GENETIC REGULATION OF INTRINSIC ENDURANCE EXERCISE CAPACITY IN MICE

A Dissertation

by

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Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2013

Major Subject: Kinesiology

ABSTRACT

Endurance exercise capacity is a powerful predictor of health status. Having low levels of endurance exercise capacity has been linked with cardiovascular disease. Variation in endurance exercise capacity, measured during a graded exercise test, has been reported across cross-section, twin, and family studies. This variation is evidence of a genetic component to the phenotype of endurance exercise capacity: however, the genetic factors responsible for explaining this variation are undefined, in part because previous research has been performed on a limited scale. Therefore, three sets of experiments were designed to identify: 1) Novel quantitative trait loci (QTL) for endurance exercise capacity in 34 strains of inbred mice using genome-wide association mapping. 2) The effect of chromosome substitution on endurance exercise capacity using linkage analysis in F_2 mice. 3) The effect of chromosome substitution on endurance exercise capacity using wild-derived mice.

The main findings of this dissertation are: 1) There are strain-specific differences in endurance exercise capacity across 34 strains of male inbred mice. Genome-wide association mapping identified novel putative QTL on chromosomes 2, 7, 11, and 13. 2) Linkage analysis identified a novel QTL on chromosome 14 at the 56 cM position for run time and work. Linkage analysis also identified a potential sex-specific QTL, with the identified QTL significant for male mice only. 3) Novel putative QTL were identified on chromosomes 3 and 14 in chromosome substitution mice from wild-derived

mice. These data suggest that chromosome 14 is an important contributor to the genetic regulation of intrinsic endurance exercise capacity. These studies support a genetic component to endurance exercise capacity by identifying strain-specific differences and novel, putative QTL.

DEDICATION

I would like to dedicate this dissertation to my family. I would be nothing without my family. I am so deeply rooted and grounded because of the strength of moral character in the family that raised me. I never ventured outside to find my heroes because everyone I looked up to or wanted to be lived in my house. The work ethic, morality, decency, and respect that are integral parts of who I am today came from my parents, brothers and sister. The hardest lessons in life were taught to me by my family and they prepared me well to face the challenges that life presents. I only obtain this level of education because I have been able to follow the successful paths they have walked ahead of me and allowed me to follow in their footsteps. And, to my beautiful wife, Andrea Laine; I love you.

ACKNOWLEDGEMENTS

I would like to acknowledge my mentors and friends who have guided and supported me through this process. It would not have been possible for me to achieve the level of education that I have reached without the support, advice, interactions and exchange of ideas with all of the people who had a hand in guiding me along this journey. I need to express my gratitude, respect and deep appreciation for all of the individuals that helped make this educational experience possible.

I must begin with my committee members, Dr. Michael Massett, Dr. Christopher Woodman, Dr. James Fluckey and Dr. Penny Riggs, it is with the deepest appreciation and gratitude to each of you for your wisdom, mentorship, friendship and support for during this research project since its inception nearly four years ago.

To my chair and mentor, Dr. Michael Massett. I cannot appropriately express my gratitude for giving me the opportunity to work in your lab at a time when I was prepared to stop this doctoral experience completely. You may never truly know what that gesture meant, but I will work diligently in my career so that you may always know that you made the right choice by taking a chance on a lost first year doctoral student. You have supported me through times when I was faced with personal, financial and health issues and that will never be forgotten as I go through the rest of my life. It has been an honor to learn and develop under your mentorship.

To Dr. Woodman. I have always looked to you for the appropriate way to conduct myself during my time as a doctoral student. You are wise in ways I have not even considered and I appreciate your direction as a teacher, committee member, and friend. I have learned much from you. You are the benchmark that I use when I teach

courses and when I counsel students. I will carry your lessons the rest of my career. I will never forget your kindness when I came home from the hospital. Thank you for your clarity when I had confusion over research, writing, or educational maters.

To Dr. Fluckey. I will never forget the TACSM meeting at Tyler in 2009. I was at a crossroads in my education. I will never forget your act of kindness and your words of wisdom. You asked me to give the faculty at Texas A&M another chance to prove to me that there were good people and good researchers in the department. I will never forget your parting words to me, “Let us prove to you that we are people that you can believe in again”. You were right. Shortly after our conversation Dr. Massett gave me the opportunity to work in his lab and I was able to assemble an amazing committee. You are truly one of the most important people to me during this experience. Your opinions and advice have always become truths for me across time. You always welcomed we into your lab family and made me feel very connected as a doctoral student. I only wish I had words to truly express how much I respect you and appreciate my time with you at Texas A&M. You are truly amazing.

To Dr. Penny Riggs. I cannot thank you enough for opening your lab to me and allowing me to conduct virtually all of my genetics work under your supervision. The research experience and research environment provided me with a clear understanding how to perform bench science in a healthy, and productive atmosphere. In my time with you, I learned how to create the lab I would hope to have in my future. I feel like I learned how to ask research questions and conduct experiments in an ideal setting. After working in your lab I truly know exactly how I want to interact with future students and colleagues. You are a phenomenal role model and I appreciate the mentorship that has

led me to this point in my career. Working in your lab is priceless and I know that I can move forward with a plan for success. I do not know how to express my respect and thankfulness to you for providing me the opportunity to work with your lab group. I carry these skills and lessons that your lab has taught me into the rest of my career. I also appreciate your advice to me when you said "You are a doctoral candidate now and you have to write a dissertation, so I do not want to hear that you cannot write well anymore. *"Just go do it"*. You were right and this document is testament to your advice. I am grateful to you for this advice. I can write!

To Marybeth Henthorne. You have gone from being my lifeline to my friend and now part of my family. I will never forget how quickly you responded when I came to say goodbye to you and to thank you for everything you had done for me during my first year at Texas A&M. You knew exactly what to do every time I needed anything. When I came to A&M I thought it was a weird cult with all these traditions and things I did not understand. You taught me what it means to be an Aggie and by your actions over the last 5 years I truly now understand what it means to be an Aggie. You are important to me and I may never be able to put it into words exactly what you mean to me. You are part of my family.

To Kelli Kochan. You are personally responsible for my development as a researcher, bench scientist, and budding geneticist. I miss our conversations more than you can possibly know. Your attention to detail and patience for all of my questions amazes me to this day. I would have virtually no data without your teachings and instruction. You are invaluable to your students, your lab, your department and to your institution. I may be leaving Texas A&M but I promise you Kelli Kochan that I will

forever be texting and emailing you for advice and asking questions. You made this dissertation possible because you taught me how to conduct research. I am forever indebted to you for your time and friendship.

To Kevin Shimkus. This day would not be possible had you not been home on the night of October 24, 2011. I have no words strong enough to express my deep thanks for getting me to the hospital when I was having that heart attack. You are a brother to me and you may never actually know how important it was to me to have you by my side as a groomsman in my wedding. The wedding would not have been complete without you and I feel this dissertation defense would not be complete without you being a part of my thoughts this day.

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CHAPTER I

INTRODUCTION

Physical inactivity increases risk of chronic disease

Blair et al. defined physical activity as a component of overall physical fitness profile (12, 13). Low levels of physical activity have long been associated with increased risk for chronic disease and all-cause mortality (14, 157). In 1986, Paffenbarger et al. reported that the level of physical activity is inversely related to death (primarily from cardiovascular and respiratory-related illness) (120). Similar findings were reported in subsequent studies for risk of cardiovascular disease (CVD) (74, 95, 142) and stroke (74, 75, 143). Erikssen et al. identified a 2 fold increase in all cause mortality in sedentary hypertensive males in comparison to normotensive exercising males (42). Low levels of physical activity are also associated with increased prevalence of metabolic syndrome and related disorders (obesity, diabetes, etc.) (46, 88, 164). More recently, increased risks of breast and colon cancer have been linked with low levels of physical activity (49, 108, 147). Holmes and colleagues reported that women that were physically active metabolic equivalent (> 9 MET-hours per week) after breast cancer diagnosis reduced their mortality risk by 6% over 10 years (67). Similarly, increases in physical activity are associated with decreased risk of death from coronary heart disease and all-causes (140, 148, 164) as well as the relative risk of cardiovascular events (148, 164). Thus, there is strong evidence that low levels of physical activity leads to increased risk for CVD, diabetes/metabolic syndrome, cancer, and all-cause mortality.

Fitness is an independent risk factor for chronic disease

In addition to the role of physical activity in reducing the risk of mortality from chronic diseases, a series of large prospective studies have underscored the importance of exercise capacity to health and chronic disease. An inverse relationship exists between relative risk of the onset of cardiovascular disease and exercise capacity (12, 13, 117). Exercise capacity or cardiorespiratory fitness measured during a graded treadmill test has been shown to be an independent predictor of cardiovascular disease and all-cause mortality in men and women (12, 13, 26, 57, 60, 61, 92, 93, 116, 117, 138, 139).

Cardiorespiratory fitness is also an independent predictor of the incidence of metabolic syndrome in men and women, showing an inverse relationship between disease incidence and fitness level (90). Low exercise capacity is comparable to elevated systolic blood pressure, obesity, diabetes, and smoking as a risk factor and predictor of future disease (93, 117, 158, 170, 171). For each 1 MET increase in exercise capacity, there is a 12-17% reduction in all-cause mortality rate in both men and women, regardless of CVD status (57, 93, 116, 117). Furthermore, Carnethon et al. reported that low fitness in young adulthood was associated with double the risk for developing hypertension, diabetes, or metabolic syndrome later in life (26). Conversely, results from 2 prospective studies suggested that improvements in cardiorespiratory fitness significantly reduced the risk of all-cause mortality (12, 13, 42). Blair et al. reported that each one minute increase in exercise capacity during a treadmill running test was associated with an 8% decrease in risk for all-cause mortality (12, 13). Collectively, these data suggest strongly that exercise capacity or cardiorespiratory fitness, per se can be predictive of future onset of CVD and other chronic diseases (12, 13, 120).

Individual differences in exercise capacity

Both maximal exercise capacity and sub-maximal endurance exercise performance are complex polygenic traits (18, 19, 20). Results from cross-sectional, twin, and prospective studies indicate that there is a large genetic component to exercise capacity (18, 19, 20). For example, the current recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine is that individuals participate in moderate-intensity physical activity, taking a walk between 50-60 % of maximal heart rate, for 30 or more minutes on most days of the week for optimal health (122). Data from multiple research studies clearly identify considerable variability in endurance exercise capacity (15, 16, 58, 130, 155). For example, the HERITAGE family study identified baseline differences in human subjects in blood pressure, HDL cholesterol, and VO_{2max} (18, 19, 20, 21). In 2011, Hagberg identified differences in cardiovascular risk factors at baseline that effect exercise capacity and may have a genetic component explaining the differences in individual exercise capacity (58). These data emphasize the high degree of individual variation in intrinsic endurance exercise capacity. The mechanisms underlying individual variation in the adaptations to endurance exercise are not completely understood, but likely involve variation at the genetic level.

Genetics of exercise – heritability and twin studies

Genetic analyses can be used to identify the genetic basis for a complex trait with no *a priori* bias toward a particular gene, protein, tissue, or organ system. Initial studies investigating the genetic basis for exercise capacity focused on familial resemblance and heritability of performance phenotypes. Based on comparisons among families, the

between family variance is 2-3 times greater than the within family variance for VO_{2max} and sub-maximal power output (17). Furthermore, heritability estimates for VO_{2max} and sub-maximal power output range from 25% to 50% or higher (18, 82, 107). Meas et al. reported heritability estimates of 67% for VO_{2max} in twins (107). Klissouras estimated heritability for maximal aerobic power to be as high as 93% in male monozygotic and dizygotic twins (82). For example, Wantanabe et al. investigated the genetic contribution to endurance exercise using 39 pair of adolescent identical twins (14 male, 25 female) (165). For endurance running, twins were strongly correlated within, but were found to be different between other twin pairs (165). There was no correlation within twins for sprint racing (165). The results of this identical twin study identify a genetic contribution to endurance exercise, but in an investigation of different phenotype, sprint racing, results did not identify a correlation (165).

Hamel et al., in 1986, performed a study using monozygotic twins and their response to endurance training (59). Six pairs of monozygotic twins performed cycle ergometry endurance exercise for fifteen weeks and muscle biopsies were taken at seven and fifteen weeks of the study (59). Several enzymes including creatine kinase, hexokinase, phosphofructokinase, lactate dehydrogenase, malate dehydrogenase, 3-hydroxyacyl CoA dehydrogenase, and oxoglutarate dehydrogenase were assayed and compared within twin pairs and between twin pairs (59). Enzyme activity for oxoglutarate dehydrogenase and creatine kinase activities and the phosphofructokinase/oxoglutarate dehydrogenase were found to be similar within twin pairs, but different between twin pairs, indicating a genetic component to endurance exercise (59).

Genetics of exercise – family studies and linkage analysis

Heritability estimates from family studies also vary between 25% and 50%, depending on sample size (16, 21, 123, 176). In the HERITAGE Family Study, maximum heritability for VO_{2max} was estimated to be at least 50% based on data from over 400 individuals (16). Perusse et.al. identified the genetic contribution to submaximal cycle ergometry by identifying familial resemblance within families tested but noted between family differences in exercise capacity (123). Aerobic endurance capacity, or VO_{2max}, was measured using a graded cycle exercise protocol and heritability was calculated upwards of 74 percent within families (123). This study showed that parent-offspring, and maternal inheritance, were strongly correlated, supporting familial resemblance and genetic contribution to submaximal endurance exercise testing within families (123). Thus, both maximal and sub-maximal exercise capacity are influenced by genetic factors. These authors also estimated maternal heritability to be about 30% for VO_{2max} and 30-48% for sub-maximal exercise performance (16, 123). A subsequent report indicated that polymorphisms in mitochondrial DNA are associated with differences in VO_{2max} in the untrained state (38). Therefore, a significant portion of the genetic component influencing variation in exercise capacity may be due to maternal inheritance. However, other maternal components such as intrauterine effects, pre-natal and post-natal environment, and maternal care need to be considered as well (38, 136).

An important method in genetics for the study of complex traits, is linkage analysis. Linkage is defined as the inheritance of genes or genetic markers in close proximity to each other on the same chromosome during a recombination event (124,

130). Exercise genetics has frequently used linkage analysis to identify the effect of chromosomal recombination on exercise capacity (58,131, 133, 134, 174, 175). In 1999, Rivera et al. from the HERITAGE family study used linkage analysis to identify a significant association in sibling pairs between the muscle specific creatine kinase gene and maximal oxygen consumption during cycle ergometry (133, 134). Rivera et al. concluded that the muscle specific creatine kinase gene was in linkage disequilibrium with a gene in close proximity and this relationship enhanced maximal endurance exercise capacity within families, but there was no relationship between families, only within (134). This use of linkage analysis identified a candidate gene as an important regulator of endurance exercise capacity for the phenotype of cycle ergometry (134).

In 2003 Rico-Sanz et al. published a linkage study in which they identified QTL for maximal oxygen consumption during an endurance exercise test (131). The researchers investigated maximal oxygen consumption in 192 sets of sibling pairs. The study included 509 markers on 22 autosomes and identified strong evidence of linkage on chromosomes 10 and 11 for maximal oxygen consumption between siblings (131). Suggestive QTL were identified on chromosomes 1, 7, 10, 13, and 18 for maximal oxygen consumption between siblings (131). Following a 20 week training program, QTL were identified on chromosomes 1 and 5 for maximal power output between siblings (131). The data from this study was strong evidence for linkage for endurance exercise between sibling pairs (131).

Another example of the use of linkage studies in exercise genetics was from Rice et al. in 2011 who identified QTL on two chromosomes, human chromosome 1 and human chromosome 8, and demonstrated an association between low density lipoprotein

(LDL) cholesterol and apolipoprotein B within families that were performing cycle ergometry (124, 130). The study incorporated both sedentary non-exercising and exercising families (124, 130).

Genetics of exercise – animal models

While studies have been performed to understand the genetic component to endurance exercise capacity, there are some issues associated with the human research model. For example, the heterozygosity of the human genome adds a complicated aspect to understanding the genetic component to endurance exercise capacity. Because it would be unethical to manipulate the human genome to elicit certain responses to graded exercise testing, limited mechanistic studies can be conducted in people. To overcome these scientific and ethical issues, researchers have long employed the use of inbred rodent models (173). Particularly both inbred rat and mouse strains and stocks. The rodent model provides a homozygous genetic environment because the strains are inbred for twenty generations and genotyped to ensure homozygosity. The inbred mouse model for endurance exercise capacity is a valuable research tool because strains differences in exercise capacity can be used to identify the genetic components underlying these differences. Futhermore, the mouse genome is approximately ninety-eight percent identical to the human genome; therefore genes contributing to differences in exercise capacity in the mouse are likely to have a similar function in the human. Mouse skeletal muscle has the same muscle structure as human muscle. These genetic, biological, and physiological likenesses to the human model make the inbred mouse an important model to study for complex traits such as endurance exercise capacity and afford generalizable comparisons to the human model. There are two inbred mouse models used to identify

the genetic contribution to intrinsic endurance exercise capacity in this study. The inbred mouse model, which includes both classical laboratory derived mice and wild-derived mice. Wild-derived mouse strains were originally mice that were caught in the wild and introduced into a laboratory setting for research purposes. The wild-derived strains have been bred for twenty generations of intercrosses to obtain homozygosity at every allele making them inbred wild-derived mouse strains.

Two strategies have been employed to investigate the genetic basis of complex traits in animal models: selective breeding for a specific trait or screening of multiple inbred strains for a specific phenotypic trait (2) followed by quantitative trait loci (QTL) analyses. Selective breeding for voluntary wheel running or treadmill exercise has been utilized in mice and rats. Outbred Hsd:ICR mice were selected for voluntary wheel running for over 14 or more generations resulting in 2.4-fold differences in daily wheel running (40, 70, 154). Heritability calculated after 10 generations of selection was 19% (154). After 11 generations of selection, high and low selected lines of rats from the heterogeneous N:NIH strain varied by ~660 m (~4.5-fold) in treadmill running endurance (83, 169). In the high selected line of rats, improved oxygen utilization was attributed to higher oxygen extraction, increased capillary density, and higher activity of oxidative metabolic enzymes (63, 71). Proteins involved in mitochondrial synthesis and function such as PPAR-γ, PGC-1α, and cytochrome c oxidase subunit 1 (COX1) are also elevated in soleus muscle from high capacity running rats (113, 114, 115, 121, 169).

Although large differences in exercise capacity have been observed between selected lines of rats and mice, the time required to produce these differences can be lengthy (10-15 generations). Alternatively, mouse strains inbred for at least 20

generations are homozygous at all loci (145). Thus, under standardized environmental conditions, phenotypic differences observed among inbred strains are due predominantly to genetic variation. Genetic differences in intrinsic exercise capacity, measured by treadmill and voluntary running have been reported for inbred mice and rats (7, 44, 97, 101, 159). A 2.5-fold difference in intrinsic exercise performance measured by treadmill running has been observed between the highest and lowest performing strains of rats (3). In mice, maximum treadmill running speed varies by approximately 1.5 to 2-fold across strains (97, 159), whereas treadmill-running distance varied by 3.6-fold during a maximal exercise test (101). In contrast, sub-maximal exercise performance, measured by number of beam breaks during a 30-min treadmill test varied by 20-fold between high (FVB) and low (BL6) performing strains (97), suggesting that the genetic contribution to sub-maximal endurance exercise performance may be greater than that for maximal exercise capacity. Voluntary running performance in inbred mice also varies widely among inbred strains (44, 97, 100, 101, 111, 112). Lightfoot et al. reported a 395% (~3.9-fold) difference in daily distance run between the highest and lowest performing strains (100). This is in agreement with previous reports that distance run per night was approximately five-fold greater in high compared to low performing strains (44, 97). Interestingly, Lerman et al. reported that BL6 mice had the highest duration, distance, and running speed during voluntary running, but the poorest performance in treadmill running (97), suggesting that the genetic factors influencing treadmill running performance are different from those determining voluntary running. Based on differences in treadmill and voluntary running performance, broad-sense heritability estimates for intrinsic exercise capacity ranged from 31% to 73% (97, 100, 101) whereas

narrow-sense heritability estimates ranged from 39% to 50% (7, 83). Collectively, these data suggest that variance in exercise capacity in mice and rats is significantly influenced by genetic components.

Limited data exists regarding quantitative trait locus mapping for intrinsic exercise capacity in rodents. Using traditional genome-wide linkage analysis, several QTL for intrinsic endurance exercise capacity have been identified in rats (2, 88) and mice (47, 52, 53). In 2002, Ways et al. investigated aerobic running capacity in rats through genome scan and linkage analysis to identify novel QTL. Ways et al. identified a QTL for aerobic running capacity on chromosome 16 with a logarithm of odds (LOD) score of 4.0 (166). Suggestive QTL ($P < 0.63$) for aerobic running capacity were also identified on chromosome 3 with a LOD score of 2.2, and chromosome 16 (LOD score of 2.9) (166). This study was important to exercise genetics because it was the first study to identify QTL for aerobic endurance running capacity in rat models (166).

Lightfoot et al. (102) used a similar graded exercise test to screen several strains of mice for endurance exercise performance. Significant QTL were identified on chromosomes 8 and X in an F_2 cross between DBA/2J and BALB/cJ mouse strains. The confidence interval for the QTL on chromosome 8 overlaps with the two QTL on rat chromosome 16 identified by Way et al. (The regions of rat chromosome 16 identified by Way et al. are homologous to regions of mouse chromosome 8). Furthermore, in 2010, Lightfoot et al. identified QTL for voluntary wheel running using haplotype association mapping across 41 strains of inbred mice (102). The study identified twelve QTL on chromosomes 5, 6, 8, 11, 12, 13, 19, and X (102). This study included both

classical and wild-derived inbred strains of mice (102), and identified several QTL as sex-specific for running capacity (102).

Massett and Berk identified QTL for responses to pre-training endurance exercise for the phenotype of work (kg·m) in F_2 (FVB x B6) mice on chromosomes 14 and 19 (110). The QTL on chromosome 14 for work was located at the 4.0 cM and had a LOD score of 3.72 (110). The QTL for work on MMU19 was located at 34.4 cM and a LOD score of 3.63 (110). Significant post-training QTL were also identified for work on MMU3 at 60 cM with a 4.66 LOD score, and MMU14 at 26 cM, LOD score of 4.99 (110). Two suggestive QTL for post-training work were identified on MMU11 (44.6 cM, 2.30 LOD), and MMU14 (36 cM, 2.25 LOD) (110). The data from these studies identify a quantitative component to endurance exercise capacity in rodent models (99, 100, 101, 102, 109, 110, 166).

Although traditional linkage analyses are useful for identifying chromosomal regions of importance, these regions are typically quite large and contain hundreds of genes. These regions also require replication in independent mouse or rat lines before they are considered true QTL. This dissertation study is both novel and important because it employed two methods (haplotype analysis, and chromosome substitution), which have not been used previously for the phenotype of intrinsic endurance exercise capacity. Genome wide association studies (GWAS) or haplotype analysis attempts to identify genetic variants across the population being studied, which may impact the phenotype of interest. A critical step in the GWAS process is genotyping using single nucleotide polymorphisms (SNP) that act as markers on chromosomes of interest. The advantage of using a GWAS for the large number of strains in this study is better

mapping resolution (smaller QTL regions) compared with traditional linkage studies. This results in a smaller list of potential candidate genes that require validation using additional populations or approaches.

The other relatively new mouse model, chromosome substitution strains (CSS), was used in this dissertation to assess the contribution of individual chromosomes to endurance exercise capacity (10, 118, 119, 144). When studying linkage in the mouse model, the use of chromosome substitution mice strains narrows the chromosomal investigation to one chromosome because CSS are mice made by substituting a single chromosome from a donor inbred strain on the genetic background of a host inbred strain. These mice can then be used in linkage studies to identify significant QTL on a single chromosome (66).

Purpose of the study

There is clearly a genetic component to exercise capacity. However, the genetic factors underlying this variation are undefined, in part because previous research has been performed using a limited number of inbred mouse strains. Therefore, I investigated the genetic contribution to intrinsic endurance exercise capacity using multiple inbred mouse strains. The hypothesis for this study is that high running capacity mice will have more aerobic profiles and associated gene expression than low running capacity mouse strains. This hypothesis was tested using two novel methods, haplotype analysis and chromosome substitution mice, to identify novel quantitative trait loci (QTL).

d

Specific aims and hypotheses

To identify a novel effect of genetic contribution to intrinsic endurance exercise phenotype, I proposed three specific aims for this study:

Aim 1. To identify strain-specific differences, and novel QTL for run time using genome-wide association study (GWAS) design.

Hypothesis: Differences in endurance exercise capacity will be strain-specific\and will allow for detection of novel QTL.

Aim 2. To identify QTL using chromosome substitution mice for intrinsic endurance exercise capacity.

Hypothesis: One significant QTL will be identified using CSS mice.

Aim 3. To identify QTL using wild-derived chromosome substitution mice for intrinsic endurance exercise capacity.

Hypothesis: One significant QTL will be identified using CSS mice.

CHAPTER II

IDENTIFICATION OF EXERCISE CAPACITY QTL USING ASSOCIATION MAPPING IN INBRED MICE*

Introduction

Exercise capacity, as assessed by exercise time during a graded treadmill test, is commonly used to assess cardiorespiratory fitness and is highly correlated to risk of cardiovascular disease (117). In sedentary humans, there are substantial individual differences in exercise capacity and it is well established that there is a genetic basis for these individual differences (21, 23, 131). Because of the health benefits associated with high levels of fitness, the physiological factors that determine exercise capacity have been widely studied. However, little is known about the underlying genetic determinants of exercise capacity (23, 69). To elucidate these genetic factors, Bouchard and colleagues used genome-wide linkage analysis to identify quantitative trait loci (QTL) for exercise capacity, quantified by maximal oxygen consumption (VO_{2max}), in the sedentary state and in response to training. Several promising and suggestive QTL for pre-training exercise capacity were reported (21, 150) and these regions differed from those identified for the VO_{2max} response to training. These findings imply that the genes that determine sedentary state VO_2 are different from those that determine the response to training (21, 23, 150). Thus, the underlying genes or DNA variants determining the genetic effect on sedentary state or intrinsic exercise capacity will need to be determined

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to understand the link between exercise capacity and susceptibility to disease.

Several rodent models have been utilized to investigate the genetic factors contributing to intrinsic endurance exercise capacity assessed by treadmill running (7, 83, 97, 99, 100, 101, 102, 103, 109, 110, 166). Using traditional genome-wide linkage analysis, several QTL for intrinsic endurance exercise capacity have been identified in rats (7, 166) and mice (109, 110). However, traditional QTL analyses are limited by the variation present in the genomes of the two mouse strains (7, 11, 97), which can reduce mapping resolution. A relatively new approach for refining large QTL regions and identifying novel QTL for disease and behavior-related traits, including physical activity is genome-wide association mapping (7, 97).

Early attempts at genome wide association mapping in mice were criticized for low statistical power and differences in population structure across inbred strains leading to a high rate of false positive associations due to limited genomic information from a small number of mouse strains (97, 160). Recent advances in single nucleotide polymorphism (SNP) discovery and genomic sequencing capabilities have led to the creation of dense SNP maps available for a large number of inbred strains (32, 50). These large SNP databases have facilitated the development of genome-wide association mapping approaches in mice that account for some of the concerns raised with earlier studies (31, 32, 43, 78).

In the current study, we employed efficient mixed model association (EMMA) mapping. EMMA relies on a kinship matrix to account for genetic relatedness and population structure in inbred strains of mice and other model organisms, which can reduce the number of false positive associations (43). EMMA has been used successfully

to identify QTL for physiological traits such as body weight, bone mineral density, HDL cholesterol, airway responsiveness, and pulmonary adenomas in inbred mice (43). Thus, given the limited information regarding the genetic determinants of intrinsic endurance exercise capacity and the improved methodology for *in silico* genetic analyses, the purpose of this study was to characterize intrinsic endurance exercise capacity in 34 strains of classical and wild-derived inbred mice and to apply association mapping to identify novel putative QTL for endurance exercise capacity.

Methods

All procedures in this study were approved by the Institutional Animal Care and Use Committee at Texas A&M University and adhered to the American Physiological Society Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training (4).

Animals. Seven week old male mice from thirty-four inbred strains (129S1/SvImJ, 129X1/SvJ, A/J, AKR/J, BALB/cByJ, BUB/BnJ, C3H/HeJ, C57BL/6J, C57BLKS/J, C57L/J, C58/J, CAST/EiJ, CBA/J, CE/J, DBA/2J, FVB/NJ, KK/HlJ, I/LnJ, LP/J, MA/MyJ, MRL/MpJ, NOD/ShiLtJ, NON/ShiLtJ, NZB/BINJ, NZO/HlLtJ, NZW/LacJ, PL/J, PWD/PhJ, PWK/PhJ, RIIIS/J, SJL/J, SM/J, SWR/J and WSB/EiJ) (n = 6 per strain) were purchased from Jackson Laboratory (Bar Harbor, ME). These strains were chosen for genetic diversity incorporating inbred and wild derived strains (26, 35). Animals were housed in group cages and kept on a twelve hour light:dark schedule (7:00 AM:7:00 PM) in a temperature controlled environment (21.0-22.0°C) with food and water provided *ad libitum*. All mice were allowed one week to become accustomed to

the housing facility before completing the exercise protocol. All exercise tests were performed between 9:00 AM and 11:00 AM.

Endurance exercise test. Intrinsic endurance exercise capacity was defined for each of the 34 strains as time (minutes) and work (kg·m) performed during a graded exercise test. Work performed (kg·m) or vertical work was calculated as a product of body weight (kg) and vertical distance (meters), where vertical distance = (distance run)(sinθ), where θ is equal to the angle of the treadmill from 0° to 15° (109, 110). To identify strain specific phenotypic differences in endurance exercise capacity, all mice performed two graded exercise endurance tests on a six-lane treadmill (Columbus Instruments, Columbus, OH). Prior to completing the exercise testing, mice were familiarized for two days running for 10 min up a 10° incline at 9.0 m/min on day one and 11.0 m/min on day two. This protocol introduced the mice to treadmill running, but was limited to avoid inducing any training adaptations. Following familiarization, intrinsic exercise capacity was measured in 8 week old mice by two graded exercise tests separated by 48 hours. The test started at a speed of 9.0 m/min for 9 minutes at 0°. After 9 minutes the speed was increased to 10 m/min and the grade was increased 5°. Thereafter, speed was increased 2.5 m/min every three minutes and the grade was increased 5° every 9 minutes to a maximum grade of 15°. The graded exercise test continued until mice maintained contact with the shock grid for more than 15 seconds continuously or could no longer be motivated to run (109, 110). For each mouse, the average exercise capacity for the two trials was used to calculate strain means and for association mapping. Individual body weights were measured before each exercise test.

Association mapping. Genome wide association mapping for exercise phenotypes and body mass was performed using an efficient mixed model algorithm (EMMA) using the UCLA web-based server (http://mouse.cs.ucla.edu/emmaserver). EMMA uses a linear mixed model algorithm to account for relatedness among inbred strains, which reduces the rate of false positive associations (11, 69, 81). Analysis of individual phenotypic data was performed using single nucleotide polymorphism (SNP) panels consisting of 132,285 SNPs (132 K) and 4 million SNPs (4 M) (11, 81). The initial analysis was conducted using the 132 K panel to identify putative loci for endurance exercise capacity. The SNP density of this panel is comparable to previously published mouse genome-wide association mapping studies conducted using a similar number of mouse strains. A second analysis then was conducted using the 4 M SNP panel to compare with the results from the 132 K panel and to identify novel loci not identified using the smaller panel. The 4 M SNP panel includes the SNPs in the 132 K panel. Association mapping was conducted using all 34 strains and after omitting phenotype data from the wild-derived inbred strains to assess the affect these strains have on QTL discovery. Genome-wide significance thresholds were calculated based on a false discovery rate of 5% (or q-value ≤ 0.05) using the R package q-value (129). Confidence intervals were determined by expanding the interval around the peak SNP to include all neighboring SNPs surpassing the significance threshold. For single SNP associations, the QTL confidence interval was set at 400 kb (200 kb on either side of the peak SNP), but only the location of the peak SNP is reported. If two QTL overlapped or were separated by < 1 Mb they were considered one QTL. All SNP and gene locations

were mapped to build 37.2 of the NCBI mouse genome. SNP associated p-values were transformed using $-\log_{10}$(p-value) for graphing association scores.

Statistical analysis. All data are represented as mean ± SE. Exercise capacity variables and body mass data from the 34 strains was analyzed using JMP 9.0 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) followed by a Tukey's post-hoc test was used to identify significant strain-specific differences across all 34 strains. To assess the allele effect for significant peak SNPs, strains were grouped by genotype at each individual SNP and exercise time compared between groups using a Mann Whitney test. The proportion of variance explained by significant peak SNPs was calculated by fitting exercise time against the genotypes using a linear model. Statistical significance was set at $P < 0.05$.

Results

Body mass in 8-week-old male mice varied significantly among inbred strains (Figure 2.1 and Table 2.1). There was an approximately 3-fold difference in body mass across strains. The strain with the lowest body mass was the wild-derived CAST/EiJ mice (13.8 ± 0.2 g), whereas the heaviest strain was the MRL/MpJ (39.1 ± 0.8 g). Because there are strain-specific differences in body mass, work performed during the graded exercise test is reported in addition to time.

Intrinsic endurance exercise capacity. A strain screen was performed to identify strain-specific differences in intrinsic exercise capacity across 34 strains of male mice using a graded exercise test. All mice completed both graded exercise tests. Both tests were conducted by the same individual and were highly reproducible (within-mouse coefficient of variation = 1.56 ± 0.14 %). The strain distribution patterns for time

and work are shown in Figure 2.2 and significant differences indicated in Table 2.1. There were significant differences across the strains for each exercise phenotype. For run time, there was a nearly three-fold difference (2.73) between the lowest (A/J: 18.4 ± 0.2 min) and highest (C58/J: 50.3 ± 0.1 min) performing strains (Figure 2.2A). The variation among strains was greater for work than for time (Figure 2.2B). There is a 16.5-fold difference between the lowest performing (A/J: 0.29 ± 0.02 kg·m) and highest performing (C58/J: 4.78 ± 0.12 kg·m) strains. The strain distribution pattern also changed when exercise capacity is expressed as work. This is abundantly clear in the 4 wild derived strains (CAST/EiJ, PWD/PhJ, PWK/PhJ and WSB/EiJ), which had high run times and low body mass, but relatively low work performed (Figure 2.2 and Table 2.1). Based on strain means, body mass did not correlate significantly with run time ($r = -0.30$, $P = 0.08$).

Genome-wide association mapping. Genome-wide association mapping for exercise phenotypes was performed using 132 K and 4 M SNP panels in EMMA. For each SNP panel, analyses were run two ways, one with all 34 strains including classical and wild-derived inbred mouse strains and a second with only the classical inbred strains. This was done to investigate the influence of the wild-derived strains on the genetic architecture of the exercise phenotypes. For the 132 K panel, significant associations for exercise time using all 34 strains were identified on Chromosomes 2, 7, 11, and 13 (Figure 2.3A and Table 2.2). When analyses were repeated using data from the classical inbred strains only, significant associations for exercise time were found on Chromosomes 2 (168.40-168.43 Mb) and 11 (21.66-22.56 Mb) (Figure 2.3B). Two associations on Chromosome 11 (~25 Mb and ~ 70 Mb) and those on Chromosomes 7

and 13 were no longer significant. Repeating association mapping for exercise time using the 4 M SNP panel identified two significant associations in the 34-strain cohort and 4 significant associations in the 30-strain cohort (Table 2.3). A novel significant association was identified on Chromosome 1 in both the 34-strain and 30 strain groups and a suggestive (FDR of 10%) association on Chromosome 11 in the 30 classical inbred strains.

Genome-wide association mapping for work using the 132 K SNP panel is shown in Figure 2.4. Only one significant association was identified for work in the 34-strain cohort (Chr 2 at 168.4 Mb). No significant associations were identified using the 30 classical inbred strains only. Similar analyses using the 4 M SNP panel yielded no significant associations (data not shown).

To assess the contribution of body mass to QTL for exercise capacity, genome-wide association mapping was conducted using body mass from the 34 inbred strains and the 30 classical inbred strains (Table 2.4). This analysis was performed to identify any body mass QTL that might overlap with QTL for exercise capacity. A significance threshold corresponding to $P = 10^{-5}$ was used for this analysis to maximize the number of significant associations identified. None of the significant associations for body mass overlapped with significant associations for time or work, suggesting that none of the exercise-related QTL were primarily due to genetic factors controlling body mass. Seven of the putative QTL identified for body mass overlapped with previously identified QTL for body mass or body mass change after an experimental intervention (*e.g.,* high fat diet) (Table 2.4), with the exception of the QTL on Chromosome 1 located at 54.08 Mb.

Two peak SNPs (rs27288988 and rs4135796) were identified in multiple analyses. To assess the effect of each allele on exercise capacity, strains were grouped according to their genotype and compared for exercise time. For rs27288988 on Chromosome 2, 25 strains carry the G allele and 9 the T allele. Strains carrying the G allele had significantly greater exercise time than strains with the T allele (G = 36.1 ± 1.2 min; T = 26.9 ± 1.9 min, $P < 0.05$). Similarly, exercise time was significantly greater for strains with the G allele (n = 23 strains) than those with the C allele (n = 8 strains) for rs4135796 on Chromosome 11 (G = 36.2 ± 1.2 min; C = 25.6 ± 2.1 min, $P < 0.05$). Based on provisional estimates using a linear model and considered individually, the locus on Chromosome 2 (rs27288988) accounts for 32.6% of the variance in exercise time and the locus on Chromosome 11 (rs4135796) accounts for 39.7% of the variance in exercise time. When both SNPs are included in the analysis, the contribution of the peak on Chromosome 11 is approximately 7.8% ($P < 0.001$), whereas the variance accounted for by the peak on Chromosome 2 is < 1.0% ($P = 0.14$).

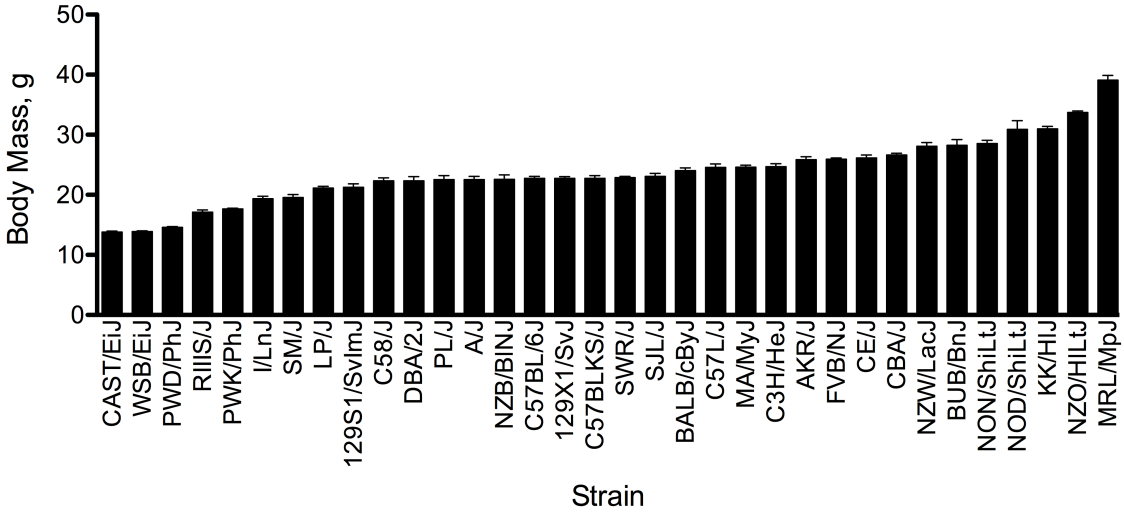


Figure 2.1. Distribution of body mass in 8-week old male mice from 34 inbred strains. Values are expressed as means ± SE. *n* = 6 mice/strain. Significant differences among strains are indicated in Table 2.1.

Table 2.1. Statistical differences among 34 inbred strain of mice for time, work, and body mass as depicted in Figures 2.1 and 2.2.

Strain	Time (Fig. 2.2A)	Work (Fig. 2.2B)	Body mass (Fig. 2.1)
C58/J	A	A	GHI
AKR/J	B	B	DEF
CAST/EiJ	BC	IJKL	M
WSB/EiJ	CD	IJKL	M
SWR/J	DE	DE	GH
PWD/PhJ	DEF	JKLM	LM
PWK/PhJ	EFG	HIJK	K
MA/MyJ	EFG	CDE	EFG
SM/J	EFG	FGHI	IJK
NON/ShiLtJ	EFG	C	CD
DBA/2J	EFG	EFGH	GHI
SJL/J	EFG	EFG	FGH
FVB/NJ	FG	DE	DEF
C57L/J	G	EF	EFG
NOD/ShiLtJ	G	CD	BC
LP/J	G	GHIJ	HIJ
CBA/J	H	FGHI	DE
BALB/cByJ	HI	IJKL	EFGH
CE/J	HI	HIJK	DE
C57BLKS/J	HI	JKLMN	GH
NZB/BlNJ	HI	KLMN	GH
129S1/SvImJ	IJ	MNO	HIJ
C3H/HeJ	IJ	KLMN	EFG
PL/J	JK	NO	GH
C57BL/6J	JK	NO	GH

Table 2.1 Continued.

Strain	Time (Fig. 2.2A)	Work (Fig. 2.2B)	Body mass (Fig. 2.1)
129X1/SvJ	K	O	GH
KK/HlJ	K	LMNO	BC
MRL/MpJ	K	IJKL	A
I/LnJ	L	PQ	JK
NZW/LacJ	L	PQ	CD
NZO/HlLtJ	L	P	B
RIIIS/J	L	Q	KL
BUB/BnJ	L	PQ	CD
A/J	M	Q	GH

Significant differences are based on results from one-way ANOVA followed by Tukey post-hoc analysis. Strains not connected by same letter are significantly different. Strains are organized based on time from the longest time (A) to the shortest time (M). The number of strain groupings varies by phenotype.

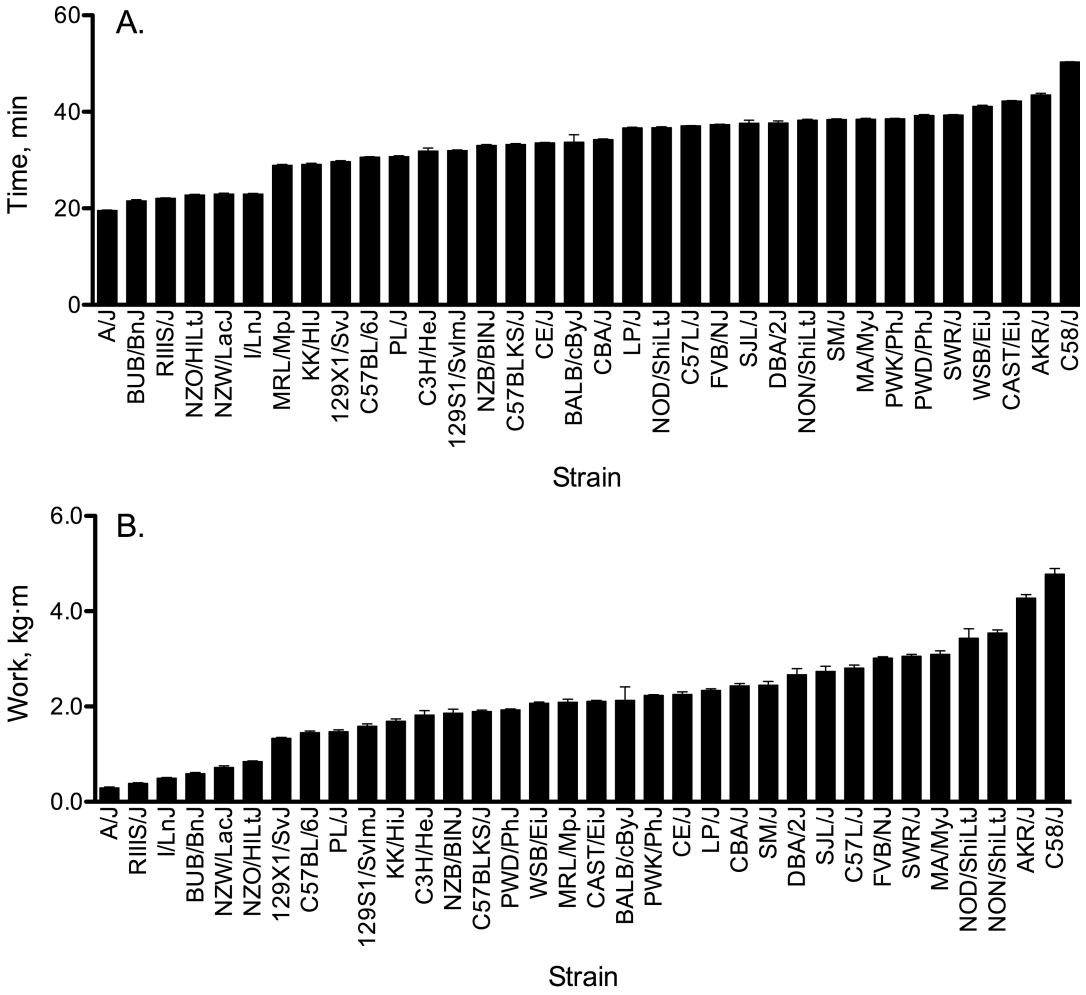


Figure 2.2. Strain survey of intrinsic endurance exercise capacity in male mice from 34 inbred strains. Exercise capacity is expressed as time (minutes) (A) and work (kg•m) (B). All mice performed 2 graded exercise tests separated by 48 hours, and individual means were used to calculate strain means. Values are expressed as means ± SE. *n*=6 mice/strain. Significant differences among strains are indicated in Table 2.1.

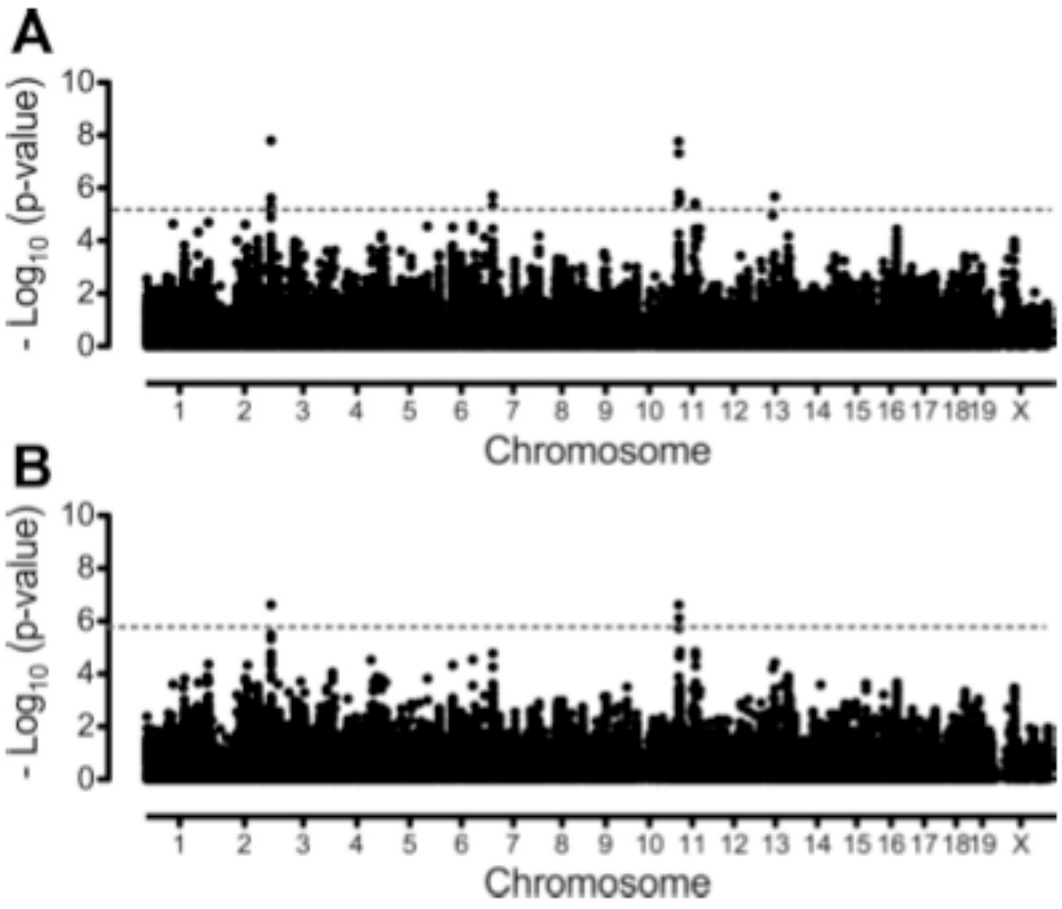


Figure 2.3. Genome-wide association mapping for endurance exercise capacity, expressed as time in minutes, in male mice from 34 classical and wild derived inbred strains (A) and 30 classical inbred strains only (B) using the 132 K SNP panel. Association mapping was conducted with an empirical mixed model algorithm (EMMA) and a 132, 285 SNP panel. The x-axis indicates genomic position divided by chromosome. Values on the y-axis are *P* values transformed using $-\log_{10}$ (*P* value). Horizontal dashed lines indicate genome-wide association significance thresholds. Significance thresholds were calculated using a 5% false discovery rate and correspond to P values of 7.21×10^{-6} and 1.87×10^{-6} for 34 strain and 30 strain cohorts, respectively.

Table 2.2. Genome-wide association mapping for exercise time in male mice from classical and wild-derived inbred strains using a 132 K SNP panel.

Chr	QTL Location, Mb	Peak SNP	P-value	Number of known genes
Classical and wild-derived inbred strains				
2	168.27-168.45	rs27288988	1.61×10^{-8}	1 (*Nfatc2*)
7	16.97-16.99	rs31142151	1.93×10^{-6}	2 (*Sae1*)
11	21.66-22.59	rs4135796	1.73×10^{-8}	9 (*AV249152*)
11	24.51-25.56	rs13480916	3.17×10^{-6}	2
11	70.26-70.81	rs3707772	3.73×10^{-6}	30 (*Med11*)
13	58.95	rs29804611	2.09×10^{-6}	2 (*Ntrk2*)
Classical inbred strains only				
2	168.40-168.43	rs27288988	2.36×10^{-7}	1 (*Nfatc2*)
11	21.65-22.56	rs4135796	2.41×10^{-7}	9 (*AV249152*)
		rs3708339	2.41×10^{-7}	(*AV249152*)

All results are significant based on genome-wide thresholds corresponding to P-values of 2.54×10^{-6} for 34-strain and 1.87×10^{-6} for 30-strain cohorts. Gene symbol in parentheses indicates the gene containing the peak SNP. Chr, chromosome; QTL, quantitative trait locus; Peak SNP, SNP with highest p-value in QTL interval; P-value, EMMA-corrected p-value; *Nfatc2*, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2; *Sea1*, SUMO-1 activating enzyme subunit 1; *AV249152*, WD repeat containing planar cell polarity effector; *Med11*, mediator of RNA polymerase II transcription, subunit 11 homolog; *Auh*, AU RNA binding protein/enoyl-coenzyme A hydratase; *Ntrk2*, neurotrophic tyrosine kinase receptor type 2.

Table 2.3. Genome-wide association mapping for exercise time in male mice from classical and wild-derived inbred strains using a 4 million SNP panel.

Chr	QTL Location, Mb	Peak SNP	P-value	Number of known genes
Classical and wild-derived inbred strains				
1	180.76	rs39502136	1.01×10^{-7}	2 (*Kif26b*)
11	21.65-22.58	rs4135796	2.75×10^{-8}	9 (*AV249152*)
		rs26852365	2.75×10^{-8}	(*Ehbp1*)
Classical inbred strains only				
1	178.54-180.76	rs39502136	2.43×10^{-8}	24 (*Kif26b*)
2	168.40-168.43	rs27288988	1.13×10^{-6}	1 (*Nfatc2*)
11	21.65-22.56	rs4135796	3.29×10^{-7}	9 (*AV249152*)
		rs3708339	3.29×10^{-7}	(*AV249152*)
		rs26852365	3.29×10^{-7}	(*Ehbp1*)
11	70.92-71.09	rs282333136	3.31×10^{-6}*	3 (*Nlrplc*)

*, P-value corresponding to a genome-wide false discovery rate of ≤ 10% (q-value of ≤ 0.10). All other results are significant based on genome-wide thresholds corresponding to P-values of
1.01×10^{-7} for 34-strain and 2.54×10^{-6} for 30-strain cohorts. Gene symbol in parentheses indicates the gene containing the peak SNP. Chr, chromosome; QTL, quantitative trait locus; Peak SNP, SNP with highest p-value in QTL interval; P-value, EMMA-corrected p-value; *Kif26b*, kinesin family member 26B; *AV249152*, WD repeat containing planar cell polarity effector; *Ehbp1,* EH domain binding protein 1; *Nfatc2*, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2; *Nlrplc*, NLR family, pyrin domain containing 1C.

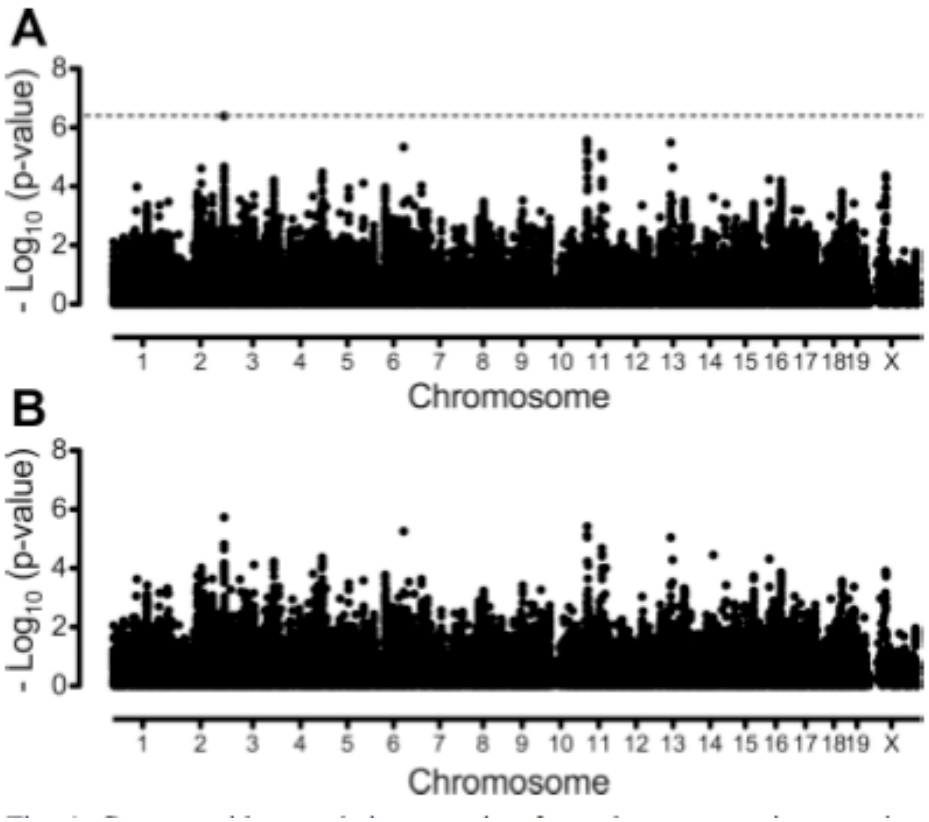


Figure 2.4. Genome-wide association mapping for endurance exercise capacity, expressed as work in kg•m, in male mice from 34 classical and wild-derived inbred strains (A) and 30 classical inbred strains only (B) using the 132 K SNP panel. Association mapping was conducted with EMMA and 132,285 SNP panel. The x-axis indicates genomic position divided by chromosome. Values on the y-axis are *P* values transformed using –log10 (*P* value). The horizontal dashed line in A indicates genome-wide significance threshold. Significance thresholds were calculated using a 5% false discovery rate and correspond to a *P* value of 4.01×10^{-7} for 34 strain cohort. *P* values were too low for the 30-strain cohort to estimate a 5% false discovery rate based on q values.

Table 2.4. Genome-wide association mapping for body mass in male mice from classical and wild-derived inbred strains.

Chr	QTL Location, Mb[a]	Peak SNP	Number of known genes	Previous QTL
Classical and wild-derived inbred strains				
1	54.08	rs6406541	1 (*Hecw2*)	
3	109.58	rs29791710	2	*Bwq8*
Classical inbred strains only				
2	59.19	rs6238344	5	*Pbwg1, Bwtq8*
4	41.42-41.69	rs6222957	13	*Wtlr3, Bwtq9, Wta1*
9	93.76-98.14	rs6360988	28 (*Acpl2*)	*Bwq6*
9	99.78	rs6387071	3	*Bwq6*
15	29.20	rs13482501	0	*Dob3*
17	48.77	rs29537880	3	*Obwq4, W10q12, W6q11, Bodwt2, Wta4*

All associations with P-value < 10^{-5} are reported. Gene symbol in parentheses indicates the gene containing the peak SNP. [a], For single SNP associations, the QTL interval for candidate gene identification was estimated to be 400 kb centered around the peak SNP; Chr, chromosome; QTL, quantitative trait locus; Peak SNP, SNP with highest p-value in QTL interval; *Hecw2*, HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2; *Acpl2*, acid phosphatase-like 2; *Bwq8*, body weight QTL 8 (39); *Pbwg1*, postnatal body weight growth 1 (35); *Bwtq8*, body weight QTL 8 (36); *Wtlr3*, weight loss response 3 (32); *Bwtq9*, body weight QTL 9 (36); *Wta1*, weight adult 1 (7); *Bwq6*, body weight, QTL 6 (30); *Dob3*, dietary obesity 3 (43); *Obwq4*, obesity and body weight QTL 4 (36); *W10q12*, weight 10 weeks QTL 12 (33); *W6q11*, weight 6 weeks QTL 11 (33); *Bodwt2*, body weight 2 (38); *Wta4*, weight adult 4 (7).

Discussion

In general, efforts to determine the genetic basis for variation in exercise capacity have been limited due to small sample size in human studies (56) or limited genetic diversity in animal studies (8, 101, 109). Advances in sequencing technology and SNP discovery have led to the development of large SNP datasets for inbred mice (43, 149). In turn, these datasets have facilitated the advancement of genome-wide association studies in mice (50, 69, 124). Therefore, the aim of this study was to identify strain-specific differences in intrinsic endurance exercise capacity across 34 strains of inbred mice and to identify significant QTL regulating intrinsic exercise capacity using genome-wide association mapping. Using this large strain set, significant variation for intrinsic endurance exercise capacity was found across strains. This variation allowed for the identification of novel QTL for exercise capacity using association mapping, including one QTL on Chromosome 2 that overlaps with a suggestive QTL for the response to exercise training (change in VO_2max) in a human linkage study. The majority of the peak SNPs for these QTL are located within genes, suggesting that these genes are prospective candidates for influencing variation in intrinsic endurance exercise capacity. Although these QTL require confirmation using traditional approaches, they provide potential new targets for identifying the underlying genetic basis for variation in endurance exercise capacity.

In the current study, phenotype data from 34 classical and wild-derived inbred mouse strains were used to conduct a genome-wide association study for intrinsic endurance exercise capacity. This large phenotype dataset represents a 3- to 4-fold expansion of exercise capacity measurements over previous reports. Using male mice

from seven inbred strains, Lerman et al. reported a 1.7-fold difference in maximal running speed across strains (89). Lightfoot et al. showed a similar degree of variation in running distance across 10 inbred strains (99). In the present study, 34 strains were utilized and included mice from all seven strain groupings from the mouse family tree (117), indicating a wide range of genetic diversity. Many of these strains were chosen based on their priority for inclusion in the Mouse Phenome Database (http://phenome.jax.org/) or their role as a founder strain for the Collaborative Cross recombinant inbred line panel (129S1/SvImJ, A/J, C57BL/6J, CAST/EiJ, NOD/LtJ, PWK/PhJ, WSB/EiJ) (http://compgen.unc.edu). There was a 2.5- to 3-fold difference between the highest and lowest performing strains with respect to time. This range is comparable to previous reports, despite differences in testing protocols and equipment. Only two strains, C57BL/6J and DBA/2J, were common amongst all three studies with the C57BL/6J strain ranking at or near the bottom in all three studies. High performing strains, FVB/NJ (89) and SWR/J (99) also performed well in our study, suggesting that these strains might be useful for future studies contrasting high (FVB, SWR) and low (C57BL/6J) performing strains.

Interestingly, despite being a low performing strain in treadmill-based exercise tests (89, 99, 109, 110), C57BL/6J mice are generally considered a high performing strain for wheel running (89, 97). The lack of correlation between treadmill running performance and voluntary wheel running performance in C57BL/6J and other strains (89) suggests that these models assess different phenotypes related to exercise. Some contributing factors could be differences in protocols for assessing exercise capacity and the exercise intensity. For example, treadmill running as described here is a measure of

acute exercise capacity, whereas wheel running is generally measured over more than one day (97). In addition, motivation to run likely has a strong influence on wheel running performance, while workload is controlled in treadmill running (1). Thus, differences in methodology, physiological responses, and contrasting performance within some inbred strains suggest that wheel running and treadmill running represent different phenotypes related to exercise performance.

The large number and genetic diversity of the strains utilized in the current study was amenable to performing a genome wide association study to identify putative QTL for intrinsic endurance exercise capacity. In the current study 8 significant or suggestive associations for intrinsic endurance exercise capacity were identified on 5 different chromosomes (Tables 2.2 and 2.3). None of the QTL from the current study overlap with previously identified QTL for intrinsic exercise capacity in mice or rats. Using traditional linkage analysis, significant QTL for running distance were identified on Chromosomes 8 and X (101). Significant QTL for pre-training work were identified on Chromosomes 14 and 19 and suggestive QTL on Chromosomes 1 (~189 Mb), 2 (~80 Mb), 3, and 8 in F_2 mice derived from FVB and B6 strains (110). Ways et al. reported several significant QTL for aerobic running capacity in rats, which map to regions of the rat genome that are syntenic with regions on mouse Chromosomes 3 and 8 (109). The lack of overlap across studies may be due to differences in the genetic diversity represented in the linkage and association studies. Traditional linkage studies in rodents are dependent on the genetic variation present in the two parental lines, whereas association mapping studies incorporate the genomes of a large number of inbred strains, allowing for greater diversity and enhanced resolution (11, 69). Furthermore, the current

study utilized only male mice whereas previous work included mice from both sexes. Sex differences in exercise performance in rodents have been reported (8, 97, 101) as have sex-specific QTL for various traits, including exercise capacity and physical activity (11, 97, 101, 109). In fact, Lightfoot et al. reported that 67% of physical activity QTL identified in a genome wide association study were sex-specific (97). Based on those results, separate studies of exercise capacity for each sex might be warranted.

Two significant associations for time (Chr 2 and Chr 11) were present in the 34-strain cohort and the 30 classical inbred strains using the 132 K panel and corroborated using the 4 M SNP panel. The putative QTL interval on Chromosome 2 is relatively small (180 Kb) and was further narrowed to ~27 Kb using the 4 M SNP panel. This interval contains only 1 gene, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 (*Nfatc2*). *Nfatc2* is a transcription factor belonging to the Nfat family (125). Nfat proteins are regulated by calcineurin, a calcium-calmodulin dependent phosphatase (125). *Nfatc2* is required for calcineurin-mediated cardiac hypertrophy (22) and also contributes to signaling pathways regulating angiogenesis (58) and skeletal muscle growth (65). Furthermore, the marker *D20S857* maps to human Chromosome 20 (position: 50.1 Mb, q13.2), which is syntenic with the putative QTL on mouse Chromosome 2. This marker was linked to the response to exercise training (change in VO_2max) in African Americans in the HERITAGE Family Study (127). Based on the potential links to endurance exercise in humans and mice, this genomic region is of interest and should be considered for more detailed analyses of genetic variants contributing to differences in intrinsic endurance exercise capacity.

The other QTL (Chr 11, ~21 Mb) associated with exercise time was identified in all analyses. This interval was relatively large (~900 Kb) and contains 9 known genes with the peak SNPs falling within *AV249152* (also identified as *Wdpcp*) and *Ehbp1*. No genes within this region have been directly linked to exercise performance. However, several other significant QTL also were identified on Chromosome 11 and these regions contain genes with potential links to exercise performance (i.e., *Eno3, Zfp3*) (30, 81). In addition, a previously reported suggestive QTL for the change in work in response to four weeks of exercise training in an F_2 population derived from FVB and B6 mice was identified on Chromosome 11 (72.6 Mb) (109). Although no direct link to exercise capacity was identified on Chromosome 11, the collective evidence suggests that this chromosome should be considered for further investigation.

Several of the high performing strains in this study are classified as wild-derived inbred strains. These strains were captured in the wild and subsequently inbred for 20 or more generations. Inclusion of these wild-derived inbred strains expands the range of strains measured for endurance exercise capacity and provides phenotype information on mouse strains that are extremely different genetically from classical inbred strains (149). Mice from the four wild-derived inbred strains had exercise times that exceeded 38 minutes with a very narrow range of times (38.5 – 42.2 min). In general this is in agreement previous reports of exercise capacity in wild mice (48, 132). Therefore, inclusion of wild-derived strains can increase both genetic and phenotypic diversity to strain screens for exercise-related phenotypes. However, including these strains in association mapping studies is somewhat controversial because wild-derived strains are so genetically dissimilar to classical inbred strains (149). In the present study,

performing association mapping with and without inclusion of wild-derived strains markedly changed the number of significant associations (Table 2.2 and 2.3). Lightfoot et al. also observed an affect of the wild-derived strains on association mapping for wheel running traits (97). Twelve QTL were identified for wheel running phenotypes using male and female mice from 38 inbred and wild-derived strains. This number was notably reduced after removing the wild-derived strains. Although the use of a large number of inbred strains and a high-density SNP map is recommended for genome wide association mapping (135), the effect of wild-derived strains on association mapping observed by Lightfoot and by us suggests that it would be prudent to conduct analyses with and without these strains to identify significant associations and the effect of population structure on the genetic architecture of complex traits.

QTL were identified for exercise capacity using EMMA to perform association mapping. One criticism of genome wide association mapping approaches including single SNP marker and haplotype mapping, which relies on a 3-SNP window to define haplotype blocks, is that it can be affected by population structure, sparse SNP data, regions of low polymorphisms, or high haplotype diversity, potentially resulting in a large number of false positives (135). Although EMMA also has limitations (150), it was selected for association mapping because it incorporates a kinship matrix that corrects for relatedness among inbred strains (69). This should correct for differences in population structure due to the inter-relatedness of inbred strains, which has been shown to reduce the number of false positive associations compared with other mapping approaches (69). Although EMMA has been used successfully to identify significant associations for a variety of physiological and disease-related traits (11, 35, 69),

traditional linkage analysis needs to be performed to confirm results from mouse genome-wide association studies (102, 131).

In summary, we performed a large strain survey for exercise capacity using a graded treadmill test. This study demonstrated that a large strain survey and association mapping could be used to identify new QTL for exercise capacity. As with most genome-wide association studies, replication studies are needed to confirm our findings. One option is to use the strain survey data presented here, coupled with large SNP databases to identify the most appropriate strains for traditional linkage analysis to confirm these QTL. In addition, the narrow QTL regions identified here contain a small number of potential candidate genes that can be systematically studied to confirm their role in modulating exercise capacity. Future studies confirming these QTL and identifying the physiological relationship between candidate gene and exercise capacity are required to establish this gene or genetic variant as being the underlying cause of differences in exercise capacity.

CHAPTER III

THE EFFECT OF CHROMOSOME SUBSTITUTION ON INTRINSIC ENDURANCE EXERCISE CAPACITY IN F_2 GENERATION MICE

Introduction

Exercise capacity, measured by exercise time during a graded treadmill test, is generally used to assess cardiorespiratory fitness and is highly correlated to risk of cardiovascular disease (117). Results from cross-sectional, twin, and prospective studies indicate that there is a large genetic component to exercise capacity (15, 16, 17, 25). Having higher levels of exercise capacity has been shown to be beneficial for reducing the onset of cardiovascular disease; the factors determining exercise capacity have been widely studied (12,13, 15, 117). Presently, the genetic contribution to intrinsic endurance exercise capacity is not completely understood. To address the genetic component of exercise capacity, several candidate genes contributing to improved exercise capacity have been proposed based on these studies (18, 19, 20, 21, 123, 144). However, no causative relationship has been currently established.

Several studies have investigated the genetic factors contributing to endurance exercise capacity using inbred rodent models (7, 36, , 97, 99, 100, 101, 102, 109, 110, 166). One method to identify the genetic factor underlying a complex trait is to screen multiple rodent strains specific phenotype followed by quantitative trait locus (QTL) analyses. This approach has been used to identify QTL for intrinsic endurance exercise capacity in rats (166) and mice (36, 100, 101, 101, 109, 110). Research from our laboratory previously identified significant and suggestive QTL on several chromosomes that may house candidate genes that influence variation in endurance exercise capacity

(36, 109, 110). These identified regions overlap with other mouse and human QTL, suggesting that these regions and/or genes are conserved among species (110). In the current study we employed a relatively new mouse model, chromosome substitution strains (CSS) to assess the contribution of individual chromosomes to endurance exercise capacity (10, 36, 118, 119, 144). CSS mice are made by substituting a single chromosome from a donor inbred strain on the genetic background of a host inbred strain (recipient). Therefore phenotypic differences between the recipient or background strain mice and CSS mice support the presence of a QTL on the substituted chromosome for the phenotype being measured. Results from a previous study identified the A/J strain as a low endurance exercise capacity strain in comparison to the C57BL/6J (B6) strain and therefore we chose to use CSS mice based on A/J and B6 inbred strains (36).

Utilizing this CSS model, the main purposes of the present study are to investigate the effect of chromosome substitution on endurance exercise capacity and to identify novel QTL regulating endurance exercise capacity in mice. We hypothesize that chromosome substitution will significantly effect exercise capacity. We also expect to identify novel QTL on chromosome 14 and to confirm the importance of chromosome 14 in the genetic contribution and regulation of endurance exercise capacity in mice.

Methods

Animals. All procedures adhered to the established National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Texas A&M University. Seven week-old inbred mice (A/J, C57BL/6J, chromosome 3 (B6.A3) and chromosome 14 substitution mice (B6.A14)) (n=12/strain, 6 male and 6 female mice) were purchased

from Jackson Laboratory (Bar Harbor, ME.). Upon arrival at Texas A&M, all mice were given one week to acclimate to their new environment. Chromosome substitution (CSS) mice were selected to confirm a previously identified QTL for endurance exercise training (110) using B6 as the genetic background strain and A/J as the donor strain. To generate the population for QTL mapping, male B6 mice were crossed with female chromosome substitution B6.A14 mice to generate F_1 mice. The F_1 mice were then intercrossed to produce 155 F_2 generation mice (67 male and 88 female mice). All mice were allowed food and water *ad libitum* and maintained on a 12 hr light:dark schedule.

Exercise performance test. At 8 weeks of age, all mice were familiarized to run on a motorized rodent treadmill (Columbus Instruments, Columbus, OH), with an electric grid at the rear of the treadmill for two days as described previously (109, 110). On day 1 mice ran for 10 min up a 10° incline at 9.0 m/min, and ran for 10 min at 11.0 m/min on day two. Each mouse then completed two graded exercise tests separated by 48 hrs. Mean values for each mouse were used for statistical analyses. For each performance test, the treadmill was started at 9.0 m/min at 0° grade for 9 minutes as a warm-up. The grade was then increased 5° every 9 minutes up to a final grade of 15° and speed was increased 2.5 m/min from a starting speed of 10 m/min every three minutes until exhaustion. Exercise continued until each mouse refused to run, defined as spending >50% of the time or >15 consecutive seconds on the grid (109, 110). Each mouse was immediately removed from the treadmill when exhaustion was determined and returned to its home cage. Exercise capacity was estimated for each animal using time (minutes) and work (kg·m). Work performed (kg·m) or vertical work was calculated as a product of body weight (kg) and vertical distance (meters), where vertical

distance = (distance run)(sinθ), where θ is equal to the angle of the treadmill from 0° to 15° (109, 110).

Genotyping. Twenty-four hours after the last graded endurance exercise test, heart, gastrocnemius, plantaris, soleus muscle and liver tissue were excised from mice, washed in ice-cold (4°C) saline, blotted dry to remove excess liquid, and snap froze in liquid nitrogen. DNA was isolated with a DNeasy Blood and Tissue kit (Qiagen Science, Germantown, Maryland) from 25 mg of liver tissue and quantified using nanodrop spectrophometry. Genotyping was performed using competitive allele-specific polymerase chain reaction (PCR) single nucleotide polymorphism (SNP) genotyping (Kbiosciences, Hoddesdon, UK) (Massett 2009). All 155 F_2 mice were genotyped using 12 SNPs spaced at approximately 5 cM intervals (124).

QTL identification. One dimensional linkage scans were performed using R/qtl program to identify novel QTL with main effects on chromosome 14. Permutation tests (1,000 repetitions) were used to identify threshold values for logarithm of odds (LOD) scores (5, 25, 31, 32). For single chromosome-wide scans with no covariates, LOD scores ≥ 2.30 and 2.23 were considered significant ($P \leq 0.05$) for time and work, respectively. For each exercise phenotype, sex was included as additive and interacting covariate in single genome scans. Differences in LOD scores (ΔLOD) ≥ 2.0 between scans that included additive and interacting covariates were considered significant. If sex was found to be a significant interacting covariate, one-dimensional scans were performed on male and female mice separately to identify potential sex-specific QTL.

Statistics. All data are presented as mean ± SE. In this study statistical significance was denoted with $P < 0.05$. Comparisons of two way ANOVA (sex x strain)

was used to determine the effect of sex on endurance exercise phenotype data, which is defined as time (minutes), or work (kg•m), and sex by genotype differences (SPSS (19.0). If significant strain-by-sex interactions were detected, males and females were analyzed separately using one-way analysis of variance. Dunnett's post hoc test was used to determine significant strain differences compared with B6.

Results

Exercise capacity – inbred strains. Exercise capacity, defined as mean run time during two graded exercise treadmill tests, for inbred and CSS strains is shown in Figure 3.1. Exercise time in A/J and B6.A14 mice were significantly less than that in B6 mice. Run time in B6.A3 mice was not different from that in B6 mice. A significant effect of sex was identified in 3 strains (A/J, B6.A14, and B6). For each strain, female mice ran significantly longer than male mice from the same strain (Figure 3.1). When exercise capacity was expressed as work, all strains were significantly different from B6, with A/J and B6.A14 performing less work and B6.A3 performing more work than B6 mice (Figure 3.2). For each strain there was also a significant effect of sex. Male mice performed more work than female mice for all strains except A/J, which showed the opposite result (Figure 3.2). The differences in work are likely due to differences in body mass (Figure 3.3). Body mass in A/J, and B6.A14 were significantly different in comparison to B6, but B6.A3 strain was not different from B6. Sex had a significant effect in all strains tested with males significantly different from females (Figure 3.3).

Exercise capacity – (B6.A14 x B6) F_2 mice. The sex-specific distributions for exercise time and work in F_2 mice are shown in Figures 3.4 and 3.5. Both time and work varied significantly between male and female F_2 mice. On average, run time was

significantly greater in female F_2 mice (31.90 ± 0.08 min) compared with males (29.27 ± 0.15 min), whereas work was not significantly different between males (1.68 ± 0.03 kg•m) and females (1.70 ± 0.02 kg•m). Body mass was significantly greater ($P < 0.05$) in males (25.0 ± 0.21 g) compared with females (19.9 ± 0.15 g). Relative to the progenitor strains, eight week old F_2 mice ran an average of 30.77 ± 0.1 min, which was significantly longer ($P < 0.05$) than B6 (30.48 ± 0.19 min) and B6.A14 (27.52 ± 0.2 min) progenitor strains. Statistical analysis indicated that F_2 (B6 x B6.A14) mice performed significantly more work (1.69 ± 0.01 kg•m, $P < 0.05$) than B6 (1.45 ± 0.03 kg•m) and B6.A14 (0.95 ± 0.15 kg•m) strains. Body mass was compared across strains and generations. F_2 (B6 x B6.A14) mice had average body mass of 22.1 ± 0.2 g which was significantly different ($P< 0.05$) compared to the progenitor B6 strain (22.7 ± 0.3 g), and the CSS progenitor B6.A14 strain (18.9 ± 0.5 g).

QTL analysis. The program Rqtl identified a novel QTL on chromosome 14 for endurance exercise capacity. QTL analyses were performed three different ways: using all mice with no covariate, all mice with sex as an additive covariate, and all mice with sex as an interactive covariate. For single genome scan, suggestive QTL ($P < 0.63$) were identified for time and work (Figure 3.6). When sex was included as an interacting covariate, significant QTL were identified (Δ LOD score $\geq$ 2.0) at 57 cM for time and work (Figure 3.7). Because significant QTL were identified using sex as an interacting covariate, chromosome-wide scans were performed on male and female mice separately. In male mice, QTL scans approached significance for time (LOD = 2.29, threshold = 2.38) and work (LOD = 2.18, threshold = 2.22) with a common peak at 56 cM (Figure 3. 8). In female mice, a suggestive QTL for work was identified at 18.5 cM, which is different from the peak observed in males (56 cM) and likely explains the lack of a significant finding in the combined population.

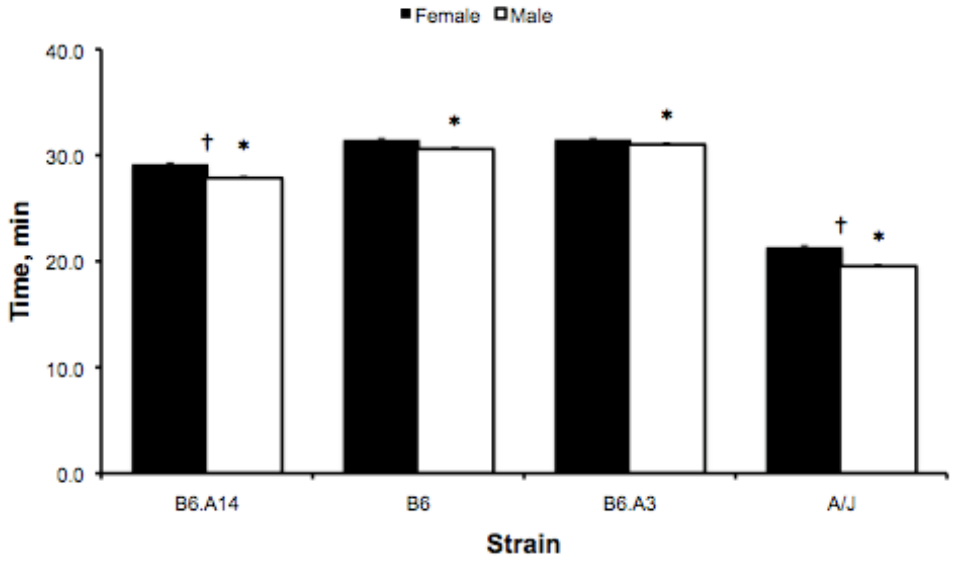


Figure 3.1. Strain-dependent differences in exercise time measured in minutes during a graded exercise test in male and female mice from B6, A/J and CSS strains. Run time was recorded as a measure of exercise capacity. Values are expressed as mean ± SE. *n*= 6 mice per sex, *n* = 12 mice per strain. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.

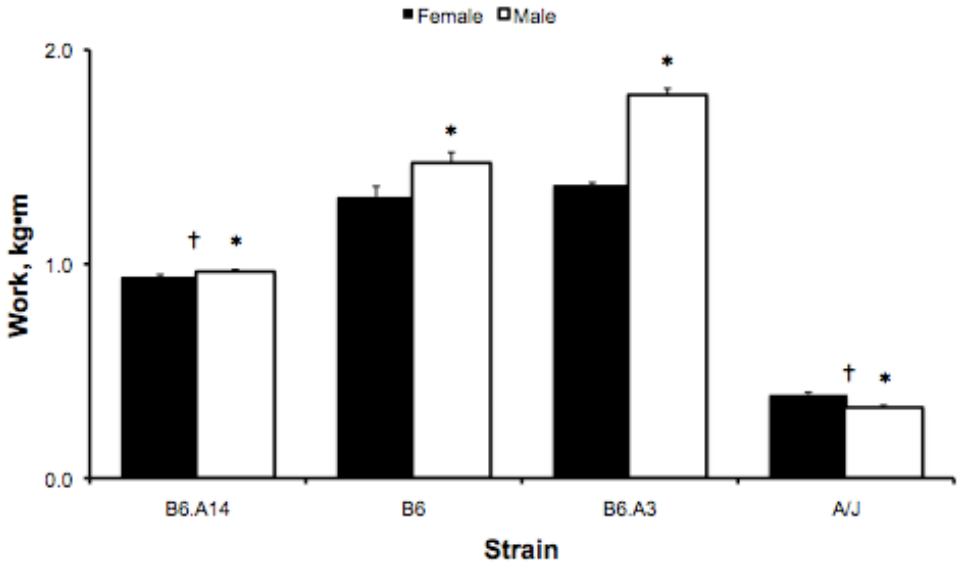


Figure 3.2. Strain-dependent differences in work performed during a graded exercise test in male and female mice from B6, A/J and CSS strains. Vertical work was measured as kg·m and incorporates body weight. Values are expressed as mean ± SE. *n*= 6 mice per sex , *n* = 12 mice per strain. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.

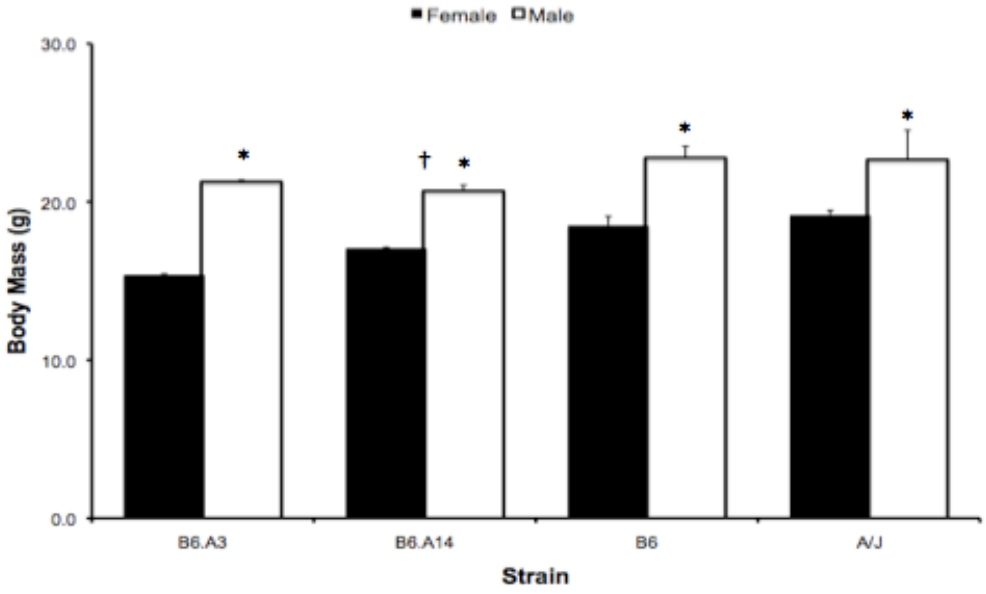


Figure 3.3. Strain-dependent differences in body mass using B6, A/J and CSS mice. Body mass was measured in grams. Values are expressed as mean ± SE. *n*= 6 mice per sex per and 12 mice per strain. Significance noted $P < 0.05$. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.

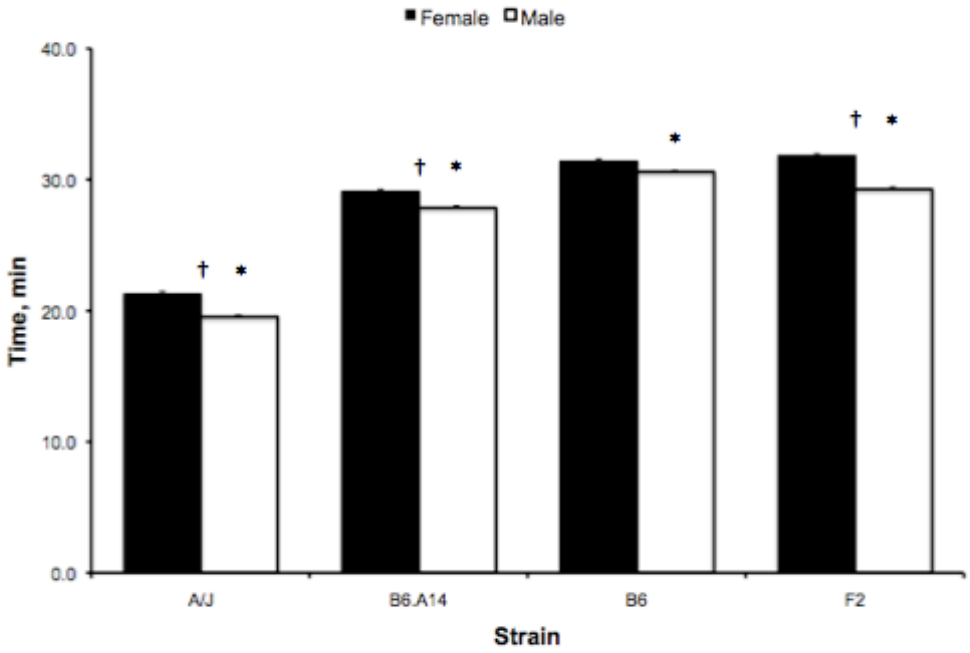


Figure 3.4**.** Run time for F_2 mice during a graded exercise test measured in minutes and displayed as a frequency distribution. Female F_2 mice had higher run times in comparison to F_2 male mice. $n = 88$ female mice and 67 male mice. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.

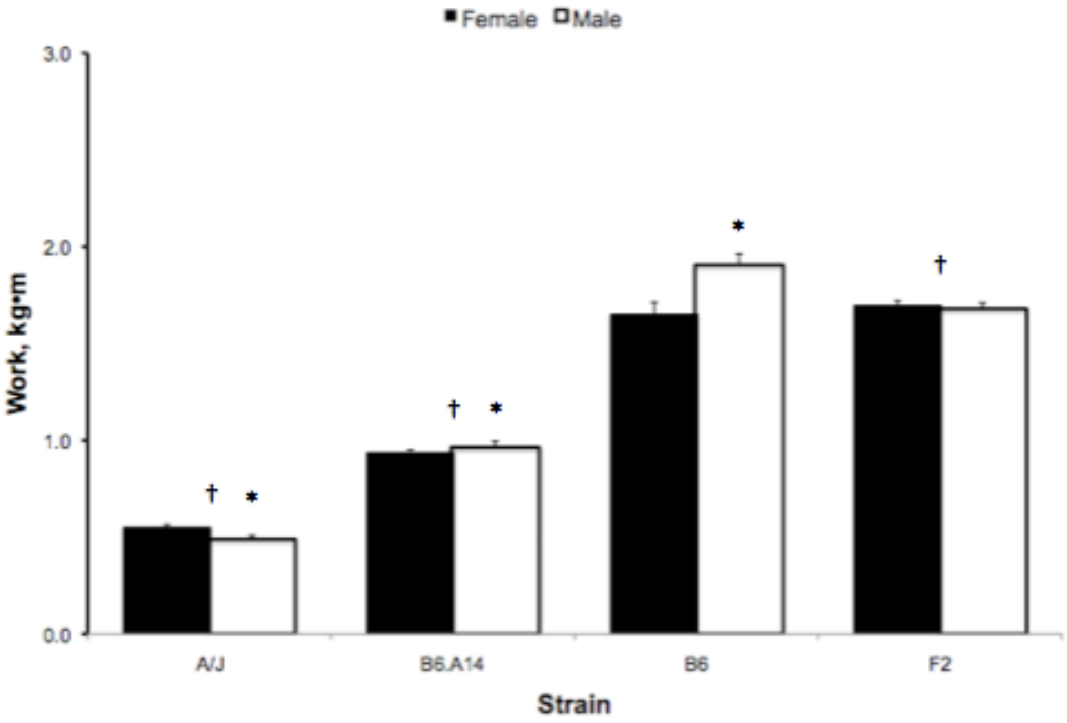


Figure 3.5. Work measured during a graded exercise test for F_2 mice and measured in kg·m. Vertical work incorporates body mass and degree of the treadmill. Body mass differences between sexes effects the measured amount of vertical work. $n = 88$ female F_2 mice and 67 male F_2 mice. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.

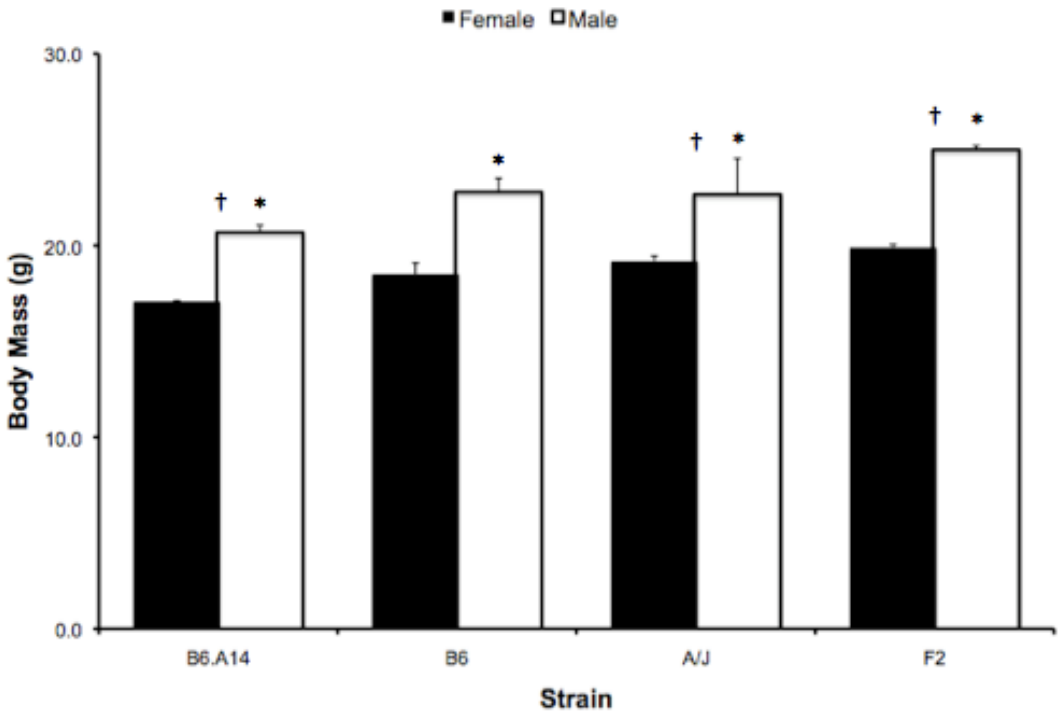
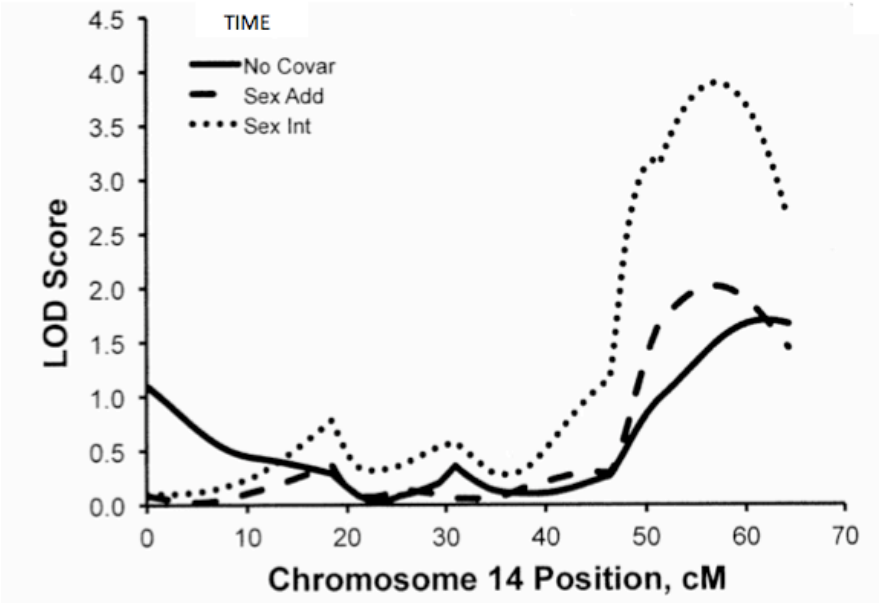


Figure 3.6. Body mass for F_2 mice measured in grams and expressed as a frequency distribution. F_2 male mice had higher body mass in comparison to F_2 female mice. n = 88 female F_2 mice and 67 male mice. * $P < 0.05$ compared to females, † $P < 0.05$ compared to B6.



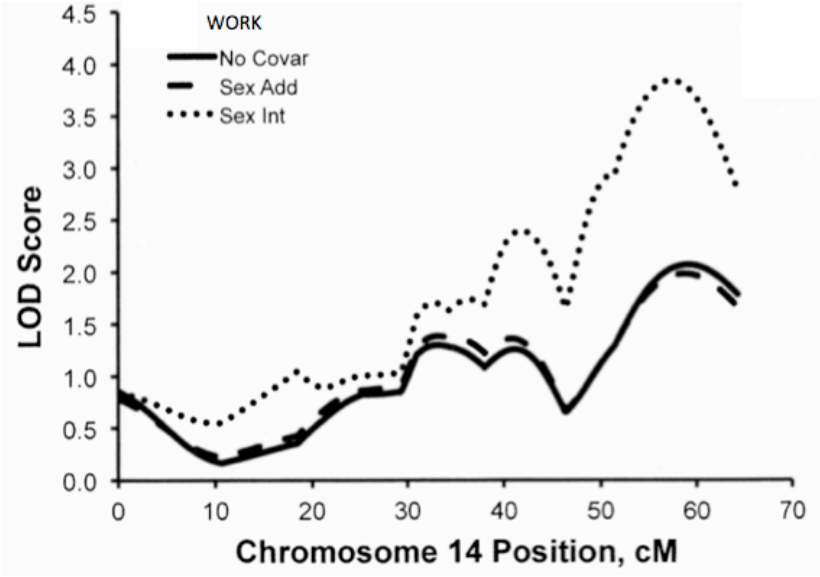


Figure 3.7. QTL analyses for intrinsic endurance capacity expressed as time (min, upper panel) and work (kg•m, lower panel) using R/qtl and incorporating all F_2 generation mice (n=155). Three analyses were performed with no covariate, sex as an additive covariate and sex as an interactive covariate. LOD scores above 0.80 and 2.30 are considered suggestive ($P < 0.63$) and significant ($P < 0.05$), respectively for time. LOD score thresholds for suggestive ($P < 0.63$) and significant ($P < 0.05$) QTL for work are 0.82 and 2.29, respectively.

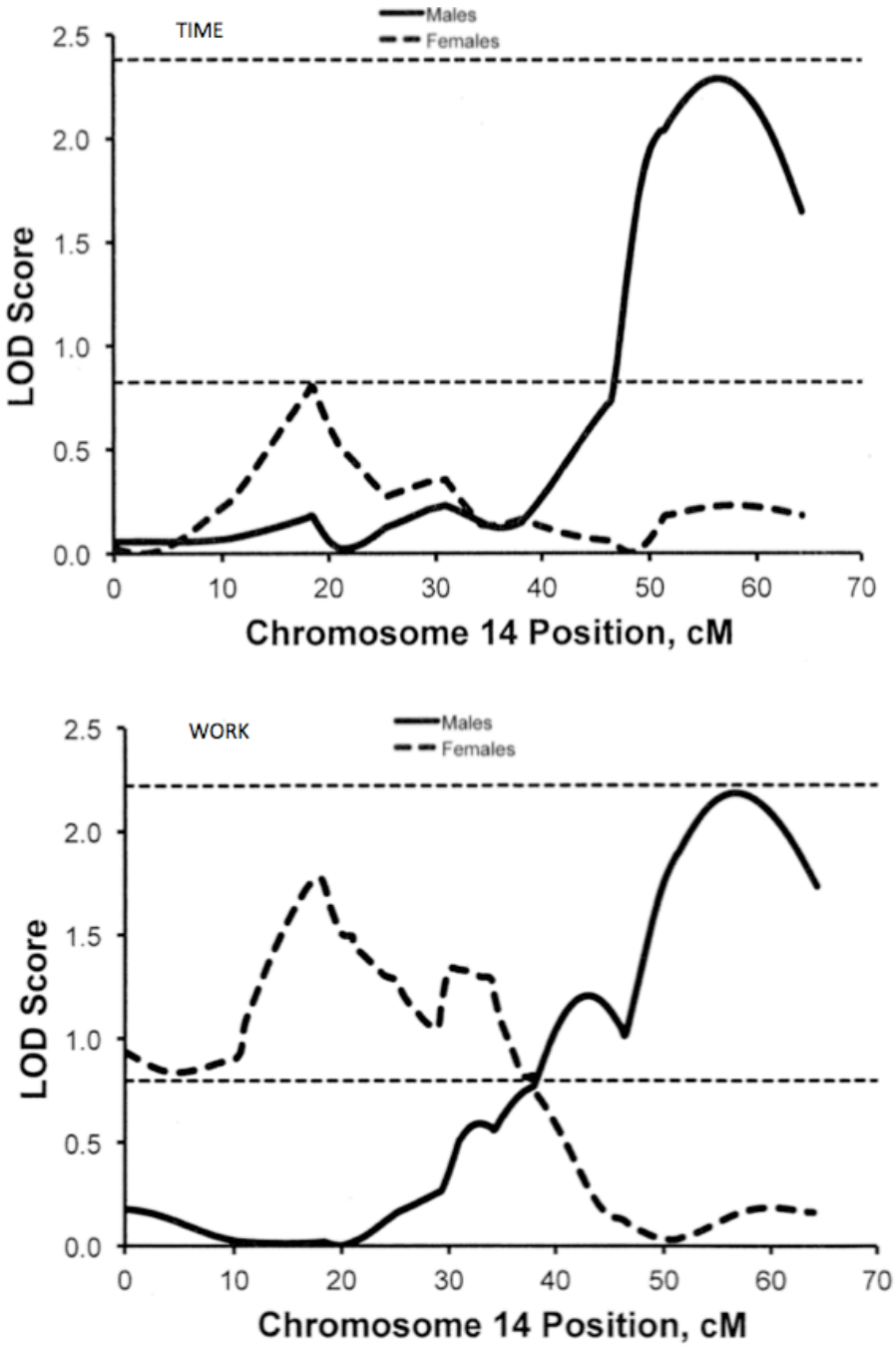


Figure 3.8. QTL analyses for the effect of sex on intrinsic endurance exercise capacity expressed as Time (minutes) and Work (kg•m). Single chromosome-wide scans for time and work were performed separately on male and female F_2 mice. Dashed lines in the upper graph represent the suggestive (LOD = 0.82, $P < 0.63$) and significant (LOD = 2.38, $P < 0.05$) thresholds for time in males. Dashed lines in the lower graph represent the suggestive (LOD = 0.80, $P < 0.63$) and significant (LOD = 2.22, $P < 0.05$) thresholds for work in males.

Discussion

QTL for intrinsic exercise capacity expressed as work were previously identified on Chromosomes 3 and 14 (110). In the present study, CSS mice based on inbred B6 and A/J strains were used to confirm the importance of these chromosomes in determining exercise capacity. A significant difference was observed between B6.A14 and B6 mice for exercise time and work, confirming the importance of chromosome 14 for determining the genetic basis for exercise capacity. Conversely, exercise time was not different between B6.A3 and B6 mice, suggesting that the previously described QTL might be population-specific. Following a comprehensive phenotypic investigation of intrinsic endurance exercise across progenitor strains and F_2 derived from B6 and B6.A14 strains, we identified potential sex-specific QTL for time (minutes), and work (kg•m) on chromosome 14. Analysis of the data also identified significant differences in endurance exercise performance between sexes. In fact our data clearly demonstrate the importance of a sex effect on differences in intrinsic endurance exercise performance because the QTL identified is male-specific.

Previous work (36) and the current study (Figures 3.1 and 3.2) identified strain-dependent differences in exercise time and work between A/J and B6 strains. B6 mice, which are the most commonly used inbred train in biomedical research, exhibited greater exercise performance during a graded exercise test than the A/J strain. Because these strains are inbred, phenotypic differences between them suggest there are genetic differences underlying this phenotypic variation. Therefore, chromosome substitution strains (CSS) based on A/J and B6 strains were utilized to assess the contribution of chromosomes 3 and 14 on endurance exercise capacity. Significant differences between

the background strain (B6) mice and B6.A14 were identified for time and work (Figures 3.1 and 3.2). These differences support the presence of a QTL on chromosome 14 for time and work. Conversely, exercise phenotypes were similar in B6 and B6.A3 mice; therefore, there is no support for an exercise QTL on chromosome 3 using this cohort. However, data from a 2009 study identified QTL on chromosome 14 for pre-training work in F_2 mice at 4.0 cM and had a LOD score of 3.72 (110). The study also identified a suggestive QTl on chromosome 14 at 36 cM, with a LOD score of 2.25 for post-training work (110). The data from this study indicated a genetic component to endurance exercise capacity and lent strong evidence to investigate chromosome 14 using CSS mice in the current study.

Although CSS mice are a good model to identify chromosomes of interest for genetic analysis, QTL identification is limited because QTL can only be localized to a specific chromosome. To better localize the QTL on chromosome 14, F_2 mice were generated from B6 and B6.A14 progenitor strains. On average, run time and work were significantly greater in (B6.A14 x B6) F_2 mice compared with the background B6 strain. However, single chromosome scans did not identify any significant QTL. One suggestive QTL for each phenotype was identified at 56 cM on chromosome 14. This QTL does not overlap with the previously identified QTL for work on chromosome 14 (4.0 cM) in F_2 mice derived from B6 and FVB strains (110). It is, however, relatively near a suggestive QTL identified for the change in work with exercise training (36.0 cM) found in this same population (110), suggesting that several exercise capacity-related QTL exist on this chromosome. Furthermore, in the current study, when sex was included as a covariate, significant QTL were identified for time and work. Further

analysis using males and females separately indicates the suggestive QTL identified in the entire F_2 population is a male-specific QTL. Collectively, the data from this study and our previous work indicate that genes on chromosome 14 are important in determining the genetic basis for exercise capacity. These data also imply that chromosome 14 might contain more than one QTL for exercise capacity.

Data from the present study for average run time (exercise capacity) during a graded exercise treadmill test indicates sex had a role in determining exercise capacity (P <0.05). Sex was identified as an important variable and this is supported by male and female mice identified as significantly different in endurance exercise capacity (p< 0.05). The differences in male and female mice illustrate sex as an important variable for identifying differences in endurance exercise capacity. Work (kg•m) performed during graded exercise tests also identifies sex as an important variable for intrinsic endurance exercise capacity. Previous research from Tarnapolsky identified important differences in muscle metabolism between sexes and this further supports the hypothesis that males and females are different for endurance exercise capacity and there is a genetic component as well as other metabolic factors driving the differences in performance between sexes for a graded endurance exercise test (137, 156).

Body mass, measured in grams, was identified as an important variable throughout this study. In comparison to the B6 reference strain, all other strains were found to be significantly different for body mass. F_2 generation mice were also found to be significantly different from B6 mice. Body weight is an important variable to investigate in this because it is a factor when calculating the amount of vertical work the mice performed during graded exercise tests. Other research studies from Lightfoot and

Massett have found body weight to be different across mouse strains from those perspective studies (99, 100, 101, 110). A strain screen published in 2012 identified strain-specific differences in body mass using both classical and wild-derived inbred strains of mice (36). Results from Courtney et al. identified mice having less body mass are more efficient runners posting longer run time in minutes in comparison to mice carrying more body mass (36).

In conclusion, the findings of the present study identify chromosome 14 as an important regulator of the phenotype intrinsic endurance exercise capacity. Novel, putative QTL were identified at the 50 cM region of chromosome 14, which were different from QTL previously identified for pre-training work (110). Sex is also an important factor to consider when identifying the genetic contribution to intrinsic endurance exercise capacity. QTL were identified on chromosome 14 when all mice were considered with sex as an interactive covariate, and when mice were analyzed by comparing males to females the novel QTL was identified as a male driven QTL. These findings from the present study, along with results from previous research confirm there is a genetic contribution to intrinsic endurance exercise capacity, and that sex has an effect on intrinsic endurance exercise capacity.

CHAPTER IV

EFFECT OF CHROMOSOME SUBSTITUTION ON ENDURANCE EXERCISE CAPACITY IN MICE

Introduction

Exercise capacity, as assessed by exercise time during a graded treadmill test, is commonly used to assess cardiorespiratory fitness and is highly correlated to risk of cardiovascular disease (12, 13, 117). Because of these beneficial effects of exercise, the factors determining exercise capacity have been widely studied. However, the genetic determinants of exercise capacity are poorly understood. Both maximal exercise capacity and sub-maximal endurance exercise performance are complex polygenic traits (17). Results from cross-sectional, twin, and prospective studies indicate that there is a large genetic component to exercise capacity (17). Several candidate genes contributing to improved exercise capacity have been proposed based on these studies (17, 123). However, no causative relationship has been established.

Several rodent models have been utilized to investigate the genetic factors contributing to endurance exercise capacity (7, 97, 102, 109, 110, 151, 166). One of these, screening of multiple inbred strains for a specific phenotypic trait followed by quantitative trait loci (QTL) analyses has been used to identify QTL for intrinsic endurance exercise capacity in rats (166) and mice (102, 110). We previously identified significant and suggestive QTL on several chromosomes that may house candidate genes that influence variation in endurance exercise capacity (110). These identified regions overlap with other mouse and human QTL, suggesting that these regions and/or genes are conserved among species (110). In the current study we employed a relatively new

mouse model, chromosome substitution strains (CSS) to assess the contribution of individual chromosomes to endurance exercise capacity (118, 119, 144, 146). CSS mice are made by substituting a single chromosome from a donor inbred strain on the genetic background of a host inbred strain (recipient). Therefore phenotypic differences between the recipient or background strain mice and CSS mice support the presence of a QTL on the substituted chromosome for the phenotype being measured. Utilizing this model, the aims of this study were to investigate the genetic basis for variation in endurance exercise capacity and to confirm the role of two previously identified chromosomes (3 and 14) in regulating endurance exercise capacity using chromosome substitution strains of mice.

Methods

Animals. All procedures adhered to the established National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Texas A&M University. We previously compared exercise capacity in male mice from 34 inbred and wild-derived strains (36). Based on results of the strain survey, female and male mice (n = 6/sex) from 2 inbred strains (B6 and PWD) were obtained for further study. These strains were chosen because of the availability of chromosome substitution strain (CSS) mice developed from these progenitors (55). Chromosome substitution mice are genetically identical to the recipient strain except for a single chromosome from the donor strain (118, 119, 144, 146). CSS mice used in this study were obtained from Jackson Laboratory (Bar Harbor, ME) and carry either chromosome 3 or 14 from the PWD (B6.PWD3 and B6.PWD14, respectively) strain on the B6 background. These chromosomes were chosen based

evidence for endurance exercise capacity QTL on these chromosomes (109, 110). Male and female mice (n = 6/sex) from the CSS and inbred progenitor strains were tested for endurance exercise performance. All mice were allowed food and water *ad libitum* and maintained on a 12 hr light:dark schedule.

Exercise performance test. Upon arrival, mice were given one week to acclimate to their new environment. At 8 weeks of age, all mice were familiarized to run on a motorized rodent treadmill (Columbus Instruments, Columbus, OH), with an electric grid at the rear of the treadmill for two days as described previously (109, 110). On day 1 mice ran for 10 min up a 10° incline at 9.0 m/min, and ran for 10 min at 11.0 m/min on day two. Each mouse then completed two graded exercise tests separated by 48 hrs. All mice completed each test. Mean values for each mouse were used for statistical analyses. For each test, the treadmill was started at 9.0 m/min at 0° grade for 9 minutes as a warm-up. The grade was then increased 5° every 9 minutes up to a final grade of 15° and speed was increased 2.5 m/min from a starting speed of 10 m/min every three minutes until exhaustion. Exercise continued until each mouse refused to run, defined as spending >50% of the time or >15 consecutive seconds on the grid (109, 110). Each mouse was immediately removed from the treadmill when exhaustion was determined and returned to its home cage. Exercise capacity was estimated for each animal using time (minutes) and work (kg·m). Work performed (kg·m) or vertical work was calculated as a product of body weight (kg) and vertical distance (meters), where vertical distance = (distance run)(sinθ), where θ is equal to the angle of the treadmill from 0° to 15° (109, 110).

Tissue samples. All mice were anesthetized by intraperitoneal injection of a ketamine (80 mg/kg) - xylazine (5 mg/kg) cocktail before tissue excision. Heart, gastrocnemius, plantaris, and soleus muscles were excised from mice, washed in ice-cold (4°C) saline, blotted dry to remove excess liquid, and weighed to obtain wet weight.

Interval-specific haplotype analysis. To narrow the QTL regions and identify potential candidate genes, interval-specific haplotype analysis for chromosomes 3 and 14 was performed using the mouse SNP strain comparison tool in the Mouse Phenome Database (The Jackson Laboratory, http://phenome.jax.org/). SNP comparisons to identify regions where four high performing strains (PWD, FVB/NJ, AKR/J, and SWR/J) are identical by state and different from four low performing strains (B6, NZW/LacJ, 129X1/SvJ, and KK/HlJ) were based on data from the Center for Genome Dynamics (CGD) imputed SNPs – Build 36 (74 strains). Only SNPs polymorphic between at least 3 high performing strains and 3 low performing strains (at least 6 strains total) were included. SNPs that were not polymorphic between groups or not present for at least 3 strains per group were omitted.

Statistical analysis. All data are presented as mean ± SE. Data were analyzed using SPSS 17.0. Differences among chromosome substitution strains for exercise and anthropometric variables were determined using a general linear model (GLM) univariate analysis of variance using strain and sex as between subject and within subject factors, respectively (33). If significant strain-by-sex interactions were detected, males and females were analyzed separately using one-way analysis of variance. Dunnett's post hoc test was used to identify strains that differed significantly from B6 mice, the recipient strain for CSS mice. A significant main effect for strain indicated the presence

of a QTL on the specific chromosome. In the single-sex analyses, a significant strain effect indicated the presence of a putative sex-specific QTL. Differences between sexes within each strain were determined using Student's T-tests. Pearson correlation analyses were performed for the CSS mice to determine the relation between exercise capacity and anthropometric variables. Statistical significance was set at $P < 0.05$.

Results

Male and female mice from the CSS and their progenitor strains completed graded exercise tests to assess intrinsic exercise capacity. As in the previously reported strain survey, PWD mice had significantly higher exercise capacity compared with inbred B6 mice (Figure 4.1). For exercise time, there was a significant main effect for strain ($P < 0.05$) with B6.PWD3 and B6.PWD14 mice having significantly greater exercise times than inbred B6 mice (Figure 4.1). There was also a significant strain-by-sex interaction ($P < 0.05$); therefore, male and female mice were analyzed separately. In males, exercise time was significantly lower in B6 mice compared with all other strains. In females, B6 mice had significantly lower exercise times compared with B6.PWD3 and PWD mice, but not female B6.PWD14 mice. These results suggest that the QTL detected on chromosome 14 might be male-specific. Between sex comparisons within each strain for exercise time revealed significant differences between male and female B6 and B6.PWD14 mice, whereas exercise time was not different between sexes for B6.PWD3 and PWD mice.

When endurance exercise capacity was expressed as work, there was a significant main effect for strain ($P < 0.05$) with PWD, B6.PWD3 and B6.PWD14 mice performing significantly greater work than inbred B6 mice (Figure 4.2). Based on the

significant strain-by-sex interaction ($P < 0.05$), separate analyses were run on male and female mice to identify putative sex-specific QTL. As with the primary analysis, PWD, B6.PWD3 and B6.PWD14 mice performed significantly greater work than inbred B6 mice regardless of sex. Within each strain, work was significantly greater in male mice compared with female mice for all strains.

Strain-by-sex comparisons were also performed for several anthropometric variables (Table 4.1). Significant main effects for strain were detected for body mass, heart mass, gastrocnemius, soleus, and plantaris muscle masses, as well as tissue mass-to-body mass ratios for heart and soleus muscle. Several significant strain-by-sex interactions were also detected suggesting the presence of putative sex-specific QTL. For body mass and heart mass, significant differences were observed between B6 mice and B6.PWD3 and PWD mice for both sexes, but no difference was observed for B6.PWD14 regardless of sex. Significant strain and strain-by-sex effects were observed for gastrocnemius mass. Strain means for gastrocnemius mass were not different between B6.PWD14 and B6 mice; however, in gender-specific comparisons gastrocnemius mass for B6.PWD14 mice was significantly different from B6 mice for both sexes. For B6.PWD3 mice a significant difference from B6 mice was only detected in males. A significant difference between male B6 and B6.PWD3 mice was also observed for heart mass-to-body mass ratio, which was not present in female mice. This ratio was significantly different between B6 and PWD mice for both sexes.

To determine if certain physical characteristics are associated with exercise time and work, correlation analyses were performed. Exercise time was significantly correlated with all physical characteristics measured except gastrocnemius mass-to-body

mass ratio and plantaris mass-to-body mass ratio (Table 4.2). In general, exercise time was negatively correlated with body mass and the mass of each tissue, suggesting that smaller mice have a higher intrinsic exercise capacity. In contrast, only gastrocnemius mass and soleus mass-to-body mass ratio were significantly correlated with work performed (Table 4.2) and these correlations were in the negative direction.

Interval-specific haplotype analysis. The Mouse Phenome Database was used to identify regions where SNPs for four high performing strains (PWD, FVB/NJ, AKR/J, and SWR/J) are identical by state and different from four low performing strains (B6, NZW/LacJ, 129X1/SvJ, and KK/HlJ). For chromosome 14, twenty-three genes contained SNPs that met the criteria (Table 4.3), 17 of which mapped to the previously identified QTL interval (0-76 Mb) (28). Three of these genes contain non-synonymous SNPs, suggesting a potential change in structure and function. These genes would be primary candidates for further investigation of sequence and expression differences among the strains tested. On chromosome 3, 49 protein coding and hypothetical genes were identified (Table 4.4). Of these 49, 20 were located within the previously identified QTL interval (97-162 Mb) (28) and only one predicted gene contained a non-synonymous SNP.

Table 4.1. Physical characteristics of female and male C57BL/6J, PWD/PhJ, and B6.PWD CSS mice.

	C57BL/6J		B6.PWD3		B6.PWD14		PWD/PhJ	
	female	male	female	male	female	male	female	male
Body mass, g	18.5 ± 0.6†	22.8 ± 0.7	15.2 ± 0.1†	18.6 ± 0.2*	18.0 ± 0.2†	22.7 ± 0.3	13.7 ± 0.3†	14.9 ± 0.4*
Heart mass, mg	104.3 ± 1.9†	122.0 ± 5.9	93.2 ± 2.6†	105.2 ± 1.9*	102.3 ± 1.5†	114.8 ± 1.8	98.7 ± 2.1	97.3 ± 2.9*
HM:BM, mg/g	5.68 ± 0.21†	5.03 ± 0.13	6.03 ± 0.17†	5.57 ± 0.10*	5.69 ± 0.06†	5.03 ± 0.03	7.05 ± 0.26	6.58 ± 0.11*
Gastocnemius mass, mg	113.3 ± 3.4†	151.8 ± 6.0	102.5 ± 2.6†	115.8 ± 3.0*	127.2 ± 3.4	134.7 ± 2.4	79.7 ± 3.1	79.7 ± 6.1*
GM:BM, mg/g	6.18 ± 0.30	6.26 ± 0.15	5.77 ± 0.97	6.13 ± 0.13	7.07 ± 0.19	5.90 ± 0.11	5.69 ± 0.20	5.37 ± 0.19
Soleus mass, mg	7.5 ± 0.3†	9.5 ± 0.2	6.7 ± 0.3†	7.8 ± 0.3*	7.5 ± 0.2†	8.8 ± 0.3	4.7 ± 0.2†	5.7 ± 0.3*
SM:BM, mg/g	0.41 ± 0.03	0.39 ± 0.01	0.43 ± 0.02	0.41 ± 0.02	0.42 ± 0.01	0.39 ± 0.01	0.33 ± 0.01	0.38 ± 0.01*
Plantaris mass, mg	17.8 ± 0.4†	20.3 ± 0.4	15.5 ± 0.7†	18.5 ± 0.6*	18.5 ± 0.2†	21.8 ± 0.7	11.2 ± 0.3	16.0 ± 3.5*
PM:BM, mg/g	0.97 ± 0.04	0.84 ± 0.02	1.00 ± 0.04	0.98 ± 0.03	1.03 ± 0.02	0.96 ± 0.03	0.80 ± 0.02	1.09 ± 0.25

Values are mean ± SE. n = 6 per group, except PWD males where n = 3-4. HM:BM, heart mass-to-body mass ratio; GM:BM, gastrocnemius mass-to-body mass ratio; SM:BM, soleus mass-to-body mass ratio; PM:BM, plantaris mass-to-body mass ratio. *, P<0.05 significant main effect for strain compared with C57BL/6J. †, P<0.05 significantly different from male mice of same strain.

Table 4.2. Pearson correlation coefficients between time and work and body mass, heart mass, and muscle masses of B6, PWD, and CSS mice.

	Time, min	Work, kg·m
Body mass, g	-0.718 **	-0.135
Heart mass, mg	-0.483 **	-0.019
HM:BM, mg/g	0.738 **	0.246
Gastorocnemius mass, mg	-0.795 **	-0.337 *
GM:BM, mg/g	-0.280	-0.272
Soleus mass, mg	-0.753 **	-0.266
SM:BM, mg/g	-0.322 *	-0.325 *
Plantaris mass, mg	-0.616 **	-0.129
PM:BM, mg/g	0.005	-0.048

HM:BM, heart mass-to-body mass ratio; GM:BM, gastrocnemius mass-to-body mass ratio; SM:BM, soleus mass-to-body mass ratio; PM:BM, plantaris mass-to-body mass ratio. *, $P<0.05$; **, $P<0.01$.

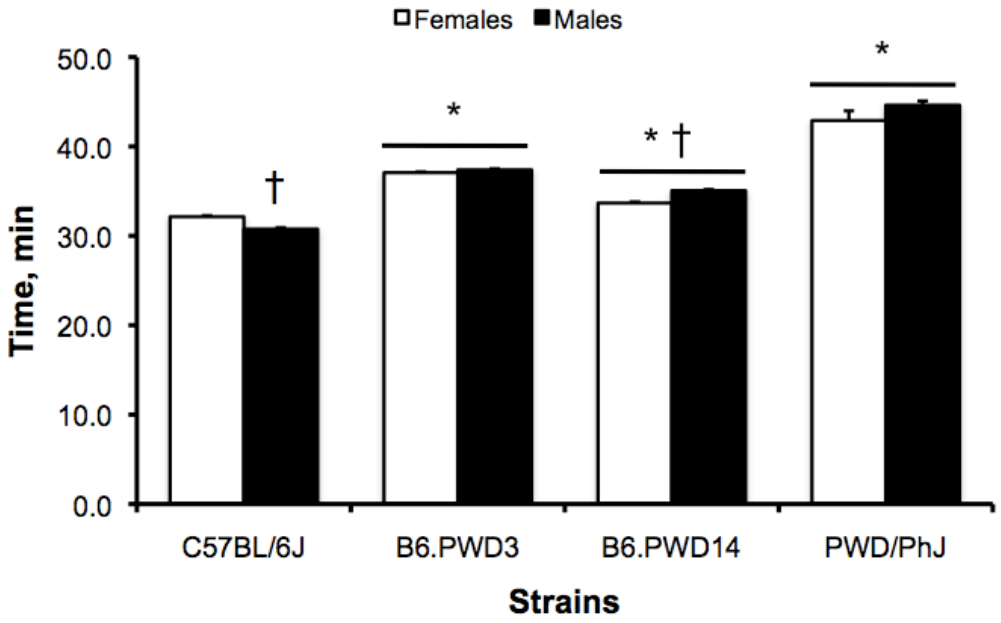


Figure 4.1. Endurance exercise capacity expressed as time (in min) in male and female inbred and CSS mice. Male and female CSS mice were derived from the chromosome donor strain (PWD) and background strain (B6). Substituted chromosomes are 3 (B6.PWD3), and 14 (B6.PWD14). Values are expressed as mean ± SE. $n = 6$ per group, except for PWD males (n = 4). * $P < 0.05$ significant strain effect vs. B6; † $P < 0.05$ significantly different from males of the same strain. Exercise time was significantly greater in PWD, B6.PWD3, and B6.PWD14 mice compared to B6.

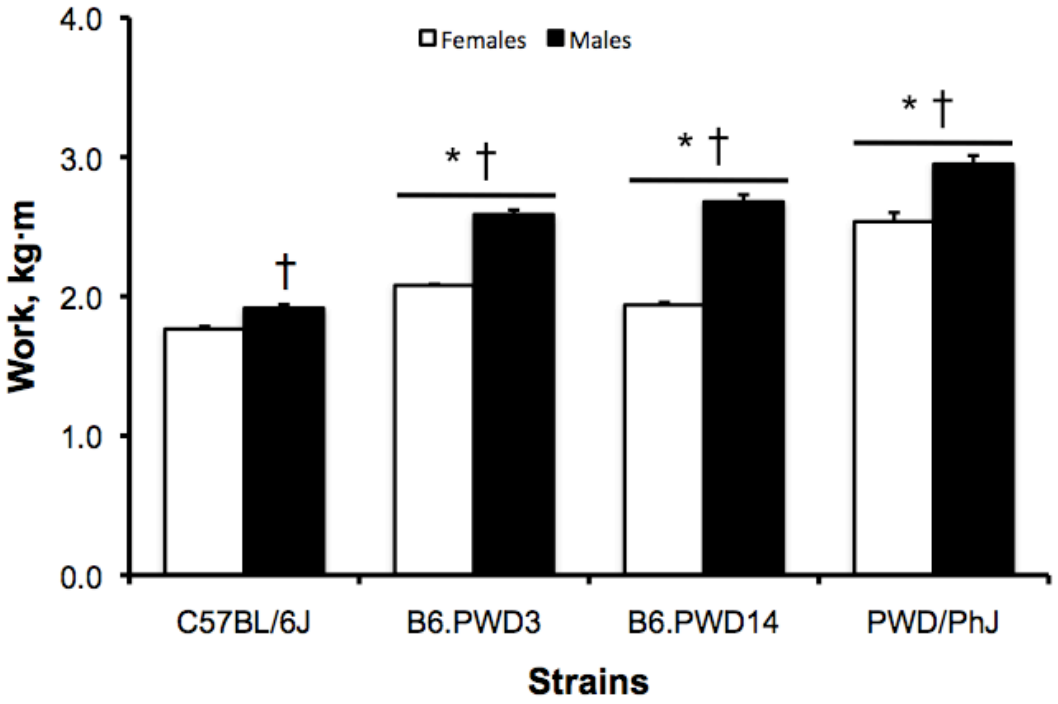


Figure 4.2. Endurance exercise capacity expressed as work (in kg·m) in male and female inbred and CSS mice. Male and female CSS mice were derived from the chromosome donor strain (PWD) and background strain (B6). Substituted chromosomes are 3 (B6.PWD3), and 14 (B6.PWD14). Values are expressed as mean ± SE. $n = 6$ per group, except for PWD males (n = 4). * $P < 0.05$ significant strain effect vs. B6; † $P < 0.05$ significantly different from males of the same strain. Work was significantly greater in PWD, B6.PWD3, and B6.PWD14 mice compared to B6.

Table 4.3. Genes on chromosome 14 identified using interval-specific haplotype analysis.

Genomic Position, bp	Gene Symbol	Description
22683477	1700112E06Rik	RIKEN cDNA 1700112E06 gene
24117983	Kcnma1	potassium large conductance calcium-activated channel, subfamily M, alpha member 1
27512760	Dnahc12	dynein, axonemal, heavy chain 12
28051225	Arhgef3	Rho guanine nucleotide exchange factor (GEF) 3
28435628	Erc2	ELKS/RAB6-interacting/CAST family member 2
29718129	Cacna2d3	calcium channel, voltage-dependent, alpha2/delta subunit 3
31362335	Tkt	Transketolase
31408555	Prkcd	protein kinase C, delta
31447399	EG665466	predicted gene 7644
31467561	Rft1	RFT1 homolog (S. cerevisiae)
31528035	Sfmbt1	Scm-like with four mbt domains 1
31638780	Tmem110	transmembrane protein 110
31692443	Mustn1	musculoskeletal, embryonic nuclear protein 1
31699678	Itih4	Inter-alpha-trypsin inhibitor, heavy chain 4
31721758	Itih3	inter-alpha trypsin inhibitor, heavy chain 3
31742366	Itih1	inter-alpha trypsin inhibitor, heavy chain 1
49064375	6720456H20Rik	RIKEN cDNA 6720456H20 gene
93412920	Pcdh9	protocadherin 9
96278500	4921530L21Rik	RIKEN cDNA 4921530L21 gene
96504484	Klhl1	kelch-like 1 (Drosophila)
100858642	LOC100043413	Gm4423
105658021	Ndfip2	Nedd4 family interacting protein 2
119537487	Hs6st3	heparan sulfate 6-O-sulfotransferase 3

The Mouse Phenome Database was used to identify regions where SNPs for four high performing strains (PWD/PhJ, FVB/NJ, AKR/J, and SWR/J) are identical by state and different from four low performing strains (C57BL/6J, NZW/LacJ, 129X1/SvJ, and KK/HlJ). SNP comparisons were based on data from CGD imputed SNPs – Build 36 (74 strains). Only SNPs polymorphic between at least 3 high performing strains and 3 low performing strains (at least 6 strains total) were included. Genomic positions are based on NCBI mouse build 37.0.

Table 4.4. Genes on chromosome 3 identified using interval-specific haplotype analysis.

Genomic Position, bp	Gene Symbol	Description
7502681	3110050N22Rik	RIKEN cDNA 3110050N22 gene
7570083	Il7	interleukin 7
15846070	LOC381484	
28160150	Tnik	TRAF2 and NCK interacting kinase
30133515	Mds1	myelodysplasia syndrome 1 homolog (human)
30443919	LOC100040727	
30643308	Samd7	sterile alpha motif domain containing 7
31800061	Kcnmb2	potassium large conductance calcium-activated channel, subfamily M, beta member 2
34093766	EG665636	predicted gene, EG665636
35963192	Acad9	acyl-Coenzyme A dehydrogenase family, member 9
48409654	1700018B24Rik	RIKEN cDNA 1700018B24 gene
50166973	Slc7a11	solute carrier family 7 (cationic amino acid transporter, y+ system), member 11
54915407	Spg20	spastic paraplegia 20, spartin (Troyer syndrome) homolog (human)
54939377	A730037C10Rik	RIKEN cDNA A730037C10 gene
55044739	Dclk1	doublecortin-like kinase 1
55429155	Nbea	Neurobeachin
56939410	LOC666585	
57089164	Tm4sf1	transmembrane 4 superfamily member 1
63097903	Mme	membrane metallo endopeptidase
63498188	Plch1	phospholipase C, eta 1
65310150	EG667093	predicted gene, EG667093
65330580	Tiparp	TCDD-inducible poly(ADP-ribose) polymerase
65857483	Veph1	ventricular zone expressed PH domain homolog 1 (zebrafish)
67176210	Mlf1	myeloid leukemia factor 1
67232107	Gfm1	G elongation factor, mitochondrial 1
67260341	Lxn	Latexin
66789594	Rsrc1	arginine/serine-rich coiled-coil 1
67280817	Rarres1	retinoic acid receptor responder (tazarotene induced) 1
69118854	Ppm1l	protein phosphatase 1 (formerly 2C)-like
116263956	Lrrc39	leucine rich repeat containing 39
116282347	Ccdc76	coiled-coil domain containing 76
116296213	sass6	spindle assembly 6 homolog (C. elegans)
117482929	Snx7	sorting nexin 7
121160464	Slc44a3	solute carrier family 44, member 3
137530292	Dnajb14	DnaJ (Hsp40) homolog, subfamily B, member 14

Table 4.4 continued

Genomic Position, bp	Gene Symbol	Description
137592420	Dapp1	dual adaptor for phosphotyrosine and 3-phosphoinositides 1
137938615	Adh1	alcohol dehydrogenase 1 (class I)
137974361	Adh6a	alcohol dehydrogenase 6A (class V)
138104571	Adh5	alcohol dehydrogenase 5 (class III), chi polypeptide
138403195	Tspan5	tetraspanin 5
141126762	Unc5c	unc-5 homolog C (C. elegans)
141498148	Bmpr1b	bone morphogenetic protein receptor, type 1B
141903624	Pdlim5	PDZ and LIM domain 5
142158201	Gbp5	guanylate nucleotide binding protein 5

The Mouse Phenome Database was used to identify regions where SNPs for four high performing strains (PWD/PhJ, FVB/NJ, AKR/J, and SWR/J) are identical by state and different from four low performing strains (C57BL/6J, NZW/LacJ, 129X1/SvJ, and KK/HlJ). SNP comparisons were based on data from CGD imputed SNPs – Build 36 (74 strains). Only SNPs polymorphic between at least 3 high performing strains and 3 low performing strains (at least 6 strains total) were included. Genomic positions are based on NCBI mouse build 37.0.

Discussion

QTL identified using inbred crosses may be population-specific and require replication to support their contribution to a trait (31, 32). Significant and suggestive QTL for intrinsic exercise capacity, expressed as work, were identified on chromosomes 14 and 3, respectively using an F_2 population derived from a cross of FVB/NJ and B6 mice (110). In a survey of exercise capacity across 34 inbred mouse strains, PWD mice were found to have an exercise capacity similar to the high performing FVB/NJ strain, and significantly greater than B6 mice. On the basis of these differences, CSS mice were used to confirm the role of these QTL in intrinsic exercise capacity. A significant difference was observed between B6.PWD14 and B6 mice for exercise time and work, confirming the significant QTL reported on chromosome 14. Exercise capacity in B6.PWD3 mice was also significantly greater than in B6 mice, confirming the presence of a QTL for exercise capacity on chromosome 14. Additional putative QTL for several anthropometric variables were also identified on chromosome 3, suggesting that there is a potential interaction between genes regulating exercise capacity and body mass. Differences between B6 and CSS strains for exercise capacity and the identification of new QTL on chromosome 3 indicate that the contributions of chromosomes 3 and 14 to intrinsic exercise capacity warrant further investigation. Furthermore, these results suggest that a gene or genes on these chromosomes contribute to variation in endurance exercise capacity.

It is well established that there is a genetic basis for differences in exercise capacity (21, 102, 131, 166). However, the genetic factors underlying this variation are undefined. In a previous study, exercise capacity was assessed in male mice from 34

inbred strains and significant differences were found for time and work. There was approximately a two-fold difference in running time between the highest and lowest performing strains, which is comparable to previous reports (97, 101). When exercise capacity was expressed as work, this difference was increased to approximately 14-fold. These data suggest that body mass might be a contributing factor to exercise performance despite the relatively low correlation between exercise time and body mass in mice from the strain survey.

Chromosome substitution strains represent a relatively novel approach to confirm previously reported QTL or identify new genomic regions that contribute to the variation in a trait (118, 119). In the present study, differences between B6.PWD14 and inbred B6 mice for exercise time and work confirms the importance of chromosome 14 in determining exercise capacity. As strain, B6.PWD14 ran about 9% longer and performed 25% more work than B6 mice. Although this difference is relatively small compared to that between the inbred PWD and B6 strains, the advantage of using CSS is that genetic variation is limited to one chromosome, thereby reducing the complexity of the genetic interactions that might influence a trait in a mixed genetic background (118, 119). The results from the present study provide a third line of evidence that genetic factors on chromosome 14 are important for determining exercise capacity. Previously, a significant QTL for exercise capacity was identified on chromosome 14 using an intercross between B6 and FVB strains (109, 110) Bouchard and colleagues (21) previously reported that VO_{2max} in the sedentary state was linked to several markers that map to mouse chromosome 14 and two fall within the mouse QTL for pre-training work (110). Further analysis revealed that the difference in exercise time between B6.PWD14

and B6 mice was only significant in males, implying that this QTL might be sex-specific. In contrast, the effect of sex was not observed when exercise capacity was expressed as work. The original QTL for work was identified in a combined population of male and female F_2 mice and separate QTL analyses were not performed on single-sex groups. However, unlike the present study, work was not significantly different between male and female F_2 mice (110). Therefore, additional analyses are required to confirm the sex-specificity of this QTL.

The QTL previously reported on chromosome 3 for exercise capacity, expressed as work, only reached the threshold for a suggestive QTL (LOD = 2.2) (110). However, using CSS mice, significant differences between B6.PWD3 and B6 mice were observed for intrinsic exercise capacity expressed as time and work (Figures 4.1 and 4.2). B6.PWD3 ran approximately 15-20% longer than B6 mice, which translates into about 17-35% more work performed. The difference between B6.PWD3 and B6 is roughly one-half that observed between the inbred B6 and PWD progenitor strains (Figure 4.2). The large effect observed in the CSS mice relative to that reported in the linkage study suggests that additional alleles present in the mixed background of the F_2 population might have masked the effects of this chromosome on exercise capacity (118, 119). Two other linkage markers for sedentary state VO_{2max} that have been reported in humans have orthologous loci on mouse chromosome 3 (21). Because QTL location cannot be determined using CSS, it is unclear whether all of these QTL represent a single QTL or multiple loci. Collectively, however, these data support the presence of a QTL for intrinsic exercise capacity on chromosome 3.

In addition to the QTL for exercise capacity, putative QTL for several body and tissue mass phenotypes were identified. The majority of these QTL were identified on chromosome 3. Body mass, heart mass, and muscle masses (gastrocnemius, soleus, and plantaris) were all significantly smaller in B6.PWD3 mice compared with B6 mice. Heart mass-to-body mass ratio was significantly higher in B6.PWD3 mice versus B6 mice. In contrast, these phenotypes were similar between B6.PWD14 and B6 mice, suggesting that these QTL are specific for chromosome 3. Several QTL for body mass at 10 weeks of age have been reported on chromosome 3 in different populations of F_2 mice (29, 30, 135). Interestingly, Shao et al. reported a significant QTL for body weight at 18 weeks on chromosome 3 using an F_2 population derived from B6 and PWK/PhJ (143). PWK mice are a wild-derived inbred strain that is genetically similar to the PWD strain used in the present study (54, 55). Two significant QTL for gastrocnemius muscle mass have also been identified on chromosome 3 (91, 103) as have two significant QTL for heart mass (29, 94). Although QTL position cannot be localized using CSS mice, the results of the present study and others support the presence of several QTL related to body and/or tissue mass on chromosome 3.

The presence of putative QTL for heart and muscle mass on chromosome 3 suggest these traits contribute to the differences in exercise capacity among inbred strains of mice. Both body mass and heart mass were significantly lower in PWD mice compared with B6 mice. Body mass, heart mass, and muscle masses were also significantly correlated with exercise time (Table 4.2). For most of these traits, smaller mass was associated with greater exercise time. A similar association was reported for aerobic endurance running performance and body mass in rats (7), implying that body mass is a

factor in determining exercise capacity. In contrast, higher heart mass-to-body mass ratio was associated with higher exercise capacity (Table 4.2). This ratio was significantly higher in PWD and B6.PWD3 mice compared with B6 mice (Table 4.1). Barbato and colleagues reported a significant correlation between running distance and cardiac output across eleven strains of inbred rats (7). Furthermore, overlapping QTL for aerobic running performance and heart weight, relative heart weight and body mass have been reported previously in rats (166). Thus, a gene or genes regulating cardiac structure and/or function might influence endurance exercise capacity directly or indirectly.

Interval-specific haplotype analysis was used to identify potential candidate genes based on SNPs that are polymorphic between high and low capacity strains. Three of the genes identified on chromosome 3, PDZ and LIM domain 5 (Pdlim5), leucine rich repeat containing 39 (Lrrc39), and myelodysplasia syndrome 1 homolog (Mds1) have been linked to cardiac structure/function. Evidence suggests that Pdlim5 and Lrrc39 are involved in stress/stretch sensing in cardiac muscle (24, 28, 168). The function of Pdlim5 has also been associated with embryonic development of the heart (172), whereas mice with a null mutation in Mds1 fail to develop a properly functioning heart (72). Multiple genes on chromosome 3 have also been associated with neuromuscular function (Spg20, Unc5c, Mlf1, Nbea, and Kcnmb2) (6, 37, 79, 95, 96, 98, 110) and bone morphogenic protein signaling (Lxn, Spg20, and Bmpr1b) (9, 77, 161). One gene, acyl-Coenzyme A dehydrogenase family, member 9 (Acad9), is has been linked to mitochondrial complex I deficiency and exercise intolerance (51). Conversely, the genes on chromosome 14 identified by haplotype analysis have less obvious links to exercise capacity or exercise-related traits and require further study. The exception is protein

kinase C, delta (Prkcd). Prkcd expression is increased in cardiac tissue after acute and chronic exercise and in coronary arteries after exercise training (27, 86). Kinase activity is also increased with training in skeletal muscle and is associated with improved insulin signaling (62). Collectively, these results identify Prckd as a component of the beneficial adaptations to exercise training and therefore are involved in determining the genetic basis for variation in intrinsic exercise capacity. However, improved localization of the QTL for exercise capacity and related phenotypes identified in the present study is necessary before potential candidate genes can be identified.

CHAPTER V

CONCLUSIONS

The data presented within this dissertation study demonstrate there are strain-specific differences and there are significant QTL regulating intrinsic endurance exercise capacity in inbred mice. Secondly, the data demonstrate the importance of chromosome 14 through the identification of novel QTL for intrinsic endurance exercise capacity using CSS F_2 generation mice. In addition, the data from this study clearly demonstrate the effect of sex on intrinsic endurance exercise capacity across 155 male and female F_2 CSS mice during graded exercise treadmill tests. For the purposes of this dissertation study, exercise capacity was defined as average run time or average work performed during graded exercise tests. Sex differences were found significant for both time and work with females having longer run times in comparison to males, and males performing more work because of differences in body mass with male mice weighing more than female mice. Interestingly, this was not the case for F_2 mice when considering work where no significant difference was identified. A GWAS study identified chromosomes 1, 2, 7, 11, and 13 as housing significant QTL for average run time. A candidate gene, Nfatc2 was identified as potentially important to the genetic regulation of intrinsic endurance exercise capacity. Data from a linkage study using low running A/J-CSS mice support the hypothesis that chromosome is important to the regulation of intrinsic endurance exercise capacity through the identification of several novel QTL at 56 cM. Data from the linkage study involving the high running PWD-CSS mice identified QTL on chromosome 14 and the gene for protein kinase C, delta (Prkcd) was

identified within the QTL region. Kinase C is linked to skeletal muscle and insulin signaling, so this may be important for intrinsic endurance exercise capacity.

The future directions for this study involve more in depth investigation of the QTL identified across the entirety of this study. The identification of novel QTL are initial critical steps, but the investigation of gene expression across the strains from this study is critical to understanding the variation in intrinsic endurance exercise capacity for all murine strains from this dissertation study. The true future of intrinsic endurance exercise capacity study is a complimentary training study using these strains for an extended training study.

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