ADVERSE RESPONSE TO EXERCISE: MOUSE MODEL DEVELOPMENT

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ABSTRACT

Rachel C McMullan: Adverse Response to Exercise: Mouse Model Development.
(Under the direction of Fernando Pardo-Manuel de Villena)

Obesity is extremely prevalent in the U.S., associated with numerous chronic diseases and an economic burden on the health care system. Exercise results in beneficial health outcomes, protects against a variety of chronic diseases and can reduce body mass and fat. U.S. exercise guidelines recommend identical exercise programs for everyone regardless of age, sex or genetic background. Furthermore, individual variation in responses to recommend exercise programs occurs across a variety of responses with some individuals experiencing adverse responses, including fat gain. In order to establish effective exercise guidelines, dissection of underlying physiological mechanisms and driving factors as well as the evaluation of potential interventions needs to occur. Experimental mouse models of exercise-induced adverse outcomes will be valuable in identification of mechanisms and evaluation of interventions while overcoming limitations in human studies. Several studies have identified individual mice exhibiting adverse fat gain following exercise, but no systematic effort has been conducted to identify and characterize models of adverse response. Strains from the Collaborative Cross (CC) genetic reference population were used due to its high levels of genetic variation, its reproducible nature, and the observation that the CC is a rich source of novel disease models, to assess the impact genetic background has on exercise responses. This thesis work aimed to identify and develop mouse models of exercise-induced adverse body composition response and to determine the effect of different factors, including age, sex, exercise program and genetic background, on body composition response. In an initial study, we assessed body composition responses to voluntary exercise in aged females from 42 CC strains. We

observed significant variation in body composition responses due to genetic background. Some strains, in particular CC027/GeniUnc, had an adverse body composition response. An additional study identified CC002/Unc as a model of voluntary exercise-induced adverse body composition response in old females. Unlike the initial screen, this study took advantage of age matched females with a case – control experimental design to account for body composition changes due to aging. Additionally, we measured body composition and metabolic responses to different forced exercise programs (HIIT and MICT) in a subset of four CC strains. We found body composition responses to different exercise programs varied by sex and further by genetic background. Overall, females had more beneficial body composition responses to HIIT than MICT programs. Across these studies we have demonstrated that genetic background has a significant effect on responses to exercise and further genetic background interacts with other factors to influence these responses. Additionally, we evaluated body composition and metabolism responses to long-term exercise during aging in C57BL/6J mice. We observed body mass and composition response trajectories to long-term exercise vary dependent on sex. Overall, exercise was protective against age related changes in body mass and composition.

This work provides novel models for studies to determine the mechanisms behind adverse metabolic responses to exercise and enables development of more rational personalized exercise recommendations based on factors such as age, sex, and genetic background.

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BMI	Body mass index
CC	Collaborative Cross
FI	Food Intake (g)
FIa	Adjusted Food Intake
HDL	High-density lipoprotein
HIIT	High intensity interval training
LDL	Low-density lipoprotein
MICT	Moderate intensity continuous training
NE	No exercise
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
VCO_2	volume carbon dioxide output
VE	Voluntary exercise
VO_2	volume oxygen dioxide intake

CHAPTER 1: INTRODUCTION

This work details the development of mouse models for exercise-induced adverse fat response[1] and the exploration of exercise-induced body composition responses across different genetic backgrounds, sexes, exercise types and ages. The primary aim of these studies was to identify genetically stable mouse models of exercise-induced adverse fat response in the Collaborative Cross (CC) mouse population. The secondary aim of these studies was to determine the contributions of genetic background, sex, type of exercise and all their interactions on exercise-induced body composition responses. Lastly, the tertiary aim of these studies and future studies was to identify potential mechanisms driving adverse fat responses within our established CC model strains. In this chapter, I give an introduction to obesity, exercise, the observations of adverse responses, the lack of mouse models for exercise-induced adverse fat responders and the CC mouse population.

1.1 Obesity

1.1.1 Prevalence and economic burden of obesity

Obesity[2] is an important health issue that requires additional study because it is a large economic and burden to society and is predicted to only increase in severity in subsequent decades. Obesity is prevalent in the U.S with ~36% of adults and 17% of youth

[1]A gain of body fat in response to exercise is an adverse response. In this work, adverse fat response is defined as a gain of body fat.

[2]Obesity is medically defined as a BMI (body mass index) ratio equal to or greater than 30 in adults. (Overweight is a BMI greater than or equal to 25 and less than 30; Normal weight is a BMI less than 25). In youth, obesity is classified as BMI greater or equal to an age- and sex- specific BMI in the 95th percentile. BMI is calculated as the squared product of weight (kg) divided by height (m) (*1*).

classified as obese from 2011-2014. The prevalence of adult obesity was greater in females (38.3%) than males (34.3%). Additionally, obesity in U.S. adults increased from 30.5% in 1999 to 37.7% in 2014 (*1*, *2*). Projections estimate an additional 65 million obese US adults by 2030 (20% of the 323.1 million U.S. population today) (*3*).

Not only is obesity prevalent in the U.S., but it also is a large economic burden. The enormous economic burden of obesity is driven by the cost of increased health liability associated with obesity. In 2008, the estimated annual medical cost of obesity was ~$147 billion. Annual medical costs for obese individuals were ~$1,429 (42%) greater than individuals of normal weight in 2006. Furthermore, the health care costs attributed to obesity and overweight are expected to double with each decade due to the increasing population of obese individuals. Obesity is expected to account for 16-18% of total U.S. healthcare expenditure by 2030. In addition, economic costs of obesity extend past medical costs and include loss of productivity incurred by associated morbidity and mortality. It has been estimated that obesity resulted in a loss of 1.7-3 million in productive person working years in U.S. adults, equivalent to a $390-580 billion loss, based on 2008 data (*3*, *4*). The economic burden of the growing obesity epidemic is due to the medical costs of diseases linked to obesity.

1.1.2 Obesity and chronic disease risk

Obesity results from excess fat storage due to an imbalance between energy intake and expenditure. This is primarily driven by excessive food intake and/or insufficient physical activity. Obesity is associated with an increased risk for chronic diseases such as type 2 diabetes, cardiovascular diseases, early death, metabolic disorders, musculoskeletal disorders and cancer (*2*, *3*, *5-11*).

Although the majority of obese individuals are at greater risk for chronic diseases, some obese individuals have no metabolic abnormalities and can be “healthy”. The location of adipose tissue, not the presence of adipose itself, is responsible for determining

dysmetabolic consequences (*12*). In particular elevated amounts of visceral fat have a higher association with metabolic disease and chronic disease risk compared to subcutaneous fat which has little to no associated risks. Subcutaneous fat is located in peripheral regions (*e.g.* hips, thighs); whereas, visceral fat is located in intra-abdominal regions. Both subcutaneous and visceral fat are composed of white adipose tissue. White adipocytes[3] store lipids and release hormones (functioning as endocrine cells). Visceral white adipocytes are susceptible to lipid overload and tend to develop a stressed state resulting in hypertrophy, death, metainflammation and other complications associated with metabolic syndrome (*12*, *13*). Infiltration of visceral adipocytes into muscle or liver tissue can further alter metabolism and result in metabolic dysfunction. For example, visceral fat accumulation is associated with insulin resistance and is likely caused by the excessive release of fatty acids into the liver from surrounding visceral fat (*14*). In conclusion, obesity and specifically visceral fat are associated with metabolic dysregulation and chronic diseases.

1.2 Exercise

1.2.1 Exercise benefits and adaptations

Physical inactivity or sedentary lifestyle results in weight and visceral fat gain, metabolic deterioration and increased risk for chronic diseases (*8*, *12*). Exercise has numerous positive health benefits including the reduction of chronic disease risks and weight and fat loss. Chronic diseases, which can be delayed or prevented by exercise, include but are not limited to psychiatric diseases, neurological diseases, metabolic diseases, cardiovascular diseases, pulmonary diseases, musculo-skeletal disorders and cancer. In addition to preventing diseases, exercise is also used as a treatment option for a

[3]White adipocytes are cells that form white adipose tissue (fat). White adipocytes are large spherical cells that store energy in lipid (triglycerides) droplets. These lipid droplets consume ~90% of the cell volume and can grow with increases in available lipids (*13*).

variety for diseases (*e.g.* coronary heart disease, osteoporosis, anxiety disorders, etc) (*8*, *15-18*).

The ability of regular exercise to protect against chronic diseases arises from subsequent physiological and metabolic adaptations acquired in response to exercise. Exercise improves insulin sensitivity, HDL levels, cardiovascular fitness, dyslipidemia and reduces blood pressure, triglyceride and LDL levels (*12*, *19*). Furthermore, exercise alters muscular and cellular metabolism, substrate oxidation, blood flow, hormone and neurotransmitter secretion, and peptides regulating appetite, satiety and gastric emptying (*16*). In both animals and humans, changes in metabolites in response to exercise have been observed in blood and skeletal muscles biopsies (*20*). Overall, engaging in physical activity reduces chronic disease risk and elicits beneficial physiological adaptations.

Exercise can not only prevent weight gain, but can also reduce fat and maintain or increase muscle mass (*15*). Multiple studies in humans have shown that exercise prevents obesity and in particular, exercise prevents obesity due to increased visceral adipose tissue (*8*). The exercise-induced reduction of fat mass occurs from the increased energy expenditure and activation of lipolysis. This assumes energy expenditure is not compensated for through increased energy intake or other compensatory behaviors (*15*). Thus, exercise has been shown to reverse obesity and many of its associated diseases (*16*). While aerobic exercise is historically well studied and recommended to adults as the primary exercise program, there are multiple forms of aerobic exercise and other types of exercise, such as muscle-strengthening exercise.

1.2.2 Emergence of exercise programs and exercise-induced adaptations

In the 2008 Physical Activity Guidelines for Americans, the U.S. Department of Health recommended 150 minutes (2.5 hours) of moderate intensity exercise (*e.g.* walking 3mph or faster) weekly to maintain and improve health (*21*). Public health guidelines generally recommend traditional exercise programs, such as moderate intensity interval

training (MICT; exercising at a continuous moderate intensity), as a method of weight management and health benefit for the whole population (*22*). Surprisingly, only ~21% of U.S. adults achieve the 2008 Physical Activity Guidelines. The most common reason for individuals not completing traditional exercise training programs is lack of time (*23*, *24*). Alternative exercise programs that focus on strategies reducing amount of exercise time necessary to see health benefits may enable many more adults to meet the weekly exercise recommendations with the same health benefits.

High intensity interval training (HIIT) programs have been used as a time efficient form of exercise and involve exercising at short intense intervals alternated with brief intervals of lower intensity for recovery. Studies have demonstrated that HIIT programs are not only a time efficient and effective alternative to traditional endurance-based training programs such as MICT, but they may also elicit physiological responses through distinct mechanisms (*22*, *25*). HIIT has been shown to induce similar or better results than MICT for a range of responses (*e.g.* physiological adaptations, performance, health-related markers) in the general population, physically inactive individuals, individuals suffering from chronic diseases and athletes. Recent human studies have demonstrated extensive benefits from HIIT include improvements in aerobic fitness, anaerobic fitness, cardiorespiratory markers, skeletal muscle oxidative capacity, aerobic energy metabolism, insulin action, physiological remodeling and reduction in risk factors for metabolic syndrome (*22-29*). Initial studies have explored the metabolic mechanism behind HIIT-induced adaptations. In particular, HIIT has been shown to increase lactate concentrations and adenine nucleotide catabolism products in both plasma and skeletal muscle indicating increased anaerobic metabolism and ATP turnover (*20*).

Preliminary studies have demonstrated HIIT can efficiently and effectively reduce body mass and body fat (*29-33*) and increase lean mass (*6*, *34*, *35*). However, individual variability in body composition responses with the presence of responders and non-

responders has been observed in HIIT, MICT and other exercise programs making it difficult to determine effective personalized exercise programs (*29*). These observations demonstrate different exercise programs can elicit distinct physiological responses and exercise programs should be designed using scientific evidence for particular desired outcomes.

Recently, mouse populations have been used to assess physiological adaptations to HIIT programs (*25*, *36*-*39*). HIIT had beneficial effects on cardiac remodeling, physical performance, insulin sensitivity and metabolic markers in mice with diet-induced obesity and reversed changes in gut microbiota associated with diet-induced obesity (*25*, *36*-*39*). Seldeen *et al*. demonstrated HIIT improved physical performance and reduced markers for frailty compared to sedentary controls in aged C57BL/6J mice (*25*). HIIT was shown to have superior metabolic adaptations (reduced body weight, body fat percentage, adipocyte size) compared to MICT in male ICR (outbred) mice with high-fat diet induced obesity (*37*). While studies in mouse populations have supported human findings and demonstrated HIIT is a physiologically effective exercise program, these studies have been limited to standard laboratory inbred strains, in particular C57BL/6J. These studies are informative but limited in genetic diversity. It is likely physiological adaptations to both HIIT and MICT exercise programs will vary significantly across genetic backgrounds. Studies utilizing mouse multi-parental populations are necessary to evaluate the physiological effectiveness of HIIT and MICT in different genetic backgrounds.

1.2.3 Sex and exercise induced responses

Previous studies have shown sex affects physical activity traits and that sex differences regulate body composition traits in both human and mouse populations (*40*-*42*). Initial data suggests that males and females vary in physiological responses when exposed to the same exercise program, including interval training (*22*)**.** The presence of sex differences varies depending on physiological response. For example, recent human evidence suggests

that men and women have similar cardiovascular response to HIIT, but men have significantly greater benefits to muscle protein synthesis and mitochondrial biogenesis compared to women (*43*). For body mass response to exercise, the prevailing view is that women lose less weight in response to exercise than men. Recently, human studies have shown when exercise-induced energy expenditure is held equal there are no sex effects on exercise induced weight loss (*44*). However, the effects of sex within the context of genetic backgrounds on body composition response to HIIT and MICT training are not clear. Specifically, it is unknown whether both sexes demonstrate the same or different body composition responses when receiving the same varied intensity training (*10*, *22*). Identifying sex-based responses and intensity-based responses to exercise in genetically characterized and reproducible mice can help address unanswered questions.

1.3 Adverse responders

1.3.1 Individual variability to exercise

Engaging in cardiovascular exercise activity is typically believed to result in weight and fat reduction, but this is not always the case. Some individuals even experience an adverse response to exercise. There is large inter-individual variation in both direction and magnitude of exercise-induced responses. Interestingly, exercise-induced weight loss is often less than expected (*16*, *18*). Individual variability in responses to exercise are observed in both human and rodent populations (*19*). It is important to note, most studies focus on the group mean for a response to a standardized exercise intervention rather than reporting individual responses. Individual variation to exercise has been observed in numerous responses including but not limited to VO_2 max, resting heart rate, heart rate during exercise, aerobic and anaerobic threshold, resting muscle glycogen and muscle enzyme activity (*45*). It is well established numerous variables contribute to individual variation in exercise-induced responses.

Multiple studies have demonstrated individual variability in exercise-induced responses is in part explained by exercise factors, compensatory behaviors and environment. In particular, insufficient exercise dose (frequency, duration, intensity), lack of program adherence and physiological and behavioral compensatory adaptations (*e.g.* alterations in energy intake, reductions in non-exercise or habitual physical activity) contribute to individual variation in exercise-induced responses and can affect exercise-induced health benefits. Furthermore, compensatory adaptations vary by exercise type (*e.g.* intensity, duration, etc) and individual factors (*e.g.* sex, age, and body weight) (*16*, *19*). Even when energy expenditure and energy intake are controlled, there still is variability in weight response to exercise (*19*). Individual variability in responses after controlling for energy expenditure and intake indicates exercise factors and compensatory factors are not the sole contributors to variability in exercise-induced responses.

Some variability in exercise-induced responses can be explained by accounting for a baseline level of activity in untrained individuals. In one study, baseline phenotypes accounted for ~1% of exercise-induced response for HDL cholesterol levels and VO2max. Whereas, other baseline phenotypes contributed more to exercise-induced responses (*e.g.* heart rate during activity) (*46*). In some cases there is little relationship between the pre-training trait and the magnitude of the resulting training response (*45*). Other factors contributing to exercise-induced responses include: variability in recovery ability, prior training status, nutritional status and measurement error (*45*).

Finally, exercise-induced responses vary not only by training background and environmental factors but also by genetic background (*8*). This is not surprising, as numerous exercise-related phenotypes prior to exercise exposure are also heritable. Prior twin and family studies have established different exercise-induced responses are heritable and particular gene variants are associated with certain exercise response phenotypes (*45*). In particular, the role of genetics in regulating weight and fat response to exercise is

becoming evident (*19*). Twin studies have demonstrated shared familial factors (*e.g.* environment and genetics) are likely significant regulators in markers of body composition response (*e.g.* fat mass, body fat percentage change) to exercise. The extent to which familial factors influence body composition responses to exercise are likely to vary by sex with factors having a greater influence on fat mass response in females and fat-free mass (e.g. muscle mass) response in males (*18*). While exercise variables, compensatory behaviors and baseline phenotypes contribute to variability in exercise-induced responses, it is becoming evident genetics and other factors are likely driving contributors.

1.3.2 Observations of adverse responses

While some individuals are high positive responders to exercise training, there are a small proportion of individuals who are non responders (no improvement) or adverse responders (deterioration) in response to exercise. Thus, particular individuals do not obtain health benefits of exercise training (*45*). Adverse responders have been observed across a variety of traits and risk factors for chronic diseases including plasma fasting insulin, HDL cholesterol levels, triglycerides, resting systolic blood pressure (*47*), VO_2 max (*45*), body weight (*48*) in humans. Bouchard *et al.* (2012) identified 31% of individuals across six separate studies had one exercise-induced adverse response in four possible traits (fasting insulin, HDL-cholesterol, triglycerides, resting systolic blood pressure) (*47*). Adverse responders have been observed across numerous traits, but we are particularly interested in adverse fat responders to exercise due to the health risks associated with obesity.

1.3.3 Adverse fat responses to exercise in humans and mice

Multiple studies have demonstrated that weight and fat response to exercise varies by individual even when controlling for compliance, energy intake and energy expenditure (*49*, *50*). Barwell *et al.* (2009) measured fat loss in response to a seven week aerobic (at 65-80% max heart rate) exercise program with 55 women and observed ~25% of the women were adverse fat responders. During the seven week intervention food weights

were self reported and used to calculate energy intake. After adjusting for energy expenditure and energy intake, ~45% of the women had an adverse fat response (*49*). Human studies of exercise induced responses are limited in numerous ways including accurate measurement of phenotypes (e.g. self reported food intake). Animal systems offer an improved way to measure body composition responses to exercise since they enable accurate measurements and the ability to standardize and control variables (*e.g.* energy intake).

Exercise-induced adverse fat response has only previously been observed in outbred and partially inbred mouse populations. Kelly *et al*. (2011) measured body composition responses to six days of voluntary exercise in outbred mice (generation four of an advanced intercross line created from reciprocal cross between high running selection line and C57BL/6J). They observed exercise-induced fat response ranged from -69.1 to 88.3% and lean mass response ranged from -17.2 to 26.3% across the whole population (n = 797) (*19*). Thus, a subset of outbred mice had exercise-induced adverse fat response. While exercise-induced adverse fat responders have been observed in outbred and partially inbred mouse populations, these mice are genetically unique individuals making it difficult to determine if genetics, experimental error or other variables are driving the adverse response. Additionally, developing mouse models by selective breeding of outbred mice is a long process.

1.4 Mouse model development

1.4.1 Need for mouse model

Complex phenotypes are often influenced by multiple genetic loci and the modification of each loci by genetic background, sex, age and environmental factors (*51*). Given the complex interactions between physical activity, energy intake, body composition, as well as other factors, it is difficult to determine successful exercise regimes that result in healthy body composition. Animal models are critical towards this success, as human

studies often suffer from many confounding variables (*e.g.* food consumption, environmental and genetic differences) and prove difficult to measure and study mechanisms associated with exercise-related phenotypes (*41*), (*52*). Inbred mouse models in particular are excellent tools to dissect complex traits while controlling and manipulating environments. Furthermore, genetically distinct mouse strains can be used to assess the magnitude of genetic responses for all of these traits (*51*, *53*, *54*). Even though it is common in human studies, especially in women, to observe exercise-induced adverse fat (gain) responses, no inbred mouse models exist which recapitulate these phenotypes.

1.4.2 Collaborative Cross

The CC is a multi-parental population of ~75 recombinant inbred mouse strains. The CC is derived from eight founder laboratory strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ) representing the three *Mus musculus* subspecies of mouse (*M. m. musculus, M. m. domesticus,* and *M. m. castaneous*). The CC was initially conceived as an improved resource for systems genetics enabling data integration across a platform of stable and reproducible sets of unique genotypes. CC strains are inbred which enables repeatability and reproducibility of experiments along with the ability to modulate variables (*e.g.* diet, sex, exercise type, etc) in the same genetic background (*55-58*) (*53*, *59*). Multiple computational and

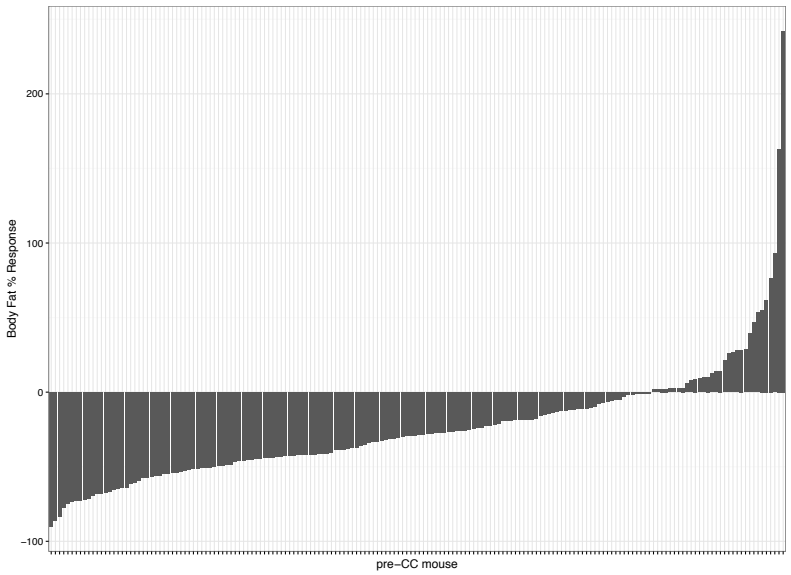


Figure 1.1 Body fat response to 12 days of voluntary exercise in pre-CC male mice. Each bar represents an individual pre-CC mouse. Body fat responses above zero represent adverse responses and below zero represent standard responses. Figure was made publically available data from MPD dataset: Mathes1

genomic tools designed for the CC enable dissection of genetic and molecular mechanisms driving phenotypes and analyzing new models (*59*). The CC population is ideal for identifying stable research models. Each strain is inbred and reproducible, but across strains there exists a high level of genetic and phenotypic diversity. The CC has expansive phenotypic diversity, phenotypic range beyond other laboratory mice and continuous distribution for numerous traits (*60*-*63*). The CC has been a rich source of models of human disease especially disease traits that do not exist or are underrepresented in standard laboratory mouse strains (*53*, *64*, *65*). The expansive phenotypic diversity and presence of outlier strains make the CC ideal for identifying strains that accurately model human disease traits. For example, Rogala *et al*. (2014) identified CC011/Unc as a new model for spontaneous colitis. Unlike existing murine models for colitis, CC011/Unc developed colitis in the absence of chemical treatment, an infectious agent or direct mutagenesis (*64*). This illustrates the potential for development of animal models with genetic disorders as opposed to animal models with environmentally controlled disease status. Finally, a prior study tested exercise-related traits in the pre-CC[4]. The pre-CC captured phenotypic variation often beyond variation observed in the CC founder inbred strains in exercise-related traits. Seventeen percent of 176 pre-CC mice had an adverse fat response to twelve days of voluntary exercise (Figure 1.1) (*66*). This observation of exercise-induced adverse fat response in the pre-CC suggests the CC might be a rich source of mouse models of exercise-induced adverse body composition response.

1.5 Thesis purpose

The main objectives of my thesis research are: 1) to demonstrate that the CC is a useful systems genetics resource for modeling human exercise and exercise-related traits 2) to identify model strains for exercise-induced adverse fat response and 3) to determine

[4] The pre-CC is defined as mice from CC strains during the inbreeding process but prior to the strains becoming fully inbred. Thus, pre-CC mice are partially inbred or outbred.

the contribution of genetic background, sex and exercise type to fat response. My thesis work will also provide a better understanding of the effect of different exercise programs (HIIT vs MICT) has on body composition response and whether males and females elicit similar body composition responses to HIIT and MICT programs in the context of different genetic background. Identifying a stable mouse model(s) of exercise-induced adverse fat response will enable genetic and mechanistic dissection of adverse fat response to exercise. These findings and the development of mouse models will enable ongoing and future studies to determine personalized exercise programs or other treatment options to prevent adverse fat response and improve physical activity guidelines in the human population.

CHAPTER 2: LONG-TERM EXERCISE IN MICE HAS SEX-DEPENDENT BENEFITS ON BODY COMPOSITION AND METABOLISM DURING AGING[5]

2.1 Overview

Aging is associated with declining exercise and unhealthy changes in body composition. Exercise ameliorates certain adverse age-related physiological changes and protects against many chronic diseases. Despite these benefits, willingness to exercise and physiological responses to exercise vary widely, and long-term exercise and its benefits are difficult and costly to measure in humans. Furthermore, physiological effects of aging in humans are confounded with changes in lifestyle and environment. We used C57BL/6J mice to examine long-term patterns of exercise during aging and its physiological effects in a well-controlled environment. One-year-old male (n = 30) and female (n = 30) mice were divided into equal size cohorts and aged for an additional year. One cohort was given access to voluntary running wheels while another was denied exercise other than home cage movement. Body mass, composition, and metabolic traits were measured before, throughout, and after 1 year of treatment. Long-term exercise significantly prevented gains in body mass and body fat, while preventing loss of lean mass. We observed sex-dependent differences in body mass and composition trajectories during aging. Wheel running (distance, speed, duration) was greater in females than males and declined with age. We conclude that long-term exercise may serve as a preventive measure against age-related weight gain and body composition changes, and that mouse inbred strains can be used to characterize effects of long-term exercise and factors (e.g.

[5] The following work was previously published: McMullan RC, Kelly SA, Hua K, Buckley BK, Faber JE, Pardo-Manuel de Villena F and D Pomp. 2016. Long-term exercise in mice has sex-dependent benefits on body composition and meatoblism during aging. *Physiological Reports*: **4**, e13011. Supporting figures are located in Appendix A.

sex, age) modulating these effects. These findings will facilitate studies on relationships between exercise and health in aging populations, including genetic predisposition and genotype-by-environment interactions.

2.2 Introduction

It has been estimated that Americans older than 65 years-age will double in number from 40 million in 2010 to 81 million by 2040 (*67*). During the aging process, individuals generally experience a gain in body fat, redistribution of body fat, increase in intramuscular fat, and a decrease in lean mass and bone density (*5*, *19*, *68*). These concomitant changes in body composition and body mass are thought to be due to alterations in resting metabolic rate StOnge:2010ba}. It is common for energy expenditure to decline due to slower metabolism, decreased lean mass, and less physical activity (*69*). Further, the loss of lean mass during aging may cause dysregulation of energy expenditure via decreased basal metabolic rate (BMR) (*70*, *71*), reduced physical fitness, and lower quality of life (*72*). Accompanying alterations in body composition and metabolism, physical activity levels decline with aging in both rodents (*73*-*75*) and humans (*76*, *77*) in part due to the changes in skeletal muscle structure and function (*67*). Meanwhile, chronic health issues, such as obesity and other metabolic and cardiac conditions, increase with age in humans (*7*).

It is well established that regular aerobic exercise results in beneficial health outcomes including among others, prevention or delay of diseases such as heart disease, stroke, certain forms of cancer, and dementia. In addition, physical activity is effective in weight management, regulation of obesity risk (*8*, *78*, *79*), and mitigation of physiological changes (e.g. muscle loss) that contribute to decline in exercise capacity with aging (*67*, *80*, *81*). Even though much literature suggests that regular exercise in elderly individuals mitigates age-related decline in health, three-fourths of older adults do not meet the recommended levels of exercise (*82*). Despite the benefits of exercise some individuals do not exercise or fail to experience positive

response to exercise treatments (*8*, *19*, *78*, *79*, *83*). Thus, developing a better understanding of the physiological effects of exercise during aging is vital for application to the growing aged human population (*67*).

Exercise traits (e.g. amount, intensity) vary depending on sex in both humans and rodents. In humans, physical activity traits are greater in males compared to females, whereas, females run further and at greater speeds than males in multiple mouse strains (*40*, *84*). Sex differences exist in body weight, composition, and energy metabolism in both humans and rodents. In both humans and rodents, females tend to maintain white adipose tissue and resist loss of these energy stores, compared to males. Human females have greater adipose mass, greater brown adipose mass, store adipose in different regions, have lower lean mass and greater circulating free fatty acids (FFA) than males. Even with these sex differences, females maintain glucose homeostasis in part because estrogen (E2) concentrations are tightly regulated between puberty and menopause (*85*). In both sexes, E2 regulates adipose development and deposition, body composition, energy balance, mitochondrial function, fatty acid transport and oxidation, and glucose metabolism. Postmenopausal women display a loss of E2, which leads to alterations in metabolism, body weight gain, and increased visceral fat in both humans and rodents (*86*-*89*).

Habitual low-intensity exercise (over 100 days) results in cardiorespiratory and metabolic adaptations, specifically the loss of fat mass and maintenance of fat-free mass (*77*). However, there is a relative lack of knowledge regarding the effects of long-term exercise (exercise > 3 months) or the effects of exercise initiated during midlife. Animal models provide a time-efficient alternative to longitudinal human studies, which are difficult and expensive to conduct. However, most rodent studies have examined the impact of habitual exercise for short periods [6 to ~60 days (*90*)] in young mice (typically 60 days old). Previous studies on long-term exercise in mice demonstrated positive outcomes on median lifespan, maintenance of motor coordination,

and altered expression of genes involved in heart and immune function (*74*, *91*, *92*). However, there is a gap in knowledge regarding long-term exercise patterns during aging, the metabolic response to long-term exercise, and the effect of sex on these traits in a controlled environment.

In this report, we examined long-term voluntary exercise patterns in an aging mouse population of C57BL/6J mice. The present results represent data collected within a larger experiment aiming to understand the impact of exercise, beginning at mid-life and extending through the aging process, in protection against disease. The C57BL/6J strain was selected because it is the most widely used in biomedical research, is the mouse reference genome (*93*), and is commercially available at mid-age. The median lifespan for C57BL/6J males is 901 days (30 months) and 866 days (28.8 months) for females (*94*). We examined physiological response to voluntary exercise in both sexes of C57BL/6J mice starting at ~1 year-age until ~2 years-age, which is equivalent to ~40–70 years in humans (*95*). We demonstrate that long-term exercise even with decreasing physical workload provides beneficial outcomes on body weight and composition during aging, and that it does so in a sex-dependent manner. In addition, our findings on metabolic response to physical activity during aging may aid establishing guidelines for exercise in the aging human population.

2.3 Methods

2.3.1 Animals

Thirty male and 30 female C57BL/6J mice were purchased from the National Institute of Aging at 1 year of age and after arrival at University of North Carolina-Chapel Hill were allowed to acclimate to the vivarium for 1 week. Mice were then assessed by Echo MRI for body weight and composition (EchoMRI-100, Echo Medical Systems, Houston, TX) and placed into individual indirect calorimetry cages (Phenomaster/Labmaster, TSE SYSTEMS, Chesterfield, MO) for 48 h with O2 consumption and CO2 production, energy expenditure and food and water consumption measured for 48 h. Respiratory exchange rate (RER, VCO2/VO2) was also

calculated.

Following initial body composition and metabolic measures, animals in the experimental cohort were individually housed with access to running wheels (Lafayette Instruments, Lafayette, IN; model 80850L), while animals in the control cohort were single-housed with access to ordinary lab-animal enrichment (mouse huts) but no access to running wheels. Voluntary running was recorded at 1-min intervals for 23–24 h a day for 12 months. The following daily exercise parameters were obtained: distance (total revolutions), time spent running (cumulative 1-min intervals in which at least 1 revolution was recorded), average speed (total revolutions/time spent running), and maximum speed (highest number of revolutions in any 1-min interval within a 24-h period) (*96*).

Body weight and composition were measured by MRI every 60 days; these measurements were conducted for all mice on the same day, except as noted below. Animals in both groups were removed from their respective cages for 48 h of indirect calorimetry assessment after 6 months and again after 1 year. At both time points, and again due to limitations in the number of indirect calorimetry cages, MRI measurement of body weight and body composition (obtained just prior to placement in the indirect calorimeters) for all 60 mice required 26 days; the initial 30 mice measured were from the experimental cohort, while the final 30 mice measured were from the control group. Throughout the paper, we refer to the age of the mice as either ~1, ~1.1, ~1.4, ~1.5, ~1.6, ~1.8 or ~2 years representing an approximate age of 372, 423, 520, 549, 606, 669, or 731 days. The approximate age at each phenotype time point was calculated as the mean age in days from the actual age in days of each individual mouse at the time of data collection.

Four mice in the experimental group died during the first 3 months of the experiment. Three of these mice died prior to the measures at ~1.1 years (~423 days) of age and the fourth prior to the measures at ~1.4 years (~520 days) of age. These mice were included in statistical

analyses of all measures prior to their death. Upon death, each of these four mice was replaced with an individual previously placed into the control group. Upon replacement and granting of wheel access these mice were placed in the experimental group for all subsequent statistical analyses. This was done to maintain sample size in the experimental cohort, and had no impact on results relative to removal of these mice from the analyses (data not shown). All procedures were approved by and conducted in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

2.3.2 Statistical analysis

Estimated marginal means and standard errors of body mass (g), percent body fat and percent lean mass were calculated at the beginning of the experiment at a mean age of ~1 year, and every other month over the next year. Percent body fat (and lean) was calculated as (fat mass/body mass) × 100. Percent change in variables [(pre–post/pre) × 100] was calculated relative to the prior measurement. Estimated marginal means and standard errors of RER were calculated at a mean age of ~1, ~1.5, and ~2 years of age. Measures represent diurnal (lights on) means on day-2 of a 2-day trial. Home cage activity was monitored for two consecutive days and mean activity levels were analyzed. General Linear Models (GLM) [Univariate GLM analysis of variance (ANOVA) (SPSS, Chicago, IL)] were utilized to examine the effects of sex (fixed effect), wheel access (fixed effect), and the sex-by-wheel access interaction on all phenotypic measurements. Statistical significance was defined as $P < 0.05$, and all P-values presented are two-tailed. GLM for data collected from TSE equipment also included the following covariates in the analysis: Activity, mean of the diurnal home cage activity on day-2 of the 2-day trial; Batch, reflects group that individuals were tested in within a given time point; VO2 (mL/kg/h), VCO2 (mL/kg/h), and RER values represent the diurnal (lights on) means on day-2 of the 2-day trial. Additionally, for a subset of the phenotypes, repeated measures ANOVAs [GLM (SPSS, Chicago, IL)] were utilized to investigate the effects of age across all

groups (sex and experimental vs. control).

Estimated marginal means and standard errors were calculated for the physical activity traits (mean revolutions per day, mean time spent running per day, mean running speed per day). Measures were represented as means across 57 weeks. Comparisons between sexes were analyzed, using GLM (SPSS, Chicago, IL). Wheel freeness was included in models as a covariate. Wheel freeness was calculated as the number of wheel revolutions following acceleration to a given velocity. Pearson partial correlations (*r*; controlling for sex) were calculated for revolutions/day (distance), 1-min intervals/day (time, cumulative 1-min intervals in which at least one revolution was recorded), and average running speed (total revolutions/time spent running) and metabolic traits at ~1, ~1.5, and ~2 years. Pearson partial correlations (*r*; controlling for sex and wheel access) were calculated for physical activity traits and body composition traits at ~1, ~1.1, ~1.4, ~1.5, ~1.6, ~1.8, ~2 years. Pearson partial correlations (*r*; controlling for sex and wheel access) were calculated for body composition and metabolic traits at ~1, ~1.5, ~2 years. Degrees of freedom ranged from 20 to 54.

2.4 Results

For simplicity, we first summarize data for metabolic changes across aging in control mice (no wheel access) to establish “baseline” phenotypes, and then describe experimental data across time for mice provided wheel access in the following sections: (1) patterns of exercise, (2) impact of exercise on metabolic changes, and (3) exercise-by-sex interactions on metabolic changes.

2.4.1 Age and sex contribute to metabolic changes in control mice

The body mass of control C57BL6/J mice increased during aging from ~1 to ~2 years, and body mass changes occurred in a sex-dependent manner (Fig. 2.1A). Over the entire period studied, males weighed significantly ($P < 0.001$) more than females (Table 2.1, Table S1, Fig. 2.1A). Additionally, there were significant sex effects on body mass changes between

measurement time points except from ~1.5 to ~1.8 years ($P < 0.05$, Fig. 2.1A, Table 2.1). In control male mice, we observed an initial increase in body mass (~1 to ~1.4 years), stable body mass levels in the midsection of our study (~1.4 to ~1.6 years) and a decline in body mass at older ages (~1.6 to ~2 years). In contrast, in control female mice, body mass remained stable early (~1 to ~1.1 years) and then increased for the remainder of the study (~1.1 to ~2 years) (Fig. 2.1A, Table S1).

Body composition also changed during aging in control mice (Fig. 2.1B, C). One-year-old females had lower body fat and higher lean mass than males, but the situation reversed itself as the mice aged. Males had greater percent body fat and lower percent lean mass than females until ~1.6 years of age when females had greater percent body fat and lower percent lean mass than males. Overall the pattern of increase in body fat in both sexes closely followed the changes in body mass (compare Fig. 2.1A and B). In females increases occurred over the length of the study, while in males there was initial increase in body fat, followed by a period of stabilization and a final decrease (Fig. 2.1B). The patterns for lean mass are similar but inverted (Fig. 2.1A, C). Significant ($P < 0.05$) sex effects on body fat (Table 2.1, Fig. 2.1B), lean mass (Table 2.1, Fig. 2.1C) and changes in percent fat and lean mass between consecutive measurements were observed for most time points (Table 2.1). The changes in both percent body fat and percent lean mass during aging in control mice occurred in a sex-dependent manner (Fig. 2.1B, C, Table S1).

Indirect calorimetry was performed three times on all mice: at the beginning of the experiment (~1 year) at ~1.5 years and at ~2 years of age (Table 2.2, Table S2). Over the period of study, RER levels ranged from 0.78 to 0.88 in control mice (Fig. 2.2, Table S2). In control males, VO2, VCO2, and RER levels were lower at ~1 year compared to ~1.5 years and greater at ~1.5 years compared to ~2 years (Table S2, Fig. 2.2). Female control mice had lower VO2, VCO2 and RER levels at ~1 year compared to ~1.5 years; whereas, females had greater VCO2 and RER levels and reduced VO2 levels at ~1.5 years compared to ~2 years (Table S2,

Fig. 2.2). We observed significant sex effects on VO2 and VCO2 at all three time points, with females having greater values ($P < 0.05$, Table S2). There was only a significant sex effect on RER levels at ~2 years ($P = 0.025$, Table S2). Finally, there was a significant difference in RER levels at ~2 years compared to ~1 and ~1.5 years in both males and females ($P < 0.01$, Fig. 2.2).

Food intake (as measured in the indirect calorimeters) was lower at ~1.5 years compared to ~1 year and greater at ~2 years compared to ~1.5 years in aging control mice. Water intake increased during aging in both sexes in the control cohorts. There were significant sex effects on food and water consumption at ~1 year and ~1.5 years ($P < 0.005$) and significant sex effects on water consumption at ~2 years ($P = 0.001$, Table 2.3). There were significant sex effects on home cage activity levels at all time points ($P < 0.05$). Female mice had greater levels of home cage activity compared to male mice (Table 2.3, Table S2).

2.4.2 Long-term physical activity varies during aging in a sex-dependent manner

In the experimental cohorts of both sexes, we observed an increase in mean revolutions per day (distance) after mice gained wheel access (weeks 1 through 5 in Fig. 2.3A). Subsequently, distance run declined over the 57 weeks of wheel access. During weeks 25 and 51, mice were removed from their home cage and placed in metabolic cages (no wheel access) for 48 h and returned to their home cage after the metabolic measurements. After both metabolic analysis time points, there was an increase in distance run for several weeks, followed by a stabilization and decline (Fig. 2.3A). Exercise duration followed similar patterns as daily distance except there was no initial increase in duration over the first few weeks of wheel access (Fig. 2.3B). Mean average running speed (rpm) also followed similar patterns as distance (Fig. 2.3C). Thus, we observed an age-related decline in all aspects of physical activity over the one-year experimental period.

Females ran longer distances, for longer duration, and at higher speeds than males throughout the 57 weeks of wheel access. The sex effects were significant ($P < 0.05$) for distance from weeks 1–45 (~1 to ~1.85 years of age) of voluntary exercise. Similarly, significant sex effects on duration were observed during weeks 1–48 and week 54 ($P < 0.05$, Fig. 2.3B). There were very few weeks with significant sex effects on speed, although females had higher rpm than males and rpm slightly decreased with age in both sexes (Fig. 2.3C). Females demonstrated slightly different patterns of exercise in the early part of the study compared to males. Females had a sharper increase in distance (weeks 1–5) and a sharper decrease in distance (weeks 5–12) compared to male mice (Fig. 2.3A).

2.4.3 Physical activity protects from age-related metabolic changes

Overall body mass was significantly lower in experimental mice compared to control mice at all time points, with experimental mice having ~16% less body mass during ~ 1.1 to ~2 years ($P < 0.05$, Table 2.1, Fig. 2.1A, Table S1). Specifically, experimental mice followed similar temporal patterns of body mass changes as control mice but at significantly lower body mass. Female experimental mice had from 6 to 22 percent less body mass than female control mice. Male experimental mice had 11–19 percent less body mass than male control mice during aging (Table 2.1, Fig. 2.1A, Table S1).

Wheel access had a significant effect on body composition. Experimental mice had ~50% less body fat and ~15% more lean mass than control mice throughout the study. There were significant effects of exercise on percent fat and lean mass and on change in percent lean between experimental time points throughout the experiment ($P < 0.001$). Female experimental mice had 34–59% less percent body fat and 6–20% more percent lean mass than female control mice during aging from ~1.1 to ~2 years-old. Thus, female mice with wheel access had ~52% less fat and ~16% more lean mass than control female mice from ~1.1 to 2 years. Male experimental mice had 44–55% less percent body fat and 9–17% more percent lean mass than male control mice during aging from ~1.1 to ~2 years. Male mice with wheel access had on

average ~49% less body fat and ~15% more lean mass than control male mice from ~1.1 to ~2 years. There was only a significant wheel effect on change in percent fat observed between ~1 and ~1.1 years ($P < 0.001$). In the experimental cohort, female mice retained greater percentage lean mass than males until ~1.6 years old (Fig. 2.1C, B, Table 2.1, Table S1). Wheel access only had a significant effect on VCO2 and RER at ~1.5 years ($P < 0.04$, Table 2.2, Table S2). RER levels in the experimental cohort increased with aging. RER levels in the experimental females increased from ~1 to ~1.5 years then remained stable from ~1.5 to ~2 years (Fig. 2.2).

Both food and water intake increased during aging in both sexes in the experimental cohort (Table S2). There was a significant wheel access effect on food and water consumption at ~1.5 years ($P < 0.005$) and on food consumption at ~2 years ($P = 0.025$, Table 2.3). Food and water intake was greater in experimental females than in experimental males. Both experimental males and females had greater food and water intake at both ~1.5 and ~2 years than control mice (Table S2). There was a slight decline in activity levels in control mice access during aging; whereas, experimental mice had an increase in home cage activity levels during aging but these effects were not significant except at ~2 years ($P = 0.004$, Table 2.3, Table S2).

2.4.4 Exercise-by-sex interactions on metabolic changes during aging

We only observed a few significant exercise-by-sex interactions on metabolic changes during the aging process. The majority of the significant interactions on metabolic phenotypes collected by indirect calorimetry were observed at ~1.5 years. Significant interactions on percent change in mass and percent change in percent lean mass existed at ~1.4 and ~1.6 years ($P < 0.05$, Table 2.1, Fig. 2.1A). There was a significant exercise-by-sex interaction on RER levels observed at ~1.5 years and on VCO2 levels at ~1.5 and ~2 years. Females with wheel access had greater RER levels than control females at ~1.5 years; whereas males in both cohorts had the same RER levels at ~1.5 years (Fig. 2.1C, Table 2.2, Table S2).

2.4.5 Phenotypic correlations between exercise and metabolic traits during aging

As expected, all physical activity phenotypes (distance, speed, time) were significantly and positively correlated at each time point during aging (Tables 2.4, 2.5). Body mass was significantly and positively correlated with percent fat and percent lean mass during aging. Distance and speed were significantly and negatively correlated with change in mass and change in percent lean mass at ~1.1 years (Table 2.5). VCO2 was significantly and positively correlated with VO2 and RER during aging. Exercise distance and time were significantly and positively correlated with RER levels at ~2 years but not at ~1 and ~1.5 years (Table 2.4). Body mass, percent fat, and percent lean mass were significantly correlated with RER at ~1 and ~1.5 years but not at ~2nyears (Table 2.6). For a complete list of correlational analyses results see Tables 2.4, 2.5, 2.6.

2.5 Discussion

2.5.1 Physical activity prevents age-related, sex-dependent changes in body mass and composition

The most interesting conclusion of this study is that regular long-term exercise starting in midlife prevents body mass and body composition alterations observed during aging in both male and female mice. Mice that exercised had 16% lower body mass, 50% lower body fat, and 15% higher lean mass than mice who were not exposed to running wheels. The changes in body mass were not significantly correlated with specific physical activity measurements (distance, time, speed) indicating that engaging in exercise, and not the specific workout load, is potentially sufficient for maintenance of body weight and composition throughout aging. This may aid formulating recommendations of exercise in the aging human population, since it demonstrates that exercise even in reduced amount may be sufficient to alleviate age-related changes in body mass and composition, at least in mice, although there could be a threshold of activity below which benefits are not achieved.

Changes in body mass and body composition during aging followed sex-dependent trajectories in both experimental and control mice. It has been established that sex differences exist in physical activity levels, body mass, body composition, and metabolism in both humans and rodents (*40*, *84*, *97*, *98*). In humans, cross-sectional studies have suggested there are different trajectories of changes in body fat depending on sex (*99*). Both longitudinal and cross-sectional studies in humans have shown men and women gain weight specifically due to greater amount of fat than lean mass when less than 60 years old. Men older than 60 years display loss of weight and body fat. The trajectory for body composition changes in women after the age of 60 years has not been established (*99*). Our findings demonstrate that there are sex differences in the trajectories of body mass and body composition changes during aging long-term. In both sexes, introducing exercise midlife prevented accumulation of age-related changes in body mass and composition. Even though work load declined during aging, participating in physical activity was still sufficient to protect against age-related changes in body mass and composition in a sex-dependent manner. There were significant exercise-by-sex interactions on changes in body mass and composition during aging, indicating exercise alters body mass and composition in a sex-dependent manner. Interestingly, similar sex-dependent trajectories of body mass and composition were observed in the experimental and control mice and could be attributed to the regulation of food intake in conjunction with increased activity. Food consumption is typically positively correlated with wheel running, but the extent of the effects can be sex dependent and contingent on initial body composition differences (*19*). Future experiments, monitoring food consumption more regularly, are needed to determine the underlying causes of sex-dependent trajectories of body mass and composition during aging.

2.5.2 Physical activity levels change during aging in a sex-dependent manner

Physical activity levels decline as humans age (*76*). The CDC surveillance data have found that 17% of adults 45–64 years-old, 23% of adults 65–74 years-old, and 36% of adults

75+ years-old remain physically inactive (*100*). In the current experiment, we observed a decline in exercise levels during voluntary participation over 12 months. There was an initial increase in distance and speed for the first 5 weeks of exercise followed by a decrease in physical activity. This also occurred after each indirect calorimetry measurement wherein mice were removed from the wheels for 48 h. These observations may extend from behavioral responses to a novel environment (new home cage, wheel access), adaption to the wheel, and potentially trained exercise ability. Novel environments induce stress response-eliciting behavioral responses, such as increased activity to rewarding stimuli (*101-103*). In particular, C57BL/6J mice respond to novel cage environment (with wheel access) by increased wheel activity (*101*).

We observed a sex effect on physical activity, in which females ran greater distance and duration than males, consistent with previous reports (*104*). These sex effects disappeared around ~46–49 weeks of exercise or ~23 months of age. However, after the initial 5 weeks of wheel access, females showed a sharp decline in physical activity and males showed a gradual decline in physical activity. There are several possible explanations for these declines. The decrease could be due to aging. Another possible explanation is loss of environmental novelty and habituation (*105*). Lastly, decline in physical activity could result from reduced E2 levels. Acyclicity in rodents has been established as the menopausal transition, which occurs in human females during aging (*106*). Although we do not have data for acyclicity, we would expect female mice to begin acyclicity at ~390–480 days (13–16 m) (*86*, *107*, *108*). E2 contributes to regulating adipose development, exercise levels, and age-related changes in body composition. Both human and animal studies have shown a decline in of E2 production causes insulin resistance, and exercise mitigates the resulting glucose intolerance and composition changes (*40*, *85*, *87*, *88*, *109*). Estrogen deficiency in postmenopausal women and ovariectomized rodents leads to higher RER levels, reduced lipid oxidation, and greater carbohydrate oxidation

during rest and exercise (*85*, *86*).

The decrease in physical activity levels, body mass changes, and percent fat changes in males that we observed during aging could also be due to hormonal changes. Reduction in testosterone levels during aging in males decreases fat-free mass and increases body fat (*110*). Additionally, physical activity levels are reduced in male rodents, including C57BL/6J, after castration (*40*). Hormonal changes during aging may also explain the sex-dependent changes in physical activity levels, body mass, body composition, and metabolism that we observed.

2.5.3 Changes in metabolism in response to aging and exercise

RER (ratio of CO2 production to O2 consumption) is used to indirectly determine relative use of carbohydrates or lipids for energy expenditure. Higher RER values (e.g. 1.0) indicate greater carbohydrate use; whereas lower RER values (e.g. 0.7) indicate greater lipid oxidation. Individuals, both rodents and humans, with sedentary lifestyles normally have higher RER values and lower fat oxidation. Physically active individuals tend to demonstrate lower RER values than untrained individuals in response to exercise (*66*, *111*). Previous studies have shown that older C57BL/6J mice (660 days, ~1.8 years), compared to young mice (90 days), have greater body mass, reduced lean mass, VO2 and RER, greater FFAs, and lower triglycerides (*112*). Sex differences in RER also exist. Females tend to store FFAs as triglycerides, which in turn assists in fat storage; whereas males oxidize circulating FFAs. Under increased energy demands from exercise, women oxidize a greater proportion of lipids versus carbohydrates than men, thus exercise results in lower RER in females (*86*). We found that aging C57BL/6J mice had higher resting RER levels, especially at ~2 years, indicating a preference toward carbohydrate utilization in aged individuals. Most interestingly, female mice with wheel access had greater resting RER levels than control females. Thus, females utilize more carbohydrates as an energy source in response to exercise; whereas, male mice had no difference in RER levels in response to exercise.

We observed a decrease in both oxygen consumption (VO2) and carbon dioxide

production (VCO2) during aging. Both VO2 consumption and VCO2 production are thought to be a proxy for the amount of metabolism occurring (e.g. more VO2 consumed and the more VCO2 produced the more metabolism occurring). We expected both VO2 and VCO2 to decrease during aging since metabolism and BMR are reduced with age (*69-71*). Although VO2 and VCO2 are used as proxies for metabolism, we cannot determine the efficiency of metabolism (e.g. the amount of ATP produced relative to oxygen intake) with only those measurements. We would expect VO2 to be positively correlated with running distance since we would expect a higher metabolism with greater running distance. It is possible the observed nonsignificant negative correlation of VO2 and running distance at 2 years of age could be due to a more efficient utilization of VO2 consumed in exercise-trained mice.

In conclusion, this study demonstrates that exercise in mice protects against age-related alterations in body mass, body composition and metabolism in both sexes. We showed that patterns of physiological changes during aging vary by sex. Additionally, sex impacts exercise abilities and physiological responses to exercise during aging. However, the benefits occur despite the significant differences in physiological and exercise ability between the sexes. We conclude that exposure to exercise from midlife on has significant benefits in mice that may extend to humans. Further studies need to determine if the benefits from long-term exercise during aging occur due to exposure to exercise at a specific time and/or for specific duration of time. Future studies should investigate the effect of exercise in other outcomes such as cognitive function, cancer, heart disease, etc. Our results support the contention that laboratory mice are a valuable model to study the effects of age and exercise. In particular, the mouse could be used to add a genetic dimension to these studies, given the plethora of existing genetic resources in this organism.

2.6 Figures

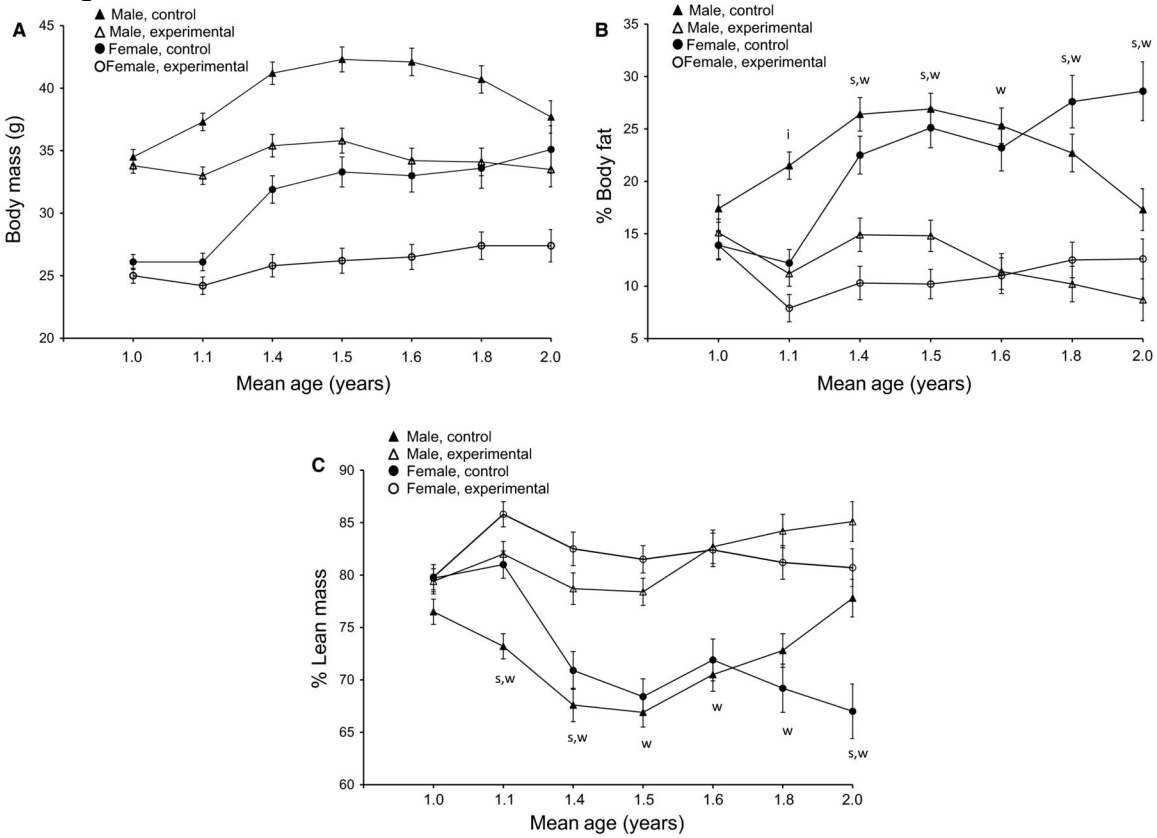


Figure 2.1

Estimated marginal means and standard errors of (A) body mass (g), (B) percent body fat, and (C) percent lean mass beginning at approximately one year of age and extending over the course of the following year. Wheel access (experimental) or no wheel access (control) was granted after the measurement at ~1 year of age. (A) At all time points, General Linear Models (GLM) revealed that males weighed significantly more than females ($P < 0.05$) and, with the exception of age ~1 year (immediately prior to wheel access), wheel access significantly reduced mass ($P < 0.05$). No significant sex-by-wheel access interactions were detected. However, at ~1.1 years of age, following the first 51 days of wheel access, the sex-by-wheel access interaction ($F1, 52 = 3.354$; $P = 0.073$) suggested that wheel access reduced body mass to a greater extent among male mice. For panels (B) and (C), at a given mean age, an "i" indicates a significant ($P < 0.05$) interaction, a "s" indicates a significant effect of sex, and a "w" indicates a significant effect of wheel access on percent body fat.

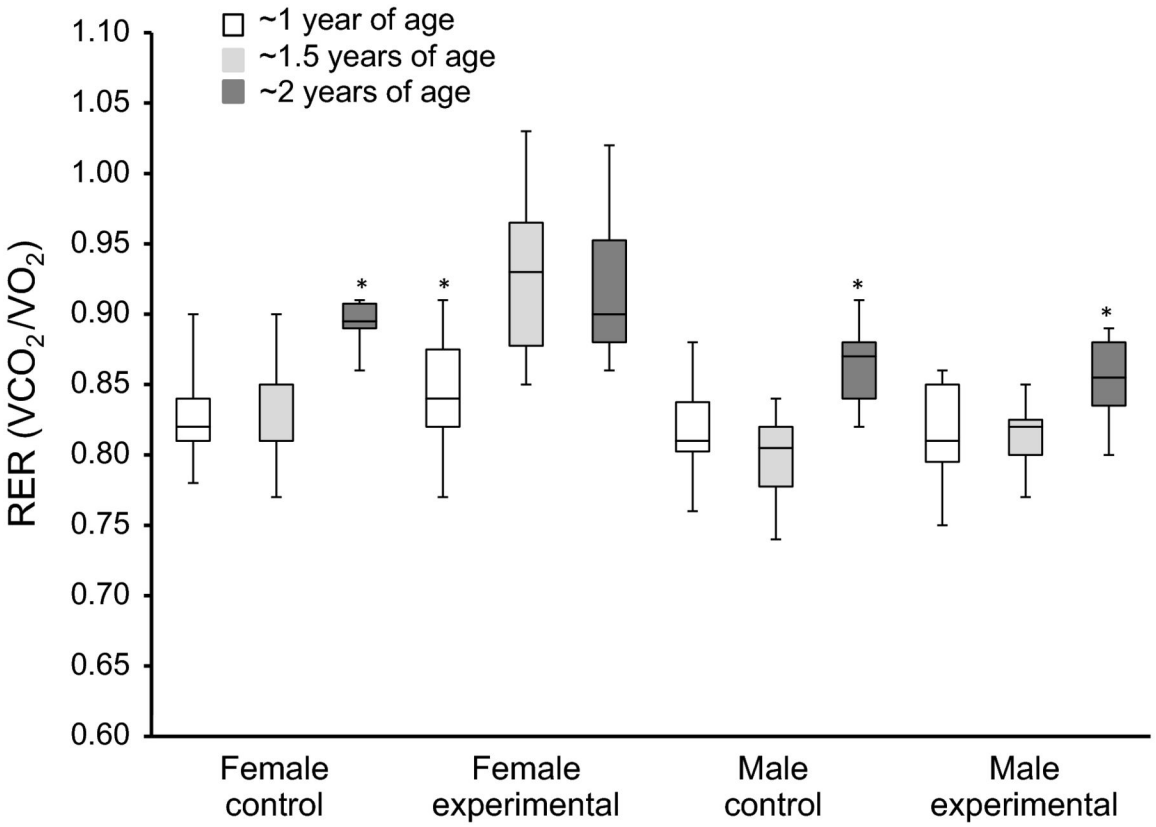


Figure 2.2

Respiratory exchange ratio during aging and across experimental groups (sex; treatment). Repeated measure analysis of variance (ANOVAs) [GLM (SPSS, Chicago, IL)] revealed a significant effect of age across all groups (sex and experimental vs. control) ($P < 0.05$). Pairwise comparisons indicated that RER at ~ 2 years was significantly higher compared to ~1 year ($P < 0.001$) or ~1.5 years ($P < 0.001$). Additionally, within each treatment group a similar trend was observed - asterisks represent results for within group pairwise comparisons.

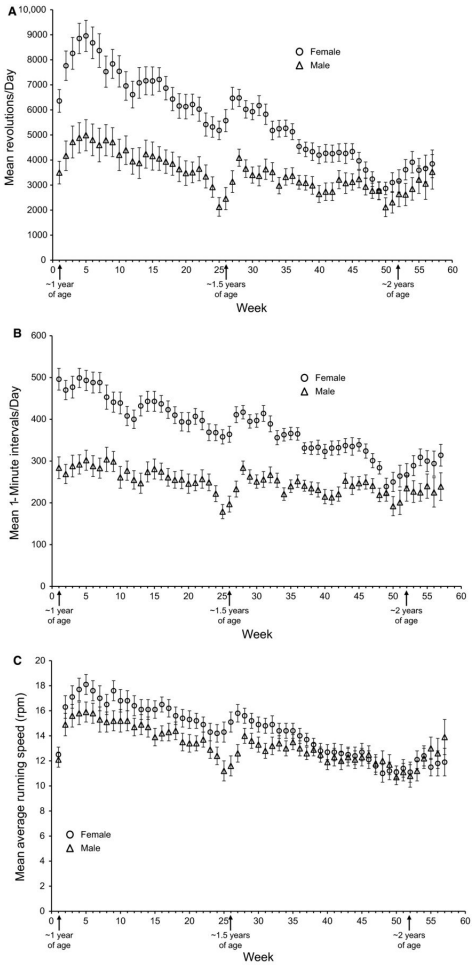


Figure 2.3 Estimated marginal means and standard errors of mean (A) revolutions per day, (B) time (i.e., cumulative 1 min intervals in which at least 1 revolution was recorded) spent running, and (C) running speed (mean revolutions/mean running time) across 57 weeks. (A) Comparisons between sexes by General Linear Models (GLM) revealed females ran significantly more than males during weeks 1–45. During weeks 46–57 there was no significant difference between the sexes. (C) Comparisons between sexes by GLM revealed females ran significantly faster only during weeks 15, 16, 25, 26, 27, and 31.

2.7 Tables

Table 2.1

Effects of sex (male vs. female) and exercise (wheel vs. no wheel) on body composition. Measures were taken beginning at approximately one year of age and extended over the course of the following year.

Trait	N	Sex	Wheel Access	Interaction
~Day 372				
Body mass (g)	59	$F_{1, 55} = 199.689$ **p < 0.001**	$F_{1, 55} = 2.127$ p = 0.150	$F_{1, 55} = 0.094$ p = 0.760
% Fat	59	$F_{1, 55} = 3.092$ p = 0.084	$F_{1, 55} = 0.767$ p = 0.385	$F_{1, 55} = 0.749$ p = 0.390
% Lean	59	$F_{1, 55} = 2.174$ p = 0.146	$F_{1, 55} = 1.400$ p = 0.242	$F_{1, 55} = 1.349$ p = 0.250
~Day 423				
Body mass (g)	56	$F_{1, 52} = 213.576$ **p < 0.001**	$F_{1, 52} = 20.102$ **p < 0.001**	$F_{1, 52} = 2.911$ p = 0.094
% Fat	56	$F_{1, 52} = 25.098$ **p < 0.001**	$F_{1, 52} = 33.781$ **p < 0.001**	$F_{1, 52} = 5.575$ **p = 0.022**
% Lean	56	$F_{1, 52} = 22.356$ **p < 0.001**	$F_{1, 52} = 30.530$ **p < 0.001**	$F_{1, 52} = 2.750$ p = 0.103
% Change in Mass	55	$F_{1, 51} = 7.896$ **p = 0.007**	$F_{1, 51} = 25.754$ **p < 0.001**	$F_{1, 51} = 1.769$ p = 0.189
% Change in % Fat	55	$F_{1, 51} = 10.332$ **p = 0.002**	$F_{1, 51} = 32.134$ **p < 0.001**	$F_{1, 51} = 1.313$ p = 0.257
% Change in % Lean	55	$F_{1, 51} = 18.918$ **p < 0.001**	$F_{1, 51} = 24.674$ **p < 0.001**	$F_{1, 51} = 1.840$ p = 0.181
~Day 520				
Body mass (g)	54	$F_{1, 50} = 97.594$ **p < 0.001**	$F_{1, 50} = 38.151$ **p < 0.001**	$F_{1, 50} = 0.040$ p = 0.842
% Fat	54	$F_{1, 50} = 6.494$ **p = 0.014**	$F_{1, 50} = 51.438$ **p < 0.001**	$F_{1, 50} = 0.053$ p = 0.818
% Lean	54	$F_{1, 50} = 4.942$ **p = 0.031**	$F_{1, 50} = 50.711$ **p < 0.001**	$F_{1, 50} = 0.042$ p = 0.839
% Change in Mass	53	$F_{1, 49} = 7.414$ **p = 0.009**	$F_{1, 49} = 37.680$ **p < 0.001**	$F_{1, 49} = 16.525$ **p < 0.001**
% Change in % Fat	53	$F_{1, 49} = 4.128$ **p = 0.048**	$F_{1, 49} = 2.212$ p = 0.143	$F_{1, 49} = 8.603$ **p = 0.005**
% Change in % Lean	53	$F_{1, 49} = 5.763$ **p = 0.020**	$F_{1, 49} = 40.322$ **p < 0.001**	$F_{1, 49} = 0.410$ p = 0.525
~Day 549				
Body mass (g)	53	$F_{1, 49} = 80.383$ **p < 0.001**	$F_{1, 49} = 42.233$ **p < 0.001**	$F_{1, 49} = 0.073$ p = 0.789
% Fat	53	$F_{1, 49} = 4.164$ **p = 0.047**	$F_{1, 49} = 74.067$ **p < 0.001**	$F_{1, 49} = 0.771$ p = 0.384
% Lean	53	$F_{1, 49} = 2.503$ p = 0.120	$F_{1, 49} = 71.116$ **p < 0.001**	$F_{1, 49} = 0.313$ p = 0.578
% Change in Mass	52	$F_{1, 48} = 0.226$ p = 0.637	$F_{1, 48} = 2.763$ p = 0.103	$F_{1, 48} = 0.449$ p = 0.506
% Change in % Fat	52	$F_{1, 48} = 0.135$ p = 0.715	$F_{1, 48} = 0.060$ p = 0.807	$F_{1, 48} = 0.178$ p =0.675
% Change in % Lean	52	$F_{1, 48} = 2.975$ p =0.091	$F_{1, 48} = 60.021$ **p < 0.001**	$F_{1, 48} = 0.258$ p = 0.614
~Day 606				
Body mass (g)	53	$F_{1, 49} = 55.311$ **p < 0.001**	$F_{1, 49} = 40.734$ **p < 0.001**	$F_{1, 49} = 0.417$ p = 0.521

% Fat	53	$F_{1, 49} = 0.502$ $p = 0.482$	$F_{1, 49} = 51.737$ **$p < 0.001$**	$F_{1, 49} = 0.199$ $p = 0.657$
% Lean	53	$F_{1, 49} = 0.091$ $p = 0.764$	$F_{1, 49} = 44.899$ **$p < 0.001$**	$F_{1, 49} = 0.277$ $p = 0.601$
% Change in Mass	53	$F_{1, 49} = 6.902$ **$p = 0.011$**	$F_{1, 49} = 0.539$ $p = 0.466$	$F_{1, 49} = 6.539$ **$p = 0.014$**
% Change in % Fat	53	$F_{1, 49} = 8.162$ **$p = 0.006$**	$F_{1, 49} = 0.383$ $p = 0.539$	$F_{1, 49} = 11.721$ **$p = 0.001$**
% Change in % Lean	53	$F_{1, 49} = 0.696$ $p = 0.408$	$F_{1, 49} = 45.924$ **$p < 0.001$**	$F_{1, 49} = 0.023$ $p = 0.880$
~Day 669				
Body mass (g)	51	$F_{1, 47} = 31.317$ **$p < 0.001$**	$F_{1, 47} = 27.705$ **$p < 0.001$**	$F_{1, 47} = 0.029$ $p = 0.866$
% Fat	51	$F_{1, 47} = 3.458$ $p = 0.069$	$F_{1, 47} = 51.524$ **$p < 0.001$**	$F_{1, 47} = 0.442$ $p = 0.509$
% Lean	51	$F_{1, 47} = 3.438$ $p = 0.070$	$F_{1, 47} = 43.578$ **$p < 0.001$**	$F_{1, 47} = 0.026$ $p = 0.873$
% Change in Mass	51	$F_{1, 47} = 2.240$ $p = 0.141$	$F_{1, 47} = 7.599$ **$p = 0.008$**	$F_{1, 47} = 0.704$ $p = 0.406$
% Change in % Fat	51	$F_{1, 47} = 13.176$ **$p = 0.001$**	$F_{1, 47} = 1.476$ $p = 0.231$	$F_{1, 47} = 1.226$ $p = 0.274$
% Change in % Lean	51	$F_{1, 47} = 1.306$ $p = 0.259$	$F_{1, 47} = 53.151$ **$p < 0.001$**	$F_{1, 47} = 0.162$ $p = 0.689$
~Day 731				
Body mass (g)	49	$F_{1, 45} = 8.647$ **$p = 0.005$**	$F_{1, 45} = 16.183$ **$p < 0.001$**	$F_{1, 45} = 1.515$ $p = 0.225$
% Fat	49	$F_{1, 45} = 11.882$ **$p = 0.001$**	$F_{1, 45} = 31.040$ **$p < 0.001$**	$F_{1, 45} = 2.794$ $p = 0.225$
% Lean	49	$F_{1, 45} = 13.721$ **$p = 0.001$**	$F_{1, 45} = 25.857$ **$p < 0.001$**	$F_{1, 45} = 2.409$ $p = 0.128$
% Change in Mass	49	$F_{1, 45} = 18.171$ **$p < 0.001$**	$F_{1, 45} = 0.512$ $p = 0.478$	$F_{1, 45} = 12.510$ **$p = 0.001$**
% Change in % Fat	49	$F_{1, 45} = 17.647$ **$p < 0.001$**	$F_{1, 45} = 0.225$ $p = 0.638$	$F_{1, 45} = 0.216$ $p = 0.644$
% Change in % Lean	49	$F_{1, 45} = 10.069$ **$p = 0.003$**	$F_{1, 45} = 35.749$ **$p < 0.001$**	$F_{1, 45} = 3.765$ $p = 0.059$

Statistical significance was judged at $P < 0.05$ (in bold), and all *P*-values presented are two-tailed.

Table 2.2.

Effects of sex (male vs. female) and exercise (wheel vs. no wheel) on metabolic parameters. Measures were taken at ~1 year (prior to running wheel exposure), ~1.5, and ~2 years of age

Trait	N	Sex	Wheel Access	Interaction	Activity	Batch
~Day 372						
VO_2 (ml/kg/h)	59	$F_{1, 53} = 189.696$ **p < 0.001**	$F_{1, 53} = 0.036$ p = 0.850	$F_{1, 53} = 2.991$ p = 0.090	$F_{1, 53} = 2.614$ p = 0.112	$F_{1, 53} = 0.007$ p = 0.936
VCO_2 (ml/kg/h)	59	$F_{1, 53} = 114.856$ **p < 0.001**	$F_{1, 53} = 0.027$ p = 0.651	$F_{1, 53} = 0.264$ p = 0.610	$F_{1, 53} = 1.482$ p = 0.229	$F_{1, 53} = 0.144$ p = 0.706
RER (VCO_2/VO_2)	59	$F_{1, 53} = 2.642$ p = 0.110	$F_{1, 53} = 0.552$ p = 0.461	$F_{1, 53} = 1.486$ p = 0.228	$F_{1, 53} = 0.106$ p = 0.746	$F_{1, 53} = 0.049$ p = 0.826
~Day 549						
VO_2 (ml/kg/h)	44	$F_{1, 38} = 93.906$ **p < 0.001**	$F_{1, 38} = 1.390$ p = 0.246	$F_{1, 38} = 0.006$ p = 0.939	$F_{1, 38} = 1.553$ p = 0.220	$F_{1, 38} = 0.085$ p = 0.772
VCO_2 (ml/kg/h)	44	$F_{1, 38} = 55.383$ **p < 0.001**	$F_{1, 38} = 6.102$ **p = 0.018**	$F_{1, 38} = 4.703$ **p = 0.036**	$F_{1, 38} = 0.010$ p = 0.921	$F_{1, 38} = 0.070$ p = 0.793
RER (VCO_2/VO_2)	44	$F_{1, 38} = 2.863$ p = 0.099	$F_{1, 38} = 9.835$ **p = 0.003**	$F_{1, 38} = 14.044$ **p = 0.001**	$F_{1, 38} = 3.037$ p = 0.089	$F_{1, 38} = 0.041$ p = 0.840
~Day 731						
VO_2 (ml/kg/h)	45	$F_{1, 39} = 5.351$ **p = 0.026**	$F_{1, 39} = 0.446$ p = 0.508	$F_{1, 39} = 3.864$ p = 0.056	$F_{1, 39} = 7.405$ **p = 0.010**	$F_{1, 39} = 1.054$ p = 0.311
VCO_2 (ml/kg/h)	45	$F_{1, 39} = 9.370$ **p = 0.004**	$F_{1, 39} = 0.038$ p = 0.846	$F_{1, 39} = 5.137$ **p = 0.029**	$F_{1, 39} = 4.147$ **p = 0.049**	$F_{1, 39} = 0.050$ p = 0.824
RER (VCO_2/VO_2)	45	$F_{1, 39} = 5.473$ **p = 0.025**	$F_{1, 39} = 3.430$ p = 0.072	$F_{1, 39} = 1.521$ p = 0.225	$F_{1, 39} = 0.402$ p = 0.530	$F_{1, 39} = 3.071$ p = 0.088

Statistical significance was judged at $P < 0.05$ (in bold), and all *P*-values presented are two-tailed.

Table 2.3.

Effects of sex (male vs. female) and exercise (wheel vs. no wheel) on home cage activity. Measures were taken at ~1 year (prior to running wheel exposure), ~1.5, and ~2 years of age

Trait	Trans	N	Sex	Wheel Access	Interaction	Batch
~Day 372						
Home Cage Activity		59	$F_{1, 54} = 26.973$ **p < 0.001**	$F_{1, 54} = 0.170$ p = 0.682	$F_{1, 54} = 0.025$ p = 0.874	$F_{1, 54} = 0.035$ p = 0.852
Food Consumption		59	$F_{1, 54} = 15.459$ **p < 0.001**	$F_{1, 54} = 0.055$ p = 0.815	$F_{1, 54} = 0.416$ p = 0.521	$F_{1, 54} = 0.281$ p = 0.598
Water Consumption	lg10	59	$F_{1, 54} = 9.912$ **p = 0.003**	$F_{1, 54} = 0.253$ p = 0.617	$F_{1, 54} = 7.284$ **p = 0.009**	$F_{1, 54} = 0.249$ p = 0.620
~Day 549						
Home Cage Activity		44	$F_{1, 39} = 6.070$ **p = 0.018**	$F_{1, 39} = 1.166$ p = 0.287	$F_{1, 39} = 10.402$ **p = 0.003**	$F_{1, 39} = 9.860$ **p = 0.003**
Food Consumption		44	$F_{1, 39} = 13.352$ **p = 0.001**	$F_{1, 39} = 11.025$ **p = 0.002**	$F_{1, 39} = 8.609$ **p = 0.006**	$F_{1, 39} = 0.724$ p = 0.400
Water Consumption		43	$F_{1, 38} = 10.837$ **p = 0.002**	$F_{1, 38} = 9.618$ **p = 0.004**	$F_{1, 38} = 0.792$ p = 0.379	$F_{1, 38} = 4.231$ **p = 0.047**
~Day 731						
Home Cage Activity		45	$F_{1, 40} = 32.582$ **p < 0.001**	$F_{1, 40} = 9.624$ **p = 0.004**	$F_{1, 40} = 1.815$ p = 0.186	$F_{1, 40} = 9.831$ **p = 0.003**
Food Consumption		45	$F_{1, 40} = 3.232$ p = 0.080	$F_{1, 40} = 5.452$ **p = 0.025**	$F_{1, 40} = 1.869$ p = 0.179	$F_{1, 40} = 1.858$ p = 0.180
Water Consumption		44	$F_{1, 39} = 14.398$ **p = 0.001**	$F_{1, 39} = 4.002$ p = 0.052	$F_{1, 39} = 0.009$ p = 0.926	$F_{1, 39} = 0.437$ p = 0.512

Statistical significance was judged at $P < 0.05$ (in bold), and all *P*-values presented are two-tailed.

Table 2.4.

Pearson partial correlations for mean running traits during week 1, week 25, and week 51 of wheel access and metabolic traits measured immediately prior to each time point. Approximate age of individuals is one year, 1.5, and 2 years

Traits	Age		
	~1 year	~1.5 years	~2 years
Distance:Time	0.932**	0.943**	0.938**
Distance:Speed	0.793**	0.827**	0.706**
Distance:VO_2	0.04	-0.097	-0.127
Distance:VCO_2	-0.002	0.049	0.26
Distance:RER	-0.042	0.165	0.538*
Time:Speed	0.554*	0.661*	0.439*
Time:VO_2	0.011	-0.067	-0.184
Time:VCO_2	0.04	0.211	0.192
Time:RER	0.07	0.374	0.486*
Speed:VO_2	0.075	-0.059	0.108
Speed:VCO_2	-0.005	-0.058	0.314
Speed:RER	-0.095	-0.024	0.387
VO_2:VCO_2	0.837**	0.753**	0.745*
VO_2:RER	0.088	0.162	0.037
VCO_2:RER	0.613**	0.766**	0.691**

Pearson partial correlations (*r*; controlling for sex) are shown. NA (Not Applicable) represents correlations that cannot be calculated since the measurements depend on a prior experimental time point. *$P < 0.05$. **$P < 0.001$.

Table 2.5.

Pearson partial correlations for mean running traits during week 1, 7, 21, 25, 33, 42, 51 of wheel access and body composition measured immediately prior to each time point.

Traits	Age						
	~1 year	~1.1 years	~1.4 years	~1.5 years	~1.6 years	~1.8 years	~2 years
Distance:Time	0.923**	0.857**	0.942**	0.917**	0.918**	0.880**	0.930**
Distance:Speed	0.762**	0.877**	0.834**	0.804**	0.857**	0.834**	0.683**
Distance:Mass	-0.108	-0.366	-0.456*	-0.266	-0.24	-0.276	-0.166
Distance:% Fat	0.042	-0.266	-0.544*	-0.267	-0.338	-0.322	-0.232
Distance:% Lean	0.002	0.029	0.483*	0.272	0.293	0.271	0.109
Distance:% Δ in Mass	NA	-0.515*	-0.278	-0.122	0.023	-0.257	0.23
Distance:% Δ in %Fat	NA	-0.487*	-0.381	0.157	-0.088	-0.103	-0.004
Distance:% Δ in % Lean	NA	0.004	0.515*	0.412*	0.282	0.322	0.172
Time:Speed	0.492*	0.567*	0.642**	0.579*	0.605*	0.504*	0.39
Time:Mass	-0.15	-0.431*	-0.454*	-0.186	-0.205	-0.119	-0.026
Time:% Fat	0.005	-0.336	-0.529*	-0.128	-0.19	-0.049	-0.093
Time:% Lean	0.035	0.099	0.447*	0.118	0.143	0.006	-0.006
Time:% Δ in Mass	NA	-0.302	-0.157	-0.055	0.001	-0.097	0.308
Time:% Δ in %Fat	NA	-0.575*	-0.311	0.163	0.032	0.075	0.002
Time:% Δ in % Lean	NA	0.067	0.484*	0.235	0.161	0.067	0.04
Speed:Mass	-0.026	-0.356	-0.421*	-0.453*	-0.307	-0.412*	-0.396
Speed:% Fat	0.037	-0.352	-0.529*	-0.563*	-0.554*	-0.558*	-0.488*
Speed:% Lean	-0.001	0.126	0.479*	0.570*	0.525*	0.509*	0.383
Speed:% Δ in Mass	NA	-0.620**	-0.269	-0.05	0.105	-0.31	0.043
Speed:% Δ in %Fat	NA	-0.345	-0.311	0.167	-0.17	-0.225	-0.099
Speed:% Δ in % Lean	NA	0.126	0.511*	0.662**	0.494*	0.548*	0.445*
Mass:% Fat	0.752**	0.882**	0.824**	0.868**	0.800**	0.799**	0.739**
Mass:% Lean	-0.766**	-0.619**	-0.833**	-0.866**	-0.784**	-0.802**	-0.751**
Mass:% Δ in Mass	NA	0.16	0.463*	0.197	0.248	0.301	0.189
Mass:% Δ in %Fat	NA	0.332	0.12	0.056	0.084	0.238	0.148
Mass:% Δ in % Lean	NA	-0.706**	-0.774**	-0.743**	-0.783**	-0.817**	-0.777**
% Fat:% Lean	-0.986**	-0.700**	-0.792**	-0.950**	-0.981**	-0.992**	-0.969**
% Fat:% Δ in Mass	NA	0.224	0.403*	0.102	0.064	0.215	0.102

% Fat:% Δ in %Fat	NA	0.396*	0.419*	0.165	0.118	0.416*	0.497*
% Fat:% Δ in % Lean	NA	-0.791**	-0.797**	-0.821**	-0.946**	-0.990**	-0.989**
% Lean:% Δ in Mass	NA	-0.411*	-0.406*	-0.231	-0.136	-0.207	-0.091
% Lean:% Δ in %Fat	NA	-0.408*	-0.16	-0.117	-0.198	-0.398*	-0.527*
% Lean:% Δ in % Lean	NA	0.961**	0.890**	0.857**	0.950**	0.990**	0.989**
% Δ in Mass:% Δ in %Fat	NA	0.591*	0.567*	0.459*	0.653**	0.536*	0.202
% Δ in Mass:% Δ in % Lean	NA	-0.299	-0.33	-0.091	-0.123	-0.164	-0.069
% Δ in %Fat:% Δ in % Lean	NA	-0.257	-0.19	0.204	-0.099	-0.346	-0.444*

Pearson partial correlations (*r*; controlling for sex) are shown. NA (Not Applicable) represents correlations that cannot be calculated since the measurements depend on a prior experimental time point. *$P < 0.05$. **$P < 0.001$.

Table 2.6.

Pearson partial correlations for mean body composition traits and metabolic traits measured immediately prior to wheel access.

Traits	Age		
	~1 year	~1.5 years	~2 years
Mass:% Fat	0.776**	0.911**	0.884**
Mass:% Lean	-0.763**	-0.894**	-0.879**
Mass:% Δ in Mass	NA	0.343*	0.567**
Mass:% Δ in % Fat	NA	0.108	0.401*
Mass:% Δ in % Lean	NA	-0.869**	-0.895**
Mass:VO_2	-0.511**	-0.655**	-0.558**
Mass:VCO_2	-0.632**	-0.600**	-0.512*
Mass:RER	-0.504**	-0.376*	-0.031
% Fat:% Lean	-0.984**	-0.991**	-0.986**
% Fat:% Δ in Mass	NA	0.320*	0.504*
% Fat:% Δ in % Fat	NA	0.181	0.580**
% Fat:% Δ in % Lean	NA	-0.921**	-0.973**
% Fat:VO_2	-0.654**	-0.619**	-0.499*
% Fat:VCO_2	-0.735**	-0.567**	-0.492*
% Fat:RER	-0.471**	-0.345*	-0.12
% Lean:% Δ in Mass	NA	-0.316*	-0.483*
% Lean:% Δ in % Fat	NA	-0.175	-0.602**
% Lean:% Δ in % Lean	NA	0.925**	0.974**
% Lean:VO_2	0.644**	0.633**	0.478*
% Lean:VCO2	0.709**	0.549**	0.448*
% Lean:RER	0.436**	0.302*	0.053
% Δ in Mass:% Δ in % Fat	NA	0.528**	0.424*
% Δ in Mass:% Δ in % Lean	NA	-0.221	-0.480*
% Δ in Mass:VO2	NA	-0.299	-0.616**
% Δ in Mass:VCO2	NA	-0.25	-0.605**
% Δ in Mass:RER	NA	-0.109	-0.139
% Δ in % Fat:% Δ in % Lean	NA	0.064	-0.480*
% Δ in % Fat:VO2	NA	-0.036	-0.247
% Δ in % Fat:VCO2	NA	-0.088	-0.202
% Δ in % Fat:RER	NA	-0.121	0.041
% Δ in % Lean:VO2	NA	0.587**	0.508*
% Δ in % Lean:VCO2	NA	0.458*	0.487*
% Δ in % Lean:RER	NA	0.211	0.087
VO2:VCO2	0.850**	0.818**	0.923**
VO2:RER	0.229	0.320*	0.117
VCO2:RER	0.701**	0.799**	0.486*

Pearson partial correlations (*r*; controlling for sex) are shown. NA (Not Applicable) represents correlations that cannot be calculated since the measurements depend on a prior experimental time point. *$P < 0.05$. **$P < 0.001$.

CHAPTER 3: THE COLLABORATIVE CROSS POPULATION IS A SYSTEM GENETICS RESOURCE TO STUDY EXERCISE AND EXERCISE-RELATED TRAITS

3.1 Introduction

Exercise and subsequent physiological responses are highly variable, complex traits influenced by genomics, environment and their interactions. Exercise has numerous health benefits and is commonly used as a therapeutic and preventative for a variety of chronic diseases (*15*, *16*). Despite the known benefits of exercise, there is still individual variability in physiological adaptations to exercise (*16*, *18*, *19*, *45*, *54*). In order to fully understand genomic and molecular mechanisms driving variation and complexity in exercise and exercise-related traits, a systems genetics based approach needs to be utilized. Human studies are often confounded by numerous variables making it difficult to identify underlying genetic mechanisms of exercise-related phenotypes. Animal models are valuable for dissecting the genetic and molecular mechanism of exercise-induced adaptations due to the ability to control genetic background, standardize phenotypic measurements and regulate environmental variables (*41*, *52*, *54*). The Collaborative Cross (CC) offers a systems genetics based platform to dissect complex traits. It overcomes the limited range of phenotypic and genotypic diversity in common murine models since the CC is derived from eight founder strains from the three *Mus musculus* subspecies. The CC enables improved accuracy of phenotypic measurement and data integration across a variety of factors (e.g. exercise type, age, sex, diet, etc) in the same genetic backgrounds due to inbred nature of the CC strains and the genomic tools available for analysis of CC strains (*54*, *55*, *58*). Mathes *et al*. (2011) examined exercise and exercise related traits in pre-CC mice (partially inbred). The pre-CC study demonstrated high phenotypic diversity in

exercise and exercise related traits. A subset of ~17% of pre-CC mice had an adverse body fat response to exercise suggesting the CC population could be a rich source of adverse physiological adaption models (*66*). The purpose of this study was to demonstrate the CC population is a useful system genetics resource for studying exercise and exercise-related traits. In order to understand the exercise abilities and responder types present in the CC population, we screened 50 CC strains for forced endurance abilities and 43 corresponding CC strains for voluntary exercise abilities and subsequent body mass and composition responses. As expected we observed phenotypic variability in exercise and exercise-related traits across the CC strains. We demonstrated the CC population to be a useful source for identifying model strains for human exercise-related traits. In particular, CC027/GeniUnc females were identified as a potential model for adverse body composition response to voluntary exercise.

3.2 Materials and Methods

3.2.1 Mice

Female mice from 50 CC strains were obtained from another project (National Institutes of Health U19AI100625) or from the System Genetics Core at UNC-Chapel Hill. The female mice in the experiment were all retired breeders and varied in life history (e.g. number of litters, most recent litter, etc) and age. Prior to and during the experiment, all mice had *ad libitum* access to standard laboratory chow (Envigo 2920 irradiated chow) and water in a temperature controlled (23° +/- 1°C) and humidity-monitored vivarium with a standard 12:12 h light:dark cycle (lights on at 0700h). All CC strains were at least 85% inbred (determined by genotyping of the most recent common ancestor born in 2011-2013) with 41 strains ~90% (or greater) inbred. Additional information regarding the CC strains can be found at http://csbio.unc.edu/CCstatus/index.py (*55*, *59*). All procedures performed within this experiment were approved by the University of North Carolina – Chapel Hill Institutional Animal Care and Use Committee.

3.2.2 Body mass and composition measurements

Body composition was assessed using whole body MRI (EchoMRI 3-in-1 Body Composition Analyzer, EchoMRI, Houston, TX) to determine fat and lean mass content (in grams) for each animal. Body mass (g) was recorded at the time of each MRI. Body fat percentage and lean mass percentage were calculated relative to body mass at each time point. All measurements were collected in the morning between 0700 and 1200h.

3.2.3 Forced Endurance Cohort

Forced endurance distance was measured in 50 CC strains (total of 232 aged mice; age range: 4.27-18.23 months; age mean: 10.39 months) across 26 treadmill batches starting in February 2015 and ending in June 2016. Mice in the forced endurance cohort were group housed with mice from the same strain in standard laboratory cages. Forced endurance was measured on Exer-3/6 treadmill (Columbus Instruments, Columbus, OH) after three adaptation days (Table 3.7) followed by one maximal performance day (Table 3.7) that measured distance traveled. Max forced endurance distance was recorded when the individual mouse failed to run either from the inability to continue treadmill running or the refusal to continue running despite extra stimulus from the shock grid and prodding. For treadmill protocols refer to Table 3.7. Body mass and composition was collected prior to the start of day 1 of treadmill acclimation.

3.2.4 Forced Endurance Cohort—Statistical Analysis

A summary of descriptive statistics (mean, variance, standard deviation, standard error) for each CC strain across all traits can be found in Table 3.1. All statistical analyses were conducted in the R environment (CRAN). Linear models were generated to assess the variance in endurance distance and contribution from strain (genetic background) and/or each potential mediator (age, baseline body mass, baseline body fat percentage, and baseline lean mass percentage). Base linear models (only one mediator as a fixed

effect; “Model F#a”), additive models (mediator and Strain; “Model F#b”) or interaction models (mediator and Strain; “Model F#c”) were generated. Nested ANOVA analysis was performed to assess statistical significance of model fit for adding additional explanatory variables. Models are as follows:

Model F1a: *distance ~ Strain*

Model F2a: *distance ~ Age*

Model F2b: *distance ~ Age + Strain*

Model F2c: *distance ~ Age*Strain*

Model F3a: *distance ~ Body Mass*

Model F3b: *distance ~ Body Mass + Strain*

Model F3c: *distance ~ Body Mass*Strain*

Model F4a: *distance ~ Body Fat %*

Model F4b: *distance ~ Body Fat % + Strain*

Model F4c: *distance ~ Body Fat %*Strain*

Model F5a: *distance ~ Lean Mass %*

Model F5b: *distance ~ Lean Mass % + Strain*

Model F5c: *distance ~ Lean Mass %*Strain*

3.2.5 Voluntary Exercise Cohort

Aged female mice (n = 186; age range: 5.27 – 22.50 months; age mean: 11.28 months) across 43 CC strains were exposed to one month of voluntary exercise in 17 batches starting in August 2015 and ending in September 2016. Mice in the voluntary exercise cohort were individually housed in standard laboratory cages with attached running wheels (1.1m circumference; Lafayette Industries Lafayette, IN). Mice were allowed *ad libitum* access to running wheels for a month (~28 days). Voluntary wheel running data was recorded continuously in 1-min intervals (as number of revolutions) over the month using an automated activity wheel monitoring program (AWM, Lafayette Industries,

Lafayette, IN). Food was weighed prior to the start of the experiment, two weeks after the start of the experiment and at the end of the experiment (one month). Any food spillage was collected and weighed (*113*). Body mass and composition was measured and recorded prior to the experiment, mid experiment (two weeks) and at the end of the experiment (one month). All measurements were collected in the morning between 07:00 and 12:00.

3.2.6 Voluntary Exercise Cohort – Calculations

Three body mass and composition responses were calculated: total response (one month), response 1 (week1-2), and response 2 (week3-4). Body mass response was calculated as [((Post body mass – Pre body mass)/ Pre body mass)*100%]. Body composition (body fat % and lean mass %) response was calculated as [((Post %– Pre %)/ Pre %)*100%]. Food intake (FI) was calculated as [Pre food weight – post food weight] for both week1-2 and week3-4. Adjusted food intake (FIa) was calculated as [FI/Pre Body Mass].

The following physical activity measurements were obtained for each day of wheel access: distance (total revolutions x 1.1 m), duration (cumulative 1-min intervals in which at least 1 revolution was recorded), and average speed (total revolutions/total duration) (*96*). For week1-2 (days1-14), week3-4 (days15-28), month (days1-28) of wheel access the following were calculated using the calculated daily physical activity measurements: total distance (km), total duration (min) and average speed (m/min).

3.2.7 Voluntary Exercise Cohort – Statistical analysis

A summary of descriptive statistics (mean, variance, standard deviation, standard error) for each trait across all CC strains can be found in Tables 3.2 and Table 3.3. Linear models were generated to assess the variance in response (body mass, body fat and lean mass response) and contribution from strain (genetic background) and/or each potential mediator (distance, duration, speed, food intake). Base linear models (only one mediator as a fixed effect; “Model V2a”), additive models (mediator and Strain; “Model V2b”) or

interaction models (mediator and Strain; “Model V2c”) were generated. Nested ANOVA framework analysis was performed to assess statistical significance of model fit for adding additional explanatory variables. Models are as follows:

Model V1a: *Response ~ Strain*

Model V2a: *Response ~ Mediator*

Model V2b: *Response ~ Mediator + Strain*

Model V2c: *Response ~ Mediator*Strain*

Phenotypic pearson’s correlations and statistical significance of each correlation was obtained using the *Hmisc* package and are provided in Table 3.6.

3.2.8 Data Availability

All raw data for both the forced endurance and voluntary exercise experiment are publically available at https://phenome.jax.org. Forced endurance data set is Mouse Phenome Database (MPD): McMullan1. Voluntary exercise data sets are MPD: McMullan2 and McMullan3. In addition for both forced endurance and voluntary exercise experiments, tables containing general descriptive statistics for each trait are located in Table 3.1, 3.2 and 3.3.

3.3 Results

3.3.1 Forced endurance distance varied by 16.5 fold across 50 CC strains.

Forced endurance distance was measured in 232 mice across 50 CC strains. Genetic background had a significant effect on forced endurance distance ($p<2.2x10^{-6}$; Model F1a). The strain mean endurance distance in the CC population varied 16.5 fold ranging from 275m to 4,819m. Thirteen CC strains had a mean endurance distance above 2,371m with CC001/Unc having the greatest strain mean endurance distance of 4,819.33m. Eleven strains had a mean endurance distance below 1,038m. Furthermore, endurance distance across the CC population had a large population variance and variance within each CC strain differed based on CC strain (Figure 3.1, Table 3.1).

Baseline body mass and composition varied significantly by genetic background ($p<2.2x10^{-16}$). Strain means for baseline body mass ranged from 18.97g to 36.02g across the 50 CC strains with an overall mean of 27.75g. Baseline body fat percentage ranged from a strain mean of 0.90% to 31.96% with an overall mean of 14.70%. CC strain means for baseline lean mass percentage ranged from 63.42% to 91.32% with an overall mean of 78.08%. Genetic background contributed more to forced endurance distance than any baseline body mass or composition mediator alone (Model F3a vs F3b $p<2.2x10^{-6}$; Model F4a vs F4b $p<2.2x10^{-6}$; Model F5a vs F5b $p<2.2x10^{-6}$) signifying genetic background was driving forced endurance distance. Thus, baseline body mass, body fat percentage, and lean mass percentage did not have a significant effect on forced endurance distance (Figure 3.2 A-C, Table 3.1). Although, there were significant correlations between endurance distance and baseline body mass ($r=-0.372$, $p=4.87x10^{-09}$), body fat percentage ($r=-0.298$, $p=3.80x10^{-06}$) and lean mass percentage ($r=0.302$, $p=2.80x10^{-06}$). Age also varied across the forced endurance cohort (Figure 3.2 D, Table 3.1). There was a significant genetic background by age interaction on endurance distance (Model F2a vs F2b $p<2.2x10^{-6}$; Model F2c vs F2b $p=0.00816$). In addition, there was a significant negative correlation between age and endurance distance ($r=-0.269$, $p=3.3x10^{-05}$) indicating older mice had lower forced endurance distance.

3.3.2 The CC population is a source of potential model strains for adverse body composition responses induced by voluntary exercise

Genetic background had a significant effect on body fat percentage response at each time point (1 month $p=6.09x10^{-09}$; week1-2 $p=6.32x10^{-08}$; week3-4 $p=6.096x10^{-09}$). Body fat percentage response to one month of voluntary exercise ranged from ranged from -64.5% to 91.0% across CC strain means with an overall mean of -17.95%. Twelve CC strains (of 43) had an adverse fat response to a month of voluntary exercise. In the first two weeks of exercise (week1-2), strain means ranged from -67.8% to 50.5% with an overall

mean of -22.8%. Five of the 43 CC strains had an adverse fat response to week1-2 of exercise. However, in the second two weeks of exercise (week3-4), fat response ranged from -23.4% to 54.4% with an overall mean of 7.3%. Interestingly, 27 of the 43 CC strains had an adverse fat response to week3-4 of exercise. This suggests that as voluntary exercise is continued, there is a greater chance of an adverse fat response. CC063/Unc, CC070/TauUnc and CC027/GeniUnc were the only strains with consistent adverse fat responses means at week1-2, week3-4 and the month (Figure 3.3, Table 3.2).

Genetic background had a significant effect on lean mass percentage response at each time point (1 month $p=3.4x10^{-04}$; week1-2 $p=7.44x10^{-10}$; week3-4 $p=3.4x10^{-04}$). Strain means for lean mass percentage response to a month of voluntary exercise ranged from -7.6% to 23.1% across the 43 CC strains with an overall mean of 5.5%. Seven CC strains had an adverse lean mass response (loss of lean mass) to one month of exercise. Lean mass response ranged from -3.2% to 20.6% (overall mean 5.6%) for week1-2 and from -13.7% to 14.3% (overall mean of -0.1%) for week3-4. There were six CC strains with adverse lean mass response to week1-2 of exercise and 20 CC strains with adverse lean mass response to week3-4 of exercise, thus in most strains there was a pattern of lean muscle response that mirrored body fat response over the course of the exercise. CC063/Unc, CC070/TauUnc, CC027/GeniUnc and CC040/TauUnc were the only strains with consistent adverse lean mass responses means at week1-2, week3-4 and one month (Figure 3.4, Table 3.2).

Finally, genetic background had a significant effect on body mass response at each time point (1 month p=0.01388; week1-2 $p=1.15x10^{-08}$; week3-4 p=0.013). Body mass response was measured for voluntary exercise in CC strains and during one month of exercise ranged from -19.4% to 23.1% across the 43 CC strains with an overall mean of -5.5%. Eight CC strains had an adverse body mass response (gain of body mass) to one month of exercise. Body mass response ranged from -20.0% to 23.2% (overall mean of -

5.6%) for week1-2 and from -19.9% to 28.6% (overall mean of 0.6%) for week3-4. There were six CC strains with adverse body mass response to week1-2 of exercise and 27 CC strains with adverse body mass response to week3-4 of exercise. CC052/GeniUnc, CC070/TauUnc, CC027/GeniUnc and CC040/TauUnc were the only strains with consistent adverse body mass responses means at week1-2, week3-4 and one month (Figure 3.5, Table 3.2).

Both baseline body mass and composition and adjusted food intake varied across the 43 CC strains in the voluntary cohort (Table 3.2, Figures 3.6 and 3.7).

3.3.3 Voluntary physical activity levels vary extensively across 43 CC strains

Over a month of voluntary exercise, total distance ranged from 6.9 to 232.9 km (Figure 3.8 A), cumulative duration ranged from 2,191 to 13,829 (Figure 3.9 A) and average speed ranged from 2.7 to 19.7 m/min (Figure 3.10 A) for CC strain means.
Total distance ranged from 4.0 to 112.2 km for week1-2 (Figure 3.8 B) and 2.8 to 117.4 km for week3-4 (Figure 3.8 C), cumulative duration ranged from 1,279 to 6,887 for week1-2 (Figure 3.9 B) and 881.3 to 6,806 for week3-4 (Figure 3.9 C) and average speed ranged from 2.6 to 20.1 m/min for week1-2 (Figure 3.10 B) and 2.7 to 21.1 m/min for week3-4 (Figure 3.10 C) for CC strain means (Table 3.3). This suggests that while there is strain variation for the three measures of physical activity (Figure 3.8, Figure 3.9, Figure 3.10), the amount of exercise is consistent across the measured time points.

3.3.4 Genetic background has a more significant contribution to body mass and composition response to one month of voluntary exercise than physical activity levels, food intake or age alone

In the voluntary exercise cohort, all mediators (distance, duration, speed, and adjusted food intake) were under genetic control at each time point (1 month, week1-2, and week3-4) (Table 3.4). To determine if these potential mediators alone can explain any of the responses, linear models of the mediator alone were compared to additive models

(mediator + genetic background) for each body mass and composition response at every time point. Then, interaction models (mediator*genetic background) were compared to the additive models for each body mass and composition response to determine if any interactions had a significant effect on observed responses. Genetic background contributed more than each mediator (distance, duration, speed, adjusted food intake) alone for body mass response. There was a significant genetic background by mediator (distance, duration, speed, adjusted food intake) interaction on body mass response at week1-2. For both body fat percentage and lean mass percentage response at 1 month and week1-2, genetic background had a more significant contribution than each mediator alone (distance, duration, speed, adjusted food intake) alone. Whereas, genetic background did not have a significant additive effect on body fat percentage and lean mass percentage response at week3-4 compared to the mediators alone (distance, duration, speed, adjusted food intake). This observation indicates that genetic background does not add any additional information for body composition response during week3-4, although all mediators were genetically regulated during week3-4. Interestingly, there were significant genetic background by mediator (distance, duration, speed, and adjusted food intake) interactions (at some or all of the time points) on body fat percentage response. Genetic background contributed more to all responses at each time point (with the exception of body fat percentage and lean mass percentage response at week3-4) than age alone (Table 3.5). Overall, genetic background drives body mass and composition responses to voluntary exercise in the CC. During week3-4, genetic background did not add any more additional information than the genetically regulated mediators.

All phenotypic correlations and their significance are located in Table 3.6. There were no significant correlations between physical activity traits and body mass and composition responses with each time point with the exception of fat response. Fat response during week1-2 was significantly and negatively correlated with duration (week1-

2; r=-0.18, p=0.015) and speed (week1-2; r=-0.15, p=0.042). Fat response during week3-4 was significantly and positively correlated with duration (week3-4; r=0.17, p=0.02). Food intake was also significantly correlated with some body mass and composition responses (Table 3.6). Therefore, lower duration and speed during week1-2 resulted in greater fat gain; whereas, greater duration during week3-4 was associated with greater fat gain.

3.4 Discussion

3.4.1 CC027/GeniUnc as a potential model for voluntary exercise-induced adverse body composition response

As expected based on exercise studies performed in the pre-CC population (*66*) and studies of other traits performed in the CC (*60-63*), the CC population has a broad phenotypic range for exercise and responses to exercise along with the presence of extreme outlier strains. It is likely that the genetic diversity of the eight founder inbred strains of the CC and unique combinations of alleles makes the CC population a rich source of new models of human disease traits. This dataset in particular is useful for selecting potential CC strains for model development of different exercise related traits. CC027/GeniUnc is a potential model for adverse body fat and lean mass response induced by voluntary exercise (at all time points). CC027/GeniUnc had an overall strain mean body fat percentage response of 93.8% to one month of voluntary exercise (46.9% week1-2; 32.1% week3-4). Additionally, CC027/GeniUnc had the highest voluntary exercise distance over one month (232.9 km strain mean). Food intake and food composition are important for body composition regulation post exercise (*114*). One possible reason for the observed muscle mass breakdown and fat mass build up could be due to extensive aerobic activity combined with the lack of appropriate (amount and/or nutritional content) food intake (resulting in reduced resting metabolic rate). Other possible reasons include preferential metabolic breakdown of carbs and proteins over fats or altered cortisol levels. These reasons are all speculative and future studies will be necessary to determine the genetic

and molecular mechanism driving the adverse body composition responses in CC027/GeniUnc. Identifying genetic and molecular architecture of physiological adaptations to exercise will be valuable in deriving personalized exercise recommendations for individuals and particular populations.

This dataset is useful for selecting CC strains for model development for other exercise-related traits such as physical inactivity. For example, CC010/GeniUnc, CC028/GeniUnc and CC059/TauUnc had mean total distance less than 15km over a month of voluntary exercise. These CC strains could be useful for studying motivation to engage in exercise (via voluntary exercise) and genetic mechanisms regulating physical activity. Potential CC model strains will be important in future research for identifying the underlying genetic and biological mechanisms regulating physical activity and subsequent adaptations in order to improve health and physical activity (*115*).

While this dataset is useful for selecting potential model strains, it is important to note this dataset contains confounding caveats. All mice in the dataset were female, varied in older age and were retired breeders. Additionally, the voluntary exercise dataset does not have a control (no exercise) cohort to compare body mass and composition responses alongside. Thus, future studies may not replicate these findings in different sexes, ages and life histories. Still, this dataset provides valuable information on a large number of CC strains and can be used to identify potential model strains for a variety of exercise-related phenotypes.

3.4.2 Increased presence of adverse responders during week 3-4 of voluntary exercise

Interestingly, more strains with exercise-induced adverse body fat responses were observed during week3-4 measurements (27 strains) than week1-2 measurements (5 strains). There were significant correlations between fat response week3-4 and duration and food intake. It is unlikely that duration is driving the adverse fat responses during

week3-4 since duration at week1-2 and week3-4 are highly correlated (r=0.79). Food intake was also significantly and positively correlated with fat response week3-4 thus could potentially explain the increase in adverse responder strains observed (Table 3.6). It is common for individuals to exhibit compensatory behaviors, such as increased food intake especially increased carbohydrate intake, in response to exercise. Increased food intake in response to exercise can reduce health benefits of exercise (*16*). Previous studies in mice have observed positive correlation between food intake and running distance (*19*).

3.4.3 The presence and magnitude of individual variation differs across CC strains

As this dataset demonstrated there is extensive phenotypic range in exercise and exercise-related traits observed in the CC population. It is important to note that while the strain means reported for each phenotype are informative, it is vital to examine the individual measurements for each individual within a strain. We observed a range of individual variation for each trait measurement and CC strain (variance within strains is reported in Table 3.1-3.3). Some CC strains had very little variance while other CC strains had large variance. For example, CC028/GeniUnc had little within strain variance for total voluntary exercise distance over one month (var=5.05, n=4) and CC055/TauUnc had large within strain variation (var=9,276, n=8) (Figure 3.8 A, Table 3.3). Thus, individual variation was observed in genetically homogeneous CC strains and variance levels fluctuated across strains and traits. While this study demonstrates genetic background is important for each of these exercise and exercise-related traits, other potential factors are influencing the same traits. It is likely environmental differences and genetic background-by-environment interactions are driving some of the observed individual variation not accounted for in these studies. CC population is ideal for dissection of complex traits due to its reproducible nature and genetic diversity.

3.4.4 Forced endurance and voluntary exercise are two distinct but complementary traits

Both forced and voluntary exercise programs are used in rodents as a method to measure exercise performance and other exercise-related traits. In rodents, voluntary exercise by means of running wheel is believed to model voluntary exercise as observed in human populations. Voluntary exercise is a self-rewarding behavior and a complex trait that not only captures physical activity habits but also represents engagement in neural and physiological mechanisms required for the behavior. While both forced and voluntary depend on common variables (e.g. physiological systems, organ function), there are distinct factors to each program including: psychological desire to run, fear, pain perception, shock avoidance, etc (*116*), (*78*), (*51*). In this study there was a significant and positive correlation between CC strains mean forced endurance distance and voluntary exercise distance ($r=0.316$, $p=0.044$). However, some strains did not have correlated forced endurance and voluntary exercise distances. For example, CC011/Unc has a strain mean of ~150km of voluntary exercise over a month yet has a low strain mean for forced endurance distance (~440m). Thus this observation indicates there are distinct factors to the voluntary and forced programs for CC011/Unc. While CC011/Unc has the physical ability to engage in exercise, it is likely a unique factor distinct to forced endurance (treadmills) preventing the strain from achieving a greater distance. Whereas, CC059/TauUnc had a strain mean of 1,864m for forced endurance but low voluntary exercise levels over a month (6.9km). Most likely, CC059/TauUnc lacks the motivation to engage in voluntary exercise or doesn't find the behavior self-rewarding; while the strain has the ability to exercise as demonstrated by forced endurance (Figure 3.11).

3.5 Figures

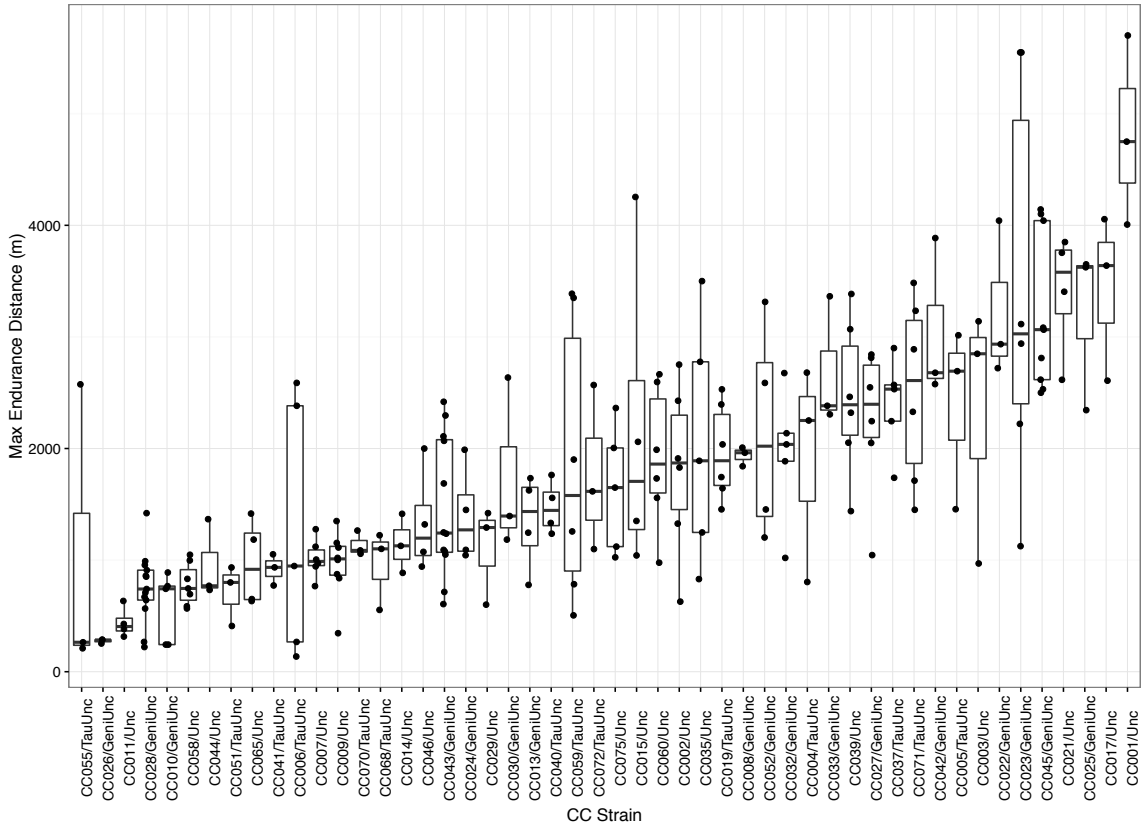


Figure 3.1. Forced Endurance Distance (m) across 50 CC strains.

Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for forced endurance distance.

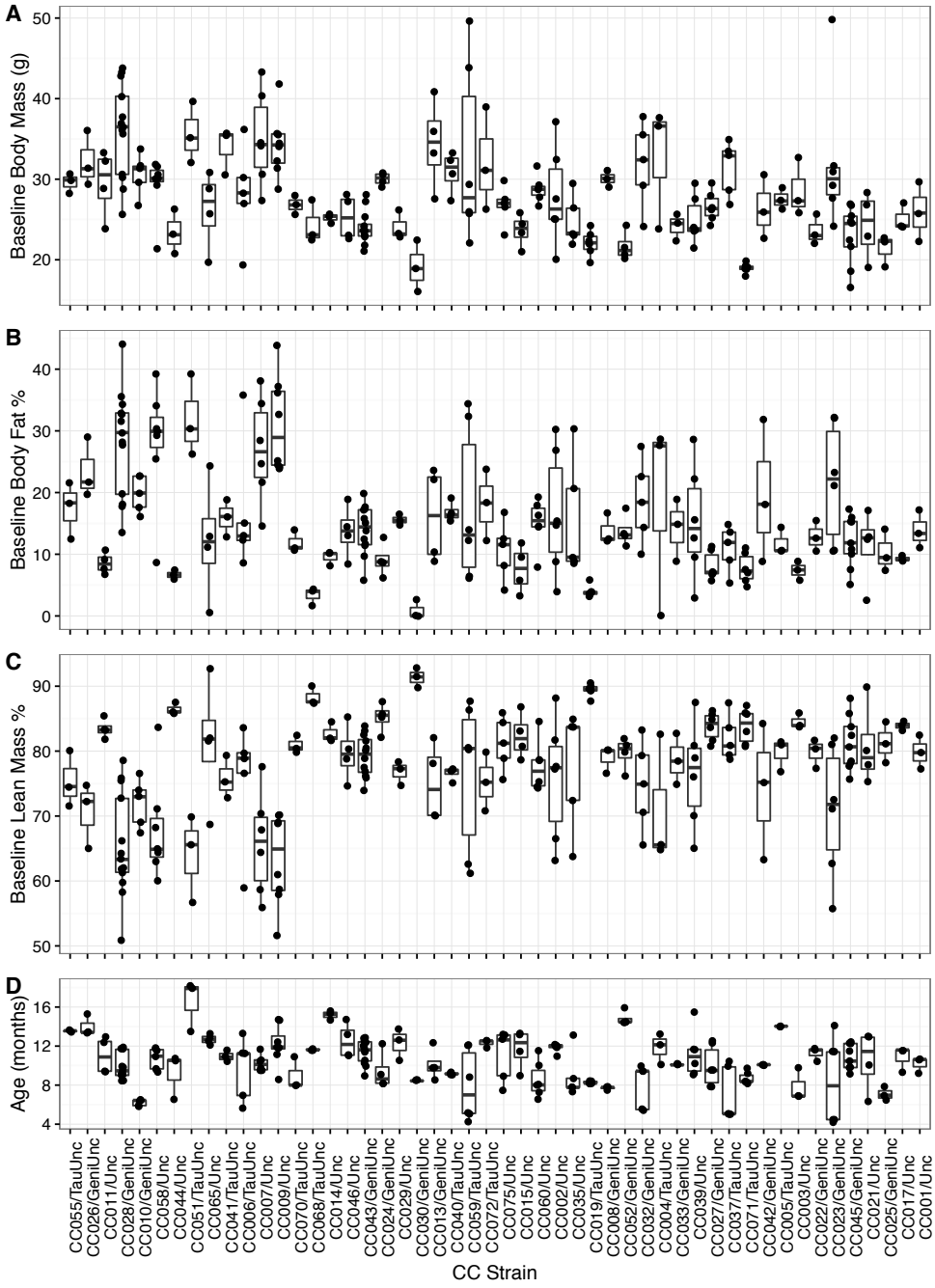


Figure 3.2. Baseline traits in the forced endurance cohort.

Baseline body mass (g) (A), baseline body fat percentage (B), baseline lean mass percentage (C) and age (m) at the start of the experiment (D) for all mice in the forced endurance cohort. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for forced endurance distance (Figure 3.1).

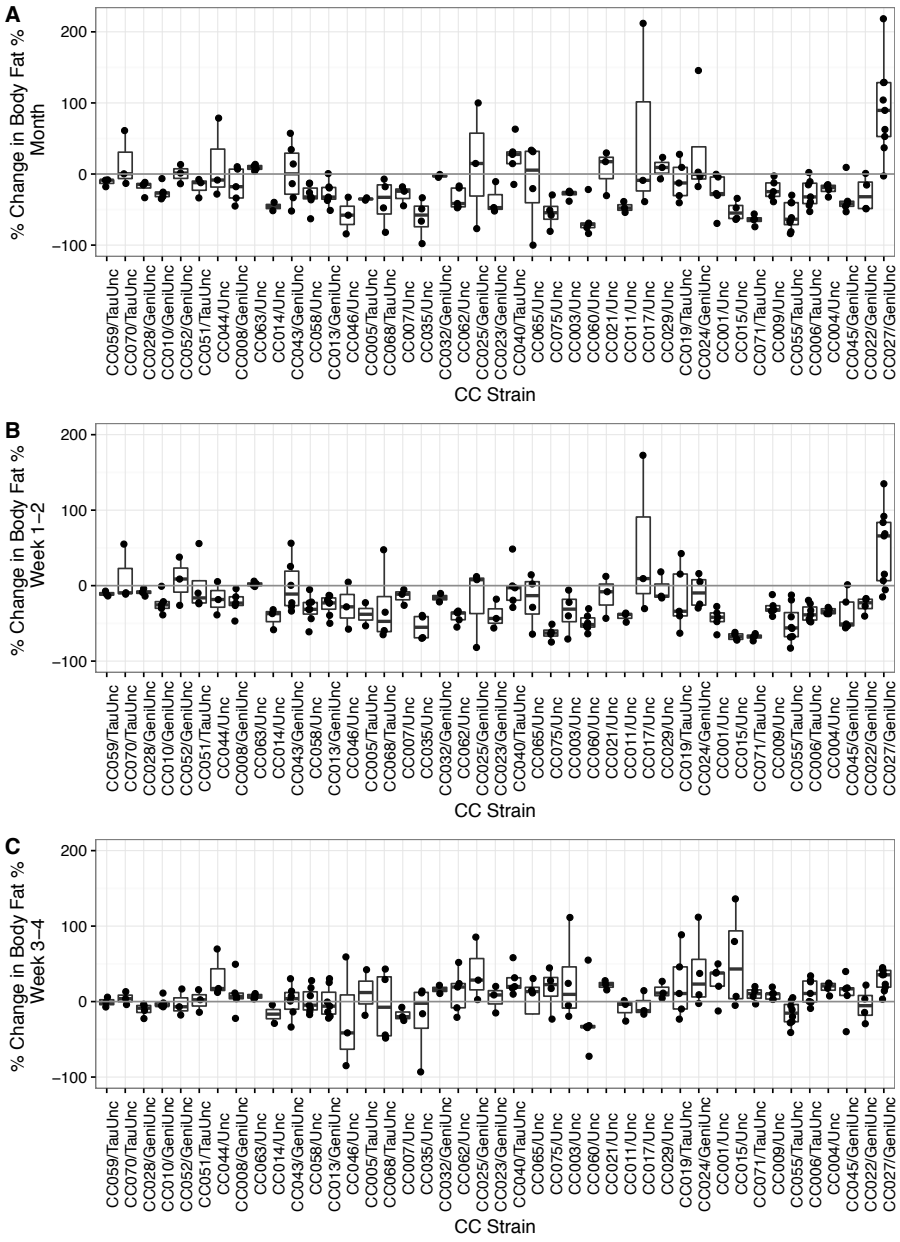


Figure 3.3. Body fat response to voluntary exercise in 43 CC strains.

Body fat response (%) to one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data above 0 represents an adverse fat response and data below 0 represents a standard response. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

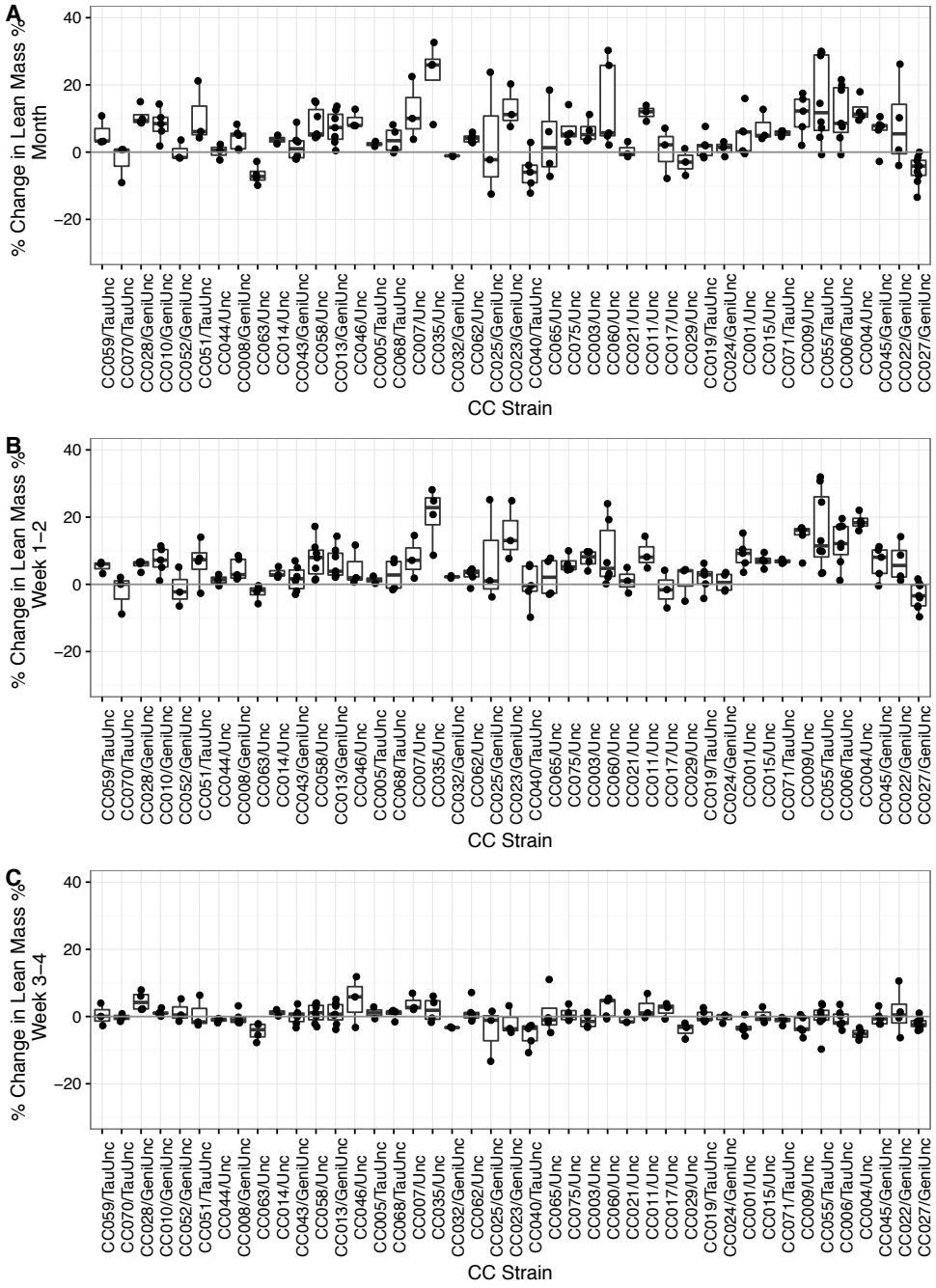


Figure 3.4. Lean mass response to voluntary exercise in 43 CC strains.

Lean mass response (%) to one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data above 0 represents a standard response and data below 0 represents an adverse lean mass response. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

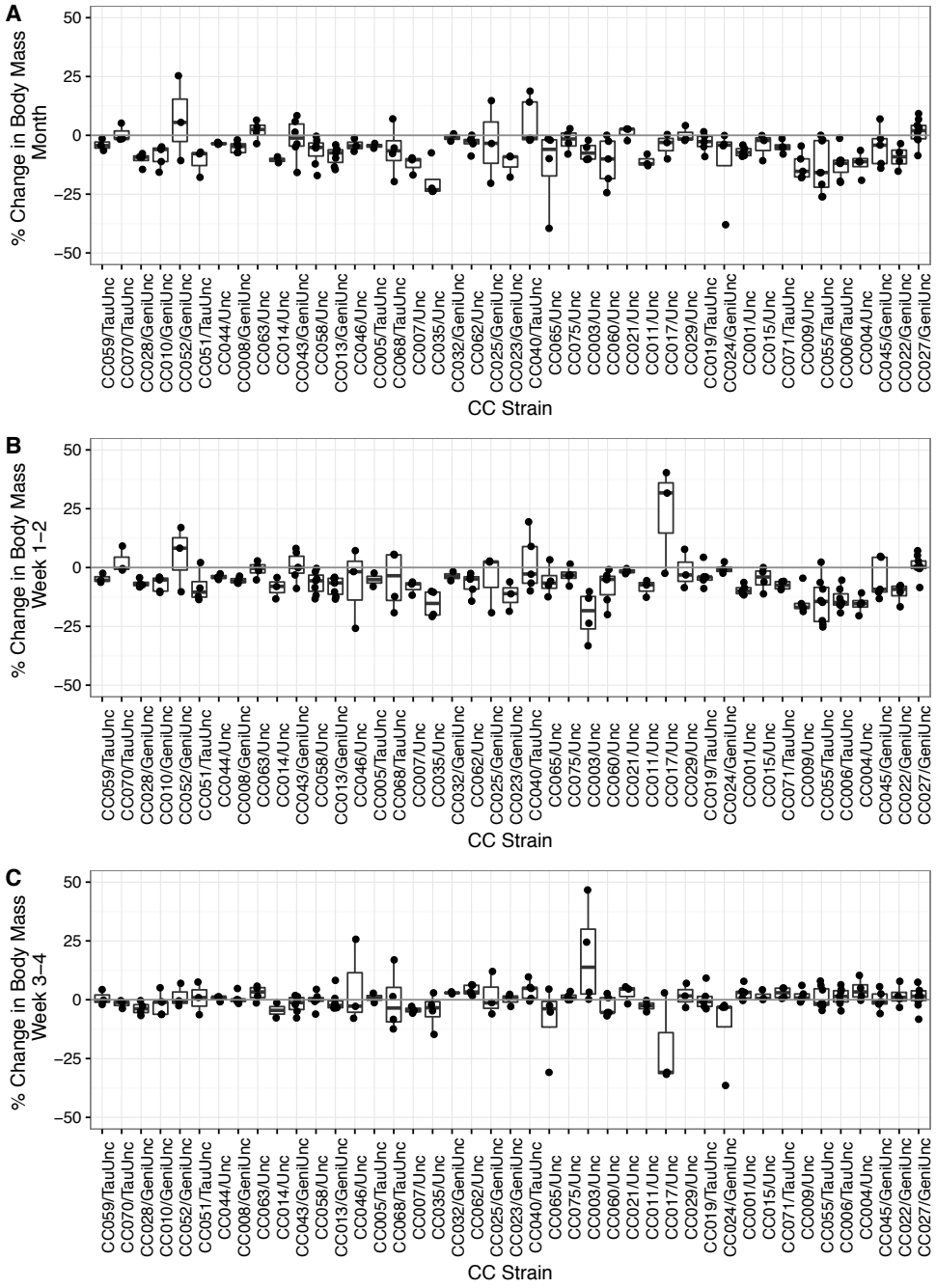


Figure 3.5. Body mass response to voluntary exercise in 43 CC strains.

Body mass response (%) to one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data above 0 represents an adverse body mass response and data below 0 represents a standard response. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

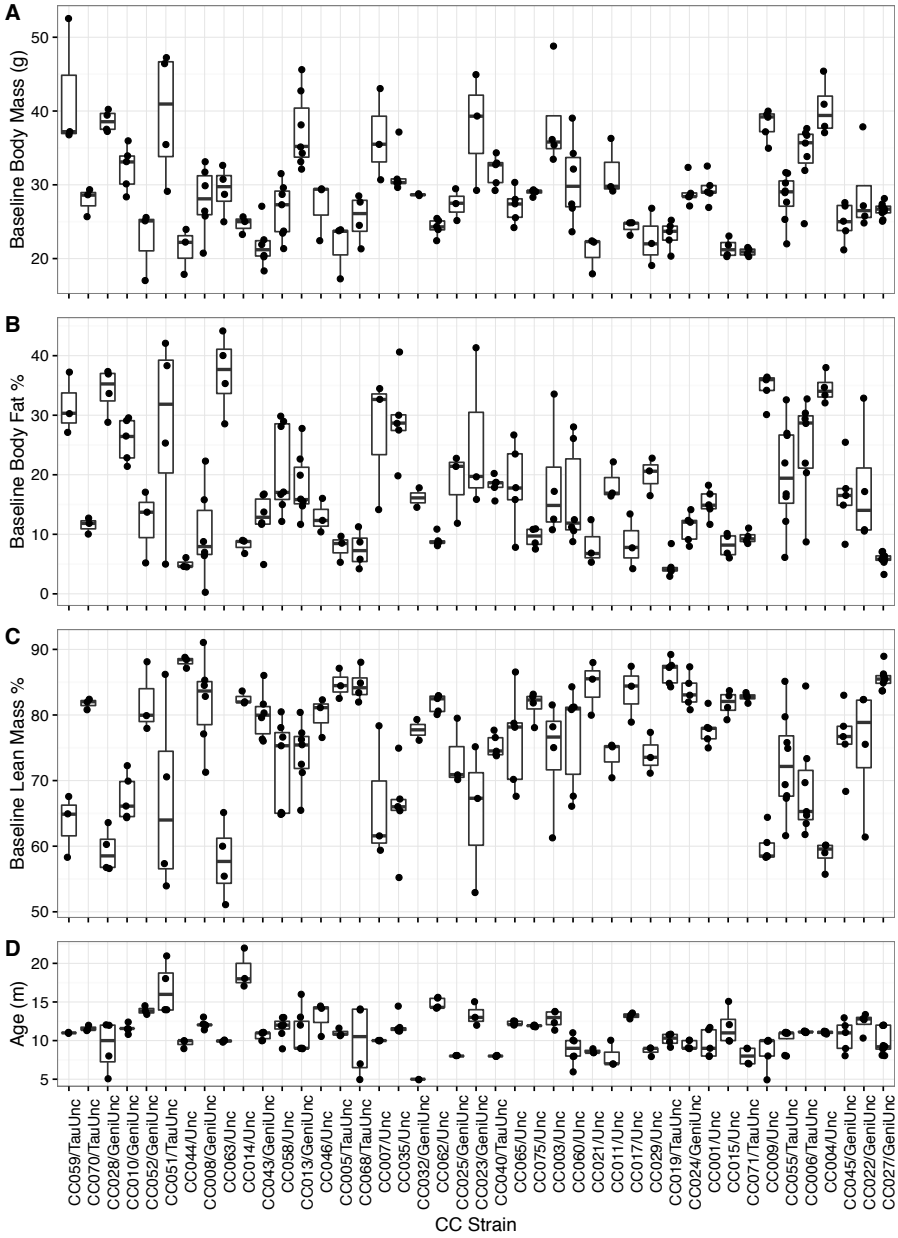


Figure 3.6. Baseline body mass and composition traits in the voluntary exercise cohort. Baseline body mass (g) (A), baseline body fat percentage (B), baseline lean mass percentage (C) prior to exercise in the voluntary exercise cohort. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

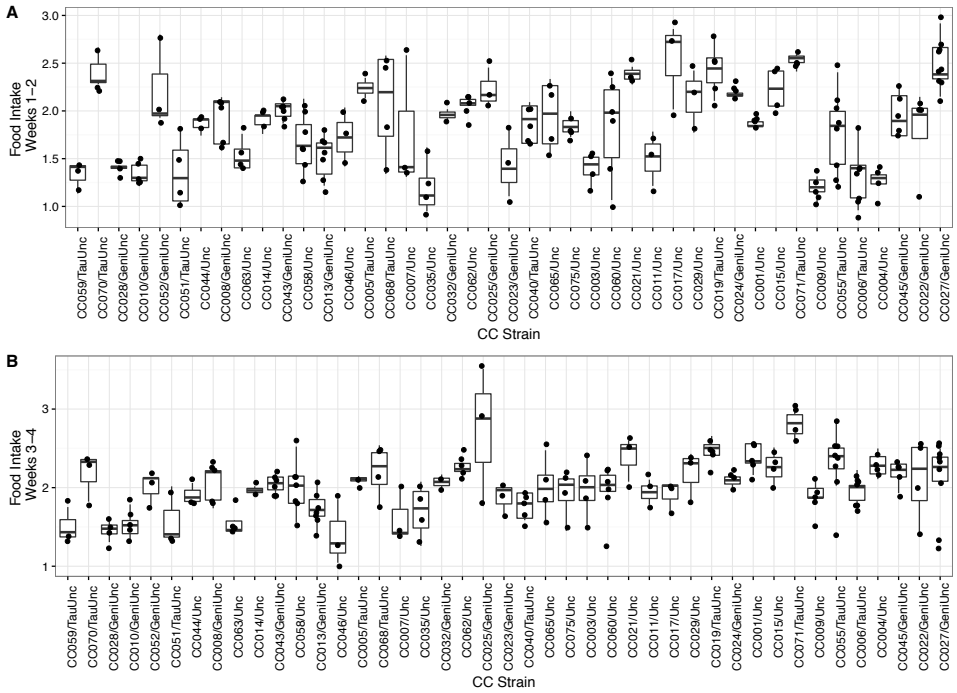


Figure 3.7. Adjusted food intake during voluntary exercise.

Adjusted food intake during week1-2 (A) and week3-4 (B) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

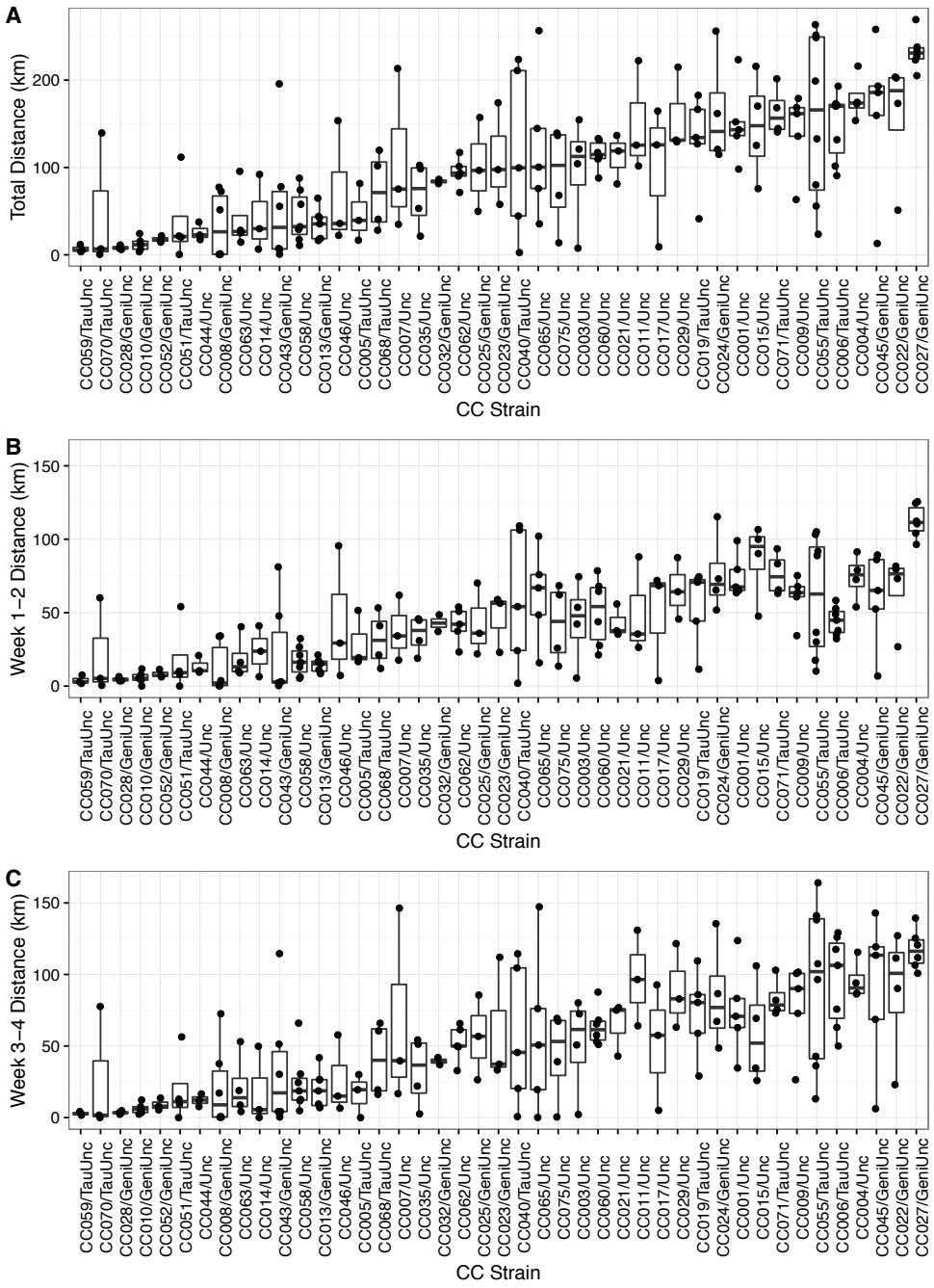


Figure 3.8. Voluntary exercise distance in 43 CC strains.

Total distance (km) during one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for total distance (A).

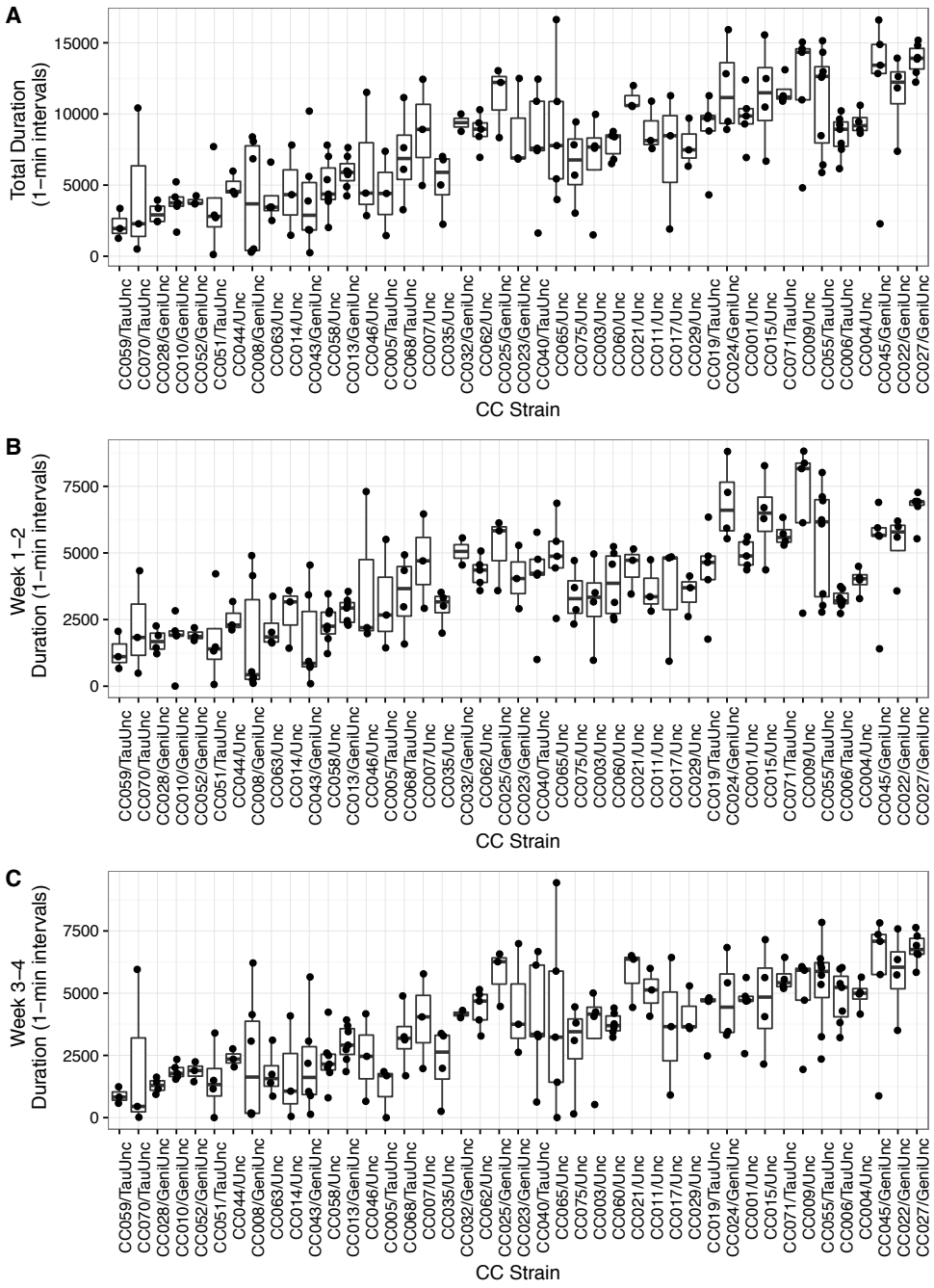


Figure 3.9. Voluntary exercise duration in 43 CC strains.

Total duration (1-min intervals) during one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

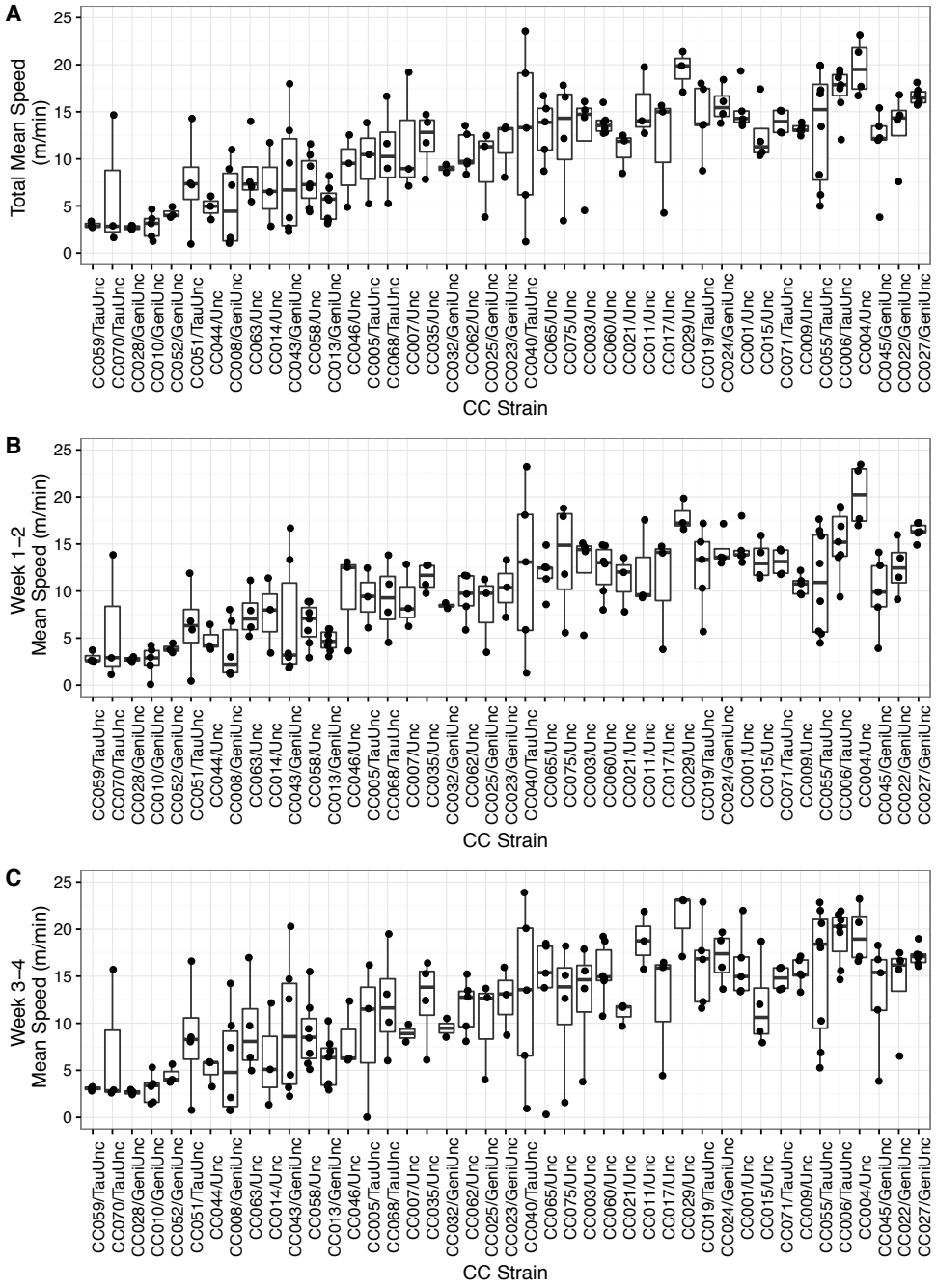


Figure 3.10. Voluntary exercise speed in 43 CC strains.

Mean speed (m/min) during one month (A), week1-2 (B), and week3-4 (C) of voluntary exercise. Each dot represents an individual mouse within a CC strain. Data is ordered by the CC strain median for total distance (Figure 3.8 A).

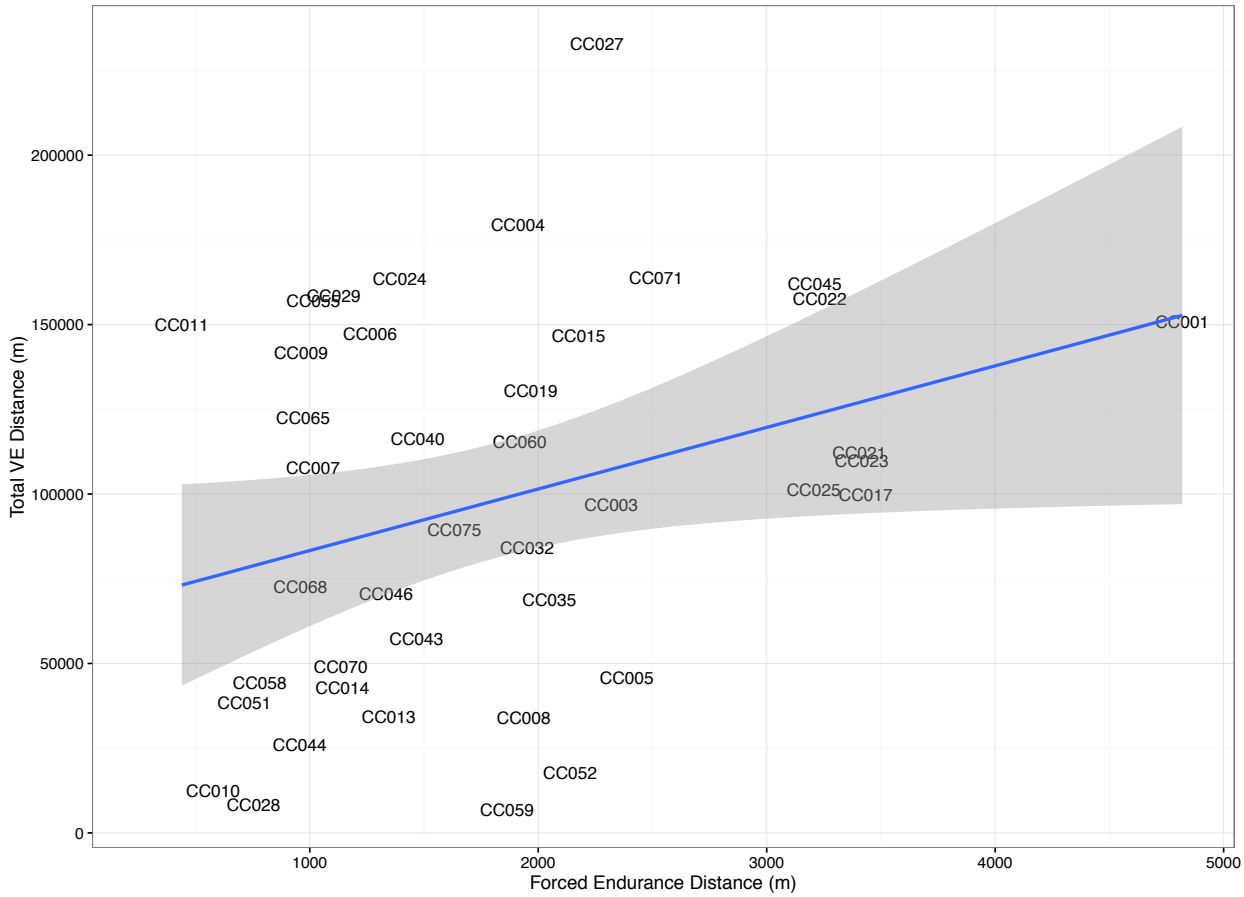


Figure 3.11. Correlation between voluntary exercise distance and forced endurance distance in 43 CC strains.

Total distance (m) is represented as a strain mean for both voluntary exercise (VE) and forced endurance. The blue line represents the best-fitted linear model between the two variables. The shaded grey region represents the 95% confidence interval.

3.6 Tables

Table 3.1. Descriptive statistics for CC strains in the forced endurance experiment.

CC Strain	n	Age (month)				Total Distance (m)				Baseline Body Mass (g)				Baseline Body Fat (%)				Baseline Lean Mass (%)			
		Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	3	10.1	0.7	0.9	0.5	4,819.3	721,014.3	849.1	490.2	25.9	13.7	3.7	2.1	13.9	9.1	3.0	1.7	79.8	6.8	2.6	1.5
CC002/Unc	6	11.9	0.2	0.4	0.2	1,812.7	582,051.9	762.9	311.5	27.9	36.6	6.1	2.5	16.7	103.2	10.2	4.1	75.7	88.2	9.4	3.8
CC003/Unc	3	7.9	2.9	1.7	1.0	2,319.7	1,387,370.3	1,177.9	680.0	28.6	12.9	3.6	2.1	7.4	2.3	1.5	0.9	84.5	1.5	1.2	0.7
CC004/TauUnc	3	11.9	2.5	1.6	0.9	1,911.7	966,204.3	983.0	567.5	32.7	59.2	7.7	4.4	18.7	263.9	16.2	9.4	71.0	101.1	10.1	5.8
CC005/TauUnc	3	14.0	0.0	0.0	0.0	2,388.0	677,389.0	823.0	475.2	27.5	1.9	1.4	0.8	11.8	4.9	2.2	1.3	79.8	6.5	2.5	1.5
CC006/TauUnc	5	9.7	10.4	3.2	1.4	1,264.3	1,342,779.1	1,158.8	518.2	28.2	36.7	6.1	2.7	16.9	116.9	10.8	4.8	75.6	93.0	9.6	4.3
CC007/Unc	6	10.3	0.7	0.8	0.3	1,014.2	29,631.8	172.1	70.3	35.0	35.6	6.0	2.4	27.0	73.6	8.6	3.5	65.8	63.0	7.9	3.2
CC008/GeniUnc	3	7.7	0.0	0.1	0.1	1,937.0	7,441.0	86.3	49.8	30.1	0.9	1.0	0.5	13.8	6.5	2.6	1.5	79.0	4.3	2.1	1.2
CC009/Unc	8	12.2	4.0	2.0	0.7	962.0	88,426.0	297.4	105.1	34.3	15.1	3.9	1.4	31.0	57.9	7.6	2.7	63.4	49.4	7.0	2.5
CC010/GeniUnc	5	6.2	0.1	0.3	0.1	577.0	96,028.0	309.9	138.6	30.6	6.8	2.6	1.2	19.8	8.6	2.9	1.3	72.0	14.1	3.8	1.7
CC011/Unc	4	11.0	3.5	1.9	0.9	439.8	18,922.9	137.6	68.8	29.6	18.2	4.3	2.1	8.6	3.0	1.7	0.9	83.4	2.2	1.5	0.7
CC013/GeniUnc	4	10.1	2.7	1.6	0.8	1,345.8	187,002.9	432.4	216.2	34.4	31.3	5.6	2.8	16.2	58.9	7.7	3.8	75.1	35.6	6.0	3.0
CC014/Unc	3	15.2	0.3	0.5	0.3	1,143.0	70,129.0	264.8	152.9	25.2	0.4	0.6	0.4	9.5	1.5	1.2	0.7	82.7	2.5	1.6	0.9
CC015/Unc	4	11.7	4.1	2.0	1.0	2,176.8	2,099,372.9	1,448.9	724.5	23.7	4.2	2.1	1.0	7.6	14.7	3.8	1.9	82.3	12.2	3.5	1.7
CC017/Unc	3	10.8	1.8	1.3	0.8	3,434.0	554,971.0	745.0	430.1	25.1	3.0	1.7	1.0	9.3	0.2	0.5	0.3	83.9	0.4	0.7	0.4
CC019/TauUnc	6	8.3	0.0	0.1	0.1	1,967.5	184,250.7	429.2	175.2	22.0	2.5	1.6	0.7	4.0	0.9	0.9	0.4	89.4	0.8	0.9	0.4
CC021/Unc	4	10.6	9.6	3.1	1.6	3,406.3	314,113.6	560.5	280.2	24.3	17.9	4.2	2.1	11.2	38.1	6.2	3.1	80.8	41.1	6.4	3.2
CC022/GeniUnc	3	11.3	0.5	0.7	0.4	3,232.3	503,226.3	709.4	409.6	23.6	3.5	1.9	1.1	12.9	6.5	2.5	1.5	79.8	4.8	2.2	1.3
CC023/GeniUnc	6	8.3	19.6	4.4	1.8	3,416.5	3,218,651.9	1,794.1	732.4	32.2	81.0	9.0	3.7	21.6	94.0	9.7	4.0	70.9	105.6	10.3	4.2
CC024/GeniUnc	4	9.4	3.7	1.9	1.0	1,393.8	190,288.3	436.2	218.1	30.0	0.6	0.8	0.4	9.1	7.4	2.7	1.4	85.2	5.1	2.3	1.1
CC025/GeniUnc	3	7.1	0.5	0.7	0.4	3,206.0	557,452.0	746.6	431.1	21.4	3.9	2.0	1.1	10.3	11.9	3.5	2.0	81.3	9.6	3.1	1.8
CC026/GeniUnc	3	14.0	1.2	1.1	0.6	275.0	378.3	19.4	11.2	32.2	11.8	3.4	2.0	23.5	24.2	4.9	2.8	70.7	26.0	5.1	2.9
CC027/GeniUnc	6	9.9	4.2	2.1	0.8	2,257.2	449,595.8	670.5	273.7	26.6	3.6	1.9	0.8	8.1	5.3	2.3	0.9	83.8	4.8	2.2	0.9
CC028/GeniUnc	13	9.9	1.8	1.3	0.4	754.2	97,080.0	311.6	86.4	36.0	33.5	5.8	1.6	28.2	74.9	8.7	2.4	65.4	64.8	8.0	2.2
CC029/Unc	3	12.3	2.8	1.7	1.0	1,104.7	194,420.3	440.9	254.6	24.1	3.3	1.8	1.1	15.6	0.7	0.8	0.5	76.8	3.3	1.8	1.0

CC030/GeniUnc	3	8.4	0.0	0.0	0.0	1,738.3	615,484.3	784.5	452.9	19.1	10.3	3.2	1.9	0.9	2.5	1.6	0.9	91.3	2.4	1.5	0.9
CC032/GeniUnc	5	8.0	5.1	2.3	1.0	1,951.2	359,650.7	599.7	268.2	31.8	28.5	5.3	2.4	18.6	47.1	6.9	3.1	74.7	49.1	7.0	3.1
CC033/GeniUnc	3	10.1	0.0	0.1	0.0	2,684.3	347,942.3	589.9	340.6	24.1	2.8	1.7	1.0	14.2	25.2	5.0	2.9	78.7	15.7	4.0	2.3
CC035/Unc	5	9.0	5.8	2.4	1.1	2,049.0	1,197,057.0	1,094.1	489.3	24.8	9.2	3.0	1.4	15.6	93.8	9.7	4.3	77.7	86.9	9.3	4.2
CC037/TauUnc	5	7.1	8.0	2.8	1.3	2,397.0	189,637.0	435.5	194.7	31.4	11.8	3.4	1.5	11.0	14.2	3.8	1.7	82.0	12.7	3.6	1.6
CC039/Unc	6	11.2	5.8	2.4	1.0	2,454.8	490,916.2	700.7	286.0	25.0	9.0	3.0	1.2	15.3	83.3	9.1	3.7	76.4	63.9	8.0	3.3
CC040/TauUnc	4	9.2	0.0	0.1	0.1	1,472.8	55,540.3	235.7	117.8	30.9	6.6	2.6	1.3	16.8	2.5	1.6	0.8	76.6	1.1	1.0	0.5
CC041/TauUnc	3	11.0	0.4	0.6	0.3	920.0	19,629.0	140.1	80.9	33.9	8.3	2.9	1.7	15.9	8.9	3.0	1.7	75.8	10.9	3.3	1.9
CC042/GeniUnc	3	10.1	0.0	0.0	0.0	3,047.3	530,122.3	728.1	420.4	26.4	15.8	4.0	2.3	19.6	134.0	11.6	6.7	74.3	111.1	10.5	6.1
CC043/GeniUnc	12	11.4	1.6	1.2	0.4	1,467.1	390,101.2	624.6	180.3	24.0	4.1	2.0	0.6	14.0	15.9	4.0	1.2	79.2	10.1	3.2	0.9
CC044/Unc	3	9.2	5.6	2.4	1.4	956.7	126,622.3	355.8	205.4	23.4	7.6	2.8	1.6	6.7	0.5	0.7	0.4	86.4	0.8	0.9	0.5
CC045/GeniUnc	9	10.8	1.7	1.3	0.4	3,210.2	483,484.2	695.3	231.8	23.0	13.0	3.6	1.2	12.1	17.2	4.1	1.4	81.2	17.0	4.1	1.4
CC046/Unc	4	12.5	3.2	1.8	0.9	1,334.0	221,672.0	470.8	235.4	25.3	8.0	2.8	1.4	13.7	18.2	4.3	2.1	79.7	19.3	4.4	2.2
CC051/TauUnc	3	16.5	7.2	2.7	1.5	714.7	74,105.3	272.2	157.2	35.6	14.7	3.8	2.2	32.0	44.2	6.6	3.8	64.1	44.7	6.7	3.9
CC052/GeniUnc	4	14.8	0.5	0.7	0.4	2,139.8	975,848.3	987.9	493.9	21.7	3.2	1.8	0.9	13.7	6.7	2.6	1.3	79.7	6.1	2.5	1.2
CC055/TauUnc	3	13.5	0.0	0.1	0.0	1,016.3	1,822,966.2	1,350.2	779.5	29.6	1.5	1.2	0.7	17.5	20.8	4.6	2.6	75.4	18.6	4.3	2.5
CC058/Unc	7	10.7	1.2	1.1	0.4	781.6	35,497.6	188.4	71.2	29.3	13.2	3.6	1.4	28.1	92.6	9.6	3.6	67.9	60.9	7.8	2.9
CC059/TauUnc	6	7.9	13.1	3.6	1.5	1,864.0	1,582,164.8	1,257.8	513.5	32.8	126.0	11.2	4.6	17.6	160.0	12.6	5.2	76.4	135.3	11.6	4.7
CC060/Unc	6	8.6	3.3	1.8	0.7	1,919.5	414,479.5	643.8	262.8	28.8	2.8	1.7	0.7	15.0	15.8	4.0	1.6	77.6	15.2	3.9	1.6
CC065/Unc	4	12.7	0.2	0.5	0.2	971.3	153,642.9	392.0	196.0	26.3	23.8	4.9	2.4	12.3	95.3	9.8	4.9	81.3	96.7	9.8	4.9
CC068/TauUnc	3	11.7	0.0	0.1	0.1	959.3	126,889.6	356.2	205.7	24.3	7.3	2.7	1.6	3.3	2.0	1.4	0.8	88.4	2.3	1.5	0.9
CC070/TauUnc	3	9.0	2.9	1.7	1.0	1,136.3	12,641.3	112.4	64.9	26.8	1.3	1.2	0.7	11.9	3.2	1.8	1.0	81.0	1.9	1.4	0.8
CC071/TauUnc	6	8.7	0.4	0.6	0.2	2,516.5	681,457.1	825.5	337.0	19.0	0.4	0.7	0.3	7.7	6.3	2.5	1.0	83.9	7.1	2.7	1.1
CC072/TauUnc	3	12.3	0.2	0.4	0.2	1,761.3	556,066.3	745.7	430.5	32.1	40.4	6.4	3.7	18.1	33.5	5.8	3.3	75.2	20.3	4.5	2.6
CC075/Unc	5	11.1	7.1	2.7	1.2	1,632.8	325,845.2	570.8	255.3	26.8	6.0	2.5	1.1	10.7	22.5	4.7	2.1	81.2	17.2	4.1	1.9

Table 3.2. Descriptive statistics for responses to voluntary exercise in CC strains.

CC Strain	n	Body Mass Response (%) - Month				Body Mass Response (%) - Week1-2				Body Mass Response (%) - Week3-4			
		Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	5	-6.91	4.13	2.03	0.91	-9.56	4.63	2.15	0.96	2.98	10.20	3.19	1.43
CC003/Unc	4	-6.86	15.75	3.97	1.98	-20.05	111.13	10.54	5.27	18.62	465.23	21.57	10.78
CC004/Unc	4	-11.99	28.14	5.30	2.65	-15.51	15.65	3.96	1.98	4.18	21.83	4.67	2.34
CC005/TauUnc	3	-4.48	2.03	1.43	0.82	-5.16	16.27	4.03	2.33	0.78	7.74	2.78	1.61
CC006/TauUnc	7	-12.23	39.60	6.29	2.38	-13.37	22.00	4.69	1.77	1.28	14.11	3.76	1.42
CC007/Unc	3	-12.28	16.23	4.03	2.33	-8.33	9.36	3.06	1.77	-4.34	2.29	1.51	0.87
CC008/GeniUnc	6	-5.00	5.73	2.39	0.98	-5.46	1.65	1.28	0.52	0.50	6.40	2.53	1.03
CC009/Unc	5	-13.12	33.18	5.76	2.58	-14.54	32.53	5.70	2.55	1.69	7.87	2.81	1.25
CC010/GeniUnc	5	-8.64	21.43	4.63	2.07	-6.89	10.04	3.17	1.42	-1.85	22.05	4.70	2.10
CC011/Unc	3	-10.98	7.27	2.70	1.56	-8.52	13.83	3.72	2.15	-2.64	5.66	2.38	1.37
CC013/GeniUnc	7	-8.78	15.40	3.92	1.48	-7.98	15.80	3.97	1.50	-0.79	17.81	4.22	1.60
CC014/Unc	3	-10.44	3.06	1.75	1.01	-8.55	20.85	4.57	2.64	-4.51	20.60	4.54	2.62
CC015/Unc	4	23.13	3,031.11	55.06	27.53	-4.82	24.64	4.96	2.48	28.57	2,918.10	54.02	27.01
CC017/Unc	3	-4.22	28.33	5.32	3.07	23.19	510.05	22.58	13.04	-19.89	389.56	19.74	11.40
CC019/TauUnc	5	-3.08	16.52	4.06	1.82	-3.54	23.87	4.89	2.18	0.62	27.40	5.23	2.34
CC021/Unc	3	1.08	8.22	2.87	1.66	-1.60	1.26	1.12	0.65	2.75	15.75	3.97	2.29
CC022/GeniUnc	4	-9.26	25.70	5.07	2.53	-10.70	17.13	4.14	2.07	1.65	20.91	4.57	2.29
CC023/GeniUnc	3	-11.94	25.85	5.08	2.94	-11.95	39.03	6.25	3.61	0.09	7.22	2.69	1.55
CC024/GeniUnc	5	-11.61	315.30	17.76	7.94	-0.53	4.51	2.12	0.95	-11.32	282.20	16.80	7.51
CC025/GeniUnc	3	-3.01	308.20	17.56	10.14	-4.72	158.85	12.60	7.28	1.58	87.66	9.36	5.41
CC027/GeniUnc	9	1.63	27.52	5.25	1.75	0.78	19.34	4.40	1.47	0.90	19.53	4.42	1.47
CC028/GeniUnc	4	-10.19	8.74	2.96	1.48	-6.76	2.98	1.73	0.86	-3.68	7.87	2.81	1.40
CC029/Unc	3	0.09	12.11	3.48	2.01	-1.34	68.98	8.31	4.80	1.73	26.60	5.16	2.98
CC032/GeniUnc	2	-0.88	4.96	2.23	1.58	-3.68	7.52	2.74	1.94	2.91	0.38	0.62	0.44
CC035/Unc	5	-19.36	63.54	7.97	3.56	-15.38	33.87	5.82	2.60	-4.64	55.80	7.47	3.34
CC040/TauUnc	5	5.57	102.00	10.10	4.52	1.81	147.95	12.16	5.44	3.96	16.75	4.09	1.83
CC043/GeniUnc	6	-1.45	76.77	8.76	3.58	0.42	39.01	6.25	2.55	-1.98	13.79	3.71	1.52
CC044/Unc	3	-3.48	0.26	0.51	0.29	-3.97	1.38	1.18	0.68	0.52	1.57	1.25	0.72
CC045/GeniUnc	5	-4.94	71.04	8.43	3.77	-4.76	74.13	8.61	3.85	-0.10	18.73	4.33	1.94
CC046/Unc	3	-4.21	7.47	2.73	1.58	-6.84	290.89	17.06	9.85	5.02	327.21	18.09	10.44
CC051/TauUnc	4	-10.92	36.20	6.02	3.01	-8.11	50.23	7.09	3.54	0.69	48.27	6.95	3.47
CC052/GeniUnc	3	6.68	325.97	18.05	10.42	4.98	195.88	14.00	8.08	1.35	25.32	5.03	2.91
CC055/TauUnc	8	-13.61	117.36	10.83	3.83	-14.25	92.94	9.64	3.41	0.70	21.65	4.65	1.65
CC058/Unc	7	-6.69	34.82	5.90	2.23	-6.56	23.42	4.84	1.83	-0.16	9.83	3.13	1.18
CC059/TauUnc	3	-4.06	6.65	2.58	1.49	-4.70	4.01	2.00	1.16	0.70	10.27	3.21	1.85
CC060/Unc	6	-11.06	106.99	10.34	4.22	-8.00	53.48	7.31	2.99	-2.87	18.38	4.29	1.75
CC062/Unc	5	-3.30	11.06	3.33	1.49	-6.94	22.79	4.77	2.13	3.99	4.58	2.14	0.96
CC063/Unc	4	1.95	17.97	4.24	2.12	-0.77	11.49	3.39	1.69	2.76	10.67	3.27	1.63
CC065/Unc	5	-13.20	320.99	17.92	8.01	-5.61	43.60	6.60	2.95	-8.49	238.06	15.43	6.90
CC068/TauUnc	4	-6.44	119.68	10.94	5.47	-5.12	157.33	12.54	6.27	-0.63	170.80	13.07	6.53
CC070/TauUnc	3	0.72	14.51	3.81	2.20	2.59	33.00	5.74	3.32	-1.76	3.14	1.77	1.02
CC071/TauUnc	4	-4.93	7.40	2.72	1.36	-7.56	3.56	1.89	0.94	2.86	5.81	2.41	1.21
CC075/Unc	4	-2.04	21.88	4.68	2.34	-3.25	14.49	3.81	1.90	1.23	2.79	1.67	0.83

CC Strain	Body Fat % Response (%) - Month				Body Fat % Response (%) - Week1-2				Body Fat % Response (%) - Week3-4			
	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	-26.33	759.67	27.56	12.33	-43.71	197.99	14.07	6.29	26.85	592.99	24.35	10.89
CC003/Unc	-28.54	42.68	6.53	3.27	-34.75	773.14	27.81	13.90	27.78	3,435.93	58.62	29.31
CC004/Unc	-21.38	63.71	7.98	3.99	-33.62	17.95	4.24	2.12	18.34	61.87	7.87	3.93
CC005/TauUnc	-35.13	4.92	2.22	1.28	-37.94	469.71	21.67	12.51	11.98	1,821.71	42.68	24.64
CC006/TauUnc	-27.41	397.35	19.93	7.53	-36.27	129.50	11.38	4.30	12.79	282.27	16.80	6.35
CC007/Unc	-28.99	193.52	13.91	8.03	-14.24	115.29	10.74	6.20	-17.57	81.25	9.01	5.20
CC008/GeniUnc	-15.88	592.33	24.34	9.94	-23.02	253.68	15.93	6.50	9.85	662.33	25.74	10.51
CC009/Unc	-22.10	211.57	14.55	6.50	-28.98	116.23	10.78	4.82	9.30	44.67	6.68	2.99
CC010/GeniUnc	-25.12	122.26	11.06	4.94	-23.24	199.71	14.13	6.32	-1.76	56.53	7.52	3.36
CC011/Unc	-46.85	59.99	7.75	4.47	-40.95	42.81	6.54	3.78	-9.42	206.31	14.36	8.29
CC013/GeniUnc	-27.37	275.31	16.59	6.27	-25.82	190.43	13.80	5.22	-0.85	440.23	20.98	7.93
CC014/Unc	-45.71	67.94	8.24	4.76	-42.46	196.52	14.02	8.09	-16.62	299.63	17.31	9.99
CC015/Unc	-51.95	193.16	13.90	6.95	-67.36	24.04	4.90	2.45	54.36	4,365.43	66.07	33.04
CC017/Unc	54.76	18,754.15	136.95	79.07	50.50	11,582.59	107.62	62.14	-4.73	280.77	16.76	9.67
CC019/TauUnc	-9.27	785.78	28.03	12.54	-15.86	1,880.91	43.37	19.40	22.42	2,046.33	45.24	20.23
CC021/Unc	5.55	1,010.65	31.79	18.35	-13.04	789.83	28.10	16.23	21.95	37.61	6.13	3.54
CC022/GeniUnc	-27.98	617.59	24.85	12.43	-25.74	115.05	10.73	5.36	-4.58	490.14	22.14	11.07
CC023/GeniUnc	-36.66	508.42	22.55	13.02	-39.19	377.43	19.43	11.22	4.65	322.02	17.95	10.36
CC024/GeniUnc	31.72	5,824.93	76.32	34.13	-8.29	496.14	22.27	9.96	38.92	2,637.82	51.36	22.97
CC025/GeniUnc	12.70	7,818.27	88.42	51.05	-20.73	2,811.84	53.03	30.62	38.87	1,799.88	42.42	24.49
CC027/GeniUnc	90.99	4,152.44	64.44	21.48	49.61	2,638.56	51.37	17.12	28.91	214.28	14.64	4.88
CC028/GeniUnc	-18.57	97.84	9.89	4.95	-8.78	17.02	4.13	2.06	-10.89	71.33	8.45	4.22
CC029/Unc	8.58	229.64	15.15	8.75	-4.00	372.09	19.29	11.14	14.17	133.10	11.54	6.66
CC032/GeniUnc	-2.69	8.03	2.83	2.00	-15.96	62.83	7.93	5.60	16.15	57.49	7.58	5.36
CC035/Unc	-61.67	764.67	27.65	12.37	-54.91	278.24	16.68	7.46	-20.91	2,509.00	50.09	22.40
CC040/TauUnc	24.32	792.45	28.15	12.59	-0.58	889.99	29.83	13.34	27.48	355.07	18.84	8.43
CC043/GeniUnc	1.11	1,732.19	41.62	16.99	-0.36	1,244.99	35.28	14.40	0.73	494.80	22.24	9.08
CC044/Unc	13.96	3,230.85	56.84	32.82	-17.31	487.62	22.08	12.75	33.02	1,010.84	31.79	18.36
CC045/GeniUnc	-33.85	613.92	24.78	11.08	-36.13	624.68	24.99	11.18	8.63	873.96	29.56	13.22
CC046/Unc	-58.25	661.59	25.72	14.85	-27.06	971.65	31.17	18.00	-22.40	5,454.99	73.86	42.64
CC051/TauUnc	-17.83	190.57	13.80	6.90	-0.22	1,429.84	37.81	18.91	1.31	224.60	14.99	7.49
CC052/GeniUnc	0.35	183.67	13.55	7.82	6.86	1,023.92	32.00	18.47	-2.62	311.00	17.64	10.18
CC055/TauUnc	-58.81	398.72	19.97	7.06	-50.41	605.26	24.60	8.70	-15.59	255.61	15.99	5.65
CC058/Unc	-31.03	287.16	16.95	6.40	-31.11	314.80	17.74	6.71	1.36	287.72	16.96	6.41
CC059/TauUnc	-11.54	29.38	5.42	3.13	-10.79	10.16	3.19	1.84	-0.78	42.51	6.52	3.76
CC060/Unc	-64.13	590.68	24.30	9.92	-48.90	139.42	11.81	4.82	-23.43	2,211.86	47.03	19.20
CC062/Unc	-34.34	221.48	14.88	6.66	-41.06	79.47	8.91	3.99	13.22	815.63	28.56	12.77
CC063/Unc	9.12	17.76	4.21	2.11	1.97	9.37	3.06	1.53	7.03	10.97	3.31	1.66
CC065/Unc	-13.92	3,917.77	62.59	27.99	-19.14	1,236.40	35.16	15.73	-10.75	3,608.98	60.07	26.87
CC068/TauUnc	-38.69	1,124.03	33.53	16.76	-28.08	2,705.30	52.01	26.01	-5.05	2,304.84	48.01	24.00
CC070/TauUnc	16.11	1,563.72	39.54	22.83	11.41	1,429.90	37.81	21.83	4.35	73.54	8.58	4.95
CC071/TauUnc	-64.50	53.87	7.34	3.67	-67.81	16.67	4.08	2.04	9.43	98.55	9.93	4.96
CC075/Unc	-54.72	446.99	21.14	10.57	-62.90	94.35	9.71	4.86	16.56	831.78	28.84	14.42

CC Strain	Lean Mass % Response (%) - Month				Lean Mass % Response (%) - Week1-2				Lean Mass % Response (%) - Week3-4			
	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	5.63	42.90	6.55	2.93	8.99	19.35	4.40	1.97	-3.15	5.33	2.31	1.03
CC003/Unc	6.25	12.25	3.50	1.75	7.52	7.23	2.69	1.34	-1.18	4.78	2.19	1.09
CC004/Unc	12.54	14.08	3.75	1.88	18.65	6.57	2.56	1.28	-5.16	2.56	1.60	0.80
CC005/TauUnc	2.38	1.03	1.01	0.58	1.32	2.20	1.48	0.86	1.07	6.15	2.48	1.43
CC006/TauUnc	11.33	74.21	8.61	3.26	12.12	43.10	6.57	2.48	-0.78	6.78	2.60	0.98
CC007/Unc	12.10	89.65	9.47	5.47	7.81	40.93	6.40	3.69	3.88	7.02	2.65	1.53
CC008/GeniUnc	4.10	10.74	3.28	1.34	4.46	10.65	3.26	1.33	-0.32	4.23	2.06	0.84
CC009/Unc	10.99	39.85	6.31	2.82	14.12	19.62	4.43	1.98	-2.78	7.93	2.82	1.26
CC010/GeniUnc	8.24	21.26	4.61	2.06	7.03	17.48	4.18	1.87	1.12	0.80	0.89	0.40
CC011/Unc	11.73	5.60	2.37	1.37	9.08	22.57	4.75	2.74	2.52	14.10	3.76	2.17
CC013/GeniUnc	7.37	24.61	4.96	1.88	6.34	22.14	4.71	1.78	1.01	10.92	3.30	1.25
CC014/Unc	3.69	3.25	1.80	1.04	3.43	2.74	1.66	0.96	1.16	1.78	1.33	0.77
CC015/Unc	-7.63	900.68	30.01	15.01	7.01	4.00	2.00	1.00	-13.68	779.77	27.92	13.96
CC017/Unc	0.49	57.51	7.58	4.38	-1.51	31.62	5.62	3.25	1.96	5.97	2.44	1.41
CC019/TauUnc	1.82	13.62	3.69	1.65	1.77	16.04	4.01	1.79	0.08	3.15	1.77	0.79
CC021/Unc	0.42	5.92	2.43	1.40	1.14	14.01	3.74	2.16	-0.68	2.72	1.65	0.95
CC022/GeniUnc	8.29	177.77	13.33	6.67	6.64	36.31	6.03	3.01	1.29	48.72	6.98	3.49
CC023/GeniUnc	13.05	42.44	6.51	3.76	15.19	77.22	8.79	5.07	-1.72	18.71	4.33	2.50
CC024/GeniUnc	14.70	746.46	27.32	12.22	0.67	8.19	2.86	1.28	14.33	875.43	29.59	13.23
CC025/GeniUnc	3.02	348.87	18.68	10.78	7.47	239.93	15.49	8.94	-4.29	62.81	7.93	4.58
CC027/GeniUnc	-5.22	16.77	4.10	1.37	-3.22	14.74	3.84	1.28	-2.06	2.69	1.64	0.55
CC028/GeniUnc	10.61	8.82	2.97	1.49	5.77	2.26	1.50	0.75	4.59	8.11	2.85	1.42
CC029/Unc	-2.94	16.32	4.04	2.33	1.15	28.89	5.38	3.10	-3.98	6.08	2.47	1.42
CC032/GeniUnc	-1.10	0.10	0.31	0.22	2.24	0.22	0.47	0.33	-3.27	0.02	0.14	0.10
CC035/Unc	23.14	109.92	10.48	4.69	20.62	72.64	8.52	3.81	2.04	13.93	3.73	1.67
CC040/TauUnc	-5.66	32.40	5.69	2.55	-0.20	40.64	6.38	2.85	-5.40	12.54	3.54	1.58
CC043/GeniUnc	1.73	18.02	4.24	1.73	1.72	15.20	3.90	1.59	0.03	6.15	2.48	1.01
CC044/Unc	0.18	5.43	2.33	1.35	1.26	2.89	1.70	0.98	-1.07	0.44	0.66	0.38
CC045/GeniUnc	6.05	26.36	5.13	2.30	6.46	24.66	4.97	2.22	-0.37	4.97	2.23	1.00
CC046/Unc	9.51	7.65	2.77	1.60	4.74	35.59	5.97	3.44	4.82	58.60	7.65	4.42
CC051/TauUnc	10.54	87.21	9.34	4.67	6.51	46.88	6.85	3.42	0.79	23.81	4.88	2.44
CC052/GeniUnc	0.10	9.62	3.10	1.79	-1.21	34.46	5.87	3.39	1.45	11.66	3.41	1.97
CC055/TauUnc	15.32	155.14	12.46	4.40	15.81	136.12	11.67	4.12	-0.41	17.84	4.22	1.49
CC058/Unc	8.56	23.95	4.89	1.85	7.59	32.05	5.66	2.14	0.97	7.76	2.78	1.05
CC059/TauUnc	5.72	18.87	4.34	2.51	5.25	3.20	1.79	1.03	0.43	11.43	3.38	1.95
CC060/Unc	13.77	173.05	13.15	5.37	9.17	99.58	9.98	4.07	2.94	8.71	2.95	1.20
CC062/Unc	4.10	1.65	1.29	0.58	2.62	5.42	2.33	1.04	1.49	11.28	3.36	1.50
CC063/Unc	-6.77	9.00	3.00	1.50	-2.41	5.45	2.33	1.17	-4.46	7.14	2.67	1.34
CC065/Unc	3.46	130.80	11.44	5.11	2.26	33.99	5.83	2.61	1.02	47.47	6.89	3.08
CC068/TauUnc	3.67	15.76	3.97	1.98	2.88	23.38	4.84	2.42	0.81	2.44	1.56	0.78
CC070/TauUnc	-2.50	31.75	5.63	3.25	-2.24	33.14	5.76	3.32	-0.26	1.24	1.11	0.64
CC071/TauUnc	5.57	0.71	0.84	0.42	6.87	0.30	0.55	0.27	-1.21	1.15	1.07	0.54
CC075/Unc	6.95	23.81	4.88	2.44	6.14	7.02	2.65	1.32	0.72	5.08	2.25	1.13

Table 3.3. Descriptive statistics for exercise traits in CC strains from the voluntary endurance experiment.

CC Strain	n	Distance - Month (km)				Distance - Week1-2 (km)				Distance - Week3-4 (km)			
		Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	5	150.69	2,077.50	45.58	20.38	74.93	219.85	14.83	6.63	75.04	1,056.14	32.50	14.53
CC003/Unc	4	96.87	3,981.46	63.10	31.55	43.91	835.91	28.91	14.46	51.36	1,232.44	35.11	17.55
CC004/Unc	4	179.32	686.58	26.20	13.10	74.22	244.80	15.65	7.82	95.72	186.54	13.66	6.83
CC005/TauUnc	3	45.92	1,084.97	32.94	19.02	29.18	374.61	19.35	11.17	16.52	232.99	15.26	8.81
CC006/TauUnc	7	147.34	1,566.83	39.58	14.96	44.29	93.25	9.66	3.65	95.42	1,033.13	32.14	12.15
CC007/Unc	3	107.79	8,745.14	93.52	53.99	37.89	500.17	22.36	12.91	67.60	4,786.73	69.19	39.94
CC008/GeniUnc	6	33.94	1,402.30	37.45	15.29	12.12	289.43	17.01	6.95	21.37	844.98	29.07	11.87
CC009/Unc	5	141.63	2,178.14	46.67	20.87	60.38	240.46	15.51	6.93	78.34	979.95	31.30	14.00
CC010/GeniUnc	5	12.31	66.12	8.13	3.64	5.79	19.07	4.37	1.95	6.34	15.08	3.88	1.74
CC011/Unc	3	149.84	4,072.64	63.82	36.84	49.95	1,105.34	33.25	19.20	97.21	1,118.28	33.44	19.31
CC013/GeniUnc	7	34.32	319.89	17.89	6.76	14.12	21.68	4.66	1.76	19.58	167.69	12.95	4.89
CC014/Unc	3	42.78	1,954.95	44.21	25.53	23.70	299.21	17.30	9.99	18.45	743.55	27.27	15.74
CC015/Unc	4	146.78	3,602.36	60.02	30.01	86.09	705.90	26.57	13.28	58.97	1,333.44	36.52	18.26
CC017/Unc	3	99.92	6,543.12	80.89	46.70	48.05	1,475.41	38.41	22.18	51.73	1,942.19	44.07	25.44
CC019/TauUnc	5	130.36	3,001.98	54.79	24.50	54.66	733.07	27.08	12.11	72.76	923.18	30.38	13.59
CC021/Unc	3	112.22	803.78	28.35	16.37	42.96	127.35	11.28	6.52	64.95	364.03	19.08	11.02
CC022/GeniUnc	4	157.60	5,235.83	72.36	36.18	65.33	674.15	25.96	12.98	87.92	2,099.35	45.82	22.91
CC023/GeniUnc	3	109.84	3,497.77	59.14	34.15	46.08	406.39	20.16	11.64	60.87	1,968.28	44.37	25.61
CC024/GeniUnc	4	163.38	4,250.05	65.19	32.60	76.39	750.65	27.40	13.70	84.45	1,396.36	37.37	18.68
CC025/GeniUnc	3	101.25	2,902.19	53.87	31.10	42.72	615.72	24.81	14.33	56.27	879.15	29.65	17.12
CC027/GeniUnc	6	232.88	450.14	21.22	8.66	112.23	129.03	11.36	4.64	117.43	196.52	14.02	5.72
CC028/GeniUnc	4	8.21	5.05	2.25	1.12	4.66	1.65	1.28	0.64	3.42	0.99	1.00	0.50
CC029/Unc	3	158.55	2,380.04	48.79	28.17	65.80	439.68	20.97	12.11	89.24	879.70	29.66	17.12
CC032/GeniUnc	2	83.99	12.62	3.55	2.51	42.86	63.91	7.99	5.65	39.43	13.16	3.63	2.57
CC035/Unc	4	68.89	1,504.01	38.78	19.39	35.11	162.78	12.76	6.38	32.51	613.76	24.77	12.39
CC040/TauUnc	5	116.26	9,703.59	98.51	44.05	59.16	2,308.85	48.05	21.49	57.11	2,555.53	50.55	22.61
CC043/GeniUnc	6	57.29	5,574.80	74.66	30.48	22.80	1,152.26	33.94	13.86	34.17	1,942.98	44.08	18.00
CC044/Unc	3	25.88	108.93	10.44	6.03	13.58	39.78	6.31	3.64	12.15	19.62	4.43	2.56
CC045/GeniUnc	5	161.93	8,240.56	90.78	40.60	59.98	1,113.09	33.36	14.92	90.12	2,927.43	54.11	24.20
CC046/Unc	3	70.58	5,220.54	72.25	41.72	44.06	2,113.46	45.97	26.54	26.41	753.28	27.45	15.85
CC051/TauUnc	4	38.51	2,481.80	49.82	24.91	18.10	594.72	24.39	12.19	19.67	629.42	25.09	12.54
CC052/GeniUnc	3	17.81	14.28	3.78	2.18	8.29	7.61	2.76	1.59	8.96	18.16	4.26	2.46
CC055/TauUnc	8	156.89	9,276.81	96.32	34.05	60.43	1,643.16	40.54	14.33	92.43	3,112.21	55.79	19.72
CC058/Unc	7	44.22	862.96	29.38	11.10	17.30	102.94	10.15	3.83	24.07	414.34	20.36	7.69
CC059/TauUnc	3	6.88	18.83	4.34	2.51	4.02	9.27	3.05	1.76	2.80	1.60	1.27	0.73
CC060/Unc	6	115.30	274.64	16.57	6.77	50.59	542.73	23.30	9.51	63.73	181.02	13.45	5.49
CC062/Unc	5	94.68	277.84	16.67	7.45	41.49	148.04	12.17	5.44	51.87	164.06	12.81	5.73
CC063/Unc	4	40.87	1,367.22	36.98	18.49	18.93	214.81	14.66	7.33	21.26	487.20	22.07	11.04
CC065/Unc	5	122.59	7,168.89	84.67	37.87	61.86	1,034.22	32.16	14.38	58.73	3,303.24	57.47	25.70
CC068/TauUnc	4	72.58	2,017.26	44.91	22.46	31.90	351.07	18.74	9.37	40.53	696.37	26.39	13.19
CC070/TauUnc	3	48.94	6,154.92	78.45	45.30	21.93	1,095.34	33.10	19.11	26.41	1,966.78	44.35	25.60
CC071/TauUnc	4	163.81	783.58	27.99	14.00	76.36	211.04	14.53	7.26	83.35	186.13	13.64	6.82
CC075/Unc	4	89.48	3,629.35	60.24	30.12	42.48	724.98	26.93	13.46	44.01	1,037.96	32.22	16.11

CC Strain	Duration - Month (min)				Duration - Week1-2 (min)				Duration - Week3-4 (min)			
	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	9,772.00	3,875,192.50	1,968.55	880.36	4,965.20	287,583.70	536.27	239.83	4,492.20	1,302,589.70	1,141.31	510.41
CC003/Unc	6,704.50	13,212,955.00	3,634.96	1,817.48	3,152.25	2,715,792.25	1,647.97	823.98	3,462.00	3,996,860.67	1,999.22	999.61
CC004/Unc	9,401.50	762,157.67	873.02	436.51	3,962.00	251,900.00	501.90	250.95	4,950.25	370,850.92	608.98	304.49
CC005/TauUnc	4,413.00	8,770,483.00	2,961.50	1,709.82	3,206.33	4,352,092.33	2,086.17	1,204.45	1,179.00	1,047,652.00	1,023.55	590.95
CC006/TauUnc	8,517.29	1,953,445.90	1,397.66	528.26	3,262.43	123,123.62	350.89	132.62	4,848.14	1,190,424.14	1,091.07	412.38
CC007/Unc	8,772.67	13,965,206.33	3,737.00	2,157.56	4,696.00	3,131,167.00	1,769.51	1,021.63	3,932.00	3,627,696.00	1,904.65	1,099.65
CC008/GeniUnc	4,078.33	16,589,837.47	4,073.06	1,662.82	1,709.83	4,831,481.77	2,198.06	897.36	2,320.17	6,599,706.97	2,568.99	1,048.79
CC009/Unc	11,952.20	18,524,760.70	4,304.04	1,924.83	6,845.60	6,333,351.30	2,516.62	1,125.46	4,928.60	3,103,047.30	1,761.55	787.79
CC010/GeniUnc	3,670.20	1,650,226.20	1,284.61	574.50	1,742.80	1,090,781.70	1,044.40	467.07	1,866.40	100,456.30	316.95	141.74
CC011/Unc	8,864.67	3,177,590.33	1,782.58	1,029.17	3,640.00	990,327.00	995.15	574.55	5,066.33	925,600.33	962.08	555.46
CC013/GeniUnc	5,914.29	1,345,372.57	1,159.90	438.40	2,835.43	235,744.95	485.54	183.52	2,988.29	561,558.24	749.37	283.24
CC014/Unc	4,538.67	10,082,185.33	3,175.25	1,833.23	2,728.00	1,317,199.00	1,147.69	662.62	1,733.67	4,415,736.33	2,101.37	1,213.22
CC015/Unc	11,302.00	13,834,408.67	3,719.46	1,859.73	6,408.00	2,583,519.33	1,607.33	803.67	4,747.00	4,592,059.33	2,142.91	1,071.45
CC017/Unc	7,222.33	23,133,602.33	4,809.74	2,776.90	3,534.00	5,054,812.00	2,248.29	1,298.05	3,666.67	7,631,476.33	2,762.51	1,594.94
CC019/TauUnc	8,795.60	7,059,313.30	2,656.94	1,188.22	4,327.20	2,786,259.20	1,669.21	746.49	4,285.80	1,022,137.70	1,011.01	452.14
CC021/Unc	11,036.00	687,817.00	829.35	478.82	4,444.67	773,810.33	879.66	507.87	5,767.67	1,349,062.33	1,161.49	670.59
CC022/GeniUnc	11,439.75	8,081,473.58	2,842.79	1,421.40	5,337.50	1,443,109.67	1,201.29	600.65	5,793.75	2,919,116.92	1,708.54	854.27
CC023/GeniUnc	8,744.33	10,569,150.33	3,251.02	1,876.98	4,077.67	1,412,465.33	1,188.47	686.16	4,456.00	5,132,836.00	2,265.58	1,308.03
CC024/GeniUnc	11,784.25	10,599,678.25	3,255.71	1,627.86	6,887.00	2,190,874.00	1,480.16	740.08	4,757.25	2,836,402.92	1,684.16	842.08
CC025/GeniUnc	11,197.33	6,311,817.33	2,512.33	1,450.50	5,182.33	1,932,966.33	1,390.31	802.70	5,768.33	1,294,126.33	1,137.60	656.79
CC027/GeniUnc	13,829.33	1,236,704.27	1,112.07	454.00	6,723.00	367,536.80	606.25	247.50	6,806.17	397,349.37	630.36	257.34
CC028/GeniUnc	3,051.25	549,164.92	741.06	370.53	1,707.50	216,902.33	465.73	232.86	1,288.00	92,756.67	304.56	152.28
CC029/Unc	7,834.00	2,942,092.00	1,715.25	990.30	3,480.33	614,122.33	783.66	452.45	4,175.67	937,769.33	968.38	559.10
CC032/GeniUnc	9,387.50	747,864.50	864.79	611.50	5,056.50	519,180.50	720.54	509.50	4,163.00	42,050.00	205.06	145.00
CC035/Unc	5,262.75	4,841,028.25	2,200.23	1,100.12	2,960.75	459,986.92	678.22	339.11	2,225.50	2,136,693.67	1,461.74	730.87
CC040/TauUnc	7,995.60	17,302,151.30	4,159.59	1,860.22	3,989.00	3,200,816.00	1,789.08	800.10	4,006.60	6,003,568.30	2,450.22	1,095.77
CC043/GeniUnc	3,942.00	12,859,929.60	3,586.07	1,464.01	1,748.83	3,209,700.17	1,791.56	731.40	2,163.00	3,996,139.60	1,999.03	816.10
CC044/Unc	4,967.67	776,986.33	881.47	508.92	2,529.33	323,857.33	569.08	328.56	2,385.67	131,844.33	363.10	209.64
CC045/GeniUnc	12,006.60	31,674,265.30	5,627.99	2,516.91	5,108.40	4,549,311.30	2,132.91	953.87	5,778.20	8,108,422.20	2,847.53	1,273.45
CC046/Unc	6,269.00	21,273,147.00	4,612.28	2,662.90	3,825.00	9,090,237.00	3,015.00	1,740.71	2,430.67	3,089,590.33	1,757.72	1,014.82
CC051/TauUnc	3,358.00	10,000,310.00	3,162.33	1,581.16	1,769.75	3,059,714.92	1,749.20	874.60	1,514.00	1,988,798.00	1,410.25	705.12
CC052/GeniUnc	3,879.33	103,842.33	322.25	186.05	1,924.00	60,781.00	246.54	142.34	1,859.67	160,384.33	400.48	231.22
CC055/TauUnc	11,064.50	13,015,752.57	3,607.74	1,275.53	5,462.25	4,225,703.36	2,055.65	726.78	5,388.00	3,133,178.29	1,770.08	625.82
CC058/Unc	4,939.14	3,905,083.81	1,976.13	746.91	2,345.29	542,727.24	736.70	278.45	2,301.57	1,077,151.95	1,037.86	392.27
CC059/TauUnc	2,190.67	1,143,882.33	1,069.52	617.49	1,279.00	507,484.00	712.38	411.29	881.33	112,272.33	335.07	193.45
CC060/Unc	7,913.50	970,537.10	985.16	402.19	3,839.33	1,534,460.27	1,238.73	505.71	3,776.67	199,673.87	446.85	182.43
CC062/Unc	8,792.00	1,537,556.50	1,239.98	554.54	4,293.40	334,467.80	578.33	258.64	4,397.00	603,143.50	776.62	347.32
CC063/Unc	4,005.50	3,223,729.67	1,795.47	897.74	2,173.25	674,072.25	821.02	410.51	1,777.75	923,468.92	960.97	480.49
CC065/Unc	8,942.00	25,263,338.00	5,026.26	2,247.81	4,832.00	2,475,916.50	1,573.50	703.69	3,997.20	14,082,068.70	3,752.61	1,678.22
CC068/TauUnc	7,038.25	10,784,023.58	3,283.90	1,641.95	3,458.75	2,230,000.92	1,493.32	746.66	3,237.75	1,719,922.25	1,311.46	655.73
CC070/TauUnc	4,402.67	27,946,546.33	5,286.45	3,052.13	2,217.00	3,806,992.00	1,951.15	1,126.50	2,140.67	10,959,160.33	3,310.46	1,911.30
CC071/TauUnc	11,595.75	1,038,284.92	1,018.96	509.48	5,697.75	211,796.25	460.21	230.11	5,618.25	323,669.58	568.92	284.46
CC075/Unc	6,502.00	7,700,642.67	2,775.00	1,387.50	3,404.25	1,080,225.58	1,039.34	519.67	2,876.25	3,604,351.58	1,898.51	949.26

CC Strain	Mean Speed - Month (m/min)				Mean Speed - Week1-2 (m/min)				Mean Speed - Week3-4 (m/min)			
	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error	Mean	Variance	Std Deviation	Std Error
CC001/Unc	15.22	5.56	2.36	1.05	14.61	3.74	1.93	0.86	16.16	12.74	3.57	1.60
CC003/Unc	12.53	28.64	5.35	2.68	12.31	21.56	4.64	2.32	12.72	38.38	6.19	3.10
CC004/Unc	19.73	9.14	3.02	1.51	20.21	11.48	3.39	1.69	19.41	9.87	3.14	1.57
CC005/TauUnc	9.87	19.16	4.38	2.53	9.31	9.76	3.12	1.80	9.25	68.31	8.26	4.77
CC006/TauUnc	17.26	6.57	2.56	0.97	15.25	11.62	3.41	1.29	19.22	8.54	2.92	1.10
CC007/Unc	11.78	42.56	6.52	3.77	9.06	11.21	3.35	1.93	14.37	90.41	9.51	5.49
CC008/GeniUnc	5.15	19.50	4.42	1.80	3.64	9.47	3.08	1.26	5.86	30.68	5.54	2.26
CC009/Unc	13.19	0.32	0.56	0.25	10.69	1.12	1.06	0.47	15.46	2.29	1.51	0.68
CC010/GeniUnc	2.92	1.96	1.40	0.63	2.60	2.54	1.59	0.71	3.09	2.46	1.57	0.70
CC011/Unc	15.51	13.87	3.72	2.15	12.14	22.04	4.69	2.71	18.77	9.49	3.08	1.78
CC013/GeniUnc	5.29	3.66	1.91	0.72	4.70	1.25	1.12	0.42	5.87	7.40	2.72	1.03
CC014/Unc	7.01	19.69	4.44	2.56	7.57	16.06	4.01	2.31	6.17	30.22	5.50	3.17
CC015/Unc	12.59	10.72	3.27	1.64	13.29	4.44	2.11	1.05	11.99	23.49	4.85	2.42
CC017/Unc	11.65	40.92	6.40	3.69	10.93	37.67	6.14	3.54	12.30	45.79	6.77	3.91
CC019/TauUnc	14.29	14.02	3.74	1.67	12.32	20.52	4.53	2.03	16.25	20.92	4.57	2.05
CC021/Unc	10.95	4.89	2.21	1.28	11.11	8.71	2.95	1.70	11.08	1.61	1.27	0.73
CC022/GeniUnc	13.26	15.87	3.98	1.99	12.51	8.44	2.90	1.45	14.09	26.46	5.14	2.57
CC023/GeniUnc	11.53	9.12	3.02	1.74	10.30	9.51	3.08	1.78	12.61	13.29	3.65	2.11
CC024/GeniUnc	15.76	4.11	2.03	1.01	14.33	3.63	1.91	0.95	17.02	7.50	2.74	1.37
CC025/GeniUnc	9.19	22.18	4.71	2.72	8.21	16.95	4.12	2.38	10.11	28.22	5.31	3.07
CC027/GeniUnc	16.65	0.81	0.90	0.37	16.36	0.77	0.87	0.36	17.17	1.08	1.04	0.42
CC028/GeniUnc	2.69	0.05	0.23	0.11	2.75	0.06	0.25	0.13	2.66	0.04	0.21	0.10
CC029/Unc	19.48	4.83	2.20	1.27	17.89	2.84	1.68	0.97	21.10	12.22	3.50	2.02
CC032/GeniUnc	8.99	0.33	0.57	0.41	8.45	0.11	0.32	0.23	9.47	2.03	1.42	1.01
CC035/Unc	12.04	9.68	3.11	1.56	11.45	2.32	1.52	0.76	12.54	21.44	4.63	2.32
CC040/TauUnc	12.66	83.74	9.15	4.09	12.29	79.29	8.90	3.98	13.02	88.50	9.41	4.21
CC043/GeniUnc	8.18	41.08	6.41	2.62	6.72	42.81	6.54	2.67	9.60	53.81	7.34	2.99
CC044/Unc	4.85	1.61	1.27	0.73	4.87	1.99	1.41	0.81	4.99	2.26	1.50	0.87
CC045/GeniUnc	11.36	20.03	4.48	2.00	9.79	15.96	3.99	1.79	13.11	33.75	5.81	2.60
CC046/Unc	8.98	15.04	3.88	2.24	9.73	27.77	5.27	3.04	8.25	12.85	3.58	2.07
CC051/TauUnc	7.46	29.66	5.45	2.72	6.25	21.73	4.66	2.33	8.47	41.93	6.48	3.24
CC052/GeniUnc	4.23	0.35	0.59	0.34	3.88	0.24	0.49	0.28	4.47	1.17	1.08	0.62
CC055/TauUnc	13.36	37.43	6.12	2.16	10.91	29.58	5.44	1.92	15.62	49.50	7.04	2.49
CC058/Unc	7.77	7.47	2.73	1.03	6.53	5.11	2.26	0.85	8.94	13.33	3.65	1.38
CC059/TauUnc	2.98	0.16	0.40	0.23	2.94	0.41	0.64	0.37	3.07	0.06	0.24	0.14
CC060/Unc	13.82	1.47	1.21	0.49	12.28	7.60	2.76	1.13	15.51	9.65	3.11	1.27
CC062/Unc	10.73	4.88	2.21	0.99	9.46	5.93	2.44	1.09	11.82	8.49	2.91	1.30
CC063/Unc	8.52	13.79	3.71	1.86	7.60	6.63	2.58	1.29	9.54	28.79	5.37	2.68
CC065/Unc	13.12	10.60	3.26	1.46	11.94	5.19	2.28	1.02	13.21	56.30	7.50	3.36
CC068/TauUnc	10.62	22.83	4.78	2.39	9.26	15.75	3.97	1.98	12.18	32.17	5.67	2.84
CC070/TauUnc	6.41	52.35	7.24	4.18	5.97	47.38	6.88	3.97	7.03	56.37	7.51	4.33
CC071/TauUnc	13.98	1.84	1.36	0.68	13.11	2.14	1.46	0.73	14.79	1.63	1.28	0.64
CC075/Unc	12.48	42.56	6.52	3.26	13.53	38.27	6.19	3.09	11.90	52.03	7.21	3.61

Table 3.4. Genetic background has a significant effect on all potential mediators of exercise-induced body composition response to voluntary exercise.

P-values from ANOVAs of linear models measuring the effect of genetic background on each potential mediator (distance, duration, speed, and adjusted food intake) at all three time points (1 month; week1-2; week3-4). Statistical significance: $p<0.05$.

Mediator	Time point		
	Month	**Week 1-2**	**Week 3-4**
Total Distance	3.09x10-12	3.25x10-12	6.64x10-10
Total Duration	2.57x10-12	9.16x10-14	5.097x10-09
Average Speed	5.08x10-13	3.37x10-14	1.167x10-09
Adjusted Food Intake	<2.2x10-16	<2.2x10-16	1.12x10-10

Table 3.5. Nested ANOVA analysis of potential mediators and genetic background on body mass and composition response to voluntary exercise. Base models included a mediator as a fixed effect. Additive models included a mediator and genetic background as additive effects. Full models included a mediator effect, genetic background effect and their interaction. Grey boxes represent significant p values.

Mediator	Response	Time point	Base vs Additive model	Additive vs Full model
Total Distance	Body Mass	Month	0.012	0.994
		Week 1-2	1.32x10-08	9.27x10-04
		Week 3-4	0.0236	0.9127
	Body Fat %	Month	6.61x10-10	0.022
		Week 1-2	1.152x10-08	0.0129
		Week 3-4	0.09038	0.0204
	Lean Mass %	Month	3.7x10-04	0.7551
		Week 1-2	9.77x10-10	0.261
		Week 3-4	0.1643	0.999
Total Duration	Body Mass	Month	0.0106	0.999
		Week 1-2	1.88x10-08	0.026
		Week 3-4	0.0286	0.996
	Body Fat %	Month	7.83x10-10	0.05196
		Week 1-2	1.569x10-08	0.0283
		Week 3-4	0.1289	0.1219
	Lean Mass %	Month	3.55x10-04	0.9806
		Week 1-2	7.79x10-10	0.4224
		Week 3-4	0.1974	0.9975
Average Speed	Body Mass	Month	0.01659	0.9907
		Week 1-2	5.049x10-09	9.54x10-04
		Week 3-4	0.01502	0.3612
	Body Fat %	Month	2.418x10-09	0.009836
		Week 1-2	4.98x10-08	0.001293
		Week 3-4	0.08204	0.2008
	Lean Mass %	Month	4.316x10-04	0.9908
		Week 1-2	2.187x10-09	0.346
		Week 3-4	0.1283	0.8192

Mediator	Response	Time point	Base vs Additive model	Additive vs Full model
Adjusted Food Intake	Body Mass	Month	0.045	0.759
		Week 1-2	3.45x10-08	0.0054
		Week 3-4	0.0355	0.763
	Body Fat %	Month	9.933x10-08	0.01839
		Week 1-2	4.936x10-07	0.00102
		Week 3-4	0.0404	0.213
	Lean Mass %	Month	0.00345	0.6629
		Week 1-2	5.416x10-08	0.319
		Week 3-4	0.1982	0.997
Baseline Age	Body Mass	Month	0.1172	0.9871
		Week 1-2	1.25x10-08	0.7791
		Week 3-4	0.037	0.7117
	Body Fat %	Month	1.216x10-8	0.9751
		Week 1-2	4.99x10-08	0.991
		Week 3-4	0.0967	0.872
	Lean Mass %	Month	0.00329	0.6741
		Week 1-2	9.705x10-10	0.8715
		Week 3-4	0.126	0.7428

Table 3.6. Phenotypic correlations and significance between body mass and composition responses and potential mediators in the voluntary exercise cohort. Grey boxes represent significant correlations.

Time Point	Mediator	Body Mass Response		Body Fat % Response		Lean Mass % Response	
		cor	p	cor	p	cor	p
Month	Age	-0.0881	0.2425	-0.0674	0.3712	0.1082	0.1506
Week 1-2	Age	-0.0704	0.3467	0.0384	0.6078	0.0231	0.7578
Week 3-4	Age	-0.0104	0.8906	-0.1048	0.1639	0.1037	0.1684
Month	Distance - month	-0.0721	0.3374	-0.0111	0.8830	0.0260	0.7294
Week 1-2	Distance - month	-0.0865	0.2455	-0.1322	0.0752	0.1464	0.0486
Week 3-4	Distance - month	-0.0067	0.9293	0.1377	0.0660	-0.1032	0.1690
Month	Distance - week 1-2	0.0476	0.5269	0.0264	0.7257	-0.0853	0.2563
Week 1-2	Distance - week 1-2	-0.0067	0.9282	-0.1084	0.1451	0.0629	0.3988
Week 3-4	Distance - week 1-2	0.0615	0.4137	0.1755	0.0188	-0.1707	0.0223
Month	Distance - week 3-4	-0.1505	0.0443	-0.0299	0.6911	0.0978	0.1926
Week 1-2	Distance - week 3-4	-0.1325	0.0746	-0.1354	0.0683	0.1875	0.0113
Week 3-4	Distance - week 3-4	-0.0559	0.4573	0.1061	0.1576	-0.0476	0.5267
Month	Duration - month	-0.0651	0.3869	-0.0322	0.6687	0.0279	0.7112
Week 1-2	Duration - month	-0.1035	0.1645	-0.1803	0.0149	0.1597	0.0312
Week 3-4	Duration - month	0.0081	0.9140	0.1679	0.0247	-0.1108	0.1397
Month	Duration - week 1-2	-0.0304	0.6861	-0.0362	0.6302	0.0108	0.8858
Week 1-2	Duration - week 1-2	-0.0718	0.3355	-0.1793	0.0155	0.1257	0.0908
Week 3-4	Duration - week 1-2	0.0187	0.8035	0.1537	0.0400	-0.0955	0.2035
Month	Duration - week 3-4	-0.0889	0.2369	-0.0135	0.8581	0.0283	0.7066
Week 1-2	Duration - week 3-4	-0.1168	0.1164	-0.1559	0.0356	0.1649	0.0261
Week 3-4	Duration - week 3-4	-0.0034	0.9635	0.1725	0.0210	-0.1197	0.1106
Month	Food Intake - week 1-2	0.1766	0.0180	0.3762	0.0000	-0.2933	0.0001
Week 1-2	Food Intake - week 1-2	0.4026	0.0000	0.3211	0.0000	-0.4190	0.0000
Week 3-4	Food Intake - week 1-2	-0.1147	0.1264	0.1572	0.0356	0.0280	0.7096
Month	Adj Food Intake - week 1-2	0.4427	0.0000	0.3185	0.0000	-0.5065	0.0000
Week 1-2	Adj Food Intake - week 1-2	0.6243	0.0000	0.2534	0.0006	-0.6140	0.0000
Week 3-4	Adj Food Intake - week 1-2	-0.0149	0.8426	0.2051	0.0059	-0.0734	0.3286
Month	Food Intake - week 3-4	-0.1515	0.0429	0.0413	0.5833	0.1450	0.0528
Week 1-2	Food Intake - week 3-4	-0.1427	0.0561	-0.1046	0.1623	0.2869	0.0001
Week 3-4	Food Intake - week 3-4	-0.0303	0.6870	0.2248	0.0025	-0.0795	0.2899
Month	Adj Food Intake - week 3-4	0.0739	0.3253	-0.0510	0.4975	-0.0736	0.3275
Week 1-2	Adj Food Intake - week 3-4	-0.1063	0.1555	-0.2583	0.0005	0.0960	0.2000
Week 3-4	Adj Food Intake - week 3-4	0.1779	0.0172	0.3668	0.0000	-0.1901	0.0108
Month	Speed - month	-0.1070	0.1539	-0.0454	0.5466	0.0836	0.2659
Week 1-2	Speed - month	-0.0731	0.3268	-0.1552	0.0364	0.1717	0.0205

Week 3-4	Speed - month	-0.0529	0.4819	0.1084	0.1487	-0.0450	0.5498
Month	Speed - week 1-2	-0.0143	0.8496	-0.0357	0.6352	0.0005	0.9944
Week 1-2	Speed - week 1-2	-0.0289	0.6984	-0.1503	0.0428	0.1258	0.0907
Week 3-4	Speed - week 1-2	0.0144	0.8483	0.1233	0.1002	-0.1127	0.1332
Month	Speed - week 3-4	-0.1713	0.0219	-0.0519	0.4900	0.1395	0.0626
Week 1-2	Speed - week 3-4	-0.1038	0.1632	-0.1521	0.0403	0.1949	0.0084
Week 3-4	Speed - week 3-4	-0.0998	0.1840	0.0900	0.2307	0.0072	0.9233

Table 3.7. Treadmill protocols for acclimation days and forced endurance testing.

Acclimation Day 1						
Angle of Inclination	5^0					
Step	Start Speed (m/min)	End Speed (m/min)	Period (sec)	Distance in this step (m)	Total distance accumulated after this step (m)	Total Running Time (at end of this step; min)
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	600	90	120	15
5	9	0	120	9	129	17
Acclimation Day 2						
Angle of Inclination	10^0					
Step	Start Speed (m/min)	End Speed (m/min)	Period (sec)	Distance in this step (m)	Total distance accumulated after this step (m)	Total Running Time (at end of this step; min)
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	600	90	120	15
5	9	16	120	25	145	17
6	16	16	120	32	177	19
7	16	0	60	8	185	20
Acclimation Day 3						
Angle of Inclination	10^0					
Step	Start Speed (m/min)	End Speed (m/min)	Period (sec)	Distance in this step (m)	Total distance accumulated after this step (m)	Total Running Time (at end of this step; min)
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	60	9	39	6
5	9	16	60	12.5	51.5	7
6	16	16	180	48	99.5	10
7	16	18	180	51	150.5	13
8	18	18	120	36	186.5	15
9	18	20	180	57	243.5	18
10	20	20	120	38	281.5	20
11	18	0	60	9	290.5	21
Endurance Test Day						
Angle of Inclination	10^0					
Step	Start Speed (m/min)	End Speed (m/min)	Period (sec)	Distance in this step (m)	Total distance accumulated after this step (m)	Total Running Time (at end of this step; min)
1	0	6	120	6	6.0	2.0
2	6	13	720	114.0	120.0	14.0
3	13	13	360	78.0	198.0	20.0
4	13	14	30	6.8	204.8	20.5
5	14	14	360	84.0	288.8	26.5

6	14	15	30	7.3	296.0	27.0
7	15	15	360	90.0	386.0	33.0
8	15	16	30	7.8	393.8	33.5
9	16	16	720	192.0	585.8	45.5
10	16	17	30	8.3	594.0	46.0
11	17	17	720	204.0	798.0	58.0
12	17	18	60	17.5	815.5	59.0
13	18	18	720	216.0	1031.5	71.0
14	18	19	60	18.5	1050.0	72.0
15	19	19	720	228.0	1278.0	84.0
16	19	20	180	58.5	1336.5	87.0
17	20	20	999	333.0	1669.5	103.7
18	20	20	999	333.0	2002.5	120.3
19	20	20	999	333.0	2335.5	137.0
20	20	21	999	341.3	2676.8	153.6
21	21	21	999	349.7	3026.5	170.3
22	21	21	999	349.7	3376.1	186.9
23	21	22	999	358.0	3734.1	203.6
24	22	22	999	366.3	4100.4	220.2
25	22	22	999	366.3	4466.7	236.9
26	22	23	999	374.6	4841.3	253.5
27	23	23	999	383.0	5224.3	270.2
28	23	23	999	383.0	5607.2	286.8
29	23	24	999	391.3	5998.5	303.5
30	24	24	999	399.6	6398.1	320.1
31	24	24	999	399.6	6797.7	336.8
32	24	24	999	399.6	7197.3	353.4
33	24	18	120	42.0	7239.3	355.4
34	18	6	120	24.0	7263.3	357.4
35	6	0	120	6.0	7269.3	359.4

CHAPTER 4: CC002/Unc FEMALES ARE MOUSE MODELS OF EXERCISE-INDUCED ADVERSE FAT RESPONSE[6]

4.1 Overview

Exercise results in beneficial health outcomes and protects against a variety of chronic diseases. However, U.S. exercise guidelines recommend identical exercise programs for everyone, despite individual variation in responses to these programs, including paradoxical fat gain. Experimental models of exercise-induced adverse outcomes may enable the dissection of underlying physiological mechanisms as well as the evaluation of potential interventions. Whereas several studies have identified individual mice exhibiting adverse fat gain following exercise, no systematic effort has been conducted to identify and characterize models of adverse response. Strains from the Collaborative Cross (CC) genetic reference population were used due to its high levels of genetic variation, its reproducible nature, and the observation that the CC is a rich source of novel disease models, to assess the impact genetic background has on exercise responses. We identified the strain CC002/Unc as a robust exercise-induced adverse fat response model in a controlled voluntary exercise study across multiple ages in female mice. We also found sex and genetic differences were consistent with this pattern in a study of forced exercise programs. These results provide a novel model for studies to determine the mechanisms behind paradoxical adverse metabolic responses to exercise, and enable development of more rational personalized exercise recommendations based on factors such as age, sex, and genetic background.

[6] This work was submitted to Physiological Genomics and is under review. The work described has been completed in collaboration with Martin T. Ferris, Timothy A. Bell, Vineet D. Menachery, Ralph S. Baric, Kunjie Hua, Daniel Pomp, Abbie E. Smith-Ryan and Fernando Pardo-Manuel de Villena. Supporting materials are located in the Appendix.

4.2 Introduction

Exercise is known to have numerous positive health benefits, plays a role in weight management and obesity prevention, and has the potential to reduce morbidity associated with chronic diseases (*16*, *117*). Although exercise reduces health risks, the effects on body composition are still unknown. Exercise is expected to reduce body mass and fat, but exercise-induced weight loss is often less than expected (*16*). In both human and rodent populations, individual variation across a multitude of exercise-induced responses occurs, with some individuals experiencing adverse responses (*45*, *47*, *49*, *118-122*). Even when exercise doses and energy expenditures are controlled, there is large individual variation in body mass and composition responses to exercise programs (*19*, *49*, *79*). In fact, some humans gain weight (*16*, *48*) and gain body fat (*49*) in response to exercise; and similar adverse responses have been observed in outbred mice (*19*). This observed variation can be partially attributed to insufficient exercise dose, lack of adherence, and physiological and behavioral compensatory adaptations (e.g. energy intake, habitual activity levels, metabolic adaptations) (*16*, *123*). Furthermore, the individual variation in response to exercise treatments suggests genetic variation contributes to differences in exercise-induced responses (*8*, *49*, *83*, *121*, *124-127*). Initial studies in humans (*121*, *124*, *125*, *127*, *128*) and rodents (*19*, *83*, *126*, *129*) have suggested that genetics contributes to exercise-induced body mass and composition responses.

U.S. guidelines for physical activity recommend the same exercise programs despite age or sex (*21*). A moderate intensity continuous training (MICT) program consists of continuous exercise at a moderate intensity and closely resembles recommended physical activity guidelines. Alternatively, exercise programs can vary in intensity, such as high intensity interval training (HIIT), which consists of exercising at intervals of high intensity followed by short periods of low intensity or rest. HIIT programs are time-efficient alternatives to MICT and have been shown to elicit rapid beneficial physiological responses

(*26*, *130*). Initial studies have demonstrated HIIT can efficiently and effectively reduce body fat (*29*, *30*) and increase lean mass (*34*, *35*). However, individual variability in body composition responses with the presence of responders and non-responders has been observed in HIIT, MICT and other exercise programs making it difficult to determine effective personalized exercise programs. Furthermore, initial evidence in humans suggests that physiological outcomes as a result of HIIT may be sex specific (*43*). It is unclear if or how genetics influences body composition in response to HIIT and MICT exercise programs (*22-29*, *35*).

Given the complex interactions between physical activity, energy intake, body composition, as well as other variables, it is difficult to determine causal factors and successful exercise regimes that elicit beneficial responses to exercise in the human population. Sets of genetically distinct inbred mouse strains can be used to assess the impact of genetic responses for all of these traits while controlling environmental variables (*51*, *54*). Even though it is common in human studies, especially in women, to observe exercise-induced body fat gain, no inbred mouse models exist which recapitulate these phenotypes. Most mouse studies (*83*, *90*, *126*, *129*) have only observed a standard response (body fat loss) to exercise, with the exception of some outbred mice presenting adverse responses (*19*). A previous study used incipient Collaborative Cross (pre-CC) mice to examine exercise-related traits and observed ~17% of the pre-CC lines had an adverse response to voluntary exercise (*66*). Since biological replicates within each pre-CC line were not tested, it remains unknown whether the observed adverse body composition responses were due to genetics, experimental noise or another underlying mechanism.

We utilized the CC population (*53*, *55*, *57-59*) to determine if there are inbred strains with consistent adverse fat response to exercise. The CC was selected because of 1) the previously reported exercise-induced adverse responses in the pre-CC (*66*); 2) its high genetic and phenotypic diversity (*62*, *65*, *131-133*); 3) the possibility to generate biological

replicates; and 4) it has proven to be a rich source for novel human disease models (*64*, *65*). We expected variation in exercise phenotypes among CC strains to be comparable to variation observed in the human population (*54*), and to provide strain(s) that can serve as models of exercise-induced adverse fat response.

Here we report the results of three independent but related experiments in CC mice: a screen for variability in responses to voluntary exercise; validation of strain CC002/Unc as a model for adverse response; and finally, an evaluation of metabolic response for two different exercise programs. These studies aimed to identify potential CC strains with exercise-induced adverse body composition response for model development and for understanding genetic background contribution on physiological responses to different exercise programs.

4.3 Materials & Methods

Each section is divided into subsections for the following experiments: 1) Voluntary Exercise Screen; 2) CC002/Unc Model Validation; 3) Exercise Program Evaluation.

4.3.1 Mice & Exercise Treatment

All mice were purchased from the Systems Genetics Core Facility (http://csbio.unc.edu/CCstatus/index.py) and housed in the Division of Comparative Medicine facilities at the University of North Carolina at Chapel Hill. All procedures performed within this experiment were approved by the University of North Carolina – Chapel Hill Institutional Animal Care and Use Committee (IACUC #15-015). Mice were housed in a temperature controlled (23° +/- 1° C) and humidity controlled vivarium with a standard 12:12h light:dark cycle (lights on at 0700h). Mice were allowed *ad libitum* access to standard laboratory chow (Envigo 2920 irradiated chow) and water.

1) Voluntary Exercise Screen. Female mice (n=173 total mice; ~9 months +/- 4 weeks) from 13 CC strains were used in this screen and are also part of an ongoing aging study at UNC. CC strains were selected based on availability of at least 15 age-matched females (born

April to October 2015). Females were selected due to the need to group house the mice during the aging process. Mice were assigned to experimental (voluntary exercise; n=93) or control (no exercise; n=80) treatment cohorts prior to the start of the experiment (n=4-8 per strain and treatment). The experiment was performed in six batches spaced approximately one month apart each. During the experiment two mice died of unrelated causes, one CC040/TauUnc from the control cohort and one CC030/GeniUnc from the experimental cohort (these mice were only used in the baseline phenotypic analysis).

Mice in the experimental cohort were individually housed in standard laboratory cages with *ad libitum* access to attached running wheels (1.1m circumference; Lafayette Industries Lafayette, IN; (*134*)). Wheel running data was recorded continuously in 1-min intervals over a two-week period using an automated activity wheel monitoring program (AWM, Lafayette Industries, Lafayette, IN). The following physical activity measurements were obtained for each day of wheel access: distance (total revolutions x 1.1 m), duration (cumulative 1-min intervals in which at least 1 revolution was recorded), and average speed (total distance/total duration; m/min) (*96*). For days 11-12 of wheel access, the mean total distance, duration and average speed were calculated for each mouse. Mice in the control cohort were group housed (or single housed in select cases when all other cage mates were assigned to the experimental cohort) for the two weeks.

2) CC002/Unc Model Validation. Female mice (n=12 per strain; born September to October 2016) from CC002/Unc and CC037/TauUnc were used to assess robustness of the CC002/Unc model at a younger age (~4 months +/- 2 weeks). Mice were assigned to control (CC002/Unc n=3; CC037/TauUnc n=5) or experimental (CC002/Unc n=9; CC037/TauUnc n=7) cohort. Both cohorts were acclimated for two weeks to single housing with attached wheels in same vivarium room but without access to wheels. After acclimation, mice in the experimental cohort were given *ad libitum* access to running wheels for eight weeks. The control cohort did not have access to running wheels.

Experimental procedures for running wheel data collection and physical activity calculations follow those detailed in the “Voluntary Exercise Screen” section above. Total weekly distance, total weekly duration and average weekly speed were calculated for each two week interval of wheel access and were labeled 1, 2, 3 and 4 (respectively: weeks 1-2, weeks 3-4, weeks 5-6, weeks 7-8 of wheel access).

3) Exercise Program Evaluation

Strain selection: Four strains were selected based on the following criteria: 1) ability to run on treadmills, 2) fat response type to voluntary exercise, 3) endurance ability (MPD datasets: McMullan1, McMullan2, McMullan3; Experiment “Voluntary Exercise Screen”; all data was collected in older females). Selected strains were: CC002/Unc adverse fat responder, low endurance; CC027/GeniUnc adverse fat responder, high endurance; CC013/GeniUnc standard fat responder, low endurance; and CC037/TauUnc standard fat responder, high endurance.

Maximum endurance speed: To assess maximum endurance speed, mice from the selected strains (n=3 per sex and strain) were group housed with the same sex and strain. Mice were acclimated to the treadmill (Exer 3/6, Columbus Instruments, Columbus, Ohio) over three days (Supporting Table 1). Then, mice were run to exhaustion using the following endurance protocol performed at 20° inclination: initial speed was 4 m/min, increased by 2 m/min every two minutes then at 12 m/min the speed increased by 1 m/min every minute. The endurance protocol was performed twice on each mouse on two separate days and maximum speed (m/min) was recorded for both days. Maximum speed was defined as the last speed the mouse was able to maintain steady treadmill running before failure. Failure was defined as the inability or refusal to run on the treadmill despite stimulus via shock grid or prodding. The mean maximum speed was calculated for each sex and strain combination (Supporting Table 2).

Exercise program protocol design: Strain and sex specific training protocols (HIIT and MICT) were designed based on the measured mean maximum speeds. There were five separate exercise groups: 1) CC002/Unc females; 2) CC013/GeniUnc females; 3) CC027/GeniUnc and CC037/TauUnc females; 4) CC002/Unc and CC013/GeniUnc males; and 5) CC027/GeniUnc and CC037/TauUnc males. The HIIT protocols consisted of five intervals with 80% max speed for four minutes, 20% max speed for one min, and ten 30 second transitions to decrease and increase speed between the different intensities. The MICT protocols were distance matched to the HIIT protocols and consisted of ~43 min duration at 50% max speed (Supporting Table 2-3).

Exercise program evaluation: Mice (n=252 total mice; n=minimum 8 per sex, strain and exercise treatment combination; age: 8-10 weeks at start; born between March to August 2016 and February to March 2017) from CC002/Unc, CC013/GeniUnc, CC027/GeniUnc and CC037/TauUnc were housed with the same strains and sex in groups of three. The experiment was performed in five batches. Every strain, sex, exercise program combination was represented at least once in each batch for batches 1-4. In order to increase biological replicates, an additional batch (batch 5) was added. Batch 5 consisted of CC002/Unc females, CC027/GeniUnc females, CC037/TauUnc females, CC027/GeniUnc males, and CC037/TauUnc males (for females HIIT n=8 & MICT n=8 per strain; for males HIIT n=3 & MICT n=3 per strain). Across all batches, within each home cage, there was one mouse randomly assigned to each of the three exercise programs (HIIT, MICT, and no exercise [NE]) to avoid confounding cage effects with exercise program effects (with the exception of batch 5 which consisted of only HIIT and MICT programs). In five cases (for batches 1-4) more than three mice were group housed in one cage.

Mice completed five weeks of exercise training on the Exer-3/6 treadmills (Columbus Instruments, Columbus, Ohio). Mice assigned to both HIIT and MICT training protocols were acclimated to the treadmills for three days during the first week (Supporting Table 1).

After acclimation, HIIT and MICT mice completed four weeks of training, three times a week of their respective training protocol (Supporting Table 3). All training occurred in the morning and mice were randomly assigned to a treadmill lane each training day. Compliance was tracked over the full 15 days of exercise training and mice with 50% or more non-compliant days were removed from statistical analysis. A mouse was considered non-compliant if it refused or was not able to continue regular treadmill running despite extra stimulus from shock and/or prodding. Ten non-compliant mice were removed from the study (two CC002/Unc females HIIT; two CC002/Unc males HIIT; three CC013/GeniUnc females HIIT; two CC027/GeniUnc females HIIT; one CC027/GeniUnc female MICT). Five mice died during the experiment and were removed from the analysis (one CC013/GeniUnc female HIIT; one CC027/GeniUnc male MICT; one CC027/GeniUnc female HIIT; two CC037/TauUnc females HIIT).

4.3.2 Metabolic measurements

In all experiments, body composition was assessed (during mornings 0700-1200h) using whole body MRI (EchoMRI 3-in-1 Body Composition Analyzer, EchoMRI, Houston, TX) to determine fat and lean mass content (in grams) for each animal.

1) Voluntary Exercise Screen. Body mass and composition were measured immediately prior to the start of the experiment, and following the two-week experiment for all cohorts. Food was weighed prior to and after the experiment for the experimental cohort. To prevent variation in food intake due to food wastage, any food spillage was collected and weighed (*113*). Food intake for the control cohort was not tracked as a result of group housing.

2) CC002/Unc Model Validation. Body mass and composition were measured every two weeks over the 10 weeks of the experiment for all cohorts. Food was weighed at the same time points as body composition for both control and experimental cohorts.

3) Exercise Program Evaluation. Metabolic measurements and body mass and composition were measured prior to the start of the experiment and upon completion of exercise

training. Metabolic measurements were assessed by indirect calorimetry (PhenoMaster, TSE systems, Chesterfield, MO). For each batch, mice were randomly assigned to a calorimetry batch (A, B, or C) and calorimetry cage (1-24). Calorimetry data recorded included: oxygen consumption (VO_2; ml/h/kg), carbon dioxide output (VCO_2; ml/h/kg), activity (counts), food weight (g), water volume (ml) and heat production (kcal/h/kg). Respiratory exchange ratio (RER; VCO_2/VO_2) was calculated at each collection point. To acclimate to single housing, mice were individually housed for 24 hours prior to the start of the indirect calorimetry. Mice were then individually housed in calorimetry cages for 24 hours and data was recorded every ~50 minutes for each calorimetry cage. After, mice were returned to their assigned group housing.

4.3.3 Metabolic Calculations

Body fat and lean mass percentages were calculated relative to body mass at each time point in every experiment. Body mass response was calculated as [(Post-mass – pre-mass)/pre-mass] x 100. Body composition percentage response was calculated as [(post-measurement % - pre-measurement %)/pre-measurement %] x 100. Body mass and composition responses for individual mice in the experimental cohort (or HIIT, MICT) were adjusted to the mean strain (or strain-by-sex) responses in the control cohort (or NE) to account for experimental variability between cohorts (e.g. adjusted body mass response = [individual body mass response – control cohort strain mean body mass response]; adjusted body fat % response = [individual body fat % response – control cohort strain mean body fat % response]). Negative values represent a loss and positive values represent a gain in response to treatment. Food intake was calculated as the differential between baseline and post exercise food weights (g). Adjusted food intake was calculated as the food intake relative to the baseline body mass.

1) Voluntary Exercise Screen. Body mass and composition responses for both cohorts were calculated for the two weeks of treatment. Adjusted body mass and composition responses were calculated for the experimental cohort.

2) CC002/Unc Model Validation. For each mouse, cumulative body mass and composition responses were calculated for every experimental time point interval (1, 2, 3, 4; respectively: weeks 0-2, weeks 0-4, weeks 0-6 and weeks 0-8 of treatment). Adjusted cumulative body mass and composition responses were calculated for the experimental cohort at each time point interval. Adjusted food intake was calculated for each interval.

3) Exercise Program Evaluation. Body mass and composition responses were calculated for the five weeks of exercise program treatment. Adjusted body mass and composition responses were calculated for HIIT and MICT mice. Calorimetry data collected from 0700-1100h (day) and 1900-2300h (nocturnal) was used to calculate the following traits for each mouse for both day and nocturnal values (at baseline and post treatment): mean VO_2 intake, mean VCO_2 output, mean RER, total activity, mean heat production, food intake and water intake.

4.3.4 Statistical Analysis

All statistical analyses were performed in the R programming environment (https://cran.r-project.org). Descriptive statistics (mean, variance, coefficient of variance, standard deviation and standard error) were calculated for phenotypes across CC strain (Supporting Table 4-8). Pearson's correlations were calculated for the relationship between body mass and composition responses and potential mediators (physical activity traits and adjusted food intake). Heritability of body mass and composition response in the voluntary exercise screen was measured by inter-class correlation (icc) and the coefficient of genetic determination (cgd) (*135*). In order to determine potential mediators of the physiological responses between strains, we utilized a nested ANOVA framework to identify the set of

explanatory variables (e.g. treatment, genetic background, sex, metabolic responses), which robustly explain variation in the physiological responses.

4.3.5 Data Availability

All data are publically available at the Mouse Phenome Database (https://phenome.jax.org). CC strain information is located at: http://csbio.unc.edu/CCstatus/index.py (*55*, *59*).

4.4 Results

4.4.1 Screen for voluntary exercise-induced adverse body composition responders in aged CC females

In a screen of 13 CC strains, body mass and composition responses to two weeks of treatment (control or experimental) were measured in ~9 month old females. Exercise had a significant effect on body mass and composition responses ($p<1.0x10^{-05}$), but genetic background had a greater contribution (Table 4.1). All responses were heritable (cgd=31.0%, icc=47.4% body mass response; cgd=22.4%, icc=36.8% body fat response; and cgd=26.3%, icc=41.7% lean mass response). Furthermore, there were significant genetic background-by-treatment interactions on body mass, body fat and lean mass responses ($p=5.1x10^{-11}$, $p=3.5x10^{-07}$, and $p=1.9x10^{-09}$, respectively, Table 4.1).

In the control cohort (no exercise), three strains had a significant standard body mass response (lost body mass), two strains had a significant standard body fat response (lost body fat) and three strains had a significant standard lean mass response (gained lean mass). In the experimental cohort (voluntary exercise), six CC strains had a significant body mass loss, seven strains had a significant standard body fat response and nine CC strains had a significant standard lean mass response (Figure 4.1, Supporting Table 4). In order to determine the effect of exercise independent of aging, the responses in the experimental cohort were adjusted to the mean strain responses in the control cohort. In the experimental cohort, six strains had a significant standard adjusted body mass response (Figure 4.2A). Eight CC strains had a significant standard adjusted body fat response to

exercise treatment. CC072/TauUnc had no significant change in mean body fat as there was great individual variability within the strain. CC002/Unc had a significant adverse adjusted fat response (mean: 25.6%; range: -5.78% to +82.06%) (Figure 4.2B). Eight CC strains had a significant standard adjusted lean mass response and one strain, CC002/Unc, had a significant adverse adjusted lean mass response (Figure 4.2C, Supporting Table 4).

Other traits varied significantly by genetic background (running distance p=0.0025; duration p=0.018; speed $p<2.7x10^{-06}$; adjusted food intake p=0.0001) (Supporting Figure 1-2, Supporting Table 5). Therefore, phenotypic correlations were used to assess whether these potential mediators were associated with body mass and composition responses. Running duration was significantly and negatively correlated with body mass (r=-0.211) and body fat response (r=-0.223). Mean speed was significantly correlated with body fat response (r=-0.206). All physical activity traits had significant positive correlations with lean mass response (distance r=0.244, duration r=0.266, speed r=0.278). Adjusted food intake was significantly correlated with body mass response (r=0.755), although this is not surprising since adjusted food intake is calculated by dividing food intake by baseline body mass. Adjusted food intake was also significantly correlated with lean mass response (r=-0.665) and body fat response (r=0.411) (Table 4.2). While potential mediators were correlated with responses, genetic background had a more significant contribution than any mediator alone to body mass and composition responses. There was a significant genetic background-by-adjusted food intake interaction on fat response (p=0.018, Table 4.3).

4.4.2 Further characterization of CC002/Unc as a model for exercise-induced adverse fat response

To determine whether the CC002/Unc model of exercise-induced adverse body composition response extended beyond aged females, the robustness of the model was tested in younger females. The experiment was performed in ~4 month old females from CC002/Unc and CC037/TauUnc, the latter strain had a standard response at ~9 months.

There was no significant effect of treatment, genetic background or genetic background-by-treatment interaction on body mass or body composition response to two weeks of treatment in young CC002/Unc and CC037/TauUnc females. Despite the lack of statistical significance, the direction and magnitude of fat response to two weeks of exercise was consistent between young and old CC002/Unc females. Young CC002/Unc females in the experimental cohort had a 28.94% mean unadjusted gain of body fat (range: -14.7% to 70.7%) and a 19.73% mean adjusted gain of body fat (range: -23.9% to 61.5%). CC002/Unc mice had a lower baseline body fat percentage in young (mean 10.4%) compared to old (mean 16.8%) females. Additionally, adjusted food intake was greater in young females (mean 2.2) than old females (mean 1.85). Younger CC002/Unc mice ran approximately the same mean distance (4.51 km), but at lower mean speed (15.7 m/min) and greater mean duration (279.1 min) than old mice (mean distance 4.49 km, speed 17.8 m/min, duration 248.6 min) on days 11-12. Initial body fat response to two weeks of voluntary exercise in young CC037/TauUnc females was not the same direction or magnitude as body fat response observed in old females (young: 6.68% unadjusted, -1.93% adjusted; old: -43.64% unadjusted, -37.85% adjusted mean body fat response) (Supporting Figure 3, Supporting Table 4-6).

Cumulative body mass and composition response was also measured over eight weeks of treatment to assess the effect of additional exercise on physiological responses. For cumulative body mass response, there was no significant effect of time point, treatment, genetic background and all their interactions. There was a significant additive effect of time point and genetic background on cumulative fat response ($p=0.0018$) and cumulative lean mass response ($p=0.0471$) demonstrating body composition responses varied by time point and genetic background. There was no significant effect of treatment on cumulative fat or lean mass response over time. Young experimental CC002/Unc females had an increase in adverse fat response over the eight weeks of wheel access with

a mean unadjusted cumulative fat response of 28.9, 20.3%, 25.6% and 32.0% for weeks 0-2, 0-4, 0-6 and 0-8. In the CC002/Unc control cohort, mean unadjusted cumulative fat response increased over time intervals (9.2%, 16.9%, 23.6% and 30.9%). CC037/Tau mean cumulative fat response fluctuated over eight weeks in experimental females (6.68%, -5.6%, 17.9% and 0.19% for weeks 0-2, 0-4, 0-6 and 0-8) and in control females (8.65%, -0.77%, 19.04% and 14.8%) (Figure 4.3, Supporting Figure 4, Supporting Table 6).

Total distance and duration in the CC002/Unc experimental females decreased during weeks 1-4 to weeks 4-8. Mean speed remained stable over eight weeks of wheel access. CC037/TauUnc experimental females had stable running duration, but increased in mean speed and distance during the eight weeks of exercise (Supporting Figure 5, Supporting Table 7). Adjusted food intake increased during the first two weeks of wheel access in both cohorts and strains relative to food intake during single housing acclimation. Fluctuations in food intake were observed over the course of treatment in both cohorts and strains (Supporting Figure 6). The fluctuations in physical activity levels and food intake over the course of treatment are important since energy expenditure and energy intake contribute to body mass and composition responses.

4.4.3 Effect of exercise program on body composition response across both sexes and different genetic backgrounds

We selected four CC strains (see materials and methods) to measure the effects of genetic background, sex and two types of forced exercise programs (HIIT and MICT) on exercise-induced metabolic responses. Overall, exercise programs, HIIT and MICT, significantly reduced body mass relative to NE programs ($p=6.82x10^{-12}$; HIIT-NE $padj<1.0x10^{-07}$; MICT-NE $padj=3.0x10^{-07}$). Body mass response in mice exposed to HIIT was not significantly different from body mass response in mice exposed to MICT. Genetic background-by-exercise program had a significant interaction on body mass response ($p=0.001$) indicating that body mass response varied by exercise program dependent on

genetic background. Body mass response was not significantly modified by sex; thus, both males and females had similar mass response (Figure 4.4A, Figure 4.5A, Supporting Table 8).

Exercise programs were suggestive of body fat percentage response (p=0.063), but the interaction between exercise program and sex had a significant effect on body fat percentage response (p=0.039). Specifically, body fat response varied between HIIT and MICT programs among females (padj=0.01). Furthermore, there was a significant genetic background-by-exercise program-by-sex interaction effect on body fat percentage response (p=0.0002). CC002/Unc females had a significant standard adjusted fat response to HIIT (mean -24.37%, p=0.002) but no significant change in adjusted fat response to MICT (mean -1.0%, p=0.906). CC027/GeniUnc females had a significant adverse adjusted fat response to MICT (mean 38.01%, p=0.028) but no significant adjusted fat response to HIIT (mean 6.55%, p=0.571). Unlike females, males demonstrated similar fat responses to both HIIT and MICT programs (Figure 4.4B, Figure 4.5B, Supporting Table 8).

Exercise program (p=0.011), and the interaction between exercise program and sex (p=0.0109), had a significant effect on lean mass percentage response. Again, the differences in lean mass response to HIIT and MICT programs in females were driving this significant interaction (padj=6.97×10^{-04}). There was a significant genetic background-by-exercise program-by-sex interaction on lean mass percentage response (p=0.009). CC027/GeniUnc females had a significant adverse lean mass response to both HIIT (adjusted mean -1.70%, p=0.004) and MICT (adjusted mean -2.78%, p=0.0002) programs (Figure 4.4C, Figure 4.5C, Supporting Table 8).

All baseline metabolic variables (RER, VO_2, VCO_2, activity, heat, food intake and water intake) during both nocturnal and daytime were under genetic control ($p<0.05$). Baseline metabolic variables were not more predictive than genetic background for body mass and composition responses to exercise programs. However, baseline nocturnal

activity and nocturnal water intake were as predictive as genetic background for body fat response (Table 4.4). Pearson's correlations revealed baseline nocturnal activity was positively and significantly correlated with body fat response (r=0.185, p=0.0035). These observations indicate baseline nocturnal activity levels are predictive of fat response to exercise. Whereas, baseline nocturnal water intake was not significantly correlated with body fat response (r=-0.067, p=0.29) indicating that water intake is not likely casual of body fat response to exercise and instead may be confounded. After exercise training, metabolic variables during both nocturnal and day time were under genetic control ($p<0.05$) with the exception of nocturnal RER (p=0.459), day VO2 (p=0.069) and day heat production (p=0.056). In some cases post metabolic traits were just as predictive as genetic background for body fat response (Table 4.4). This observation is likely due to the fact that the metabolic traits are genetically regulated.

4.5 Discussion

4.5.1 CC002/Unc is a model for exercise-induced adverse body composition response

One previous study that reported an exercise-induced adverse response in 17% of partially inbred mice (*66*), only examined one individual mouse per genotype in the pre-CC population, which limited the ability to assess genetic control of this trait. In contrast, our study used replicate inbred animals from the CC population with both sedentary control and voluntary exercise cohorts. The most significant finding was that CC002/Unc, one of the 13 CC strains screened in the initial study, had a voluntary exercise-induced adverse body composition response among old females. It is possible that CC002/Unc overcompensates with food intake in response to exercise driving the observed adverse response, but this may not be only factor or driving factor contributing to the adverse response. Food intake varied significantly by genetic background and there was a significant genetic background-by-adjusted food intake interaction on fat response. Thus, these findings suggest genetic

background, including food intake, are driving the adverse response in old CC002/Unc females. For example, a standard response was observed in CC042/GeniUnc, which had similar levels of adjusted food intake and physical activity. Furthermore, a standard response occurred in CC039/Unc, even though CC039/Unc had similar adjusted food intake levels but lower physical activity levels than CC002/Unc.

Young CC002/Unc females had consistent direction and magnitude of fat response to two weeks of voluntary exercise with old CC002/Unc females; however the treatment effect was not statistically significant at ~4 months. This is likely due to the variability present in the control and experimental treatment groups leading to overlap in the body composition response measurements between the groups in the younger mice. Interestingly, there was higher variance in body fat responses at ~4 months than at ~9 months. The higher levels of variability could be due to the ongoing alterations in body composition occurring at this age. The younger CC002/Unc females had a lower baseline body fat percentage than the older mice (mean 10.4% and 16.8%, respectively). This is not surprising, as it is known that aging typically results in increased body fat, alterations in body composition and redistribution of fat (*68*, *134*). The younger CC002/Unc females could have been undergoing alterations in body composition associated with aging over the eight weeks of treatment. Other factors could have contributed to the higher level of variability in the young mice and lack of significant effect. Unlike the older control mice, the young control cohort was housed in the same vivarium and cages as the experimental cohort. The control cohort for the young CC002/Unc and CC037/TauUnc females could have been exposed to stress or other factors impacting body composition responses confounded in the experiment.

Young CC002/Unc females exposed to forced exercise programs (HIIT and MICT) did not have an adverse body composition response, as observed in the old and young CC002/Unc females exposed to voluntary exercise. Instead, CC002/Unc females had a

significant standard adjusted fat response to HIIT and no significant change in adjusted fat response to MICT. It is important to note the forced endurance programs were performed three times a week over the course of four weeks and totaled ~480m per session for CC002/Unc females; whereas, CC002/Unc females in the voluntary exercise treatment were running ~4.5km on days 11-12 of wheel access. Thus, CC002/Unc females exposed to voluntary exercise had a greater frequency, duration and distance and varying intensity of exercise over two weeks than the females exposed to forced exercise programs. In rodents, both forced and voluntary exercise programs are used as a method to measure exercise abilities, exercise performance and other exercise-related traits. Voluntary exercise is a self-rewarding behavior and a complex trait that not only captures physical activity habits but also represents engagement in neural and physiological mechanisms required for the behavior (*51*). While both forced and voluntary depend on common variables (e.g. physiological systems, organ function), there are distinct factors to each program including: psychological desire to run, fear, pain perception, shock avoidance, etc (*51*, *78*, *116*). Factors unique to voluntary exercise and their interaction with the CC002/Unc genetic background could be driving the observed exercise-induced adverse body composition response. In conclusion, CC002/Unc females are a novel model mouse strain for voluntary exercise-induced adverse body composition response. These findings strongly suggest that this response is due to unique physiological and metabolic conditions under genetic control.

4.5.2 Females have different body composition responses depending on exercise program and genetic background

Body composition responses to HIIT, MICT and NE programs were examined in both males and females in four different genetic backgrounds. There was a significant genetic background-by-sex-by-exercise program interaction on both body fat and lean mass response to program. Specifically, females responded differently to HIIT and MICT

programs, and the response further varied by genetic background. This finding indicates genetic background, sex and exercise factors (e.g. intensity, duration) should be considered in design of exercise programs for humans. From this study, additional CC strains as potential models of adverse body composition responses to exercise were identified. In particular, both CC027/GeniUnc and CC037/TauUnc females had a significant adverse adjusted fat response to MICT programs. Additional studies utilizing these identified models of adverse responders and non-responders will be necessary to identify underlying mechanistic pathways and genetic biomarkers predicting response to particular exercise programs. These studies will be important for informing the design of effective exercise programs for particular genetic populations and individuals.

In addition, the current study demonstrated that baseline metabolism, including RER, did not predict body mass and composition response. Genetic background instead predicted body mass and composition response in the four CC strains. RER is commonly used to indirectly determine the contribution of carbohydrates and lipids to energy expenditure. The contribution of these fuels can be affected by diet, muscle glycogen presence, exercise factors (intensity, duration) and training status (*111*, *120*). Individual variation in substrate oxidation during exercise has been observed in both trained and untrained individuals. In this study, baseline RER was not associated body composition response to exercise supporting prior findings in humans (*118*).

4.5.3 Within strain individual variability was observed across phenotypes

Even though these studies were performed in mouse strains that were almost fully inbred (*55*), variance in all phenotypes and variability in levels of variance across CC strains was observed (Supporting Table 4-8). This is not surprising since individual variability in exercise-related phenotypes in inbred strains has previously been observed (*66*, *116*). In particular, large individual variability in body fat percentage response, physical activity traits, and adjusted food intake occurred within CC072/TauUnc. It is possible that the

differences in adjusted food intake, in combination with the differences in physical activity levels were driving the observed differences in fat response. In addition, CC072/TauUnc may be more susceptible to environmental influences (e.g. life history) or epigenetic influence (e.g. *in utero* environment) that were unaccounted for in the study design. The large individual variability observed in CC072/TauUnc was unlikely caused by segregating regions of the strain's genome since the only 0.8% of the genome was segregating in this strain two years ago (*55*).

4.5.4 Concluding Remarks

Despite significant health burdens and public interest in understanding and optimizing exercise regimes within the human population, relatively little is known about the genetic architecture and control of the diverse behavioral, metabolic and physiological responses that converge to drive successful response to exercise. This study used mouse strains from the CC population to identify and develop mouse model(s) of exercise-induced adverse fat responders. Genetic variation in the CC resulted in phenotypic diversity in exercise-related traits. The presence of extreme outliers in body composition response to exercise in a small subset of CC strains, further supports that the CC population is a rich source for new models of human traits (*54*, *64*).

CC002/Unc was identified as a model for adverse body composition response under certain conditions (females, voluntary exercise, significance of effect varies by age). Voluntary exercise-induced body mass and composition responses were driven by genetic background independent of physical activity levels further supporting the importance of genetic background on exercise-induced responses (*126*). Lastly, this study demonstrated a significant genetic background-by-sex-by-exercise program interaction on body composition response. Specifically, HIIT elicited more beneficial body composition responses than MICT programs in females dependent on genetic background. It will be vital to consider genetic

background, sex and age in the design of effective exercise programs in the human populations.

4.6 Figures

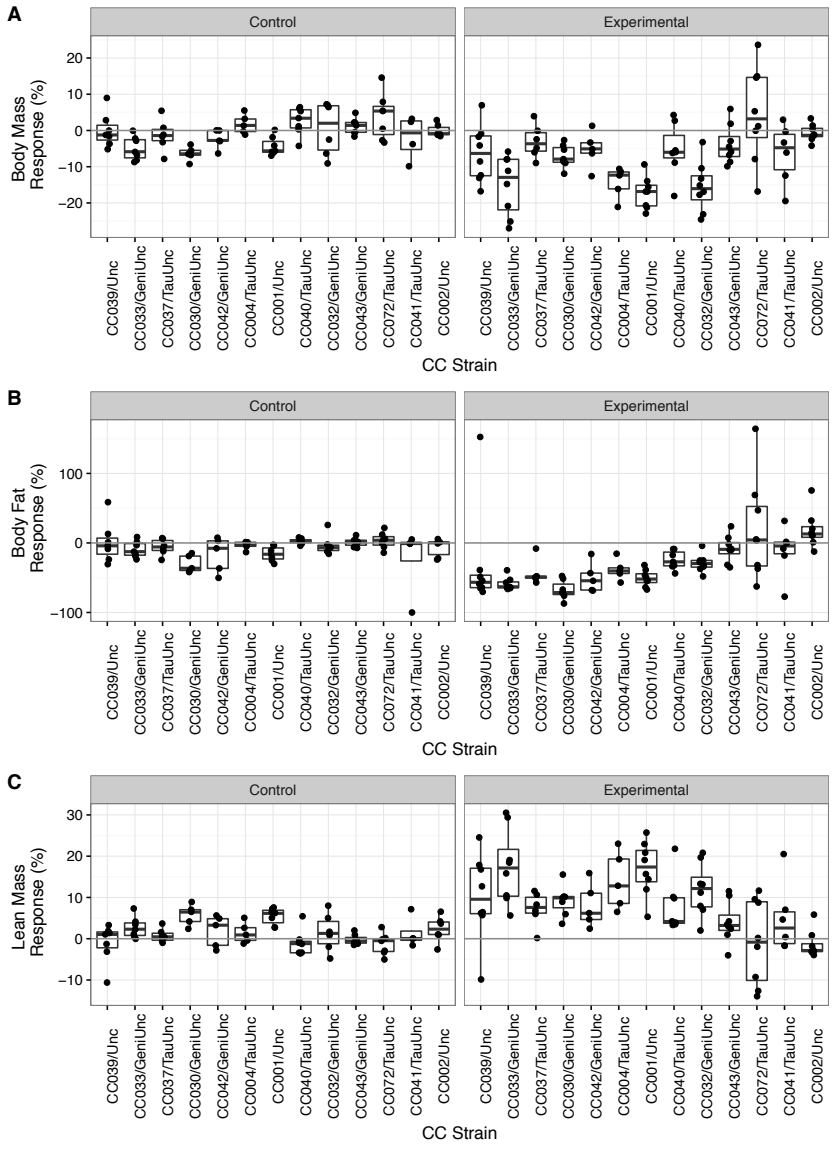


Figure 4.1. Body mass and composition response to two weeks of treatment in aged females across 13 CC strains. Body mass response (%) (A), body fat percentage response (%) (B) and lean mass percentage response (%) (C) over two weeks of treatment in both control and experimental treatment cohorts. Each dot represents an individual female mouse. Strains are ordered by median adjusted fat response.

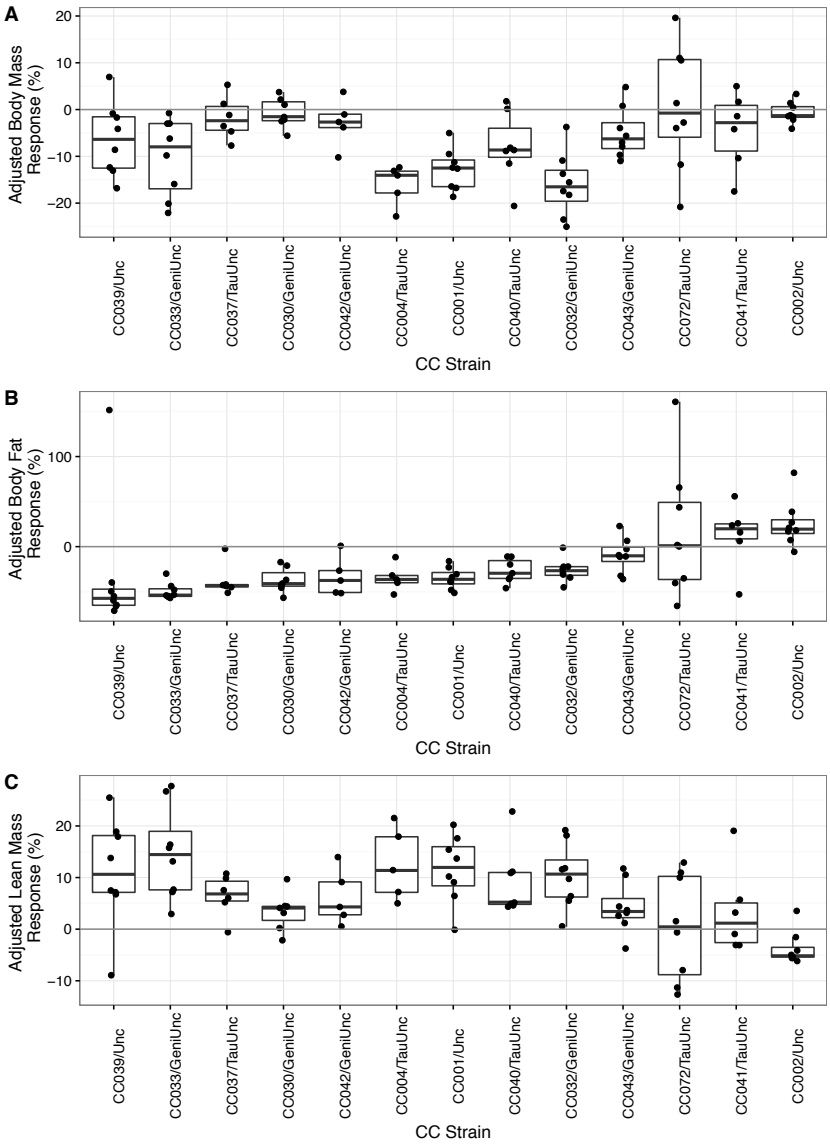


Figure 4.2. Exercise-induced body mass and composition response in aged females across 13 CC strains. Adjusted body mass response (%) (A), adjusted body fat percentage response (%) (B) and adjusted lean mass percentage response (%) (C) to two weeks of wheel access in the experimental cohort. Responses are adjusted to strain mean responses in the control cohort. Each dot represents an individual female mouse. Strains are ordered by median adjusted fat response.

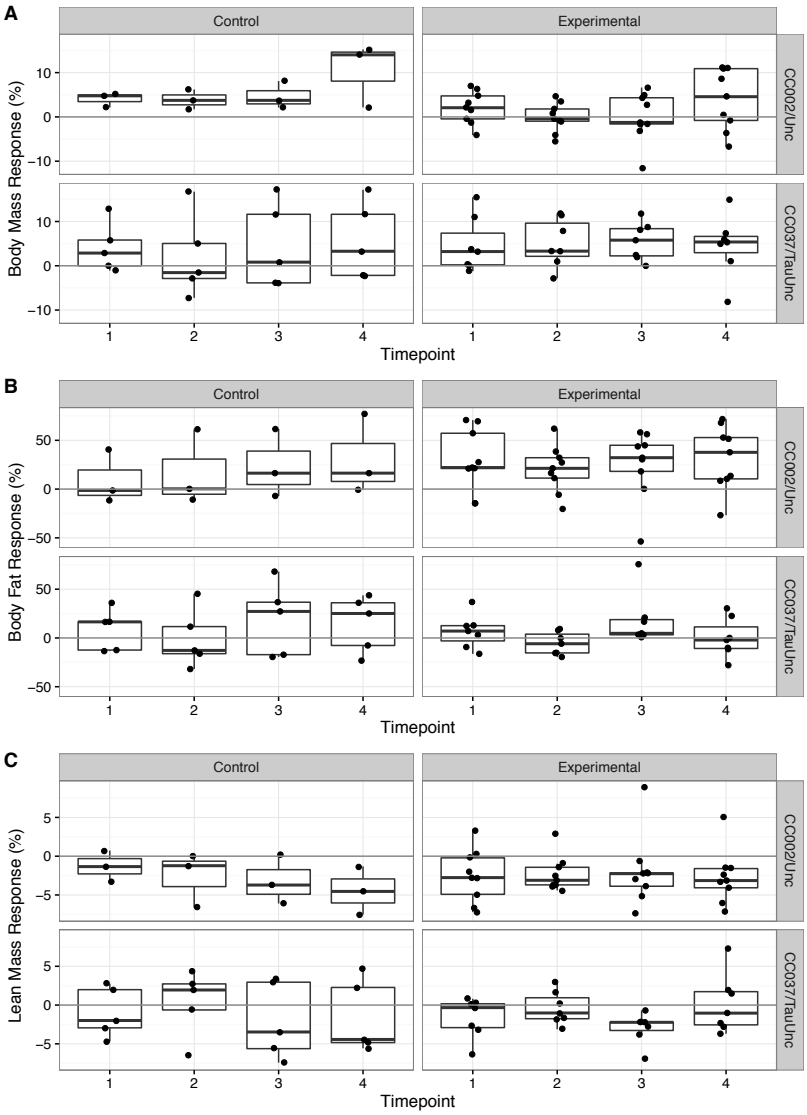


Figure 4.3. Cumulative body mass and composition response to treatment over 8 weeks in CC002/Unc and CC037/TauUnc. Body mass response (%) (A), body fat percentage response (%) (B) and lean mass percentage response (%) (C) for each two week interval of the experiment in both control and experimental treatment cohorts. Each dot represents an individual female mouse. Each response is calculated for a two week interval. Responses are represented as timepoint intervals: timepoint 0 (single housing acclimation), 1 (weeks 0-2 of treatment), 2 (weeks 2-4 of treatment), 3 (weeks 4-6 of treatment) and 4 (weeks 6-8 of treatment).

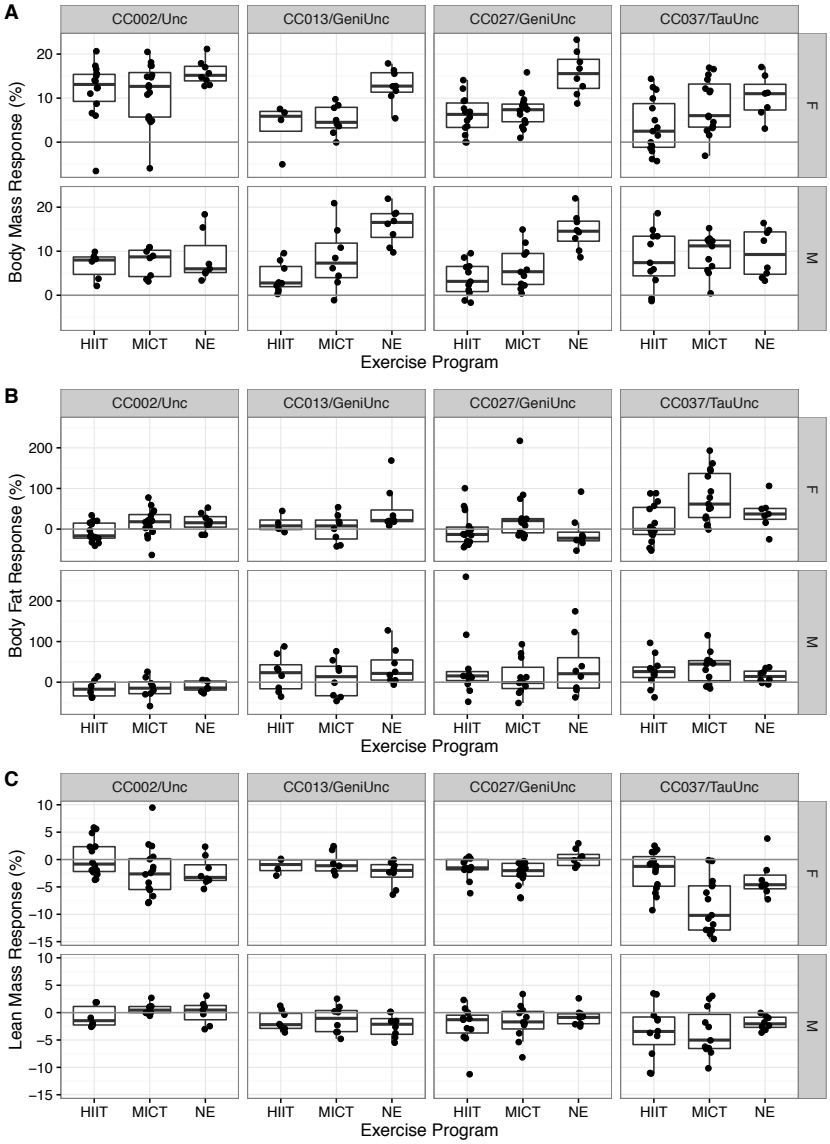


Figure 4.4. Body mass and composition response to exercise programs in four CC strains. Body mass response (%) (A), body fat percentage response (%) (B) and lean mass percentage response (%) (C) to five weeks of exercise program training. Top panels are only female mice (F) and bottom panels are only male mice (M). Each dot represents an individual mouse.

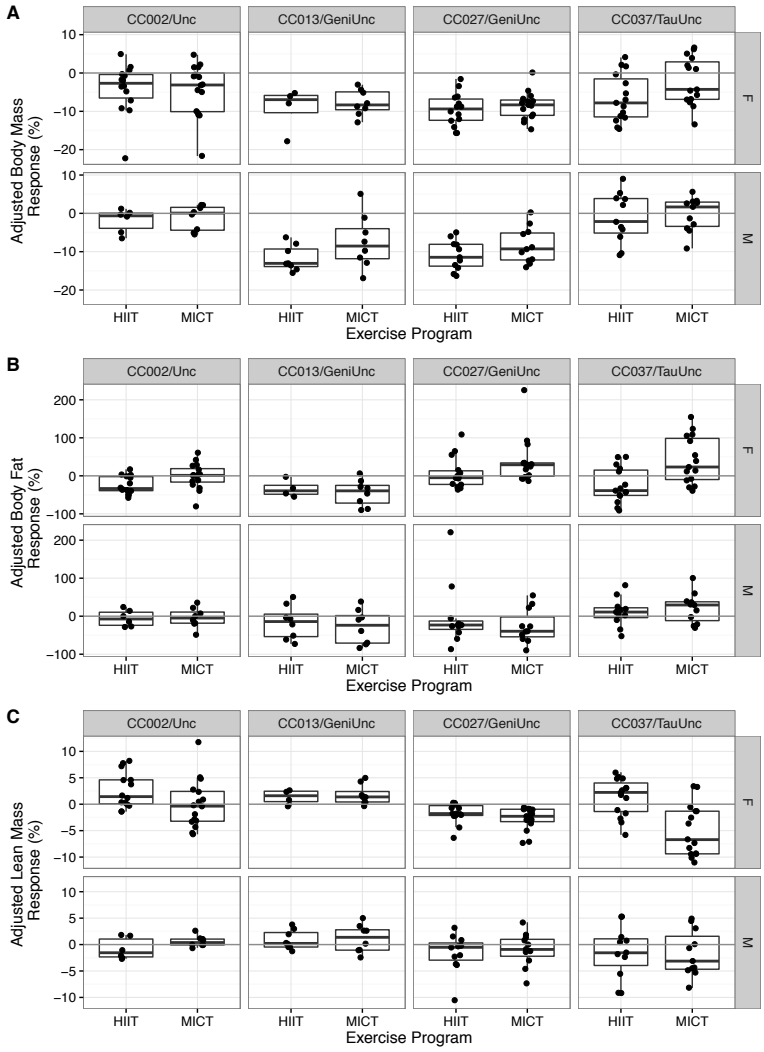


Figure 4.5. Adjusted body mass and composition response to exercise programs in four CC strains. Adjusted body mass response (%) (A), adjusted body fat percentage response (%) (B) and adjusted lean mass percentage response (%) (C) to five weeks of exercise program training. Exercise programs include HIIT (high intensity interval training) and MICT (moderate intensity continuous training). Responses are adjusted to mean responses in matching strain and sex NE (no exercise) cohort. Top panels are only female mice (F) and bottom panels are only male mice (M). Each dot represents an individual mouse.

4.7 Tables

Table 4.1. P-values from nested ANOVA analysis of treatment cohort and genetic background effect on body mass and composition response. Base models included treatment cohort as a fixed effect. Additive models included treatment cohort and genetic background as an additive effect. Full models included treatment cohort effect, genetic background effect and their interaction.

		base vs additive model	base vs full model	additive vs full model
Response	**Body Mass**	1.35x10-09	5.14x10-11	8.18x10-04
	Body Fat %	1.19x10-06	3.53x10-07	0.011
	Lean Mass %	7.7x10-08	1.91x10-09	8.74x10-04

Table 4.2. Pearson's correlations between body mass and composition response and potential mediators. Significant correlations ($p<0.05$) are highlighted in grey.

Responses	Body Mass	Body Fat %	Lean Mass %
Distance (Days 11-12)	-0.144	-0.202	0.244
Duration (Days 11-12)	-0.211	-0.223	0.266
Speed (Days 11-12)	-0.17	-0.206	0.278
Adjusted Food Intake	0.755	0.411	-0.665
Food Intake	0.584	0.514	-0.581

Table 4.3. P-values from nested ANOVA analysis of potential mediators and genetic background on body mass and composition response within the experimental cohort. Base models included a mediator as a fixed effect. Additive models included a mediator and genetic background as additive effects. Full models included a mediator effect, genetic background effect and their interaction.

		Mediator: distance		
		base vs additive model	**base vs full model**	**additive vs full model**
Response	**Body Mass**	2.33x10-08	3.70x10-07	0.095
	Body Fat %	7.16x10-06	0.0012	0.807
	Lean Mass %	4.325x10-07	3.56x10-05	0.42
		Mediator: duration		
		base vs additive model	**base vs full model**	**additive vs full model**
Response	**Body Mass**	5.35x10-08	3.78x10-07	0.06
	Body Fat %	8.27x10-06	0.0019	0.889
	Lean Mass %	1.52x10-06	4.66x10-06	0.056
		Mediator: speed		
		base vs additive model	**base vs full model**	**additive vs full model**
Response	**Body Mass**	4.697x10-08	9.53x10-07	0.126
	Body Fat %	2.633x10-05	0.0051	0.924
	Lean Mass %	5.426x10-07	1.39x10-05	0.219
		Mediator: adjusted food intake		
		base vs additive model	**base vs full model**	**additive vs full model**
Response	**Body Mass**	4.45x10-08	9.30x10-06	0.498
	Body Fat %	6.09x10-07	5.90x10-07	0.018
	Lean Mass %	1.606x10-09	5.19x10-06	0.905

Table 4.4. Analysis of metabolic traits and genetic background on body mass and composition response. P-values from partial F tests of a nested ANOVA analysis was performed comparing the best fit model with each metabolic variable subbed in for genetic background vs the best fit model including each metabolic variable. Best fit model are as follows: exercise program*genetic background (body mass response) or exercise program*sex*genetic background (body fat and lean mass response). Grey boxes represent non-significant p-values (p>0.05).

Baseline Metabolic Trait	Body Mass Response	Body Fat % Response	Lean Mass % Response	Post Metabolic Trait	Body Mass Response	Body Fat % Response	Lean Mass % Response
RER nocturnal	0.0048	0.0034	5.15x10-05	**RER nocturnal**	0.0176	0.0004	5.558x10-10
RER day	0.0068	0.0034	0.0001	**RER day**	0.0150	0.3552	0.0862
VO2 nocturnal	0.0017	0.0002	0.0001	**VO2 nocturnal**	9.357x10-05	0.0024	9.512x10-05
VO2 day	0.0171	0.0197	0.0026	**VO2 day**	0.0035	0.0092	8.007x10-05
VCO2 nocturnal	0.0005	0.0044	0.0010	**VCO2 nocturnal**	0.0057	0.0005	6.387x10-07
VCO2 day	0.0192	0.0121	9.226x10-05	**VCO2 day**	0.0144	0.1937	0.0054
Heat nocturnal	0.0013	0.0003	0.0017	**Heat nocturnal**	0.0003	0.0018	4.223x10-05
Heat day	0.0188	0.0208	0.0002	**Heat day**	0.0042	0.0194	0.0001
Activity nocturnal	0.0266	0.0680	0.0006	**Activity nocturnal**	0.0290	0.0200	0.0051
Activity day	0.0009	0.0170	6.118x10-07	**Activity day**	0.0074	0.0221	2.351x10-05
Food intake nocturnal	0.0394	0.0100	4.452x10-05	**Food intake nocturnal**	0.2746	0.0003	1.174x10-07
Food intake day	0.0007	0.0209	0.0041	**Food intake day**	0.0410	0.0639	0.0233
Water intake nocturnal	0.0072	0.1074	0.0007	**Water intake nocturnal**	0.0040	0.1490	0.0006
Water intake day	0.0006	0.0064	0.0014	**Water intake day**	0.0405	0.0622	0.0042

CHAPTER 5: CONCLUSIONS

Exercise is often recommended as a health preventative and treatment. Current guidelines recommend the same exercise programs for every U.S. adult regardless of age, sex or genetics (*21*). These “one size fits all” public health recommendations for exercise and other lifestyle interventions have been insufficient for the diverse human population. A large diversity of response types, including adverse responders, has been observed across a broad range of traits to the recommended exercise program. In addition to the large heterogeneity in exercise-induced responses, factors contributing to heterogeneity remain unknown. In order to establish effective physical activity guidelines, the driving mechanism and sources of individual variation need to be identified (*16*, *136*).

5.1 Contributions to advancement of the field

While large individual variation in exercise-induced responses is observed in the human population, it is difficult to determine sources of variation since it is hard to control, track and measure potential contributing factors (e.g. genetic background, environmental variables, etc). My thesis utilized a multi-parental mouse population due to the ability to standardize measurements, regulate environmental variables and determine contributing cofactors to exercise-induced responses across a wide range of genetic backgrounds. My thesis aimed to establish mouse models of both exercise-induced positive and adverse fat response and to use these models to determine the role of genetic background, sex, and type of exercise on body composition.

This complete work has provided multiple mouse models for exercise-induced standard and adverse body composition response, highlighted the importance of genetic background in exercise-induced metabolic responses and demonstrated the complexity of

exercise-induced responses. Additionally, this work has demonstrated that body composition responses vary by the type of exercise program, specifically in comparing HIIT and MICT programs, and these responses are also dependent on sex and genetic background. CC002/Unc was identified as a mouse model of exercise-induced adverse body composition response to voluntary exercise in females and CC027/GeniUnc as a model of exercise-induced adverse body composition response to voluntary exercise in aged females and HIIT programs in young females. In addition, CC013/GeniUnc was identified as a model of exercise-induced standard body composition response to multiple exercise programs and in both sexes. The presented findings further support the utility of the CC population for identifying extreme phenotypic outliers in exercise response that can serve as natural models of human traits in response to exercise.

Each CC strain is inbred allowing for biologic replicates, which enables studies to be repeated and allows for individual variables to be tested across multiple studies (*54*, *55*). There are also extensive genomic and computational tools for analysis of the CC strains (*59*) enabling studies to connect genotype to phenotype in the CC. Future studies using these models will assist in identifying genes and metabolic pathways (identified through different 'omics' approaches) contributing to both adverse responders and standard responders to exercise. These models provide the opportunity to further determine the cofactors contributing to exercise-induced responses. Furthermore, these models will enable studies to test potential interventions and treatments for reversal of adverse fat response.

5.2 Personalized medicine to personalized exercise approach

Historically, medicines and treatments were designed with the intention of treating everyone with few exceptions. It is now accepted that diseases are heterogeneous and each individual's disease is unique. Personalized medicine aims to identify specific factors, such as 'omics' or environment that vary across individuals with the same disease. This will

enable therapeutic agents or preventions to be specifically designed to an individual or a defined population that shares the same underlying factors. Currently, the personalized genomic medicine approach has been successfully utilized in the treatment of diseases in the fields of oncology, psychiatry, and cardiovascular conditions. There are currently three main objectives to personalized medicine: identifying polymorphisms contributing to certain adverse drug events, no response or outlier drug responses; identifying potential biomarkers for specific diseases; and assessing clinical response in combination with targeted therapeutics (*137*). Although, the majority of efforts to this date have focused on understanding heterogeneity in drug responsiveness among human populations (*138*). Personalized medicine has the potential to extend beyond drug response and is applicable to other forms of health management, such as exercise or diet response.

Individual variation in responses to standard exercise programs indicates that there should be no single standard exercise guidelines, but instead exercise should be designed and utilized in the same personalized medicine approach that is used to treat the diseases mentioned above (*48*, *138*). Each individual is unique due to genetic background, environmental factors, epigenetics, gene-by-environment interactions and more. All of these modify nutritional requirement, metabolism, predisposition to disease and response to drug or lifestyle intervention (*136*). Therefore, personalized exercise programs should be tailored to each individual based on defined factors (e.g. genetics, age, sex, prior training, etc). Establishment of individual tailored approaches for exercise prescription will improve exercise-induced health benefits across the population, minimize side effects and optimized efficacy (*8*, *16*, *136*, *139*). Additionally, effective policies, personalized lifestyle interventions and exercise guidelines designed to promote healthier body composition and lifestyle not only will improve the obesity epidemic, but will also have an enormous economic benefit on society (*3*, *140*).

5.3 Next steps for development of personalized exercise programs

Diagnostics of adverse responders and effective intervention options need to be developed in order to implement personalized exercise programs. Additionally, ways to ensure education and adherence to programs need to be developed alongside personalized exercise programs.

5.3.1 Identifying diagnostics of responder type

The genetic and mechanistic pathways driving exercise-induced adverse body composition response remain unknown. Determining mechanistic pathways, driving factors and predictors of adverse, standard and non-responders will be vital for the development of diagnostics for response type. Multi-omic profiling, including genomic and metabolomics profiling of blood or other tissue, can be utilized for discovery of genetic or metabolic biomarkers. Biomarkers predictive of adverse responders and biomarkers for intervention program selection need to be established. Once these biomarkers are scientifically validated, assays and other tools can be developed and applied in a clinical setting to identify individuals more or less receptive to each exercise program prior to initiating exercise (*16*, *29*, *47*, *139*, *141*). Development of interventions needs to occur alongside the identification and development of companion biomarkers for classification of responder type.

5.3.2 Intervention development and optimization

Future studies aimed at intervention development for low and adverse responders are necessary for establishing personalized treatments to promote healthy outcomes. Exercise is one form of intervention that can be varied by multiple factors (*45*) and induces individual variation in responses. Knowledge of sources of heterogeneity in exercise responses and a physiological understanding of mechanisms regulating exercise responsiveness are necessary for designing interventions and informing clinical trials to evaluate personalized strategies (*138*). Exercise varies by modality (type), intensity,

frequency, duration of exercise and other factors. In order to design effective exercise interventions for specific individuals and populations, additional studies will be important for identifying the optimal mode, frequency, intensity and duration of exercise and intervention length for inducing beneficial responses (*22*, *29*, *45*).

Other forms of intervention, such as dietary and nutrition based interventions, should be studied. Food intake is often a compensatory behavior in response to exercise and can alter exercise-induced body composition responses. Studies have demonstrated food composition can alter metabolism mechanism and adaptations to exercise (*22*) and exercise combined with diet interventions significantly reduce body mass (*142*). Evaluation of dietary interventions in combination with exercise will be vital for the development of personalized lifestyle approaches aimed to improve body composition and reduce chronic disease risk.

5.3.3 Future studies utilizing Collaborative Cross models

Based on the need to develop personalized exercise programs to ensure beneficial health outcomes, CC mouse models will be valuable for performing three future studies: biomarker identification, mechanistic pathway and intervention design.

First, biomarkers need to be identified in both CC model strains for exercise-induced adverse body composition response and standard response. Metabolomics (or other omics platforms) performed in model strains prior to exercise exposure will enable identification of prognostic biomarkers unique to either standard or adverse body composition responders. These prognostic biomarkers may be shared between adverse responder strains or may be unique to one adverse responder model strain. Next studies utilizing multiple technologies (metabolomics, RNA-sequencing, etc) can be used to identify genetic and molecular pathways driving adverse responses in CC model strains. Additionally, biomarkers can be monitored before, during and after exercise interventions to develop an understanding of

how the prognostic biomarkers respond to each intervention. Lastly, future studies utilizing the CC model strains should focus on development of intervention. Studies should examine exercise interventions alone, diet interventions alone and synergistic exercise-diet combinations in CC models before and after adverse responses have occurred. Exercise interventions tested should vary in mode (forced, voluntary), duration, intensity and intervention length. Diet interventions tested should be comparable to diets commonly consumed and studied in the human population such as ketogenic, Mediterranean and paleo. These studies will be vital for the advancement of precision medicine in the human population.

5.4 Commercialization and challenges to consider

The ultimate goal is to be able to translate scientific findings to inform the design of effective physical activity guidelines that improve health responses and adaptations to exercise across multiple populations. During the translation process, there will be opportunities for commercialization of genetic tests, biomarker assays and/or other tools. There are commercially available genetic tests (direct to consumer) for training program guidance and athlete selection. Thirty-nine companies exist today with direct to consumer genetic tests for exercise performance and injury (*143*). However, these commercial tests face numerous issues and challenges due to the rigor, robustness and reproducibility of scientific findings supporting the products. These challenges include reproducibility across studies and populations, limited sample size, small effect sizes and weak associations of identified genes with phenotypes. In many cases, identified association between genotype and training response findings have not been replicated in additional studies. Additionally, there are many unknowns in the complex relationship between genotype and training response. For example, it is unknown whether genetic variation associated with variation in exercise response will remain stable across different exercise programs (e.g. types of exercise, duration, intensity) and populations (e.g. genetically different populations, healthy

vs diseased populations, etc) (*45*, *139*). Furthermore, it is unlikely that one genetic variant will explain a large portion of the trait. For example, ACTN3 genotype is associated with power performance, but only predicts a small portion of athletic performance. Additionally, ACTN3 genotype is not predictive or necessary for elite-level competition (*139*). Thus, the identification of multiple genetic variants predictive of training responses and with strong scientific evidence will be necessary for the development of genetic screening technologies for adverse responders.

5.5 Implementation of personalized medicine in healthcare

With the emergence of personalized exercise programs and other interventions it will be vital to incorporate the healthcare infrastructure necessary for successful implementation and treatment of patients. Proper healthcare infrastructure will enable large datasets to accurately model the biological complexity and incorporate patient specific factors (e.g. history, health status, etc). Computational infrastructure and complex datasets will be important for precise and predictive outcomes personalized to the individual patient (*137*). Additionally, health care professionals will need to be trained on how to design personalized exercise programs and trained to consult individuals identified as low or adverse responders to ensure these individuals engage in the most effective exercise program and experience health benefits (*8*, *139*).

Implementation of personalized lifestyle medicine extends beyond the health care and into public policy and regulatory branches. For successful implementation the following changes must occur: policy changes for system-wide adoption, alterations in regulatory policy (e.g. FDA), reimbursement policy must be established (e.g. who will pay for the costs of tests and treatments), legislative initiatives (e.g. genetic privacy laws), etc (*144*).

APPENDIX A: SUPPLEMENTAL MATERIALS

A-1 Chapter 2: Supporting tables

Supporting Table 1. Estimated marginal means and standard errors corresponding to tests presented in Table 2.1.

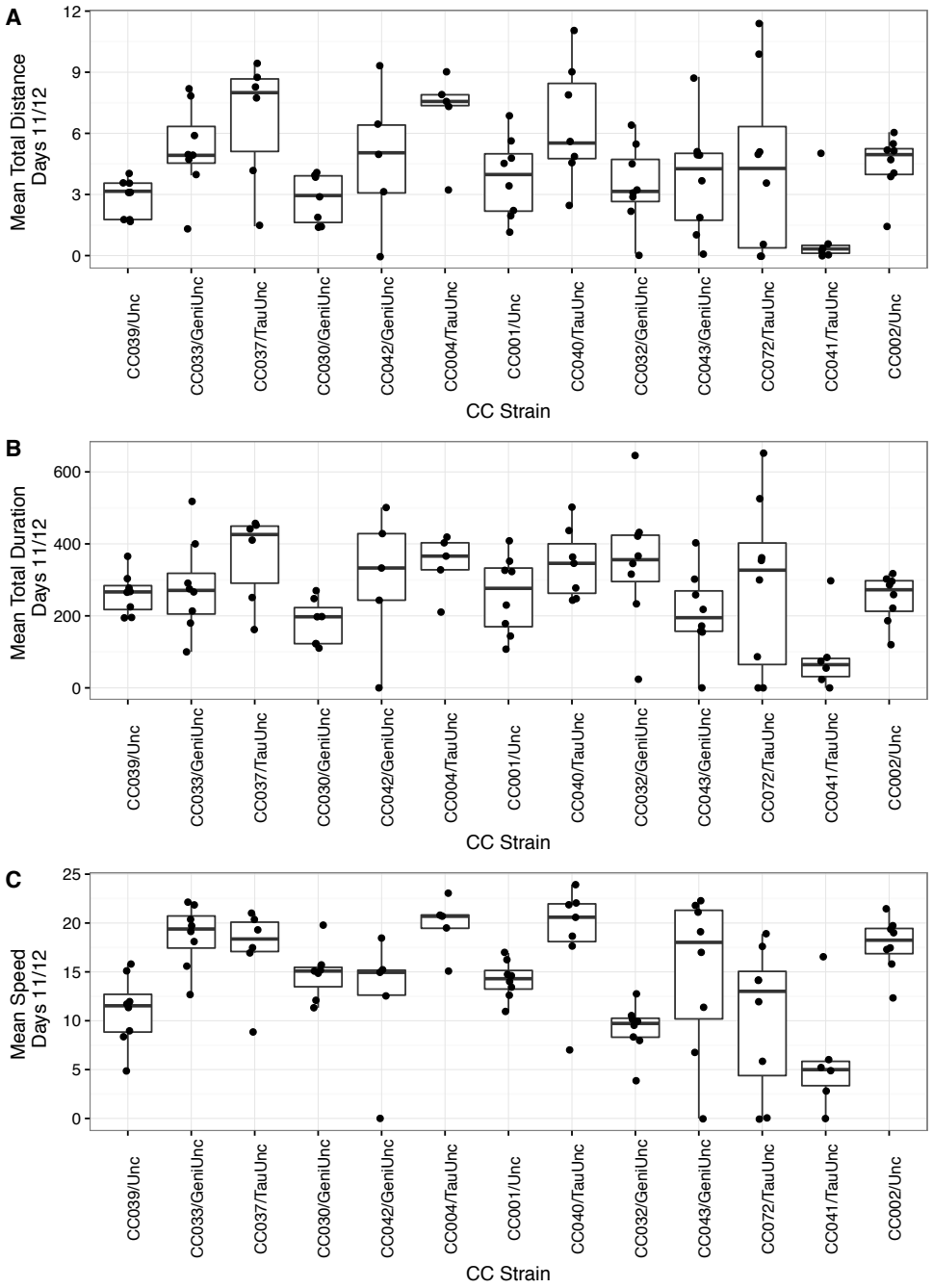
Trait	Female				Male			
	Control		Experimental		Control		Experimental	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
~Year 1								
Body mass (g)	26.1	0.6	25.0	0.6	34.5	0.6	33.8	0.6
% Fat	13.9	1.4	13.9	1.3	17.4	1.3	15.1	1.3
% Lean	79.7	1.3	79.8	1.2	76.5	1.2	79.4	1.2
~Year 1.1								
Body mass (g)	26.1	0.7	24.2	0.7	37.3	0.7	33.0	0.7
% Fat	12.2	1.3	7.9	1.3	21.5	1.3	11.2	1.2
% Lean	81.0	1.3	85.8	1.2	73.2	1.2	82.0	1.2
% Change in Mass	1.5	1.4	-3.3	1.3	6.9	1.3	-1.4	1.2
% Change in % Fat	-5.8	7.6	-37.9	7.0	25.2	7.0	-23.2	6.8
% Change in % Lean	83.1	2.0	89.9	1.9	72.3	1.9	84.2	1.8
~Year 1.4								
Body mass (g)	31.9	1.1	25.8	0.9	41.2	0.9	35.4	0.9
% Fat	22.5	1.8	10.3	1.6	26.4	1.6	14.9	1.6
% Lean	70.9	1.8	82.5	1.6	67.6	1.6	78.7	1.5
% Change in Mass	19.5	1.5	5.5	1.4	10.1	1.3	7.3	1.3
% Change in % Fat	72.1	10.8	28.2	10.0	22.8	9.6	37.1	9.3
% Change in % Lean	71.1	3.1	87.1	2.8	62.6	2.7	82.2	2.6
~Year 1.5								
Body mass (g)	33.3	1.2	26.2	1.0	42.3	1.0	35.8	1.0
% Fat	25.1	1.9	10.2	1.4	26.9	1.5	14.8	1.4
% Lean	68.4	1.7	81.5	1.3	66.9	1.4	78.4	1.3
% Change in Mass	1.9	0.9	1.2	0.7	2.8	0.7	1.1	0.7
% Change in % Fat	1.9	8.5	6.6	6.8	2.2	6.8	1.0	6.6
% Change in % Lean	62.6	3.4	86.6	2.7	59.1	2.7	80.1	2.6
~Year 1.6								
Body mass (g)	33.0	1.3	26.5	1.0	42.1	1.1	34.2	1.0
% Fat	23.2	2.2	11.0	1.7	25.3	1.7	11.4	1.7
% Lean	71.9	2.0	82.4	1.6	70.5	1.6	82.7	1.6
% Change in Mass	-0.7	1.4	1.4	1.0	-0.8	1.1	-4.6	1.0
% Change in % Fat	-9.8	5.8	10.0	4.5	-7.0	4.6	-20.7	4.5
% Change in % Lean	67.3	3.8	88.3	2.9	64.1	3.0	86.2	2.9
~Year 1.8								
Body mass (g)	33.6	1.6	27.4	1.1	40.7	1.1	34.1	1.1
% Fat	27.6	2.5	12.5	1.7	22.7	1.8	10.2	1.7

% Lean	69.2	2.3	81.2	1.6	72.8	1.6	84.2	1.6
% Change in Mass	-2.2	1.9	3.2	1.3	-3.2	1.4	-0.3	1.3
% Change in % Fat	1.0	6.9	13.5	4.7	-12.7	4.9	-12.1	4.7
% Change in % Lean	60.9	4.1	85.2	2.8	65.8	2.9	87.5	2.8
~Year 2								
Body mass (g)	35.1	1.9	27.4	1.3	37.7	1.3	33.5	1.4
% Fat	28.6	2.8	12.6	1.9	17.3	2.0	8.7	2.0
% Lean	67.0	2.6	80.7	1.8	77.8	1.8	85.1	1.9
% Change in Mass	4.3	2.0	-0.1	1.3	-7.8	1.4	-1.2	1.4
% Change in % Fat	3.6	8.7	3.6	5.9	-28.6	6.2	-22.1	6.3
% Change in % Lean	55.9	4.5	83.9	3.0	74.0	3.2	88.2	3.2

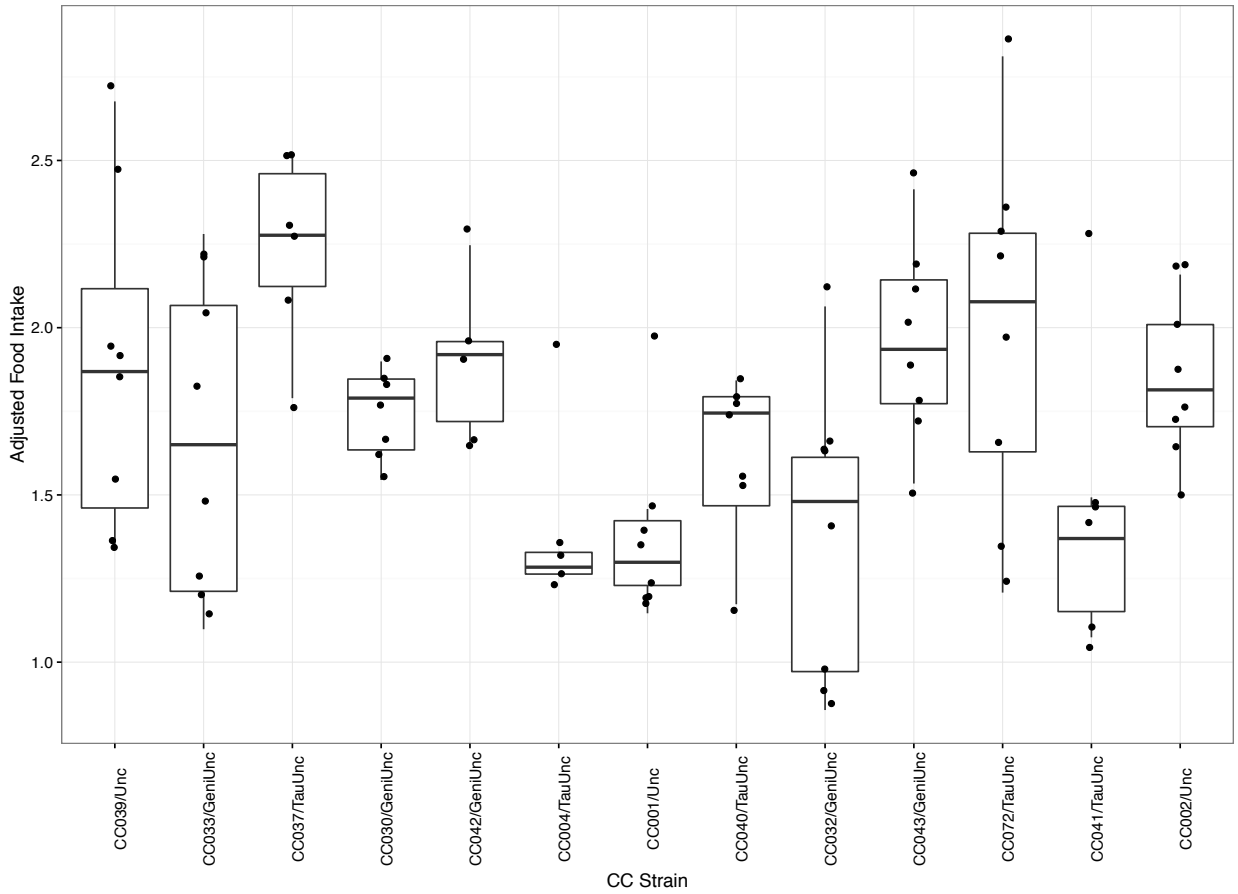
Supporting Table 2. Estimated marginal means and standard errors corresponding to tests presented in Table 2.2 and 2.3.

Trait	Tran	Female				Male			
		Control		Experimental		Control		Experimental	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
~Year 1									
VO_2 (ml/kg/h)		3423.5	74.5	3326.5	47.9	2537.9	56.3	2600.9	68.6
VCO_2 (ml/kg/h)		2820.5	86	2840	55.2	2069.7	64.9	2144	79.1
RER (VCO_2/VO_2)		0.82	0.02	0.85	0.01	0.82	0.01	0.82	0.01
Home Cage Activity		2501.9	185	2391.4	113.9	1802.7	139.7	1728.5	165.2
Food Consumption		3.83	0.34	3.79	0.21	2.73	0.26	2.97	0.31
Water Consumption	lg10	0.578	0.043	0.532	0.026	0.41	0.032	0.508	0.038
~Year 1.5									
VO_2 (ml/kg/h)		3042.4	110	3174.5	60.1	2152.3	82.1	2295	83.6
VCO_2 (ml/kg/h)		2409.8	136.7	2954.5	74.7	1751.9	102	1922.9	103.9
RER (VCO_2/VO_2)		0.78	0.03	0.93	0.01	0.81	0.02	0.83	0.02
Home Cage Activity		2021.4	194.1	1931.7	132.1	1332.5	146.1	1983.1	184.8
Food Consumption		2.88	0.49	5.88	0.33	2.54	0.37	3.85	0.46
Water Consumption		2.95	0.4	4.8	0.27	2.28	0.31	3.71	0.37
~Year 2									
VO_2 (ml/kg/h)		2909.4	164.8	2959.9	117.7	2704.8	131.7	2410.9	131.8
VCO_2 (ml/kg/h)		2562.4	162.7	2793.4	116.2	2266.1	130	2105.2	130.1
RER (VCO_2/VO_2)		0.88	0.02	0.93	0.02	0.84	0.02	0.87	0.02
Home Cage Activity		2097	188.6	2633.6	99.6	1279.1	132.1	2103	150.4
Food Consumption		3.67	0.46	5.27	0.24	3.51	0.32	4.4	0.37
Water Consumption		3.96	0.4	4.86	0.21	2.99	0.29	3.93	0.31

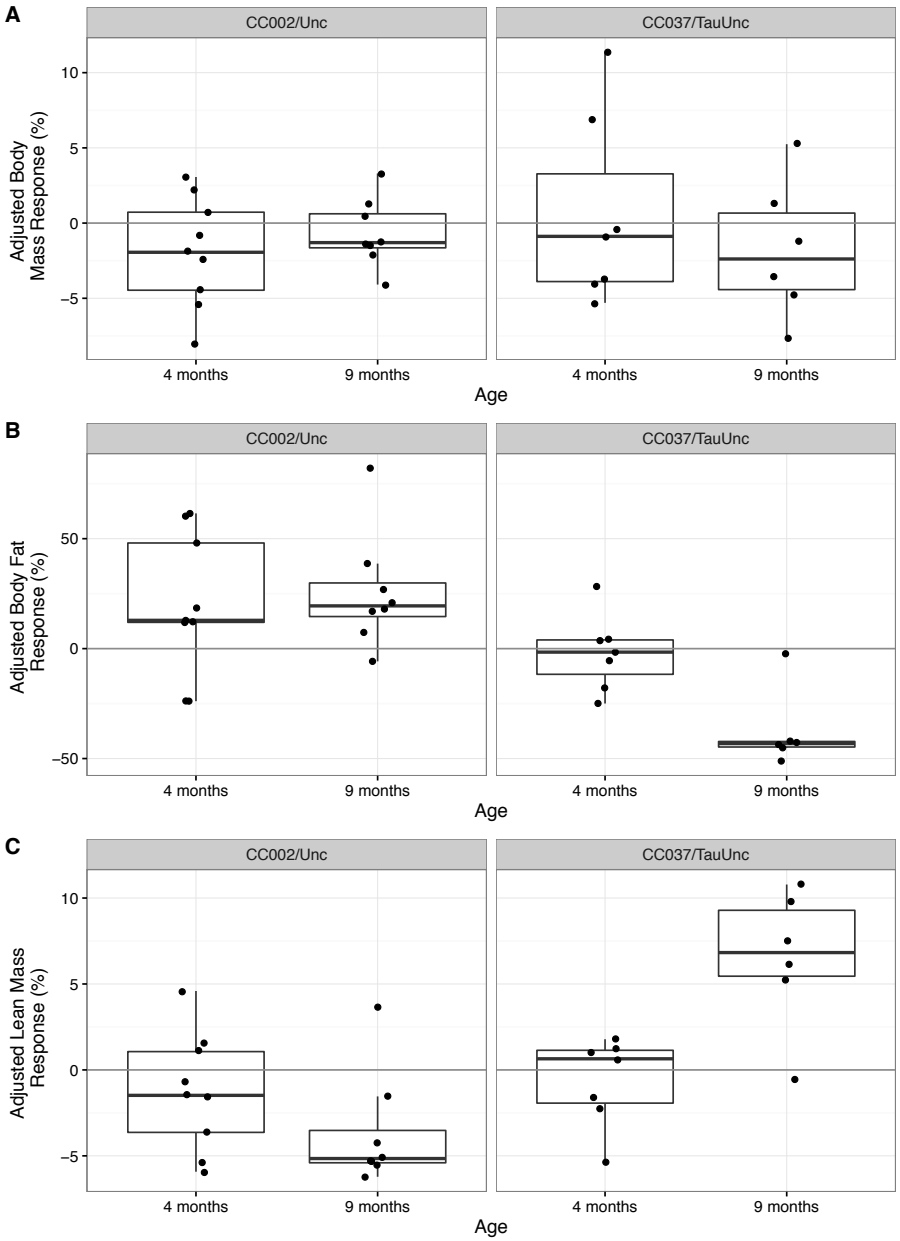
A-2 Chapter 4: Supporting figures



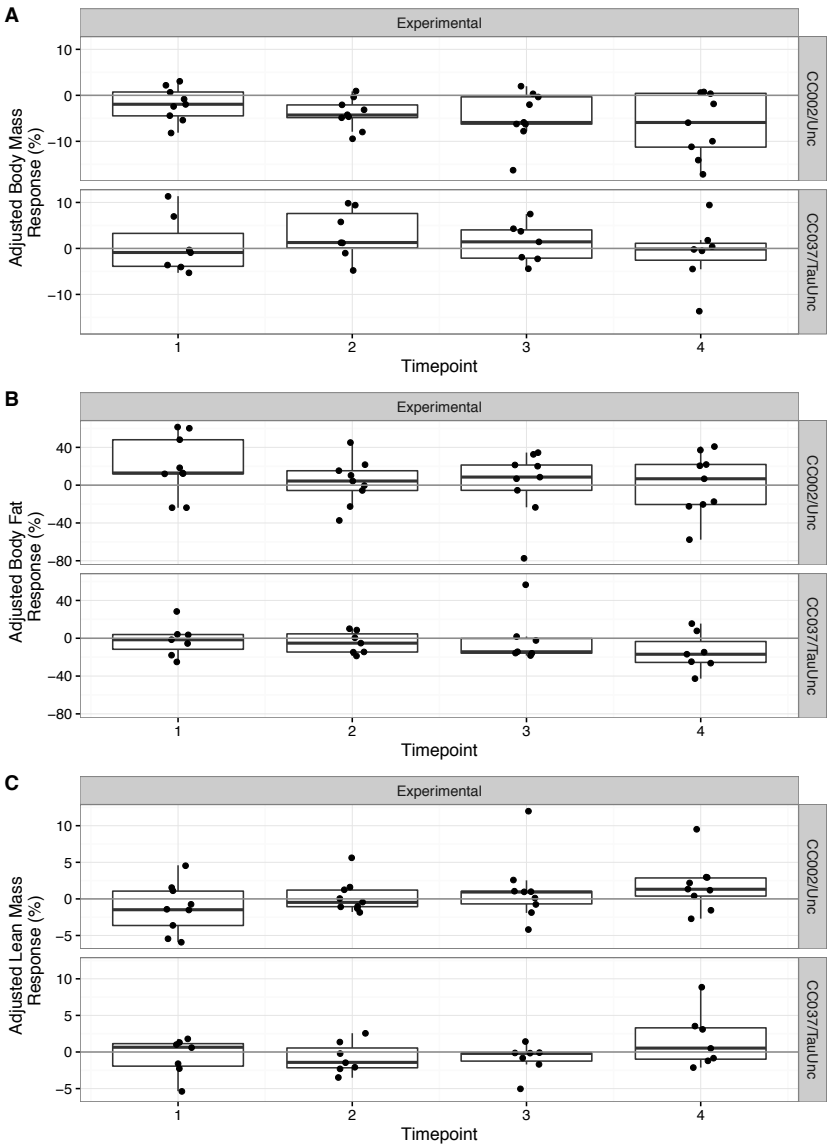
Supporting Figure 1: Physical activity traits in aged females across 13 CC strains. Mean total distance (km) (A), mean total duration (1-min intervals) (B) and mean speed (m/min) (C) for days 11/12 of wheel access. Each dot represents and individual female mouse. Strains are ordered by median adjusted fat response.



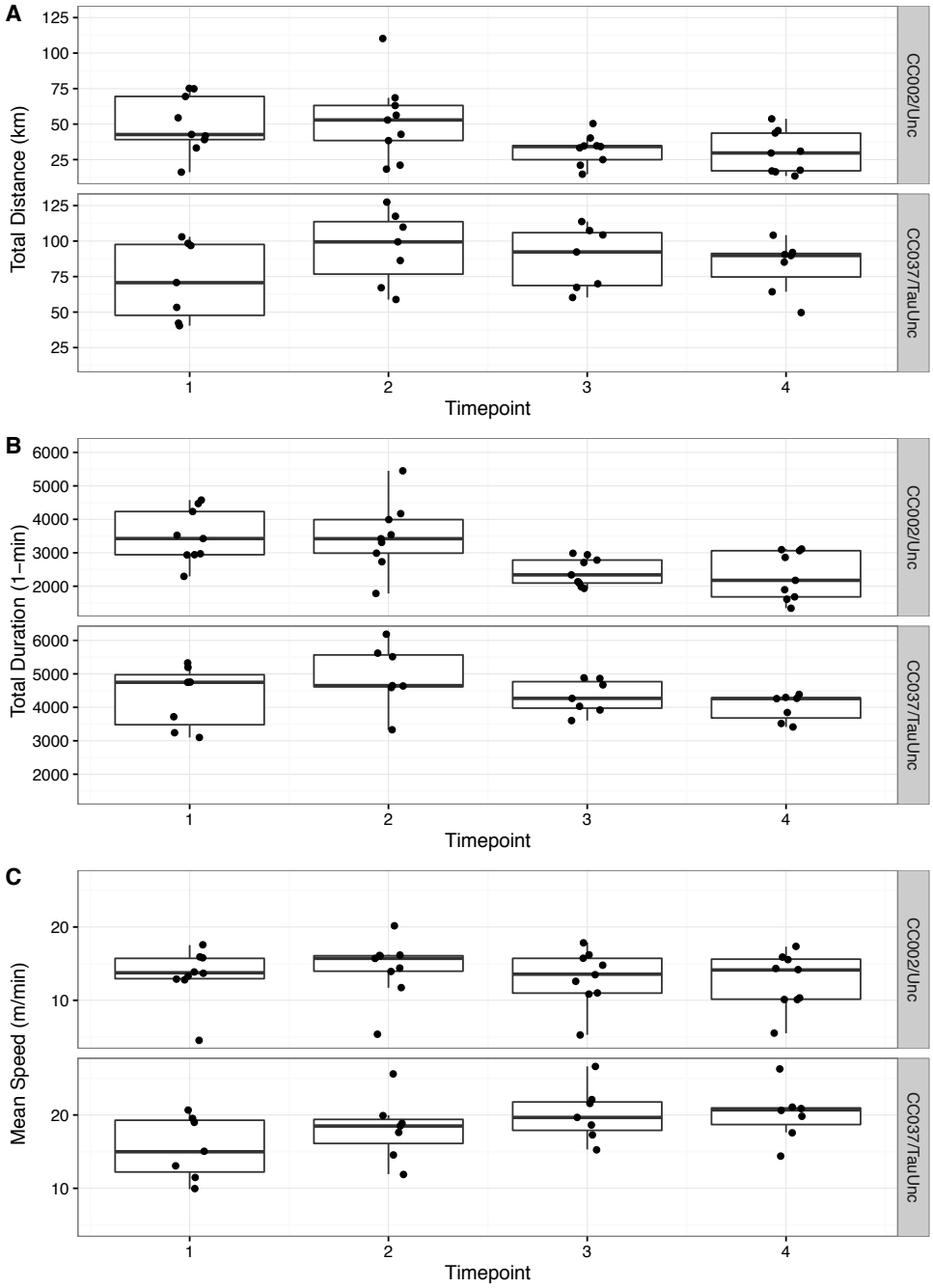
Supporting Figure 2: Adjusted food intake in experimental cohort across 13 CC strains. Adjusted food intake to two weeks of treatment in mice from the experimental cohort. Each dot represents an individual female mouse. Strains are ordered by median adjusted fat response.



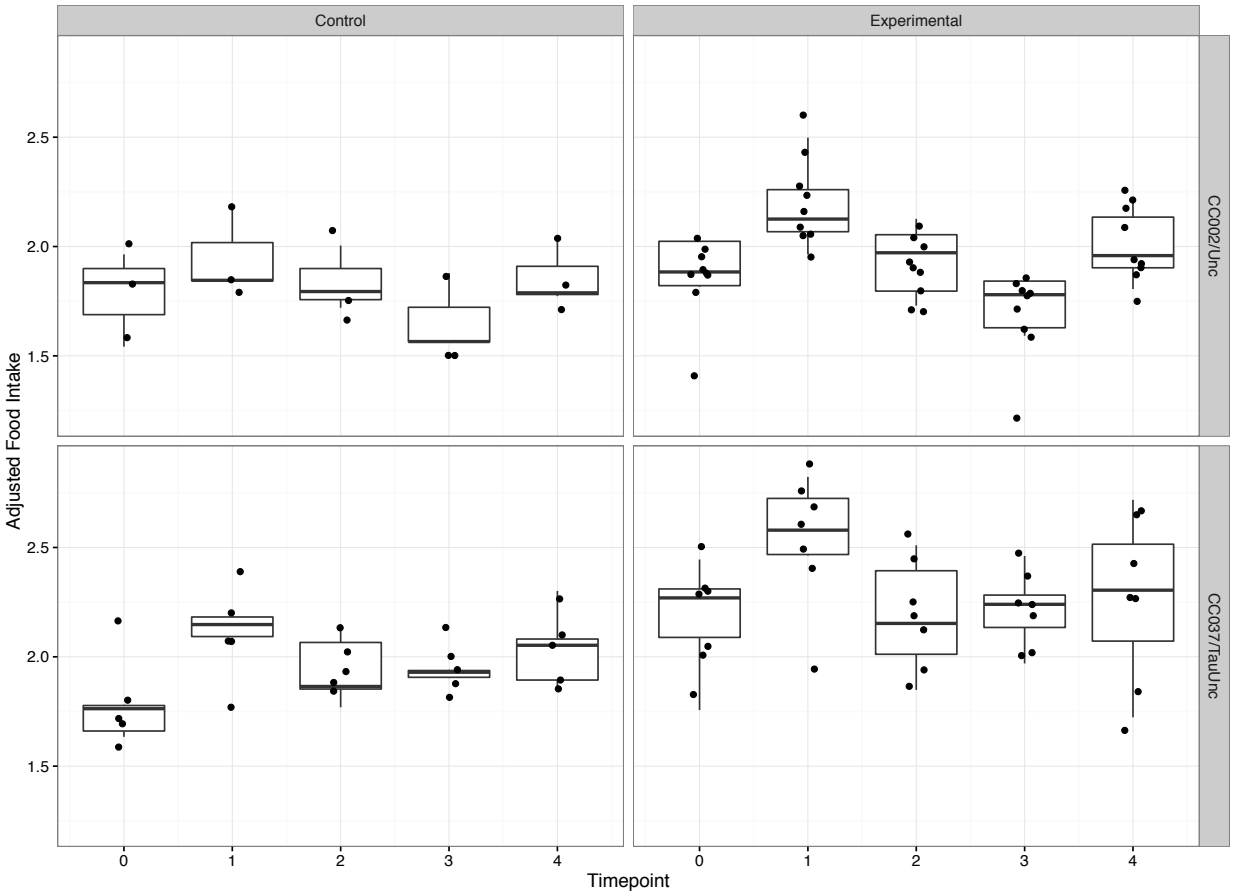
Supporting Figure 3: Adjusted body mass and composition response to two weeks of exercise in young and old CC002/Unc and CC037/TauUnc female mice. Adjusted body mass response (%) (A), adjusted body fat percentage response (%) (B) and adjusted lean mass percentage response (%) (C) to two weeks of wheel access in the experimental cohorts. Responses are adjusted to mean responses in the control cohort. Each dot represents an individual female mouse.



Supporting Figure 4: Cumulative adjusted exercise-induced body mass and composition response over 8 weeks in CC002/Unc and CC037/TauUnc female mice. Adjusted body mass response (%) (A), adjusted body fat percentage response (%) (B) and adjusted lean mass percentage response (%) (C) for each two week interval of the experiment in the experimental treatment cohort. Responses are adjusted to mean responses in the control cohort. Each dot represents an individual female mouse. Each response is calculated for a two week interval. Responses are represented as timepoint intervals: timepoint 0 (single housing acclimation), 1 (weeks 0-2 of treatment), 2 (weeks 2-4 of treatment), 3 (weeks 4-6 of treatment) and 4 (weeks 6-8 of treatment).



Supporting Figure 5: Physical activity traits over 8 weeks of wheel access in CC002/Unc and CC037/TauUnc female mice. Total distance (km) (A), total duration (1-min intervals) (B) and mean speed (m/min) (C) are represented in two week intervals over 8 weeks of wheel access. Each dot represents and individual female mouse. Timepoint intervals: timepoint 1 (weeks 0-2 of wheel access), 2 (weeks 2-4 of wheel access), 3 (weeks 4-6 of wheel access) and 4 (weeks 6-8 of wheel access).



Supporting Figure 6: Adjusted food intake over 10 weeks of treatment in CC002/Unc and CC037/TauUnc female mice. Adjusted food intake for each two week intervals of treatment (control or experimental). Each dot represents an individual female mouse. Timepoint intervals are timepoint 0 (single housing acclimation), 1 (weeks 0-2 of treatment), 2 (weeks 2-4 of treatment), 3 (weeks 4-6 of treatment) and 4 (weeks 6-8 of treatment).

A-3 Chapter 4: Supporting tables

Supporting Table 1: Treadmill protocols for three days of acclimation. Interval length represents the length of each interval in seconds. Start speed is the speed (m/min) at which the interval begins at and end speed is the speed at which the interval ends. Interval distance is the distance (m) covered during each interval. Day 1 covers 129 m total, day 2 covers 185m total and day 3 covers 290.5m.

Acclimation Day 1						
Angle of Inclination	5^0					
Step	**Start Speed (m/min)**	**End Speed (m/min)**	**Interval Length (s)**	**Interval Distance (m)**	**Total distance accumulated after this step (m)**	**Total Running Time (at end of this step; min)**
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	600	90	120	15
5	9	0	120	9	129	17
Acclimation Day 2						
Angle of Inclination	10^0					
Step	**Start Speed (m/min)**	**End Speed (m/min)**	**Interval Length (s)**	**Interval Distance (m)**	**Total distance accumulated after this step (m)**	**Total Running Time (at end of this step; min)**
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	600	90	120	15
5	9	16	120	25	145	17
6	16	16	120	32	177	19
7	16	0	60	8	185	20
Acclimation Day 3						
Angle of Inclination	15^0					
Step	**Start Speed (m/min)**	**End Speed (m/min)**	**Interval Length (s)**	**Interval Distance (m)**	**Total distance accumulated after this step (m)**	**Total Running Time (at end of this step; min)**
1	0	3	30	0.75	0.75	0.5
2	3	6	90	6.75	7.5	2
3	6	9	180	22.5	30	5
4	9	9	60	9	39	6
5	9	16	60	12.5	51.5	7

6	16	16	180	48	99.5	10
7	16	18	180	51	150.5	13
8	18	18	120	36	186.5	15
9	18	20	180	57	243.5	18
10	20	20	120	38	281.5	20
11	18	0	60	9	290.5	21

Supporting Table 2: Maximum endurance speed for each CC strain and sex. Max Speed (m/min) for each mouse is reported for both testing days. Mean strain speed (m/min) was calculated as a mean for each strain and sex across all maximum endurance speeds collected within the strain-by-sex groups. Protocol group represents the strains and sexes that will be running through exercise programs together and are matched by sex and maximum endurance speed abilities. Protocol group mean speed (m/min) is calculated as the mean speed for protocol groups with more than one strain. Speeds for the training programs are calculated using the protocol group mean speed.

CC Strain	Sex	Endurance Testing Day	Max Speed (m/min)			Mean Strain Speed (m/min)	Protocol Group	Protocol Group Mean Speed (m/min)	Calculated Speeds for Training Protocols		
			Mouse 1	Mouse 2	Mouse 3				80%	20%	50%
CC002/Unc	F	1	23	25	26	24.17	1	24.17	19.33	4.83	12.08
		2	25	23	23						
CC013/GeniUnc	F	1	39	28	30	33.67	2	33.67	26.93	6.73	16.83
		2	39	38	28						
CC027/GeniUnc	F	1	41	40	39	40.50	3	38.42	30.73	7.68	19.21
		2	43	38	42						
CC037/TauUnc	F	1	38	32	33	36.33	3				
		2	39	38	38						
CC002/Unc	M	1	26	21	25	24.00	4	25.58	20.47	5.12	12.79
		2	27	20	25						
CC013/GeniUnc	M	1	27	29	29	27.17	4				
		2	25	27	26						
CC027/GeniUnc	M	1	28	27	27	27.33	5	28.08	22.47	5.62	14.04
		2	30	23	29						
CC037/TauUnc	M	1	32	30	26	28.83	5				
		2	31	29	25						

Supporting Table 3: Exercise Program Treadmill Protocols for HIIT and MICT for each of the 5 exercise groups. Interval length represents the length of each interval in seconds. Start speed is the speed (m/min) at which the interval begins at and end speed is the speed at which the interval ends. Interval distance is the distance (m) covered during each interval. For each exercise group the total time (minutes) and distance (m) is listed for both HIIT (33min) and MICT programs (42min). Total distance (for both HIIT and MICT programs) for each exercise group are as follows: 1) 480m; 2) 670m; 3) 764m; 4) 509m; 5) 559m.

HIIT Protocols

Protocol Group	Interval Length (s)	Start Speed (m/min)	1 End Speed (m/min)	Interval Distance	Start Speed (m/min)	2 End Speed (m/min)	Interval Distance	Start Speed (m/min)	3 End Speed (m/min)	Interval Distance	Start Speed (m/min)	4 End Speed (m/min)	Interval Distance	Start Speed (m/min)	5 End Speed (m/min)	Interval Distance
Intervals 1 - 5	60	0.00	4.83	2.42	0.00	6.73	3.37	0.00	7.68	3.84	0.00	5.12	2.56	0.00	5.62	2.81
	60	4.83	4.83	4.83	6.73	6.73	6.73	7.68	7.68	7.68	5.12	5.12	5.12	5.62	5.62	5.62
	30	4.83	19.30	6.03	6.73	26.93	8.42	7.68	30.73	9.60	5.12	20.47	6.40	5.62	22.47	7.02
	240	19.30	19.30	77.20	26.93	26.93	107.72	30.73	30.73	122.92	20.47	20.47	81.88	22.47	22.47	89.88
	30	19.30	4.83	6.03	26.93	6.73	8.42	30.73	7.68	9.60	20.47	5.12	6.40	22.47	5.62	7.02
	60	4.83	4.83	4.83	6.73	6.73	6.73	7.68	7.68	7.68	5.12	5.12	5.12	5.62	5.62	5.62
	60	4.83	0.00	2.42	6.73	0.00	3.37	7.68	0.00	3.84	5.12	0.00	2.56	5.62	0.00	2.81

MICT Protocols

Protocol Group	Interval Length (s)	Start Speed (m/min)	1 End Speed (m/min)	Interval Distance	Start Speed (m/min)	2 End Speed (m/min)	Interval Distance	Start Speed (m/min)	3 End Speed (m/min)	Interval Distance	Start Speed (m/min)	4 End Speed (m/min)	Interval Distance	Start Speed (m/min)	5 End Speed (m/min)	Interval Distance
Interval 1	120	0.00	12.08	12.08	0.00	16.83	16.83	0.00	19.21	19.21	0.00	12.79	12.79	0.00	14.04	14.04
	2295	12.08	12.08	462.06	16.83	16.83	643.75	19.21	19.21	734.78	12.79	12.79	489.22	14.04	14.04	537.03
	60	12.08	0.00	6.04	16.83	0.00	8.42	19.21	0.00	9.61	12.79	0.00	6.40	14.04	0.00	7.02

Supporting Table 4. Descriptive statistics for body mass and composition responses across 13 CC strains.

			Body Mass Response					Body Fat % Response					Lean Mass % Response				
CCStrain	Treatment	n	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error
CC001/Unc	Control	7	-4.33	6.82	-1.57	2.61	0.99	-15.64	111.94	-7.16	10.58	4.00	5.41	4.18	0.77	2.04	0.77
CC002/Unc	Control	6	-0.03	3.18	-117.97	1.78	0.73	-6.48	168.53	-26.03	12.98	5.30	2.30	9.91	4.31	3.15	1.29
CC004/TauUnc	Control	5	1.72	7.17	4.16	2.68	1.20	-3.82	34.20	-8.94	5.85	2.62	1.42	6.15	4.34	2.48	1.11
CC030/GeniUnc	Control	5	-6.37	3.71	-0.58	1.93	0.86	-30.30	157.02	-5.18	12.53	5.60	5.80	6.40	1.10	2.53	1.13
CC032/GeniUnc	Control	6	0.47	54.06	115.57	7.35	3.00	-2.96	221.58	-74.82	14.89	6.08	1.49	21.02	14.13	4.58	1.87
CC033/GeniUnc	Control	7	-4.97	10.99	-2.21	3.31	1.25	-9.23	138.09	-14.96	11.75	4.44	2.69	6.37	2.37	2.52	0.95
CC037/TauUnc	Control	6	-1.28	19.23	-15.06	4.39	1.79	-5.80	138.22	-23.85	11.76	4.80	0.75	2.89	3.86	1.70	0.69
CC039/Unc	Control	7	0.04	22.43	577.93	4.74	1.79	0.80	866.84	1080.80	29.44	11.13	-1.06	22.16	-20.90	4.71	1.78
CC040/TauUnc	Control	8	2.64	14.73	5.59	3.84	1.36	2.37	16.53	6.98	4.07	1.44	-1.06	10.19	-9.65	3.19	1.13
CC041/TauUnc	Control	4	-1.96	37.10	-18.95	6.09	3.05	-24.17	2563.64	-106.06	50.63	25.32	1.43	15.28	10.70	3.91	1.95
CC042/GeniUnc	Control	5	-2.40	6.83	-2.85	2.61	1.17	-16.82	644.75	-38.32	25.39	11.36	1.86	14.80	7.93	3.85	1.72
CC043/GeniUnc	Control	7	1.15	4.80	4.19	2.19	0.83	0.97	39.91	41.14	6.32	2.39	-0.21	1.59	-7.44	1.26	0.48
CC072/TauUnc	Control	7	3.97	40.49	10.21	6.36	2.40	3.35	136.61	40.84	11.69	4.42	-1.28	6.76	-5.29	2.60	0.98
CC001/Unc	Experimental	8	-17.15	19.63	-1.14	4.43	1.57	-50.75	141.58	-2.79	11.90	4.21	16.99	42.78	2.52	6.54	2.31
CC002/Unc	Experimental	8	-0.70	5.17	-7.43	2.27	0.80	19.17	690.32	36.02	26.27	9.29	-1.40	10.64	-7.63	3.26	1.15
CC004/TauUnc	Experimental	5	-14.32	18.76	-1.31	4.33	1.94	-38.49	227.00	-5.90	15.07	6.74	14.01	49.17	3.51	7.01	3.14
CC030/GeniUnc	Experimental	8	-7.07	10.36	-1.47	3.22	1.14	-67.54	192.33	-2.85	13.87	4.90	9.20	13.93	1.51	3.73	1.32
CC032/GeniUnc	Experimental	8	-15.55	47.25	-3.04	6.87	2.43	-29.13	159.90	-5.49	12.65	4.47	11.88	40.13	3.38	6.33	2.24
CC033/GeniUnc	Experimental	8	-15.07	68.62	-4.55	8.28	2.93	-58.86	82.49	-1.40	9.08	3.21	17.39	80.84	4.65	8.99	3.18
CC037/TauUnc	Experimental	6	-3.04	21.08	-6.92	4.59	1.87	-43.64	313.47	-7.18	17.71	7.23	7.25	16.40	2.26	4.05	1.65
CC039/Unc	Experimental	8	-6.31	61.27	-9.71	7.83	2.77	-31.03	5594.76	-180.28	74.80	26.45	10.03	109.66	10.93	10.47	3.70
CC040/TauUnc	Experimental	7	-5.37	55.68	-10.37	7.46	2.82	-24.53	177.63	-7.24	13.33	5.04	8.11	45.09	5.56	6.72	2.54
CC041/TauUnc	Experimental	6	-6.43	68.22	-10.60	8.26	3.37	-11.77	1307.25	-111.10	36.16	14.76	4.88	71.00	14.54	8.43	3.44
CC042/GeniUnc	Experimental	5	-5.21	25.76	-4.95	5.08	2.27	-49.98	470.84	-9.42	21.70	9.70	8.04	29.33	3.65	5.42	2.42
CC043/GeniUnc	Experimental	8	-3.81	28.77	-7.54	5.36	1.90	-8.23	369.16	-44.85	19.21	6.79	3.99	24.99	6.26	5.00	1.77
CC072/TauUnc	Experimental	8	4.35	173.68	39.94	13.18	4.66	19.69	5309.06	269.65	72.86	25.76	-0.95	105.86	-111.75	10.29	3.64

Supporting Table 5. Descriptive statistics for physical activity traits in the experimental cohort across 13 CC strains.

			Mean Distance Days 11-12					Mean Duration Days 11-12					Mean Speed Days 11-12				
CCStrain	Treatment	n	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error
CC001/Unc	Experimental	8	3.84	3.95	1.03	1.99	0.70	258.75	11,878.50	45.91	108.99	38.53	14.20	3.60	0.25	1.90	0.67
CC002/Unc	Experimental	8	4.49	2.06	0.46	1.44	0.51	248.63	4,647.84	18.69	68.18	24.10	17.79	8.07	0.45	2.84	1.00
CC004/TauUnc	Experimental	5	7.00	4.89	0.70	2.21	0.99	345.30	6,914.95	20.03	83.16	37.19	19.81	8.53	0.43	2.92	1.31
CC030/GeniUnc	Experimental	8	2.78	1.52	0.55	1.23	0.44	181.36	4,121.14	22.72	64.20	22.70	14.86	7.43	0.50	2.73	0.96
CC032/GeniUnc	Experimental	8	3.47	3.93	1.13	1.98	0.70	348.06	31,489.03	90.47	177.45	62.74	9.16	6.53	0.71	2.56	0.90
CC033/GeniUnc	Experimental	8	5.20	4.80	0.92	2.19	0.77	280.50	16,884.64	60.19	129.94	45.94	18.68	10.47	0.56	3.24	1.14
CC037/TauUnc	Experimental	6	6.65	9.81	1.47	3.13	1.28	362.33	15,623.57	43.12	124.99	51.03	17.31	19.60	1.13	4.43	1.81
CC039/Unc	Experimental	8	2.83	0.92	0.32	0.96	0.34	261.81	3,294.92	12.59	57.40	20.29	11.01	12.93	1.17	3.60	1.27
CC040/TauUnc	Experimental	7	6.49	8.85	1.36	2.98	1.12	345.36	9,620.48	27.86	98.08	37.07	18.80	32.03	1.70	5.66	2.14
CC041/TauUnc	Experimental	6	1.05	3.83	3.65	1.96	0.80	89.08	11,416.94	128.16	106.85	43.62	5.90	31.62	5.36	5.62	2.30
CC042/GeniUnc	Experimental	5	4.77	12.19	2.56	3.49	1.56	301.20	37,796.83	125.49	194.41	86.94	12.26	51.39	4.19	7.17	3.21
CC043/GeniUnc	Experimental	8	3.81	7.76	2.04	2.79	0.98	208.31	14,197.07	68.15	119.15	42.13	14.93	66.16	4.43	8.13	2.88
CC072/TauUnc	Experimental	8	4.45	19.42	4.37	4.41	1.56	284.94	57,888.53	203.16	240.60	85.07	10.32	55.86	5.41	7.47	2.64

Supporting Table 6. Descriptive statistics for cumulative body mass and composition response over eight weeks of treatment in young CC002/Unc and CC037/TauUnc females.

				Cumulative Body Mass Response					Cumulative Body Fat % Response					Cumulative Lean Mass % Response				
CC Strain	Treatment	Timepoint	n	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error
CC002/Unc	Control	1	3	4.02	2.66	0.66	1.63	0.94	9.21	763.23	82.89	27.63	15.95	-1.28	3.87	-3.02	1.97	1.14
CC002/Unc	Control	2	3	3.88	5.02	1.29	2.24	1.29	16.90	1,506.17	89.15	38.81	22.41	-2.64	12.22	-4.64	3.50	2.02
CC002/Unc	Control	3	3	4.66	9.49	2.04	3.08	1.78	23.63	1,215.70	51.44	34.87	20.13	-3.19	10.28	-3.22	3.21	1.85
CC002/Unc	Control	4	3	10.47	52.31	5.00	7.23	4.18	30.92	1,673.27	54.11	40.91	23.62	-4.46	9.63	-2.16	3.10	1.79
CC002/Unc	Experimental	1	9	2.11	13.41	6.34	3.66	1.22	28.94	1,018.77	35.21	31.92	10.64	-2.55	11.63	-4.55	3.41	1.14
CC002/Unc	Experimental	2	9	-0.11	11.02	-104.03	3.32	1.11	20.30	585.70	28.85	24.20	8.07	-2.30	5.23	-2.27	2.29	0.76
CC002/Unc	Experimental	3	9	-0.07	30.55	-467.03	5.53	1.84	25.61	1,221.03	47.68	34.94	11.65	-1.98	20.35	-10.26	4.51	1.50
CC002/Unc	Experimental	4	9	3.97	47.43	11.94	6.89	2.30	31.95	1,067.06	33.39	32.67	10.89	-2.67	12.15	-4.54	3.49	1.16
CC037/TauUnc	Control	1	5	4.09	31.29	7.64	5.59	2.50	8.65	451.28	52.20	21.24	9.50	-0.98	10.34	-10.59	3.22	1.44
CC037/TauUnc	Control	2	5	2.02	87.05	43.02	9.33	4.17	-0.77	903.66	-1,170.73	30.06	13.44	0.40	18.22	45.91	4.27	1.91
CC037/TauUnc	Control	3	5	4.35	91.65	21.05	9.57	4.28	19.04	1,393.71	73.22	37.33	16.70	-2.03	24.58	-12.08	4.96	2.22
CC037/TauUnc	Control	4	5	5.52	74.65	13.53	8.64	3.86	14.77	837.61	56.71	28.94	12.94	-1.57	22.28	-14.17	4.72	2.11
CC037/TauUnc	Experimental	1	7	4.66	39.03	8.38	6.25	2.36	6.68	296.53	44.38	17.22	6.51	-1.62	6.60	-4.08	2.57	0.97
CC037/TauUnc	Experimental	2	7	5.12	29.80	5.83	5.46	2.06	-5.60	136.33	-24.36	11.68	4.41	-0.40	4.59	-11.43	2.14	0.81
CC037/TauUnc	Experimental	3	7	5.55	18.08	3.26	4.25	1.61	17.88	706.26	39.50	26.58	10.04	-2.98	3.96	-1.33	1.99	0.75
CC037/TauUnc	Experimental	4	7	4.50	48.58	10.80	6.97	2.63	0.19	407.91	2,145.86	20.20	7.63	0.14	14.66	106.55	3.83	1.45

Supporting Table 7. Descriptive statistics of physical activity traits in CC002/Unc and CC037/TauUnc young females.

				Total Distance (km)					Total Duration (1-min)					Mean Speed (m/min)				
CC Strain	Treatment	Timepoint	n	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error
CC002/Unc	Experimental	1	9	49.66	415.77	8.37	20.39	6.80	3,485.89	625,308.36	179.38	790.76	263.59	13.38	13.50	1.01	3.67	1.22
CC002/Unc	Experimental	2	9	52.42	770.45	14.70	27.76	9.25	3,487.44	1,041,438.53	298.63	1,020.51	340.17	14.41	16.65	1.16	4.08	1.36
CC002/Unc	Experimental	3	9	32.01	111.79	3.49	10.57	3.52	2,434.67	177,645.50	72.97	421.48	140.49	13.09	14.19	1.08	3.77	1.26
CC002/Unc	Experimental	4	9	29.77	220.83	7.42	14.86	4.95	2,315.56	516,770.03	223.17	718.87	239.62	12.59	14.42	1.15	3.80	1.27
CC037/TauUnc	Experimental	1	7	72.13	752.68	10.44	27.44	10.37	4,297.86	860,606.48	200.24	927.69	350.63	15.50	18.30	1.18	4.28	1.62
CC037/TauUnc	Experimental	2	7	95.22	660.17	6.93	25.69	9.71	4,932.43	871,797.95	176.75	933.70	352.91	18.15	18.54	1.02	4.31	1.63
CC037/TauUnc	Experimental	3	7	87.94	473.71	5.39	21.76	8.23	4,319.86	248,753.14	57.58	498.75	188.51	20.14	13.71	0.68	3.70	1.40
CC037/TauUnc	Experimental	4	7	82.23	349.63	4.25	18.70	7.07	3,998.43	163,387.62	40.86	404.21	152.78	20.10	13.06	0.65	3.61	1.37

Supporting Table 8. Descriptive statistics for body mass and composition response across four CC strains, both sexes and three exercise training programs.

				Body Mass Response					Body Fat % Response					Lean Mass % Response				
CC Strain	Sex	Intensity	n	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error	Mean	Variance	Coefficient of Variance	Std Deviation	Std Error
CC002/Unc	F	HIIT	14	11.68	44.22	3.79	6.65	1.78	-7.53	565.02	-75.07	23.77	6.35	0.34	11.39	33.87	3.37	0.90
CC002/Unc	F	MICT	16	11.28	46.33	4.11	6.81	1.70	15.84	1,146.68	72.39	33.86	8.47	-2.25	20.83	-9.28	4.56	1.14
CC002/Unc	F	NE	8	15.79	7.78	0.49	2.79	0.99	16.85	555.60	32.98	23.57	8.33	-2.27	6.91	-3.05	2.63	0.93
CC002/Unc	M	HIIT	6	6.76	9.45	1.40	3.07	1.26	-15.18	471.42	-31.06	21.71	8.86	-0.70	4.23	-6.08	2.06	0.84
CC002/Unc	M	MICT	8	7.54	10.80	1.43	3.29	1.16	-13.80	696.30	-50.47	26.39	9.33	0.66	1.01	1.53	1.00	0.36
CC002/Unc	M	NE	7	8.64	33.43	3.87	5.78	2.19	-9.85	181.14	-18.39	13.46	5.09	0.08	4.76	62.36	2.18	0.82
CC013/GeniUnc	F	HIIT	4	3.58	34.07	9.52	5.84	2.92	13.34	523.78	39.25	22.89	11.44	-1.16	2.09	-1.79	1.44	0.72
CC013/GeniUnc	F	MICT	8	5.09	11.11	2.18	3.33	1.18	2.54	1,186.18	466.31	34.44	12.18	-0.71	3.65	-5.12	1.91	0.68
CC013/GeniUnc	F	NE	8	12.83	15.18	1.18	3.90	1.38	47.35	3,024.06	63.87	54.99	19.44	-2.52	5.24	-2.08	2.29	0.81
CC013/GeniUnc	M	HIIT	8	4.11	11.14	2.71	3.34	1.18	20.78	1,948.33	93.77	44.14	15.61	-1.53	3.30	-2.16	1.82	0.64
CC013/GeniUnc	M	MICT	8	8.41	49.16	5.84	7.01	2.48	9.59	2,065.23	215.24	45.44	16.07	-1.26	6.71	-5.32	2.59	0.92
CC013/GeniUnc	M	NE	8	15.83	17.08	1.08	4.13	1.46	37.52	2,054.29	54.76	45.32	16.02	-2.42	4.29	-1.78	2.07	0.73
CC027/GeniUnc	F	HIIT	14	6.12	18.34	3.00	4.28	1.14	-1.69	1,782.88	-1,056.28	42.22	11.28	-1.45	3.41	-2.36	1.85	0.49
CC027/GeniUnc	F	MICT	15	7.18	13.38	1.86	3.66	0.94	29.77	3,627.30	121.86	60.23	15.55	-2.54	4.74	-1.87	2.18	0.56
CC027/GeniUnc	F	NE	8	15.69	24.30	1.55	4.93	1.74	-8.24	2,022.77	-245.37	44.98	15.90	0.25	2.47	9.79	1.57	0.56
CC027/GeniUnc	M	HIIT	11	3.70	14.95	4.04	3.87	1.17	37.36	7,058.99	188.93	84.02	25.33	-2.40	13.20	-5.51	3.63	1.10
CC027/GeniUnc	M	MICT	11	6.20	21.81	3.52	4.67	1.41	12.63	1,989.01	157.45	44.60	13.45	-1.72	10.18	-5.91	3.19	0.96
CC027/GeniUnc	M	NE	8	14.61	18.06	1.24	4.25	1.50	38.68	5,447.14	140.83	73.80	26.09	-0.78	2.66	-3.43	1.63	0.58
CC037/TauUnc	F	HIIT	15	3.74	38.43	10.27	6.20	1.60	13.68	2,118.10	154.83	46.02	11.88	-2.12	12.55	-5.92	3.54	0.91
CC037/TauUnc	F	MICT	15	8.17	38.67	4.73	6.22	1.61	78.98	3,900.85	49.39	62.46	16.13	-8.46	23.65	-2.80	4.86	1.26
CC037/TauUnc	F	NE	7	10.29	23.78	2.31	4.88	1.84	38.34	1,583.78	41.31	39.80	15.04	-3.49	12.90	-3.69	3.59	1.36
CC037/TauUnc	M	HIIT	11	8.37	42.83	5.12	6.54	1.97	26.14	1,410.58	53.96	37.56	11.32	-3.36	24.44	-7.27	4.94	1.49
CC037/TauUnc	M	MICT	11	9.30	19.86	2.14	4.46	1.34	36.31	1,576.17	43.41	39.70	11.97	-3.57	19.01	-5.32	4.36	1.31
CC037/TauUnc	M	NE	8	9.54	29.66	3.11	5.45	1.93	15.26	267.58	17.54	16.36	5.78	-1.89	1.63	-0.86	1.28	0.45

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