ASSOCIATION BETWEEN GENETIC BACKGROUND AND THE RESPONSE TO EXERCISE TRAINING

A Dissertation

by

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ABSTRACT

Cardiorespiratory fitness has been shown to increase with physical activity regardless of intrinsic exercise capacity, age, sex, and race. However, the variation in the response to training is heterogeneous, including individuals who do not respond and even those who respond negatively. Exercise is known to produce a cascade of beneficial physiological adaptations including the modulation of mitochondrial biogenesis, oxidative phosphorylation, and glucose transport in skeletal muscle. However, non-responders fail to gain the benefits that increased exercise capacity provides. Many variables in physical activity are adjustable (including intensity, duration, and volume) thereby providing means to attempt to optimize training to cardiorespiratory fitness in non-responders, if the response to exercise is not genetically set. Therefore, two sets of experiments were designed to: 1) characterize differences in training responses across a large number of inbred mouse strains and identify quantitative trait loci associated with the response to exercise training using genome-wide association mapping; 2) determine the interaction between genetic background and training volume (intensity x duration) on exercise performance and skeletal muscle proteins in four inbred mouse strains.

The main findings of this dissertation are: 1) The response to exercise training was heterogeneous with some strains being non-responders. Genome-wide association mapping identified QTL regions associated with the training response for both time and

work with peak SNP residing in candidate genes. Overlap in previously identified genes and QTL within human and rodent studies were confirmed as well; 2) Body weight, delta time and delta work were significant for main effects for strain and intensity. Significant interactions for all three phenotypes include strain x duration, and strain x duration x intensity. Protein content did not follow any specific trends associated with changes in exercise capacity. However, increases in proteins that were expected to increase with exercise capacity did yield higher concentrations more frequently with four-week continuous moderate exercise. In summation, four-week low continuous exercise appears to be the optimal training paradigm for increasing exercise capacity in a mouse model.

DEDICATION

To my loving wife Cassity Avila, my muse. Her dedication to my success is without comparison.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Cardiorespiratory fitness and all-cause mortality

Cardiorespiratory fitness is an important index of overall health status (89, 209, 232, 234) and is defined as the ability of the cardiovascular, respiratory, and skeletal muscle systems to supply oxygen during physical activity. Cardiorespiratory fitness is often reported in metabolic equivalents (METs) or maximal oxygen consumption (VO_{2max}). One MET is equivalent to the amount of oxygen utilized while at rest; ~ 3.5 ml of O_2 per kg of body weight each minute (109). VO_{2max} is generally measured during a graded exercise test on a treadmill. Cardiorespiratory fitness or VO_{2max} is an independent predictor of cardiovascular disease and all-cause mortality (126, 127). Furthermore, strong evidence exists that having average or high baseline (intrinsic) cardiorespiratory fitness significantly reduces the relative risk of all-cause mortality and prevalence of cardiovascular disease (CVD) in both sexes (20, 76, 127, 139, 168, 172). For example, Wei et al. followed 25,714 men to assess the contribution that cardiorespiratory fitness had on CVD and all-cause mortality over a 10 year period (233). The investigators found that low fitness levels were associated with a higher risk of death than diabetes, elevated cholesterol, hypertension and smoking status. Additionally, the presence of CVD at the start of the study was the strongest predictor of death, but low cardiorespiratory fitness had a similar predictive value as CVD for mortality. In a study beginning in the 1970's, the Cooper Institute compiled an extensive

data set which included indices of cardiorespiratory fitness on 60,000 men and women (113). Results indicate that cardiorespiratory fitness was strongly correlated with traditional risk factors such as obesity, metabolic syndrome, diabetes, and high blood pressure (17, 19, 20, 45-47, 212) for CVD and all-cause mortality, data that are in agreement with Wei et al. (233). Additionally, high levels of cardiorespiratory fitness mitigates the relative risk of death (17, 19, 20, 45-47, 212). It should also be highlighted that individuals with elevated risk factors for coronary heart disease (CHD) but high cardiorespiratory fitness, exhibited lower incidences of death than those with low cardiorespiratory fitness and low CHD risk factors (138).

Improving cardiorespiratory fitness via increased physical activity is an achievable goal for many individuals. Improvements in cardiorespiratory fitness can significantly reduce the risk of all-cause mortality (18, 62). Each one minute increase in exercise capacity during a treadmill running test is associated with an 8% decrease in risk for all-cause mortality (18). Erikssen et al. (62) measured baseline cardiorespiratory fitness in middle-aged men and noted that increased cardiorespiratory fitness within a seven-year time frame resulted in lower rates of all-cause mortality during follow up regardless of initial cardiorespiratory fitness. Extrapolating this data, Kodama et al. (123) noted that with each MET increase in cardiorespiratory fitness, the risk for all-cause mortality decreased accordingly regardless of age, sex, smoking status, CHD risk factors. Such improvements can contribute to as much as a 10-25% improvement in mortality compared to those with low cardiorespiratory fitness (154, 173).

Although physical activity and cardiorespiratory fitness are linked, (increasing physical activity can lead to increased fitness) the association between measures of physical activity and cardiorespiratory fitness are relatively low, with correlations ranging from 0.09 to 0.81 (171, 236), suggesting that these 2 parameters are somewhat independent (231). In an extensive meta-analysis, Williams concluded that the relationship between the risk for CVD and cardiorespiratory fitness was significantly different from that between CVD risk and physical activity, with the relative risk reduction for fitness being greater than that for physical activity (236). Others have supported this finding (171, 231). Myers et al. in a direct comparison of the predictive value of physical activity and exercise capacity reported that exercise capacity was a much stronger predictor of mortality than physical activity (hazard ratio: 0.70 vs. 0.53) (171). Furthermore, being unfit was associated with a higher mortality risk regardless of activity level, again supporting the supposition that these two measures are somewhat independent. These data suggest that individuals that increase their physical activity levels but do not improve their fitness levels ("non-responder") may not reduce their relative risk of mortality as much as active individuals that do improve their fitness ("responder").

1.2 Variation in responses to exercise training

Combating chronic diseases is of paramount importance. In 2013, in the United States alone the cost of treating chronic diseases increased 3.6% to a net expenditure of $2.9 trillion and is forecasted to continue to rise at a rate of 5.7% until 2023 (217). To

address this urgent health care problem, position statements highlighting exercise as a treatment and in the prevention of chronic disorders emerged (49, 67, 71). The American College of Sports Medicine (ACSM) provides evidence-based guidelines on the frequency (≥ 3-5 days/week), intensity (moderate/vigorous), duration (30-60 min/day or 150 min/week), and volume (≥500-1,000 MET/min/wk) of exercise for optimal fitness (71). The benefits of exercise on improving quality of life and reducing all-cause mortality has been well documented (21, 108, 135-137, 155, 205, 206).

The standard convention for exercise training studies is to report the mean response (e.g., change in VO_{2max}) and standard deviation for cohorts. Bouchard et al. reported that a group mean increase of ~25% in endurance capacity does not accurately reflect the true responses to training (29). In fact, some individuals yield results that are either much higher or lower. Within the realm of human studies, it is common for normally distributed phenotypic data to be ≥ 1 standard deviation from the mean (27, 48). Literature shows that exercise capacity, as well as the response to exercise, is highly variable with some individuals having no change in exercise capacity in response to exercise training (19, 22, 216). Health and exercise performance-related phenotypes including blood pressure, heart rate, and cholesterol levels also show highly variable responses to exercise training (36). These data emphasize the high degree of individual variation in adaptation to exercise training.

1.3 Genetics of exercise training

The mechanisms underlying individual variation in the adaptations to exercise training are not known, but likely involve variation at the genetic level. To address this, training studies were conducted in monozygotic twins (56, 81, 187, 215). Monozygotic twins show a high resemblance ($R^2 = 0.69$) in training responses expressed as changes in VO_{2max}. In twin studies, the variance in changes in VO_{2max} between genotypes has been reported to be approximately 6-9 times greater than the variance within genotypes in response to controlled training programs. The HERITAGE family study, in an attempt to address the aforementioned twin study, significantly increased their study population by recruiting ~130 families from a diverse ethnic background and assessing the variation in response to 20-weeks of standardized exercised training (25). Heritability for baseline VO_{2max} (23), and heart rate during sub-maximal exercise (6) was estimated to be 50% and 59% respectively. The notion that intrinsic exercise related phenotype values might be able to predict the response to exercise became a consideration. As such, the association between intrinsic values and their response was investigated utilizing data from the HERITAGE family study. In an attempt to predict the affect intrinsic values have on the response to exercise, intrinsic values for high-density lipoprotein cholesterol were shown to predict only 1.2% of exercise induced changes while intrinsic VO_{2max} was found to account for ~1%; other attempts to predict responses based on intrinsic values yielded low associations (87, 124, 208).

Rico-Sanz et al. (193) utilized a linkage study approach to identify the association between a genetic marker and phenotype across generations. Linkage

studies are useful in identifying regions of a chromosome with a gene(s) influencing a phenotype, even without prior knowledge of a gene (33). The investigation examined 419 pairs of siblings and their response to exercise training for 20 weeks. Results indicated that quantitative trait loci (QTL), or regions in which genes are linked to a measurable trait (164), were identified on five chromosomes for oxygen consumption (Chrs 1, 4, 7, 13, 16, 20) and differed from QTL identified for pre-training oxygen consumption between siblings (193), indicating that genetic background is a strong component of exercise capacity.

Because traditional linkage studies lack mapping resolution and candidate gene approaches are based on *a priori* hypothesis on pathways linked to the response to training, genome-wide association studies (GWAS) are being used to identify genetic variants and potential candidate genes for complex traits. These approaches take advantage of resources provided by sequencing the human and mouse genomes. Genome wide association studies (GWAS) are an analytical method based on the concept that a causal genomic variant or allele is found at a higher frequency in individuals with the phenotype of interest (40, 105) compared with those having a different phenotype and genotype. By utilizing a larger number of variants and individuals, mapping resolution or localization is much better than traditional linkage studies (211). For example, confidence intervals for QTL are on the order of 10-100 kb in GWAS studies in comparison to linkage studies in which mapping resolution spans 10 Mb or more, suggesting that candidate genes identification should be improved.

However, GWAS have not been fruitful in identifying genes underlying the variation in response to training in humans, in part because so few studies have been performed and replicated. Furthermore, the replication of GWAS studies in additional and independent samples is critical (175) and often difficult in human populations (240). A genome-wide association study was conducted (31) to determine the genetic basis for changes in VO_{2max} with training. This study utilized approximately 320,000 SNPs (32) and 1,012 individuals from three exercise intervention trials including the HERITAGE family cohort. Although no genome-wide significant associations were identified, 39 single nucleotide polymorphisms (SNPs) were associated with changes in VO_{2max} in response to exercise training. Twenty-one SNPs accounted for 49% of the VO_{2max} response to training and a sub-set were validated in another exercise training cohort. In addition, Timmons et al. (218) used genome-wide expression analysis (i.e., microarray) to identify transcripts that could be used as predictors for the response to training. Predictor genes were identified by comparing gene expression differences at baseline in skeletal muscle samples from individuals classified as high or low responders to exercise training. Interestingly, none of these predictor genes showed a change in expression in response to exercise training. Although not a true GWAS, none of these predictor genes were confirmed in the HERITAGE genome-wide association study (32). These results suggest that putative candidate genes can be identified in humans, but variation in training paradigms, environment, and the human genome make replication difficult (80).

1.4 Animal models of genetics and exercise training

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 (3) followed by quantitative trait loci (QTL) analyses. The use of inbred rodents, especially mice has several advantages in research incorporating physiology and genetic components. Mice have short gestation periods, reach sexual maturity at 6 to 8 weeks of age, are relatively inexpensive to house, and take up less space than most mammals. Therefore, completing studies with large numbers of individuals in a relatively short time period is more feasible than similar studies in humans. Additional benefits of using a mouse model include strict environmental control (70) and the ability to study relevant tissue that is often prohibitive in human subjects. Furthermore, mouse strains inbred for at least 20 generations are homozygous at all loci (59). Thus, under standardized environmental conditions, phenotypic differences observed among inbred strains are due predominantly to genetic variation. Based on the homology between mice and humans, areas of interest identified in mice can be implicated to regions of the human genome (55), which is a vital part in translating discoveries found in a mouse model to humans.

Little is known about training responses across inbred strains of mice or rats. A comparison of inbred strains of rats reported that strain-differences persisted whether training was performed at the same relative or absolute workload (121). In that study, Koch and colleagues examined the effect of absolute vs. relative training intensity on responses to training in inbred rats having low intrinsic exercise capacity (COP) and

high intrinsic exercise capacity (DA). COP rats showed little or no improvement in exercise capacity with either mode of training whereas, DA rats showed significant changes with both modes of training (121). These results suggest that responses to exercise training might be independent of exercise intensity and genetically set. Furthermore, examination of citrate synthase (CS), which is a key enzyme within the TCA cycle and is used as a mitochondrial marker for training status (54, 95, 188), did not change in DA rats that significantly increased their exercise capacity while the COP rats, which showed no improvement in exercise capacity, had a significant increase in CS levels. This lends itself that changes in exercise capacity might not always be reflected in changes in component phenotypes (141). Massett et al. (160) reported that responses to the same relative workload varied significantly across 3 inbred and 3 F_1 hybrid mouse strains. Among the inbred strains, the response to treadmill training was greatest in inbred FVB/NJ and smallest in inbred BALB/cJ mice. The C57BL/6J strain had a low response to training that was relatively similar to BALB/cJ and significantly less than FVB/NJ. Estimated broad-sense heritability for post-training values for distance run in mice ranged from 47% to 65%, suggesting that variance in adaptation responses to exercise in mice are significantly influenced by genotypic variance. However, in contrast to Koch et al. (121) skeletal muscle protein contents for cytochrome c and endothelial nitric oxide synthase were elevated the most in the highest responding strain (FVB). Using an alternative model of exercise, swim training, Kilikevicius et al. (114) compared changes in swimming endurance in six inbred strains of mice. Similar to Massett et al. the BALB/cByJ as well as the A/J were identified as

low responding strains. The C57BL/6J and DBA/2J were the highest responding strains. There was an approximately 6.5-fold difference in exercise capacity after training between the highest and lowest performing strains. In addition to the heterogeneity in exercise capacity skeletal muscle adaptions also varied across strains, soleus weight was approximately two-fold higher in the inbred C57Bl/6J compared to the wild derived PWD/PhJ. Furthermore cross-sectional area was greater by 30% in the C57BL/6J mice as well (114). While citrate synthase activity increased with endurance training within the gastrocnemius muscle, changes in exercise capacity were based on genetic background. Collectively, these results indicate that training responses vary among inbred strains and the magnitude of response is determined, in part, by genetic background.

Based on significant strain differences in intrinsic exercise capacity and responses to exercise training, linkage and association studies have been conducted to identify underlying QTL and/or candidate genes. Ways and colleagues utilized two inbred strains of rats identified as having high and low intrinsic exercise capacity to create an F_2 cross (COPxDA) to identify QTLs for aerobic exercise capacity. Results from that study implicated chromosome 16 (RNO16) and a portion of chromosome 3 (RNO3). To further their investigation, the group created a congenic model by backcrossing RNO16 and RNO3 from high performing dark agouti (DA) rats to the low performing Copenhagen (COP) rats. Results indicated that COP.DA (chr 16) rats significantly improved their performance over COP rats (696.7 ± 38.2 vs. 571.9 ±27.5 m, $P<0.05$). COP.DA (chr 3) rats improved their performance but not significantly from

COP controls (230). Several QTL also have been identified for intrinsic exercise capacity defined as pre-training work in mice (50, 148, 160). Using an F_2 cross between BALB/cJ and DBA/2J mice Lightfoot et al. (148) identified two chromosomes (8 and X) with QTL for endurance exercise performance. Alternatively, Courtney and Massett utilized both inbred mouse strains and wild-derived mice to identify novel putative QTLs for intrinsic exercise capacity using GWAS. They reported significant heterogeneity in intrinsic exercise capacity with the difference between the lowest and highest run time being ~2.7-fold. When work was analyzed (a component including body mass as a factor), there was a 16.5 fold-difference between the lowest and highest ranked mouse strains. Significant associations were found on five chromosomes. One significant association overlapped with a genomic marker linked to training responses in humans (50). Collectively, these data support the genetic basis for intrinsic exercise capacity. Furthermore, they indicate the feasibility of utilizing genetic approaches in model organisms to identify genes related to exercise capacity.

To identify the genetic basis for responses to training, Massett and colleagues utilized a linkage approach based on an intercross between the low responding C57BL/6J and high responding FVB/NJ strains. Mice performed 4-weeks of treadmill training and changes in exercise capacity were recorded. For intrinsic or pre-training work, two significant QTLs were identified on Chromosomes 14 at 4.0 cM (3.72 LOD) and 19 at 34.4 cM (3.63 LOD). Post-training work QTLs were identified at chromosome 3 at 60 cM (4.66 LOD) and 14 at 26 cM (4.99 LOD). No overlap was found between QTL pre- and post-training for work indicating that genes associated with the response

to exercise training is different from those for intrinsic exercise capacity (160). Finally, two suggestive QTL were identified for the response to training. This limited data suggest that genomic regions linked to exercise training responses can be identified. However, these data require replication experiments before candidate genes can be identified.

1.5 Skeletal muscle responses to exercise training

The structural and functional characteristics of skeletal muscle impact exercise capacity in multiple ways. Skeletal muscle capillary density contributes to oxygen delivery, while skeletal muscle fiber type and metabolic profile determine oxygen utilization. The interaction of these two components is a primary factor in determining VO_{2max} and exercise capacity (85, 91, 203). Skeletal muscle is immensely plastic and adapts to stressors from motor neuron activity (82). Positive changes in muscle metabolism and morphology have been shown to be induced with exercise training, resulting in an increase in aerobic capacity (7, 15, 24, 38, 83, 84, 104, 128, 129, 135, 149, 150, 174, 229). Furthermore, the enhanced demand for energy is met by increases in enzymes and transporters for glucose and free fatty acid metabolism. Exercise training can lead to changes in skeletal muscle oxidative enzyme capacity, mitochondrial function, and fiber type. These adaptations occur in response to repeated bouts of exercise, likely as the result of transient changes in transcript levels. Repeated bouts of exercise, i.e., endurance training, results in the signaling of genes which in turn leads to phenotypic adaptations including fiber type differentiation, and mitochondrial biogenesis

(2). Several molecular factors regulating these adaptations have been identified, namely the PGC-1alpha – NRF – Tfam pathway. However, it is unclear whether the skeletal muscle adaptations to exercise training are altered by genetic background. Therefore, it is important to understand the mechanisms leading to skeletal muscle adaptations to training and then determine their response in genetic models of exercise.

One of the molecules consistently associated with skeletal muscle adaptations to exercise training is peroxisome proliferator-activated receptor **γ** coactivator 1α (PGC-1alpha), a transcriptional coactivator proposed to be a master regulator in skeletal muscle mitochondrial biogenesis (95, 96, 131, 197, 214). While PGC-1alpha was originally discovered to be associated with thermogenesis, the transcriptional changes made by PGC-1alpha have been shown to be induced by motor neuron signaling in skeletal muscle (82, 119, 199). Thus, PGC-1alpha was found to have properties linked to adaptations seen with training such as glucose metabolism, mitochondrial biogenesis, and fiber type differentiation (119). For example, Chow et al. conducted an experiment in which mice performed treadmill running 5 days/week at 80% of peak oxygen consumption for 8 weeks. Endurance training improved mitochondrial function in skeletal muscle, mitochondrial enzyme activities, increased mitochondrial ATP synthesis rates, mtDNA copy numbers, and increased expression of PGC-1alpha (44). More specifically, over-expression of PGC-1alpha causes an increase in mtDNA copy number, mitochondria biogenesis, and respiratory capacity (39, 235). Both voluntary wheel running and treadmill endurance performance are greater in mice overexpressing PGC-1alpha in skeletal muscle. In contrast, muscle-specific deletion of PGC-1alpha has no

affect on voluntary wheel running distance (72) but impairs treadmill endurance performance (82, 235). In addition, Geng and colleagues (72) knocked out PGC-1alpha in muscle in an attempt to elucidate the effect it has on skeletal muscle in response to endurance training. In the absence of PGC-1alpha, mice subjected to voluntary wheel running had attenuated levels of mitochondrial enzymes and platelet endothelial cell adhesion molecule-1 compared with wild type mice, suggesting that PGC-1alpha contributes to exercise-induced mitochondrial biogenesis and angiogenesis. However, exercise-induced fiber type transformation from IIb-to-IIa was not affected in muscle-specific PGC-1alpha knockout mice. These results suggest that PGC-1alpha does not play a role in fiber type transformation (72), but is important for exercise-induced mitochondrial biogenesis and function.

PGC-1alpha has a pleiotropic effect on modifying mitochondrial biogenesis due to the interaction with nuclear receptors along with transcription factors, such as Transcription Factor A, Mitochondrial (Tfam) Transcription Factor A, Mitochondrial, nuclear respiratory factor 1 (NRF-1), and nuclear respiratory factor-2 (NRF-2), which in turn stimulate mtDNA replication and gene expression. Nuclear respiratory factor 1 and NRF-2 are transcription factors that regulate oxidative gene expression and are expected to change in a manner similar to PGC-1alpha. (142). NRF is involved in the process of encoding genes involved in respiratory enzymes, mitochondrial enzymes, and mtDNA transcription and replication (34, 42, 63, 200, 227, 228). Baar et al. generated transgenic mice overexpressing NRF in skeletal muscle and noted that mitochondrial proteins increased approximately 60% and cytochrome c increased two-fold in the gastrocnemius

muscle. Additionally, a novel finding was made that NRF transgenic mice exhibited an increase in MEF2A and Glut4 and an increase in insulin-stimulated glucose transport (10). Gumede and Ojuka (77) also showed in C2C12 myotubes that overexpression of NRF leads to an increase in Glut4 expression via MEF2A. These results suggest that NRF-1 is not only involved in mitochondrial biogenesis via its interaction with PGC-1alpha, but also contributes to regulating glucose transport. Because exercise increases PGC-1alpha, NRF-1, and NRF-2, it is likely that the interaction among these molecules regulates both exercise related changes in mitochondrial biogenesis and glucose transport (11).

NRF serves as an intermediate signal in the process of mitochondrial biogenesis. NRF therefore interacts with TFAM, previously known as mtTFA, and binds specifically to the upstream major transcriptional promoters, the heavy- and light-strand promoters, and results in mtDNA replication (66). Additionally, TFAM has been shown to be pivotal in providing maintenance and stabilization of the mitochondria chromosome (65, 66). TFAM is also vital for mtDNA function and respiratory function (57). Previous studies have shown that knock out of TFAM in mice results in lethality, as a consequence of low levels of mtDNA (134). This led to the discovery that TFAM is positively correlated with mtDNA. Mice overexpressing TFAM have an increase in mtDNA copy number which is strongly correlated with TFAM expression levels (61). However, the connection between TFAM and exercise is less direct. Park et al. reported that an acute bout of exercise leads to an increase in TFAM in wild type and p53 knockout mice. However, p53 knockout mice have reduced expression of TFAM as well

as decreased mtDNA and exercise capacity (179). In addition, Nogueira et al. (177) reported that treatment with an exercise mimetic (-)-epicatechin increased exercise performance in mice (177). This treatment was associated with increased expression of TFAM, increased mitochondrial volume, and increased oxidative phosphorylation-complex proteins. Collectively, these results suggest that TFAM is a component of the adaptive response to exercise training. However, its contribution remains unclear.

PGC-1alpha has a mechanistic link with Silent mating type information regulation 2 homolog (SIRT1), a member of the sirtuin family. SIRT1 was first discovered by Nemoto et al.(176), who illustrated the interaction between the PGC-1alpha and SIRT1. Mice lacking SIRT1 show decreased expression of PGC-1alpha. Conversely, PGC-1alpha expression is increased in myotubes overexpressing SIRT1(5). SIRT1 deacetylates PGC-1alpha which in turn activates PGC-1alpha causing the signaling cascade through transcription factor A, mitochondrial (TFAM) which begins mitochondrial gene transcription. Thus, data support the role of SIRT1 in regulating the transcriptional activity of PGC-1alpha. However, the contribution of SIRT1 to exercise-induced changes in PGC-1alpha signaling is unclear. Philip et al. investigated the role of SIRT1 on mitochondrial biogenesis by generating a knock out mouse lacking SIRT1 in muscle. Following 20 days of voluntary wheel running, endurance was not impaired nor were PGC-1alpha levels, indicating no difference between mice with SIRT1 and knock out mice (186). However a study by Suwa et al. in rats which underwent 14 days on treadmill endurance running at various intensities including steady-state continuous running and high-intensity interval training resulted in increased endurance capacity

with both training paradigms along with increased expression of SIRT1, Hexokinase, and Glut4. However, rats in the high-intensity group did not exhibit increases in PGC-1alpha (214). In contrast to the work of Philip et al. (186), Chalkiadaki and colleagues utilized SIRT1 transgenic mice overexpressing SIRT1 and saw changes in fiber type distribution from fast twitch to slow twitch. Increases in PGC-1alpha were coupled with increased oxidative metabolism and mitochondrial biogenesis and increased endurance capacity (41). Collectively these results show that exercise induces a signaling cascade leading to skeletal muscle adaptations that favor increased oxidative metabolism and endurance performance, but the contribution of SIRT1 to these adaptations has not been clearly elucidated.

1.6 Genetics and skeletal muscle responses

To date, the majority of the exercise-related research focusing on the genetic basis for exercise capacity or training responses has used the candidate gene approach. As described above, transgenic or knockout mice have been generated to alter the expression level of genes already known to be relevant to acute or chronic exercise. In most cases, deletion or over-expression of genes globally or in heart or skeletal muscle results in marked changes in exercise performance or responses to training (64, 86, 115, 157, 165, 166). However, it is less clear if these candidate genes contribute to the genetic basis for variation in exercise capacity and responses to training in other genetic models of exercise training.

In rats selectively bred for endurance running performance, improved oxygen utilization was attributed to higher oxygen extraction, increased capillary density, and higher activity of oxidative metabolic enzymes (90, 103). In this same genetic model, a panel of 239 genes (OXPHOS, TCA cycle, PPRAR) in skeletal muscle were analyzed and found to be highly correlated with high exercise capacity and disease risk phenotypes (117). 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 (237). Additionally, genes for the metabolic enzymes PPAR-α and LPL are putative candidate genes for a QTL related to treadmill running in inbred rats (93). In humans, QTLs for VO_{2max}, changes in VO_{2max}, and maximal power output during a cycle ergometer test encompass genes related to skeletal muscle metabolism, growth, and function, including PGC-1α, AMPK, CaMK4, calcineurin B, calmodulin 2, and the transcription factor MyoD (28, 193). Studies have also linked polymorphisms in skeletal muscle creatine kinase and actinin, alpha 3 to endurance exercise performance (69, 194, 195). Furthermore, sequence variation in mtDNA was associated with individual differences in VO_{2max} and responses to training in previously sedentary men (58). Collectively, these data from human and animal studies of the genetic basis for exercise capacity have identified structural and functional characteristics of skeletal muscle as potential contributing factors to genetic differences in exercise capacity.

1.7 Modification of traditional training paradigms

Many variables in an exercise training program are modifiable including intensity, duration, and volume, which raises questions as to whether exercise training can be optimized to yield an increase in exercise capacity in individuals characterized as non-responders. For example, it is not fully understood if non-responders have no response to a standardized training protocol or simply have a different threshold to elicit a response.

Most of the literature regarding non-response to exercise is based on a standardized exercise-training program. To investigate the effects of training volume on the non-response to training, Sisson et al. compared responses to six months of training in post-menopausal women (207). Women completed 30 min of exercise at 50% of VO_{2max} designed to expend 4-, 8-, or 12-kcal $\cdot kg^{-1} \cdot wk^{-1}$. The effect of increasing the volume of exercise resulted in improved health outcomes and a lower frequency of non-response to training. Similarly, Church *et al.* reported that the same exercise protocol elicited graded changes in fitness in obese or overweight post-menopausal women after six months of training. Collectively, these data suggest that increasing training volume can elicit dose-response changes in fitness and limit the non-response to exercise. However, the interaction between training volume and genetic background is not well established.

To investigate the interaction of intensity and duration on the skeletal muscle responses to exercise, Hildebrandt and colleagues (92) compared metabolic gene expression in gastrocnemius muscle from rats performing an acute bout of exercise. Rats

performed low-intensity treadmill exercise at 50% of VO_{2max} for 45 or 180 min, or high intensity treadmill exercise at 75% of VO_{2max} for 45 min. Results indicated that pyruvate dehydrogenase kinase-4 (PDK4) and uncoupling protein-3 (UCP3) increased ~10-fold with high-intensity interval training (HIIT), 40-80-fold with 45 min of low-intensity and more >200-fold with 180 minutes of low-intensity training. These results indicated that intensity and duration have differential effects on gene expression in skeletal muscle. However, the study did not examine the effects of chronic endurance training or a genetic basis for these differential responses.

High-intensity interval training has been proposed as an alternative to moderate intensity continuous exercise as an effective and efficient means of improving fitness (74, 88, 92, 137, 178, 221). HIIT is comprised of bouts of vigorous exertion for a short duration, coupled with an active rest at a lower moderate intensity and repeated until exhaustion (12, 60, 73, 98, 150, 192). In studies that have compared interval training with continuous exercise training, the majority have reported increases in VO_{2max} with both types of training (170, 198, 201, 219, 238). When training protocols resulted in comparable energy expenditure, the increase in VO_{2max} with interval training was similar to or greater than continuous training despite training for a shorter duration. In healthy adults, HIIT has been reported to increase muscle mitochondrial biogenesis, improve glucose tolerance, and insulin sensitivity (73, 167, 192). Furthermore, HIIT has been reported to increase skeletal muscle PGC-1alpha in patients with heart failure (237) or metabolic syndrome (219), and obese individuals (201) to a greater extent than continuous training. Collectively, these data suggest that interval training can induce

similar changes in VO_{2max} and greater changes in skeletal muscle gene and protein expression than comparable levels of continuous exercise.

Similar to humans, HIIT has been used in rodents to elicit changes in exercise capacity and skeletal muscle structure and function. And similar to humans, HIIT has mixed effects on exercise capacity and skeletal muscle adaptations when compared with moderate intensity continuous exercise training. Several studies indicate that HIIT produces greater increases in exercise capacity than continuous exercise training (83, 169). However, others have shown no difference between HIIT and continuous exercise training for improving exercise capacity (79, 184). Skeletal muscle adaptations also have been shown to be greater with HIIT (220) or not different from those induced by continuous exercise training (53, 79, 97, 169, 184). In one study, comparable adaptations occurred in skeletal muscle despite greater increases in exercise capacity with HIIT (169). Interestingly, the effects of HIIT can vary based on skeletal muscle fiber type and protein/gene of interest. For example, Holloway *et al*. showed similar changes in succinate dehydrogenase (SDH) activity and mitochondrial electron transport chain protein content in red gastrocnemius muscle after HIIT or continuous exercise training (97). However, in white gastrocnemius muscle SDH activity was decreased in a fiber type specific manner after HIIT. Similarly, training-induced changes in SDH activity and superoxide dismutase protein were equivalent after HIIT and continuous exercise training in rat skeletal muscle; however, glutathione peroxidase activity was higher in soleus muscle after HIIT. In addition, in C57BL/6J mice performing HIIT or continuous low intensity exercise training, both paradigms resulted in improved exercise

capacity when compared to their sedentary control. However, mice who performed HIIT demonstrated a decrease in fatty acid oxidation and an increase in glucose oxidation (77), suggesting that HIIT might alter substrate utilization in mice. Collectively, these results suggest that HIIT can induce changes in exercise capacity and skeletal muscle adaptations in rodents. However, the functional and morphological changes can be highly variable and dependent on training protocol, skeletal muscle, and gene or protein of interest.

1.8 Purpose and hypotheses

The purpose of this study was to identify the genetic determinant(s) mediating the adaptations to exercise training and to identify component phenotypes associated with adaptions to exercise training in an attempt to identify responsible candidate genes. It is clear that the variation in response to exercise training is in part driven by genetic background. However, due to the interaction of multiple genes, environment and large sample size required it has been difficult to identify candidate genes in the human population that are associated with the variation in the response to exercise. Therefore, I utilized a mouse model to investigate the genetic contribution to the response to exercise. Two hypotheses were therefore proposed:

1) The variation in the response to exercise training is influenced by genetic factors that differ between strains.

2) The variation in response to training is independent of training paradigm (intensity or duration).

CHAPTER II

RESPONSE TO EXERCISE TRAINING IS GENETICALLY DETERMINED IN INBRED MOUSE STRAINS

2.1 Introduction

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 for 30 or more minutes on most days of the week for optimal health (180). However, changes in cardiorespiratory fitness in response to a standardized exercise training protocol are highly variable. In a highly controlled training study of 710 individuals, Bouchard et al. reported changes in maximal oxygen consumption (VO_{2max}) ranging from 0 to 1,000 ml O_2/min after 20 weeks of exercise (22, 30). Lortie et al. (151) and Kohrt et al. (125) reported similar degrees of variation in maximal aerobic capacity (16-97%) or VO_{2max} (0-42%), respectively, in response to a standardized training program for up to 12 months. Furthermore, changes in phenotypes associated with exercise training such as submaximal blood pressure and heart rate and HDL cholesterol are also highly variable among individuals (30). These data emphasize the high degree of individual variation in adaptation to exercise training. The mechanisms underlying individual variation in the adaptations to exercise training are not known, but likely involve variation at the genetic level.

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 (24).

Furthermore, heritability estimates for VO_{2max} and sub-maximal power output vary from 25% to 50% or higher for twin and family studies (23, 28, 81, 118, 153, 185). In the HERITAGE Family Study, maximum heritability for VO_{2max} was estimated to be at least 50% based on data from over 400 individuals (23). Higher estimates for heritability (50-75%) were reported by this group for sub-maximal exercise performance (26, 185). Thus, both maximal and sub-maximal exercise capacity are influenced by genetic factors. Less is known about the genetic basis for training responses. However, results from cross-sectional, twin, and prospective studies indicate that genetics contribute significantly to individual variation in responses to training as well. The HERITAGE Family Study reported a heritability of 47% for training responses in 98 two-generation families (22). Furthermore, monozygotic twins show a high resemblance ($R^2 = 0.69$) in training responses expressed as changes in VO_{2max}. In twin studies, the variance in changes in VO_{2max} between genotypes has been reported to be approximately 6-9 times greater than the variance within genotypes in response to controlled training programs. Collectively, these data indicate that variation in both intrinsic exercise capacity and exercise training responses is dependent, in part, on genotype.

To date, data from animal models regarding the genetic variation in the responses to exercise training is lacking (121, 222). In rats selectively bred for high intrinsic exercise capacity, modest treadmill training for 24 days improved treadmill running capacity by 58% compared with the low selected line (222). Narrow-sense heritability was estimated to be 43% (222). Massett et al. reported that training responses varied significantly across inbred and hybrid mouse strains (159). Broad-sense heritability

estimates for changes in exercise capacity in mice ranged from 47% to 65%, suggesting that variation in adaptation responses to exercise in mice are significantly influenced by genetic factors. A subsequent study in mice reported that several quantitative trait loci influence the variation in exercise capacity and training responses in mice, providing further evidence that genetic factors contribute to the responses to training. However, traditional linkage analysis provides a limited view of the potential genetic diversity based on the number of mouse strains utilized for such a study. Furthermore, traditional linkage studies generally identify large regions of the genome containing hundreds of genes, limiting the ability to determine quantitative trait genes. Thus, the genetic factors responsible for the variation in the responses to exercise training remain unclear. Therefore, the aims of this study were to 1) characterize differences in training responses across a large number inbred mouse strains; 2) estimate the genetic contribution to the responses to exercise training; and 3) identify genes influencing the responses to exercise training using genome wide association mapping.

2.2 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. Male mice from 25 inbred strains ($n \approx 6$/strain) were purchased from Jackson Laboratories and acclimatize to their housing upon arrival. The following strains were used: 129S1/SvImJ, 129X1/SvJ, A/J, AKR/J, BALB/cByJ, C3H/HeJ, C57BL/6J, C57BR/cdj, CBA/J, CE/J,

DBA/2J, FVB/NJ, I/LnJ, KK/HIJ, LG/J, LP/J, MA/MyJ, NOD/LtJ, NON/LtJ, NZO/HILtJ, NZW/LacJ, PL/J, SJL/J, SM/J, SWR/J. Mice from these strains were chosen based on genetic diversity and the availability of sequence data. All mice were allowed food and water ad libitum and maintained on a 12-hr light:dark schedule.

Exercise performance test. At eight weeks of age, all mice were familiarized to running on a motorized rodent treadmill (Columbus Instruments, Columbus, OH) for two days as described previously (159, 160). Each session was approximately 10 min in duration and mice ran at 9 and 10 meters per minute (m/min) up a 10° incline. After familiarization, mice performed two graded exercise performance tests separated by 48 hours, the average of the two trials for each mouse were used in strain mean calculations. Tests started at 9 m/min for 9 minutes then increased from 10 m/min by 2.5 m/min every 3 minutes. The starting incline was 0° and was raised by 5° every 9 minutes, with a maximal incline of 15°. Exercise continued until exhaustion, defined as spending greater than 15 consecutive seconds on the electric grid at the rear of the treadmill (159, 160). Exercise capacity was expressed in time (min) and work (kg ·m). Work performed (kg · m) was calculated as the 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° (50, 159, 160). A second pair of exercise performance tests were completed after training and changes in exercise capacity were calculated to determine the response to training.

Exercise training. Mice performed four weeks of exercise training on a rodent treadmill running 5 days/wk, 60 min/day at a final intensity equivalent to ~65% of the

maximal workload (speed and incline) attained during the exercise performance test. The intensity of 65% of maximal workload was chosen to ensure that all strains would be able to complete the protocol over four weeks. The relative workload was chosen to account for differences in intrinsic exercise capacity between strains. During the first two weeks of exercise training the speed and duration were gradually increased until the desired training workload could be sustained.

Body and tissue mass. Body mass was recorded in grams before, weekly during, and after the training period. After completing training (~at least 24 hr after the last exercise bout) all mice were weighed and anesthetized with an intraperitoneal injection with Ketamine (80 mg/kg) – Xylazine (5 mg/kg) cocktail. Heart, gastrocnemius, plantaris and the soleus muscle were harvested, washed in ice-cold (4°C) saline, weighed (wet weight in mg), and flash-frozen in liquid nitrogen and stored at -80°C for future analysis.

Heritability. Estimates of broad sense heritability were calculated based on intraclass correlation (r_I), which is an estimate of the proportion of the total variation that is accounted for by differences between strains, and the coefficient of genetic determination (g^2), which accounts for the doubling of the additive genetic variance that occurs with inbreeding (1-3). The following equations were used to calculate r_I and g^2: $r_I = (MS_B - MS_W)/[MS_B + (n - 1)MS_W]$ and $g^2 = (MS_B - MS_W)/[MS_B + (2n - 1)MS_W]$, where MS_B and MS_W are the between- and within- mean square, respectively, and n is the number of animals per strain.

Association mapping. Genome-wide association mapping for exercise phenotypes was performed utilizing Efficient Mixed Model Association (EMMA) on a web-based server (http://mouse.cs.ucla.edu/). Association scans were performed with a panel of approximately 4 million single nucleotide polymorphisms (SNP) (16, 116). Thresholds for significance for each phenotype were set using a false discovery rate (FDR) of 5%, while suggestive thresholds were based on an FDR of 10%. Thresholds were calculated using the "q value" package in R statistical software (210). Confidence intervals for significant and suggestive associations were defined as regions spanning 200 kilobase (kb) in each direction from the peak SNP or the SNP with the strongest association. If two intervals overlapped or were within 1 megabase (Mb), they were considered one interval (75, 110). All significant SNPs found in each interval were mapped against Build 37.2 of the National Center for Biotechnology Information mouse genome (http://www.ncbi.nlm.nih.gov/genome).

Statistical analysis. All data is presented as mean ± SE. Strain comparisons among the 25 inbred strains were made using a one-way ANOVA followed by Tukey post-hoc analysis. Interactions were performed using full factorial analysis with intensity, duration and strain as construct model effects (JMP, Version 11. SAS Institute Inc., Cary, NC, 1989-2015). Statistical significance was set at $P < 0.05$.

2.3 Results

Exercise endurance. Strain distributions for exercise capacity expressed as time and work are shown in Figs 1 and 2 respectively. Pre-training exercise capacity showed

a two-fold difference between the highest and lowest performing strains (Fig. 1-1A). The lowest performing strain was the A/J with a mean run time of 21.1 ± 0.6 min, whereas, the highest performing strain was the NOD/LtJ with a mean run time of 42.1 ± 1.6 min. Significant differences among strains are shown in Table 1.1. These data indicate that genetic background influences intrinsic exercise capacity. After four weeks of exercise training, the A/J strain remained the lowest performing strain with a mean run time of 23.5 ± 2.0 min, whereas the SJL/J strain ran for the longest time with a mean time of 48.0 ± 2.9 min. Similar to the intrinsic exercise capacity, there was an approximately two-fold difference between the highest and lowest performing strains. Significant differences for post-training are indicated in Table 1.1. When examining the effect that training had on performance, expressed as change in time, the LG/J strain had an average -1.6 ± 1.2 min response to training (Fig. 1-1C). The SJL/J strain had the highest response to training with a mean increase of 7.4 ± 2.7 min (Fig. 1-1C and Table 1.1). The wide range in training response; there are some strains that respond negatively and others positively, indicates that the response to training is determined, in part, by genetics.

Significant differences also were found for work across the inbred strains (Table 1.2). For pre-training work, there was a 9-fold difference between the lowest performing strain (A/J: 0.43 ± 0.10 kg·m) and the highest performing strain (AKR/J: 3.90 ± 0.40 kg·m) (Fig. 1-2A). Similar to the trend seen in pre-training, there was an 8.4-fold difference for post-training among strains (Fig.1-2B and Table 1.2). The A/J and NZW/LacJ, the two strains with the lowest pre-training work, remained at the same rank

for post-work (Fig. 1-2B and Table 1.2). Variation for the change in work was similar to post-training work. The lowest response by NON (-0.24 ± 0.40 kg·m) was approximately 8.4-fold lower than the highest response by FVB (2.10 ± 0.50 kg·m)(Fig. 1-2C and Table 1.2). The large strain-dependent differences in training responses among inbred mouse strains could be the basis for future genetic analyses of adaptation to exercise training.

Broad-sense heritability. Heritability estimates were calculated for each measure of exercise capacity. The intraclass correlations (r_I) for post-training time and the change in run time were 0.69 and 0.08, respectively. The coefficients of genetic determination (g^2) for these phenotypes were 0.51 and 0.04. Broad-sense heritability was somewhat higher for post-training work ($r_I = 0.66$ and $g^2 = 0.48$) and the change in work ($r_I = 0.22$ and $g^2 = 0.11$) than for time. The heritability for changes in body weight were similar to those of time with $r_I = 0.09$ and $g^2 = 0.04$.

Genome-wide association mapping. We have previously published GWAS results for pre-training or intrinsic exercise capacity using a larger number of inbred strains (50, 51). Therefore, this analysis will focus on post-training exercise capacity and the response to training. Genome-wide association mapping results for post-training time and the change in time are shown in Fig. 1-3 and Tables 1.3 and 1.4.

Five significant associations ($P \leq 1.88 \times 10^{-6}$) for post-training exercise time were located on four chromosomes: 3 (55.81-56.70 Mb), 6 (116.59 -116.77 Mb), 12 (7.97 - 8.06 Mb), and 14 (72.47-73.44 Mb, 73.48-73.96 Mb) (Fig. 1-3A and Table 1.3). Additionally, twenty-one suggestive QTL ($P \leq 5.44 \times 10^{-5}$) for post-training time were found on 11 different chromosomes: 1-6, 11-12, 17, 19, and X (Table 1.7). When

examining the change from pre- to post-training run time, one significant ($P \leq 1.51 \times 10^{-6}$) locus was identified on Chromosome 10 (125.60-126.17 Mb). One suggestive ($P \leq 5.83 \times 10^{-6}$) locus was identified on Chromosome 9 (100.81-101.21 Mb) (Fig. 1-3B and Table 1.4). No overlap in QTL for post-training time and the change in time were observed.

Genome-wide association mapping for post-training work and the change in work were performed to account for the variation in body mass on exercise capacity. Thirty-seven significant ($P \leq 4.81 \times 10^{-5}$) associations on 14 chromosomes were identified for post-training work (Fig. 1-4A & Table 1.5). Fifteen additional suggestive ($P \leq 1.25 \times 10^{-4}$) loci were found on chromosomes: 3-7, 12, 14-15, 17, 19, and X (Table 1.8).

Eleven chromosomes were identified as having significant associations for change in work: 1 - 3, 5, 9, 10, 12, 14, 15, and 17 -18 (Fig 1-4B and Table 1.6). No overlap in QTL were found for pre-training and response to training for time or work, indicating that none of the QTL responsible for intrinsic exercise capacity has a role in the response to training. Twenty-three significant unique loci were identified for change in work. Of these, 10 surpassed the 1% FDR level ($P \leq 1.10 \times 10^{-6}$) on eight chromosomes: 1 (174.83-174.85 Mb), 2 (76.37-76.75 Mb, 77.54-77.94 Mb), 3 (48.41-48.43 Mb), 10 (125.60-126.32 Mb), 12 (69.0-69.40 Mb), 14 (23.86-24.29 Mb), 15 (70.88-71.28 Mb), 17 (3.93-4.31 Mb, 86.12-86.68 Mb) (Fig.1-4B and Table 1.6). At the 5% FDR level ($P \leq 1.1 \times 10^{-6}$) thirteen loci were found on seven chromosomes: 1 (53.43-53.83 Mb, 163.12-163.52 Mb), 3 (54.89-55.79 Mb, 55.85-56.25 Mb, 140.45-140.85

Mb), 5 (129.05-129.57 Mb), 9 (100.81-101.21 Mb), 12 (72.59-72.60 Mb), 17 (27.48-27.49 Mb, 69.91-70.31 Mb), 18 (66.44-67.11 Mb, 79.09-79.11 Mb). Eight loci for change in work overlapped with loci for post training work: Chr 1 (53.43-53.83 Mb), 2 (76.37-76.75 Mb, 77.54-77.94 Mb), 3 (55.81-56.70 Mb), 5 (66.73-66.74 Mb), 14 (23.79-24.43 Mb), 15 (71.08-71.38 Mb), 18 (66.44-67.11 Mb). In total, 26 genes were common to both post-training and the change in work loci with 7 genes (Osbpl6, Cwc22, Kcnma1, Dnah7a, Spg20, Apbb2, Mc4r) having a peak SNP within the coding region.

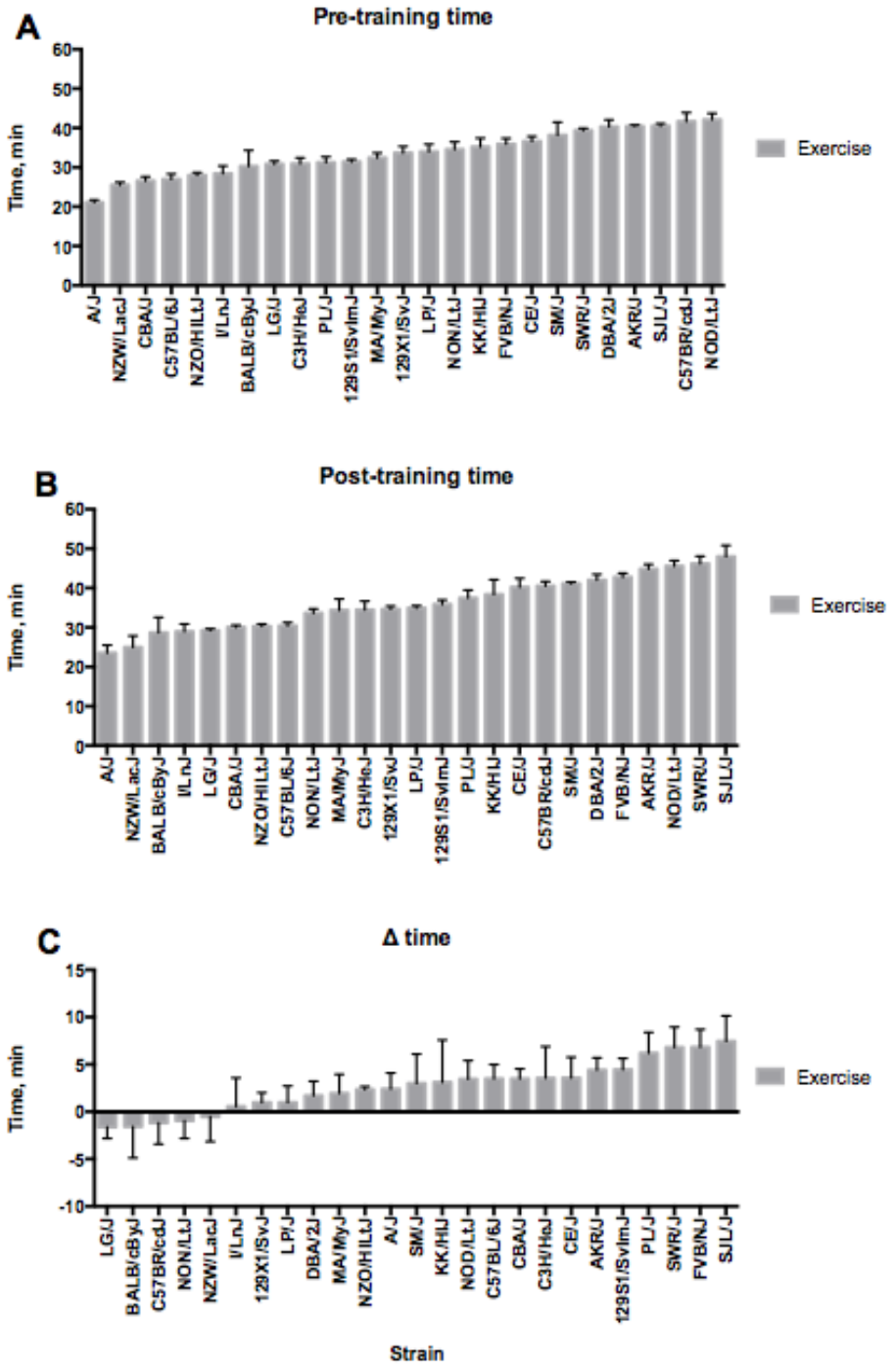

Figure 1.1. Strain distribution pattern for pre-training time (A), post-training time (B) and change in time (C) in minutes for 25 inbred strains of mice. Values are expressed as mean ± SE.

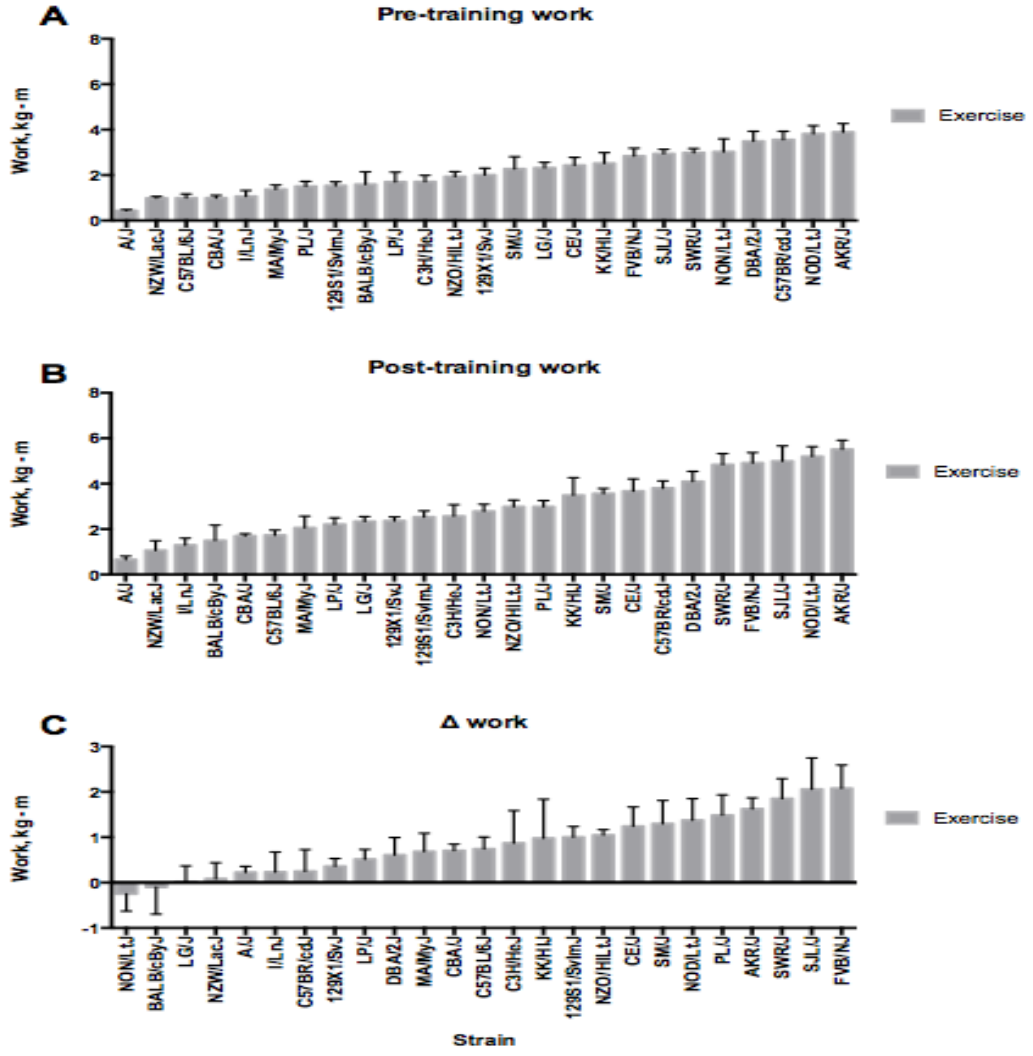

Figure 1.2. Strain distribution pattern for pre-training work (A), post-training work (B) and change in work (C) in kg·m for 25 inbred strains of mice. Values are expressed as mean ± SE.

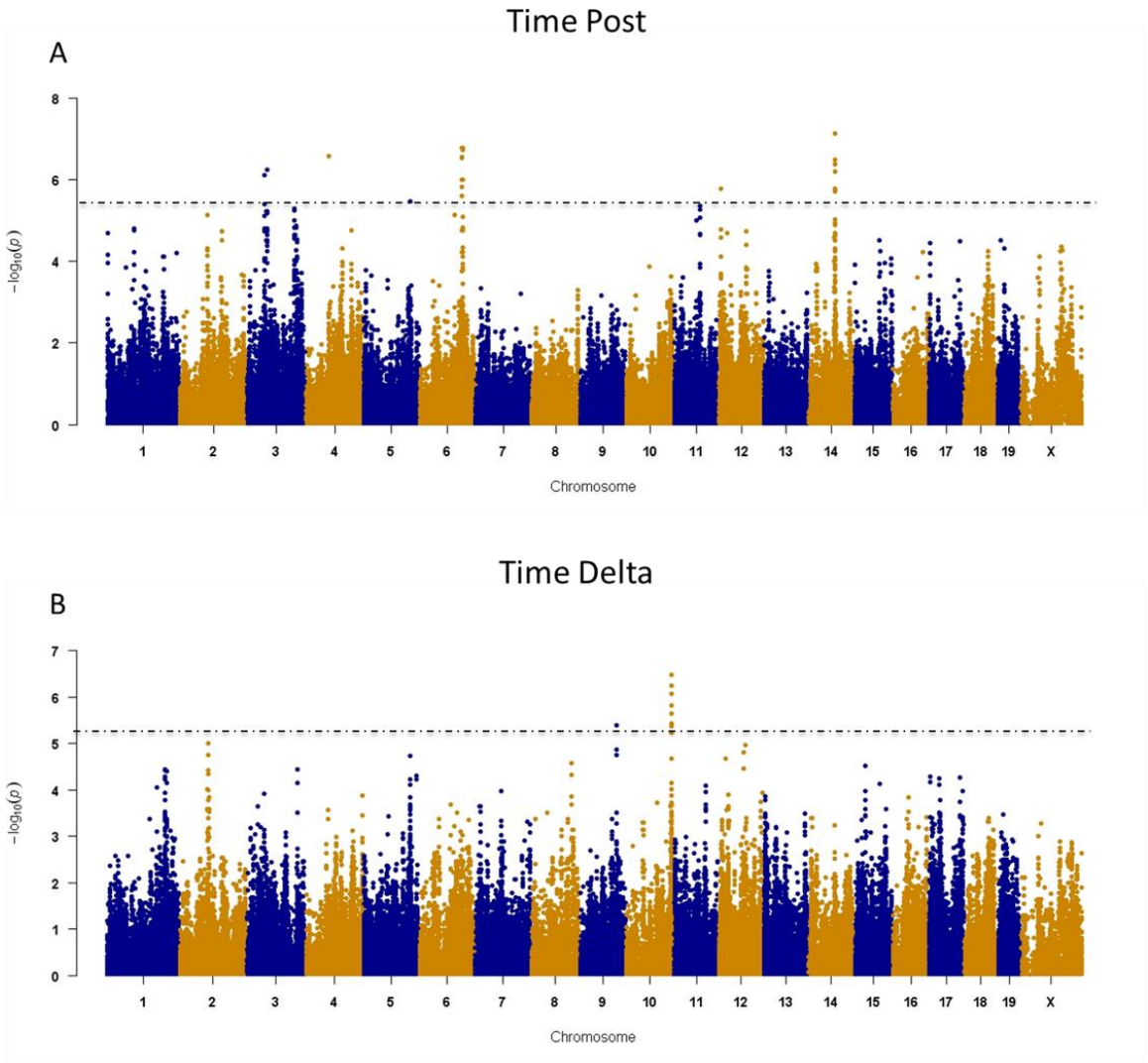

Figure 1.3. Genome-wide association results for (A) post-training time and (B) change in time for 25 inbred strains performing four weeks of exercise training. GWAS was performed utilizing an efficient mixed model algorithm with a 4 million SNP panel. Chromosomes are located on the x-axis and the y-axis is indicative of P-values transformed by $-\log^{10}$. For post-training time the dashed horizontal line indicates a significant cut-off threshold (P-value $\leqq 1.88 \times 10^{-6}$) (A). For Delta time the dashed line indicated a significant cut-off threshold (P-value $\leqq 5.83 \times 10^{-6}$) (B).

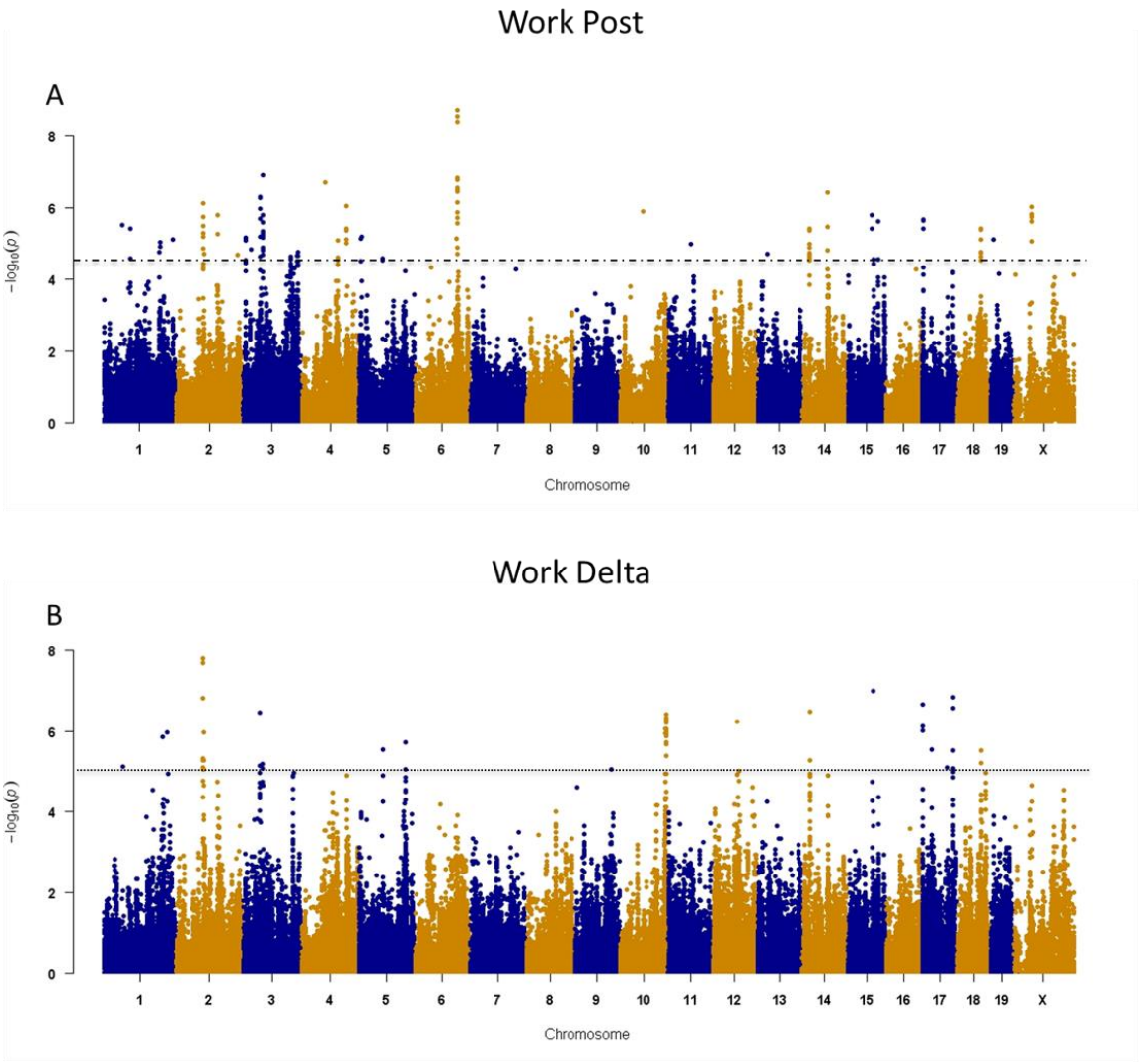

Figure 1.4. Genome-wide association mapping results for (A) post-training training work and (B) change in work for 25 inbred strains performing four weeks of exercise training. GWAS was performed utilizing an efficient mixed model algorithm with a 4 million SNP panel. Chromosomes are located on the x-axis and the y-axis is indicative of P-values transformed by $-\log^{10}$. For post-training work the dashed horizontal line indicates a significant cut-off threshold (P-value $\leqq 4.81 \times 10^{-5}$) (A). For Delta work the dashed line indicated a significant cut-off threshold (P-value $\leqq 1.10 \times 10^{-6}$) (B).

Table 1.1. Statistical differences among 25 inbred strains for time

Strain	Time, Pre (Fig. 1A)	Time, Post (Fig. 1B)	Time, Δ (Fig. 1C)
NOD/LtJ	A	ABC	ABCDEFG
C57BR/cdj	A	DEFG	FG
SJL/J	A	A	A
AKR/J	AB	ABCD	ABCDE
DBA/2J	AB	BCDEF	CDEFG
SWR/J	ABC	AB	ABC
SM/J	ABCD	CDEFG	ABCDEFG
CE/J	BCDE	EFG	ABCDEF
FVB/NJ	CDEF	BCDE	AB
KK/HIJ	DEF	FGH	ABCDEFG
NON/LtJ	DEFG	IJKL	FG
LP/J	EFGH	HIJ	DEFG
129X1/SvJ	EFGH	HIJK	DEFG
MA/MyJ	FGH	HIJK	BCDEFG
129S1/SvImJ	GHI	HI	ABCD
PL/J	GHI	GHI	ABC
C3H/HeJ	GHI	HIJK	ABCDEF
LG/J	GHIJ	LMN	FG
BALB/cByJ	HIJK	MN	G
I/LnJ	IJKL	MN	DEFG
NZO/HiLtJ	IJKL	JKLM	ABCDEFG
C57BL/6J	JKL	KLM	ABCDEF
CBA/J	KL	LM	ABCDEF
NZW/LacJ	L	NO	EFG
A/J	M	O	ABCDEFG

Statistical differences was determined by a One-way ANOVA followed by Tukey's post-hoc test. Strains not connected by the same letter are significant different ($P<0.05$). Strains are organized based on pre-training time from the longest run time (A) to the shortest time (M). The number of strain groups varies by phenotype.

Table 1.2. Statistical differences among 25 inbred strains for work

Strain	Work, Pre (Fig. 2A)	Work, Post (Fig. 2B)	Work, Δ (Fig. 2C)
AKR/J	A	A	ABC
NOD/LtJ	A	A	ABCDE
C57BR/cdJ	AB	CD	GHI
DBA/2J	AB	BC	CDEFGHI
NON/LtJ	BC	EFG	I
SWR/J	BCD	AB	AB
SJL/J	BCD	AB	A
FVB/NJ	BCD	AB	A
KK/HIJ	CDE	CDE	BCDEFG
CE/J	CDEF	CDE	ABCDEF
LG/J	CDEFGH	FGHI	GHI
SM/J	DEFG	CDE	ABCDE
129X1/SvJ	EFGHI	FGHI	EFGHI
NZO/HILtJ	FGHI	DEFG	ABCDEFG
C3H/HeJ	FGHIJ	FGH	BCDEFGH
LP/J	GHIJ	FGHIJ	DEFGHI
BALB/cByJ	GHIJ	IJKL	HI
129S1/SvImJ	HIJ	FGH	BCDEFG
PL/J	HIJ	DEF	ABCD
MA/MyJ	IJ	GHIJ	CDEFGHI
I/LnJ	JK	JKL	GHI
CBA/J	JK	HIJK	CDEFGHI
C57BL/6J	JK	HIJK	CDEFGH
NZW/LacJ	JK	KL	GHI
A/J	K	L	FGHI

Statistical differences was determined by a One-way ANOVA followed by Tukey's post-hoc test. Strains not connected by the same letter are significant different ($P<0.05$). Strains are organized based on pre-training work from the highest work performance (A) to the least amount of work performed (K). The number of strain groups varies by phenotype.

Table 1.3. Genome-wide significant associations for post-training exercise time in 25 inbred mouse strains

Chr	Position, Mb	Peak SNP	P Value	Known Genes, n
3	55.85 – 56.70	rs30692485	5.75E-07	2
6	116.59 – 116.77	rs37554461	1.66E-07	3
12	7.97 – 8.06	rs29135975	1.62E-06	1
14	72.47 – 73.44	rs30287744	7.30E-08	2
14	73.48 – 73.96	rs30990007	3.23E-07	7 (Nudt15)

The significance threshold was defined as P values $\leq 1.88 \times 10^{-6}$ (5% FDR) . Confidence intervals were determined by expanding 200 kb around a single peak SNP. If two intervals overlapped or were within 1 megabase (Mb), they were considered one interval. Peak markers were identified with SNP (rs) numbers and cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome; SNP, single nucleotide polymorphism.

Table 1.4. Significant and suggestive genome-wide associations for the change in exercise time

Chr	Position, Mb	Peak SNP	P Value	Known Genes, n
9	100.81 – 101.21	rs36599278	4.13E-06	4 (Ppp2r3a)
10	125.60 – 126.17	rs36240918	5.83E-06	0

The significance threshold was defined as $P \leq 1.51 \times 10^{-6}$ (5% FDR) and the suggestive threshold defined as $P \leq 5.83 \times 10^{-6}$ (10% FDR). Confidence intervals were determined by expanding 200 kb around a single peak SNP. If two intervals overlapped or were within 1 megabase (Mb), they were considered one interval Peak markers were identified with SNP (rs) numbers cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome; SNP, single nucleotide polymorphism.

Table 1.5. Significant genome-wide associations for post-training work in 25 inbred strains of mice

Chr.	Position, Mb	Peak SNP	P Value	Known Genes, n
1	53.43 – 53.83	rs32767656	3.03E-06	2 (Dnah7a)
1	75.29 – 75.34	rs32025312	3.77E-06	1 (Dnpep)
1	153.32 – 153.54	rs30993819	1.70E-05	2 (Rnf2)
1	154.84 – 154.97	rs31173780	9.36E-06	2 (Nmnat2)
1	190.35 – 190.75	rs37379622	7.77E-06	1 (Ush2a)
2	76.37 – 76.75	rs27966858	7.61E-07	3 (Osbpl6)
2	77.54 – 77.94	rs27980868	1.92E-05	2 (Cwc22)
2	115.17 – 115.42	rs27426179	1.64E-06	1 (BC052040)
2	168.23 – 168.63	rs27288927	2.01E-05	3 (Nfatc2)
3	8.55 – 9.22	rs29665833	6.94E-06	4
3	24.14 – 24.54	rs13477021	1.42E-05	1 (Naaladl2)
3	47.95 – 48.47	rs31692821	5.35E-07	1
3	49.81 – 50.21	rs30047036	6.30E-06	1
3	54.81 – 55.81	rs30550735	1.64E-06	7 (spg20)
3	55.81 – 56.70	rs30692485	1.22E-07	2
3	129.99 – 130.99	rs3660884	2.35E-05	6 (Lef1)
3	131.02 – 131.35	rs36605283	2.75E-05	2
3	134.28 – 135.16	rs30958250	4.30E-05	6 (Ebe2d3)
4	98.93 – 98.97	rs28093881	2.46E-05	2
4	100.42 – 100.42	rs28180112	8.22E-06	0
4	124.44 – 124.98	rs27530247	8.87E-07	13
5	8.70 – 9.05	rs31412528	6.37E-06	4 (Abcb1b)
5	66.73 – 66.74	rs6334689	2.61E-05	1 (Apbb2)
6	45.41 – 45.42	rs36977921	4.61E-05	1 (Cntnap)
6	114.23 – 114.63	rs48377525	7.29E-06	3 (Hrh1)
6	116.59 – 116.77	rs30562936	1.82E-09	3 (Rassf4)
6	118.02 – 118.69	rs30659320	1.45E-07	8 (Rasgef1a)
10	64.43 – 64.83	rs46123113	1.30E-06	1
11	62.23 – 62.63	rs26970405	1.04E-05	7
13	27.72 – 28.12	rs30049120	1.92E-05	8
14	23.79 – 24.43	rs50727178	3.81E-06	2 (Kcnma1)
14	72.47 – 73.30	rs30287744	3.75E-07	1
15	71.08 – 71.38	rs45977326	2.79E-05	1 (Fam135b)
15	84.83 – 85.36	rs31795100	2.44E-06	8 (Fam118a)
17	86.34 – 86.63	rs46866929	6.01E-05	2 (Prkce)
18	66.44 – 67.11	rs38018854	3.95E-06	3

Table 1.5 Continued

Chr.	Position, Mb	Peak SNP	P Value	Known Genes, n
19	10.08 – 10.48	rs30672343	7.58E-06	9
X	54.16 – 54.29	rs29059781	9.54E-07	1

The significance threshold was defined as P values $\leq 4.81 \times 10^{-6}$ (5% FDR). Confidence intervals were determined by expanding 200 kb around a single peak SNP. If two intervals overlapped or were within 1 megabase (Mb), they were considered one interval. Peak markers were identified with SNP (rs) numbers cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome; SNP, single nucleotide polymorphism.

Table 1.6. Significant genome-wide associations for change in work in 25 inbred strains of mice

Chr.	Position, Mb	Peak SNP	P Value	Known Genes, n
1	53.43 – 53.83	rs32767656	7.59E-06	2 (Dnah7a)
1	163.12 – 163.52	rs33739542	1.40E-06	3 (Tnfsf4)
1	174.83 – 174.85	rs49102850	1.07E-06*	1
2	76.37 – 76.75	rs6285328	1.58E-08*	6 (Osbpl6)
2	77.54 – 77.94	rs27980868	1.09E-06*	2 (Cwc22)
3	48.41 – 48.43	rs30189414	3.50E-07*	1
3	54.89 – 55.79	rs30549853	6.39E-06	7 (Spg20)
3	55.85 – 56.25	rs30648206	6.39E-06	1
3	140.45-140.85	rs37503768	1.07E-05	0
5	66.54 – 66.94	rs6334689	2.84E-06	3 (Apbb2)
5	129.05 – 129.57	rs52564865	1.91E-06	5
9	100.81 – 101.21	rs36599278	8.73E-06	4 (Pp2r3a)
10	125.60 – 126.32	rs29346528	3.71E-07*	1
12	69.00-69.40	rs29203182	5.76E-07*	0
12	72.59 – 72.60	rs36540084	9.75E-06	0
14	23.86 – 24.29	rs51963737	3.31E-07*	2 (Kcnma1)
15	70-88 – 71.28	rs31397922	9.81E-08*	1
17	3.93 – 4.31	rs33635708	2.11E-07*	0
17	27.48 – 27.49	rs33796002	2.84E-06	0
17	69.91 – 70.31	rs46970879	7.71E-06	1
17	86.12 – 86.68	rs47148259	1.44E-07*	3
18	66.44 – 67.11	rs37660744	2.99E-06	3 (Mc4r)
18	79.09 – 79.11	rs29562361	1.06E-05	1 (Setbp1)

The significance threshold was defined as P values $\leq 1.16 \times 10^{-5}$ (5% FDR). * denotes significance at $P \leq 1.10 \times 10^{-6}$ (1% FDR). Confidence intervals were determined by expanding 200 kb around a single peak SNP. If two intervals overlapped or were within 1 megabase (Mb), they were considered one interval. Peak markers were identified with SNP (rs) numbers cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome; SNP, single nucleotide polymorphism.

Table 1.7. Suggestive genome-wide association mapping for time post in inbred mice using a 4 million SNP panel

Chr.	Location, Mb	Peak SNP	P Value	Known Genes, n
1	2.81 – 3.21	rs31192577	2.01E-05	1
1	75.29 – 75.34	rs37486293	1.53E-05	1
2	76.37 – 76.42	rs6285328	7.18E-06	1 (Osbpl6)
2	115.17 – 115.42	rs27426179	1.79E-05	1 (BC052040)
3	54.81 – 55.81	rs30549853	5.80E-06	6 (Spg20)
3	130.50 – 131.04	rs30260078	5.12E-06	4
3	131.06 – 131.35	rs50807877	2.18E-05	2 (sgms2)
3	134.28 – 135.16	rs30958250	1.35E-05	8 (Ube2d3)
3	137.65 – 137.83	rs30018158	2.83E-05	5 (4930579F01Rik)
4	98.77 – 99.17	rs32864413	4.81E-05	4
4	124.78 – 125.18	rs27530247	1.73E-05	4
5	127.87 – 128.27	rs29621092	3.36E-06	5
6	95.55 – 95.95	rs31159908	7.40E-06	1
6	118.02 – 118.49	rs30659320	1.48E-05	7 (Rasgef1a)
11	62.23 – 62.63	rs26970405	9.91E-06	12
12	24.89 – 25.29	rs31608859	2.03E-05	4
12	76.16 – 76.21	rs32374624	1.82E-05	1 (Kcnh5)
17	86.14 – 86.54	rs47148259	3.18E-05	1
19	10.08 – 10.48	rs30672343	2.98E-05	9
X	113.33 – 113.69	rs29105606	4.33E-05	1
X	114.35 – 114.75	Rs29103184	5.43E-05	0

Quantitative trait loci (QTL) having P values $\leq 5.44 \times 10^{-4}$ (10% FDR) were considered significant. QTL intervals were determined by expanding 200 kb around a single peak SNP. For multiple SNP residing in less than a Mb, they were grouped as one QTL. Peak markers were identified with SNP (rs) numbers cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome.

Table 1.8. Suggestive genome-wide association mapping for work post in inbred mice using a 4 million SNP Panel

Chr.	Location, Mb	Peak SNP	P Value	Known Genes, n
3	137.63 – 138.03	rs30018158	7.01E-05	8 (4930579F01Rik)
4	95.50 – 95.53	rs28106603	1.11E-04	1 (fggy)
5	127.87 – 128.27	rs29621092	5.98E-05	4
6	95.55 – 95.95	rs31159908	1.15E-04	1
7	36.26 – 36.66	rs31561779	9.11E-05	8
7	125.91 – 126.31	rs51440010	5.25E-05	5 (Gprc5b)
12	75.99 – 76.39	rs29140562	1.14E-04	1 (Kcnh6)
14	73.70 – 74.10	rs30595135	8.08E-05	5
15	3.18 – 3.33	rs32023583	7.73E-05	3 (Ghr)
15	102.52 – 102.78	rs31760735	8.73E-05	4 (Calcoco1)
17	86.34 – 86.63	rs46866929	6.01E-05	2 (Prkce)
19	24.82 – 24.82	rs37818092	6.91E-05	1 (Pgm5)
X	7.31 – 7.38	rs33370285	7.19E-05	3 (Ccdc120)
X	114.35 – 114.75	rs29103184	8.60E-05	0
X	166.22 – 166.62	rs31827822	7.19E-05	2

Quantitative trait loci (QTL) having P values $\leq 1.25 \times 10^{-4}$ (10% FDR) were considered suggestive. QTL intervals were determined by expanding 200 kb around a single peak SNP. For multiple SNP residing in less than a Mb, they were grouped as one QTL. Peak markers were identified with SNP (rs) numbers cross referenced against the UCSC genome browser database. Genes within parentheses indicates gene in which the peak SNP resides. Chr., chromosome.

2.4 Discussion

Exercise capacity is a complex polygenic trait that has been studied in both humans and rodents with strong evidence of a known genetic basis (28). To date, the majority of studies have focused on the variation in intrinsic capacity. While the response to exercise training has been studied, limited results have been inconclusive due to relatively small sample sizes in human studies which limits reproducibility (33). Training studies in rodents have been conducted using genetic crosses (159, 160) and rodents artificially selected for high and low exercise capacity (120). However, the limited genetic diversity and lack of mapping resolution in these studies have constrained the ability to determine the genetic basis for the responses to exercise training. We, therefore, assessed exercise capacity expressed as time and work in 25

inbred mouse strains after four weeks of exercise training. Utilizing the variation in response to training, we employed genome-wide association mapping to identify loci associated with post-training exercise capacity and the response to training. The main findings from this study were: 1) significant variation in response to exercise training exists across 25 inbred mouse strains; 2) significant and suggestive associations were identified for all phenotypes measured; and 3) proposed candidate genes for future investigation have been identified.

To date, data from animal models regarding the genetic variation in the responses to exercise training are lacking. However, the limited data indicate that training responses vary among inbred strains and genetically heterogeneous populations of rodents. In the current study, mice from 25 inbred strains completed exercise training. There was an ~ 4.6-fold difference between the highest and lowest performing strains when changes are expressed as time. The difference is greater (8.5-fold) when changes in exercise capacity are expressed as work. Overall, this range of responses is similar to those reported for rats and mice. For example, Kilikevicius et al. (114) assessed adaptations to 5 weeks of swim training in 6 inbred strains of mice. There was an approximately 6-fold difference between strains for post-training endurance time with C57BL/6J mice having the greatest endurance and BALB/cByJ mice having the least. Massett et al. (159) previously demonstrated significant differences in the responses to treadmill training in mice from 3 inbred and 3 hybrid strains. Mice from these strains completed a training protocol comparable to the current study and showed significant variation among strains with FVB/NJ > C57BL/6J > BALB/cJ. Furthermore, the range of responses to 4 weeks

of exercise training in an F_2 population based on FVB/NJ and C57BL/6J strains varied by 3.5-fold from highest to lowest (160). Koch et al. compared the change in running capacity in rats from 10 inbred strains in response to 8 weeks of treadmill running at the same absolute workload (122). There was a 3.9 fold difference in the change in running distance between the highest and lowest performing strains. In the same study, the authors reported that the change in distance varied from -339 m to +627 m in a genetically heterogeneous population of rats. These results build upon their previous finding that strain-differences in the response to exercise training persisted whether training was performed at the same relative or absolute workload (121). Collectively, these data indicate that exercise training responses vary across inbred and genetically heterogeneous populations of rodents. Furthermore, the significant differences between inbred strains of mice or rats suggest that changes in exercise capacity in response to exercise training is significantly influenced by genetic factors.

To provide some insight into the genetic contribution to the responses to exercise training, two estimates of broad sense heritability were calculated for post-training exercise capacity and the change in exercise capacity. The heritability estimates for post-training exercise time were $r_I = 0.69$ and $g^2 = 0.51$, respectively. These estimates were similar ($r_I = 0.65$ and $g^2 = 0.46$) for post-training work. The heritability estimates for post-training time and work are comparable to those reported by Massett et al. (159) in a smaller number of mouse strains. Broad sense heritability estimates for the change in exercise capacity were considerably smaller than for post-training exercise capacity. The estimates for the current study are also lower than those previously reported for

changes in time (0.38 and 0.55) and work (0.38 and 0.55) in inbred and hybrid mouse strains. However, Koch et al. (122) reported a heritability estimate of 0.13 for the change in distance from 10 inbred strains of rats, which is similar to the current study. Heritability estimates account for the influence of genetic background to the phenotype of interest. The low heritability estimates for change in exercise capacity could be explained by the environmental influences in the training protocol that we utilized. Mice were repeatedly handled on an almost daily basis for the duration of the study and subjected to multiple training sessions.

Genome-wide association mapping revealed five significant QTL for post-training time on four chromosomes. Two significant associations were located on chromosome 14. These loci fall within a quantitative trait locus (QTL) for post-training time previously identified by Massett et al. (160) in an F_2 cross based on C57BL/6J and FVB/NJ strains. The peak SNP for one significant locus on Chromosome 14 was in Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15 (*Nudt15*). *Nudt15* was found via gene expression profiles to be differentially expressed in hindlimb muscle in rats that were artificially selected for high and low intrinsic running capacity (117). Transmembrane protein 72 (Tmem72) was identified on chromosome 6, which is a kidney specific secreting protein. This gene was previously identified by Lee et al. (140) via microarray to be differentially regulated between healthy individuals and those with type 2 diabetes mellitus (T2DM). This link is important as individuals with T2DM and metabolic syndrome have been shown to have reduced exercise capacity (172, 219).

The variation in the change in time was such that seven unique strain distribution

groups were present with an approximately a 4.65 fold-difference between the lowest time change (LG/J) to the highest (SJL/J). Despite the wide variation among strains, only one significant and one suggestive locus were identified for the change in time. Neither of these loci overlapped with the significant associations for post-training time. No genes were found in the significant locus on chromosome 10. On chromosome 9, the suggestive interval contained 4 genes. The peak SNP was located within protein phosphatase 2, regulatory subunit B alpha (*Ppp2r3a*). *Ppp2r3a* is associated with somatic muscle development. A study by Brown et al. (37) examining the effects of exercise associated DNA methylation changes revealed that DNA methylation of *PPP2R3A* in humans is increased after exercise and is associated with regulation of anatomical structure morphogenesis. Methylation changes are of emerging interest to exercise biology as genes that are imprinted have been shown to regulate a cascade of relevant processes such adiposity, energy metabolism, and glucose regulation (37).

When exercise capacity was expressed as post-training work twelve distinct strain groupings were identified (Table 1.2). Genome-wide association mapping identified 37 significant associations. Fourteen of these loci overlap with QTL for post-training work reported by Massett et al. (160). Furthermore, our laboratory published results on intrinsic exercise capacity across multiple inbred strains (50) and identified several significant associations. One significant locus overlaps with a significant association for post-training work on chromosome 2 at 168.23-168.63 Mb. The peak SNP for this locus falls within nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 2 (*Nfatc2*), which is thought to have a wide array of functions

ranging from differentiation of tissue (99) to energy balance (94). Kostrzewa et al. (130) found that when *Nfatc2* is knocked out in mice, wheel running activity is significantly impaired. There is overlap between significant loci for post-training work and post-training time on the following chromosomes: 3 (55.81-56.70 Mb), 6 (116.59-116.77 Mb), and 14 (72.47-73.30 Mb).

Several genes of interest reside in loci associated with post-training work. For instance, spastic paraplegia 20, spartin (*Spg 20*), identified on chromosome 3 (54.81-55.81 Mb), is typically associated with Troyer syndrome because of the role is has in neuromuscular processes and adipose tissue development (13, 14, 181, 196). Amyloid beta (A4) precursor protein-binding, family B, member 2 (*Apbb2*) is involved in protein binding stabilization and apoptotic processes. Tucsek et al. (223) examined the effects of *Apba2* in young and old C57BL/6J mice on a regular chow and high-fat chow diet and showed that obesity decreased *Apba2* expression in the hippocampus. Based on microarray data from GEO (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM429920) RasGEF domain family, member 1A (*Rasgef1a*) is a potential candidate as well. *Rasgef1a* is a protein coding gene which codes the RAS proteins and is involved in biological processes associated with vascular endothelial growth factor signaling pathway and cell migration. Kivela et al. (117) utilized rats selectively bred as high- and low- capacity runners and found that *Rasfef1a* is differently expressed in the gastrocnemius muscle between the two strains. *Rasgef1a* warrants further investigation as these two strains differ greatly in their risk for metabolic disease.

In contrast to post-training work, change in work exhibited a smaller number of significant strain groupings resulting in a total of nine. Association mapping identified significant loci at two FDR levels (1% and 5%). In total, 10 significant associations were identified on 7 chromosomes. It should be noted that all loci with peak SNP residing in genes for change in work at an FRD of 1% were also identified in post-training work. There was no overlap in associations for any of the phenotypes associated with time but four loci overlapped with post-training work chromosomes: 2 (76.37-76.75 Mb, 77.54-77.94 Mb), 14 (23.86-24.29 Mb), 15 (70.88-71.28 Mb).

One loci common to both post-training work and the change in work is on chromosome 2. This loci at 76.37 - 76.75 Mb harbors the gene Titan (*Ttn*). *Ttn* plays a pivotal role in striated muscle and muscle contractility, which was also observed by Timmons et al. (218) in Caucasian young adults through a regression analysis to predict oxygen consumption after training, Another gene in that interval is Oxysterol Binding Protein-Like 6 *(Osbpl6). Osbpl6* is a part of the oxysterol-binding protein (OSBP) family, which serves as an intracellular lipid receptor (143). *Osbpl6* is associated with cholesterol hemostasis (107, 132, 143). When cholesterol is oxidized, oxysterols are formed which in turn can activate liver X receptors (106), which through a cascade of event regulating processes include cholesterol efflux, (191, 225, 226) and absorption along with the synthesis of fatty acids (190, 202). Mathes et al. (161) observed differential in gene expression of another OSBP family member, *Osbpl1a*, in the nucleus accumbens from mice selectively bred for high wheel running compared to ICR mice.

Another gene of interest is potassium large conductance calcium-activated

channel, subfamily M, alpha member 1 *(Kcnma1)* was found on chromosome 14 (23.86-24.29 Mb). This loci is also common to both post-training work and change in work. *Kcnma1* has been implicated in insulin secretion, calcium-, potassium- channels, and renin secretion. Li et al. (147) investigated the effect of exercise training on thoracic smooth muscle in spontaneously hypertensive rats after eight weeks of endurance training and noted that blood pressure decreased to levels below that of the non-hypertensive control rats. This decrease in blood pressure was associated with lower levels of *Kcnma1* gene expression. Furthermore, Shi and collogues (204) examined the effects of aerobic exercise training in old male Wistar rats and concluded that exercise training reverses the down regulation of *Kcnma1* with age, which reinforces the benefits of exercise on vasculature system and could potentially be a mechanism which explains adaptations to exercise training.

In conclusion, we confirmed that the variation in the response to exercise is heterogeneous among inbred mouse strains as seen in humans (30) and identified significant associations for post-training exercise capacity and the change in exercise capacity. Several of the intervals support QTL that were identified in previous animal studies (50, 160) and contain genes identified in human studies of the genetic basis for exercise training responses. The identification of punitive candidate genes through techniques such as GWAS provides a foundation for future studies utilizing techniques such as knockout studies; however, caution should be exercised because with polygenic traits like exercise, the phenotype of interest is likely the results of the contribution of multiple genes (68).

CHAPTER III

INTERACTION BETWEEN GENETIC BACKGROUND AND TRAINING INTENSITY INFLUENCES TRAINING RESPONSES

3.1 Introduction

Cardiorespiratory fitness has been shown to increase with physical activity regardless of intrinsic exercise capacity, age, sex, and race (22, 62). However, individual variation in adaptations to exercise is heterogeneous including individuals that do not improve their fitness in response to exercise training. Bouchard et al., investigating the response of maximal oxygen consumption (VO_2 max) in men and women to 20 weeks of standardized training, reported a tremendous heterogeneity for changes in maximal oxygen consumption ranging from 0 – 1,000 mL O_2 (22). Similar variation in maximal aerobic capacity or VO_{2max} in response to a 12 month standardized training program also have been reported for healthy, sedentary adults (125, 151). These data highlight the magnitude of individual variation in adaptation to exercise training. However, the mechanisms underlying this individual variation are unknown.

Several factors can contribute to the adaptations to exercise training. Overall, intrinsic exercise capacity is thought to contribute only 1% to the response to exercise with sex contributing approximately 5% to the response seen with exercise training (22, 30). Results from cross-sectional, twin, and prospective studies indicate that genetics contribute significantly to individual variation in responses to training as well. The HERITAGE Family Study reported a heritability of 47% for training responses in 98

two-generation families (6). In twin studies, the variation in responses to aerobic training range from 6 – 9 times greater between, than within pairs (187). These data indicate that genetic factors modulate the response to exercise training.

In addition, several components of an exercise training program are modifiable including intensity, duration, and volume. Thus, the question arises as to whether these variables can be optimized to yield an increase in exercise capacity in individuals characterized as non-responders. In two reports from the Dose-Response to Exercise in postmenopausal Women (DREW) study, the frequency of non-response to training was inversely proportional to the training volume (46, 207). In that study women completed 30 min of exercise at 50% of VO_{2max} with the goal of expending 4-, 8-, or 12-kcal $\cdot kg^{-1} \cdot wk^{-1}$. Furthermore, Astorino et al. (9) demonstrated that 95% of participants increased VO_2max in response to 12 weeks of high intensity interval training (60-90% max). Collectively, these data suggest that increasing training volume or intensity can decrease or minimize the non-response to exercise training.

High-intensity interval training (HIIT) is comprised of bouts of vigorous exertion for a short duration, coupled with an active rest at a lower moderate intensity and repeated until physical activity ceases (12, 60, 73, 98, 150, 192). The majority of studies that have compared interval training with continuous exercise training have reported increases in VO_{2max} with both types of training (170, 198, 201, 219, 238). The increase in VO_{2max} with interval training was similar to or greater than continuous training despite training for a shorter duration even when training protocols are matched for energy expenditure. Furthermore, HIIT has been reported to increase muscle mitochondrial

biogenesis, improve glucose tolerance, and insulin sensitivity (73, 167, 192). In patients with heart failure (237) or metabolic syndrome (219), and obese individuals (201), HIIT also has been reported to increase skeletal muscle signaling for mitochondrial biogenesis to a greater extent than continuous training. Thus, it appears that interval training when compared to similar levels of continuous exercise can induce similar changes in VO_{2max} and greater adaptations in skeletal muscle. Therefore, the purpose of this study was two-fold: first, to identify the influence of intensity and duration on non/low–responders; and second, to delineate changes in proteins known to be enhanced with exercise via the PGC-1alpha (*Ppargc1a*) pathway in inbred mouse strains.

3.2 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. Male mice (n=36/strain, 7 wks) from four inbred strains: C57BL/6J (B6), 129S1/SvImJ (129S1), SJL/J (SJL), and NON/LtJ (NON) were purchased from Jackson Laboratories (Bar Harbor, ME). Strains were chosen based on phenotypic differences (Chapter II), genetic diversity, and the availability of sequence data. All animals were group housed, with food and water provided *ad libitum.* Mice were maintained on a 12-hr light:dark schedule (7:00 AM – 7:00 PM) in a temperature-controlled environment (21.0 – 22.0°C).

Exercise performance test (RTE). Mice were given at least a one-week period to acclimatize to their housing upon arrival. At 8 weeks of age, all mice completed a 2-day familiarization protocol on a motorized rodent treadmill with an electric grid at the rear of the treadmill (Columbus Instruments, Columbus, OH), as described previously (159, 160). Following familiarization, mice completed two exercise performance tests separated by 48 hours before training. The graded exercise tests started at 9 m/min for 9 minutes then increased from 10 m/min by 2.5 m/min every 3 minutes. The starting incline was 0° and was raised by 5° every 9 minutes, with a maximal incline of 15°. Exercise continued until exhaustion, defined as staying on the shock grid and not responding to external motivation, defined as spending greater than 15 consecutive seconds on the grid (159, 160). Exercise capacity was expressed in time (min), distance (m), and work (kg ·m). Work performed (kg · m) was calculated as the product of body mass (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° (50, 159, 160). Two additional exercise performance tests were repeated after training to determine changes in exercise capacity. Both before and after training, the two trials were averaged for each mouse and the average used in strain mean calculations.

Moderate intensity continuous training (MOD). MOD consisted of treadmill running 5/day/week, ~ 70 min/day at 10° incline at a final intensity equivalent to ~65% of the maximal workload (speed and incline) performed during the exercise performance test for 4 or 8 weeks. In the 8-week training group exercise capacity was assessed after the first 4-weeks to adjust running speed for the last half of training. The intensity of

65% of maximal workload was chosen as a result of all mice strains being able to complete this protocol over 4 weeks. During the first two weeks, the speed and duration were gradually increased until the desired training protocol could be sustained.

High-intensity interval training (HIIT). HIIT consisted of treadmill running 5/day/wk, 60 min/day at 10° incline. High-intensity training periods were comprised of 6 sets of 8 minutes of high intensity (85%) followed by 2 minutes of lower intensity (50%) running for 4 or 8 weeks. Total distance was matched between low-intensity and HIIT. In the 8-week training group exercise capacity was assessed after the first 4-weeks to adjust running speed for the last half of training.

Body and tissue mass. All mice had body mass recorded in grams prior to the start and end of training. Weekly weights were recorded for each mouse during the duration of training for both exercise and control mice. Approximately 48 hours after the final bout of exercise, all mice were weighed and anesthetized with an intraperitoneal injection of Ketamine (80 mg/kg) – Xylazine (5 mg/kg) cocktail. Heart, gastrocnemius, plantaris and the soleus muscle were harvested, washed in ice-cold (4°C) saline, weighed (wet weight in mg), flash-frozen in liquid nitrogen and stored at -80°C for future analysis.

Muscle lysate preparation. Plantaris and gastrocnemius muscle lysates were prepared in Cell Extraction Buffer FNN0011 (Invitrogen) with Protease Inhibitor Cocktail (Roche #11836145) following manufacturer's instructions. Tissues were homogenized with the FastPrep-24 using stainless steel bead lysing matrix (MP Biomedicals, Solon, OH) for 60 seconds at 6.0 m/s. Homogenate was centrifuged at

10,000 RPM at 4°C for 5 minutes to pellet tissue debris. Supernatant was collected, aliquoted and frozen at -80°C.

Protein assay. Protein content was measured in triplicate and quantified utilizing the bicinchoninic acid (BCA) (23227, Thermo Scientific, Waltham, MA) protein assay with commercially available Bovine Serum Albumin pre-diluted standards (23208, Thermo Scientific, Waltham, MA).

Immunoblotting. Muscle protein lysates were separated and identified using NuPage Novex 4-12% Bis-Tris Protein Gels (1.0 mm). Molecular weight identification was visualized through a mixture of 5 µl of SeeBlue Plus 2 (LC5925, Life Technologies) and 10 µl of MagicMark XP (LC5602, Life Technologies). For each tissue sample, 30 µg of protein was loaded in each lane for separation by electrophoresis at 200 V for 60 minutes at room temperature in Xcell SureLock Mini-Cell according to manufacturer's instruction utilizing a MOPS SDS running buffer supplemented with antioxidant (Life Technologies). Proteins were transferred to a 0.45 µm polyvinylidene dilfluoride (PVDF) membrane via a semi-wet process (XCell II, Life Technologies) for 90 minutes at 30 V at room temperature. Membranes were blocked for 1 hour (5% BSA or 5% non-fat dry milk [NFDM] in Tris-buffered saline with Tween 20) at room temperature.

Antibodies. Membranes were incubated on a rocker overnight at 4°C against the following: Anti-Hexokinase II (ab76959; Abcam) at 1:1000 in BSA, Anti-PGC-1Alpha (ab54481, Abcam) at 1:1000 in BSA, NRF-2 H-300 (SC-13032, Santa Cruz Biotechnology) at 1:200 in BSA, mtTFA E-16 (SC-20963 Santa Cruz Biotechnology) at 1:200 in NFDM, Purified Mouse Anti-Cytochrome C Clone 7H8.2C12 (556433, BD

Biosciences) at 1:1000 in BSA, Glut-4 C-20 (SC-1608, Santa Cruz Biotechnology) at 1:200 in BSA, SirT1 D60E1 (3931, Cell Signaling Technology) at 1:1000 in NFDM, α-Tubulin (2144, Cell Signaling Technology) at 1:1000 in BSA. Membranes were washed three times at five minutes each with a total of 15 ml of TBS-T then incubated at room temperature with their respective species-specific secondary horseradish peroxidase-conjugated antibody for one hour in NFDM. Membranes were subsequently washed prior to enhanced chemiluminescent substrate (Pierce ECL Western Blotting Substrate, 32105, Thermo Scientific) being applied, and visualized on a FujiFilm LAS-4000 imager (HE Healthcare Life Sciences, Piscataway, NJ). Bands were quantified image studio light (LI-COR, Lincoln, NE).

Statistical analysis. All data is presented as mean ± SE. Comparisons within strains and across training paradigms were made using a one-way ANOVA followed by Tukey post-hoc analysis for body mass, time, and work. Full factorials were performed within strain to assess the interaction for main effects. Protein analysis was examined within strain and for each duration by non-parametric multiple comparison analysis via Steel-Dwaas with control set to each duration's sedentary group. Protein correlations were performed within strains and training durations. All statistical analysis was performed using JMP 11 (SAS Institute., Cary, NC 1989-2015) with statistical significance set at $P<0.05$ for correlations.

3.3 Results

Intrinsic exercise. Exercise capacity was assessed for all mice utilizing a run to exhaustion test. Prior to exercise training, body mass, exercise time, and work were recorded for each training paradigm within strains. No statistical differences were observed between groups in three of the four strains. The NON strain exhibited a significant difference in body mass in the 4-week continuous moderate exercise group compared to the rest of the training paradigms (Table 2.1).

Body mass. Within the 4-week duration all mice increased body mass during training, but only the B6 HIIT mice exhibited a body mass that was significantly different from sedentary post training (4-week HIIT 26.2 ± 0.4 g vs 4-week SED 28.7 ± 0.3 g, $P<0.05$). For NON, the moderate intensity mice had a significantly smaller change in body mass compared to sedentary (0.1 ± 0.6 g vs 2.9 ± 0.2 g, $P<0.05$). In SJL mice, both the HIIT and moderate-intensity mice had significantly smaller changes in body mass than sedentary mice (3.2 ± 0.4 g, 3.4 ± 0.2 g vs 5.0 ± 0.5 g, $P<0.5$). For the 8-week duration, within B6 there was a significant difference for change in body mass with HIIT gaining less weight then sedentary (1.9 ± 0.6 g vs 4.2 ± 0.6 g, $P<0.05$). For NON, moderate-intensity-trained mice weighed significantly less post-training than sedentary mice (29.8 ± 0.3 g vs 34.4 ± 1.3 g, $P<0.05$). Moderate-intensity and HIIT mice within SJL weighed less than sedentary mice post-training (24.9 ± 0.7, 25.6 ± 0.7 vs 28.3 ± 0.7 g, $P<0.05$); these same mice gained significantly less weight compared to sedentary (5.3 ± 0.6 g, 4.8 ± 0.8 g vs 7.5 ± 0.4 g, $P<0.05$).

Table 2.1. Pre, post, and change in body mass with exercise training for inbred mouse strains

129S1	4 SED	4 MOD	4 HIIT	8 SED	8 MOD	8 HIIT	Duration	Intensity	D x I
BW, g Pre	21.48 ±0.67	21.67 ± 0.81	23.12 ± 0.55	22.45 ±1.63	22.98 ± 0.90	22.55 ± 0.69	N/A	N/A	N/S
BW, g Post	24.6 ± 0.64	24.31 ± 0.87	25.98 ± 0.44	26.15 ±0.77	27.48 ± 0.69	28.63 ± 1.08	0.0005	0.0494	0.5727
BW, g Delta	3.12 ± 0.07	2.64 ± 0.30	2.86 ± 0.27	3.7 ± 1.10	4.5 ± 0.41	6.08 ± 1.31	0.0038	N/A	N/S
B6									
BW, g Pre	24.29 ±0.47	23.24 ±0.45	23.23 ± 0.39	23.73 ±0.93	23.42 ± 0.83	24.93 ± 0.32	N/A	N/A	N/S
BW, g Post	28.71 ±0.31	26.63 ±0.77	26.18 ±0.42*	27.93 ±0.79	27.82 ± 1.12	26.79 ± 0.41	0.5795	0.0691	0.4249
BW, g Delta	4.43 ± 0.31	3.38 ± 0.37	2.94 ± 0.72	4.19 ± 0.57	4.40 ± 0.77	1.86 ± 0.60*	0.8448	0.0111	0.2258
NON									
BW, g Pre	30.62 ±1.20	32.00 ± 0.69	25.80 ± 2.22	28.38 ±0.67	27.38 ± 0.83	28.86 ± 0.59	N/A	N/A	N/S
BW, g Post	33.56 ±1.30	32.11 ± 0.77	29.36 ± 1.37	34.38 ±1.29	29.81 ± 0.28*	32.03 ± 0.73	0.6373	0.0047	0.0617
BW, g Delta	2.94 ± 0.19	0.11 ± 0.64*	3.56 ± 1.11	5.99 ± 0.63	2.43 ± 0.59*	3.17 ± 0.33*	0.0039	0.0001	0.0312
SJL									
BW, g Pre	20.84 ±1.29	20.91 ± 0.70	21.52 ± 0.46	20.75 ±0.76	19.63 ± 0.56	20.79 ± 1.10	N/A	N/A	N/S
BW, g Post	25.88 ±1.05	24.26 ±0.64	24.7 ± 0.36	28.26 ±0.73	24.94 ±0.66*	25.55 ±0.72*	0.0284	0.0045	0.4368
BW, g Delta	5.04 ± 0.49	3.35 ± 0.32*	3.18 ± 0.34*	7.51 ± 0.41	5.31 ± 0.55*	4.76 ± 0.79*	<0.0001	0.0004	0.7064

Full factorial analysis with strain, intensity, strain x duration, strain x intensity, duration x intensity, strain x duration x intensity. Significant P-values reported, non-significant P-values indicated as NS. Delta, change in body mass from pre- to post-training.

Time. Post-training exercise capacity, expressed as time for 129S1 mice, was significantly different across training paradigms (p=0.0110) (Table 2.2). The variation was such that two significantly different groups were present when assessed by a one-way ANOVA. A 1.23-fold difference was found between the lowest performing and highest performing cohorts. The 4-week sedentary was the lowest performing cohort with a mean time of 29.2 ± 0.7 min in comparison to the highest performing cohort, the 8-week HIIT, with a mean time of 23.5 ± 1.9 min. Similarly, change in time exhibited the same pattern as post-training time with ~10-fold difference between 4-week sedentary; change in time of -0.4 ± 0.3 min, and the highest cohort; 8-week HIIT, with a mean change in time of 4.4 ± 1.4 min (Figure 2-1A). Full factorial analysis of change in time demonstrated a significant interaction between duration and intensity (P=0.0120) and a main effect for intensity (P=0.0258)(Figure 2-1A).

Post training time for B6 mice across training paradigms was significantly different; the variation showed three distinct groupings (Table 2.2). Four week sedentary training had the lowest post exercise time of 26.4 ± 0.5 min, with the highest post exercise time resulting from four weeks of moderate continuous exercise training 30.6 ± 0.3 min, a 1.15 fold difference from lowest to highest performers. When examining change in time, two significantly different groups were present (Figure 2-1B). The highest change in time, similar to post time, was 4-weeks moderate continuous exercise at 3.1 ± 0.5 min.

Table 2.2. Pre- and post-training exercise time and work in inbred mouse strains

129S1	4 SED	4 MOD	4 HIIT	8 SED	8 MOD	8 HIIT	Duration	Intensity	D x I
Time									
Pre	29.6 ± 0.5	30.1 ± 0.8	28.7± 0.4	30.0 ± 1.2	31.4 ± 0.4	30.1 ± 0.7	N/S	N/S	N/S
Post	29.2 ± 0.3^{B}	33.0 ± 1.2^{A,B}	29.5 ± 0.7^{B}	30.0 ± 0.7^{A,B}	31.5 ± 1.2^{A,B}	34.5 ± 1.9^{A}	N/S	0.0469*	0.0205*
Work									
Pre	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.0	1.4 ± 0.1	1.6 ± 0.1	1.40 ± 0.08	0.0199*	N/S	N/S
Post	1.4± 0.1^{B}	2.02 ± 0.2^{A,B}	1.5 ± 0.1^{B}	1.6 ± 0.1^{B}	2.0 ± 0.2^{A,B}	2.8 ± 0.5^{A}	0.0196*	0.0270*	0.0217*
B6									
Time									
Pre	26.4 ± 0.6	27.4 ± 0.7	28.3 ±0.7	27.7 ± 0.8	28.5 ± 0.3	26.6 ± 0.4	N/S	N/S	0.0317*
Post	26.4 ± 0.5^{C}	30.6 ± 0.3^{A}	27.6 ± 0.7^{B,C}	26.7 ± 0.3^{C}	29.5 ± 0.7^{A,B}	27.1 ± 0.3^{C}	N/S	<0.0001*	N/S
Work									
Pre	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.20± 0.1	1.0 ± 0.1	N/S	N/S	N/S
Post	1.1 ± 0.1^{B,C}	1.7 ± 0.1^{A}	1.2 ± 0.1^{B,C}	1.14± 0.1^{C}	1.6 ± 0.2^{A,B}	1.1 ± 0.0^{C}	N/S	<0.0001*	N/S
NON									
Time									
Pre	33.4 ± 0.2	34.6 ± 0.8	32.4 ± 1.2	33.9 ± 0.2	33.3 ± 0.7	33.2 ± 0.6	N/S	N/S	N/S
Post	29.3 ± 0.9^{B}	33.6 ± 0.5^{A}	33.2 ± 1.3^{A,B}	31.2 ± 0.7^{A,B}	32.8 ± 1.0^{A,B}	31.5 ± 0.9^{A,B}	N/S	0.0097*	N/S
Work									
Pre	2.6 ± 0.1	3.0 ± 0.2	2.5 ± 0.1	2.5 ± 0.1	2.3 ± 0.2	2.4 ± 0.1	0.0193*	N/S	N/S
Post	2.0 ± 0.2^{A,B}	2.5± 0.1^{A}	2.8 ± 0.1^{A,B}	2.1 ± 0.2^{A,B}	2.3 ± 0.3^{B}	2.3 ± 0.3^{A,B}	N/S	N/S	N/S
SJL									
Time									
Pre	27.0 ± 3.7	29.7 ± 0.3	31.2 ±0.8	33.1 ± 1.7	29.9 ± 0.4	33. 0 ± 1.8	N/S	N/S	N/S
Post	31.2 ± 1.1^{A,B}	33.4 ± 1.3^{A}	32.1 ± 1.2^{A,B}	31.8 ± 1.3^{A,B}	25.8 ± 1.6^{B}	31.7 ± 2.3^{A,B}	N/S	N/S	N/S
Work									
Pre	1.4 ± 0.2	1.23 ± 0.07	1.5± 0.1	1.8 ± 0.31	1.2 ± 0.1	1.8 ± 0.4	N/S	N/S	N/S
Post	1.8 ± 0.2	2.1 ± 0.2	1.9 ± 0.2	2.1 ± 0.3	1.0 ± 0.2	1.9 ± 0.47	N/S	N/S	N/S

Full factorial analysis with strain, intensity, strain x duration, strain x intensity, duration x intensity, strain x duration x intensity. Significant P-values reported, non-significant P-values indicated as NS. Training paradigms not connected by the same letter are significantly different.

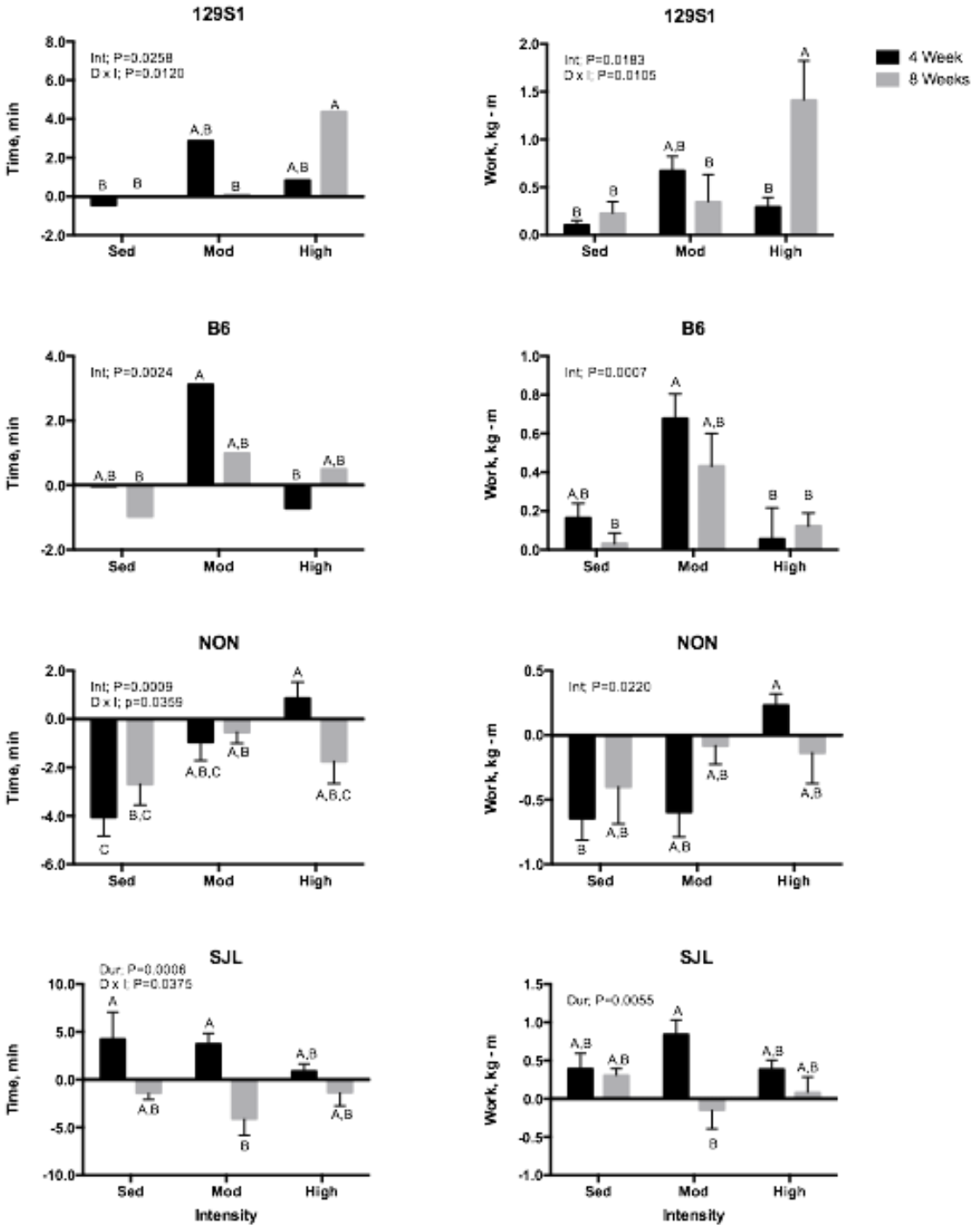

Figure 2.1. Changes in exercise capacity expressed as change in time and change in work with full factorial analysis for inbred mouse strains. Change in time expressed in minutes: (A) 129S1, (B) B6, (C) NON, (D), SJL. Change in work expressed in kg –m: (E) 129S1, (F) B6, (G), NON, (H) SJL. Significant interactions are reported as D x I with P-value, main effect for intensity = Int, main effect for duration = Dur. Letters not connected are significantly different (P<0.05).

However, the lowest change in time occurred in the 8-week sedentary mice, which had a -1.0± 0.6 min change, approximately a 3.2 fold difference. Full factorial analysis indicated that a significant main effect for intensity was present (P=0.0001)(Table 2.2).

NON mice post training time was significantly different with enough variation for two groups (Table 2.2). Four-week sedentary animals ran the least at 29.3 ± 0.9 min with the 4-week moderate yielding the highest run time at 33.6 ± 0.5 min, indicating a 1.15 fold difference between the highest and lowest training paradigm. Within the NON strain, change in time for the lowest performing cohort remained the same as the previous strains (Figure 2-1C). The 4-week sedentary change in time mean was -4.1 ± 0.8 min with the highest change occurring within the 4-weeks of high intensity interval training at 0.9 ± 0.7 min, which resulted in a 5.78 fold difference between the lowest and highest changes in running time. Full factorial analysis uncovered a significant interaction between duration and intensity (P=0.0359) as well as a main effect for intensity (P=0.0009), for change in time (Figure 2-1C).

The lowest and highest cohorts for both post training time and the change in time were the same for SJL mice. The 4-week moderate continuous exercise had the highest post training time at 33.4 ± 1.3 min (Table 2.2) and a change in time of 3.7 ± 1.1 min (Figure 2-1D). The lowest post training time was in the 8-week moderate training cohort at 25.8 ± 1.6 min and the lowest change occurred in the 8-week sedentary group at -1.4 ± 0.7 min, indicating ~ 1.29 fold difference for post training time and a ~1.09 fold difference for change in time. A significant interaction was found between duration and

intensity (P-0.0375) ; in addition a significant main effect was present for duration (P=0.0006)(Figure 2-1D).

Work. To account for the influence that body mass has on exercise capacity, a second phenotype, work (kg ·m), was calculated. Within the 129S1 strain, 4-week sedentary mice performed the least amount of work after training with a mean of 1.36 ± 0.05 kg·m, while 8-week high-intensity interval training performed the most work post-training at 2.81 ± 0.48 kg·m, indicating a ~ 2-fold difference between lowest and highest performers (Table 2.2). Change in work was significantly different in the 129S1 strain across training paradigms. Similar to post training time, 8-week high-intensity had the highest change in work at 1.41 ± 0.42 kg·m and 4-week sedentary had the smallest change in work at 0.10 ± 0.05 kg·m, indicating ~14 fold difference between the two training paradigms (Figure 2-1E). Interactions for 129S1 indicate a significant interaction between duration and intensity (P=0.0105) and a main effect for intensity (P=0.0183)(Figure 2-1E).

Within B6 mice, a significant difference was found in post training work (P=0.0001). Four-week moderate intensity group mice performed the highest amount of work at 1.72 ± 0.10 kg·m, and the lowest work was performed by the 4-week sedentary at 1.12 ± 0.07 kg·m, a ~ 1.5 fold difference from lowest to highest (Table 2.2). Change in work showed a pattern similar to post-training work (Figure 2-1F). No significant interaction was found between duration and intensity for change in work, however a significant main effect was found for intensity (P=0.0007)(Figure 2-1F).

In NON mice, there were no significant differences between groups for post-training work, however when change was assessed, significant differences were present (P=0.0291)(Figure 2-1G). Four-week HIIT mice had the greatest change in work at 0.23 ± 0.09 kg·m while 4-week sedentary mice had a negative change in work of -0.64 ± 0.17, resulting in a 0.35 fold difference from lowest to highest (Figure 2-1G). No interactions were found but a main effect for intensity was significant (P=0.0220)(Figure 2-1G).

SJL mice exhibited no differences in post-training work (Table 2.2), however when examining change in work a significant difference was found between training paradigms (P=0.0160)(Figure 2-1H). The 4-week moderate intensity group had the greatest change in work with 0.84 ± 0.19 kg·m, while the 8-week moderate group had the smallest change with -0.14 ± 0.25 kg·m. A 6 fold difference between 8-week moderate intensity and 4-week moderate-intensity was present. No interactions were found for change in work, however a significant main effect was found for duration (P=0.0055)(Figure 2-1H).

Across strains, full factorial analysis was completed to assess the contribution of strain, duration, intensity and their interaction on change in body mass, change in time, and change in work (Table 2.3). There was a significant main effect of strain for all three phenotypes (P<0.001), duration was a significant main effect for change in body mass (P<0.001), along with change in time (P=0.0048), but not for change in work. Intensity was a significant main effect for all three phenotypes. A significant interaction for strain x duration was observed for all three phenotypes. The interaction between strain x intensity was significant for change in body mass (P<0.001) and change in work

Table 2.3. Full factorial analysis for strain, duration, and intensity for training responses in inbred mouse strains

Factor	Change in body mass	Change in time	Change in work
Strain	P<0.0001	P<0.0001	P<0.0001
Duration	P<0.0001	P=0.0008	NS
Intensity	P=0.0002	P=0.0243	P=0.0079
Strain x Duration	P=0.0196	P<0.0001	P=0.0050
Strain x Intensity	P<0.0001	P=0.0043	P=0.0038
Duration x Intensity	NS	P=0.0092	NS
Strain x Duration x Intensity	P=0.0422	P=0.0196	P=0.0013

Full factorial analysis with strain, intensity, strain x duration, strain x intensity, duration x intensity, strain x duration x intensity. Significant P-values reported, non-significant P-values indicated as NS.

(P=0.0038), but not change in time. Duration x intensity interaction was the opposite with change in time being the only significant interaction (P=0.0018). The interaction between strain x duration x intensity was significant on all accounts.

Protein expression. 129S1 have average intrinsic exercise capacity and modest responses to training (Table 2.2 and Figure 2-1A). Protein contents for all groups are shown in Figure 2.2. TFAM and PGC-1alpha for 4-weeks moderate followed the trend in change in time but are not significantly different from sedentary. Four-week training yielded significant differences (P<0.05) for both moderate-intensity and high-intensity interval training compared to sedentary for SIRT1. TFAM was significantly different (P<0.05) in 8 weeks of training when moderate intensity was compared to sedentary, but the protein expression trends for intensity did not follow the change in time trends. Glut4 and Hexokinase exhibited the same patterns for protein expression across both 4- and 8-week training as seen in their change in time. Significant correlations exist for three proteins for exercise related phenotypes. Within the 4-week duration, Glut4 was significantly

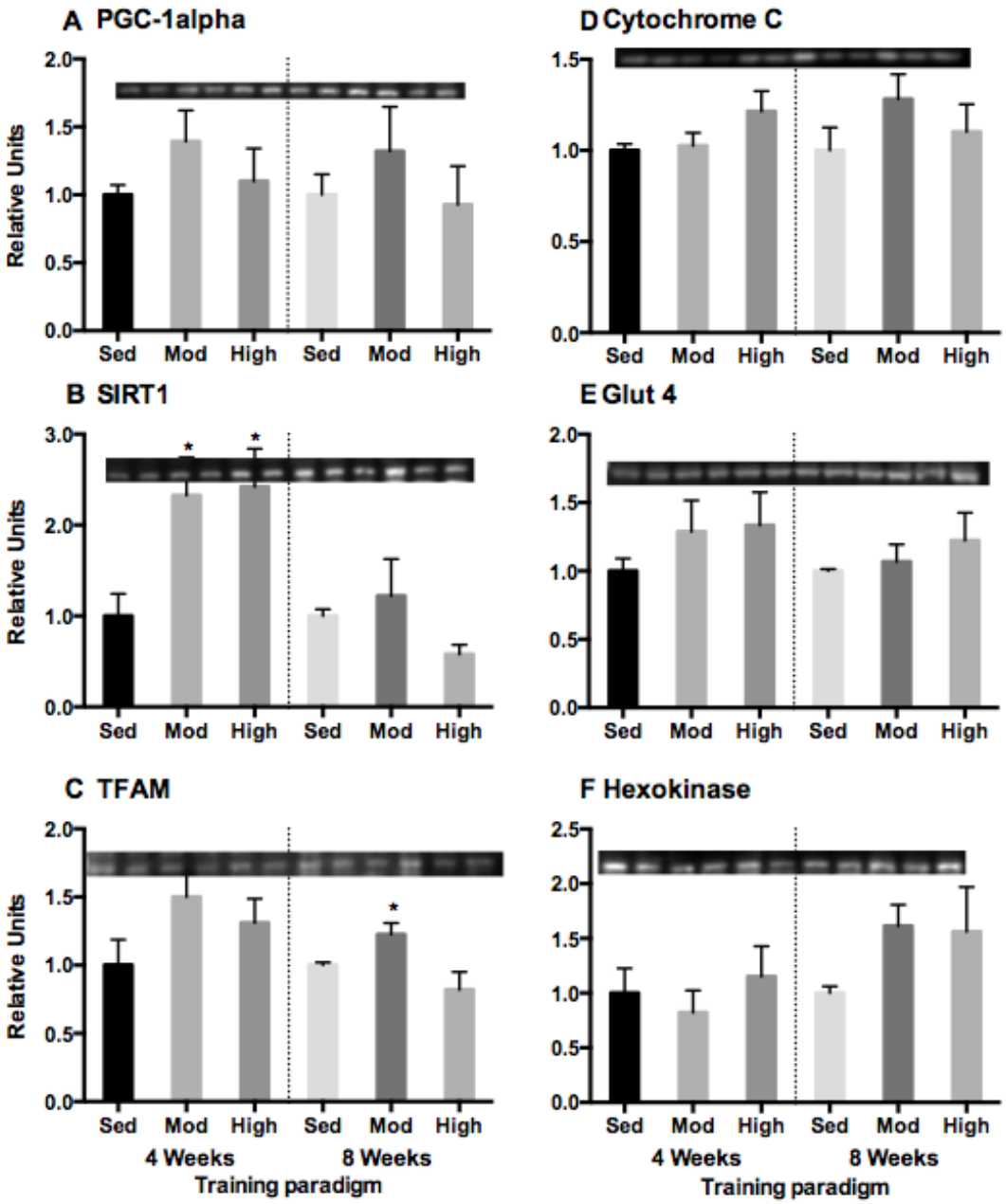

Figure 2-2. 129S1 inbred mice protein expression response to exercise intensity separated by duration, expressed as relative units for six proteins: (A) PGC-1Alpha, (B) SIRT1, (C) TFAM, (D) Cytochrome C, (E) Glut4, (F) Hexokinase. * Indicates a significant difference from respective sedentary group ($P<0.05$).

correlated with the change in body mass (r=-0.5749, P<0.05), TFAM was significantly correlated with change in time (r=0.4687, P<0.0496), and hexokinase was significantly correlated with post-training time (r=-0.508, P<0.05), change in time (r=-0.4931, P<0.05), post-training work (r=-0.503, P<0.0396), and change in work (r=-0.5356, P<0.05). After 8-weeks of training, TFAM was significantly correlated with change in time (r=-0.5162, P<0.05), and change in work (r=-0.5492, P<0.05).

B6 mice have the lowest intrinsic exercise capacity when compared to the other strains (Table 2.2). Protein data (Figure 2-3) in the 4-week duration yield one significant difference for SIRT1 where protein was lower in mice that performed moderate continuous exercise compared to sedentary (P<0.05). No significant differences in 8-week training were found, although PGC-1alpha (Figure 2-3A) exhibited a trend consistent with change in time data. Two proteins were significantly correlated with body mass or exercise phenotypes. Within 4-weeks, PGC was correlated with post training body mass (r=-0.529, P<0.05), change in time (r=-0.6953, P<0.01), and change in work (r=-0.6536, P<0.05). Glut4 was significantly correlated with post training body mass (r=0.5926, P<0.05) within 4 weeks of training. For 8-weeks both proteins were also significantly correlated with exercise phenotypes: Glut4 with pre training body mass (r=0.5762, P<0.05) and PGC-1alpha with the change in body mass (r=-0.5352, P<0.05).

NON mice had a trend of improving exercise capacity as intensity increased within the four week training period. This pattern holds true with protein expression for PGC-1alpha, SIRT1, TFAM, Cytochrome C, and Hexokinase (Figure 2-4). Hexokinase

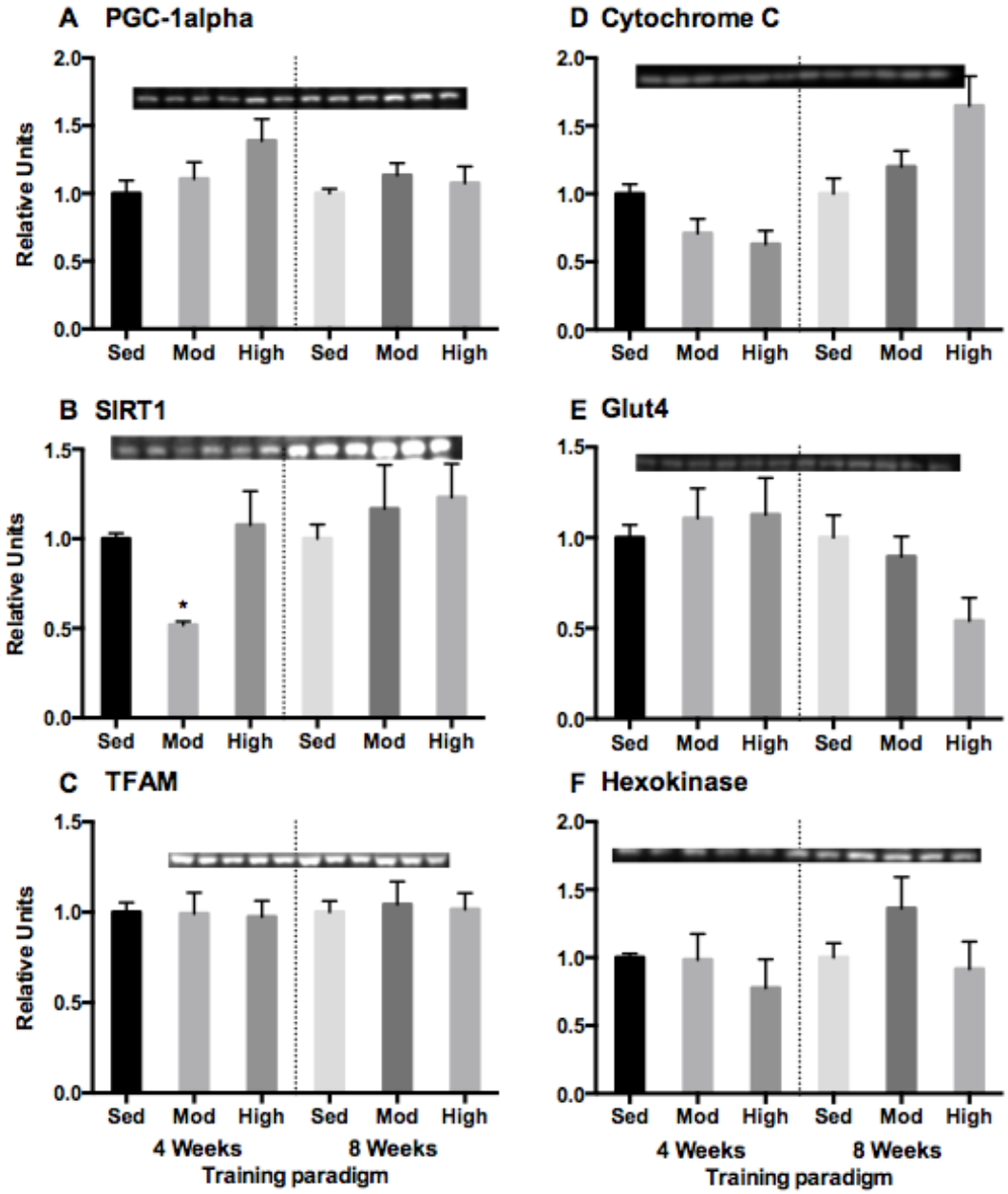

Figure 2.3. B6 inbred mouse protein expression response to exercise intensity separated by duration, expressed as relative units for six proteins: (A) PGC-1Alpha, (B) SIRT1, (C) TFAM, (D) Cytochrome C, (E) Glut4, (F) Hexokinase. * Indicates a significant difference from respective sedentary group ($P<0.05$).

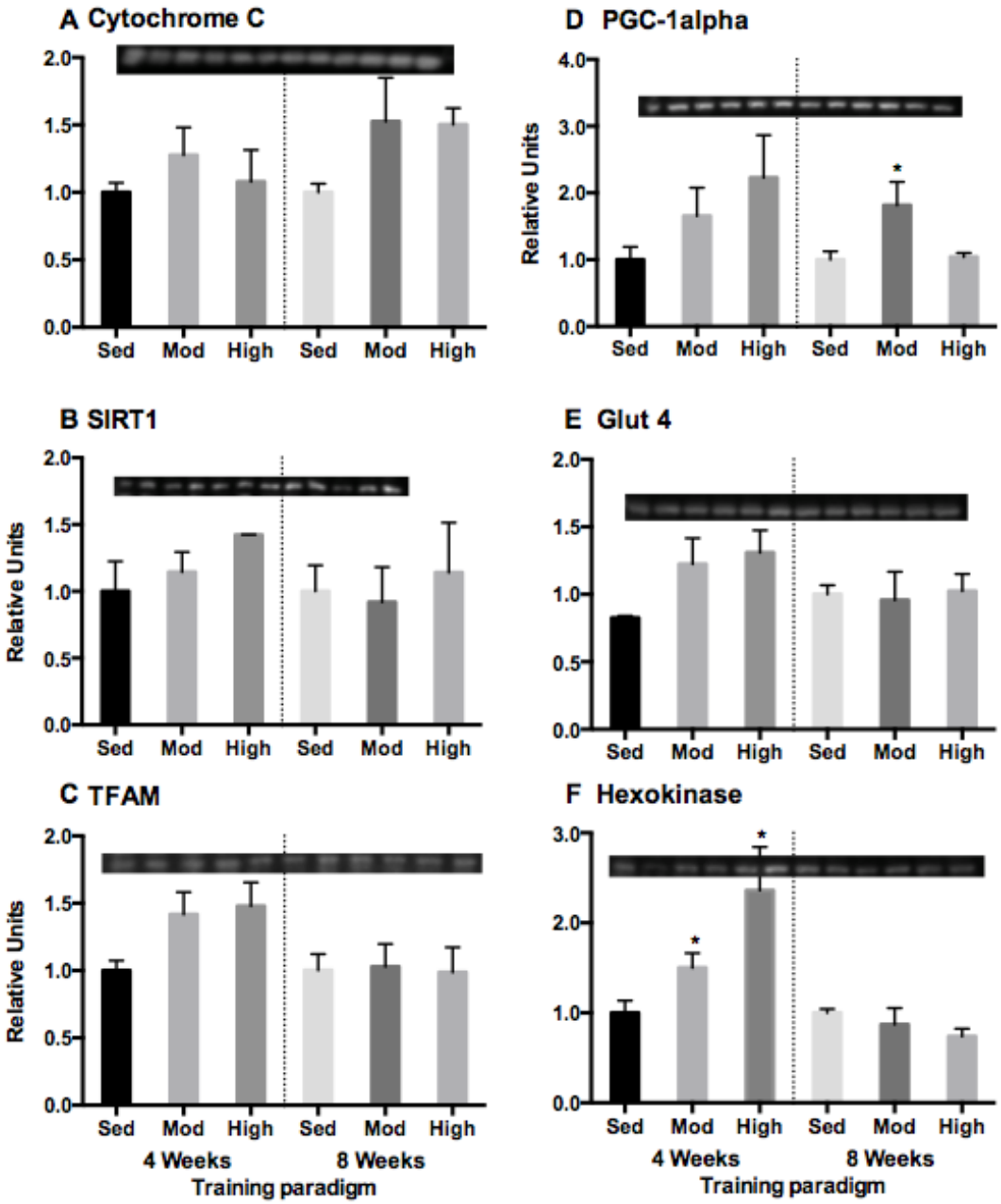

Figure 2.4. NON inbred mouse protein expression response to exercise intensity separated by duration, expressed as relative units for six proteins: (A) PGC-1Alpha, (B) SIRT1, (C) TFAM, (D) Cytochrome C, (E) Glut4, (F) Hexokinase. * Indicates a significant difference from respective sedentary group ($P<0.05$).

was the only protein within the 4-week duration that had a significant difference with both moderate and high being different from sedentary ($P<0.05$). For the 8-week duration, two of the six proteins, PGC-1alpha and Cytochrome C mimicked the variation pattern for change in time (Figure 2-4) with PGC-1alpha moderate intensity being significantly different from sedentary. Additionally, proteins were correlated to exercise capacity phenotypes for both 4- and 8- weeks of training. Within the 4-week duration, PGC-1alpha was significantly correlated with pre-training body mass ($r=-0.5959$, $P<0.05$) and change in body mass ($r=0.5018$, $P<0.05$). SIRT1 was significantly correlated with post-training time ($r=0.571$, $P<0.0262$), change in time ($r=0.534$, $P<0.0403$), and post-training work ($r=0.564$, $P<0.0285$). Within the 8-week duration, pre-training body mass was correlated with Glut4 ($r=0.5762$, $P<0.05$) and change in body mass with PGC-1alpha ($r=-0.5451$, $P<0.05$) (data not shown).

SJL mice had high intrinsic exercise capacity when expressed as time in minutes; and are also high responders to training (Table 2.3). In the 4-week training groups, there were no significant differences in protein content for any of the proteins measured. However, PGC-1alpha, SIRT1, TFAM, and Cytochrome C protein content tended to be higher in the 4-week moderate training compared to 4 week sedentary and high groups. PGC-1alpha was significantly higher in the moderate and high intensity groups after 8 weeks. No other proteins showed significant differences across groups (Figure 2-5). No significant correlations were found between exercise phenotypes and proteins for 4-week training duration.

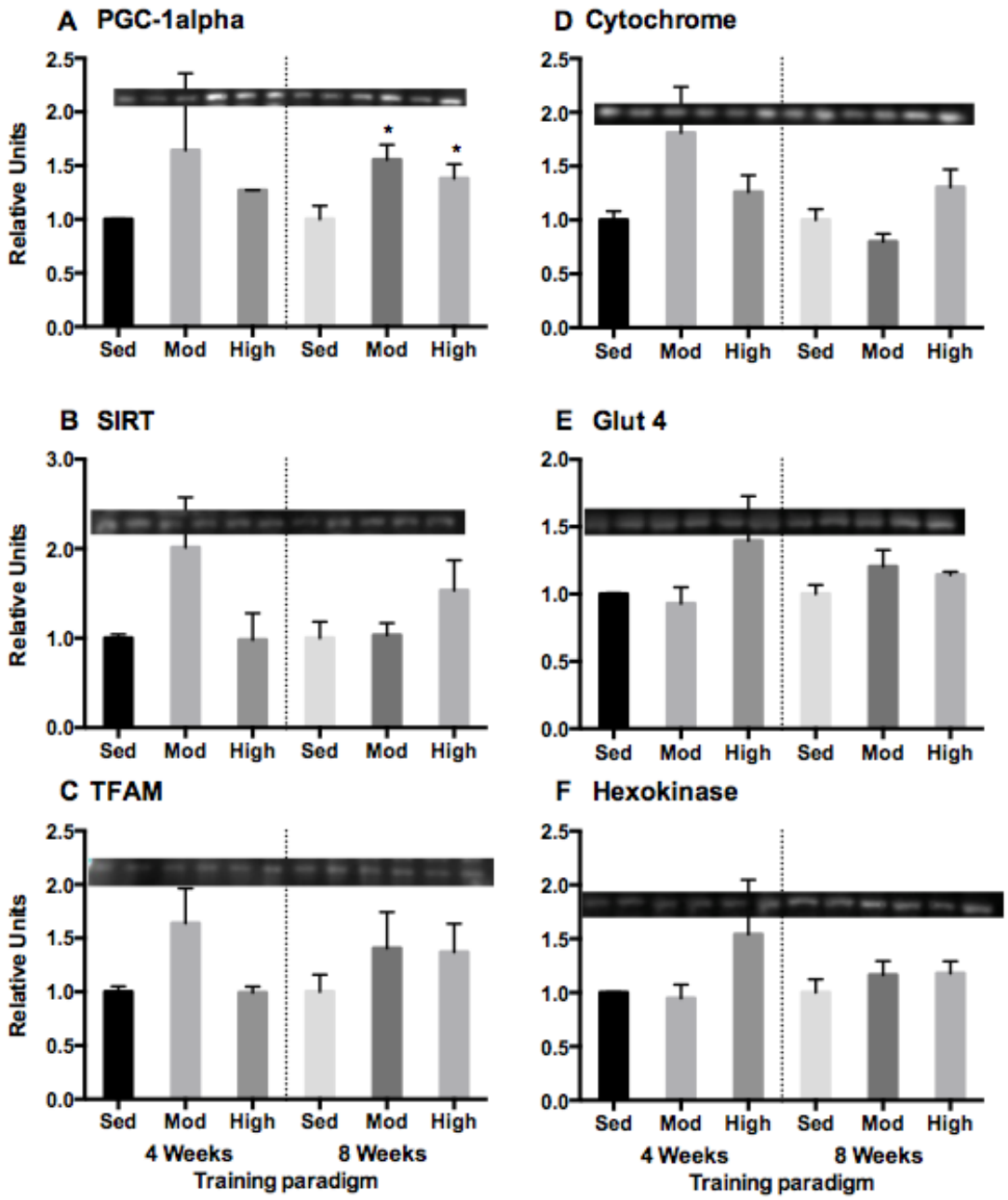

Figure 2.5. SJL inbred mice protein expression response to exercise intensity separated by duration, expressed as relative units for six proteins: (A) PGC-1Alpha, (B) SIRT1, (C) TFAM, (D) Cytochrome C, (E) Glut4, (F) Hexokinase. * Indicates a significant difference from respective sedentary group ($P<0.05$).

However, in 8 week groups significant correlations were found between TFAM and the change in work ($r= -0.5492$, $P<0.05$) and change in time ($r= -0.5164$, $P<0.05$) (data not shown).

3.4 Discussion

The response to exercise is highly variable, such that responses to a standardized training program can range from positive to negative. Genetic background, intrinsic exercise capacity, exercise intensity and duration are known to influence the responses to training. However, it is unknown how these factors interact to determine responses to training. Identifying how manipulation of intensity and duration on various genetic backgrounds is paramount in understanding the complex underpinning genetic background has on exercise capacity. Therefore, inbred mouse strains with a wide range of intrinsic exercise capacity and responses to training (Chapter II) were selected to assess the interaction of these variables on the responses to training. Treadmill training was used to investigate both intrinsic exercise capacity and the response to moderate intensity and HIIT in four inbred mouse strains to determine if the response to exercise is genetically determined and how protein expression is affected.

Delineating the optimal training paradigm to improve exercise capacity is a complex undertaking with multiple variables exerting influences. The primary finding of this study is that genetic background has a significant effect on adaptations to exercise training which was the same as study 1 (Chapter II). When all mice were analyzed together, there was a significant main effect for strain on the changes in time and work.

Factorial analysis also identified main effects for intensity and duration for the change in time, but only a main effect for intensity for the change in work. However, it is interesting to note that changes in exercise capacity were greater with four weeks of training than 8 weeks and exercise at lower intensity elicited greater responses than HIIT. When examining changes in time and work in strains independently, intensity was an important factor in all strains, except SJL. For SJL, a main effect for duration was found for both changes in time and work, suggesting that changes in exercise intensity had little effect on the responses to training in SJL mice. However, overall 4-week moderate intensity training appears to be optimal for increasing exercise capacity because 4-week moderate intensity resulted in the highest overall increases in exercise capacity more frequently and when high-intensity interval training was the highest there was often no significant difference between the 4-week moderate intensity and the high-intensity interval cohort. This continuous training resulted in either the highest absolute change across all intensities and durations or there was no significant difference between 4-weeks moderate and the highest absolute change in time. This trend held true for both measures of exercise capacity expressed as time and work. These results suggest for the strains tested that moderate continuous exercise is preferable compared to HIIT when total volume of exercise is matched. In general, the greatest changes in exercise capacity occurred in response to 4 weeks of continuous exercise training. Although the magnitude of responses in the 4-week moderate groups were similar to previous reports by our laboratory (158, 160) (Chapter II), the lack of improvement with a longer training period or higher intensity of exercise was unexpected. Training at ~60% of maximal

lactate steady state workload (15 m/min, 0% grade) has been shown to increase exercise capacity after 4 weeks of training and further increased exercise capacity after 8-weeks of training (182, 183). These data suggest that longer duration training is associated with greater improvements in exercise capacity. Furthermore, Haram, et al. (83) utilized rats selectively bred for low intrinsic exercise capacity, which present with phenotypes similar to metabolic syndrome and are prone to obesity, insulin resistance, hypertension dyslipidemia, and impaired glucose utilization, to assess the effect of exercise intensity on responses to training (120). Rats completed interval training (85-90%) or steady state (70%) training based on a percentage of VO_2 max. While both groups covered the same distance, interval training rats ran approximately 1 hour while steady state animals run up to a total of two hours. High-intensity increased VO_2max by 45%, whereas lower intensity training increased VO_2max by 10%, suggesting that high intensity training is more effective in a genetic model of low intrinsic exercise capacity (83). Previous studies in mice have also reported that HIIT resulted in significant increases in VO_2max (78, 79, 112). Kemi et al. (112) observed a 29% increase in VO_2max in male mice after completing 8 weeks of high intensity treadmill running for 2 hours per day and 5 days per week. Hafstad et al. (78, 79) utilized a HIIT protocol modified from Kemi et al. (112) and also reported significantly higher VO_2max in HIIT trained mice compared to sedentary controls after 8 weeks of training. However, when the effect of HIIT was compared with lower-intensity training in B6 mice for 8-10 weeks, both training paradigms increased exercise capacity relative to sedentary mice, but there was no difference in aerobic capacity between low-intensity and HIIT groups (79). HIIT did

however improve glucose tolerance while low-intensity training had no significant impact. Collectively, these data indicate that 8 weeks of high intensity training can increase exercise capacity. However, these data also suggest that both high and moderate intensity training might elicit comparable changes in exercise capacity depending on the animal model. Within the training paradigms that were tested, we saw that our 8-week groups failed to show improvements, it is possible that for some strains adjusting intensity and duration resulted in overtraining. While we do not have any physiological markers of overtraining in this study, results from such measurements could aid in determining if overtraining drove poor response.

Protein expression. Endurance exercise is a powerful modulator in a continuum of physiological adaptations, including but not limited, to fatty acid oxidation, oxidative phosphorylation, glucose oxidation and mitochondrial gene transcription and replication. As such, protein targets have been selected to identify adaptations to chronic endurance exercise within strains and across training paradigms to identify variations in response to training. Due to significant interactions found between training intensity and duration for changes in exercise capacity, analysis of protein expression was separated by duration.

One of the proteins associated with skeletal muscle adaptations to exercise training is the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1alpha), a proposed a master regulator in skeletal muscle mitochondrial biogenesis (95, 96, 131, 197, 214). With 4-weeks of exercise training, the greatest changes in exercise capacity were observed with moderate intensity training.

There were no significant differences in PGC-1alpha content in 4-week trained groups. In the 8-week groups, PGC-1alpha was higher in exercise groups from the NON and SJL strains (Figures 2-4 and 2-5). In all strains, PGC-1alpha content tended to follow changes in exercise capacity. However, PGC-1alpha content was not significantly correlated with post-training exercise capacity. In many studies, exercise training has been shown to increase PGC-1alpha mRNA in skeletal muscle (100, 145, 146, 213, 239). In addition to gene expression, several groups have shown that PGC-1alpha protein is higher in skeletal muscles from exercise trained mice (1, 8, 43, 239) and rats (101, 133, 156), whereas others have shown that 4-12 weeks of exercise training did not affect PGC-1alpha protein content in skeletal muscle (100, 145, 146, 156, 162, 213). We demonstrated that two strains (NON and SJL) had significant differences in protein expression with 8-weeks of training. The remaining two strains (129S1 and B6) did not reach statistical significance which could be the result of protein content returning to baseline. Selecting several time points including immediately after the last bout of exercise would reveal when protein content was highest across strains. In the event that the optimal time to capture elevated protein expression was missed, additional proteins along the pathway were examined to determine the effect of the upstream signal has downstream.

SIRT1 and Tfam, proteins associated with PGC-1alpha and mitochondrial biogenesis also were measured in gastrocnemius muscle. As with PGC-1alpha, there were no consistent effects of exercise training on SIRT1 or Tfam protein content in the current study. Only the 4-week moderate and high exercise groups from the 129S1

strain showed significantly higher SIRT1 protein content than sedentary controls. These inconsistent results are in agreement with the literature. Exercise training has been shown to have no effect or increase SIRT1 protein in skeletal muscle (35, 145, 146, 156, 162). Limited data from exercise training studies in rodents also suggests that Tfam protein content is variably affected by exercise training in skeletal muscle (52, 133, 156). Thus, although Tfam is positively correlated with mtDNA copy number, which is thought to increase with exercise training, the contribution of Tfam to the adaptive response to exercise training remains unclear.

Exercise training can lead to changes in skeletal muscle oxidative enzyme capacity, mitochondrial function, and fiber type. Therefore, protein levels of cytochrome c, Glut4, and hexokinase were measured to provide some insight into the interaction of exercise intensity and duration on skeletal muscle metabolism. In general, there were no significant differences for any of these proteins across training intensities or duration. The lone exception was the significantly higher hexokinase protein content in muscle from 4-week trained NON mice. The protein content was higher in muscles from both training groups compared with sedentary controls. Hexokinase content tended to be greater in the 4-week HIIT group (Figure 2-4), which matches the changes in exercise capacity in this strain. It is generally expected that the content of all of these proteins might increase with exercise training (189). For example, we have previously demonstrated that cytochrome c protein expression in plantaris muscle is increased after 4 weeks of moderate intensity training (159). This increase was not uniform across all strains tested, suggesting that the protein content might be strain-specific. Other studies

also have reported increases in cytochrome c protein expression with exercise training (1, 144), but not all (35, 133). Leick et al. showed higher cytochrome c protein in mouse soleus, quadriceps, and white gastrocnemius muscle after 5 weeks of exercise training (144). They also reported significantly higher levels of hexokinase in skeletal muscle from trained mice compared with sedentary controls, suggesting that their training program was sufficient to elicit adaptations in skeletal muscle. Although their exercise-training program was comparable to the moderate intensity training used in the current study, only hexokinase protein content showed any differences across training groups. This suggests that adaptations in skeletal might be strain and intensity dependent. However, few studies have reported exercise-training induced changes in hexokinase protein in mouse skeletal muscle and therefore future research is needed. Furthermore, the increase expression of these proteins have been reported to return to baseline shortly after the cessation of exercise (102, 111). It is possible the optimal time to collect samples was missed and protein content had already returned to or was returning to baseline levels. Additionally changes in these proteins are often more subtle in mice that are exercise trained compared to humans (4).

In summary, our data demonstrates that moderate intensity exercise training yields the greatest improvements in exercise capacity during short duration training. The interaction between genetic background, intensity, and duration significantly contributes to the response to training. The adaptations to training in skeletal muscle are not directly related to changes in exercise capacity.

CHAPTER IV

SUMMARY AND CONCLUSIONS

4.1 Summary

The data presented within this dissertation collectively help to address gaps in the area of genetic background and the response to exercise training. Two studies were designed. The first was to characterize the response to training in a large number of inbred mouse strains to continuous moderate exercise training. The exercise intensity was used similar to human exercise studies at 65% of maximal effort. An additional component of this study was to identify QTL/candidate genes associated with the response to exercise training. The second study utilized four strains with various training responses to four weeks of exercise training. The aim was to investigate if manipulation of duration and intensity of the training stimulus exerted influence over the response to training. This study was designed to determine if modifying intensity and duration of a training protocol could yield a significant increase in exercise capacity compared to standardized training protocol (Chapter II) or if the response is independent of training paradigm.

Exercise capacity is a complex polygenic trait that has been studied in both humans and rodents (50, 51) The genetic basis for intrinsic exercise capacity has been well described. However, the genetic factors responsible for the response and variation to exercise training remain unclear (121). Therefore, the purpose of the first study was to characterize differences in training responses across a large number inbred mouse

strains, estimate the genetic contribution to the response to exercise training, and identify genes using genome wide association mapping. Exercise capacity defined as time and work was measured in male mice (n=6) from 25 classical inbred strains performing a run to exhaustion test before and after four weeks of exercise training. Male mice were used exclusively due to the lack of an estrous cycle; utilizing female mice would create additional variability considering the duration of training and the age of the mice. Genome-wide association mapping was performed utilizing an efficient mixed model (EMMA) algorithm. Exercise capacity was significantly different among strains for pre and post-training time and work. There was a 2-fold difference between the lowest (A/J) and the highest running strain (SJL/J) after exercise training. The changes in exercise capacity were also different among strains. The response to training varied by a 4.7-fold between the lowest (LG) and the highest (AKR/J) responding strains. Genome-wide association mapping identified multiple significant loci for post-training time, work, and changes in time and work. Several significant associations overlapped with previously identified genomic regions for exercise capacity or responses to training. These results suggest that genome wide association mapping can be used for exercise training responses. Furthermore, these results provide candidate loci for genetic studies of the responses to exercise training. Additionally, broad sense heritability estimates were calculated to highlight the genetic contribution on the trainability of inbred mice strains. Heritability estimates for post-training exercise time were 0.68 and 0.50 and were similar for post-training work at 0.66 and 0.47. Estimates for change in exercise capacity were smaller than for post-training exercise capacity. Koch et al. also reported

smaller heritability estimates for changes in exercise capacity than for exercise capacity before and after training in rats (122). Koch explains that the low heritability seen for the change in an exercise phenotype does not indicate a small additive genetic variance, instead they point to the notion that that a small portion of the variance in the exercise phenotype is likely a result of genotype.

This study confirms that the variation within inbred mouse strains is heterogeneous as seen in humans. Furthermore, several of the identified QTL were found in previous animal studies utilizing fewer strains. The addition of the strains in this study allowed for increased genetic diversity as well as increased mapping resolution. The previously identified loci were reduced to a smaller region on the genome within this study. Moreover, the identified QTL contain genes, which have been identified in human exercise training studies, suggesting that some genes influencing exercise training responses are similar between humans and mice.

Delineating the optimal training paradigm to improve exercise capacity is a complex undertaking with multiple variables exerting influences. However, four-week moderate intensity training appears to the optimal for a variety of reasons. For example, the change in exercise capacity was greater at four-weeks than after eight-weeks and lower intensity exercise training resulted in the more profound positive increases in exercise performance and skeletal muscle markers of training. First, four weeks of moderate intensity training resulted in either the highest absolute change across all intensities and durations or a change comparable to other intensities or durations of training. Second, this trend held true for both measures of exercise capacity - time and

work. These results suggest that moderate continuous exercise is preferable compared to HIIT when total volume of exercise is matched. This data demonstrate that moderate intensity exercise training yields the greatest improvements in exercise capacity during short duration training. Furthermore, the interaction between genetic background, intensity, and duration significantly contributes to the response to training.

High-intensity interval training was utilized as an alternative training paradigm to standardized continuous moderate intensity exercise on a match-work basis or equivalent basis. Previous studies have shown a similar or more robust benefit of HIIT compared with continuous training in healthy and disease populations (152, 163, 219, 224). A previous study demonstrated that C57BL/6J mice performing either HIIT or continuous moderate intensity exercise training had improved exercise capacity when compared to their sedentary control. However, mice who performed HIIT demonstrated a decrease in cardiac fatty acid oxidation and an increase in cardiac glucose oxidation, suggesting that HIIT might have specific effects on cardiac metabolism (78). A subsequent study using mice with diet-induced obesity reported similar changes in cardiac substrate utilization (79). These authors suggested that changes in cardiac signaling and the metabolic effects of exercise are intensity dependent. Although they did not examine changes in skeletal muscle metabolism or signaling, intensity-dependent changes in skeletal muscle have been reported (73, 167, 192) In contrast to those findings, exercise intensity did not have a consistent effect on skeletal muscle signaling in the strains utilized within this dissertation. In fact, it can be suggested that manipulation of exercise duration and

intensity elicits different physiological thresholds for adaptations to endurance training based on genetic background.

4.2 Limitations

One of the limitations in a study examining the effects of exercise training is the lack of a quantitative measurement of oxygen consumption. However, pilot data indicated that testing a single mouse in a single lane enclosed treadmill was inefficient, unreliable, and not reproducible. For instance, approximately 45 minutes to an hour was needed after mice were placed in the VO_{2max} treadmill chamber to relax to a steady state. Furthermore, since the treadmill was fully enclosed to appropriately measure gas exchange, it was not possible to externally stimulate the mouse to run. This would result in a refusal to run prior to reaching volitional exhaustion or VO_{2max}, resulting in incomplete results. In addition, only young male mice were examined as a first step in examining the influence HIIT has on exercise capacity. In this initial study, it serves as an important first step in establishing the safety and effectiveness in optimizing the response to exercise training before adapting it to aging and disease populations for compassion.

Muscle morphological data for immunohistochemistry was originally proposed however, technical problems were encountered while attempting these studies. Cryosections for skeletal muscle were performed by the Department of Veterinary Pathobiology to ensure proficient sectioning and reduce any loss of vital tissue. A technical issue arose which resulted in poor section quality. For example, at least one

muscle in the three muscle complex of the hindlimb complex would present with freeze artifact or distorted morphology. Additionally, it was common for at least one muscle group curling up and refusing to lay flat. As a tertiary approach of morphological data myosin heavy chain isoform separation through gel electrophoresis was attempted. However even with consult with the protein chemistry lab at Texas A&M, positive identification of molecular weight bands was problematic and therefore an unreliable.

4.3 Conclusion

Collectively the data presented within this dissertation indicate that the adaptation to exercise training is modulated in part by genetic background. This is supported by the results of our first experiment in which all mice ran at a fixed intensity and presented with a varied range in responses to exercise training. The response to exercise training is also sensitive to the manipulation of both intensity and duration of training (environmental effect). Thus, experimental design of exercise training studies in mice should consider the obvious factors of exercise intensity and duration, but also strain as a major factor determining the response to training.

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