *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* 303:101-111.
- Turner, N., C. R. Bruce, S. M. Beale, K. L. Hoehn, T. So, M. S. Rolph, and G. J. Cooney. 2007. Excess Lipid Availability Increases Mitochondrial Fatty Acid Oxidative Capacity in Muscle: Evidence Against a Role for Reduced Fatty Acid Oxidation in Lipid-Induced Insulin Resistance in Rodents. *Diabetes* 56:2085-2092.
- Vaanholt, L. M., I. Jonas, M. Doornbos, K. A. Schubert, C. Nyakas, T. Garland, G. H. Visser, and G. van Dijk. 2008. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int. J. Obes.* 32:1566-1575.
- Weber, J. M. 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. *Journal of Experimental Biology* 212:593-597.
- Westerterp, K. R. 2010. Physical activity, food intake, and body weight regulation: insights from doubly labeled water studies. *Nutr Rev* 68:148-154.

Chapter 3

Effects of Western diet on activity levels, food consumption, and respiratory exchange ratio during voluntary wheel running in selectively bred High Runner mice

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ABSTRACT

Exercise behavior and metabolism can be influenced by both genetic and environmental factors, including diet. Previous studies demonstrate that male mice from four replicate lines bred for high levels of wheel-running behavior during a 6-day exposure as young adults (high runner or HR lines) can be stimulated to even higher levels of voluntary exercise by chronic exposure to a Western diet (high in fat and sucrose). We hypothesized that the high voluntary exercise of HR mice, as compared with those from four non-selected control (C) lines, would be reflected in a lower respiratory exchange ratio (RER) during exercise and possibly at rest, indicative of a higher reliance on lipids, although such differences might only occur on Western diet. We studied females because they generally run faster and for greater total distances per day than males. Mice were acclimated to cages with attached wheels for five days before transfer to metabolic chambers that contained identical cages and wheels. Oxygen consumption (O_2) and carbon dioxide (CO_2) production were measured in 1-min intervals for one day (day 6) with standard chow and then one (day 7) with Western diet. High Runner and C mice did not differ statistically in body mass. Mass-adjusted food consumption (grams/day) was significantly higher (+18%) in HR than C on days 1-5 of the experiment, but the differential was reduced to non-significance while in the metabolic chambers on days 6 (+0.01%) and 7 (+17%). Oxygen consumption at rest on days 5 and 6 did not differ statistically between HR and C mice (lowest values observed over 5, 10, 20, 30, 60, 120 minutes), nor did integrated O_2 consumption over 23-hours differ, on either day (consistent with the lack of difference in food consumption).

As expected, HR mice ran significantly more total revolutions/day than C mice, with higher mean and maximal 1-min running speeds, and also increased running duration, on both days 6 and 7. In contrast, home-cage activity (measured with passive infrared photocells) was similar for HR and C on both days. Consistent with their higher levels of wheel running, with body mass as a covariate HR mice had significantly higher maximum voluntary VO_2 over all time intervals considered (1, 2, 5, 10, 20, 30, 60, 120 minutes), on both diets. Adding the distance run on wheels during the time interval as a covariate, statistical differences in maximum voluntary VO_2 were eliminated, suggesting similar running efficiencies for HR and C mice.

On day 6, while consuming regular chow, mass-adjusted respiratory exchange ratios (RER) during peak values of VO_2 were always higher in HR mice than in C, contrary to our expectation, but the difference never reached statistical significance for any calculated time interval (1, 2, 5, 10, 20, 30, 60, 120 min). While consuming Western diet (day 7), mass-adjusted RER during peak values of VO_2 again did not differ statistically between HR and C, although now HR tended to have lower values than C. Again, results were similar when amount of wheel running and home-cage activity were included as additional covariates. Overall, these results suggest no difference between HR and C mice in patterns of carbohydrate vs. lipid usage during voluntary exercise. Mass-adjusted RER during rest also did not differ statistically between HR and C on either diet or for any time interval (5, 10, 20, 30, 60, 120 minutes), whether or not wheel running and home-cage activity were included as covariates. RER integrated over 23 hours was not statistically different between linetypes.

HIGHLIGHTS

- Genetically High Runner (HR) mice had higher voluntary VO₂max than Control (C) mice
- With wheel access, home-cage activity was similar between HR and C mice
- Respiratory exchange ratio (RER) was similar for HR and C at rest and during exercise
- Substituting Western diet for standard chow for 24 h did not significantly alter RER
- With Western diet, RER remained similar for HR and C at rest and during exercise

1. Introduction

Exercise requires energy expenditure for muscular force production, including in the cardiovascular system. In vertebrates, short-duration exercise involves mainly the oxidation of carbohydrates, whereas long-term exercise requires increased fat metabolism. The type of fuel used (usually fat and/or carbohydrate) depends on both prior nutrition and intensity and duration of the exercise. Endurance training promotes lipid oxidation (Brooks and Mercier 1994; Jeukendrup et al. 1998). Because lipids supply more energy per gram of storage, they may often be a preferred source of energy, especially for long-distance migrating birds. For example, prior to migration, sandpipers that migrate from Canada to South America consume a crustacean-rich diet that is high in polyunsaturated fatty acids, thus increasing fluidity and permeability across cellular membranes, which is believed to facilitate lipid oxidation (Weber 2009). Other migratory birds also prefer to consume diets high in fatty acids, providing further evidence that perhaps lipid utilization is better for long-distance travels (Pierce et al. 2005).

In rodents, some evidence shows that a high-fat diet (HFD) can stimulate physical activity, depending on genetic background (see also Meek et al. 2010). For example, mice selectively bred for high or low body fat ("fat" mice had 22% body fat, "lean" mice had 4% body fat) were fed either a HFD or a regular rodent diet for 42 days. The lean mice ran more than the fat mice throughout the 42 days, but fat mice on a high-fat diet significantly increased their wheel running as compared to fat mice on the regular diet (Simoncic et al. 2008). Aside from long-distance migrators and certain rodents, trained

human endurance athletes generally have higher rates of fat oxidation and lower circulating levels of free fatty acids than non-trained humans (FFA) (Klein et al. 1994).

In mammals, lipids and carbohydrates are the main fuel sources. At low-intensity exercise, ATP production is primarily derived from fat oxidation (Askew 1984; Achten and Jeukendrup 2004; Weber and Haman 2004). As exercise intensity increases (65-80% VO_2max), carbohydrates become the primary fuel source. (Askew 1984; Brooks and Mercier 1994; McClelland 2004). In addition, fat oxidation is suppressed and the rate of glycogen metabolism is increased in skeletal muscle, furthering the shift from lipid utilization to carbohydrate usage (Horowitz and Klein 2000).

Measuring carbon dioxide (CO_2) production relative to oxygen (O_2) consumption, or the respiratory exchange ratio ((RER) = CO_2 production/ O_2 consumption), is an indirect method to estimate fuel use. As exercise intensity increases, so does RER measured at the whole-animal level (Jansson 1982; Askew 1984). An RER of 1.0 indicates carbohydrates are the main fuel source, whereas a value closer to 0.7 indicates primarily lipid usage. An RER greater than 1.0 can be reached if an animal is storing fat, but it is dependent on its diet. For resting mammals on standard diets, RER is typically about 0.85. RER at rest, over the course of a day (e.g., (Novak et al. 2010)), and during exercise has been observed to change in conjunction with changes in fat and carbohydrate content of the diet (Jansson 1982; Burke LM et al. 2002; Novak et al. 2010).

At low-intensity exercise, increasing duration of activity is accompanied by increases in triacylglycerol oxidation, fatty acid uptake, and lipolysis (Horowitz and Klein 2000). Endurance training is also known to increase fat oxidation during

submaximal exercise. For example, endurance-trained rats had lower RERs than untrained rats when running at submaximal exercise for 12 minutes, indicating that the former used greater amounts of lipid relative to carbohydrate (Helge et al. 1998). Diet, training history, and genetics can also interact to affect RER. For example, high-capacity-running rats had lower RER than low-capacity-running rats during a 60-minute test at 7 m/minute after long-term feeding on a high-fat diet, but not when fed standard chow (Novak et al. 2010).

The increase in fat oxidation from rest to moderate exercise intensities is mainly the result of increased FA availability and the increased muscle mitochondria caused by training. With sufficient endurance training, most human athletes can sustain exercise at more than 70% of their maximal oxygen consumption (VO_{2max} , indicating maximum aerobic power output). Elite athletes become more dependent on carbohydrates for energy, and when glycogen stores become depleted, their primary fuel becomes lipids (Conley and Krahenbuhl 1980; Brooks and Mercier 1994).

A study comparing dogs and goats established a similar pattern, in which fat oxidation supplied energy for low-intensity exercise and carbohydrate oxidation increased with increasing exercise intensity (Roberts et al. 1996). Overall, patterns of fuel usage during exercise in mammals depend on exercise intensity and duration, as well as individual or interspecific variation in muscle fiber type and the nervous system (Askew 1984; Brooks and Mercier 1994; Roberts et al. 1996).

Few studies have examined fuel usage in animals that have been specifically bred for athletic performance. In a 2011 review, horses and dogs were compared with humans

with respect to the structural and functional features that enable these mammals to support their extreme oxidative and athletic capabilities (Poole and Erickson 2011). Both dogs and horses were established to have extraordinary muscle mass that excels in diffusive oxygen transport and utilization (Poole and Erickson 2011). In Koch and Britton rats bred for high-capacity or low capacity running, $VO_2\text{max}$ has evolved to be 12% higher in high-capacity running rats than their counterparts (Gonzalez et al. 2006). After 8 weeks of exercise training in a treadmill, both high-capacity and low-capacity running rats significantly increase their running speeds (Bye et al. 2008).

Here, we examined RER across a daily cycle in the experimental evolution model of high voluntary wheel-running mice and their control counterparts (Swallow et al. 1998b; Swallow et al. 2005; Garland et al. 2011). Four lines of laboratory house mice have been selectively bred for increased wheel-running behavior (HR), while 4 non-selected lines of mice serve as the Controls (C) (Swallow et al. 1998b). HR mice have consistently run up to 3-fold more than C mice on a daily basis since reaching selection limits at approximately generation 17-25 (Careau et al. 2013), with the experiment currently at generation 76. Both sexes of HR mice have been reported to have elevated maximal oxygen consumption ($VO_2\text{max}$) during forced exercise (Rezende et al. 2005; Vaanholt et al. 2007; Rezende et al. 2009; Templeman et al. 2012) and greater endurance during forced treadmill exercise (Meek et al. 2009), compared to C mice. HR mice also run voluntarily on wheels at a higher fraction of their $VO_2\text{max}$ (Rezende et al. 2005). Males (females not studied) also have increased insulin-stimulated glucose uptake in some muscles (Dumke et al. 2001), and higher GLUT-4 levels in gastrocnemius after 5

days of voluntary wheel running (Gomes et al. 2009). In addition, skeletal muscles of HR mice have sometimes shown higher mass-specific activities of enzymes involved in energy metabolism, depending on sex, measurement conditions, and presence of the mini-muscle phenotype (Houle-Leroy et al. 2000; Gomes et al. 2009; Templeman et al. 2012). HR males also run more on wheels than C males while being fed a diet high in fat and sucrose than with standard chow (Meek et al. 2010), Chapter 2).

However, it is not known if the HR mice demonstrate higher fat utilization at the level of whole-animal RER during voluntary wheel running, nor whether a Western diet (high in fat and sucrose) might affect any differences between HR and C mice. Thus, the purpose of our study was to determine the voluntary maximal oxygen consumption during wheel running, resting metabolic rate, and respiratory exchange ratios of mice selectively bred for high voluntary wheel running and to test for effects of a Western diet on these physiological characteristics. We hypothesized that the high voluntary exercise of HR mice, as compared with those from four non-selected C lines, would be reflected in a lower respiratory exchange ratio (RER) during exercise and possibly at rest, indicative of a higher reliance on lipids, although such differences might only occur on Western diet. We also expected HR mice to attain higher levels of oxygen consumption during voluntary wheel running and over a daily cycle.

2. Material and Methods

2.1 *Experimental animals*

Mice used were from generation 63 (LG64) of an artificial selection experiment that breeds for high voluntary wheel-running activity (for more details on the selection process see (Swallow et al. 1998a; Careau et al. 2013). Briefly, the base population was outbred, genetically variable Hsd:ICR house mice (*Mus domesticus*). Ten 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 unselected control (C) lines, bred without regard to wheel running. During the normal selection experiment process, mice approximately 6-8 week old are individually housed in standard cages attached to a Wahman-type activity wheel (1.12m circumference, 35.7 cm diameter, 10 cm wide running surface). Mice can voluntarily run in the wheels or not. Wheels are interfaced to a computer that 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 for days 5 and 6. For the high-running lines, the highest-running males and females within each family are chosen as breeders, whereas breeders are chosen randomly from within families for the control lines (disallowing sib mating in all lines). Room temperatures are always maintained at approximately 22°C. Lights were on at 0700 with a photoperiod of 12:12h. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604) were available *ad libitum*. Pregnant dams are given a breeder diet (Harlan Teklad Mouse Breeder Diet [S-2335] 7004) through weaning.

2.2 *Wheel running & respirometry*

Females from generation 63 were weaned at 21 days of age and placed into standard cages until they were adults (6 weeks old). Mice were individually given wheel access for 5 days prior to entering the metabolic wheel chambers. At the end of day 5 (~noon, i.e., beginning of day 6), two mice were placed into individual air-tight chambers ((Chappell et al. 2004; Rezende et al. 2005)) that contained both a standard cage and a wheel, and given the standard diet (Harlan Teklad TD.8604). On day 7 (~noon), both mice were switched to the Western diet (Harlan Teklad TD.88137) and continued to undergo the same measurements (see experimental timeline in **Fig. 1**). Because only two animals could be measured at once, measurements were randomly scheduled across lines, except that we always measured one HR and one C mouse per test.

In the metabolic chambers, wheel revolutions were counted with a small generator that produced voltage proportional to rotation speed ((Chappell et al. 2004; Rezende et al. 2005)). Infrared sensors measured home-cage activity (Copes et al. 2015). Air flow was measured using paired incurrent and excurrent ports, and an internal fan recirculated air within the enclosures. Oxygen and carbon dioxide flows were recorded with open-system respirometry, along with the running and home-cage activity, every 1.5 seconds for 23 hours (from 1 p.m. – noon). One hour was allotted to download and backup data, weigh mice, and weigh food hoppers, for 2 consecutive days.

Open flow-through respirometry and activity data were recorded by a Macintosh computer equipped with LabHelper software (Warthog, <http://www.warthog.ucr.edu>) as described elsewhere (Chappell et al. 2004; Rezende et al. 2006a). Airflow was maintained at 2,500mL/min \pm 1%, with mass flow controllers and reference readings were

obtained every 45 minutes with an automated system. Oxygen and carbon dioxide readings were baseline corrected using the references readings as a base point to determine rates of oxygen consumption ($\dot{V}O_2$), along with carbon dioxide production during their voluntary exercise as described in detail elsewhere (Chappell et al. 2004). Briefly, an “Oxzilla” dual-channel O_2 analyzer and two Sable Systems CA-2A analyzers were used to measure O_2 and CO_2 values (see Appendix A for Respirometry Schematic Drawing). Data were then corrected for baseline measurements and transformed to instantaneous values using:

$$\dot{V}O_2 = STP * FR * [(F_iO_2 - F_eO_2) - F_eO_2 * (F_eCO_2 - F_iCO_2)] / (1 - F_eO_2)$$

$$\dot{V}CO_2 = STP * FR * [(F_eCO_2 - F_iCO_2) - F_eCO_2 * (F_iO_2 - F_eO_2)] / (1 - F_eO_2).$$

2.3 Statistical analyses

Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects. Linetype (HR or C) and diet (if applicable) were treated as fixed effects; line was nested within line type as a random effect. Effects of linetype and diet and their respective interactions were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6. Covariates depended on the trait being analyzed and included age, body mass, wheel freeness (an inverse measure of rotational resistance), home-cage sensor calibration (measure of sensor motion sensitivity), and wheel running (revolutions). Dependent variables, independent variables, and/or

covariates were transformed as necessary to improve the homogeneity of the spread of the covariates, linearity of relations, and normality of the residuals.

3. Results

3.1 Food consumption during the wheel acclimation phase

Mice from the HR lines tended to be smaller than those from C lines (Table S1). Adjusting for variation in body mass, HR mice consumed significantly more food than C mice over the five days of wheel acclimation (Table S2: $P=0.0001$). As shown in **Figure 2**, one of the HR lines (#3) deviated from the general pattern and tended to have lower food consumption. This particular line is fixed for a Mendelian recessive gene of major effect ("mini muscle") that reduces hindlimb muscle mass by approximately 50% and has numerous other pleiotropic effects (Garland et al. 2002);(Rezende et al. 2006b); (Kelly et al. 2013)).

3.2 Food consumption and physical activity in wheel metabolic chambers

After mice were placed into the wheel metabolic chambers, HR and C mice showed no difference in mass-adjusted food consumption during either day 6 (standard chow) (**Fig. 3A**, Table S2: $P=0.9991$) or day 7 (Western diet) (**Fig. 3B**, Table S2: $P=0.2120$). As shown in **Figures 3B and 3C**, a subset of six individuals (three in C lines) consumed very little food (< 2 g) on day 7, an aspect of individual variation ("non-responders") that has been found previously for these lines of mice (see Chapter 2 and Discussion).

As expected from numerous previous studies, wheel-running activity was significantly higher for HR than C mice on both days 6 and 7 (**Fig. 4A, 4B**). Deleting the six mice that ate “too little” on day 7 (see previous paragraph) and one who ate too much (standardized residual >4), a repeated-measures ANCOVA with age as the covariate ($P=0.1594$) indicated no overall effect of day ($P=0.1816$), an effect of linetype ($P=0.0004$), and an interaction ($P=0.0796$), such that C mice ran slightly more on day 7 ($5,665 \pm 763$) than on day 6 ($5,309 \pm 763$), where as HR mice ran substantially less on day 7 with Western diet ($10,292 \pm 700$) than on day 6 ($12,449 \pm 700$).

Home-cage activity (HCA) was significantly lower on day 7 for both HR and C mice (**Fig. 5**, $P=0.0245$). Deleting the mice that ate too little or too much, a repeated-measures ANCOVA with age as a covariate ($P=0.0389$) indicated a significant difference between HR and C mice ($P=0.0317$) and an effect of day ($P=0.0245$). The day*linetype interaction was not significant ($P>0.41$). HR mice were substantially less active in their home-cages on day 7 with Western diet (49.60 ± 7.22) than on day 6 (64.70 ± 7.22). C mice were substantially less active in their home-cages on day 7 with Western diet (65.77 ± 8.10) than on day 6 (93.63 ± 8.10).

On day 6 when consumption of standard chow was analyzed with activity levels as additional independent variables ($N = 45$), it was positively related to body mass ($P=0.0365$) and to total home-cage activity ($P=0.0081$), but was unrelated to total wheel running ($P=0.6342$) and did not differ between linetypes ($P=0.4639$). Results were similar for day 7 with Western diet (body mass $P=0.0002$, home-cage activity $P=0.1049$, wheel running $P=0.8723$, linetype $P=0.2673$).

3.3 Oxygen consumption with standard chow

During day 6 with standard chow, overall daily oxygen consumption (measured over 23 hours) was positively correlated with body mass ($P=0.0476$), age ($P=0.5185$), and tended to be higher in HR than C mice ($P=0.0836$) (**Fig. 6**) (Least Squares Means and Standard Errors: HR = 1.6919 ± 0.07235 ml/min; C = 1.5155 ± 0.0405). In a model that included activity levels, daily oxygen consumption was positively related to body mass ($P=0.0001$), total wheel running ($P<0.0001$), and total home-cage activity ($P=0.0515$), but not linetype ($P=0.6007$). Thus, the higher daily oxygen consumption of HR mice can be explained (statistically) in large part by their higher levels of physical activity.

Minimal oxygen consumption during rest (a.k.a. resting metabolic rate or RMR, measured over 10 minutes) was positively associated with body mass ($P=0.0048$) and negatively with age ($P=0.0135$), but did not significantly differ between HR and C mice ($P=0.5855$) (**Fig. 7**). Results were similar over other time intervals, as shown in Table S3.

Voluntary maximal oxygen consumption was positively related to body mass and significantly higher in HR mice than C mice over 1-, 2-, 5-, 10-, 20-, 30-, 60-, and 120-minute intervals (**Fig. 8**, Table S4).

3.4 Oxygen consumption with Western diet

During day 7 with Western diet, overall daily oxygen consumption was positively correlated with body mass ($P=0.0002$) and age ($P=0.2076$), but did not differ between

HR and C mice ($P=0.1568$) (**Fig. 9**) (Least Squares Means and Standard Errors: HR = 1.6343 ± 0.05757 ml/min; C = 1.4617 ± 0.08709). In a model that included activity levels, daily oxygen consumption depended on body mass ($P=0.0001$), total wheel running ($P<0.0002$), and total home-cage activity ($P=0.0116$), but again not on linetype ($P=0.6830$).

Minimal oxygen consumption during rest (a.k.a. resting metabolic rate or RMR) over 10 minutes was positively associated with body mass ($P=0.0006$) and negatively associated with age ($P=0.0183$), but did not significantly differ between HR and C mice ($P=0.9457$) (**Fig. 10**). Results were similar over other time intervals, as shown in Table S3.

Mass-adjusted voluntary maximal oxygen consumption was significantly higher in HR mice than in C mice over 1-, 2-, 5-, 10-, 20-, 30-, 60-, and 120-minute intervals (**Fig. 11**, Table S4).

3.5 Carbon dioxide and Calculated RER with Standard Chow

Carbon dioxide produced calculated over 23 hours was positively correlated with body mass ($P=0.0145$), but did not differ between HR and C mice ($P=0.1388$). In a model that included activity levels, daily carbon dioxide production depended positively on body mass ($P=0.0011$), total wheel running ($P<0.0001$), and total home-cage activity ($P=0.0053$), but not on linetype ($P=0.9669$). Respiratory exchange ratio (carbon dioxide produced/oxygen consumed) calculated over 23 hours was not significantly different between HR and C mice ($P=0.9246$) on standard chow. Mass- and age-adjusted RER on

standard chow averaged (0.8774 ± 0.0126) for control mice and (0.8756 ± 0.0122) for HR mice (Least Squares Means and Standard Errors: $P = 0.9246$) (**Fig. 12**, Table S5).

On day 6 with standard chow, carbon dioxide production corresponding to minimal values of oxygen consumption over 10 minutes was positively correlated with body mass ($P < 0.0001$) and negatively associated with age ($P = 0.0055$), but did not differ between HR and C mice ($P = 0.6166$). Calculated RER over these 10 minutes was not significantly different between HR and C mice ($P = 0.1349$: HR = 1.1265 ± 0.04704 ml/min; C = 1.0040 ± 0.04848).

On day 6 with standard chow, carbon dioxide production corresponding to voluntary maximal oxygen consumption values was significantly higher in HR mice than in C mice for all time intervals considered (Table S6). RER calculated over the maximum oxygen consumption and corresponding CO₂ over 1 minute was not significantly different between HR and C mice ($P = 0.3855$) (Table S5).

3.6 Carbon dioxide and Calculated RER with Western Chow

Carbon dioxide produced calculated over 23 hours on day 7 was positively correlated with body mass ($P = 0.0002$), but did not differ between HR and C mice ($P = 0.2301$). In a model that included activity levels, daily carbon dioxide production depended positively on body mass ($P < 0.0001$), total wheel running ($P = 0.0180$), total home-cage activity ($P = 0.0052$), but not on linetype ($P = 0.9764$). Respiratory exchange ratio (carbon dioxide produced/oxygen consumed) calculated over 23 hours was not significantly different between HR and C mice ($P = 0.6668$) on Western diet. Mass- and

age-adjusted RER on standard chow averaged (0.8695 ± 0.0193) for control mice and (0.8814 ± 0.0163) for HR mice (Least Squares Means and Standard Errors: $P = 0.6668$) (**Fig. 13**, Table S5).

On day 7 with Western diet, carbon dioxide production corresponding to minimal values of oxygen consumption over 10 minutes was positively correlated with body mass ($P=0.0005$), did not correlate with age ($P=0.9516$), and did not differ between HR and C mice ($P=0.9211$). RER calculated over these 10 minutes was not significantly different between HR and C mice ($P=0.7133$: HR = 0.9603 ± 0.0645 C = 0.9271 ± 0.0534).

On day 7 with Western diet, carbon dioxide production corresponding to voluntary maximal oxygen consumption values was higher in HR mice than in C mice for all time intervals considered, but this difference did not reach significance in most cases (Table S6). RER was not significantly different between HR and C mice for any time interval (Table S5). **Figure 14** compares RER values measured over 23 hours on days 6 and 7. SAS repeated-measures ANCOVA determined HR and C mice did not differ in their RER on either day 6 (HR = 0.8777 ± 0.0147 ; C = 0.8912 ± 0.0157) or day 7 (HR = 0.8933 ± 0.0145 ; C = 0.9029 ± 0.0157). Values used excluded six mice that ate too little and one that ate too much on day 7; keeping those mice in the analysis did not change the P markedly. If body mass was added to the model, P values were similar (values not shown).

4. Discussion

The primary goal of the present study was to test for a difference in respiratory exchange ratio (RER) between selectively bred (63 generations) High Runner and non-selected Control lines of mice, which would indicate a difference in the relative amount of fats and carbohydrates being oxidized. As expected, HR mice had increased voluntary maximal oxygen consumption, as a consequence of their greater levels of wheel running (Rezende et al. 2006b; Rezende et al. 2006c). However, contrary to our predictions, we found no differences in RER between HR and C mice at rest or during voluntary wheel running, on either standard chow or when fed a Western diet for one day. These results are generally consistent with those of (Templeman et al. 2012), who used forced treadmill exercise. In that study, during treadmill exercise at ~66% of VO_{2max} , approximately 70% of the energy expended was estimated to be supplied via the oxidation of lipids and the remainder (30%) by carbohydrate oxidation, with no difference between HR and C mice in the estimated proportional mix of fuels sustaining exercise (% total VO_2). At ~78% of VO_{2max} , all mice relied more on carbohydrate oxidation (>40%) than at the lower exercise intensity, but again no statistical difference between HR and C mice was observed in proportional fuel usage (Templeman et al. 2012). RER at VO_{2max} during forced treadmill exercise was measured in these mice at generation 10 (Swallow et al. 1998b) and 36 (Rezende et al. 2006a), with no statistical differences between HR and C mice reported. Having the same fuel-type usage during forced exercise helps explain why HR and C mice would have similar RER values in the present study under all conditions measured.

We expected that feeding mice a Western diet would increase the contribution of energy production from fat oxidation because previous studies have established that both endurance training and consumption of a high-fat diet will lead to higher rates of fat oxidation in humans (Lambert et al. 1997; Horowitz and Klein 2000; Helge et al. 2001; Achten and Jeukendrup 2004), mice (Turner et al. 2007; Jonas et al. 2010), and rats (Turner et al. 2007). However, contrary to our expectations, RER was not significantly altered during experimental day 7, when mice were fed Western diet and had experienced six days of prior wheel access and hence some degree of endurance training (e.g., see (Gomes et al. 2009)).

Prior to placement in the wheel metabolic chambers, both linetypes received the same opportunity for exercise training (i.e., five days of wheel access), but presumably HR mice were running more than C mice during that time period, which would account for their significantly greater food consumption (Table S2) (Rezende et al. 2005; Meek et al. 2009; Meek et al. 2010; Acosta et al. 2015; Copes et al. 2015). Surprisingly, HR did not eat more food than C mice on day 6 (Table S2, Least Squares Means of 6.56 versus 6.56 grams), perhaps because of a novelty or stress effect that stimulated eating in the C lines, related to transfer into the wheel metabolic chambers. On day 7, food consumption by C lines had declined relative to day 6 (5.44 grams) and tended to be lower than for HR lines (6.56 grams). Interestingly, six individuals (Fig. 3C) did not eat much of the Western diet (“non-responders”), consistent with previous studies of these mice (Chapter 2). Possible explanations involve texture, smell, and taste differences between the

Western diet and standard chow. We have sometimes observed individuals to examine Western diet extensively, but not consume much.

Contrary to our expectations, based on previous studies of these mice ((Meek et al. 2010);(Meek et al. 2012); Chapter 2), WD did not increase wheel running (**Fig. 4**), nor did it change voluntary VO_2 max (**Figs. 8, 12**), oxygen consumption at rest (**Figs. 7, 10**) or RER as measured over any time period or condition (**Fig. 12-14**). A possible explanation for the lack of WD effect may be the short exposure duration. Giving mice WD for one day may have been insufficient to reveal its metabolic or behavioral effects, and moreover may have led to some novelty effects that could potentially mask or interact with effects of the diet *per se*. It would obviously be of interest to repeat these studies with longer periods of Western-diet feeding (e.g., see (Novak et al. 2010), but in the present study we were attempting to mimic the time course of wheel access (6 days) over which mice are routinely tested when choosing breeders for the next generation.

Figure 3.1 Experimental timeline

Figure indicating when body mass and food mass were recorded. Mice had five days of acclimation to wheels with ad lib standard chow, then one day in the wheel metabolic chamber with standard chow and finally a seventh day in the metabolic chamber with Western diet.

RER Timeline

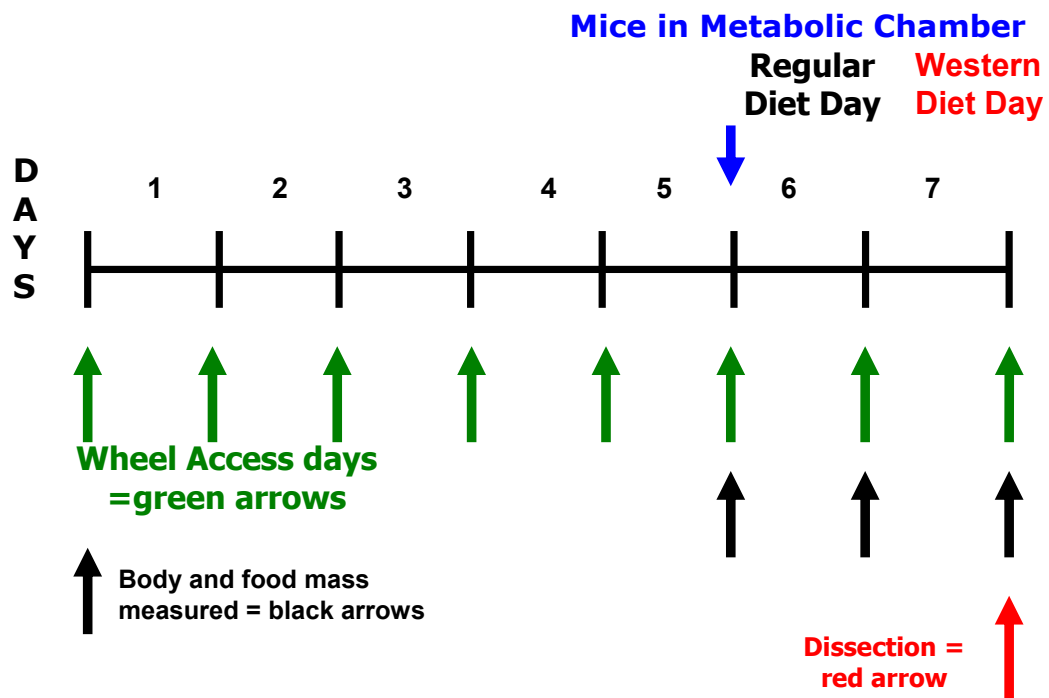


Figure 3.2 Food consumption days 1-5

High Runner (HR) lines (dark-filled shapes) consume more food than Control (C) lines of mice over 5 days with wheels. For all following food-consumption figures, unless stated otherwise, body mass (grams (g)) are shown on the x-axis and food consumption (g) are listed on the y-axis. Linetype P = 0.0001, Body mass P < 0.0001

Daily Food Consumption over days 1-5 with Wheels

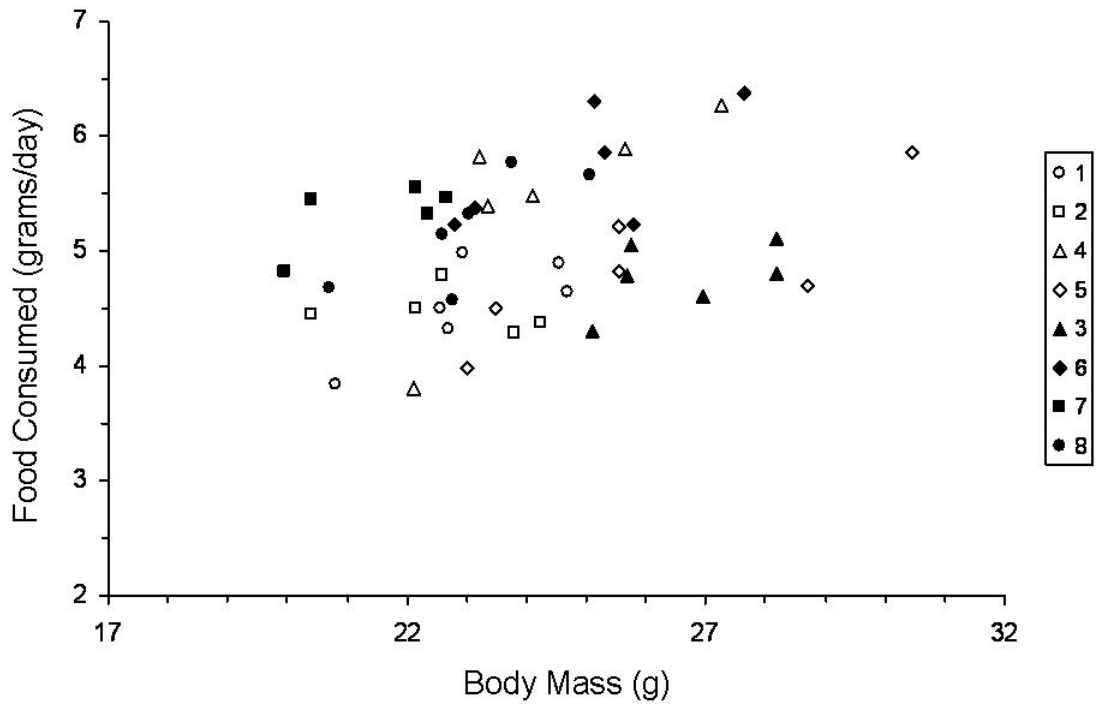


Figure 3.3 Food consumption days 6 and 7

A.) HR and C lines consumed similar amounts of standard chow in the wheel chamber on Day 6.
(Linetype P = 0.9991)

B.) HR and C lines consumed similar amounts of Western diet in the wheel chamber on Day 7 (Linetype P = 0.2120) See table S2 for further explanation

A)



B)



Figure 3.3 Food consumption days 6 and 7-continued

C.)Day 6 food consumption vs. Day 7 food consumption
See table S2 for further explanation

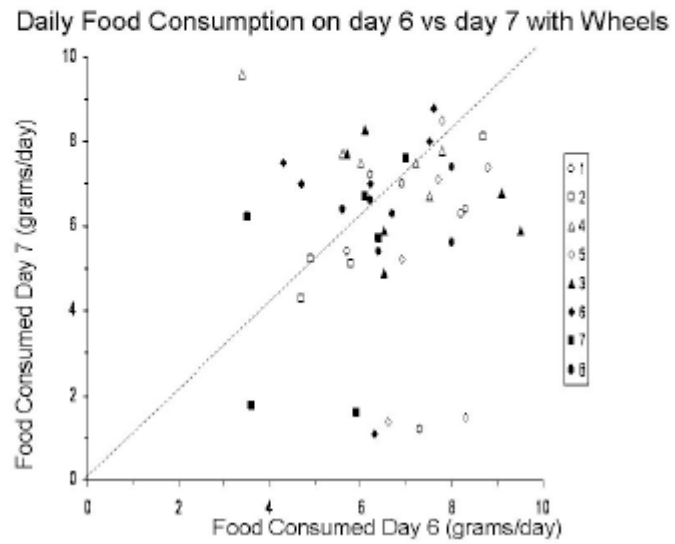
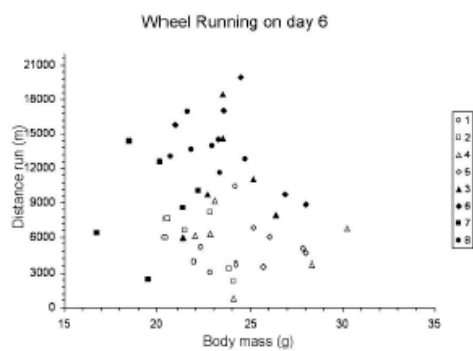


Figure 3.4 Wheel running days 6 and 7

A) Wheel running over a 23-hour period while mice were fed standard diet (day 6) was significantly higher in HR lines than in C lines (N = 44, Linetype P=0.0021; Age P = 0.4817). LSM \pm SE were 12,273 \pm 1,154 for HR mice and 5,306 \pm 703 for C mice.

B) Wheel running over a 23-hour period while mice were fed Western diet (day 7) was significantly higher in HR lines than in C lines (N = 44, Linetype P=0.0061; Age P = 0.2636). LSM \pm SE were 10,189 \pm 830 for HR mice and 5,261 \pm 853 for C mice.

A)



B)

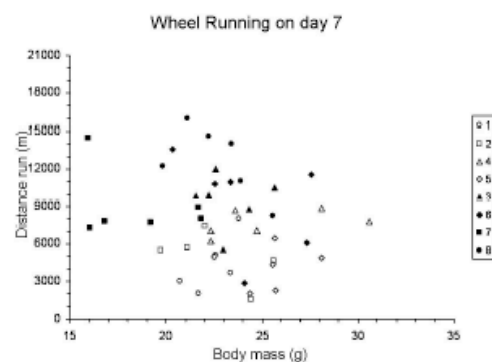


Figure 3.4 Wheel running days 6 and 7-continued

C) Comparison of wheel running on day 6 and 7 (N = 38 mice: see text for statistical results).

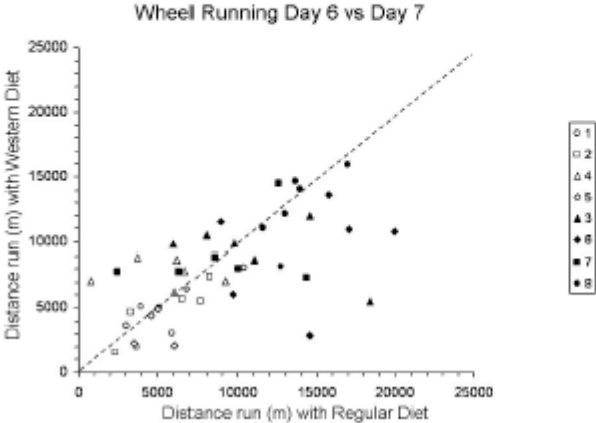


Figure 3.5 Total home-cage activity

A) Total home-cage activity over a 23-hour period while mice were fed a standard diet (day 6) was similar in HR and C lines (N=44 Linetype P=0.1732; Age P=0.0944). LSM \pm SE were 70.1844 ± 6.5736 for HR mice and 85.2564 ± 6.6850 for C mice.

B) Total home-cage activity over a 23-hour period while mice were fed a Western diet (day 7) was similar in HR and C lines (N=45 Linetype P=0.9658; Age P=0.6786). LSM \pm SE were 53.6220 ± 8.4022 for HR mice and 54.2178 ± 9.5547 for C mice

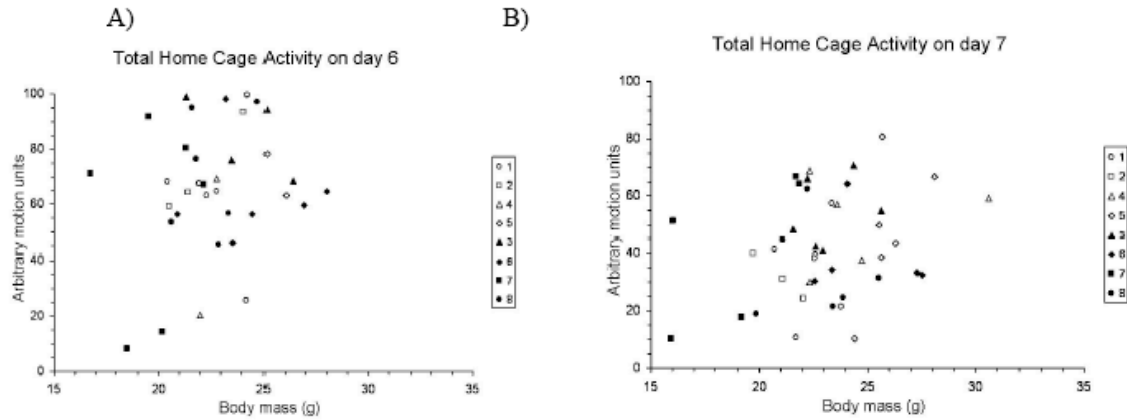


Figure 3.5 Total home-cage activity-continued

C) Comparison of total home-cage activity on day 6 and 7 (N=38: see text for statistical results)

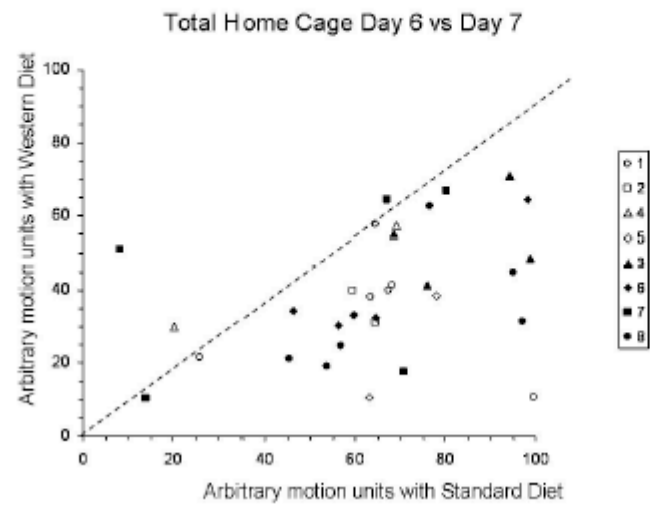


Figure 3.6 Daily oxygen consumption day 6

Figure shows 23-hour oxygen consumption on standard diet (day 6). Oxygen consumption with wheel running, total HCA, body mass and age in the model was higher in larger mice and in mice that ran more. We found no effect of linetype ($P=0.6007$), but body mass ($P=0.0001$) and total distance run ($P < 0.0001$) had significant effects on oxygen consumption, and total home-cage activity ($P = 0.0515$) had a near-significant effect

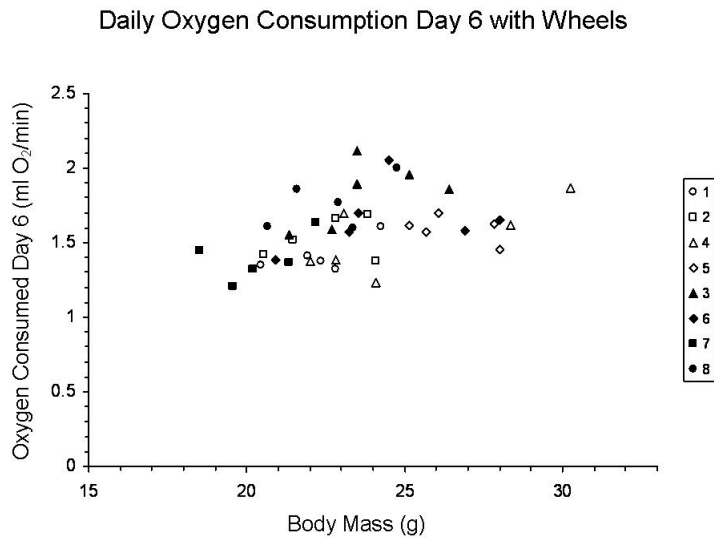


Figure 3.7 Minimal oxygen consumption (resting metabolic rate) **day 6** over 10 minutes did not differ between HR and C mice with standard chow. Linetype was not a significant factor in minimal oxygen consumption ($P=0.5855$), but effects of body mass ($P=0.0048$) and age ($P=0.0135$) were significant. Body mass had a positive effect on RMR, while age had a negative effect.

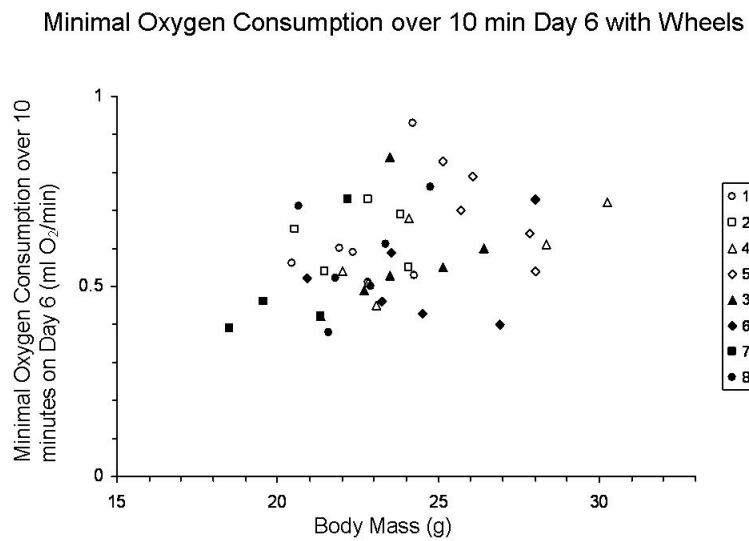


Figure 3.8. Voluntary maximal oxygen consumption (VO₂) day 6

The following panels show Voluntary maximal oxygen consumption (VO₂) over 1-, 5-, 10, 120 minute intervals. Voluntary maximal oxygen consumption was always higher in HR lines of mice than in C lines of mice. LSM and P values for all time intervals measured are shown in (Table S4).

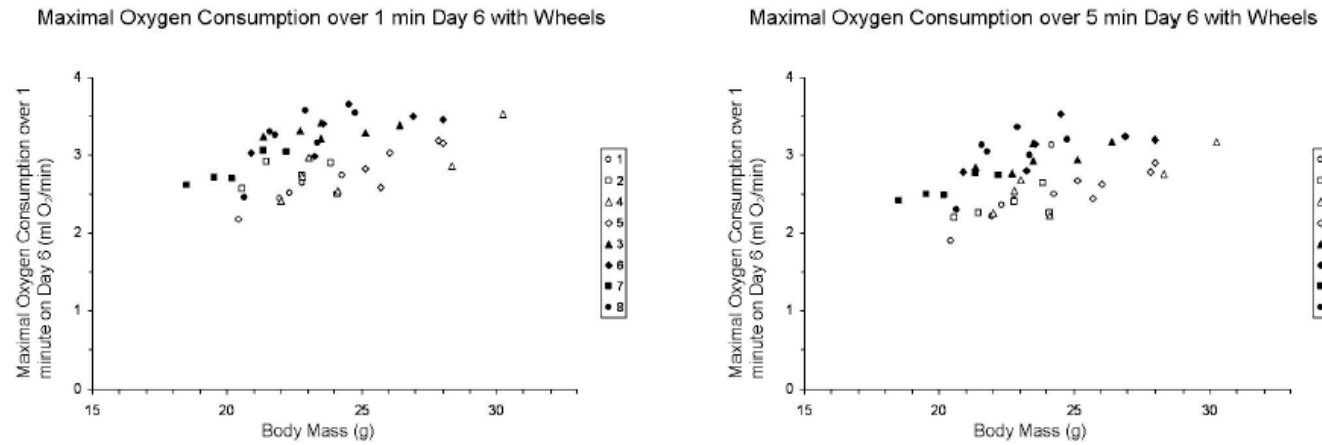
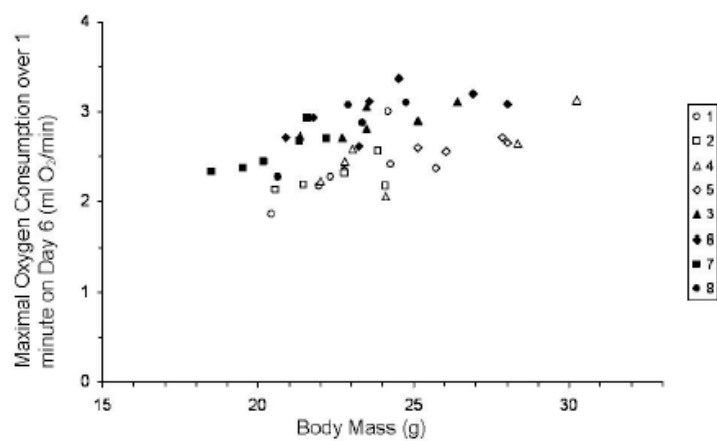


Figure 3.8. Voluntary maximal oxygen consumption (VO_2) day 6-continued

Maximal Oxygen Consumption over 10 min Day 6 with Wheels



Maximal Oxygen Consumption over 120 min Day 6 with Wheels

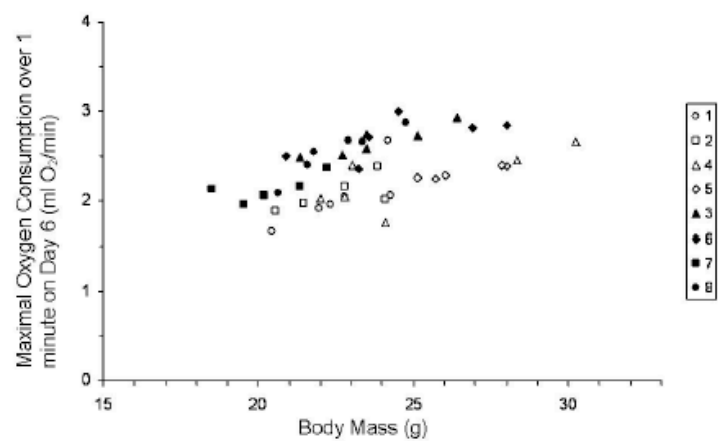


Figure 3.9 Daily oxygen consumption day 7

Daily oxygen consumption on a Western diet (day 7). Oxygen consumption was similar between HR and C lines of mice ($P=0.6830$), but was significantly affected by body mass ($P < .0001$), total distance run ($P = .0002$) and total home-cage activity ($P = .0116$). LSM + SE for HR was $1.5313 \pm .06529$ and for C mice it was $1.5736 \pm .06229$. Three line 7 mice (HR line) that had low body masses and did not consume as much Western diet.

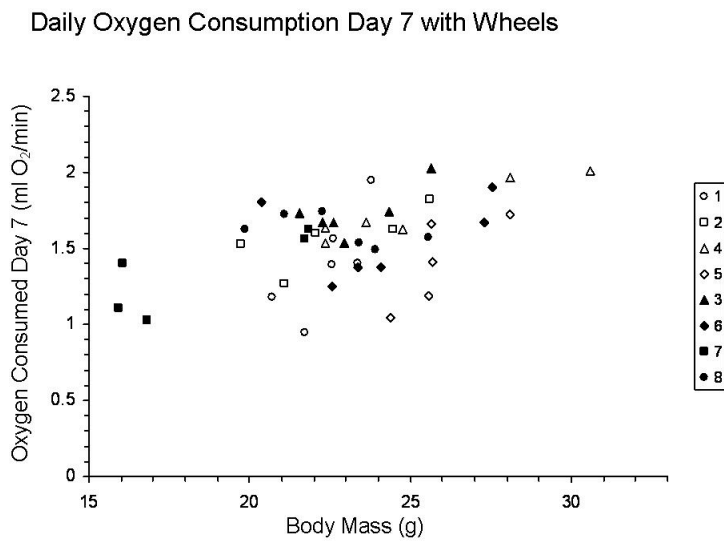


Figure 3.10 Minimal oxygen consumption day 7

Minimal oxygen consumption (resting metabolic rate) of mice fed a Western diet (day 7). Minimal oxygen consumption over a 10-minute period was similar between HR and C lines of mice ($P=0.9457$) but was significantly influenced by body mass ($P=0.0006$) and age ($P=0.0183$). Body mass had a positive effect on RMR, whereas age had a negative effect.

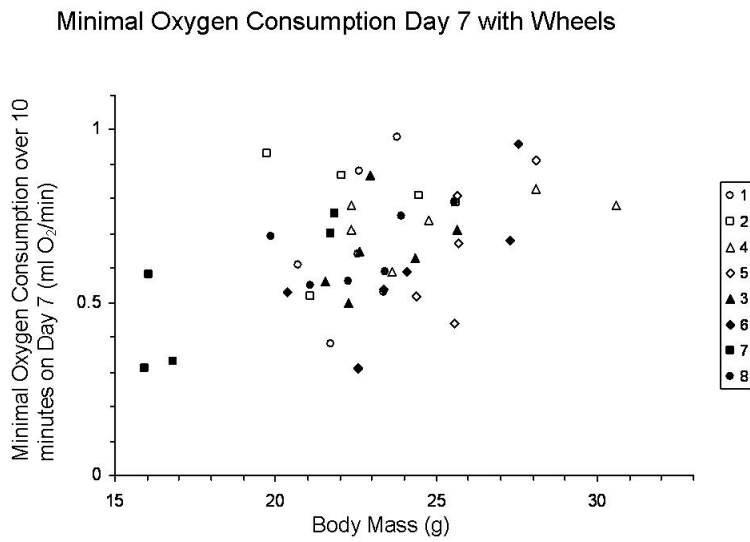


Figure 3.11 Voluntary maximal oxygen consumption (VO₂) day 7

The following panels show Voluntary maximal oxygen consumption (VO₂) over 1-, 5-, 10, 120 minute intervals. Voluntary maximal oxygen consumption was always higher in HR lines of mice than in C lines of mice. LSM and P values for all time intervals measured are shown in (Table S4).

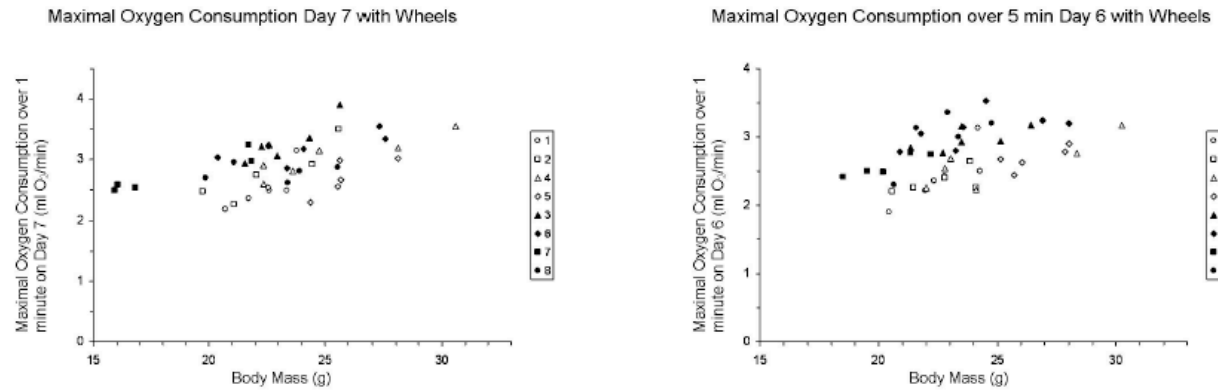


Figure 3.11 Voluntary maximal oxygen consumption (VO₂) day 7- continued

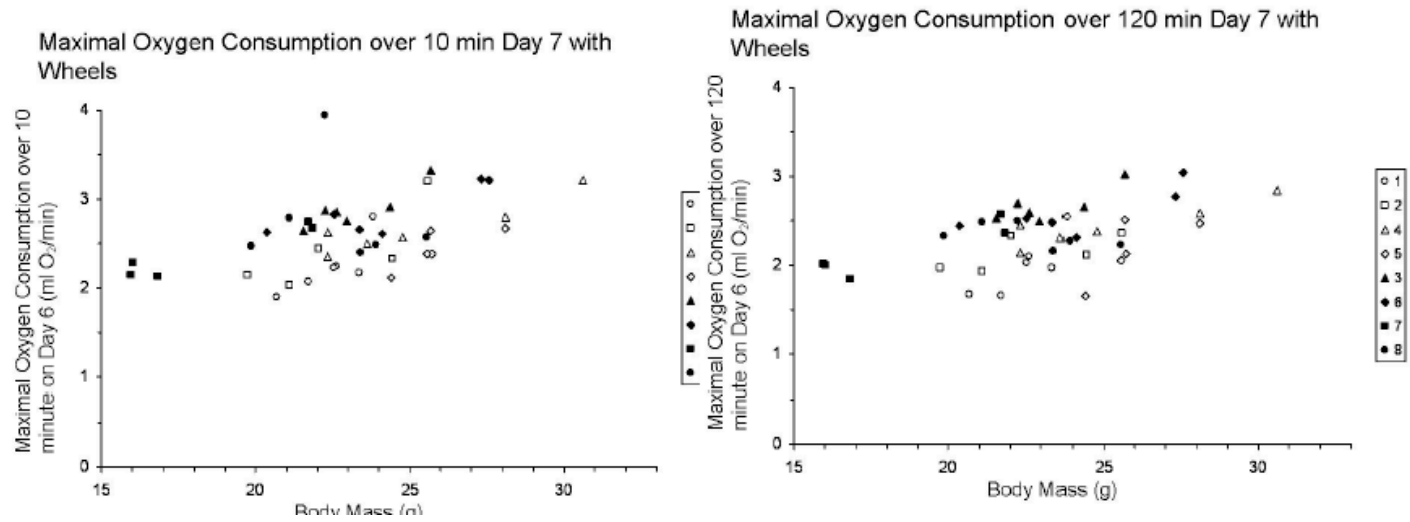


Figure 3.12 RER during day 6

RER during 23 hours on day 6 was negatively affected by wheel running distance ($P=0.0331$) and positively affected total home-cage activity ($P=0.0014$) (not shown in graph). Wheel running or home-cage activity did not affect RER at any other time interval (5, 10, 20, 30, 60, 120 minutes). There was also no significant differences between HR and C lines of mice ($P= .1088$)

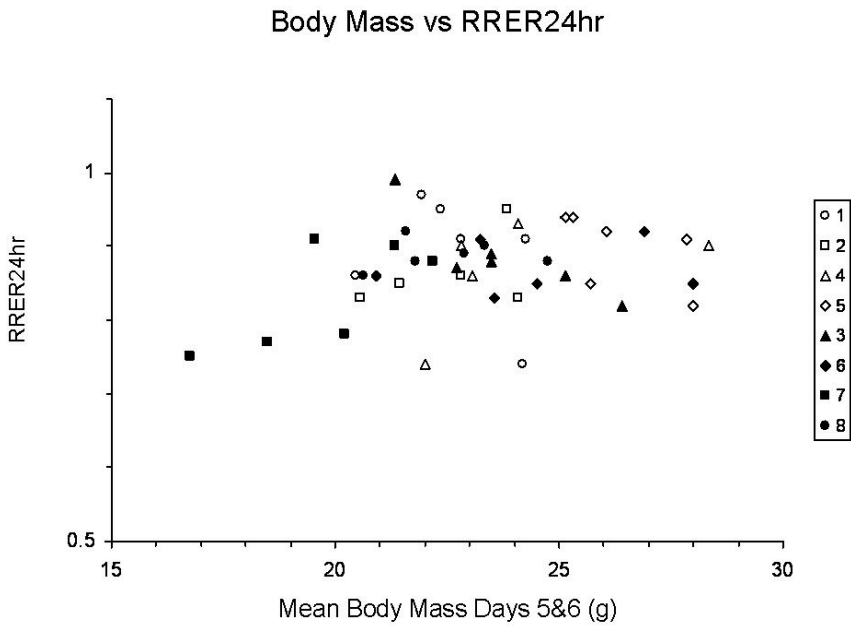


Figure 3.13 RER during day 7

RER during 23 hours on day 7 while mice were in wheel metabolic chambers with Western diet was unaffected by linetype ($P=0.5389$), but it was positively affected by body mass ($P=0.0086$) and total home-cage activity ($P=0.0188$). RER also did not significantly differ between HR and C mice for any other time interval considered (5, 10, 20, 30, 60, 120 minutes)

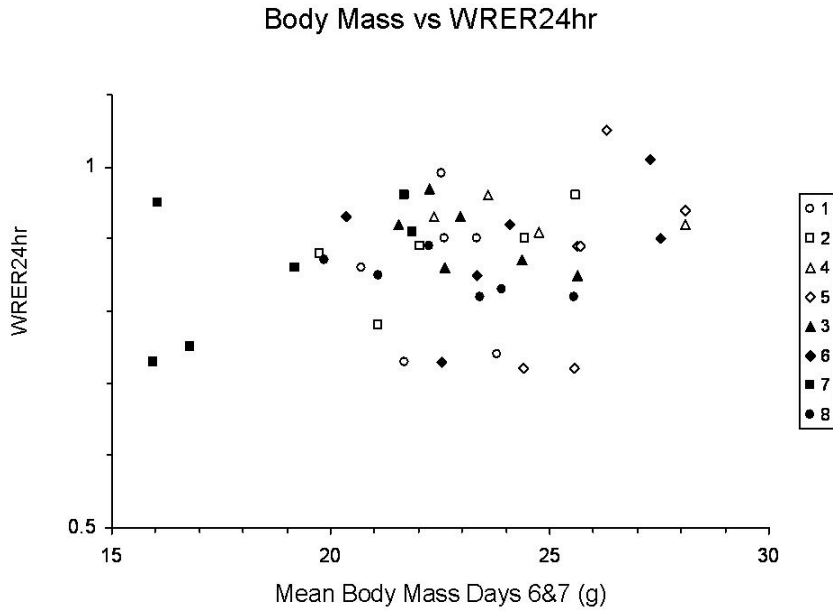


Figure 3.14 RER Day 6 vs. Day 7

HR and C mice did not differ in their RER on either day 6 (HR = 0.8777 ± 0.0147 ; C = 0.8912 ± 0.0157) or day 7 (HR = 0.8933 ± 0.0145 ; C = 0.9029 ± 0.0157).

(Linetype P=0.5447) (Day P=0.3101) (Day*Linetype P= 0.8803) (Age P= 0.8104)

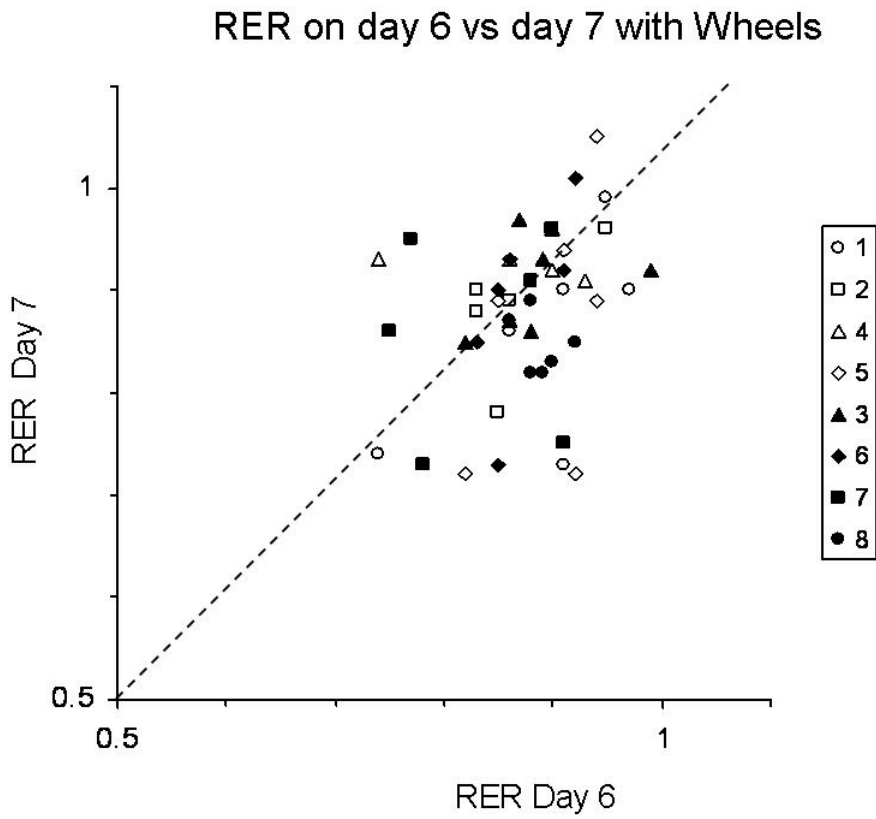


Table 3.1 Body mass

Body masses (g) throughout experiment. Values shown are LSM \pm SE, adjusting for age, from SAS procedure Mixed.

Linetype	Weaning	Days1-5	Days5-6	Days6-7
0	12.0305 \pm 0.4516	24.7199 \pm 0.8281	24.2745 \pm 0.6881	24.1074 \pm 0.6690
1	11.0014 \pm 0.5471	23.4411 \pm 0.3566	22.7991 \pm 0.4471	22.1541 \pm 0.8847
N	45	45	45	45
P linetype	0.1971	0.2059	0.1224	0.1287
P age	-	<0.0001	<0.0001	0.0012

Table 3.2 Food consumption

Food consumed (g) throughout experiment. Values shown are LSM \pm SE, adjusting for body mass and age.

*Indicates significant difference between linetypes.

Linetype	Days1-5	Day6	Day7
0	4.5823 \pm 0.07234	6.5616 \pm 0.3626	5.4401 \pm 0.6907
1	5.5798 \pm 0.08741*	6.5622 \pm 0.3699	6.5621 \pm 0.3786
N	44	45	44
P linetype	0.0001	0.9991	0.2120
P body mass	<0.0001	0.0174	0.0002
P age	0.8090	0.8103	0.0175

Table 3.3 Resting metabolic rate (mls O₂/min) for Day 6 and Day 7.Values shown are LSM \pm SE, adjusting for body mass and age.

Day 6: Regular Diet LSM \pm SE					
Minimal Oxygen Consumption (ml/min)					
	Control mice	HR mice	PLinetype	PMass	PAge
RMR ₁₀	0.5916 \pm 0.02447	0.5694 \pm 0.02768	0.5855	0.0048	0.0135
RMR ₂₀	0.6733 \pm 0.02387	0.6454 \pm 0.02925	0.5031	0.0024	0.0695
RMR ₃₀	0.7089 \pm 0.02814	0.6811 \pm 0.03246	0.5526	0.0012	0.1071
RMR ₆₀	0.7542 \pm 0.02863	0.7384 \pm 0.04508	0.7825	0.0122	0.3366
RMR ₁₂₀	0.8273 \pm 0.04990	0.8268 \pm 0.06569	0.9954	0.0110	0.2235
N=45					

Day 7: Western Diet LSM \pm SE					
Minimal Oxygen Consumption (ml/min)					
	Control mice	HR mice	PLinetype	PMass	PAge
RMR ₁₀	0.6643 \pm 0.04052	0.6606 \pm 0.02945	0.9457	0.0006	0.0183
RMR ₂₀	0.7268 \pm 0.05671	0.7290 \pm 0.03270	0.9746	0.0009	0.0718
RMR ₃₀	0.7608 \pm 0.06308	0.7701 \pm 0.03373	0.9035	0.0006	0.0841
RMR ₆₀	0.8200 \pm 0.06045	0.8089 \pm 0.03564	0.8829	0.0008	0.1177
RMR ₁₂₀	0.8967 \pm 0.07180	0.8776 \pm 0.03865	0.8266	0.0024	0.1668
N=45					

Table 3.4 Maximal Voluntary Oxygen Consumption (mls O₂/min) for Day 6 and Day 7.

Values shown are LSM ± SE, adjusting for body mass and age.

*Indicates significant difference between linetypes.

	Day 6: Regular Diet LSM ± SE					
	Control mice	HR mice	N	PLinetype	PMass	PAge
VO₂₁	2.7348 ± 0.05191	3.237 ± 0.05036*	45	0.0006	<0.0001	0.5193
VO₂₂	2.5870 ± 0.04976	3.1114 ± 0.04836*	45	0.0004	<0.0001	0.5742
VO₂₅	2.4670 ± 0.04705	2.9813 ± 0.05492*	45	0.0005	<0.0001	0.4913
VO₂₁₀	2.3751 ± 0.04427	2.8828 ± 0.04296*	45	0.0002	<0.0001	0.5852
VO₂₂₀	2.3057 ± 0.04170	2.7856 ± 0.04046*	45	0.0002	<0.0001	0.4863
VO₂₃₀	2.2628 ± 0.04035	2.7394 ± 0.03915*	45	0.0002	<0.0001	0.4194
VO₂₆₀	2.1970 ± 0.03876	2.6485 ± 0.03945*	45	0.0002	<0.0001	0.1957
VO₂₁₂₀	2.1121 ± 0.03773	2.5715 ± 0.05209*	45	0.0005	<0.0001	0.1393
	Day 7: Western Diet LSM ± SE					
	Control mice	HR mice	N	PLinetype	PMass	PAge
VO₂₁	2.7252 ± 0.09154	3.1576 ± 0.08114*	45	0.0149	0.0005	0.6476
VO₂₂	2.5950 ± 0.08583	3.0226 ± 0.08329*	45	0.0144	0.0015	0.3685
VO₂₅	2.4803 ± 0.07704	2.9040 ± 0.07477*	45	0.0094	0.0003	0.5236
VO₂₁₀	2.3951 ± 0.07178	2.7984 ± 0.06193*	45	0.0066	<0.0001	0.5426
VO₂₂₀	2.3235 ± 0.06945	2.7123 ± 0.05304*	45	0.0052	<0.0001	0.6745
VO₂₃₀	2.2839 ± 0.06881	2.6660 ± 0.04806*	45	0.0046	<0.0001	0.6632
VO₂₆₀	2.2038 ± 0.05893	2.5874 ± 0.05401*	45	0.0035	<0.0001	0.8426
VO₂₁₂₀	2.1273 ± 0.07143	2.5110 ± 0.06647*	45	0.0087	<0.0001	0.5149

Table 3.5 Respiratory Exchange Ratio (RER) corresponding to Maximal Voluntary Oxygen Consumption and corresponding Carbon Dioxide values for Day 6 and Day 7.

Values shown are LSM \pm SE, adjusting for body mass and age.

*Indicates significant difference between linetypes.

Day 6: Regular Diet						
	Control mice	HR mice	Plinetype	PMass	PAge	
RER ₁	0.8035 \pm 0.01680	0.8265 \pm 0.01630	0.3855	0.3956	0.2751	
RER ₂	0.8310 \pm 0.01373	0.8533 \pm 0.01332	0.3092	0.0140	0.1533	
RER ₅	0.8414 \pm 0.01452	0.8697 \pm 0.01409	0.2301	0.4673	0.2375	
RER ₁₀	0.8730 \pm 0.01145	0.8847 \pm 0.01110	0.5133	0.0523	0.2964	
RER ₂₀	0.8702 \pm 0.01302	0.8797 \pm 0.01264	0.6376	0.1841	0.3703	
RER ₃₀	0.8735 \pm 0.01240	0.8805 \pm 0.01203	0.7110	0.5945	0.6542	
RER ₆₀	0.8719 \pm 0.01295	0.8753 \pm 0.01256	0.8620	0.6137	0.4542	
RER ₁₂₀	0.8774 \pm 0.01454	0.8825 \pm 0.01411	0.8187	0.3132	0.1372	
RER _{23hr}	0.8774 \pm 0.01257	0.8756 \pm 0.01219	0.9246	0.2532	0.4566	
Day 7: Western Diet LSM \pm SE						
	Control mice	HR mice	Plinetype	PMass	PAge	
RER ₁	0.7970 \pm 0.02646	0.8010 \pm 0.03061	0.9282	0.0874	0.3604	
RER ₂	0.8406 \pm 0.02573	0.8274 \pm 0.03955	0.7949	0.1799	0.2184	
RER ₅	0.8524 \pm 0.02530	0.8458 \pm 0.03671	0.8908	0.2807	0.1235	
RER ₁₀	0.8641 \pm 0.02413	0.8594 \pm 0.03531	0.9194	0.2781	0.1134	
RER ₂₀	0.8795 \pm 0.02319	0.8673 \pm 0.03255	0.7794	0.1514	0.0906	
RER ₃₀	0.8806 \pm 0.02240	0.8730 \pm 0.03185	0.8572	0.1201	0.0713	
RER ₆₀	0.8932 \pm 0.01974	0.8811 \pm 0.02915	0.7506	0.1022	0.1241	
RER ₁₂₀	0.9049 \pm 0.02024	0.8810 \pm 0.02210	0.4751	0.2092	0.3134	
RER _{23hr}	0.8695 \pm 0.01926	0.8814 \pm 0.01625	0.6668	0.0138	0.1409	

Table 3.6 Carbon Dioxide values corresponding to Maximal Voluntary Oxygen Consumption (mls O₂/min) for Day 6 and Day 7.

Values shown are LSM ± SE, adjusting for body mass and age.

*Indicates significant difference between linetypes.

Day 6: Regular Diet LSM ± SE						
	Control mice	HR mice	N	Plinetype	PMass	PAge
CO ₂ ₁	2.1920 ± 0.05842	2.6722 ± 0.05668*	45	0.0014	0.0005	0.6747
CO ₂ ₂	2.1508 ± 0.05434	2.6562 ± 0.05272*	45	0.0007	<0.0001	0.6163
CO ₂ ₅	2.0678 ± 0.05185	2.5941 ± 0.05031*	45	0.0004	0.0001	0.7517
CO ₂ ₁₀	2.0739 ± 0.04201	2.5488 ± 0.04076*	45	0.0002	<0.0001	0.8726
CO ₂ ₂₀	2.0059 ± 0.04240	2.4478 ± 0.04114*	45	0.0004	<0.0001	0.9838
CO ₂ ₃₀	1.9761 ± 0.04091	2.4085 ± 0.03969*	45	0.0004	<0.0001	0.6424
CO ₂ ₆₀	1.9150 ± 0.03973	2.3139 ± 0.03855*	45	0.0005	<0.0001	0.4942
CO ₂ ₁₂₀	1.8504 ± 0.04315	2.2668 ± 0.05746*	45	0.0014	<0.0001	0.9598
Day 7: Western Diet LSM ± SE						
	Control mice	HR mice	N	Plinetype	PMass	PAge
CO ₂ ₁	2.1557 ± 0.09868	2.5224 ± 0.12840	45	0.0696	<0.0001	0.3817
CO ₂ ₂	2.1649 ± 0.08284	2.4837 ± 0.13460	45	0.0965	<0.0001	0.5153
CO ₂ ₅	2.0923 ± 0.07979	2.4437 ± 0.11450*	45	0.0498	<0.0001	0.2654
CO ₂ ₁₀	2.0530 ± 0.07588	2.3991 ± 0.11060*	45	0.0459	<0.0001	0.2425
CO ₂ ₂₀	2.0299 ± 0.08528	2.3562 ± 0.10410	45	0.0562	<0.0001	0.1985
CO ₂ ₃₀	1.9991 ± 0.08929	2.3372 ± 0.11000	45	0.0588	<0.0001	0.1881
CO ₂ ₆₀	1.9657 ± 0.08961	2.2973 ± 0.11790	45	0.0714	<0.0001	0.2337
CO ₂ ₁₂₀	1.9317 ± 0.09517	2.2270 ± 0.10690	45	0.0916	0.0004	0.3199

References

- Achten, J., and A. E. Jeukendrup. 2004. Optimizing fat oxidation through exercise and diet. *Nutrition*. 20:716-727.
- Acosta, W., T. H. Meek, H. Schutz, E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2015. Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice. *Physiol Behav* 149:279-286.
- Askew, E. W. 1984. Role of fat metabolism in exercise. *Clinics in sports medicine* 3:605-621.
- Brooks, G. A., and J. Mercier. 1994. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept.
- Burke LM, Hawley JA, Angus DJ, Cox GR, Clark SA, Cummings NK, Desbrow B, and H. M. 2002. Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med Sci Sports Exerc* 34:83-91.
- Bye, A., M. A. Hoydal, D. Catalucci, M. Langaas, O. J. Kemi, V. Beisvag, L. G. Koch, S. L. Britton, O. Ellingsen, and U. Wisloff. 2008. Gene expression profiling of skeletal muscle in exercise-trained and sedentary rats with inborn high and low VO₂max. *Physiol Genomics*. 35:213-221. doi: 10.1152/physiolgenomics.90282.92008. Epub 92008 Sep 90289.
- Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2013. Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. *Evolution* 67:3102-3119.
- Chappell, M. A., T. Garland, Jr., E. L. Rezende, and F. R. Gomes. 2004. Voluntary running in deer mice: speed, distance, energy costs and temperature effects. *J Exp Biol*. 207:3839-3854.
- Conley, D. L., and G. S. Krahenbuhl. 1980. Running economy and distance running performance of highly trained athletes. *Med Sci Sports Exerc*. 12:357-360.
- Copes, L. E., H. Schutz, E. M. Dlugosz, W. Acosta, M. A. Chappell, and T. Garland Jr. 2015. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. *Physiol. Behav*. 149:86-94.
- Dumke, C. L., J. S. Rhodes, T. Garland, E. Maslowski, J. G. Swallow, A. C. Wetter, and G. D. Cartee. 2001. Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake.

- Garland, T., Jr., M. T. Morgan, J. G. Swallow, J. S. Rhodes, I. Girard, J. G. Belter, and P. A. Carter. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. *Evolution*. 56:1267-1275.
- Garland, T., Jr., H. Schutz, M. A. Chappell, B. K. Keeney, T. H. Meek, L. E. Copes, W. Acosta, C. Drenowatz, R. C. Maciel, G. van Dijk, C. M. Kotz, and J. C. Eisenmann. 2011. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *Journal of Experimental Biology* 214:206-229.
- Gomes, F. R., E. L. Rezende, J. L. Malisch, S. K. Lee, D. A. Rivas, S. A. Kelly, C. Lytle, B. B. r. Yaspelkis, and T. Garland, Jr. 2009. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology* 212:238-248.
- Gonzalez, N. C., S. D. Kirkton, R. A. Howlett, S. L. Britton, L. G. Koch, H. E. Wagner, and P. D. Wagner. 2006. Continued divergence in VO₂max of rats artificially selected for running endurance is mediated by greater convective blood O₂ delivery. 101:1288-1296. Epub 2006 Jun 1215.
- Helge, J. r. W., K. Ayre, S. Chaunchaiyakul, A. J. Hulbert, B. Kiens, and L. H. Storlien. 1998. Endurance in high-fat-fed rats: effects of carbohydrate content and fatty acid profile. *Journal of Applied Physiology* 85:1342-1348.
- Helge, J. W., P. W. Watt, E. A. Richter, M. J. Rennie, and B. Kiens. 2001. Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. *J Physiol*. 537:1009-1020.
- Horowitz, J. F., and S. Klein. 2000. Lipid metabolism during endurance exercise. *The American Journal of Clinical Nutrition* 72:558s-563s.
- Houle-Leroy, P., T. Garland, J. G. Swallow, and H. Guderley. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. *Journal of Applied Physiology* 89:1608-1616.
- Jansson, E. 1982. On the significance of the respiratory exchange ratio after different diets during exercise in man. *Acta Physiol. Scand.* 114:103-110.
- Jeukendrup, A. E., W. H. M. Saris, and A. J. M. Wagenmakers. 1998. Fat metabolism during exercise: a review. *Int J Sports Med* 19:371-379.
- Jonas, I., L. M. Vaanholt, M. Doornbos, T. Garland, A. J. W. Scheurink, C. Nyakas, and G. van Dijk. 2010. Effects of selective breeding for increased wheel-running

- behavior on circadian timing of substrate oxidation and ingestive behavior. *Physiol. Behav.* 99:549-554.
- Kelly, S. A., T. A. Bell, S. R. Selitsky, R. J. Buus, K. Hua, G. M. Weinstock, T. Garland, Jr., F. Pardo-Manuel de Villena, and D. Pomp. 2013. A novel intronic single nucleotide polymorphism in the *Myosin heavy polypeptide 4* gene is responsible for the mini-muscle phenotype characterized by major reduction in hind-limb muscle mass in mice. *Genetics* 195:1385-1395.
- Klein, S., E. F. Coyle, and R. R. Wolfe. 1994. Fat metabolism during low-intensity exercise in endurance-trained and untrained men.
- Lambert, E. V., J. A. Hawley, J. Goedecke, T. D. Noakes, and S. C. Dennis. 1997. Nutritional strategies for promoting fat utilization and delaying the onset of fatigue during prolonged exercise. *J Sports Sci.* 15:315-324.
- McClelland, G. B. 2004. Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. *Comp. Biochem. Physiol. B-Biochem. Mol. Biol.* 139:443-460.
- Meek, T., J. Eisenmann, and T. Garland, Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *Int. J. Obes.* 34:960-969.
- Meek, T. H., E. M. Dlugosz, K. T. Vu, and T. Garland, Jr. 2012. Effects of leptin treatment and Western diet on wheel running in selectively bred high runner mice. *Physiol Behav* 106:252-258.
- Meek, T. H., B. P. Lonquich, R. M. Hannon, and T. Garland. 2009. Endurance capacity of mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology* 212:2908-2917.
- Novak, C. M., C. Escande, P. R. Burghardt, M. Z. Zhang, M. T. Barbosa, E. N. Chini, S. L. Britton, L. G. Koch, H. Akil, and J. A. Levine. 2010. Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity. *Hormones and Behavior* 58:355-367.
- Pierce, B. J., S. R. McWilliams, T. P. O'Connor, A. R. Place, and C. G. Guglielmo. 2005. Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *Journal of Experimental Biology* 208:1277-1285.
- Poole, D. C., and H. H. Erickson. 2011. Highly athletic terrestrial mammals: horses and dogs. *Compr Physiol* 1:1-37.

- Rezende, E. L., M. A. Chappell, F. R. Gomes, J. L. Malisch, and T. Garland, Jr. 2005. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *Journal of Experimental Biology* 208:2447-2458.
- Rezende, E. L., T. Garland, Jr., M. A. Chappell, J. L. Malisch, and F. R. Gomes. 2006a. Maximum aerobic performance in lines of *Mus* selected for high wheel-running activity: effects of selection, oxygen availability and the mini-muscle phenotype. *J Exp Biol.* 209:115-127.
- Rezende, E. L., F. R. Gomes, M. A. Chappell, and T. Garland, Jr. 2009. Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. *Physiol. Biochem. Zool.* 82:662-679.
- Rezende, E. L., F. R. Gomes, J. L. Malisch, M. A. Chappell, and T. Garland. 2006b. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. *Journal of Applied Physiology* 101:477-485.
- Rezende, E. L., S. A. Kelly, F. R. Gomes, M. A. Chappell, and J. Theodore Garland. 2006c. Effects of size, sex, and voluntary running speeds on costs of locomotion in lines of laboratory mice selectively bred for high wheel running activity. *Physiol. Biochem. Zool.* 79:83-99.
- Roberts, T. J., J. M. Weber, H. Hoppeler, E. R. Weibel, and C. R. Taylor. 1996. Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. *The Journal of Experimental Biology* 199:1651-1658.
- Simoncic, M., S. Horvat, P. L. Stevenson, L. Bungler, M. C. Holmes, C. J. Kenyon, J. R. Speakman, and N. M. Morton. 2008. Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. *Behavior Genetics* 38:292-300.
- Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics* 28:227-237.
- Swallow, J. G., T. Garland, P. A. Carter, W. Z. Zhan, and G. C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *Journal of Applied Physiology* 84:69-76.
- Swallow, J. G., J. S. Rhodes, and T. Garland, Jr. 2005. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. *Integr. Comp. Biol.* 45:426-437.

- Templeman, N. M., H. Schutz, T. Garland, Jr., and G. B. McClelland. 2012. Do mice bred selectively for high locomotor activity have a greater reliance on lipids to power submaximal aerobic exercise? *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* 303:101-111.
- Turner, N., C. R. Bruce, S. M. Beale, K. L. Hoehn, T. So, M. S. Rolph, and G. J. Cooney. 2007. Excess Lipid Availability Increases Mitochondrial Fatty Acid Oxidative Capacity in Muscle: Evidence Against a Role for Reduced Fatty Acid Oxidation in Lipid-Induced Insulin Resistance in Rodents. *Diabetes* 56:2085-2092.
- Vaanholt, L. M., T. Garland, Jr., S. Daan, and G. H. Visser. 2007. Wheel-running activity and energy metabolism in relation to ambient temperature in mice selected for high wheel-running activity. *J. Comp. Physiol. B-Biochem. Syst. Environ. Physiol.* 177:109-118.
- Weber, J.-M., and F. o. Haman. 2004. Oxidative fuel selection: adjusting mix and flux to stay alive. *International Congress Series* 1275:22-31.
- Weber, J. M. 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. *Journal of Experimental Biology* 212:593-597.

Concluding Remarks

This dissertation provides evidence that higher-order traits such as wheel-running behavior may be influenced by dietary choices and early-life exercise. Because obesity is a prevalent chronic disease affecting more than 40 percent of the adult U.S. population, using a mouse model seemingly resistant to obesity may be key to combat this increasing epidemic. Despite some contention, exercise reduction and the consumption of high-caloric diets are the two principle reasons for the increased obesity prevalence.

Using mice selectively for their high voluntary wheel running (HR) and their non-selected control (C) counterparts as an anti-obesity model, I investigated early-life exercise effects, dietary choices and its effects on adult physical activity. I also used food type to determine how fuel usage differed between the linetypes. In my first chapter, early-exercise opportunity increased adult wheel running but no statistical effect on spontaneous physical activity (SPA) in their home cages. The early-exercise effect was not long-lasting on wheel running, but the reduction of body mass it caused, was kept throughout the experiment's entirety. Early-exercise also caused a difference in plasma leptin levels between HR and C mice and overall, seemed to be a beneficial thing to do in terms of warding off obesity. To the author's knowledge, this was the first experiment to directly test the propensity to run as adult with early-exercise opportunity across rodent or human literature.

My second dissertation chapter investigated food preferences and its locomotion effects on HR and C mice. As expected, HR and C mice highly preferred the Western diet (WD) over the standard chow. HR had an increased preference for WD, which may

have indirectly increased their wheel running and decreased their SPA. To help decipher if WD directly increased wheel running, I gave half of the mice WD and determined, WD's positive effect depends on sex and linetype. Most mouse groups increased except, C male mice. This chapter's experiments were important because we continued to support the findings that WD had positive effects on wheel running in HR mice that have been at a selection plateau since approximately generation 16. Moreover, we found WD is highly preferred by most mice, but perhaps more interestingly, I found non-responder mice that did not consume much of the WD and need further investigation. Possible explanations for the lack of WD consumption involve texture, smell, and taste differences between the Western diet and standard chow. We have sometimes observed individuals to examine Western diet extensively, but not consume much.

My third dissertation chapter examined fuel usage in HR and C mice as indicated by whole-animal respirometry. Because we knew WD had a sometimes positive effect on wheel running, we wanted to determine if it was because of the increased lipid content in the diet and perhaps an innate ability by the HR mice to metabolize it more efficiently than C mice. Contrary to my expectations, based on the role of fats in decreasing respiratory exchange ratios (RER), I found no evidence to suggest HR mice have a greater reliance on lipids for their fuel usage using whole-body respirometry.

Together, my research provides evidence for the important sex and genetic differences between the HR and C mice, to consider for anti-obesity mouse models. Understanding the complex physiological mechanisms that resist obesity is important and using these well-known, high-performing animals may be an effective novel way of

battling the obesity epidemic. My dissertation provided longitudinal studies elucidating details of the relations between components of energy balance, changes in body composition and weight among individuals. We also used an integrative approach of biological and physiological factors that influenced energy balance, such as effects of food/meals and exercise. Although, we did not provide mechanistic approaches, we provided evidence on how factors influence energy intake, and energy balance. In my dissertation, I attempted to use early life (perhaps long-term) exercise, food preferences and effects on body mass to get a snapshot of how this exercise model system has as-a-byproduct evolved to avoid obesogenic characteristics.

Appendix A

Figure A. 1 Schematic drawing of the voluntary-wheel-respirometry setup used for oxygen and carbon dioxide measurements presented in Chapter 3.

Push Mode Flow System-Respirometry

