ACUTE EXERCISE EFFECTS ON CARDIAC GENE EXPRESSION IN PHYSICALLY ACTIVE AND INACTIVE RATS

By Michelle Lynn Simonsen

Acute exercise may affect health-associated gene expressions and phenotypes differently depending on whether the individual exercises regularly. One-month old male Sprague-Dawley rats (n=72) were divided into SED (standard laboratory cage, n=24), PA (large box, n=24), and EX (running wheel inside standard cage, n=24). At three months, half from each group were sacrificed at rest and half following 30 minutes of acute exercise. RNA was extracted from cardiac tissue. Microarray analysis was performed on 27,000 genes and qPCR on select genes. Of the 1.9% genes that were differentially expressed (p<0.05), 37 unique and identifiable genes were ≥ 2-fold. The genes *Atf3*, *Fos*, *Apold1*, and *Pxdn* were expressed differently among SED, PA and EX following acute exercise, with a clear separation of the magnitude in gene expression of SED > PA > EX. Some genes have regulatory effects on blood lipids and body weight, with healthier phenotypes in the more active groups.
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IN PHYSICALLY ACTIVE AND INACTIVE RATS

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DEDICATION

This work is dedicated to my families, both old and new, for their never ending love and support.
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CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

The development of a new public health initiative, Healthy People 2020, will build upon data collected over the past twenty years about behaviors and underlying factors that contribute to a long, healthy life. Advances in genome research have allowed scientists to identify genes as well as monitor gene expressions associated with specific health and disease conditions. This knowledge has enabled us to understand that certain genes are regulated by lifestyle, including what we do and what we do not do. Whether or not an individual is sedentary or physically active may have a significant impact on specific gene expression levels and phenotypes associated with health and disease. This is especially important in animal research whereby the typical laboratory cage restricts access to physical activity and causes animals to be physically inactive throughout their life spans.

Figure 1.1. The interaction of environment/lifestyle and genes on health related phenotypes. Important to this study is the influence of environment and lifestyle on changes in gene expression, which lead to changes in phenotypes related to health and disease.

Results from human studies have underscored the importance of regular physical activity to health and longevity. Longitudinal studies have generated interest in understanding how physical activity and inactivity affect gene expression and associated phenotypes related to health and disease. In 1948 the National Heart Institute launched the Framingham Heart Study, and examined over 5,000 men in a long-term study to identify risk factors for cardiovascular disease, the leading cause of death and illness in the United States. Among the list of cardiovascular disease risk factors was physical inactivity (Kannel & Gordon, 1978). Results from the Harvard Alumni Study, another large-scale investigation, indicated that physically inactive individuals lived shorter, less healthy lives, while regular physical activity was associated with longer life spans and healthier resting heart rate, blood pressure, body fat, blood
sugar, insulin, LDL, and HDL (Sesso, Paffenbarger & Lee, 2000; Lee & Paffenbarger, 2000). Animal studies have also highlighted the deleterious effects of physical inactivity that leads to chronic disease (Booth et al, 2008, Alessio et al, 2005). These findings emphasize the importance of physical activity on biomarkers of health and enhanced life span as well as the consequences of lifelong physical inactivity on increased risk for disease.

The use of animal models provides an expeditious way to collect lifetime data due to the relatively shorter life spans of most animals compared with humans. Animal models are useful because tissue samples are relatively easier to obtain and they provide some analyses that are not possible in most human studies. Despite the dramatic difference in size compared to humans, rodents share over 95% of the same genes as well as most of the same health and disease pathways as humans. Animal models present a very efficient and effective way to investigate the influence of physical activity and inactivity over time.

Researchers have been able to study the effect of chronic physical inactivity and activity on phenotypes associated with health and disease over the typical life span of rodents. In a study by Alessio et al (2005) male rats were divided into three groups and given access to varying levels of physical activity. Measurements such as movement (by distance covered daily), body mass, blood lipid profiles, and the number and size of tumors developed were evaluated. After 16 months, male rats that exercised daily exhibited improved biomarkers of health compared to sedentary rats. The exercise (EX) group covered the greatest distance by running on an exercise wheel, covering approximately 10 fold more distance each day compared with the sedentary (SED) group. Compared with EX animals, the male SED group had a higher mean body weight, a larger number of tumors, and larger mean tumor size (Alessio et al, 2009). The physical activity (PA) group did not show significant differences from the SED group. It was suggested that lifelong physical inactivity, as is typical for animals housed in standard laboratory cages, placed male rats at risk for obesity and cancer.

Chronic exercise also leads to adaptive changes that can maintain or improve metabolic function in skeletal muscle, whereas physically inactive and untrained muscle fails to exhibit adaptive metabolic properties. Cellular differences between physically active and physically inactive individuals can be observed even after a relatively short (e.g. 5 days) training period (Pilegaard et al, 2000). It remains unclear how changes in gene expression, due to either physical activity or inactivity, influence select phenotypes associated with health. In a study that
investigated the differences between trained and untrained human skeletal muscle, cellular and metabolic characteristics were compared before and after acute exercise (Pilegaard et al, 2000). Gene transcription was determined by polymerase chain reaction (PCR) amplification by using primer pairs constructed from human specific sequence data. Results from this study found significant increases in the transcription of genes believed to be involved with fat and glucose metabolism in muscle from both the chronically trained and acutely exercised animals compared with untrained and rested muscle. Of interest were findings that an acute bout of exercise activated transcription of several metabolically regulated genes in skeletal muscle. These transcription levels continued to increase for several hours following activity, and then returned to near baseline levels after about 22 hours. It was concluded that these changes contributed to an increase in metabolic efficiency and an improvement in biological and physiological biomarkers of health. Chronic physical inactivity, on the other hand, may prevent the beneficial cellular adaption of metabolically related genes observed following acute exercise (Pilegaard et al, 2000).

The current investigation examines the effect of an acute bout of exercise on gene expression in sedentary, physically active, and exercised animals. A previous study focused on the effect of an acute bout of exercise on gene transcription in untrained, ten week old female Sprague-Dawley rats (McKenzie & Goldfarb, 2007). Sixteen sedentary rats were randomly divided into an exercise or a sedentary control group. The exercise group performed a single acute bout of exercise for two hours at an intensity equivalent to 65% VO₂max. One hour after termination of exercise, the exercise group was sacrificed, and the sedentary control group was sacrificed at a matched time. DNA microarrays were used to examine the soleus muscle, and four genes thought to be involved in stress response to exercise were analyzed by real-time (RT) - PCR. In addition, two proteins were confirmed by Western blot analysis. In the exercise group, expression of 52 genes was significantly altered one hour post-exercise. Of the genes with significantly different transcriptional levels, gene clusters were identified that related to metabolism (~10%), apoptosis/cell cycle (~8%), muscle contraction (~10%), transcription/cell signaling (~17%), tissue generation (~15.5%), and inflammation (~10%). These results suggest that one hour after an acute bout of exercise in untrained rats, skeletal muscle undergoes significant changes in gene transcription (McKenzie & Goldfarb, 2007).
Other research suggests that the post-exercise cellular changes observed in the McKenzie and Goldfarb (2007) study may be caused by an adaptive response to the stress of exercise in an attempt to regain homeostasis (Mahoney et al, 2005). In their study, global mRNA expression was measured from skeletal muscle of healthy, young, and sedentary male human subjects. Muscle samples were taken before, three hours after, and 48 hours after an exhaustive bout of high-intensity aerobic exercise. Following microarray analysis, the data revealed 118 genes up- and 8 genes down-regulated at 3 hours post-exercise, while 29 genes were up- and 5 genes down-regulated 48 hours post-exercise (fold-change ≥2, p<0.05,). RT-PCR was performed on select genes to validate results. Based on these results, Mahoney et al (2005) suggest that an acute bout of endurance exercise initially may rapidly increase transcription of a relatively large number of genes, and decrease transcription of a relatively small number of genes. However, the differential expression may be a transient response, since relatively few transcriptional changes were observed at 48 hours post-exercise. It is important to note that the acute bout of exercise induced transcriptional changes in genes involved in metabolism, mitochondrial biogenesis, oxidant stress management and signaling, electrolyte transport, and various other functions. These findings may indicate how cells immediately recover from an acute stress of exercise, and that transcriptional changes due to an acute bout of exercise may be relatively temporary (Mahoney et al, 2005), yet initiate lasting phenotypic effects.

Obvious changes in phenotype may not always be directly influenced by an equivalent adaptation in the expression of a single gene. Physiological outcomes in response to physical activity or inactivity may be caused by subtle changes in expression of multiple genes, or because of changes in expression of just a few key regulatory genes. Clusters of genes or gene networks may involve a handful of genes with related functions that influence gene expression changes of other genes within a given pathway. Some can be up-regulated while others may be down-regulated depending on their specific function and response to environment, such as physical activity. A study by Chen et al (2002) identified phenotype changes resulting from clusters of genes containing both up-regulated and down-regulated gene expressions. Following electrically induced muscular contractions, muscles from 6-7 week old female rats were removed and flash frozen at both 1 and 6 hours post exercise. Total mRNA was later extracted for gene expression profiling. Northern blots were used to measure transcriptional activation, and both Western blots and SDS-PAGE were used to measure translational activation. Results indicated
numerous gene expression changes that were more than two-fold and statistically significant (p < 0.001) when compared at 1 and 6 hours. Chen et al. reported that multiple genes expressed at slightly altered levels may create observable changes in phenotype. Over time, responses in gene expression due to acute exercise may cause multiple, small, gradual adaptations at the cellular level which enable the body to respond more efficiently to a similar stimulus in the future. These putative changes due to a constant, repetitive stress may exhibit a hormetic effect and include beneficial adaptations on select phenotypes.

Figure 1.2. Representation of acute bouts of exercise whereby repeated stress over time begins to accumulate, causing small, gradual adaptations in gene expression by increasing the amount of up- or down- regulation.

Previous studies indicate that health is influenced by age, chronic physical exercise or chronic physical inactivity (e.g. Kannel & Gordon, 1978; Booth, Chakravarthy, & Spangenburg, 2002; Alessio et al, 2005; Hamilton, Hamilton, & Zderic, 2007). However, to date no study has systematically investigated gene expressions and associated phenotypes related to health in physically inactive and physically active animals before and after acute exercise. Certain changes in gene expression are expected to occur naturally as a result of physiological maturation and physical development. Other changes in gene expression may be age-related changes that lead to senescence, or are associated with chronic physical inactivity or physical activity. The importance of acute exercise is that it provides a stimulus that can trigger a change in the
transcription of key target genes. While a change in the transcription of any particular gene does not necessarily translate directly into a corresponding change in gene product, exercise or physical activity performed over time may result in an accumulation of transient changes in transcription that ultimately contribute to observable changes in mRNA or the protein for particular genes (Pilgaard et al, 2000).

The mechanisms by which health and disease-related phenotypes are associated with gene expressions in physically active or inactive animals are still unclear. It is likely that repeated bouts of acute exercise may temporarily influence selective gene expressions that play a role in metabolic processes, ultimately accumulating over time. This may then contribute to a hormetic effect where these regular mild or submaximal episodes of exercise induce adaptations that ultimately enhance health and function of the organism (Ji et al, 2006; Alessio et al, 2005). It is of interest to learn which gene expressions change as a result of both acute and chronic exercise, the direction of change, and how gene expression changes compare in animals that are physically inactive and physically active. In this study, gene expression levels were monitored before and after an acute bout of 30 minutes of swimming exercise in rats that were sedentary, physically active, or exercised daily. The purpose of this study was to compare gene expressions and associated phenotypes associated with health and disease in young rats before and after a strenuous bout of acute swimming exercise.

1.2 REFERENCES


CHAPTER 2: ACUTE EXERCISE EFFECTS ON CARDIAC GENE EXPRESSION IN PHYSICALLY ACTIVE AND INACTIVE RATS

2.1 INTRODUCTION

Environmental stimulation of gene expression alters phenotypes related to health and disease (Nestler and Hyman, 2002). Exercise is one type of environmental stimulation that may activate or inhibit gene expression. Certain genes are regulated by either chronic physical activity or physical inactivity (Booth et al., 2008; Hamilton, Hamilton, & Zderic, 2007; Alessio et al., 2005; Booth, Chakravarthy, & Spangenberg, 2002; Kannel & Gordon, 1978). The up- or down-regulation of these particular genes may result in different phenotypes such as body weight, cholesterol, glucose, and triglycerides (Alessio et al., 2005). In addition to the well-known effects of chronic exercise, we speculate that one bout of acute exercise may affect a number of gene expressions depending on whether or not the individual is accustomed to regular exercise or physical activity.

To date few studies have systematically investigated gene expressions and associated phenotypes in physically inactive and active animals before and after a single episode of acute exercise. One study reported that an hour following vigorous exercise, sedentary female rat muscle exhibited 52 significant changes in gene transcription compared with muscle collected from resting animals (McKenzie & Goldfarb, 2007). It is likely that multiple bouts of vigorous exercise may influence selective gene expressions that play a role in metabolic processes, ultimately accumulating over time, and affecting certain phenotypes. When performed regularly, exercise can produce a hormetic effect, with beneficial adaptations occurring as a result of regular, tolerable stress (e.g. Alessio, 2006; Ji, Gomez-Cabrera, & Vina, 2006). While a change in the transcription of any particular gene does not necessarily translate directly into a corresponding change in gene product or a particular phenotype, successive bouts of regular exercise over time have resulted in an accumulation of transient changes in transcription that contribute to observable changes in mRNA and protein for particular genes (Pilegaard et al., 2000). It has also been theorized that the absence of vigorous exercise can either silence or unleash different genes due to long periods of very low physical stimulation (Booth et al., 2002). This could have important implications in the use of animal models for research, whereby the standard laboratory cage allows for little movement, forcing an animal into a sedentary lifestyle.
As such, the sedentary model may have different physiological responses to treatment, and may not necessarily be representative of a regularly active population.

The purpose of this study was to investigate whether gene expressions change as a result of acute exercise in animals that are physically inactive or physically active. In this study, gene expression levels were assessed before and after an acute bout of 30 minutes of swimming exercise in three-month old male rats that were sedentary (SED), physically active for two months (PA), or regularly exercised for two months (EX) on a running wheel. Body weight, blood cholesterol and triglycerides were also compared in all animals.

2.2 METHODS

Ethical approval. EX and SED animals were housed in pairs and the PA animals in groups of eight in climate-controlled rooms (24 ± 2 °C) with a 12-hour light/dark cycle. All animals had free access to water and food (LabDiet 5001 Rodent Chow, Purina, St Louis, MO). The health surveillance program included comprehensive serology: samples were sent quarterly from each rat colony to the Research Animal Diagnostic Laboratory at the University of Missouri (Columbia, MO). The study was conducted in accordance with ethical procedures and policies and was approved by the Miami University Institutional Animal Care and Use Committee.

Animals. Seventy-two four week old male Sprague-Dawley rats were randomly divided into three groups and given access to varying levels of physical activity or chronic exercise: 1) EX: voluntary access to wheel running exercise, 2) PA: a large activity box, and 3) SED: sedentary control with no access to physical activity outside of a standard cage.

EX and SED animals were housed in standard plastic cages (0.454 x 0.238 m), with the exception of the EX group having access every other day to voluntary wheel running access. The PA animals were housed in a large plastic box (1.2 x 0.6 m) that had ledges to encourage climbing and tubes to encourage exploration. Video recordings over 24 h were used to determine distance covered and intensity of exercise for all groups. The exercise wheels (Nalgene, Rochester, New York) recorded revolutions per day, which were translated into meters per day for analysis. All animals were handled and weighed weekly.

Rats were sacrificed after two months of activity treatment at age three months. Animals from each treatment group (EX, PA, SED) were randomly divided into two sets before sacrifice. One subset of animals (rest) from each treatment group was sacrificed at rest, and the other
subset (swim) after exhaustive swimming (approximately 30 minutes, water temperature = 35-38°C). All animals were sacrificed by decapitation between 0800 and 1200 h. Whole blood was collected, chilled and centrifuged to recover serum. Heart, liver, kidneys, the soleus, and red and white muscle tissue from the quadriceps were removed immediately and flash frozen in liquid nitrogen. All serum, organs, and tissues were deep frozen (-80°C) for future assays and RNA extraction.

**Blood cholesterol and triglycerides.** Cholesterol was measured by an enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase (Wako Cholesterol CII, Wako Chemicals USA, Inc., Richmond, VA). Triglycerides were measured by an enzymatic method using glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase (L-Type TG H, Wako Chemicals USA, Inc., Richmond, VA).

**RNA extraction.** The left ventricles (20-30mg) from 72 heart tissue samples (rest EX, n=12; rest PA, n=12; rest SED, n=12; swim EX, n=12, swim PA, n=12; swim SED, n=12) were used for all RNA extractions and gene analysis. The RNA extraction was performed using Qiagen RNeasy (Valencia, CA) RNA extraction kits. The absorbance ratio (A₂₆₀/A₂₈₀) was determined using a ND-1000 UV/VIS spectrophotometer (Nanodrop Technologies, Inc., Montchanin DE) and was used to verify RNA purity. RNA integrity was assessed with an Agilent BioAnalyzer system by the Biomedical Genomics Core at the Research Institute at Nationwide Children’s Hospital (Columbus, OH). Samples passing quality control were pooled within each treatment group to yield 4 gene chips per treatment group, with RNA from 3 animals on each chip, to ensure correct quantity of total RNA. Samples were labeled with the Affymetrix® Whole Transcript Labelling system and then hybridized to the Affymetrix GeneChip Rat Gene 1.0 ST Array (Santa Clara, CA). The GeneChip® Rat Gene 1.0 ST Array is the latest product in the family of Affymetrix expression arrays offering whole-transcript coverage. The design of the Rat Gene 1.0 ST Array is based primarily on a subset of GeneChip® Rat Exon 1.0 ST Array probes that map to well-supported exons of known genes. The array comprised of more than 700,000 unique 25-mer oligonucleotide features constituting more than 27,000 gene-level probe sets. Data was preprocessed using the RMA approach for background correction, normalization and probe set summarization using the Bioconductor affy package in R.

**qPCR.** Real Time RT-PCR gene expression analysis was performed at the SABiosciences laboratory with a RT² qPCR Primer Assays kit (SABiosciences, Fredrick, MD).
Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as a control and the genes *Atf3*, *Fos*, *Apold1*, *Pxdn*, and *Creb1* were analyzed. The first strand reaction for each sample was carried out with 1μg of RNA, and a dissociation (melting) curve was run for quality control.

**Gene networks.** Gene networks were generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). A gene network is a graphical representation of the molecular relationships between molecules. Molecules are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). All edges are supported by at least 1 reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product. Microarray data from the current study has been overlaid onto these constructed networks to illustrate relationships between genes that were differentially expressed in response to acute exercise.

**Statistical Analysis.** Phenotype data are presented as means ± S.E.M. One-way ANOVAs were used to evaluate differences among the groups and a Bonferroni correction was applied for multiple comparisons. Significance Analysis of Microarrays (SAM) used the Bioconductor siggenes package to identify differentially expressed genes between rest and swim conditions for each treatment group. A two-class unpaired analysis with a false discovery rate (FDR) of 10% was used to maximize sensitivity and minimize the effect on accuracy. Analysis between conditions was used to determine the effect of acute exercise on gene expression. Comparisons were made between 3 mo swim EX vs 3 mo rest EX, 3 mo swim PA vs 3 mo rest PA, and 3 mo swim SED vs 3 mo rest SED. Statistical analysis of the QPCR delta-Ct values was performed using the Mann-Whitney Rank Sum test. Comparisons with a fold change ≥1.5 and a p <0.05 were considered significantly different.

### 2.3 RESULTS

**Phenotype data.** All reported phenotype differences are among SED, PA, and EX groups, combining both rest and swim conditions, and are presented in Table 1. Weight differences were observed between all three groups. Body weight for the animals in the EX group was lower than for animals in the SED group (p<0.005) and the PA group (p=0.084). The differences between PA and SED were not found to be significant (p=0.793).
Differences in average distance covered per day were also observed. The EX group covered a greater distance per day than the PA and SED groups (p<0.001), but there were no differences between the PA and SED groups (p=1.000).

The SED group had higher blood cholesterol levels than PA and EX (p<0.050). Blood triglycerides followed the same trend where EX<PA<SED, however the differences did not reach statistical significance due to large variance.

**Microarray.** For each treatment, a relatively small number of genes (466) were up- or down-regulated (10% FDR) in the swim group compared to rest (Table 2) as determined by Significance Analyses of Microarrays (SAM). For the current study, SAM analysis was implemented using the Bioconductor siggenes package. A two-class unpaired analysis with a FDR of 10% was used to maximize sensitivity without significantly impacting accuracy. Less than 1% of the total 27,000 probe sets (113) expressed fold changes greater than 2. Only the genes that have been linked to expression of a known protein are listed in Tables 3 and 4, which brought the number of unique identifiable genes that were differentially expressed to 37. All the genes differentially expressed by at least two-fold and the respective fold change values for selected genes can be found in Tables 3 and 4 and Figure 1.

**qPCR.** qPCR was performed on five genes in addition to the standard control Gapdh. These genes were selected for investigation based on relatively high fold changes in microarray, reported functions relating to health, and/or position within complex gene network systems (Figures 3a and 3b). The SED group was found to be significantly different from both the PA and EX groups (p<0.05). For the genes Atf3, Fos, and Apold1 the direction of the fold change was the same as microarray data, with an increase in gene expression in acutely exercised rats relative to control. The magnitude of change was similar to microarray data except for Fos in the SED group, which had a 7.5-fold increase in microarray but a 50-fold increase with qPCR. Differential expression of Pdxn as detected by microarray was not confirmed with qPCR. For Creb1, both microarray analysis and qPCR confirmed no change in gene expression levels in any of the three groups. Thus, qPCR confirmed microarray results at a level closely matching the 10% FDR used as a statistical cutoff. The qPCR results are reported with corresponding microarray data in Table 3 and Figures 1 and 2.

**Gene networks.** Microarray data was overlaid on gene networks to show genes that were up- or down-regulated following acute exercise among the treatment groups in this study (Figures 3a
and 3b). These pathways visually describe the relative magnitude and direction of gene expression changes specific to this study. The gene networks in Figures 3a and 3b include genes that were differentially expressed when comparing acute swim to rest, as well as those genes that did not change with treatment. Often, a gene that was not differentially expressed, such as Creb1, was found to interact with several genes that were significantly up or down-regulated. This may suggest a change in function of this gene at the protein level. In other instances, several genes that were differentially expressed are all linked together in a common chain, such as seen with Fos, Atf3, Egr1, and Apold1.

2.4 DISCUSSION

The interaction of genes and environment may influence certain health and disease related phenotypes (Alessio et al, 2005, Kannel & Gordon, 1978). It was hypothesized that an acute bout of exercise would have different effects on gene expression depending on the animal’s previous access to exercise and physical activity. Research has suggested that regular exercise may enhance the health and function of an organism by influencing selective gene expressions that play a role in metabolic processes (Alessio 2006; Ji, Gomez-Cabrera, & Vina, 2006). Thus, depending on how regularly inactive or active an animal is, acute exercise could influence the level of expression of some genes differently. These genes may then either control other genes, or directly influence the translation of certain proteins associated with important health-related phenotypes. Physical inactivity or activity may also influence gene expression by modifying chromatin and opening up core promotor regions of the DNA for the binding of regulatory proteins (Nestler and Hyman, 2002). Following an acute bout of exercise, key regulatory proteins may come in contact with each other causing transcription factor complexes to form and phosphorylation to occur that affect how proteins bind to one another. These events, possibly stimulated by exercise, are likely to activate or repress transcription of responsive target genes that may ultimately influence phenotypes associated with cardiovascular health and metabolic disease (Nestler and Hyman, 2002).

Even at a relatively young age of three months, data presented here shows differences in body weight, blood cholesterol, and triglycerides among the different treatment groups in this study (Table 1). The SED group, with no access to physical activity, had the highest cholesterol, and the PA group, with freedom to roam a large activity box, had cholesterol similar to EX.
Triglyceride levels showed similar trends, although due to large variation, differences did not reach statistical significance. Thus, it seems that inactivity has a detrimental effect on some phenotypes, whereas relatively moderate to high levels of physical activity work to prevent these deleterious changes. Previous studies have directly related levels of physical activity to health related phenotypes such as weight, HDL cholesterol, blood pressure, adiposity, blood glucose, triglycerides, and tumor development (e.g. Alessio et al, 2009; Hamilton, Hamilton & Zderic, 2007; Alessio et al, 2005). In our previous research the health-related phenotypes in the PA group were similar to those of the SED group, while in this study the PA group more closely reflects the EX group (Alessio et al, 2005). This discrepancy between PA results may be attributed to increased level of activity for the PA group in the current study. The PA treatment for the previous study allowed for only one hour of roaming the PA box twice weekly, where the PA treatment in this study housed the rats in the PA box allowing daily unlimited roaming. After only two months of residing in different environments, the weight, cholesterol, and triglyceride results from this study confirm previous research and indicate whether or not an animal is physically active can affect certain health related phenotypes. These phenotypes are associated with many of the key functions and regulatory roles of differentially expressed genes found in this study.

Gene networks are used to portray the interactions between genes in multiple related pathways and have been generated by studies of specific gene functions and causal connections (D’haeseleer, Liang, & Somogyi, 2000). Figures 3a and 3b show examples of networks portraying expression changes in RNA data obtained from rat cardiac tissue in the current study. Essentially, these networks are a snapshot in time of gene expression levels when comparing rats sacrificed immediately following acute exercise with referent-control rats sacrificed at rest. As shown (Figures 3a and 3b), many genes seem to influence other closely related genes in interconnecting pathways within a given network. For example, one network produced in the EX group gene array revealed Creb1 as a central gene connected to pathways linking Fos, Atf3, Egr1, and Apold1. While Creb1 was not differentially expressed, other genes linked in the pathway were up-regulated to a significant degree following acute exercise.

One explanation for this pattern may be the nature of cell signaling mechanisms in particular pathways. Changes in gene expression are not always associated with similar changes in protein expression patterns. Bey and Hamilton (2003) reported a decreased skeletal muscle
lipoprotein lipase (LPL) during physical inactivity that was independent of any change in LPL mRNA. Hamilton et al (2008) reported increased SOCS2 and SOCS3 mRNA but no difference in the protein expression. However, as seen here, a single bout of exercise can be stressful enough to increase or decrease gene expression beyond a threshold for a limited but critical amount of time, which then may result in changes in translation of associated proteins or activation of other genes in a particular network, thereby affecting an associated phenotype. Since the displayed network is only a capture of a single moment in time, it could be that gene expression changes are transient responses to environmental interaction. Following acute exercise, certain genes such as the ones we have explored, may be up- or down-regulated for a limited period of time. If fold changes in either direction are great enough, they may cross some critical threshold at which point translation of certain proteins may be increased or decreased. It is unknown what this “critical threshold” may be for any given gene, and it is likely that this threshold is unique to each gene. Thus, multiple bouts of exercise may result in some genes experiencing a hormetic effect whereby exposure to relatively low stress over time causes subtle changes in key gene expressions that ultimately bring about favorable biological responses (Calabrese & Baldwin, 2002).

We investigated genes of interest based on their specific functions in general health and their metabolic response to exercise. Selections were also based on relatively high fold changes when comparing groups with different access to physical activity before and after acute exercise (e.g. Atf3, Fos, Apold1, and Pxdn), or because of their strategically placed position within a complex gene network thought to be related to the regulation of cardiac function (Creb1). However, the relationships and connections among genes found in gene maps and pathways are complex and include redundant, distinct, and synergistic effects. Genes within specific pathways in a network may play key regulatory roles when expressed differently, affecting change in other connected genes or otherwise preventing change.

Responding to stress, Atf3 (Activating transcription factor 3) is considered an “adaptive response” gene, and its over-expression may suppress cell growth (Yin, Dewille, & Hai, 2008; Tamura et al, 2005; Fan et al., 2002). While increased expression of Atf3 is linked to tumor cell suppression, it may also cause the over-expression of genes known to enhance the growth of cancer cells. Thus, it is uncertain whether the up-regulation of Atf3 has an advantageous or deleterious effect (Yin, Dewille, & Hai, 2008). Induced cell damage has been found to
transiently increase its production, suggesting a protective role in cell injury (Fan et al, 2002). Never the less, Atf3 expression was found to respond differently to an acute bout of exercise in all three conditions. The present results show that acute exercise-induced expression changes were smallest in the EX group and slightly higher in the PA and EX groups with about a 5-10 fold up-regulation. Due to its protective role in cell injury, it may be possible that expression of this gene is increased in all groups following a stressful episode of exercise in response to cell damage that has occurred, with the EX group experiencing the least and the SED group experiencing the most exercise-induced cell damage.

Fos (FBJ murine osteosarcoma viral oncogene homolog) followed the same pattern as Atf3 with about a 5-10 fold increase with SED>PA>EX. In a study investigating the response of Fos to acute exercise, it was found that chronically exercised rats developed better coping mechanisms to acute stress than sedentary rats (Collins et al, 2009). However, in their study the researchers found Fos to be elevated after acute exercise in the chronically exercised rats, and not in the sedentary rats. The current study shows the opposite, where Fos was up-regulated the most in SED, and to a lesser degree in PA and EX groups. The reason for the different results may be because the Collins et al (2009) study sacrificed their animals two hours post-acute swimming- a time they thought to be peak time of Fos induction. In our study, however, animals were sacrificed immediately post-acute swim, at which point levels of Fos expression may not have had time to reach peak production. The link between Fos and lipid metabolism suggests that it has an important regulatory role in phospholipid and glycolipid production, which seems to occur in response to cell requirements (Crespo et al, 2008). A difference in cell requirements among the SED, PA, and EX groups may be one factor contributing to the cholesterol and triglyceride levels observed.

Apold1 (apolipoprotein) was selected because of its fold changes among the groups and its functions related to health and disease. Apold1 has been reported to regulate endothelial cell differentiation, activation, and cell signaling, and may also be related to vascular function (Regard et al, 2004). One study identified Apold1 as closely related to Verge (vascular early response gene), which together may be regulators of endothelial cell signaling and vascular function (Regard et. al., 2004). One study reported the association of the Apold gene cluster with triglyceride and high density lipoprotein (HDL) cholesterol levels (Lu et al, 2006), while another related it to HDL and atherosclerosis regulation (Plump, Scott, & Breslow, 1994).
study, *Apold1* was similarly over-expressed in all groups following acute exercise. The trends are similar in all three groups and the numerical differences are small, but the trend of SED > PA > EX for nearly all exercise-induced gene expressions have potential significance. It is also possible that some genes require a higher degree of stimulation for differences to become apparent in gene expression. In this case, it seems that the amount of physical activity acquired by the PA group in their box was not enough to differentiate it from the sedentary lifestyle of the SED group.

*Pxdn* (peroxidasin homolog) is involved in peroxidase activity and is associated with oxidation reduction and response to oxidative stress (Cheng et al, 2008). While it is a part of a group of peroxidases involved with hormone biosynthesis, the specific function of *Pxdn* is presently unknown. One study suggests it may play an important physiological role in an extracellular matrix formation pathway (Peterfi et al, 2009). In the present study we found that following acute exercise, *Pxdn* was down-regulated in all three groups about 2-4 fold. However, we hypothesize that the whole transcript microarray used in this study may be capturing a change in differential splicing of the *Pdxn* transcript that may not be captured by qPCR analysis which typically targets a single 3’ region of the mRNA.

Studies suggest that the activation of *Creb1* (cAMP-responsive element binding protein) plays a prominent role in various types of cancer, including breast cancer (e.g. Sakamoto & Frank, 2009; Chhabra et al, 2007). It has been found to be over-expressed and phosphorylated when examined in cancer tissue, and has thus been identified as a key target gene in cancer research and therapy (Sakamoto & Frank, 2009). Specifically, one study indicated *TNF-alpha* as a major participant in the down-regulation of *Creb1* (Todorov et al, 2005). *Creb1* may also be involved with the leptin signaling pathway and has been associated with obesity related endometrial cancer (Catalano et al, 2009). In the current study, this gene was investigated because of its strategically placed position within gene network pathways, with connections to several key regulatory genes within the network. Despite its central role in the gene network, its expression status did not change following acute exercise, implying that gene networks do not always work via enhanced or suppressed transcription.

Following analysis of genes among the three groups before and after an acute bout of exercise, we found only about 1.9% of the total rat genome to be differentially expressed and even fewer when considering genes with known functions. For example, *Atf3* was found to be
up-regulated following acute exercise in SED (8.7-fold), PA (7.3 fold), and EX (4.4-fold). \textit{Pxdn} was down-regulated following acute exercise in SED (-3.5), PA (-2.7), and EX (-2.0). The majority of genes exhibited expression changes in the same direction across all three groups, implying that an acute bout of exercise elicited a similar gene response regardless whether an animal was regularly inactive or active. However, the trend of these changes were consistently SED > PA > EX, indicating that regular physical inactivity makes a large and potentially lasting impact on gene expressions, while regular exercise attenuated the magnitude of the response of these genes to acute stress. This trend was observed in both microarray and qPCR, although the magnitude of the gene expression fold changes were typically higher in qPCR. This provides compelling evidence that a sedentary lifestyle may activate or suppress genes in such a way that contributes to unhealthy phenotypes, whereas being moderate to highly active may prevent some of these deleterious changes. Only a handful of genes responded differently to acute swimming among the three groups. Four genes (\textit{Dyrk3}, \textit{Alb}, \textit{Ptgs2}, \textit{Sox18}) responded to acute exercise only in the SED group, three genes (\textit{Myl7}, \textit{Car3}, \textit{Arl4a}) changed following acute exercise only in the PA group, and three other genes (\textit{Rgs1}, \textit{Cyp1a1}, \textit{Reg3b}) changed following acute exercise only in the EX group. These changes are likely due to whether the animal was sedentary, regularly active or had access to an exercise wheel. All genes that had at least a two -fold change in expression and their specific fold change values for each group can be found in Tables 3 and 4.

Results of this study are consistent with previous studies (e.g. Lambertucci et al., 2006; Bronikowski et al., 2002) that reported marked differences in a relatively small number of genes between untrained and trained groups of animals at rest. Additionally, select genes were observed in this study where similar gene expression changes occurred following an acute swimming exercise, among SED, PA, and EX groups. While different access to physical activity or exercise did not seem to affect most gene expressions, healthier phenotypes were found in animals that regularly exercised over a period of 2 months. Differences in phenotypes have been observed between sedentary and physically active groups of animals in biomarkers of health such as weight, cholesterol, blood glucose, triglycerides (Alessio et al, 2005), and tumor development (Alessio et al, 2009). It is possible that these phenotypic differences are a result of modified gene expression in response to regular physical inactivity or activity. An animal exposed to one acute bout of exercise may experience an increase or decrease in cardiac gene expression for a short amount of time. Animals that are chronically exercised would be exposed
to frequent bouts of acute exercise that may contribute to regular alterations in the expression of
certain genes. If these acute exercise-induced gene expressions are up or down-regulated
regularly, protein translation may be repeatedly increased or decreased. Over time, this could
lead to significant changes not only in gene response to continuous bouts of exercise, but also in
resulting phenotypes related to health and disease, including body weight and blood lipids.

Upon analyzing the entire rat genome, Atf3, Fos, Apold1, and Pxdn were expressed
differently among SED, PA and EX groups following an acute bout of swimming exercise, with
a very clear separation of the magnitude in gene expression of SED > PA > EX. This small
number of genes was found to be responsive to acute exercise with the SED group demonstrating
the most exercise-induced changes in expression. Favorable phenotypes including body weight,
total blood cholesterol, and blood triglycerides were observed in animals that voluntarily
exercised regularly on a running wheel compared with animals residing in a large box and
animals that resided in a standard cage. Differences in gene expression levels among the three
groups may have important regulatory roles in gene networks and ultimately health-associated
phenotypes related to physical inactivity and activity.

2.5 REFERENCES
A.E. Hagerman, Oxidative stress in exercise and aging. London: Imperial College

*Physiology & Behavior, 84*(1), 65-72.

Revisiting influences on tumor development: Focusing on laboratory housing. *Journal of
the American Association for Laboratory Animal Science, 48*, 1-5.

during physical inactivity: a molecular reason to maintain daily low-intensity activity.
*Journal of Physiology, 551*(2), 673-682.

Booth, F.W., Chakravarthy, M.V., & Spangenburg, E.E. (2002). Exercise and gene expression:
Physiological regulation of the human genome through physical activity. *Journal of
Physiology, 543*(2), 399-411.


# Tables

Table 2.1. Phenotype data comparisons among SED, PA, and EX groups

<table>
<thead>
<tr>
<th>SED</th>
<th>Body Weight (g)</th>
<th>Distance (m/day)</th>
<th>Cholesterol (mg·dl⁻¹)</th>
<th>Triglycerides (mg·dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>408 ± 7</td>
<td>130 ± 2</td>
<td>24 ± 1</td>
<td>152 ± 11</td>
</tr>
<tr>
<td>PA</td>
<td>397 ± 5</td>
<td>209 ± 3</td>
<td>21 ± 1</td>
<td>140 ± 16</td>
</tr>
<tr>
<td>EX</td>
<td>375 ± 8</td>
<td>11900 ± 2335</td>
<td>20 ± 1</td>
<td>139 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. SED ≠ PA, SED ≠ EX; p<0.05.
Table 2.2. Comparison of the number of significantly (10% FDR) up- and down-regulated genes in 3 month rats across all conditions

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Test</th>
<th>Control</th>
<th>Up-regulated</th>
<th>Down-regulated</th>
<th>Up-regulated at least 2 fold</th>
<th>Down-regulated at least 2 fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Swim EX</td>
<td>Rest EX</td>
<td>73</td>
<td>85</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Swim PA</td>
<td>Rest PA</td>
<td>39</td>
<td>27</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Swim SED</td>
<td>Rest SED</td>
<td>102</td>
<td>140</td>
<td>32</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 2.3. Microarray and qPCR for exercise-induced gene expression changes, in alphabetical order, for select genes with at least a two-fold change

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene Name</th>
<th>microarray</th>
<th>qPCR</th>
<th>microarray</th>
<th>qPCR</th>
<th>microarray</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apold1</td>
<td>apolipoprotein L domain containing 1</td>
<td>5.1</td>
<td>7.1</td>
<td>5.1</td>
<td>3.1</td>
<td>6.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Atf3</td>
<td>activating transcription factor 3</td>
<td>8.7</td>
<td>15.0</td>
<td>7.3</td>
<td>5.1</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Creb1</td>
<td>cAMP responsive element binding protein 1</td>
<td>0.0</td>
<td>1.5</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>-1.1</td>
</tr>
<tr>
<td>Fos</td>
<td>FBJ osteosarcoma oncogene</td>
<td>7.5</td>
<td>50.0</td>
<td>7.2</td>
<td>8.0</td>
<td>6.6</td>
<td>8.1</td>
</tr>
<tr>
<td>Pxdn</td>
<td>peroxidasin homolog (Drosophila)</td>
<td>-3.5</td>
<td>1.7</td>
<td>-2.7</td>
<td>-1.1</td>
<td>-2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fold changes in gene expression are for SWIM compared to REST within each group.
Table 2.4. Microarray results for exercise-induced gene expression changes, in alphabetical order for remaining identifiable genes with at least a two-fold change

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene Name</th>
<th>SED</th>
<th>PA</th>
<th>EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abra</td>
<td>actin-binding Rho activating protein</td>
<td>2.8</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Adams1</td>
<td>ADAM metallopeptidase with thrombospondin type 1 motif, 1</td>
<td>2.6</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Alb</td>
<td>albumin</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arl4a</td>
<td>ADP-ribosylation factor-like 4A</td>
<td>2.0</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Btg2</td>
<td>B-cell translocation gene 2, anti-proliferative</td>
<td>3.9</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Car3</td>
<td>carbonic anhydrase 3</td>
<td>-2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyp1a1</td>
<td>cytochrome P450, family 1, subfamily a, polypeptide 1</td>
<td></td>
<td></td>
<td>-2.0</td>
</tr>
<tr>
<td>Cyr61</td>
<td>cysteine-rich, angiogenic inducer, 61</td>
<td>4.9</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Dusp1</td>
<td>dual specificity phosphatase 1</td>
<td>2.0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Dyrk3</td>
<td>dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egr1</td>
<td>early growth response 1</td>
<td>4.5</td>
<td>3.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Egr2</td>
<td>early growth response 2</td>
<td>2.1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Enc1</td>
<td>ectodermal-neural cortex 1</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Errfi1</td>
<td>ERBB receptor feedback inhibitor 1</td>
<td>3.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Fosb</td>
<td>FBJ osteosarcoma oncogene B</td>
<td>4.6</td>
<td>3.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Gadd45g</td>
<td>growth arrest and DNA-damage-inducible, gamma</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Jun</td>
<td>Jun oncogene</td>
<td>2.1</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Junb</td>
<td>jun B proto-oncogene</td>
<td>2.5</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>LOC500300</td>
<td>similar to hypothetical protein MGC6835</td>
<td>2.4</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Myl7</td>
<td>myosin, light chain 7, regulatory</td>
<td></td>
<td></td>
<td>-6.0</td>
</tr>
<tr>
<td>Nr4a1</td>
<td>nuclear receptor subfamily 4, group A, member 1</td>
<td>8.1</td>
<td>5.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Nr4a2</td>
<td>nuclear receptor subfamily 4, group A, member 2</td>
<td>7.4</td>
<td>6.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Nr4a3</td>
<td>nuclear receptor subfamily 4, group A, member 3</td>
<td>3.3</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Otud1</td>
<td>OTU domain containing 1</td>
<td>6.7</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Ptgs2</td>
<td>prostaglandin-endoperoxide synthase 2</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reg3b</td>
<td>regenerating islet-derived 3 beta</td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Rgs1</td>
<td>regulator of G-protein signaling 1</td>
<td></td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>Rgs2</td>
<td>regulator of G-protein signaling 2</td>
<td>2.2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Serpine1</td>
<td>serine (or cysteine) peptidase inhibitor, clade E, member 1</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Sgk1</td>
<td>serum/glucocorticoid regulated kinase 1</td>
<td>2.0</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Snf1lk</td>
<td>SNF1-like kinase</td>
<td>2.8</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Sox18</td>
<td>SRY (sex determining region Y)-box 18</td>
<td></td>
<td></td>
<td>-2.1</td>
</tr>
</tbody>
</table>

Fold changes in gene expression are for SWIM compared to REST within each group.
Figure 2.1. Microarray values of significantly differentially expressed genes following acute exercise in SED, PA, and EX experimental groups. Fold changes for Creb1 did not differ following acute exercise. Reported as log2 transform. n=6, p<0.05
Figure 2.2. qPCR values of significantly differentially expressed genes following acute exercise in SED, PA, and EX experimental groups. Reported as log2 transform. n=6, * indicates significance p<0.05
Figure 2.3a. Ingenuity pathway analysis of differentially expressed genes in 3 month-old male rats at rest and following acute exercise. Example of previously established gene maps overlaid with EX microarray data showing gene interaction and differential gene expression. Where indicated, values in parenthesis represent microarray fold changes followed by qPCR fold changes, when available. The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product.
Figure 2.3b. Ingenuity pathway analysis of differentially expressed genes in 3 month-old male rats at rest and following acute exercise. Example of previously established gene maps overlaid with EX microarray data showing gene interaction and differential gene expression. The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product.