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The purpose of this study was to identify changes in gene transcription that occur in the soleus muscle of untrained, 10 week old rats following a single aerobic exercise bout of 2 hours. Of particular interest were genes involved in the production or protection from RONS. Rats were either run (experimental) for 2 hours or were rested (controls) and were killed 1 hour post-exercise with controls killed at a matched time. The soleus muscles from each animal were pooled and examined using DNA microarray technology. The microarray identified 52 genes significantly different between the conditions. The major gene families altered were metabolism (~10% of all genes altered), apoptosis (~8% of all genes altered), muscle contraction (~10% of all genes altered), transcription/cell signaling (~17%of all genes altered), tissue generation (~15.5% of all genes altered), and inflammation (~10% of all genes altered).

To confirm the findings of specific genes from the microarray, real time PCR was performed on 4 genes of interest (NFκB, TNFα, Atf3, and Mgst1). The results from the PCR analysis on these 4 genes were consistent with the microarray results. NFκB and TNFα were not significantly altered on the microarray or PCR analysis, whereas Atf3 (up-regulated) and Mgst1 (down-regulated) were found to be significantly altered in exercised animals by both methods. Genes related to RONS protection were down-regulated.

Western Blot analysis was used to check if protein level was correspondingly altered. The process from gene transcription to protein translational is a complex process that may not coincide. NFκB protein level was not different between the 2 groups, matching the results obtained with NFκB gene level. Atf-3 protein level was elevated in the exercise group, matching the results obtained with Atf-3 gene level.

These data suggest that one hour after a 2 hour run at ~65% of $VO_2$ max, the soleus muscle undergoes changes in gene expression. These significant changes occurred in the areas of metabolism, apoptosis, muscle contraction, transcription/cell signaling, tissue generation, and inflammatory genes. Transcription of many genes involved in production of RONS was elevated, while that of genes involved in RONS protection were either depressed or unaltered.

# EFFECTS OF A SINGLE AEROBIC EXERCISE BOUT ON GENE REGULATION IN THE SOLEUS MUSCLE OF SPRAGUE-DAWLEY RATS

By

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APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

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# CHAPTER I

# INTRODUCTION

The balance between prooxidants and antioxidants is critical for determining whether a cell will live or die. Oxidative stress can occur in all cells when the production of prooxidants exceeds the physiologic capacity of the antioxidant defense system to neutralize or inactivate these prooxidants. Prooxidants can be countered by means of the body's endogenous antioxidant defense system, in conjunction with exogenous antioxidants consumed through the diet [50]. Most cellular oxidation is mediated by reactive oxygen/nitrogen species (RONS), which are molecules with an unpaired electron that makes them unstable. RONS seek to either donate or capture another electron to become stable, often at the expense of another molecule, causing "damage" to the molecules affected. The effect of these pro- and antioxidants on gene regulation is not fully understood.

Generation of RONS occurs as a result of normal cellular metabolism, but is often increased during periods of stress [194]. Therefore RONS are typically thought of as part of the stress response signal. Some typical RONS found in the body include, but are not limited to, the following: singlet oxygen ($^{\cdot}O$), superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$) [64]. While exercise does have many documented health benefits, it also can be an intense physical stress leading to increased RONS production

[96, 194], likely modifying several macromolecules, including proteins, lipids, and nucleic acids [64].

Damage to macromolecules can alter how the cell functions and performs. In fact, a single bout of aerobic exercise for as little 20 minutes increases blood and skeletal muscle markers of oxidative stress above baseline [4].

It is clear however, that although RONS production is increased, protection from RONS is also increased with exercise [49, 160]. In addition, trained individuals show less damage to the same exercise bout than untrained persons. This training adaptation may help attenuate the rise in protein, lipid, and nucleic acid oxidation noted post-exercise[85, 167, 193].

The exact intracellular signals controlling the reduction/oxidation (redox) status of the cell following exercise have not been fully elucidated. Many transcription factors are altered in response to exercise. Several apoptotic and anti-apoptotic factors are also altered following an exercise bout [22, 107, 153, 233]. Identifying the genes that are activated or inhibited, along with how these interact with each other is of great importance for developing interventive strategies for controlling oxidative stress damage in a variety of settings.

## Purpose of the Study

The purpose of this study was to examine the response of the rat transcriptome to a single bout of aerobic exercise.

## Specific Aims

The specific Aims of this investigation are as follows:

**Specific Aim 1- To identify the transcriptional response of prooxidant genes to a single session of aerobic exercise one hour after exercise.**

It was hypothesized that genes involved in the production of prooxidants in the body (e.g. NfkappaB, activating transcription factor 3 (Atf3), Tumor Necrosis Factor (TNF-α)) will be activated less in the exercisers than the controls one hour after an aerobic exercise bout. This aim will be tested using a DNA microarray, and select genes will also be tested using PCR analysis.

**Specific Aim 2- To identify the transcriptional response of antioxidant genes to a single session of aerobic exercise one hour after exercise.**

It was hypothesized that genes involved in protecting the body from oxidative stress (e.g. superoxide dismutase, glutathione peroxidase, catalase, microsomal glutathione S transferase 1 (Mgst1)) will be activated more in the exercisers than in controls one hour after an aerobic exercise bout. This aim will be tested using a DNA microarray, and select genes will also be tested using PCR analysis.

**Specific Aim 3- To determine if reported gene transcription level will match protein level following a single session of exercise one hour after exercise.**

It was hypothesized that since the process of gene transcription to protein translation is a complicated pathway, which often takes several hours to complete, that at the one hour post-exercise time point, gene and protein levels will not match for genes which have been reported altered. The protein level will be determined using a Western Blot analysis with specific antibodies for each protein of interest.

## Significance of Study

Identifying the genes altered in response to exercise-induced stress will allow future research to focus on the role of these genes in altering the defense mechanisms (enzymes, cell signals, proteins etc.) activated by acute exercise. If transcriptional changes reflect the changes in protein levels, future research may focus on intervention strategies related to enhancing antioxidant enzymes or various transcription factors. This research will provide a snapshot of the activation/inhibition of the genes at the 1-hour post-exercise time point (many changes in proteins and inflammation are reported to increase at this time [94, 161]). It is possible that the changes in mRNA and protein levels will not mimic the altered genes meaning there are translational modifications. These experiments will serve as the beginning of a time course experiment to see if these gene alterations are transient, or if there are transcription or translation alterations occurring in the muscle. This could lead to identifying factors that may contribute to the dissociation of the transcriptional or translational efficiency of some genes and proteins. By

understanding this complex process, we may begin to understand what redox signals influence cells to live or die, and how to better manipulate these signals in both normal and diseased cells in order for optimal cellular functions to occur.

## Limitations

1. Currently, statistically significant differences between 2 groups is defined as a 1.5 fold difference between genes [66, 182, 183].
2. mRNA from multiple animals will be pooled on each chip, instead of each animal having their own chip, this could cause one animal which is an outlier to alter the expression levels reported from that chip [14]since only average gene alterations will be reported for each group.
3. Although a one hour time point will be used, the possibility that some of the genes involved in the inflammatory process and oxidative status of the cell will be altered before or after this 1 hour time is possible.
4. Some of the genes which are altered at this 1 hour time point may only be transiently altered or might have not been altered in the same manner at a different time point.
5. Changes in gene expression levels do not always reflect changes in protein levels, and therefore do not necessarily lead to a biological response.

## Delimitations

1. Gene chips (or cDNA microarrays) will be used, allowing simultaneous viewing of the entire rat transcriptome instead of a single gene by gene analysis.
2. Rat muscles share over 90% homology with human skeletal muscles in regards to muscle fiber types and structure and are therefore the species of choice in skeletal muscle microarray studies.
3. The entire soleus muscle of the animal will be used as opposed to a muscle biopsy. These samples will represent what is happening in the entire muscle as opposed to just a particular random point in the muscle, which is a more accurate assessment of the entire muscular response. It has also been suggested that since muscle biopsies are a surgical procedure, that this could activate the inflammatory process and alter gene response.
4. Rats will be fasted for at least 12 hours prior to removal of their muscles, thus limiting the influence diet may have on oxidative stress levels reported.

# CHAPTER II

# REVIEW OF LITERATURE

This chapter presents a review of recent related literature in the area of oxidative stress and exercise. It is organized into the following sections: background of reactive oxygen and nitrogen species, cellular damage, cellular protection, aerobic exercise induced oxidative stress, aerobic exercise induced protection, and oxidative stress associated cell signaling.

## Introduction

Oxidative stress is a condition in the body in which the cellular production of prooxidants exceeds the physiologic capacity of the body to render these prooxidants inactive. The generation of reactive oxygen/nitrogen species (RONS) occurs under normal physiologic conditions due to normal cellular metabolism and other cellular processes such as immune function and cell signaling. RONS normally cause little damage in unstressed conditions due to the ability of endogenous antioxidants as well as exogenous antioxidants consumed through the diet to protect the body from RONS associated damage. RONS may also be produced due to environmental (smoke, ozone) and physiological (exercise, mental stress) disruptions to homeostasis.

In some cases, the antioxidant protection of the body may not be adequate to overcome the RONS accumulation. This can lead to a situation in which cellular components, such as proteins, lipids, and nucleic acids are damaged. This process has been implicated in aging, disease progression, and exercise. Although correlations have been made between oxidative stress markers and several diseases, it is unknown if RONS cause any particular disease, or are a consequence of the disease process.

## Reactive Oxygen and Nitrogen Species

Oxidative stress, which occurs in all cells, ultimately may determine the life cycle of a cell. RONS have been implicated in apoptosis [170], necrosis [9], aging [161], and other normal cellular functions [57]. Clearly, RONS can be produced under homeostatic conditions, but any stress, such as aerobic exercise, has the potential to increase RONS production, which might be above the antioxidant capacity and may lead to several biological problems.

A brief review of RONS (or free radical) chemistry is needed at this point. RONS are molecules with unpaired electrons or thathave electrons that are not in the most stable arrangement. Electrons within compounds are typically kept in orbitals, with each orbital containing two paired electrons. Molecules or compounds that have two paired electrons in these orbitals are considered non-radicals. If the orbital only contains one electron, it is said to be free and a radical (hence the term free radical) which is capable of independent existence [64]. This unpaired electron can become a free radical (also known as a reactive species) in one of three ways: by homolysizing a covalent bond,

adding a single electron to a neutral atom (donation), or losing a single electron from a neutral atom [96] (abstraction). These free radicals are reactive because they seek to fill their orbital with an electron from another molecule. This electron donation (or abstraction) can lead to the formation of additional free radicals (reactive species) until the free radical is reacted with an antioxidant or becomes balanced.

One of the most widely studied sources of free radical formation is the oxygen molecule ($O_2$). $O_2$ has two unpaired electron, causing it to be classified as a radical. Under normal cellular conditions, $O_2$ is reduced to water during the electron transport chain in the mitochondria by way of cytochrome oxidase. During this process $O_2$ is broken down into two singlet oxygen molecules. It has been purported that 2-5% of the singlet oxygen produced leaks out of the respiratory chain and becomes free to react with other molecules (typically in the form of a superoxide molecule). The site of this electron leakage is currently under debate, but ubiquinone has been suggested [141]. RONS are also produced through a variety of other pathways as well, and the most investigated RONS will be discussed.

## Common Radicals

One of the major free radicals produced from oxygen is the superoxide radical ($O_2^{\cdot -}$). Superoxide is formed when singlet oxygen ($O^-$) binds to molecular oxygen typically in the electron transport chain, which is produced mainly from mitochondrial leakage seen during oxidative phosphorylation [50]. This is not the only source of superoxide production. Sen et al. hypothesized that superoxides are also formed from

peroxyl radicals through the electron transport chain [194]. Additionally, respiratory bursts of phagocytic cells like neutrophils, monocytes, and macrophages can lead to superoxide formation [62]. One of the major reasons that superoxides are viewed as dangerous and harmful radicals is their ability to convert into other reactive molecules, such as hydrogen peroxide ($H_2O_2$).

Hydrogen peroxide itself is not considered a strong free radical. However, hydrogen peroxide can be converted into the hydroxyl radical ($^{\cdot}OH$), through the Fenton reaction, and this $^{\cdot}OH$ is the most reactive species in the body. Using iron or any reduced transition metal, the Fenton reaction is as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + {}^{\cdot}OH.$$

Additionally, the Haber-Weiss reaction can form hydroxyl radicals as follows:

$$O_2^{-} + H_2O_2 \rightarrow O_2 + OH + {}^{\cdot}OH.$$

The hydroxyl radical is considered by many to be the most reactive molecule. This radical will react with any molecule from which it can borrow an electron, whether it is protein, lipid, or DNA [167]. The $^{\cdot}OH$ radical is considered highly reactive because it will react with the first compound in close proximity and abstract an electron from that molecule [64].

Some lesser reactive species can also play a role in oxidative modifications to macromolecules in the body. These include the peroxyl radical ($ROO^{\cdot}$), which is the result of any carbon-centered free radical ($R^{\cdot}$), and can also alter proteins, lipids, or DNA, depending on where it is produced [64]. Nitrogen species can also be formed and act as free radicals, hence the transition from the term ROS to RONS. Nitric oxide can react with

transition metals and a superoxide to form peroxynitrite ($ONO_2^-$) and other nitrogen centered radicals. A sample list of common free radicals is listed in Table 1.

Table 1: Common RONS

| Name | Symbol |
|---|---|
| Singlet Oxygen | $O^-$ |
| Superoxide | $O_2^{\cdot -}$ |
| Hydroxyl | $OH^{\cdot}$ |
| Peroxyl | $RO_2^{\cdot}$ |
| Alkoxyl | $RO^{\cdot}$ |
| Hydroperoxyl | $HO_2^{\cdot}$ |
| Hydrogen Peroxide | $H_2O_2$ |
| Peroxynitrite | $ONOO^-$ |

Adapted from Halliwell and Gutteridge [64]

RONS Production

RONS can be produced from several primary and secondary sources. These will be discussed in full detail in a later section, but it warrants mentioning here as well. Primary sources of RONS production include: electron transport chain leakage, prostanoid metabolism, catecholamine autooxidation, xanthine oxidase, and NADPH oxidase formation. Several secondary mechanisms also exist, and these include:

phagocytic cells, disruption of iron containing proteins, and excessive calcium accumulation (or loss of calcium regulation) [64].

## RONS and Cellular Damage

The extent and amount of cellular damage that occurs due to RONS is dependent on several factors. First, not all RONS molecules have the same potential to disrupt or interact with macromolecules. Another factor is the macromolecule that is attacked, as well as the location of any prooxidants. Still another factor is the frequency, and duration of the RONS, or how much and often the RONS is present in the tissue. Finally, the amount and type of antioxidants within the area present to quench the RONS can affect the amount of damage seen.

The major cellular targets of prooxidant attack appear to be proteins, lipids, and DNA. When proteins are damaged, many enzymes undergo modifications, which have implications for normal cellular function. Lipids may undergo lipid peroxidation, and cellular membranes seem to be a major site for lipid peroxidation [64]. When cell membrane lipids become peroxidized this can lead to changes in membrane integrity. If sufficient membrane changes occur this can alter what enters and exits the cell and may lead to further damage to cellular components normally protected by the membrane. Genetic material (DNA and RNA) may be damaged as well. Consequences of DNA damage are strand breakage and alterations in nucleotide bases. Clearly, this has important implications as incorrect base pairs can lead to incorrect protein formation, and

perhaps lead to further mutagenesis. DNA oxidation has been reported to occur in response to oxidative stress.

## Protein Oxidation

Many investigators in recent years have documented oxidative modification to proteins, and some of these proteins have been identified as enzymes. RONS have the ability to directly alter protein structure, or may alter structure through secondary mechanisms. A common mechanism for protein oxidation occurs when transition metal ions are present (iron, copper) as these tend to damage either peptide bonds or the protein side chains [64]. Oxidation of proteins leads to changes in structure and/or catalytic function and has been shown to make proteins more susceptible to proteolytic attack (likely by way of the ubiquitin 26S proteosome) and degradation [105]. Protein oxidation seems to be especially prevalent in diseased tissue, where oxidized protein may account for up to half of the total protein content of the tissue [96]. Clearly, whether a cause or a consequence of a disease, altered proteins are significantly correlated to certain disease [63, 96, 161]

RONS normally affect proteins in one of three ways: fragmentation, aggregation, or proteolytic digestion [58]. Fragmentation by RONS causes the protein to breakdown into several smaller fragments. Fragmentation problems can occur during protein synthesis and may alter translational events and result in formation of incomplete proteins [198]. Albumin and collagen are two proteins highly susceptible to this type of attack [121, 223] because they each contain numerous proline residues. Proline residues are highly

susceptible to hydroxyl radical oxidation [200]. Amino acids that are bound to transition metals are also highly susceptible to fragmentation since these metals serve as catalysts in hydroxyl radical formation [228].

During aggregation, RONS can cause proteins to denature and these fragments can aggregate together [228]. Squier hypothesized that RONS causes post-translational problems leading to an increase in the number of incorrectly folded proteins [198]. These incorrect proteins aggregate together, which often disrupts normal cellular functions, and can bind to chaperone proteins and proteases in a manner that leads to the formation of additional improperly folded proteins.

Oxidized proteins are more susceptible to proteolytic degradation. Since fragmentation and aggregation usually cause gross deformities in proteins, they become more susceptible to proteolytic attack by various pathways (ATP-ubiquitin, calpains) [159]. Furthermore, at sites of RONS production, there is evidence of increased proteolytic activity [228].

In terms of measuring protein oxidation in the cell, it appears as though the formation of protein carbonyls (PC) is the predominant measure used [20, 39, 52, 53, 84, 162, 196, 230]. Carbonyls react with 2,4-dinitrophenylhydrazine (DNPH), which can be detected by absorbance spectrophotometry [35] and then quantified.

## Lipid Peroxidation

Lipid alterations due to RONS damage has been termed lipid peroxidation. In this situation, carbon is attacked by either hydroxyl radicals or other lipid radicals, causing a

carbon with an unpaired electron to be present. This carbon can combine with oxygen leaked from mitochondrial respiration, forming the peroxyl radical [5]. Peroxyl radicals can then abstracthydrogens from other lipids, forming lipid hydroperoxides. A particularly damaging feature of peroxyl radicals is their ability to travel through the bloodstream, thus allowing them to attack not only locally, but also at distant sites [64].

Alessio defined three phases of lipid peroxidation; initiation, propagation, and termination [5]. During initiation, one or more hydrogens are removed from the polyunsaturated fatty acid contained in the membrane, forming a conjugated diene. At this point, propagation starts as the carbon centered fatty acid radical combines with oxygen forming a peroxyl radical. The peroxylradical begins a chain reaction attacking more polyunsaturated fatty acids. Termination occurs as the peroxyl radicals are inactivated forming lipid hydroperoxides. This lipid hydroperoxide, in the presence of transition metals, can produce RONS that can result in further damage. The consequences of lipid peroxidation appear to occur mainly as alterations to cell or organelle membranes. Some common alterations observed to occur include; loss of membrane fluidity, increased membrane permeability (likely altering enzyme function) and apoptosis [177].

Several measures of lipid peroxidation currently exist, however there is no single method that is universally used. Lipid hydroperoxides (LOOH) are often quantified using the ferrous orange assay (FOX). The measurement of thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) have also been extensively used. TBARS is a non-specific but sensitive marker of lipid peroxidation. TBARS has

therefore been used as an indicator of lipid peroxidation, but can also reflect non-lipid components. MDA is one end product of lipid peroxidation which can be translocated from one site to another [43]. Therefore, MDA measurements may only reflect the balance of production, from both elimination and clearance. MDA represents around 20% of all the lipid peroxidation that has occurred [43]. In addition, not all lipid hydroperoxides will produce MDA [80]. Other intermediates of lipid peroxidation have also been determined such as conjugated dienes [179]. It is important to note that most studies have had mixed responses determining changes in these intermediates of lipid peroxidation. Recently it has been proposed that 8-isoprostane ($F_2$ isoprostanes) measured by HPLC is a good indicator of lipid peroxidation as an oxidative stress marker [134].

DNA Oxidation

DNA damage can be manifested in several different ways including oxidative damage. It appears that most oxidative modification occurs due to damage to single base pairs, which in itself can have a variety of consequences. Damage to a single base pair could lead to strand breaks, or heteroduplex DNA (incorrect base pairs) being formed. Incorrect base pairs could create a premature stop signal and lead to aberrant protein formation having a variety of consequences from apoptosis to tumor growth [64, 107].

Recent studies have used formation of the marker 8-hydroxy-2`-deoxyguanosine (8-OHdG) as an indicator of nucleic acid oxidative modification [21]. It has been reported that this measure may actually only account for about 10% of total DNA oxidation[21]. Nonetheless, high levels of 8-OhdG have been correlated with many disease states, as

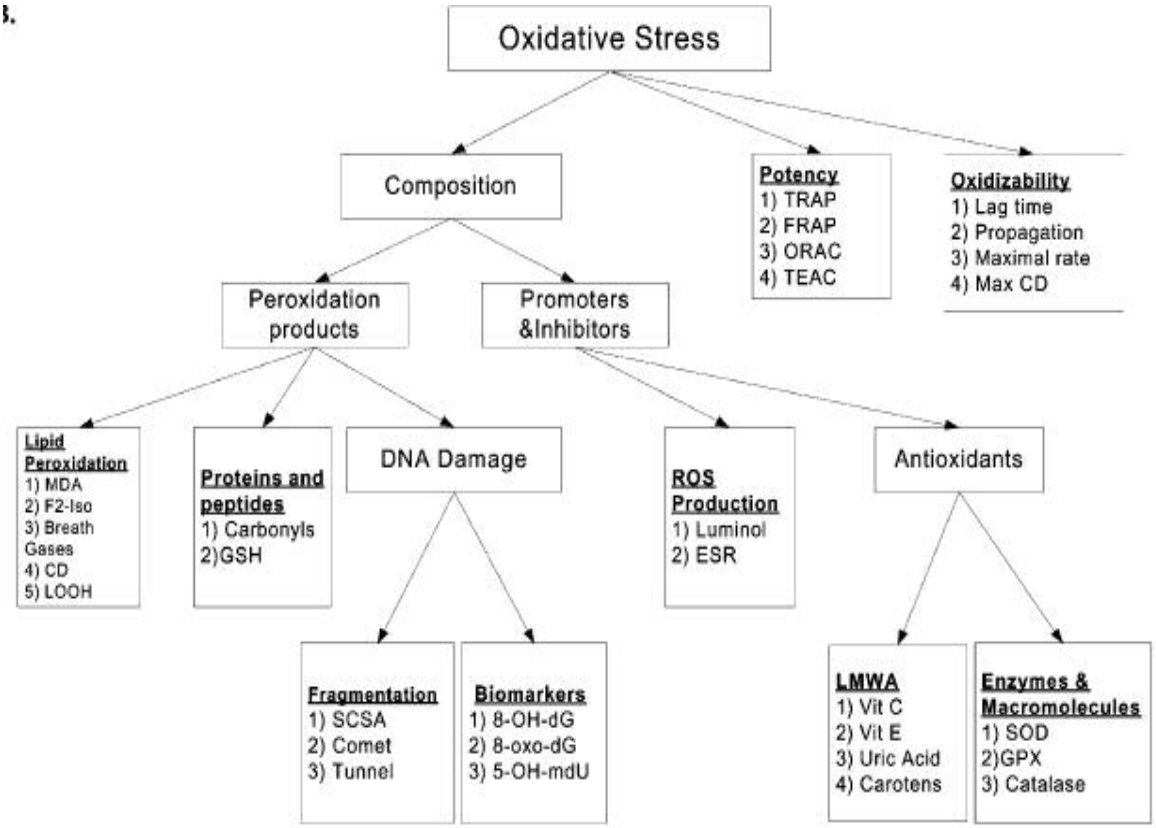
well as aging [112]. 8-OHdG increases have not always been reported in response to exercise [112, 221].

## Measurement of Oxidative Stress Markers

Many methods are currently available for one to quantify oxidative stress. Although markers can be measured following a variety of conditions, this review will mainly focus on markers following exercise. The literature has reported mixed results depending on the marker and type of exercise used. A major obstacle in oxidative stress research is that any measurement taken is only a snapshot of what is occurring at that particular moment in that particular tissue. Blood, serum or plasma levels are often assayed but the majority of the production of RONS appears to originate in the tissues. The balance between production and removal of these RONS in blood can result in an increase in oxidative stress in response to exercise of sufficient intensity and duration. However, in a recent report [227] the correlation between markers of oxidative stress in plasma and tissue was not high. Although makers of oxidative stress increased in both blood and skeletal muscles, the highest correlations for these increases were between muscles doing similar muscle actions. It has also been reported that not all markers of RONS increase in a similar manner to a specific oxidative stress [43]. It has therefore been recommended that oxidative stress be measured using several markers of RONS. In addition, there may be temporal variation in changes in the markers of RONS. Therefore, multiple time points should be determined to ascertain what is actually occurring in the body in response to this type of stress [80]. This list of measurements was not meant to be

all inclusive. For an extensive list of RONS associated measurements, please see Figure 1.

Figure 1: Methods for Determining RONS Associated Damage (adapted from [43])




## Cellular Protection Against RONS

The balance or ratio of prooxidants to antioxidants determines whether or not oxidative stress will occur in the cell. The antioxidant defenses exist in order to minimize

oxidative stress, and to protect macromolecules from oxidative attack. Table 2 lists some common antioxidants routinely studied in oxidative stress studies.

Table 2: Common Antioxidants

| Name | Type |
|---|---|
| Cooper Zinc SOD (Cu-Zn SOD) | Antioxidant Enzyme |
| Manganese SOD (Mn SOD) | Antioxidant Enzyme |
| Catalase (CAT) | Antioxidant Enzyme |
| Glutathione Peroxidase (GPX)<br>Glutathione Reductase (GR)<br>Glutathione (GSH/GSSG) | Antioxidant Enzyme<br>Antioxidant Enzyme<br>Thiol – hydrogen ion donator or acceptor |
| Vitamin C (ascorbate) | Antioxidant Scavenger<br>hydrogen ion donator |
| Vitamin E (α-tocopherol) | Antioxidant Scavenger<br>hydrogen ion donator |
| Carotenoids | Antioxidant Scavenger |

Adapted from Halliwell and Gutteridge [64]

Enzymatic Antioxidants

One of the major antioxidant enzymes is superoxide dismutase (SOD). As the table indicates, SOD actually exists in two separate forms, copper-zinc SOD, which is also referred to as SOD I, and is mainly found in the cytoplasm, and manganese SOD (Mn SOD) which, is also referred to as SOD II and is found primarily in the mitochondria. SOD is an enzyme that aids in the conversion of the highly reactive superoxide to hydrogen peroxide. The reaction looks like this:

$$O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2.$$

The hydrogen peroxide that is produced has the ability, in the presence of transition metals, to convert into the hydroxyl radical. The main function of glutathione peroxidase (GPx) and catalase (CAT) is to inactivate hydrogen peroxide before it can form the hydroxyl radical. GPx exists as both a selenium independent and selenium dependent enzyme [194]. This explains why selenium is commonly added to antioxidant cocktails in the literature [50, 53]. If adequate reduced glutathione (GSH) is present, GPx aids in the conversion of hydrogen peroxide to water and oxidized glutathione (GSSG). The reaction is:

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$$

For this reason, the amount of total glutathione (TGSH), GSSG, GSH, and the GSH to GSSG ratio have been measured to assay oxidative stress [20, 52, 53, 87]. The enzyme glutathione reductase (GR) is used to convert GSSG back to GSH in the presence of NADPH.

Mgst1 is the gene believed to be of great importance in the glutathione defense system. The Mgst1gene functions to code both glutathione transferase (GST) and glutathione peroxidase (GPx) [104]. Clearly, GST and GPx levels are important in maintaining the protective abilities of the glutathione system. It has been previously reported that Mgst1 plays a role in cancer defense [45], cell survival [116], and in response to RONS exposure [111]. This protein appears vital in proper glutathione pathway function and warrants further investigation.

The antioxidant enzyme CAT also functions to convert hydrogen peroxide into water and oxygen. The reaction is as follows in the presence of CAT [64]:

$$H_2O_2 \rightarrow H_2O + O_2.$$

Non-enzymatic Antioxidants

The most common types of antioxidants that are non-enzymatic come from the diet in the form of various vitamins (e.g. C and E), minerals (e.g. selenium, zinc, manganese), carotenoids (e.g. beta-carotene), and flavonoids. The exact amount of dietary intake for these antioxidants is unknown and likely is dependent on the training and health status of the individual. Several investigations have concluded that regular training increases endogenous antioxidants [38, 85, 160, 179]. However, oxidative stress/damage can still occur even with increased basal levels of endogenous protection. Several investigators have shown that antioxidant supplementation can cause additional protection to either reduce or prevent oxidative stress [49, 50, 53].

The two most studied dietary antioxidants are vitamins C and E. These vitamins work together to combat RONS. Vitamin C primarily works in the aqueous areas of the cell whereas vitamin E works on the lipid portions of the cell. They can interact with each other as well. Vitamin E (α-tocopherol) can help reduce lipid peroxidation, as it acts on peroxyl radicals, forming a vitamin E radical as a byproduct. This vitamin E radical is less reactive than the peroxyl radical and then can be either quenched or reverted to a reduced state with a hydrogen ion donor. Ascorbate (vitamin C) can regenerate vitamin E by accepting an electron from the vitamin E radical (or donating a hydrogen ion), thus converting it back into active or reduced α-tocopherol. Vitamin C radicals can either be converted back into ascorbate by GSH or excreted in the urine. In addition vitamin C can help to reduce oxidized glutathione (GSSG) to the reduced form of this thiol (GSH). Therefore, antioxidants are interrelated and can help reduce oxidative stress in both the lipid and aqueous portions of the cell [49, 50].

Many other non-enzymatic antioxidants function in the body as well. Metal-binding proteins bind to transition metals, denying them the opportunity to react with hydrogen peroxide to form the hydroxyl radical. Dietary minerals can also be important in the antioxidant defense system due to their role as critical components of a reaction or compound such as manganese, copper, zinc, and selenium in the enzymes Mn SOD, Cu-Zn SOD, and GPx, respectively. The vitamin A precursor beta-carotene has recently been under investigation for its potential role as an antioxidant, as it appears to bind to singlet oxygen [40]. These carotenoids can revert back to their normal antioxidant status by vibrational actions as well as being reduced with thiols. Various flavonoids consumed

through the diet may play a role in antioxidant protection, however the exact compound and mechanism of action remains to be elucidated [19]. There are a great number of flavonoids and their role as antioxidants in vivo has not been adequately investigated.

Chaperone molecules also seem to play a role in antioxidant defenses as they may protect macromolecules from oxidative damage. One of the most widely studied chaperone molecules are the heat shock proteins (HSP). For example, HSP 72 helps to synthesize new proteins by allowing them to fold and function correctly [127]. Antioxidants therefore function to decrease the amount of radical formation, or limit the progression of RONS associated damage.

## Aerobic Exercise Induced Oxidative Stress

Several primary and secondary sources of RONS production have been identified in response to exercise. Primary sources are those that actually produce RONS in response to a particular stress (e.g. exercise). Secondary sources are those in which there is a delay in RONS production due to the response of the stress often producing RONS through another mechanism. This includes damage associated with muscle inflammation and infiltration of macrophages and neutrophils to help degrade damaged muscle.

### Primary Sources of RONS Production

It appears as though the major source of radical production is the electron transport chain that uses molecular oxygen to produce ATP. Most oxygen is converted to

water at the last step of the electron transport chain. However, 3-4% of the oxygen used in the electron transport chain leaks out as singlet oxygen at rest [4, 79, 212]. It has been purported that as oxygen consumption increases, such as would occur during exercise that singlet oxygen leakage would increase proportionally. During an exercise bout, oxygen uptake may increase by up to 15 fold above resting values. Reid et al., provided evidence of this when they reported increased markers of RONS in the interstitial fluid following repeated muscle contractions (similar to exercise) [172, 173].

Another potential primary source of RONS production is prostanoid metabolism. Prostaglandins are released from cells within the muscle in response to a stress (i.e. exercise). It was reported that skeletal muscles subjected to excessive contractile activity released prostaglandins [126, 176]. One of the intermediates produced during prostaglandin metabolism is a RONS. In addition, arachidonic acid, a prostaglandin precursor, can be converted into active RONS via lipoxygenase and one of the products, 8-isoprostane, has been shown to increase with exercise [134].

During exercise, catecholamines (epinephrine and norepinephrine) are released in order to increase heart rate and control blood flow as well as to increase metabolism of lipids and carbohydrates. These hormones help prepare the body for the "fight or flight" response. It is currently believed that these catecholamines autooxidize, resulting in increased RONS formation [79, 83].

The enzyme xanthine oxidase has been reported to increase RONS levels [79, 96]. As ATP is degraded (as would be the case during exercise) incidents of ischemia, followed by reperfusion (often termed I-R injury) leads to increased ADP formation.

ADP degradation leads to the conversion of xanthine dehydrogenase to xanthine oxidase, which is a superoxide producer [131]. This situation usually occurs in the presence of hypoxanthine, which can be a substrate for either xanthine dehydrogenase or xanthine oxidase. This situation usually manifests itself when calcium homeostasis is altered [125]. This likely happens due to repeated skeletal muscle contractions.

NADPH oxidase, primarily found in neutrophils, can also be a RONS producer. Whether or not this happens in skeletal muscle appears to be speculative, as the presence of NADPH oxidase in skeletal muscle is undocumented.

Secondary Sources of RONS Production

As previously discussed, RONS can also be generated as byproducts of tissue injury-related processes. These RONS are likely key factors in determining the degree of secondary damage to the muscle, and may be a necessary component for muscle repair and remodeling [26]. Secondary sources of RONS include phago cytic cells, disruption of iron-containing proteins, and excessive calcium accumulation (or loss of calcium regulation). Damaged fibers have been shown to have increased levels of phagocytic cells (e.g. neutrophils, monocytes, macrophages) within the affected muscle fibers [114]. The phagocytic cells, such as neutrophils, can release superoxide radicals. Neutrophils act in part by degrading material (byway of superoxide radicals) and its activity is often determined by what is known as neutrophil burst activity. RONS production, in this case can serve a useful purpose at the level of damaged tissue, as these superoxide radicals help in degrading severely damaged tissue so that regeneration of new and healthy tissue

can occur. However, these RONS have also been known to attack some of the surrounding healthy tissue causing damage where it is not intended [119].

Iron disruption from iron-containing proteins can increase free iron [62, 63, 81, 82]. Excessive free iron can stimulate reactions that can result in protein and nucleic acid degradation as well as peroxidative damage to lipids. The Fenton reaction can lead to hydroxyl radical formation. The increase in free iron can stimulate the amount of hydrogen peroxide generated into the hydroxyl radical. In addition, the iron catalyzed Haber-Weiss reaction can form the hydroxyl radical. Certain exercises (primarily eccentric type actions) can cause muscle injury, which likely damages iron containing (and other transition metal) proteins within the muscle (e.g. myoglobin) and releases free iron [174], making it available for these reactions. In addition, erythrocytes within the vasculature of these muscles could be damaged and release free iron. This could result in oxidative stress within the muscle and the blood.

A third source for secondary production of RONS is likely due to an imbalance in calcium homeostasis [10, 125]. Calcium may directly activate calcium dependent proteases, such as the calpains, that stimulate protein degradation [10]. In addition, calcium overload can lead to the dissociation of ATP production within the mitochondria leading to increased superoxide levels through the ADP mechanism discussed previously [128].

Since secondary sources of RONS manifest themselves through the inflammatory process, Atf3 is believed to be of importance. Atf3 is an activating factor with several proposed functions. However, its main functions appear to be in regard to cell cycle. It appears as though this gene acts as both an apoptotic factor [137] and as a cell proliferation

factor [206]. It has been hypothesized that this gene is a stress response gene [206]. Atf3 is induced by a variety of different physiological stimuli including ischemia, reperfusion, wounding, and other stress signals [61]. It appears as though any time a stress is induced, the cell will respond with increased Atf3 levels [1, 29, 109, 148]. Therefore, it appears that At3 levels may be an important factor in determining the extent of inflammatory injury present. Further research is needed to confirm this finding.

In conclusion, RONS can be produced from both primary and secondary sources and lead to a variety of maladies that can ultimately lead to the production of muscle degradation and injury and often a loss of muscle function [130]. These systems can be stimulated during or following exercise depending on the type of exercise and the intensity of the exercise. This stimulation is likely due to increased oxygen consumption with aerobic exercise, due primarily to ischemia-reperfusion and vascular factors with instances of repeated high intensity exercise and probably secondary sources during muscle damage. Establishing the mechanism by which radicals are produced will be difficult. It is likely that RONS production is due to the collective efforts of several pathways, simultaneously activated in response to exercise.

## Markers of Aerobic Exercise Induced Oxidative Stress

Aerobic exercise has consistently shown increased markers of oxidative stress in both blood and muscle in numerous investigations [4, 5, 21, 51, 54]. The actual magnitude of the increase in the markers of RONS is dependent on several factors, including the exercise protocol, the measurement taken, the source of analysis, subject characteristics,

as well as the intensity and duration of the exercise and when the measures were obtained. It appears that for significant increases in the markers of RONS to occur, the muscle must be sufficiently recruited (sufficient time and intensity) [49]. One difficulty in measuring oxidative stress after exercise is that varying degrees of activation of the markers of RONS can be noted. For example the activation of glutathione from the reduced to the oxidized form is rapid [52]. In contrast, the production of a lipid peroxidation end product such as MDA takes time [43]. Clearly, when one reads the oxidative stress literature this needs to be kept in mind. Since most studies use an individually selected exercise protocol, cross study comparisons can prove problematic.

It has been well established that regular aerobic exercise is beneficial at reducing morbidity and mortality. In fact, the American College of Sports Medicine has recommended that individuals should perform at least thirty minutes of mild intensity aerobic exercise on most days of the week [6]. However, it is also known that moderate to high intensity exercise (usually $\geq$ 65% of $VO_2$ max) will likely increase markers of oxidative stress [49, 50, 167]. Table 3 presents a brief summary of several findings related to exercise induced changes in markers of oxidative stress.

Table 3: Sample of Reported Oxidative Stress Following Aerobic Exercise

| Measure | Exercise | Change | Reference |
|---|---|---|---|
| MDA | 20 minute run at 60% max | ↑62% | [4] |
| PC | 60 minute run at 60% max | ↑50% | [52] |
| 8-OHdG | 30 minutes cycling at 70% max | No change | [21] |
| 8-OHdG | 3 km run | ↑ 21% | [145] |
| Lipid Hydroperoxides | 45 minute run at 75% max | ↑ 30% | [163] |
| Isoprostanes | 12 hour Triathlon at 80% max | ↑ 181% | [140] |
| GSSG/GSH | 40 minutes cycling at 60% max | ↑ 50% | [98] |

It appears as though aerobic exercise can significantly increase PC generation. A recent study by Alessio et al.[5] reported PC increased 67% immediately after exercise with values returning to baseline by one hour post-exercise. These findings have been supported by several authors, including Sen et al., who reported an ~85% increase in PC immediately after a treadmill run to exhaustion in rats [191]. Additionally, our lab has

reported increases in PC in both males and females at both 60% and 80% of $VO_2$ max, with 80% having a greater increase in PC compared to 60% $VO_2$ max [54]. A common denominator reported in these studies was a return to baseline within one hour post-exercise of the PC response. The limited research in this measurement associated with acute aerobic exercise suggests that PC can be elevated by aerobic exercise of $\geq$ 60% of $VO_2$ max. Also, PC values had returned to baseline within one hour post-exercise [5, 52, 191]. It is unclear if this is typical for all types of aerobic exercise. Further research is needed to establish whether this increased PC content has any long term physiological effects. In conclusion, it that in order to have significant changes in PC levels, one must exercise for at least 30 minutes at an intensity of at least 60% of max, and that this increase is transient and often returns to baseline within one hour post-exercise [21, 52-54]. Clearly, diet and training status of the individual may alter these responses; however this does appear to be the minimum exercise prescription to consistently report PC increases.

Several investigators have reported findings dealing with lipid peroxidation following an acute bout of aerobic exercise. It should be noted that not all of these studies have determined the same marker and not all studies report increases in lipid peroxidation. It should also be understood that the measurement of TBARS is nonspecific and caution should be used when interpreting changes in this marker. In addition, studies have involved both animals and humans.

In subjects who performed a treadmill run for 30 minutes at 80% $VO_2$ max, a 33% increase in plasma TBARS was reported 30 minutes post-exercise [3]. Additionally, a maximal run to exhaustion elicited a 26% increase in plasma MDA compared to baseline

[113]. Kanter reported increases in serum MDA when the subjects ran for 35 min at 60-90% $VO_2$ max [92]. Subjects that ran at 75% of their HR max for 45 minutes also demonstrated increases in serum MDA [122]. In contrast, low intensity exercise has typically not demonstrated increases in MDA in human [44, 113].

Some investigators have measured markers of lipid peroxidation in skeletal muscle. Most of these studies have utilized rats. In the hind limb muscles of rats run to exhaustion, TBARS content was increased up to 30%, dependent on the tissue analyzed [191]. Swimming increased MDA almost 100% in rat skeletal muscle [169], however, it should be noted that rat swimming may not be a good exercise model for humans, as rats don't swim in a typical fashion. When rats swim, they tend to bounce back and forth from the bottom to the top to keep their head above water. Alessio et al. [4] reported that MDA content in rat hind limb muscles increased to moderate intensity exercise for 20 minutes. The increase was modest and depended on the type of muscle. In a follow up study these authors reported that higher intensity exercise could increase MDA within muscle to a greater extent [2]. Li and coworkers also reported increased MDA in rats to moderate intensity running for 20 minutes [86]. Recently You et al. reported that 90 minutes of downhill running increased MDA 2 hours after the exercise in specific muscles of the rat hind limb [227].

These studies support the fact that exercise can significantly increase markers of lipid peroxidation following an acute bout of aerobic exercise of sufficient intensity and duration. The literature repeatedly has reported increases in markers of lipid peroxidation following as few as 20 minutes of exercise at a moderate intensity (at least 60% of max).

Again, these values typically returned to baseline within one hour (if this was measured). It is unclear if these increases in lipid peroxidation markers lead to a physiological response and this warrants further investigation.

Several published studies refute the aforementioned studies and have reported no changes in markers of lipid peroxidation after exercise. It was reported that 45 minutes of treadmill running at 75% of age predicted max heart rate did not alter plasma MDA content post-exercise [179]. In agreement with this study, two other studies have shown no changes in plasma MDA following one hour of step aerobics [124], or changes in TBARS following voluntary wheel running in rodents [188].

While many published studies do show increases in markers of lipid peroxidation following acute aerobic exercise, some studies have reported no changes. The differences in the results to lipid peroxidation are probably due to several factors that include: subject status, assay utilized, and differences in intensity and duration of exercise. Further studies are needed to establish at what minimum intensity and duration these markers will become elevated. As previously mentioned, some studies report significant alterations in lipid peroxidation following as few as 20 minutes of exercise at intensities as low as 60% of $VO_2$ max. Diet, training status, and marker measured likely account for differences seen in this measure. Similar to PC, these alterations appear to return to baseline within an hour.

Similar to lipid peroxidation research, the results of studies measuring DNA oxidation are mixed. One major discrepancy is that some studies have measured 8-OHdG in blood, while others have measured it in urine. In addition, some studies have

measured DNA strand breaks using the comet assay. This assay uses whole blood or isolated lymphocytes and examines the dispersion of DNA in agarose. The migration appears as the shape of a comet. The amount of DNA migration indicates the amount of DNA breakage in the cell. Using the comet assay several exercise studies have reported increases in DNA damage in human leukocytes [193]. These studies have used incremental treadmill runs to exhaustion and half-marathons and triathlons as the exercise stimulus. In all these studies humans were the subjects and the increase in the DNA damage was detected several hours after the exercise. For the triathlon study the peak increase was 72 hours after the competition. Another method is fluorometric analysis of DNA unwinding (FADU). Sen and coworkers reported that a single bout of 30 minutes of exercise induced DNA strand breaks in leukocytes [194]. In contrast to the above assays, 8-OHdG as the marker has shown mostly negative results. No alterations in human lymphocytes was noted in 10 trained runners after an 8 day training camp [145] and decreases were reported in dogs run on a treadmill for 7 hours in lymphocytes [146]. It was noted that lymphocyte levels increased in response to the exercise and this might have led to the decrease. Following a 30 kilometer run, urinary 8-OHdG was increased 21% above baseline [145]. In agreement with the previous study, DNA oxidation was 33% higher post exercise in men exercising approximately 10 hours per day [156]. Radak et al., reported increases in 8-OHdG of approximately 30% following runs of either 56, 59, 93, or 120 kilometers [166]. Several studies have not shown any changes in 8-OHdG in urine to multiple days of cycling, and distance running [193]. One should use caution when interpreting results of

urine changes in regards to DNA damage as it may only represent a small fraction of actual DNA damage [21].

In contrast to these extreme aerobic bout studies, several shorter duration studies have shown no alterations in 8-OHdG. Two studies published by the same researchers reported no increases in 8-OHdG following either an incremental treadmill test or a20 kilometer run [203, 204]. Our laboratory has also reported no changes in serum 8-OHdG following 30 minutes of cycling at 75% $VO_2$ max [21] or 30 minutes of running at 75-80% $VO_2$ max in either men and women [19].

In conclusion, it appears that to consistently induce increases in DNA oxidation, exercise must be of a sufficient duration (a very long duration for 8-OHdG). Also, studies need to be completed linking the amount of 8-OHdG in the blood, muscle tissue and urine in order to properly evaluate these results. Leukocyte DNA damage in the blood may not reflect what damage if any is occurring within the muscle. It does appear that if one is using a sensitive measure of DNA oxidation (e.g. FADU, comet), as little as 30 minutes of exercise at a moderate intensity may increase markers of DNA oxidation. However, the only studies to consistently show increases in DNA oxidation have been extremely long durations (marathons and longer). No real time course for these measurements has been established.

Glutathione status has also been used as a marker of oxidative stress following aerobic exercise. It appears as though alterations in glutathione status are directly related to the intensity of the exercise session [190]. Laakonsonen et al. reported an increase in GSSG by 50%, while also reporting a decrease in GSH by 13% following 40 minutes of

cycling at 60% of $VO_2$ max [98]. Similar results have been reported following 90 minutes of cycling at 65% of $VO_2$ max [218]. Both of the aforementioned studies showed immediate alterations in glutathione levels, with levels returning to baseline within 30 minutes post-exercise. Published studies have also reported no changes in glutathione status. There were no reported changes in GSH or GSSG following 35 minutes of running at 60% of $VO_2$ max [27]. Also, it has been reported that trained subjects had no alterations in blood glutathione levels following a triathlon [120]. Clearly, these studies used different subjects, at different intensities, and different durations, which could explain the discrepancies seen in the data.

As seen with many other measures, glutathione exhibits mixed results as well. It does appear that as little as 30 minutes of exercise at a moderate intensity (60% max) may alter this marker. These changes appear to diminish as one approached 30 minutes post-exercise. Again, diet and training status of the individual can alter these results.

Taking all measures into account, it does appear as though exercise will increase some markers of oxidative stress following exercise if the bout is of sufficient intensity and duration. At present time, sufficient intensity and duration has not been defined and it likely to vary depending on the marker measured. Differences in the literature can likely be attributed to different training regiments, diet, as well as different types of subjects. Additionally, no study has ever measured every marker of oxidative stress. The possibility that some measures may show no change, while other measures are altered, clearly exists. However, most studies do seem to show increases in markers of oxidative stress in at least one measurement at one time point following aerobic exercise.

Markers of Aerobic Exercise Induced Oxidative Stress Protection

The aim of this project is to study both the prooxidant response and the antioxidant response to a single bout of aerobic exercise. Many studies have shown that exercise, while increasing oxidative stress, also increases the ability of the body to subsequently combat these stresses [85, 160, 212]. If exercise is of sufficient intensity and duration, many positive changes in the levels of endogenous antioxidants are observed. This is likely related to the exercise bout producing a sufficient amount of RONS. It is believed that the amount of protection is directly related to the amount of RONS incurred stress [167] during the bout. The more one undertakes aerobic exercise, the more protected the systems appear to be from subsequent RONS associated damage [85, 87, 160]. This could be due to either decreasing RONS production, or increasing enzymatic protection. Research seems to support the contention that aerobic exercise will induce an increase in SOD, GPx, and GSH, with mixed results in reporting alterations in CAT activity [160]. This has been documented in changes in the blood, muscle, and some measurements in mRNA levels [38, 84, 85, 157, 160]. Also, some investigators have not measured the source of protection, yet have focused on reporting decreased markers of RONS damage in response to endurance training [85, 103, 158, 160, 165, 177].

Before viewing changes in actual antioxidants, a review of selected publications showing antioxidant protection following exercise is warranted. These studies are often difficult to compare due to the differences in training intensities, durations, and exercise protocols. This list is not meant to be exhaustive, only to highlight several key studies showing protection. In elderly men run for 30 minutes, three times a week, for three

months. They reported that plasma MDA had decreased by 9% at rest and by 16% post-exercise, compared to controls [47]. In agreement with this, teenage soccer players who underwent normal sport training, had lower baseline and post-exercise blood MDA than age matched controls [132]. Radak et al. reported that regular exercise training decreased PC levels post exercise by 25% in rat muscle [167]. This same group also reported that swim trained rats (nine weeks 60 minutes/day, five times/week) showed a decrease in both PC and 8-OHdG compared to age matched controls post-exercise [165]. Also, rats undergoing ischemia-reperfusion injury in the myocardium following a single session of aerobic exercise had significantly less I-R associated damage than untrained rats [161]. This study suggested that protection may be elevated with as little as one bout of exercise.

In conclusion, it appears as though regular aerobic exercise can decrease PC levels, lipid peroxidation measures and may decrease 8-OHdG levels as well. Again, this measurement is dependent on the tissue involved and the time of measurement, as well as the training regimen and type of subject used.

It is generally believed that aerobic exercise training will result in an up-regulation of SOD, GPx, and GSH, with little or no change in CAT (see Tables 4-6). This appears to be tissue and intensity specific. For example, a recent study examining GPx levels following 10 weeks of treadmill training found increases in both GPx activity, as well as GPx mRNA, with no changes in CAT [222]. A similar study by Gore et al. reported increases in SOD activity and mRNA levels, with no changes in SOD protein levels following 10 weeks of treadmill training in the heart and some selected skeletal

muscles [55]. This raises the possibility that some alterations are transcriptional in nature, while others may be translational or posttranslational.

Glutathione status may also be altered. Aerobic training reportedly will increase resting GSH levels [190]. This increase in GSH may help lower oxidative stress associated measures, as it was reported that trained subjects had higher GSH at rest and this was correlated to lower lipid peroxidation levels post-exercise [98]. This suggests that subjects with elevated GSH at rest due to prior aerobic exercise bouts may be less prone to experience exercise induced oxidative stress.

The following three tables highlight much of the research in regards to activity of SOD (Table 4), GPx (Table 5), and CAT (Table 6) in response to training. Generally there is an increase in SOD and GPx activity in response to acute exercise in humans [160] and these findings have been observed in blood [143] as well as muscle [147]. These results have also been shown in rats, but are more mixed, likely because the type of muscle examined and the type of exercises used to induce the change were not standardized. In fact, studies have shown an increase in SOD activity following one acute aerobic exercise bout [101], as well as increases in SOD mRNA following a single aerobic bout [73, 74]. GPx activity has also been shown to be increased following a single aerobic exercise bout [89, 102]. CAT results are inconclusive, as researchers have published findings showing increases [60], decreases [207], and no alterations [87].

Table 4: Training Induced Alterations in SOD Activity

| Training | Muscle | SOD Activity | Reference |
|---|---|---|---|
| 10 wk treadmill | Vastus Lateralis | Increase | [103] |
| 12 wk treadmill | Soleus | Increase | [38] |
| 10 wk treadmill | Soleus | Increase | [158] |
| 18 wk treadmill | Vastus Lateralis | No Change | [2] |

Adapted from Powers et al. [160], all the above results were in rats.

Table 5: Training Induced Alterations in GPx Activity

| Training | Muscle | Activity | Reference |
|---|---|---|---|
| 5 wk treadmill | Gastroc | Increase | [192] |
| 12 wk treadmill | Soleus | Increase | [38] |
| 10 wk treadmill | Soleus | Increase | [157] |
| 12 wk treadmill | Soleus | Increase | [100] |

Adapted from Powers et al. [160], all the above results were in rats.

Table 6: Training Induced Alterations in CAT Activity

| Training | Muscle | Activity | Reference |
|---|---|---|---|
| 10 wk treadmill | Soleus | No Change | [157] |
| 18 wk treadmill | Vastus Lateralis | Decrease | [4] |
| 10 wk treadmill | Soleus | Decrease | [103] |
| 8 wk treadmill | Gastroc | Increase | [154] |

Adapted from Powers et al. [160], all the above results were in rats.

In summary, aerobic exercise, while increasing oxidative stress levels, can also increase protection from oxidative stress by either activating the antioxidant enzymes or possibly upregulating the concentration of some of these enzymes. For any adaptation to occur, the muscle must be recruited at a sufficient intensity and duration. Again, comparing studies often proves difficult since authors use different subjects, exercise protocols and measurements. It is unclear what the signals for the induction of these changes are within the muscle. Several redox signals have been suggested to activate nuclear material (e.g. NFkappaB, JNK, etc.) It is not known how acute exercise up-regulates the antioxidant protection thatlower s markers of oxidative stress and increases in the antioxidant enzymes.

## RONS and Cell Signaling

RONS have been implicated in apoptosis [170], necrosis [9], aging [161], and other normal cellular functions [57]. RONS have been linked to several diseases as well.

Although nobody has directly been able to prove that RONS can cause a disease, the fact that several diseases have shown increased RONS levels does warrant further investigation into this topic. For some diseases linked to RONS in humans, see Table 7.

Table 7: Sample of Diseases and Processes Linked to RONS

| Disease | Reference |
|---|---|
| Aging | [161] |
| Cancer | [214] |
| Diabetes | [219] |
| Arthritis | [69] |
| Coronary Artery Disease | [32] |
| Alzheimer's Disease | [186] |
| Muscular Dystrophy | [46] |
| Chronic Obstructive Pulmonary Disease | [56] |

Clearly, RONS can exert effects on cells through very complex signaling pathways. The exact intracellular signals controlling the reduction/oxidation (redox) status of the cell following exercise has not been fully elucidated. Also, many transcription factors are altered in response to exercise [23, 68, 155]. The transcription factor NFkappaB (NFκB), is an important factor in regulating the stress response. Oxidative stress conditions activate NFκB [107]. This signal is important in the cell cycle as it

modulates cell survival and appears to be a central transcription factor. Several other transcription factors including tumor necrosis factors (TNF-α, TNF-β), MEKK1 and MEKK3 all seem to play a role in activating NFκB [107]. C-Jun-terminal kinases (JNK) were reported to be activated during exercise [22]. It was purported that JNK can directly oppose NFκB [233]. Since JNK activation can lead to apoptosis [153], the balance between JNK and NFκB activity appears important for cell viability in response to oxidative stress. This is just a small sample of the numerous transcription factors that may be activated after an acute exercise bout. Identifying the transcription factors that are activated/inhibited, along with how these potentially interact with each other is of great importance for developing interventive strategies to prevent oxidative damage.

The role of NFκB and JNK were recently examined, and it was concluded that the two appear to be antagonists of each other [233]. Several studies have shown several areas of cross talk between NFκB and JNK that includes IL-1-RAK, MAP, ERKKK, and protein kinase C. In addition, it appears as though NFκB activated TNF-α was necessary for JNK activation [233]. This study suggests that NFκB may also indirectly activate JNK. In contrast, the antagonistic role of JNK on NFκB is under investigation. It has recently been shown that prolonged NFκB inhibition can lead to increased JNK activation [31], leading to increased apoptosis. Specifically, it is believed that RONS production leads to an imbalance in the cross talk between NFκB and JNK. It has been hypothesized that RONS may actually oxidize activated NFκB [133], thus leading to increased JNK expression. This has been confirmed in recent studies in which prolonged hydrogen

peroxide exposure lead to prolonged JNK activation [30, 180]. The mechanism for how RONS alter JNK is currently under investigation.

Antioxidant enzyme activity (concentration) has also been implicated in cell signaling. SOD levels were unchanged by NFκB inhibition [30], but GPx levels were decreased following NFκB inhibition [180]. It is unclear if the increases in SOD with endurance training are a result of changes in cell signaling through either NFκB or through JNK or some other factors. If NFκB inhibition resulted in an increase concentration of GPx it is likely that JNK and other cell signaling factors are also involved.

It has been well documented that NFκB inhibition increases apoptosis [233], however, the apoptotic activity of JNK is not well understood, as JNK can activate both apoptotic and antiapoptotic pathways. These authors have suggested that further research is needed in order to determine the exact role of the signals [233].

There are many factors which may alter NFκB or apoptosis as well. It has been reported that inhibitor protein kinase beta ($IK_\beta$) must be present in adequate amounts, and phosphorylated by an IKK complex in order for NFκB to become activated and elicit its response [71]. The factor pBAD (**B**cl-2/Bcl-XL-**a**ntagonist, causing cell **d**eath), when activated by calcium, can translocate from the cytosol to the mitochondria and cause cytochrome c release, thereby increasing RONS levels, eventually leading to cell death [197]. This is not meant to be all inclusive, as there are several factors not mentioned that have the ability to alter NFκB or apoptosis not mentioned (i.e. p50, p65)

As previously mentioned, NFκB plays a major role in the stress response to many events, such as exercise [107]. The role NFκB has in cell survival especially in relation to exercise has not been elucidated. It has been established that NFκB can oppose apoptosis in most situations; however, several stimuli that activate NFκB also can lead to apoptosis. This is apparent with TNF activation. TNF has repeatedly been shown to up-regulate apoptosis, and activate NFκB. However, the activation of NFκB leads to the activation of the antiapoptotic gene products such as TRAF1, TRAF2, and FLIP [107]. While NFκB may seem like it protects the cell, the over expression of NFκB , through the cell cycle progression regulators c-myc and cyclinD1 has been associated with cell proliferation and tumor progression [171]. Researchers have concluded that the balance between cell survival and cell death likely is dependent on NFκB working on several cell signaling factors [107].

A recent study examining the relationship between cell signaling and exercise was completed by Boluyt et al. [22]. The investigators were specifically concerned with JNK protein activation in the heart by exercise. Untrained rats were assigned to different exercise groups; low, medium, and high intensity, as well as a long duration group. Additionally, one group also underwent 6 weeks of aerobic exercise training. In the untrained rats, the higher the intensity, the greater the JNK response, with the high intensity group showing an increase of 250% over untrained controls. Chronic training down-regulated the JNK response as trained animals showed no differences above baseline in JNK activation. JNK is activated in response to cellular stress events. When JNK is activated, it leads to the activation of the following DNA binding proteins: c-Jun,

JunB, JunD, ATF2, and Elk1 [202]. Contractile activity has also been shown to increase JNK levels [11, 24]. These authors believed that JNK was somewhat responsible for the protection seen after exercise as it has the ability to activate DNA by several different mechanisms [22]. It was hypothesized that JNK activation phosphorylates heat shock factor 1, which activates various heat shock proteins [149]. However, JNK activation is a necessary step in mitochondrial apoptosis [22]. Further research is clearly needed to distinguish the extent to which JNK activation plays in both cell protection and cell death.

Another study showing the apoptotic effects of JNK was completed by Peng et al. [153]. Parkinson's disease has recently been characterized by high levels of JNK and phosphorylated c-Jun. It appears as though prolonged activation of the JNK/c-Jun pathway results in apoptosis both in vivo and in vitro [153]. An interesting finding from this study was that antioxidants (using a specific manganese antioxidant) could lower JNK activation by 66% [153]. The authors noted that JNK was activated in response to stress. It is unclear which pathways are up-regulated and how these cell signaling pathways interact to result in better protection against stress.

## Nuclear Factor Kappa Beta and Aerobic Exercise

Several studies have identified alterations in NFκB protein levels. Some of these studies have actually used exercise as a model to induce these changes, while others have simulated exercise through ischemia/reperfusion (I-R) or heat induction. This review is not meant to be all inclusive, but to only highlight some published literature.

It has been previously established that during the exercise response, the inflammatory process is activated. Viedt et al. reported thatNF κB activity increased 125% when inflammatory processes are activated [217] as would be seen during exercise. Other experiments have used heating to induce NFκB. It was reported that rats exposed to 41°C for 30 minutes not only demonstrated significantly altered muscular MDA content (by 200%) and GSH/GSSG ratio (by 50%), but had almost a three fold increase in NFκB activity one hour post-heat exposure [231].

As previously mentioned, I-R is commonly used as a model in experiments which simulates exercise as well. It has been reported that 90 minutes of I-R exposure will significantly increase NFκB protein activity levels by one hour post-exercise. This same study also reported that levels had returned to baseline within 4 hours post I-R [224]. Another group reported NFκB alterations following I-R injury. Sung et al. reported that 60 minutes of ischemia significantly elevated NFκB activity by 100% above baseline. Further, the activity remained elevated above baseline during reperfusion through one hour (increase of 150%), with the greatest increase seen 15 minutes into reperfusion [205]. No measurements were made between one hour and two hours of reperfusion.

Some investigators have used exercise as their stress model to induce NFκB alterations. Radak et al. reported that aerobic exercise not only significantly altered the GSH/GSSG ratio, but also significantly elevated NFκB levels and activity. This study also reported that younger animals (18 month old) had significantly lower levels of NFκB compared to that that of aged rats matched for time and percent of maximal effort [164]. Ji et al. reported alterations in both NFκB activity and levels following a one hour run at 25

m/min at 5% grade in 4 month old rats (this corresponds to about 75% of max). This group reported increase of 60% in NFκB activity and 250% in NFκB levels immediately post-exercise, suggesting a link between NFκB and oxidative stress [88]. In addition, this group has previously shown increase in NFκB binding to its DNA binding domain on the MnSOD gene [74]. Taken together, the authors speculate that this likely means that since MnSOD is a known antioxidant gene activated by RONS from a contracting muscle, that NFκB signaling is therefore directly related in the signal transduction process to increase antioxidant genes following exercise [88]. Finally, a recent study published by Atherton et al. studied whether a fiber type difference in NFκB levels existed. These researchers noted that NFκB levels were 300% higher in the type I soleus muscle over the type IIx and IIb extensor digitorum longus (EDL). These authors concluded that type I fibers were more dependent on NFκB signaling in their signal transduction and alterations seen in this fiber type are more likely to involve NFκB dependent pathways [13].

In conclusion, clearly NFκB plays a major role in the response to various stresses. Exercise is a stress model, and many other models that have been used simulate stress contain an aspect which would occur during exercise (e.g. I-R during contraction, heat). Repeatedly, these stress models report increases in both NFκB levels and activity.

## Conclusions

In conclusion, exercise can increase oxidative stress if the session is of sufficient intensity and duration. This exercise session also has the ability to increase protection

from oxidative stress. It is unclear how this increase in production of RONS will influence the cells viability. There are many cell signaling processes that appear to be influenced by exercise. It is unclear how these cell signaling pathways act to activate certain genes or inhibit other genes. There is a need to determine the genetic factors that are activated and inhibited by exercise induced oxidative stress. By understanding the signals affected, better therapies and treatments can be devised to help protect cells against oxidative stress.

# CHAPTER III

# METHODS AND PROCEDURES

This chapter presents the methods and materials utilized for the completion of this dissertation. It is organized into the following sections: Subjects, Experimental design and protocol, Genetic analysis, and Protein analysis.

## Subjects

Sixteen 10 week old Sprague-Dawleyfemale rats from the same litter (obtained from Charles River Laboratories, Wilmington, MA) were used in this experiment. Rats from the same litter (from the same F1) were used in order to limit genetic variation between the animals at baseline. All animals were individually housed and were fed and given water *ad libitum* in the animal facility in the Stone Building. Additionally, rats were housed for 2 weeks prior to any intervention in order to eliminate the stress response of transport and a new housing environment. All rats were handled daily and put on the rat treadmill daily for 10 min and run at a very mild intensity (10 m/min) in order to limit their apprehension due to these stresses. This intensity of running was reported to have no training adaptations in oxidative capacity (unpublished observations). Rat cages were cleaned daily, and rat chow was replaced daily up until 12 hours prior to the experiment at which time the rats began fasting. The animals were housed (one per

cage) in a standard plastic cage using a 12:12 hour reverse light cycle in a temperature (21 $\pm$ 1 ºC) and humidity (30-40%) controlled room.

## Experimental Design and Protocol

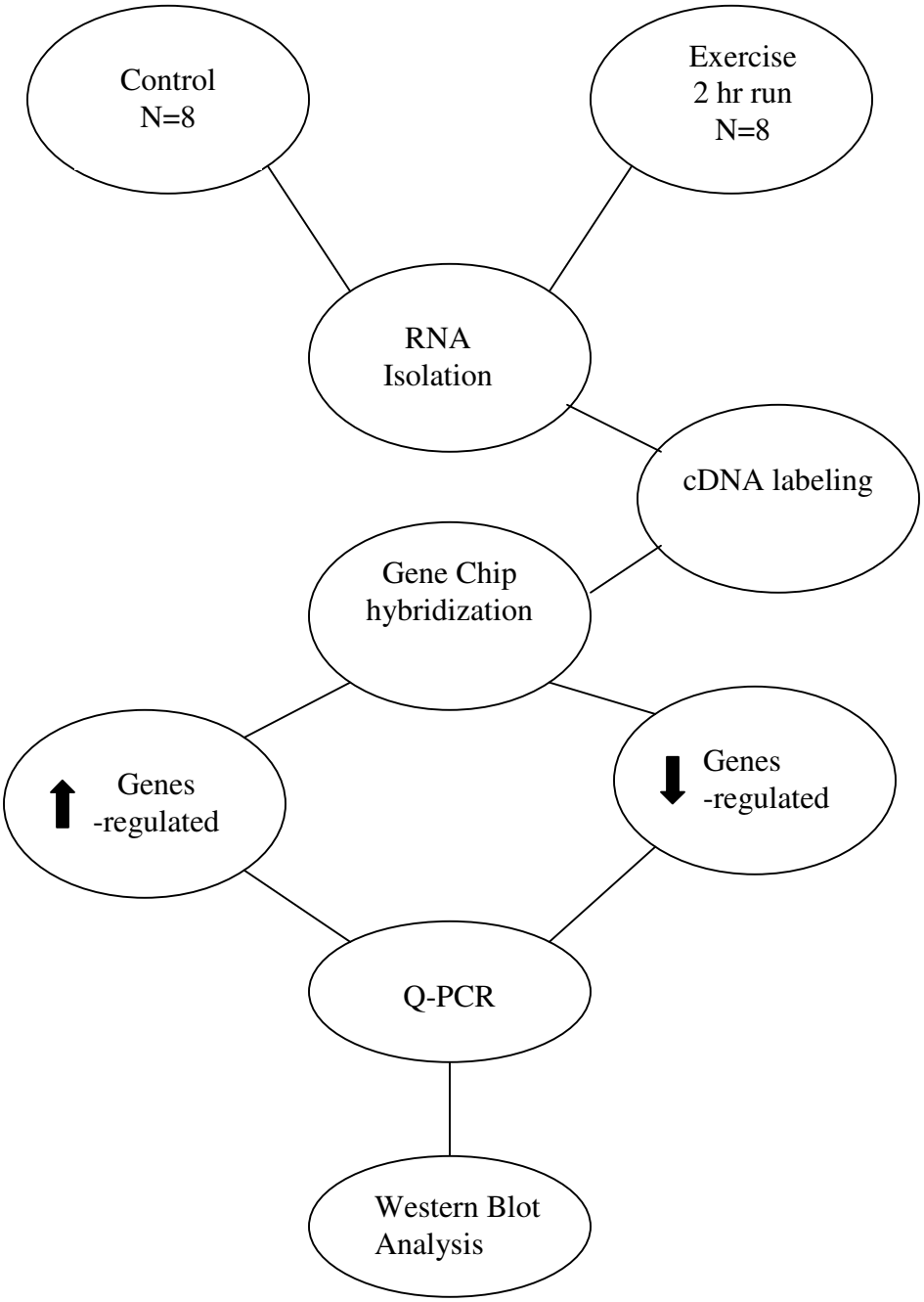
The exact protocol used was approved by the UNCG institutional animal care and use committee (IACUC). On day 15, after 2 weeks of housing, rats were randomly assigned to an exercise (E) group (n=8), or a control (C) group (n=8). The E group was fasted for 12 hours and then ran on the motorized treadmill for 2 hours. The 2 hour duration was selected based on work by Hildebrandt et al. who reported that transcriptional activation following exercise is intensity and duration dependent [68]. The 2 hour run at 22 m/min was of sufficient intensity and duration according to these authors who reported many adaptations at times ranging from 45 minutes to 3 hours. The treadmill contained a slight electrical shock at the rear of the treadmill for motivation which was only used if the animal stopped running. All exercised animals were able to complete the 2 hour run. Mild electrical shock at the back of the treadmill is commonly used to induce or stimulate the rat to run. The shock was calibrated to insure it was in the appropriate range for stimulating but not too intense. During the treadmill run the rats may have had electrical shock (3.9 volts and 0-2 mA in a pulse manner) applied to their tails to stimulate them to run. The rats only got a shock if their tails came in contact with the grid in the back of the treadmill. Typically, the rats learned to stay away from the grid. The purpose of having the rats familiarized with the treadmill and running 2 times per week was to minimize contact with the electrical shock. The rats ran at 22 m/min at

0% grade, which is considered a mild exercise intensity that was been shown to increase protein carbonyls in blood and decrease reduced glutathione and increase oxidized glutathione from rats exercised in our lab [227].

The one hour post-exercise time point was chosen as a result of personal communications with investigators who have recently completed similar experiments as well as previously published exercise data that found changes in gene expression at this time point [23, 41, 94, 155]. Rats were sacrificed by decapitation one hour post-exercise for the E group, and at a matched time for rats in the C group. Decapitation was used as previous experiments have shown that anesthesia can significantly alter the gene expression profile of a rat [17]. Upon sacrifice, the soleus muscles from both legs was rapidly removed from each animal and were placed in RNALater (Sigma-Aldrich R0901) and immediately frozen in liquid nitrogen between cooled aluminum blocks and stored at - 80ºC until assayed. The soleus muscle was chosen because it primarily contains slow twitch muscle fibers and therefore will be sufficiently recruited during the run [102].

The experimental design is shown in Figure 2.

Figure 2: Experimental Protocol



## RNA Isolation

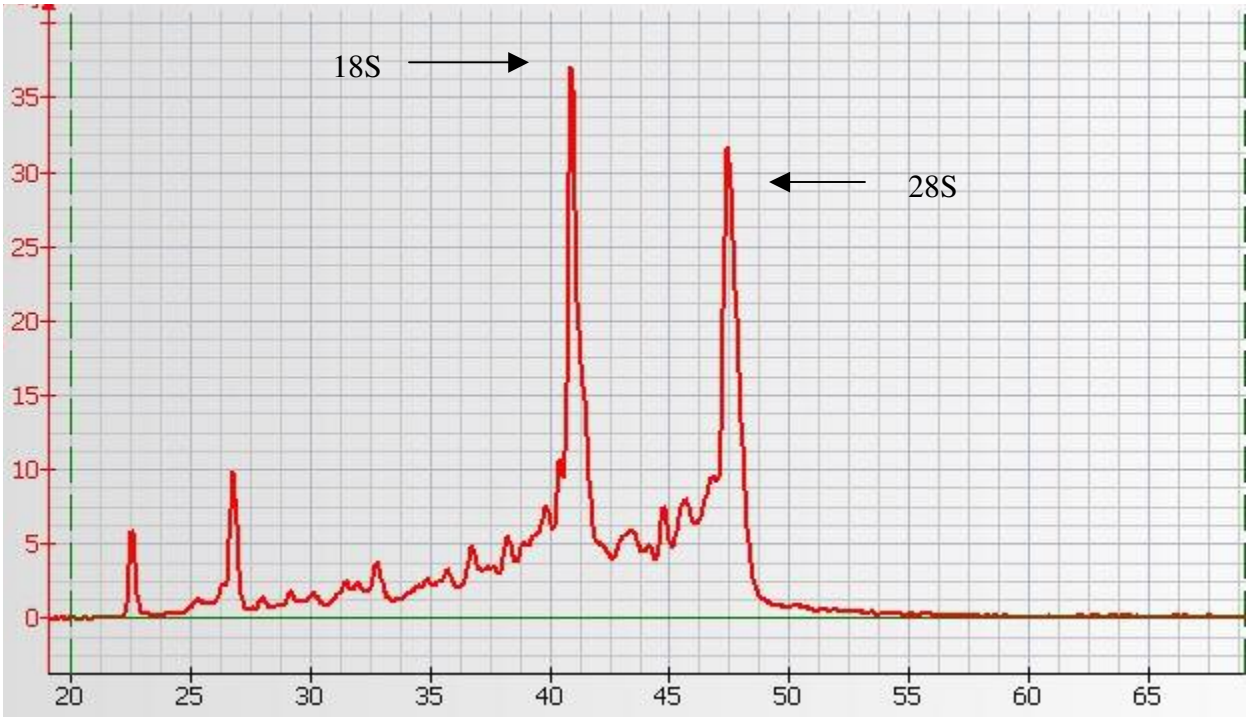
RNA was isolated from the soleus muscles using a modified Chomczynski technique [36] by using the RNA Isolation Kit from Fluka (#83913 Sigma-Aldrich, St. Louis, MO). All instructions were followed using a standard of 10mgof wet weight per soleus muscle (most muscles weighed ~10mg) and all solutions were supplied in the kit Fresh soleus muscles that had been clamped in precooled aluminum blocks were weighed and the muscle placed into solution D (solution D was made by mixing 10µl of β-mercaptoethanol with 1.4 ml of denaturing solution). The soleus muscle was then homogenized in Trizol using a Virtus Polytron homogenizer (Gardiner, NY). Approximately 10 mg of soleus muscle was used from each animal. Once homogenized, samples were quickly frozen in liquid nitrogen and stored at -80°C until assayed.

Samples were thawed and 100µl of 2M sodium acetate (pH 4.0) was added and the sample was mixed by inversion. Next, 1ml of phenol saturated with water was added and the sample was again, mixed by inversion. 200µl of chloroform:isoamylalcohol mixture (49:1) was then added and the sample was shaken vigorously for 10 seconds before being placed on ice for 15 minutes. At this point all samples were centrifuged at 10,000 g for 20 minutes at 4°C. The top aqueous layer was then transferred into a new tube as RNA is found in the upper aqueous phase. In contrast, DNA and proteins are left in the interphase and phenol phase respectively. Roughly 1ml of isopropanol (equal volume to the aqueous layer) was added and the samples were well mixed. The samples were then stored at -20°C for 60 minutes in order to precipitate the RNA. These samples were again centrifuged at 10,000 g at 4°C for 20 minutes to recover the precipitated

RNA. The supernatant was removed and the pellet was saved. The pellet was then resuspended in 300µl of solution D and mildly mixed using a pipette. 300 µl of isopropanol was then added and the samples were mixed and placed at -20°C for 60 minutes. The samples were then centrifuged at 10,000g for 10 minutes at 4°C. The supernatants were removed and 300µl of pre cooled 75% ethanolwas added and mixed. The samples were then sedimented by centrifugation at 10,000g and 4°C for 15 minutes. The samples were then centrifuged for 45 minutes at room temperature in a vacuumed centrifuge until the RNA pellet was dried. RNAse free water (50µL) was added and the samples were incubated at 65°C for 10 minutes to resuspend the RNA. At this point, approximately 45 µl of the RNA from each sample was frozen at -80 °C for further analysis, and 5 µl was used for the subsequent quality analysis.

The concentration of nucleic acids to amino acids was measured in OD buffer by an optical density ratio ($A_{260}/A_{280}$) via an Eppendorf Biophotometer (Eppendorpf-Brinkman; Westbury, NY). A ratio of 1.8 or better is acceptable for this ratio. All samples were checked for RNA quality using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). This machine is now being used in place of running the samples on agarose gels [59, 77, 110, 187]. A sample with 2 clear spikes at 28S an 18S on the Bioanalyzer readout is considered pure. Briefly, this machine takes RNA samples (5 ng of total RNA) loaded on a chip (Agilent catalog #5067-1511) and over the course of 30 minutes, electrophoretically separates the loaded components. The machine then takes the fluorescence and translates them into gel like bands and electropherograms (peaks, see Figure 3).

Figure 3: Sample Readout from Bioanalyzer




Statistical Analysis for RNA Concentration

Group means were analyzed using an independent T-test. Significance was set *a priori* at $p \leq 0.05$.

Microarray Analysis

For aminoallyl labeling, 10 µg of DNase I-treated and purified total RNA was combined with 2 µl of random DNA hexamers and RNase free water for a final volume of 17µl. The samples were then mixed well and incubated at 70°C for 10 minutes, and then at 4°C for 5 minutes. 13 µl of Master Mix (5x Buffer, 0.1M DTT, 20x dUTP, and

Superscript II RT transcriptase) was then added to each sample and each sample was then mixed and incubated at 42°C for 2 hours. To hydrolyze the RNA present, 1µl of RNase was added to each sample and each sample was incubated for 30 minutes at room temperature.

For reaction purification (removal of unincorporated aa-dUTP and free amines), the Qiagen QIAquick PCR purification kit was used (catalog # 28104) per manufacturers instructions. Each sample was mixed with 150 µl buffer PB and transferred to a QIAquick column. The column was placed in a 2 ml collection tube (Qiagen supplied) and centrifuged at ~13,000 rpm for 1 minute. The column was then washed with 750 µl of phosphate wash buffer and centrifuged at ~13,000 rpm for 1 minute. The collection tube was emptied and rewashed and recentrifuged. The column was then transferred to a new 1.5 ml microcentrifuge tube marked with the appropriate dye color and 30 µl phosphate elution buffer was added to the center of the column membrane. This was then incubated for 10 minutes at room temperature. The samples were then centrifuged at ~13,000 rpm for 1 minute. The samples were then dyed a second time by repeating the last 2 steps for a final volume of 60 µl and 1.5 µl from each sample was checked for purity using a spectrophotometer. The sample was then dried in a speed vacuum centrifuge.

To couple the aa-cDNA to the Cy Dye Ester, the aminoallyl-labeled cDNA was resuspended in 5 µl 0.1 M sodium carbonate buffer ($Na_2CO_3$, pH 9.0) and 5 µl of the appropriate NHS-ester Cy dye was added (Cy3- or Cy5 ). Each sample was then incubated for 1 hour in the dark at room temperature. Each sample then had 5 µl of 4 M

hydroxylamine added and each sample was then incubated for 15 minutes in the dark at room temperature.

For the second reaction purification (removal of uncoupled dye)**,** 35 µL of 100 mM NaOAc (pH 5.2), and 250 µl (5X reaction volume) of Buffer PB was added to each reaction tube. A QIAquick spin column was placed in a 2 ml collection tube and the sample was applied to the column, and centrifuged at ~13,000 for 1 minute. The collection tube was then emptied and washed using 0.75 ml of Buffer PE to the column and centrifuging at ~13,000 for 1 minute. The column was then placed in a clean 1.5 ml microcentrifuge tube and 30 µl of Buffer EB was added to the center of the column membrane. The column was then incubated for 10 minutes at room temperature. The column was centrifuged at ~13,000 rpm for 1 minute. The last 2 steps were repeated twice until a final volume of ~60 µl was present. From each sample, 1.5 µl was checked for purity using with a spectrophotometer. The sample was then dried in a speed vacuum centrifuge.

For hybridization, the pellet was resuspended in 190µl nuclease-free water, and then heated for 3 min at 98$^{o}$C, and then cooled to room temperature. The cDNA target hybridization solution was prepared with 215µl of cDNA, 35µl of 10x control targets and 250µl of 2x hybridization buffer for a total volume per array of 450 µl (rat microarrays catalog # G4130A from Agilent in Palo Alto, California). The samples were then mixed by pipetting and by mildly spinning. The array slide was placed into the hybridization mix with the numeric barcode side up and hybridized at 60$^{o}$C for 17 hours. All slides were then washed with wash solution 1 for 10 minutes at room temperature, and then

finally washed in wash solution 2 for 5 minutes at 4°C. All slides were then dried and analyzed using an Axon GenePix Pro 5.1 scanner (Union City, CA) with computer support for comparative and clustering analyses involving this array output.

## Statistical Analysis for Microarray

Early experiments set the bar for significance in microarray data as any gene which had a 1.5 fold increase or decrease as being considered significant [14, 66, 182, 183]. This criterion was used for this study. In addition, to verify the results of the microarray, real time polymerase chain reactions (Q-PCR) was performed on 4 genes. For the arrays, the samples were split in half, as samples E1-E4 were run against C1-C4 on arrays 1 and 2. Samples E5-E8 were run against C5-C8 on arrays 3 and 4. In order to make sure dye incorporation was not a factor is these experiments, dye swapping was performed. On array 1 and array 3, the exercised soleus muscles were incorporated with Cy3- dye, and on array 2 and array 4, exercised soleus muscles were incorporated with Cy5- dye. In order for a gene to be classified as significantly different between the 2 groups, the gene dye intensity had to be at least 1.5 fold different on all 4 arrays.

## Real Time PCR

Q-PCR was used to validate the microarray because it allows for cycle by cycle comparisons of RNA levels. Before this analysis could be completed, the RNA had to be converted to cDNA using reverse transcription (RT). This was performed using the

purified RNA isolated from the soleus muscle of each animal. The Qiagen Omniscript Reverse Transcription Kit (#205111, Valencia, CA) was used for this step based on the kit instructions. Briefly, 1µg of total RNA was added to RNase free water (#95284 Sigma Aldrich, St. Louis, MO) for a total volume of 6.5µl. A total of 3.5µl of Master Mix (using supplied chemicals) was added to each sample. The Master Mix contained 1µl of 10x buffer, 1µl of 5mM DNTs, 1µl of 10 µM gene specific reverse primer, and 0.5µl of reverse transcriptase. All samples were then loaded into a PTC-100 Thermocycler (#PTC-1196 Bio-Rad, Waltham, MA) for 60 minutes at 37° C.

For the PCR step, Taqman probe analysis was used. Using a specific probe for the gene to be tested helps ensure that the signal intensity reported is from the gene in question. It helps to eliminate non-specific binding. All samples were loaded onto an ABI 96 well plate (#436767, Foster City, CA) and the procedure followed the manufacturer's instructions. Each well contained the following: 5µl of cDNA, 25µl of PCR master mix (10x buffer, MgCl2, dNTPs, Taq enzyme), 5µl of forward primer, 5µl of reverse primer, 5µl of gene specific probe, and 5µl of RNase free water. For exact primer and probe sequences, please see Table 8. All primers were of known sequence [139]. All samples were run using the following conditions: 1) 95°C for 10 minutes 2) 95 °C for 15 seconds 3) 60° for 1 minute 4) repeat steps 2 and 3 for 40 cycles. The polymerase chain reaction serves to copy DNA.

When the Taqman cleaves the probe as more double stranded DNA is formed, it liberates the quenching molecule thus increasing the intensity of the fluorescent emission increases. As more double strands are produced, the signal will increase. This allowed

direct comparisons between the exercised and the control samples at any cycle. All of these reactions took place in an ABI 7900 HT Fast Real Time PCR System (#4351405, Foster City, CA).

Table 8: Primers and Probes used for Real Time PCR Analysis

| Gene | Genebank# | Primer | Probe |
|---|---|---|---|
| NFκB | NM_199267 | F-GTCTCAAACCAAACAGCCTCAC<br>R-CAGTGTCTTCCTCGACATGGAT | CCCCAAGCCAGCACCCCAGCCCTAT |
| TNFα | NM_012675 | F-GTTTCAGTTCTCAGGGTCCTA<br>R-CAGGATTCTGTGGCAATCTGG | AAAGGGATGAGAAGTTCCCAAATGG |
| Atf-3 | NM_012912 | F-CATCAGACCTGATTTCCGAGAGT<br>R- AACAGTTTGTAGCCAAGGACAGC | CTGCAGAAGGAGTCAGAGAAACTGG |
| Mgst-1 | NM_134349 | F- AGGCCGGTCATGTTAAATGTCTA<br>R- ACCGTCACCCTCTGATTGATTTA | ACGCGTGCGAAGAGCCCACCTGAAT |

Statistical Analyses for Q-PCR

Group differences were analyzed by an independent T-test using the $2^{-\Delta\Delta C_T}$ method. This is the analysis most frequently cited in the literature [25, 150-152]. This analysis uses the difference between the housekeeping gene and the control sample as $\Delta_1$ and the difference between the experimental group and the control group as $\Delta_2$. Significance was set *a priori* at $p \leq 0.05$. In this experiment, GAPDH (glyceraldehydes-3-phospahte dehydrogenase) was used as the housekeeping gene. GAPDH is commonly used as a housekeeping gene, and recent experiments by Mahoney et al. have confirmed that it is the housekeeping gene of choice for any experiment utilizing endurance exercise, as GAPDH remains unchanged through 48 hours post-exercise [117, 136].

## Protein Isolation

Muscle samples were homogenized in lysis buffer containing, 20 mM Tris (pH 7.4), 150 mM sodium chloride, 1% triton X-100, 0.5% non idet P-40 (NP-40), 1 mM ethylene diamine-tetraacetate (EDTA), and 1mM ethylene glycol-tetraacetic acid (EGTA). In addition, appropriate protease (0.3 µM aprotinin, 20 µM leupeptin, 1 µM pepstatin A, and 0.5 mM phenylmethylsulfonyl fluoride) and phosphatase inhibitors (2 µM sodium vanadate, 10 mM sodium fluoride, and 20 mM β-glycerophosphate) were included. Homogenates were then centrifuged at 10,000 rpm for 10 minutes at 4 °C to remove insoluble material. The supernatant was then placed in microcentrifuge tubes and protein concentrations of each soleus muscle was determined using the Lowry et al. [115] method. This method compares values to known protein standards determined on a spectrophotometer at a wavelength of 700 nm. Each sample (20µl) was combined with 1980 µl of distilled water and mixed to make a master mix for each sample. An aliquot of the master mix (20µl) was then added to a cuvette and 500µl of Lowry reagent and 480µl of distilled water were added. Each sample was then shaken and incubated at room temperature for 20 minutes. Then, 250µl of Folin's reagent was added to each sample, and the sample was shaken and incubated at room temperature for 30 minutes. Each sample was then read at a wavelength of 700 and the sample was then compared against known protein standards from bovine serum albumin standards. All samples were performed in duplicate.

## Western Blot Analysis

Western Blot analysis was performed using the previously published methods [99, 142]. NuPAGE 4x lithium dodecyl sulfate sample buffer (#NP0008, Invitrogen, Carlsbad, CA) and NuPAGE 10x reducing agent (#NP0004, Invitrogen, Carlsbad, CA) were added to each sample. A totalprotein of 8.3 μg per sample was loaded onto a NuPAGE Novex 10% Bis-Tris polyacrylamide gel (#NP0307BOX, Invitrogen, Carlsbad, CA). Gels were processed at 175 volts for four hours to separate proteins. The gels were then transferred onto a nitrocellulous membrane at 200 mAovernight at 4 °C in transfer buffer. After transfer, the membrane was placed in blocking buffer (4% non-fat dry milk in PBST) for 45 minutes followed by a serial wash with PBST (3 x 5 min). The nitrocellulose membrane was then incubated for 2 hours at 4 °C with anti-NFκB P-65 (#sc-8008, Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Atf3 C-terminus (#sc-188, Santa Cruz Biotechnology) antibody diluted 1:400 in non fat dried milk. After incubation with the primary antibodymembranes underwent a serial wash (3 x 10 min) with blocking buffer (4% non-fat dry milk in PBST). Membranes were probed with an anti-rabbit IgG secondary antibody (#sc-2372, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:2000in 2% non fat dried mile in TTBS for 1 hour in blocking buffer. An enhanced chemiluminescence (ECL) reagent was used to detect protein content and was visualized when exposed to Kodak autoradiographic film. Kodak densitometry software was used to quantify results based on band pixel intensity.

Statistical Analysis for Protein Concentration and Western Blot Analysis

Group means were normalized to the C group mean since optical density is an arbitrary unit and were analyzed using an independent T-test. Significance was set *a priori* at $p \leq 0.05$. Band intensity was normalized to the control group band intensity so that all differences are in arbitrary units in relation to protein levels in the control soleus.

# CHAPTER IV

# RESULTS

The results section is organized as follows: Animals, RNA Isolation, DNA Microarray, Real Time PCR, Protein Isolation, and Western Blot Analysis. All values in this section are presented as the mean ± the standard error the mean (SEM).

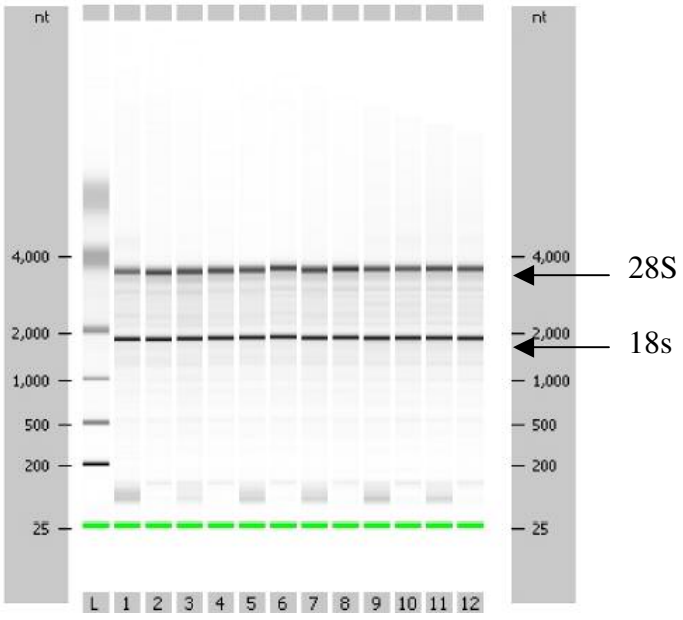
## Animals

Eighteen, two week old rats obtained from Charles River labs (originally weighing 100-120 g) were randomly assigned into either the exercise or the control group. All rats were housed in a temperature controlled (21 $\pm$ 1 $C^{o}$) room, on a 12 hour dark-light cycle and at a relative humidity of 45%. Rats were provided food and water ad libitum. All rats were familiarized to the treadmill and exercised for 10-15 minutes at least 5 days prior to the experiment. There were no differences in the body weights of the animals at the time of the experiment (E =175.3 ± 6.6g, C =170.9 ± 6.7g), or the weights of the soleus muscle (E =86 ± 3 mg, C = 84 ± 3 mg). Rats that were randomly assigned to run for the two hours all successfully completed the 2 hour run at 22 $m \cdot min^{-1}$. At one hour after the run the exercised animal and a control animal were killed by decapitation and the soleus muscles of both legs were rapidly removed.

## RNA Isolation

All RNA isolates met the criteria for high quality RNA as measured by the OD at 260/280, and as analyzed using an Agilent 2100 Bioanalyzer. No significant differences existed between the 2 groups in regards to RNA quality as measure by the 260/280 ratio (E = 2.01±.045, C = 1.94±.029), or RNA concentration (E = 1524±177, C = 1617±160 ng/ul). For a sample of the gel output by the Bioanalyzer, please see Figure 4.

Figure 4: Gel Output from Bioanalyzer




## DNA Microarray

A total of 52 genes were at least 1.5 fold different between the exercise and control group on all 4 arrays. The results are listed below in Table 9. All genes are

listed by their Genbank Accension Number, the Gene Symbol (if one is known), their average fold change across all 4 arrays, as well as the SEM. All genes shaded in red showed greater expression in the E animals, while genes shaded in green showed greater expression in C animals. All values are in fold change from control. Please see Appendix A for a complete list of every gene altered on the arrays, and Appendix B for the complete gene list organized by family.

Table 9: DNA Microarray Results

| Genbank Number | Gene Symbol | Mean Change | SEM |
|---|---|---|---|
| NM_012543 | Dbp | -1.66 | 0.02 |
| XM_215375 | LOC294337 | -1.95 | 0.05 |
| NM_021989 | Timp2 | -1.54 | 0.02 |
| AW918612 | | -1.82 | 0.05 |
| NM_012656 | Sparc | -1.78 | 0.05 |
| NM_031628 | Nr4a3 | 17.47 | 2.70 |
| XM_227446 | LOC310670 | -1.60 | 0.05 |
| XM_223124 | Col9a1_predicted | -2.20 | 0.13 |
| NM_031347 | Ppargc1a | 2.11 | 0.14 |
| NM_053356 | Col1a2 | -2.35 | 0.18 |
| NM_024388 | Nr4a1 | 5.26 | 0.90 |
| XM_213440 | Col1a1 | -2.59 | 0.23 |
| NM_138828 | Apoe | -2.12 | 0.15 |
| XM_225173 | Pfn3_predicted | -2.01 | 0.15 |
| NM_012912 | Atf3 | 4.87 | 0.73 |
| XM_346881 | LOC360478 | -2.10 | 0.17 |
| AA964296 | | 2.42 | 0.27 |
| TC483914 | LOC310395 | 2.15 | 0.19 |
| NM_021693 | Snf1lk | 3.67 | 0.59 |
| M12199 | | -2.27 | 0.27 |
| XM_343169 | Adn | -4.50 | 0.92 |
| NM_013086 | Crem | 2.41 | 0.32 |
| BF544403 | | 4.92 | 1.29 |
| AA858639 | Comt | -2.21 | 0.29 |
| AI013671 | | 2.84 | 0.46 |
| TC487987 | | 2.86 | 0.40 |
| NM_144744 | Acdc | -2.72 | 0.42 |
| NM_017151 | Rps15 | -2.10 | 0.25 |

| | | | |
|---|---|---|---|
| BF549650 | | 2.65 | 0.41 |
| NM_017240 | Myh7 | -2.35 | 0.35 |
| NM_001002850 | Prm1 | -2.21 | 0.32 |
| NM_017259 | Btg2 | 2.53 | 0.47 |
| NM_181368 | Mustn1 | 4.05 | 1.16 |
| XM_216062 | Smarcd3_predicted | -2.08 | 0.34 |
| NM_017239 | Myh6 | -2.08 | 0.35 |
| AW918430 | Lpin1_predicted | 3.13 | 0.71 |
| AI008316 | | 2.64 | 0.62 |
| NM_212505 | RGD:1303321 | -2.31 | 0.48 |
| NM_012604 | Myh3 | -2.43 | 0.49 |
| XM_341642 | Dok4_predicted | -2.32 | 0.46 |
| NM_175844 | Stars | 3.24 | 1.01 |
| NM_017184 | Tnni1 | -2.73 | 0.62 |
| NM_024349 | Ak1 | 2.16 | 0.48 |
| XM_340817 | Myh4 | 3.29 | 0.93 |
| XM_342599 | Col9a3_predicted | -1.95 | 0.33 |
| NM_030826 | Gpx1 | -1.76 | 0.13 |
| NM_019143 | Fn1 | -2.14 | 0.54 |
| XM_228900 | LOC317454 | -4.22 | 1.49 |
| XM_341671 | Tradd | -2.34 | 0.63 |
| NM_012935 | Cryab | -2.10 | 0.48 |
| NM_013172 | Myf6 | 2.46 | 0.62 |
| NM_134349 | Mgst1 | -1.91 | 0.29 |

Gene changes in regards to family are listed in Table 10. Genes known to be in multiple families may be listed more than once. The same coding sequence as Table 9 is used in Table 10.

Table 10: DNA Microarray Results Sorted by Gene Family

| **Family** | **Genebank Number** | **Symbol** | **Mean Change** | **SEM** |
|---|---|---|---|---|
| dopamine catabolism | | | | |
| | AA858639 | Comt | -2.21 | 0.29 |
| metabolism | | | | |
| | **NM_019143** | Fn1 | -2.58 | 0.58 |
| | **NM_012912** | Atf3 | 4.87 | 0.73 |

| | | | |
|---|---|---|---|
| **NM_144744** | Acdc | -2.72 | 0.42 |
| **NM_024349** | Ak1 | 2.16 | 0.48 |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| response to wounding | | | |
| **NM_181368** | Mustn1 | 4.05 | 1.16 |
| **NM_019143** | Fn1 | -2.58 | 0.58 |
| Protein amino acid phosphorylation | | | |
| **NM_021693** | Snf1lk | 3.67 | 0.59 |
| regulation of transcription from RNA polymerase II promoter | | | |
| **NM_021693** | Snf1lk | 3.67 | 0.59 |
| **NM_012543** | Dbp | -1.66 | 0.02 |
| **NM_013172** | Myf6 | 2.36 | 0.67 |
| circulation | | | |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| response to oxidative stress | | | |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| **NM_030826** | Gpx1 | -1.76 | 0.13 |
| **NM_134349** | Mgst1 | -1.91 | 0.29 |
| apoptosis | | | |
| **NM_030826** | Gpx1 | -1.76 | 0.13 |
| **XM_341671** | Tradd | -2.29 | 0.65 |
| **NM_024388** | Nr4a1 | 5.26 | 0.90 |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| response to stress | | | |
| **NM_019143** | Fn1 | -2.58 | 0.58 |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| circadian rhythm | | | |
| **NM_012543** | Dbp | -1.66 | 0.02 |
| visceral muscle development | | | |
| **NM_017239** | Myh6 | -2.08 | 0.35 |
| Muscle contraction | | | |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| **NM_012604** | Myh3 | -2.43 | 0.49 |
| **NM_017240** | Myh7 | -2.35 | 0.35 |
| **NM_017184** | Tnni1 | -2.73 | 0.62 |
| **NM_017239** | Myh6 | -2.08 | 0.35 |
| skeletal development | | | |
| **XM_213440** | Col1a1 | -2.59 | 0.23 |
| **NM_053356** | Col1a2 | -2.35 | 0.18 |
| ossification | | | |
| **NM_012656** | Sparc | -1.78 | 0.05 |
| perception of sound | | | |
| **XM_213440** | Col1a1 | -2.59 | 0.23 |
| **NM_053356** | Col1a2 | -2.35 | 0.18 |
| cell cycle regulation | | | |
| **NM_012543** | Dbp | -1.66 | 0.02 |
| **NM_013172** | Myf6 | 2.36 | 0.67 |
| **NM_021693** | Snf1lk | 3.67 | 0.59 |

| | | | |
|---|---|---|---|
| **NM_024349** | Ak1 | 2.16 | 0.48 |
| neurotransmitter catabolism | | | |
| **AA858639** | Comt | -2.21 | 0.29 |
| myogenesis | | | |
| **NM_013172** | Myf6 | 2.46 | 0.62 |
| cytoskeleton organization and biogenesis | | | |
| **NM_012604** | Myh3 | -2.43 | 0.49 |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| **NM_017239** | Myh6 | -2.08 | 0.35 |
| transcription | | | |
| **NM_013086** | Crem | 2.41 | 0.32 |
| **NM_024388** | Nr4a1 | 5.26 | 0.90 |
| **NM_031628** | Nr4a3 | 17.47 | 2.70 |
| **NM_012543** | Dbp | -1.66 | 0.02 |
| **NM_012912** | Atf3 | 4.87 | 0.73 |
| **NM_013172** | Myf6 | 2.46 | 0.62 |
| **NM_031347** | Ppargc1a | 2.11 | 0.14 |
| adenine metabolism | | | |
| **NM_024349** | Ak1 | 2.16 | 0.48 |
| sensory organ development | | | |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| development | | | |
| **NM_013172** | Myf6 | 2.46 | 0.62 |
| spermatogenesis | | | |
| **NM_013086** | Crem | 2.41 | 0.32 |
| **NM_021989** | Timp2 | -1.54 | 0.02 |
| **AW918612** | Timp2 | -1.82 | 0.05 |
| eye morphogenesis | | | |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| Protein biosynthesis | | | |
| **NM_017151** | Rps15 | -2.10 | 0.25 |
| cell-substrate junction assembly | | | |
| **NM_019143** | Fn1 | -2.58 | 0.58 |
| cholesterol homeostasis | | | |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| electron transport | | | |
| **NM_031347** | Ppargc1a | 2.11 | 0.14 |
| regulation of synaptic activity | | | |
| **XM_341642** | Dok4_predicted | -2.32 | 0.46 |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| proteolysis and peptidolysis | | | |
| **XM_343169** | Adn | -4.50 | 0.92 |
| caspase activity | | | |
| **NM_024388** | Nr4a1 | 5.26 | 0.90 |
| catecholamine metabolism | | | |
| **AA858639** | Comt | -2.21 | 0.29 |
| tissue generation | | | |

| | | | |
|---|---|---|---|
| **XM_223124** | Col9a1_predicted | -2.20 | 0.13 |
| **NM_017184** | Tnni1 | -2.73 | 0.62 |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| **NM_012604** | Myh3 | -2.43 | 0.49 |
| **NM_017240** | Myh7 | -2.35 | 0.35 |
| **NM_017239** | Myh6 | -2.08 | 0.35 |
| **NM_013172** | Myf6 | 2.46 | 0.62 |
| **NM_181368** | Mustn1 | 4.05 | 1.16 |
| cell adhesion | | | |
| **XM_342599** | Col9a3_predicted | -1.95 | 0.33 |
| **XM_213440** | Col1a1 | -2.59 | 0.23 |
| **XM_223124** | Col9a1_predicted | -2.20 | 0.13 |
| **NM_053356** | Col1a2 | -2.35 | 0.18 |
| **NM_019143** | Fn1 | -2.58 | 0.58 |
| embryonic limb morphogenesis | | | |
| **NM_181368** | Mustn1 | 4.05 | 1.16 |
| epidermis development | | | |
| **XM_213440** | Col1a1 | -2.59 | 0.23 |
| cell migration | | | |
| **NM_019143** | Fn1 | -2.58 | 0.58 |
| learning and/or memory | | | |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| intracellular transport | | | |
| **NM_138828** | Apoe | -2.12 | 0.15 |
| Protein homooligomerization | | | |
| **NM_144744** | Acdc | -2.72 | 0.42 |
| signal transduction | | | |
| **NM_144744** | Acdc | -2.72 | 0.42 |
| **NM_013086** | Crem | 2.41 | 0.32 |
| **XM_341642** | Dok4_predicted | -2.32 | 0.46 |
| **NM_024388** | Nr4a1 | 5.26 | 0.90 |
| Protein folding | | | |
| **NM_012935** | Cryab | -2.10 | 0.48 |
| complement activation, alternative pathway | | | |
| **XM_343169** | Adn | -4.50 | 0.92 |
| regulation of T cell proliferation | | | |
| **NM_212505** | RGD:1303321 | -2.31 | 0.48 |
| intracellular phosphate transport | | | |
| **NM_021693** | Snf1lk | 3.67 | 0.59 |
| **NM_144744** | Acdc | -2.72 | 0.42 |
| **XM_342599** | Col9a3_predicted | -1.95 | 0.33 |
| **XM_213440** | Col1a1 | -2.59 | 0.23 |
| **XM_223124** | Col9a1_predicted | -2.20 | 0.13 |
| **NM_053356** | Col1a2 | -2.35 | 0.18 |
| mesoderm formation | | | |
| **NM_031628** | Nr4a3 | 17.47 | 2.70 |
| ribosome biogenesis | | | |

| | | | | |
|---|---|---|---|---|
| | **NM_017151** | Rps15 | -2.10 | 0.25 |
| unknown | | | | |
| | **XM_215375** | LOC294337 | -1.95 | 0.05 |
| | **NM_017259** | Btg2 | 2.53 | 0.47 |
| | **NM_175844** | RGD:708493 | 3.24 | 1.01 |
| | **XM_346881** | LOC360478 | -2.10 | 0.17 |
| | **XM_227446** | LOC310670 | -1.60 | 0.05 |
| | **XM_225173** | | -2.01 | 0.15 |
| | **AA964296** | | 2.42 | 0.27 |
| | **TC483914** | | 2.15 | 0.19 |
| | **M12199** | | -2.27 | 0.27 |
| | **BF544403** | | 4.92 | 1.29 |
| | **AI013671** | | 2.84 | 0.46 |
| | **TC487987** | | 2.86 | 0.40 |
| | **BF549650** | | 2.65 | 0.41 |
| | **NM_001002850** | | -2.21 | 0.32 |
| | **XM_216062** | | -2.08 | 0.34 |
| | **AW918430** | | 3.13 | 0.71 |
| | **AI008316** | | 2.64 | 0.62 |
| | **XM_340817** | | 3.29 | 0.94 |
| | **XM_228900** | | -4.22 | 1.49 |

Table 11 contains fluorescence data for all genes which were reported as significantly altered. While it is not possible to accurately measure exact gene transcript number using microarray analysis, this type of analysis can measure the signal intensity from the dye incorporated within the cDNA of each gene. Therefore, it appears as though signal intensity (or fluorescence) is a good method with which to extrapolate this information. The same coding sequence is used in Table 11.

Table 11: Fluorescence Data for all Significantly Altered Genes

| **Genbank Number** | **Gene Symbol** | **E Fluor** | **SEM** | **C Fluor** | **SEM** |
|---|---|---|---|---|---|
| NM_012543 | Dbp | 726.0 | 173.9 | 1226.8 | 249.5 |
| XM_215375 | LOC294337 | 290.3 | 62.5 | 538.8 | 100.8 |
| NM_021989 | Timp2 | 461.3 | 101.1 | 654.5 | 106.5 |
| AW918612 | | 181.3 | 35.7 | 325.0 | 62.5 |

| | | | | | |
|---|---|---|---|---|---|
| NM_012656 | Sparc | 3209.8 | 618.2 | 5210.8 | 1057.0 |
| NM_031628 | Nr4a3 | 1363.5 | 366.6 | 81.5 | 20.8 |
| XM_227446 | LOC310670 | 136.8 | 24.2 | 216.3 | 31.4 |
| XM_223124 | Col9a1_predicted | 224.0 | 59.8 | 508.0 | 156.7 |
| NM_031347 | Ppargc1a | 247.0 | 53.7 | 114.5 | 20.4 |
| NM_053356 | Col1a2 | 2265.5 | 460.8 | 4854.5 | 1386.9 |
| NM_024388 | Nr4a1 | 979.0 | 214.6 | 185.0 | 43.5 |
| XM_213440 | Col1a1 | 11741.3 | 2488.2 | 24496.8 | 5112.2 |
| NM_138828 | Apoe | 448.8 | 105.6 | 900.3 | 170.4 |
| XM_225173 | Pfn3_predicted | 658.5 | 180.4 | 1158.5 | 345.1 |
| NM_012912 | Atf3 | 555.3 | 169.2 | 115.5 | 27.6 |
| XM_346881 | LOC360478 | 406.8 | 120.6 | 859.3 | 307.2 |
| AA964296 | | 512.0 | 117.4 | 186.3 | 25.1 |
| TC483914 | LOC310395 | 324.0 | 95.0 | 148.0 | 35.0 |
| NM_021693 | Snf1lk | 860.8 | 197.2 | 251.8 | 74.4 |
| M12199 | | 15758.0 | 2955.9 | 34760.0 | 8493.0 |
| XM_343169 | Adn | 201.0 | 67.7 | 832.5 | 245.7 |
| NM_013086 | Crem | 150.8 | 32.1 | 64.0 | 17.2 |
| BF544403 | | 295.3 | 75.6 | 83.3 | 24.5 |
| AA858639 | Comt | 1139.8 | 358.4 | 2380.8 | 755.7 |
| AI013671 | | 183.8 | 32.9 | 71.5 | 20.5 |
| TC487987 | | 742.8 | 124.1 | 386.3 | 100.6 |
| NM_144744 | Acdc | 119.8 | 40.6 | 293.8 | 67.1 |
| NM_017151 | Rps15 | 6976.5 | 2417.5 | 13158.8 | 3989.2 |
| BF549650 | | 151.5 | 33.1 | 63.5 | 18.6 |
| NM_017240 | Myh7 | 19131.3 | 4507.4 | 35173.8 | 5483.4 |
| NM_001002850 | Prm1 | 9204.3 | 3358.4 | 17291.5 | 5219.6 |
| NM_017259 | Btg2 | 357.5 | 71.9 | 154.0 | 39.5 |
| NM_181368 | Mustn1 | 1753.3 | 462.5 | 444.0 | 113.3 |
| XM_216062 | Smarcd3_predicted | 6236.3 | 2654.4 | 12113.0 | 4479.6 |
| NM_017239 | Myh6 | 19753.3 | 4364.5 | 32931.3 | 6467.9 |
| AW918430 | Lpin1_predicted | 235.0 | 43.6 | 71.8 | 18.1 |
| AI008316 | | 208.5 | 49.9 | 82.3 | 21.8 |
| NM_212505 | RGD:1303321 | 122.3 | 37.0 | 244.8 | 37.3 |
| NM_012604 | Myh3 | 7001.0 | 4181.9 | 9163.0 | 4895.8 |
| XM_341642 | Dok4_predicted | 49.0 | 9.8 | 62.8 | 13.2 |
| NM_175844 | Stars | 3418.5 | 987.5 | 1046.3 | 308.7 |
| NM_017184 | Tnni1 | 7748.5 | 2865.5 | 15443.0 | 2814.7 |
| NM_024349 | Ak1 | 139.5 | 44.8 | 61.8 | 17.8 |
| XM_340817 | Myh4 | 9670.3 | 3335.8 | 2388.3 | 581.8 |
| XM_342599 | Col9a3_predicted | 276.5 | 196.3 | 382.5 | 243.9 |
| NM_030826 | Gpx1 | 1552.3 | 479.9 | 2209.3 | 473.5 |
| NM_019143 | Fn1 | 285.0 | 74.6 | 704.0 | 225.8 |
| XM_228900 | LOC317454 | 958.0 | 454.1 | 2413.8 | 357.0 |
| XM_341671 | Tradd | 191.3 | 57.2 | 262.8 | 80.7 |
| NM_012935 | Cryab | 20608.0 | 5314.8 | 32182.5 | 5347.3 |
| NM_013172 | Myf6 | 854.5 | 205.9 | 388.5 | 101.2 |
| NM_134349 | Mgst1 | 79.3 | 18.9 | 146.5 | 32.7 |

## Real Time PCR

In order to validate the genes of interest analyzed on the microarray, real time PCR was performed on 4 selected genes known to be involved in either the pro- or antioxidant response of the muscle. The results of the microarray were consistent with what was found using real time PCR. Table 12 compares the differences reported in Atf3, Mgst1, NFκB and TNFα between the microarray and PCR analysis.

Table 12: Gene Fold Differences as Reported by Microarray and PCR Analysis

| Gene | Array | Q-PCR |
|---|---|---|
| Atf3 | 4.87 ± 0.73 * | 4.97 ± 1.01 * |
| Mgst1 | -1.91 ± 0.29 * | -2.25 ± 0.06 * |
| NFκB | -1.05 ± 0.34 | -1.2 ± 0.14 |
| TNFα | 1.12 ± 0.83 | 1.30 ± 0.34 |

All values are mean ± SEM. The * denotes significance at a 1.5 fold difference on the array, or $p \leq 0.05$ using an independent T-test for PCR results. Negative numbers indicate higher transcript levels in the control animals.

For Mgst1, the array reported an average fold difference of -1.91 (or 0.52) when comparing the E group to C group whereas the real time PCR revealed a -2.25 (or 0.20) fold difference. This gene was significantly lowered in response to the exercise bout. For Atf-3, the array reported an average fold difference of 4.87 when comparing the E group to C group whereas the real time PCR revealed a 4.97 fold difference. This gene was significantly up-regulated in response to the exercise bout. For NFκB, the array

reported no difference when comparing the E group to C group whereas the real time PCR revealed a -0.15 fold difference. This gene was not significantly altered in response to the exercise bout. For TNFα, the array reported no difference when comparing the E group to C group whereas the real time PCR revealed a 0.3 the fold difference. This gene was not significantly altered in response to the exercise bout.

## Protein Isolation

There was no difference in the total protein content of the soleus muscle between the 2 groups. The E group had a content of 10.2 ± 6.1 µg/µl, while the C group had a protein content of 11.6 ± .40 µg/µl.

## Western Blot Analysis

Western Blot analysis was performed on the p65 sub-unit of NFκB, and Atf-3 in order to determine if the gene levels would match the protein levels. Consistent with the gene levels, there was no difference in protein levels of NFκB between the E and C groups, and there was a significant increase in Atf-3 protein levels between the E and C groups. For this analysis, all values were normalized to the C group optical density (OD). Please refer to Figures 5, 6, 7, and 8 for results.

Figure 5: Sample Western Blot of NFκB

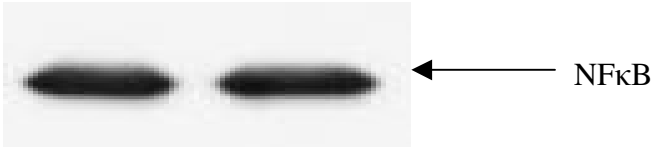
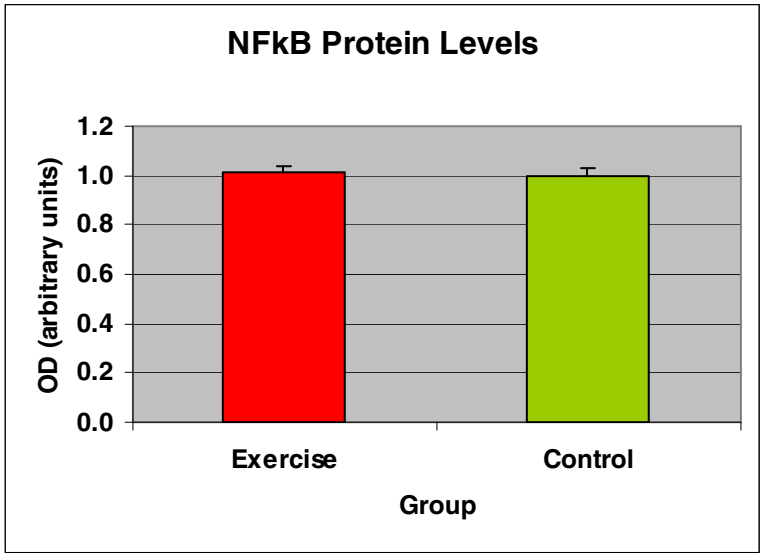



Figure 6: NFκB Protein Expression Levels




All values are mean ± SEM and are normalized to the C group.

Figure 7: Sample Western Blot of Atf-3

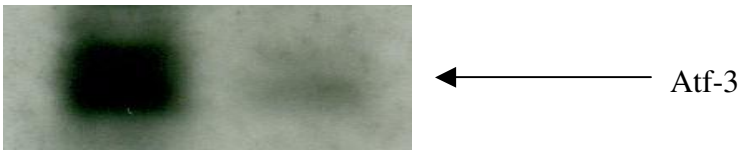
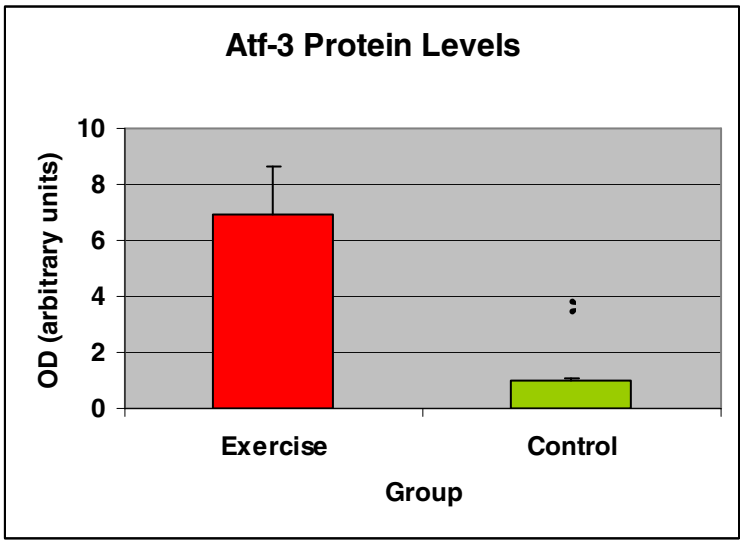


Figure 8: Atf-3 Protein Expression Levels




All values are mean ± SEM and are normalized to the C group. The * denotes significance at $p \leq 0.05$ using an independent T-test comparing group means.

# CHAPTER V

# DISCUSSION AND CONCLUSIONS

This study was completed in order to compare mRNA alterations in the working skeletal muscle (the soleus in this case) following a single bout of aerobic exercise. Another aim of this particular study was to validate some of the mRNA changes, as well as seeing if these gene changes match protein changes. To our knowledge, this is the first study to use microarray analysis one hour following a single bout of aerobic exercise that specifically looked at the working limb muscle and also compared gene changes with protein changes. Several previous investigators have looked at the response of cardiac muscle following exercise (e.g. [42, 76, 201]), but not skeletal muscle. It was hypothesized that at the one hour post-exercise time point genes involved in RONS production would be down-regulated in the E group, and that genes involved in RONS protection would be up-regulated in the E group. It was also hypothesized that gene and protein levels would not match since the process from gene transcription to protein translation is a complex process. These hypotheses were not accepted as since the current study found the opposite effect occurring in the soleus muscle. The major families of genes reported as changed on the microarray were metabolism, apoptosis, muscle contraction, transcription/cell signaling, tissue generation, and inflammatory genes (a known secondary source of RONS production), and gene and protein levels that were measured matched.

Thus, the main findings of this experiment reveal that: 1) According to the microarray, genes involved in the stress response were up-regulated in the E group, while genes involved in stress protection were mainly down-regulated in the E group, 2) the 4 genes tested with real time PCR (TNFα, NFκB, Mgst1, and Atf3) matched the results seen in the microarray and 3) the protein levels of NFκB and Atf-3 match the gene levels seen under these particular conditions.

These data suggest that in the soleus muscle 1 hour after a 2 hour run at 22 m/min, down-regulation of genes involved in the protection from oxidative stress one hour post-exercise occurred. Conversely, genes involved in stress production are up-regulated as several genes involved in the inflammatory process (a known secondary source of RONS production) are up-regulated [10, 62, 63, 80, 81, 119, 125, 128, 174]. This experiment also provides evidence that future investigators concerned with RONS protection should investigate some time point after this one hour time point used. This is also the first known investigation to measure gene changes of Atf3 and Mgst1 following exercise.

This chapter discusses the results from the present study in comparison to previous published research. Based on the results of this study, the rejection of the hypotheses is discussed. Finally, recommendations for future research within this area are proposed.

## Exercise Session

The rats utilized in this study were all approximately 10 weeks old at the time of the exercise session and sample collection. Since all rats walked slowly on the treadmill

prior to the exercise session for short periods of time, and were handled daily, the influence of stress due to animal handling and treadmill apprehension wereminimized . Since all animals were fasted for at least 12 hours at the time of decapitation, dietary influences were likely equal between the 2 groups. Finally, body weights and muscle weights were not different between the 2 groups. It therefore appears that no training effect took place in regards to the aforementioned measures.

## DNA Microarray

As previously indicated, 52 genes were reported as significantly altered due to a single session of exercise on all 4 arrays. According to the array, of the families of genes from which the 52 genes which were reported as significantly altered were metabolism (~10% of all genes altered), apoptosis (~8% of all genes altered), muscle contraction (~10% of all genes altered), transcription/cell signaling (~17%of all genes altered), tissue generation (~15.5% of all genes altered), and inflammatory genes (~10% of all genes altered). This suggests that at this time point, genes involved in the inflammatory and pro-stress processes are up-regulated, while stress protection genes are either unaltered or down-regulated. Clearly, in order to confirm whether or not these gene changes actually occurred, PCR analysis would need to be performed on each of the genes in question. Therefore, the results of this array will be compared to families of genes as grouped by other investigators in other array experiments, and not necessarily on a gene by gene basis.

The results from the current microarray are consistent with a recent study published by Mahoney et al. [118]. In this particular study, 14 untrained males cycled to exhaustion for ~75 minutes. Muscle biopsies were taken 3 hours post-exercise and RNA was isolated and 3 DNA microarrays were performed on the muscle samples. It should be noted that in their study, muscle samples were taken from the vastus lateralis (VL, a mixed quadriceps muscle). Previous EMG analysis has shown that the VL is recruited during cycling exercise [184]. However, since muscle biopsies take only a small sample of the entire muscle, these biopsies may not reflex the entire muscle's response, and biopsies may initiate the inflammatory response. In their study, 173 genes were altered due to the exercise bout. In agreement with the present study, the families of genes most altered were metabolism genes, stress response genes, apoptosis genes, tissue regeneration genes, and transcriptional genes. In the Mahoney study, 8% of the altered genes were metabolism genes, 10% of the genes altered were stress response genes, 2.5% were apoptosis related, 6% were tissue regeneration genes, and 3.5% were transcription related genes. While not exact, these percentages are similar to the current study despite differences in species (humans vs. rats), muscle (VL vs. soleus), and time point measured (3 hours post vs. 1 hour post).

Timmons et al. also examined the gene response in human VL muscles following 6 weeks of cycle training at 65% of max [210]. In this particular study, the microarray was performed on tissues taken 24 hours after the last exercise session. Subjects were also classified post-exercise as high or low exercise responders, and the microarray was only run on tissue from the high responders. There were 387 genes were significantly altered

due to exercise training. In this study, ~6% of the genes altered were related to metabolism, ~13% were stress response genes, and 14% were related to cell signaling. The authors did not report any information on apoptosis or tissue regeneration genes. There were also several differences between this study and the present study. As with the Mahoney article, their study used a different species, a different muscle, and a different time point to test gene expression. The biggest difference however is that Timmons et al. used trained subjects, and only high exercise responders were included for array analysis. It is unknown if the changes reported in this study were due to the cumulative effect of 6 weeks of training, or the last single exercise session. This is one possibility as to why no genes of tissue regeneration or apoptosis were reported as the trained subjects muscles may have already adapted to the repeated stress of the exercise session. This study did not report which exact genes were altered, thus, comparing the exact gene responses to another study is impossible. Although several gene "families" did change in similar fashion to our study it is unknown if this is a chronic training adaptation, or an acute exercise bout response, as this was not measured. Trained muscle was reported to respond differently than untrained muscle to exercise stress [87, 157, 158, 160] so comparisons may not be consistent.

Another study examined the influence of aerobic exercise training using elderly men thatwere aerobically trained on a cycle ergometer for 12 weeks . The VL muscle was biopsied for subsequent microarray analysis 72 hours after completion of the last exercise bout [168]. They reported 397 genes as different following the 12 week training program. In this study, ~44% of the genes altered were related to metabolism. Another ~5% were

tissue regeneration genes, and the authors grouped 103 genes or ~26% as physiological processes, which may include some of the other functions previously listed from the present study. This study used trained subjects, a different species, a different muscle, and used elderly subjects compared to the present study, which could account for the differences.

While a few other studies involving aerobic exercise and microarray analysis exist, many continue to use long term training in conjunction with certain disease processes like insulin sensitivity [208], and cardiovascular disease [213]. It is clear that the muscle responds differently to aerobic training than it does to resistance training, but both appear to alter the oxidative stress response of the muscle [21, 51, 53, 54, 84, 85, 87], Therefore, some published articles using microarrays and resistance training warrants mentioning.

A study by Zambon et al. recently examined the gene response to a single session of exhaustive resistance exercise in the VL of middle aged male adult humans [229]. In this study, 6 hours and 18 hours after training, 608 genes were altered. ~10% of the genes altered were metabolic genes, ~10% were related to tissue regeneration, ~9% were apoptosis related, ~4% were stress response genes, and ~6% were transcriptional related genes. The groupings of genes altered are similar to the results from current study. Again, differences in expression of specific genes maybe due to species (humans vs. rats), the type of exercise (resistance vs. aerobic), and the muscle (VL vs. soleus) used as well as the time (6-18 hours vs. 1 hour) for analysis.

Roth et al. published a study that examined the effects of resistance training on the VL muscle of both young and old, men and women. In their study, subjects trained at

least 3 days a week for 9 weeks and muscle biopsiesarrays were performed 48-72 hours after the last training session for array analysis. Comparisons with this study prove difficult, as the authors break down comparisons, by age, gender, and training. In order for a gene to count as altered due to training, it had to be altered in all 4 groups: young men, older men, young women, older women. Since aged muscle likely responds differently than young muscle [67, 220, 234], this clearly would limit the number of genes altered in all 4 groups. Taking this under consideration, 54 genes were altered in all 4 groups after training with most genes being altered grouped as either metabolism genes or tissue regeneration genes. Along with several other factors which have been previously mentioned, the fact that aged skeletal muscle was used clearly accounts for some differences.

In 2003, Chen et al. [34] reported the effects of a single session of exhaustive exercise; one leg underwent concentric contractions only, while the opposite leg underwent eccentric and concentric exercise. It is well established that eccentric exercise causes much more damage and soreness than concentric exercise [20, 51, 53]. Three adult, young men had biopsies taken from their VL ~4 hours post-exercise. The authors in this study were concerned with the difference in the gene response between the two types of exercise. The authors concluded that the 28 genes which were differentially expressed were of the family of genes responsible for tissue regeneration, the stress response, metabolism, and cell signaling. Despite differences in their study to the present investigation (such as concentric contractions only), the genes that were expressed are consistent in group function to the present study.

Resistance training, although resulting in adaptation differences within the working muscle, can show similar alterations in the family of genes. Therefore, future work needs to identify the specific genes that are altered, and determine the time course of these changes with the protein changes observed within the working muscle.

Clearly, not all genes which the array identified as altered could be confirmed using PCR analysis. However, some select other gene alterations warrants mentioning. The transcript levels of the genes Nr4a3 and Nr4a1 were reported as significantly elevated due to exercise. These genes are from a family of nuclear receptors and are classified as early response genes. It has been repeatedly hypothesized that this family of genes is critical in early apoptosis signaling [93, 106, 118, 123]. This suggests that one-hour post exercise the apoptosis process is clearly activated. This is supported by Atf3 findings in the present study. Another gene which warrants mentioning is ApoE which is related to apoptosis [12], oxidative stress [215] and is critical for proper cholesterol metabolism [16, 185]. In the present investigation, this gene was significantly decreased one hour following exercise. Recently, ApoE has gained notoriety for its role in Alzheimer's disease [129], as a variation in this gene seems to increase the occurrence of this disease. The role of AopE in regards to Alzheimer's disease and exercise warrants further investigation. One final gene which warrants discussion is Snf Ik (**s**plicing **n**ecessary **f**actor). This gene which has been identified for protein splicing was significantly elevated in the present investigation following exercise. In addition, it is believed that this gene is critical for apoptotic signaling to occur [95, 175], suggesting that condition are favorable for apoptosis one hour post-exercise.

In conclusion, it appears that any type of exercise is a major modulator of genes related to metabolism, apoptosis, muscle contraction, transcription/cell signaling, tissue generation, and inflammatory genes. It does seem that there is some consistency with exercise across subjects, exercise type, and training status but these needs to be substantiated with the specific genes within these categories.

## Real Time PCR

To confirm that a gene that truly altered on the microarray, PCR analysis was used to substantiate that changes actually occurred. In the present investigation, real time PCR was performed on 4 genes, 2 genes which our array identified as altered and known to play a role in the oxidative stress response, and 2 genes which were unchanged, yet have been reported in some conditions to play a role in the stress response. In this experiment, the results of the 4 genes tested using real time PCR matched the results from the microarray. The microarray reported a significant down-regulation of the gene Mgst1 (microsomal glutathione S-transferase 1), an up-regulation of the gene Atf3 (activating transcription factor 3), and no significant differences in NFκB (nuclear factor kappa beta) and TNFα (tumor necrosis factor alpha).

### Mgst1

In the present study, Mgst1 was reported as significantly down-regulated one hour after exercise by both the microarray and PCR analysis. The gene Mgst1 is known to

play a role in the glutathione system of the muscle. This gene is important in defense from RONS [90, 91, 135]. Mgst1is responsible for the production of the membrane bound enzyme which functions as both a glutathione transferase (GST) and a glutathione peroxidase (GPx) [104]. GST acts to catalyze the reaction between the SH group of GSH and the alkylating agent. This neutralizes their electrophilic site leaving them more water soluble which allows GSH to carry out its detoxifying effects. GPx (in the presence of GSH) acts to convert hydrogen peroxide into water and GSSG [194]. Clearly, this is an important gene for the body's endogenous antioxidant defense system. However, in our study, this gene was down-regulated, implying that the glutathione defense system was expressed less than in controls.

Several previous studies have reported altered Mgst1 levels, however none of them have used exercise as their model. Mgst1 levels were shown to be up-regulated in cells which were exposed to various types of tumor cells [45]. The authors speculated that Mgst1 is important in the body's ability to fight off conditions like cancer which increase RONS through the glutathione defense system. In a different study, rats were exposed to high levels of toxic gases known to increase RONS levels for 2 weeks for 2 hours per day. Mgst1 was up-regulated 24 hours after the last exposure [111]. This was also reported by Maeda et al. in which two types of cell cultures were exposed to RONS, one type of cells contained Mgst1 siRNA (this essentially knocks down the gene levels), while the other group was wild type. Glutathione activity and subsequent cell survival was over 40% lower in the cells with Mgst1 siRNA, again, suggesting that RONS will activate this gene [116] and that it is likely important for cell survival.

Clearly, the above results differ from what was seen in the current experiment. Three possibilities therefore exist as to why Mgst1 levels were down-regulated: 1) exercise causes a different response than other RONS producing mediums, 2) at the one hour time point chosen, the antioxidant defense system was not yet up-regulated, or 3) this is not the rate limiting factor for glutathione pathways. Since exercise has repeatedly been shown to increase the body's ability to defend against RONS using glutathione pathways [52-54, 86, 87, 89, 116], option one seems unlikely. It therefore appears that had a later time point been chosen, Mgst1 levels may have been elevated in the exercise group. This is the first known study measuring Mgst1 levels using exercise as the stressor. Since Mgst1 is a known RONS protector and the levels were down-regulated, this suggests that the muscles from the current study are susceptible to attack and apoptosis. This clearly contributes to the rejection of the hypothesis that genes involved in antioxidant protection would be up-regulated, as this very important protector is actually significantly down-regulated.

Atf3

In the present study, Atf3 levels were significantly higher one hour following exercise compared to the controls according to both microarray and PCR analysis. Atf3 is an activating factor with several proposed functions. However, its main functions appear to be in regard to cell cycle. It appears as though this gene acts as both an apoptotic factor [137] and as a cell proliferation factor [206]. It has been hypothesized that this gene is a stress response gene [206]. Atf3 is induced upon a variety of different

physiological stimuli including ischemia, reperfusion, wounding, and other stress signals [61]. In the present experiment, many genes involved in cell cycle controls were altered, and Atf3 certainly fits in this category.

Yan et al. reported that Atf3 is essential for proper function of the tumor suppressor p53, which plays a major role in cell cycle arrest and apoptosis [225]. This group reported that in most types of cancer, Atf3 levels were down-regulated, suggesting the critical role Atf3 plays in cell proliferation and apoptosis.

Consistent with the previous study, Hua et al. reported that rat cells which over expressed Atf3 directly inhibited NFκB dependent apoptosis. The authors believed that this regulation is through a TNFα response, as it has been suggested that Atf3 levels allow TNFα induced apoptosis to occur [75, 78].

Atf3 also appears to be critical for proper cell growth. It has been reported that even in cells lacking other cell proliferation factors (such as c-Myc), that if Atf3 levels are stimulated, close to normal cell proliferation can occur. However, in Atf3 knockout cells, cell proliferation was only about 50% of controls. This suggests that in order for optimal proliferation to occur, Atf3 and c-Myc must both be present, but Atf3 appears much more critical [206].

Abe et al. stimulated an inflammatory response in rat hind limb muscle by applying a tourniquet. One hour after the tourniquet was removed, Atf3 levels were significantly higher than controls. The authors also reported several other inflammatory/tissue regeneration genes altered in response to this stress as well and concluded that Atf3 was important in the muscles stress response [1].

It does appear Atf3 may play a role in protecting proteins from degradation as well. It is believed that Atf3 can work with JNK and c-Jun as previously described and lead to increased HSP 27 levels [109]. HSPs function to help proteins properly fold so that they do not undergo degradation. It therefore appears Atf3 does indeed provide some direct protection to cells as is related to oxidative stress.

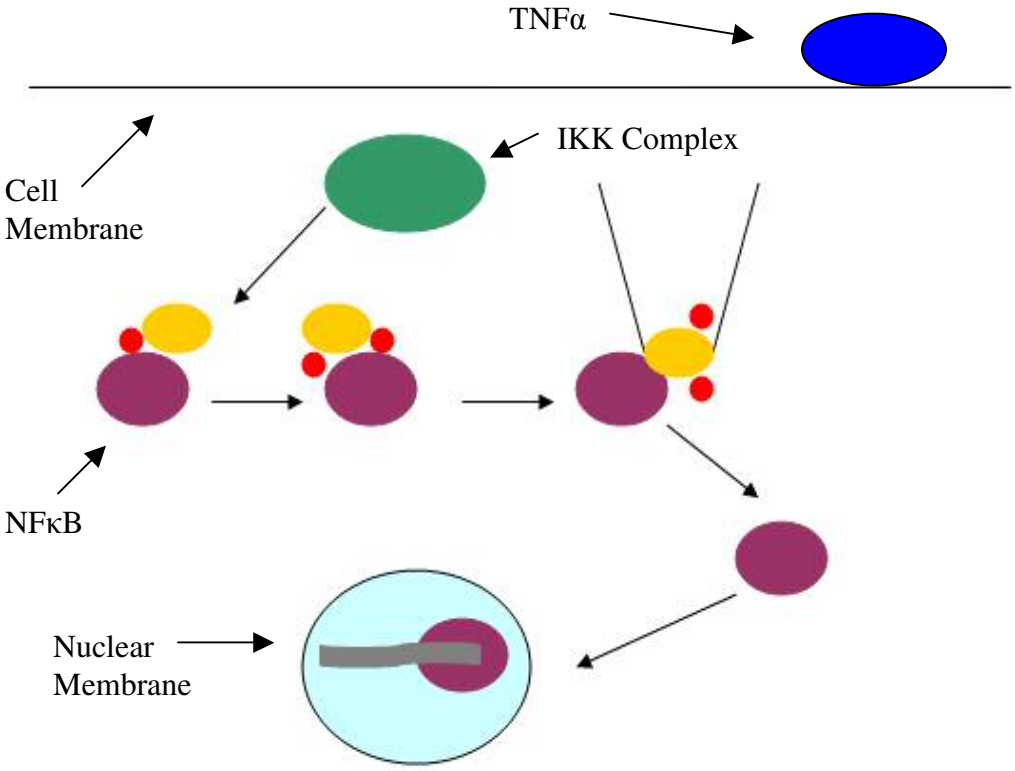
In conclusion, Atf3 has been shown to be active in a variety of pathways whether it is apoptotic, or antiapoptotic. The one constant factor that appears to be present in all cases is that Atf3 is up-regulated in response to stress, and this coincides with results from the present experiment with both the microarray and PCR results. Since the apoptotic role of Atf3 is well established, and the fact that several other inflammatory and apoptotic genes were up-regulated, it appears as though conditions in the soleus muscle of the current study favor apoptosis. Future studies may measure various apoptotic measures and compare these measures to gene levels.

NFκB

In the present study, no difference was noted in NFκB levels between exercised rats or control rats one hour after exercise using either microarray or PCR analysis. NFκB (nuclear factor kappa beta) is also known as the Rela gene. NFκB is known to be present in a variety of circumstances including ischemia/reperfusion injury [224], the inflammatory process [217], apoptosis [88] and in response to oxidative stress [28]. Almost all published studies address NFκB protein activity, and not necessarily changes in the gene or protein levels. It is known that NFκB is partly responsible for NFAT activation [72, 195],

which is a cytolsolic protein which when dephosphorylated crosses the nuclear membrane and is responsible for gene transcription. The NFκB pathway is very complex. Although gene levels of NFκB were not altered, NFκB activation may have taken place as one of several inhibitors could have been altered (IKBalpha phosphorylation, IKK levels down-regulated). Clearly this is a complicated process as indicated in Figure 9. In hindsight, it appears to be NFκB activity that may be altered and that NFκB levels may not be the most important aspect. This needs to be substantiated with further studies.

Figure 9: NFκB Activation Pathway




Adapted from Biocarta [18]

In Figure 9 the legend is as follows: the dark blue circle represents TNFα, the green circle represents the IKK complex, the yellow circle represents IKβ, the purple circle represents NFκB, the small red circles represent phosphorylation groups, the thin black lines represent ubiquitin, the light blue circle is the nucleus, and the gray inside the nucleus is DNA. Briefly, the IKK complex has the ability to phosphorylate the IKβ complex which is joined with NFκB, thus further deactivating it. Ubiquitin has the ability to bind to IKβ and remove the phosphorylation, as well as the IKβ complex, activating NFκB. Once NFκB is active, it can cross the nuclear membrane where it binds to DNA and acts as a transcription factor.

TNFα

In the present study, no differences were noted in TNFα (tumor necrosis factor alpha) levels between the exercised animals or the control animals using either microarray or PCR analysis. TNFα is well defined as an integral part of the inflammatory process as it has been specifically linked to being an activator of apoptosis [48] and as being activated as part of the inflammatory process [7]. TNFα is also thought to play a role in altering insulin sensitivity [178]. TNFα has been shown to directly altering NFκB activity by altering IKK levels [181, 211].

Paton et al. examined TNFα gene levels in elderly subjects trained aerobically for 6 months at 50-70% and blood TNFα expression levels were tested. After a single aerobic exercise bout, there was a slight increase in TNFα levels 2 hours post-exercise. The levels were even higher 4 hours post-exercise. Following the 6 months of aerobic

training, TNFα levels were again significantly higher again at both the 2 and 4 hour time points, however, the trained subjects had significantly lower TNFα than the untrained controls at rest as well as post-exercise [150]. The authors suggested that the original exercise bout caused increases in TNFα levels due to the induction of the inflammatory process. They also concluded that since TNFα levels decrease with training, one major training response is an attenuation of the inflammation process.

However, a study by Colbert et al. refutes the above study's findings In this particular study, mice ran for either 60 minutes or to exhaustion and tissue was collected immediately post-exercise, 1.5 hours post-exercise, or 3 hours post-exercise. There were no significant differences in TNFα levels at any time point, however, other known inflammatory markers were elevated at various time points measured [37]. Perhaps TNFα is not essential for the inflammatory process as there are several factors that activate this process. Perhaps there are post-translational modifications that may be activated by the inflammation response. Finally, both the present investigation and the Colbert study did not report changes in TNFα in rodent tissues after exercise. It is possible that the rodent model may not reflect a similar change in TNFα compared to humans.

Thomas et al. used a cell culture model to examine the effects of short term and long term oxidative stress exposure [209]. Cells were exposed to a low level of stress and then several inflammatory markers were measured 24 hours after exposure. The authors reported increased TNFα after both acute and chronic exposure to stress. The cells with increased TNFα levels also had higher levels of other inflammatory markers. The authors concluded that cells exposed to oxidative stress, whether acutely, or chronically

are at greater risk for alterations or damage than other cells, likely through a TNFα mediated pathway [209].

Li et al. reported the importance of TNFα in the stress response, as cells were exposed to several apoptotic activators and inhibitors. One of the major pathways of apoptosis activation is through the ASK1-JNK-p38 pathway (apoptosis signal regulating kinase, c-Jun-terminal-kinase). When the cells were exposed to TNFα inhibitors, they had significantly less apoptosis. This led the authors to conclude that in order for optimal apoptosis to occur through the ASK1-JNK-p38 pathway, TNFα must be present to induce these factors [108].

An I/R (ischemia/reperfusion) injury study examined both TNFα and NFκB levels. Rat cardiac tissue was exposed to 5 minutes of ischemia followed by 30 minutes of reperfusion. The authors reported force decrements of nearly 50% following the I/R bout. TNFα expression was greater after I/R than in control hearts, as was, NFκB activity. This led the authors to conclude that both TNFα and NFκB likely play an important role in I/R associated injury in the heart [232]. It is not known if this same response would occur within skeletal muscle.

In conclusion, the present study reports no difference in TNFα expression levels between the exercise and control groups in soleus muscle. This is consistent with the mice data from Colbert et al. [37] but somewhat inconsistent with some of the published literature with cell culture [108, 209, 232]and human research [150]. These inconsistencies may be related to the model used to examine the response, or the time point examined and the extent of stress. It is possible that: 1) the one hour time point was not the optimal time to

see changes in TNFα expression, 2) even though several apoptotic genes were up-regulated, it is possible that apoptotic genes were activated by factors other than TNFα, and 3) the levels of TNFα in the working soleusskeletal limb mus cle may respond differently than what is seen in other tissues like the heart, and 4) the rodent model may demonstrate differences to humans. Studies need to be performed to address these issues. Again, in looking at the published literature, it is likely that a later time point would have been better for optimal analysis of changes in TNFα levels.

## Western Blot Analysis

To confirm that protein changes occurred, Western Blot analysis was used. In the present investigation, Western Blot analysis was performed on 2 proteins (NFκB and Atf-3) which previously had their gene levels measured using PCR analysis. In this experiment, the results of the 2 proteins tested Western Blot analysis matched the results from gene PCR analysis. The Western Blots reported no significant differences in NFκB protein levels and whereas Atf-3 protein levels were significantly elevated in the E group.

### NFκB

NFκB (p 65 subunit) protein content was determined using western blot analysis. There was no difference detected between the E and C groups one hour after exercise with this particular protein from the soleus muscle homogenates. This is consistent with the results from NFκB gene level, as this gene was not significantly activated at this time

point comparing the two groups. It is currently known that in most instances, a gene that is activated will result in gene transcription. This process is normally followed by protein translation. These processes are usually coupled, although there may be instances when these processes are not always coupled. It is possible for a gene to be up-regulated or down-regulated, without any change in protein concentration. It is also possible to see protein concentration changes without gene alterations occurring. This may occur as a result in changes often referred to as post-translational modifications. For example, a gene may be activated to form a protein but another factor may modify its actual production within the cell. In addition, other factors beside protein synthesis can modify protein concentration. Protein concentration can be modified by altering degradation rates. For example, a slowing of the degradation rate for a protein without altering the synthesis rate will lead to increased protein concentration. Furthermore, enhancing degradation rates without changes in protein synthesis would lead to lower protein concentration. Therefore, not only gene expression but alteration of degradative processes and post-translational modification factors need to be considered when determining the factors to alter protein concentration. This is especially true when only determining the concentration of a protein at a single time point. Finally, the time to activate the processes noted above may not always match.

NFκB protein concentration following exercise was examined by Sriwijitkamol et al. It should be noted that this particular study used diabetic patients, so they may respond to exercise differently than normal healthy subjects. Subjects trained 4 times a week at 75% of $VO_2$ max for 45 minutes for 8 weeks and had a biopsy taken from their

VL muscle both before and after the 8 weeks of exercise training. NFκB levels were unchanged following exercise training at the 24 hour time point. However, IκBα and IκBβ protein levels were both shown to be about double pre exercise values [199]. Figure 9, which was previously shown, illustrates how IκB is an inhibitor of NFκB translocation to the nucleus. This suggests that an adaptation seen with training is an inhibition of NFκB levels through IκB. It could be hypothesized that NFκB protein levels following a single exercise session should be elevated to stimulate this inhibitory response seen later after repeated bouts. However, their study did not measure changes following one bout so the acute change is still an unanswered question. Clearly, there are several differences between this study and the current study. The subjects in the Sriwijitkamol study were diabetic, the VL muscle was used, and their study examined the training response of NFκB, not the single session response. Additionally, samples were taken 24 hours after exercise whereas the current study collected samples 1 hour post-exercise. This is consistent with the hypothesis of Sen et al. which stated that intense acute aerobic exercise caused oxidation of glutathione and therefore increased NFκB levels. However, regular, repeated exercise can increase glutathione levels and will therefore decrease NFκB levels [189]. In addition, other antioxidant protective enzymes are activated with training.

Vider et al. examined NFκB levels following exercise as human subjects exercised at 80% of $VO_2$ for 60 minutes and blood was taken from the antecubital vein both prior to and immediately following exercise. NFκB protein levels were ~50% higher following a single session of exercise in blood lymphocytes. The authors further

measured an increase in lipid peroxidation following exercise and suggested that NFκB levels are likely at least in part regulated by RONS [216]. The authors also acknowledged that NFκB levels are often transiently increased and variable, as 3 of their 12 subjects had no change in NFκB in the blood lymphocytes. There are several major differences between their study and the current study. Obviously, it is possible that the rats used in the current study may respond differently than humans reported in Vider et al. Also, Vider et al. reported changes in blood lymphocytes. Blood lymphocyte levels will increase with exercise [216]. Others have reported differences in NFκB levels between different working limb muscles [13], so it would seem possible that blood lymphocytes and muscle NFκB levels could be different. It is also possible that in order to elevate NFκB concentration inside a muscle cell that many different processes and signals are involved. Cleary, this would have to be investigated further to confirm this. Finally, the time factor for the present study was one hour after exercise and the blood was collected immediately after exercise in the Vider et al. study.

Additionally, Ho et al. examined NFκB protein activityfollowing exercise in male Wistar rats. Rats aerobically exercised on a treadmill for 15, 30, or 60 minutes at a moderate intensity and had their gastrocnemius muscle removed 1 hour, 3.5 hours, or 5 hours post-exercise. NFκB nuclear binding activity was 50% higher post exercise at both the 1 hour and 3.5 hour times [70]. It should be noted that this study did not measure NFκB protein, but NFκB protein expression, which are separate measures.

Ji et al. ran rats on a treadmill at 75% of max for 2 hours and killed immediately following the exercise bout and the VL was removed. NFκB activity was 66% higher

following exercise compared to controls. These authors concluded that NFκB activity is redox regulated, and that NFκB activity is a very complex pathway with many factors having input [88]. Again, it should be noted that NFκB protein levels were not measured. However, this group examined the time course of soleus NFκB activation in rats and reported that despite an activation at 1, 2 and 4 hours after exercise the increase in NFκB activity did not translate into an increase in protein until 24 hours after the exercise [88]. This suggests that the process of gene transcription and protein translation is not necessarily matched and that in order to determine whether these processes are linked, investigators may need to measure protein changes at a later time point. It should be noted that the nuclear fraction concentration of NFκB was increased and not the total muscle homogenate in their study.

Recently, Bar-Shai et al. examined NFκB protein levels, except immobilization was used as their model and not exercise. It was previously reported that both exercise and immobilization do increase RONS levels. In their study, 6 month or 24 month old rats underwent hind limb immobilization for up to 4 weeks. NFκB levels were elevated in the quadriceps of both young and old rats, but it took 3 weeks of immobilization in young rats and 4 weeks in old rats. This suggests that it is possible that the age of the subjects can influence their NFκB response as young and old subjects may not respond the same [15]. Clearly, this study is different from the current study in that it used an immobilization model and not exercise, but, as previously noted, both of these models are known to alter RONS and therefore the NFκB response is similar. It is also worth noting that the quadriceps muscles (a mixed group of muscles) were used in their study.

One additional study examined NFκB both in vivo and in vitro. Aoi et al. exposed L6 myoblasts to hydrogen peroxide for up to 3 hours and examined NFκB levels. They reported that by one hour NFκB levels had doubled compared to baseline levels and that by 3 hours the NFκB levels had increased by 400% by 3 hours. The authors concluded that RONS can activate NFκB protein levels in isolated myoblasts [8]. In order to see if the results from a cell culture experiment would hold true to a real world example, NFκB protein levels were measured following a single 60 minute run at 80% of max in male Sprague Dawley rats. The gastrocnemius muscle showed a 50% increase in NFκB protein levels one hour post-exercise and a 125% increase at 24 hours post-exercise. The authors also reported increased lipid peroxidation suggesting a role for RONS in this process. This suggests that NFκB protein levels likely increase for at least 24 hours following a single bout of aerobic exercise in the gastrocnemius muscle [8]. The major difference between this experiment and the present experiment is the muscle group analyzed and the intensity and duration of exercise. Since it is well established that the gastrocnemius is primarily a fast twitch muscle and the soleus is primarily composed of slow twitch muscles, some differences may be expected. The fact that they used a higher intensity of exercise may have helped recruit more of the gastrocnemius in their study and perhaps observed the increase at one hour after exercise. Also, several of the above studies have mentioned a possible role for RONS in NFκB protein levels. It has been repeatedly shown that slow twitch fibers contain more antioxidant enzymes and are better able to deal with RONS related injuries than fast twitch fibers [87, 157, 160].

NFκB protein concentration results have varied depending on the model used (human vs. rat vs. cell culture), the time of measurement and the type of tissue examined. The current study reports that one-hour after moderate exercise for two hours that NFκB concentration in the soleus is not significantly modified in muscle homogenates. The present study is consistent with some investigations that suggest that one-hour after exercise NFκB content is not significantly altered in soleus muscle [74]. It appears it takes some time to increase the content of this molecule but the activation of this molecule can be rather rapid. This response is most likely due to 3 main factors as: 1) it appears that NFκB protein concentration can increase in response to stress, but it may not occur for some time (at least 24 to 48 hours). It is possible that had the animals in the current study been sacrificed at a later time point that NFκB levels might have increased; 2) several studies measured NFκB activity and not NFκB protein levels, which might be a better measure because it takes into account all factors which may play a role in altering NFκB (such as IκB, Iκκ, and phosphorylation status among others) and takes into account whether a biological response is likely to occur; and 3) the soleus muscle is known to be a slow twitch muscle which has higher concentrations of antioxidant enzymes than fast twitch muscles. It is possible that the soleus muscle has a different time course of increasing the NFκB concentration compared to the gastrocnemius but this factor needs to be confirmed. However, the present study did not show activation of the NFκB gene and the NFκB protein concentration one-hour after the exercise. The process of gene transcription to protein translation to active protein with post translational modification does not always match.

Atf-3

Atf-3 gene levels were previously reported as significantly altered between the E and C groups by both microarray and PCR analysis. This group difference was also present using western blot analysis for protein concentration. As previously mentioned, the process of gene transcription to protein translation is an intricate pathway and transcription does not always lead to translation. Therefore, it can not be assumed that up-regulated gene transcript levels will always match protein levels. Ji et al. reported a 24-hour time lag from significant gene up-regulation to significant changes in protein concentration of NFκB [88]. This suggests that the time from transcription to translation may take some time and it may be different for each protein. This study only measured Atf-3 at the one-hour post-exercise time point. Further research is needed to determine the exact time course of protein translation, as well as the length of the response of Atf-3 protein levels. The current investigation is the first known study to examine Atf-3 levels in rats following an exercise bout. In the following paragraphs, the results from the current study will be compared to other studies which have used various stresses (as exercise is a known stress). Therefore, it should be taken into consideration that the stress response to exercise may differ from these other stress responses.

Okamoto et al. recently examined Atf-3 in diabetic endothethial cells in which hyperglycemia was used to induce RONS. Atf-3 levels were significantly elevated post exposure [144]. No marker of specific RONS damage was determined. This study suggested that in situations in which RONS may become elevated, Atf-3 will likely become elevated as well after RONS exposure. Clearly, since this study used diabetic

cells for their model, there are several striking differences. It appears as though cells and whole rat muscle respond the same to stress in regards to Atf-3 gene activation. However, it warrants mentioning that the whole muscle measurement used muscle that was part of a working physiological system, whereas isolated cells are not from such a system. In addition, whole muscle cells undergo physiological levels of various stimuli, whereas cell culture studies typically use RONS levels above what would be considered physiological from the working system. Also, diabetic cells are not normal, healthy cells and may therefore respond differently.

Human foreskin fibroblasts were examined by Kool et al. In this study cells were exposed to ionizing radiation. By 2 hours post radiation exposure, cells had significant increases in Atf-3 protein levels [97]. Increases in Atf-3 were present at all time points measured up to the final measurement at 8 hours. Again, this study examined isolated fibroblast cells instead of a muscle cells. Additionally, protein levels were measured 2 hours through 8 hours post exposure, so there were no 1 hour post-stress measures determined at this time. Since the present study only examined through one-hour post exercise, whether or not these time points match remains to be elucidated.

Hashimoto et al. examined Atf-3 protein levels in human umbilical vein endothelial cells (HUVECs). These cells were exposed to various RONS producers and Atf-3 protein levels were significantly elevated immediately following RONS exposure [65]. The results from Hashimoto et al. agree with the present study. As previously mentioned, differences between cell cultures and whole muscles can not be ignored.

Future studies are needed to determine if the time course following exercise would match this study since the present study did not measure Atf-3 immediately following exercise.

Tamura et al. examined the Atf-3 protein response in rat embryonic cells which were exposed to radiation or RONS producing medium for 48 hours. Both conditions resulted in significant increases in Atf-3 protein levels immediately following exposure [206]. This study demonstrated increases in rat embryonic cells; this may not match skeletal muscle changes.

Additionally, Yan et al. examined the effects of exposing mouse embryonic fibroblast cells to various genotoxic stresses. Atf-3 protein levels were measured at the following time points post-stress: 0h, 0.5h, 1h, 2h, and 4h and found significantly increased protein levels at all time points [226]. This again suggests the role of Atf-3 in the early stress response of cells. Yan et al. measured protein levels at a variety of time points and saw significant increases at all time points, suggesting that this is not a transient increase. The present study only measured Atf-3 at the one hour post-exercise time point. Clearly, further measurements would need to be made post exercise in the soleus muscle in order to determine if the whole skeletal muscle will match the time course reported here by Yan et al. It also remains to be elucidated whether or not this increase is transient.

In conclusion, it appears as though Atf-3 protein levels are elevated in response to a variety of stress induced RONS situations. It was previously mentioned that Atf-3 is known to play a role in both apoptosis [137] and cell proliferation [206]. The exact role of Atf-3 in response to exercise remains unknown as it appears to act in a different role in

response to various stressors. It does however appear that Atf-3 will become significantly elevated in response to stress. Since Atf-3 protein levels were elevated in response to the aerobic exercise bout 2 possible explanations seem likely: 1) Atf-3 responds to exercise in a similar manner to other stresses, and 2) the time course from gene transcription to protein translation is different for every protein and this process occurs very quickly in regards to Atf-3. Since Atf-3 has repeatedly been reported to be elevated due to a variety of stresses rapidly, it has been called an early stress responder [29, 33, 138]. This is consistent with the findings of the present study which reported increase in both Atf-3 at the gene and protein level following a significant stress to the muscle. The present study is the first known investigation to examine Atf-3 following aerobic exercise, and can serve as the beginning of a time course investigation as to the levels of this protein.

## Summary

The microarray results indicate that 52 genes were significantly altered at the one hour post-exercise time point by the single session of aerobic exercise. The genes affected were mainly in the families of metabolism, apoptosis, muscle contraction, transcription/cell signaling, tissue generation, and inflammation. The PCR analysis confirmed the findings of the micro arrays for 4 genes: Atf3, Mgst1, NFκB and TNFα. Atf3 is known to be activated in response to stress and was up-regulated in the present experiment. Mgst1 is part of the glutathione defense system and helps protect the body from RONS damage. In the current experiment it was found to be down regulated one

hour post-exercise. Additionally, both NFκB and TNFα were found to be unchanged one hour after exercise. Finally, NFκB protein concentration was also unchanged and matched the result observed with the transcript level. Based on these findings, it appears that at the one hour time point after the exercise, the stress response was still active in the soleus muscle and that the antioxidant defense system had not been altered at the gene or protein level.

## Recommendations

Further research is required to fully understand the gene response to exercise. This is an important topic to study as more and more people begin to realize the benefits of exercise and start regular exercise programs. A better understanding of the oxidative response to exercise will allow for more optimal exercise programs to be designed. Additionally, the knowledge gained from the antioxidant defense system could perhaps be used to treat other conditions in which RONS are known to play a role.

Recommendations for future research are as follows:

1. The present experiment should be repeated over a longer period of time for observing changes in gene and protein levels. As previously mentioned, this experiment only measured gene changes one hour post-exercise. It would be beneficial to ascertain if changes at this intensity of exercise would alter the gene expression and protein concentration at some later time as suggested by the Hollander study [74].

2. It would also be of interest to carry out a time course experiment following several different training periods to compare the differences at various times of a single session against weeks, or months of repeated bouts to get a better understanding of the genetic changes that lead to adaptations from exercise.
3. Aerobic exercise is only 1 type of exercise training. All of the current experiments, as well as future experiments could be carried out using resistance training to better understand differences in the muscular response to different types of exercise.
4. This experiment only utilized the soleus muscle, which is known to be a type I or slow twitch fiber. Understanding the differences in the exercise response in different types of muscles (using mixed or fast twitch muscles) could aid in the prescription of various exercise programs.
5. It is well established that various antioxidants protect the body from RONS damage. Repeating the current experiments with an antioxidant group could help clarify the mechanism behind this protection.
6. It is well known that exercise, while causing RONS damage, increases RONS defense as well. It would be of interest to determine how various treatments (exercise) or pharmaceutical interventions can alter other disease processes (cancer, HIV) in which elevated RONS have been implicated.
7. Finally, understanding which genes are altered could lead to pharmaceutical or nutritional interventions being developed that might help up-regulate specific

genes that increase RONS defenses while other treatments could be developed that decrease RONS producers. This is what some have called the “exercise pill.”

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APPENDIX A

DNA MICROARRY RESULTS FOR ALL ARRAYS

| GenBank | Gene Symbol | Array 1 | Array 2 | Array 3 | Array 4 |
|---|---|---|---|---|---|
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| XM_215375 | LOC294337 | -1.96 | -1.82 | -1.95 | -2.05 |
| NM_021989 | Timp2 | -1.52 | -1.59 | -1.47 | -1.52 |
| AW918612 | | -1.79 | -1.73 | -1.95 | -1.81 |
| NM_012656 | Sparc | -1.67 | -1.85 | -1.71 | -1.87 |
| NM_019237 | Pcolce | -1.44 | -1.45 | -1.50 | -1.57 |
| NM_031628 | Nr4a3 | 15.16 | 18.09 | 11.99 | 24.66 |
| XM_227446 | LOC310670 | -1.50 | -1.72 | -1.53 | -1.65 |
| NM_012588 | Igfbp3 | -1.61 | -1.74 | -1.48 | -1.59 |
| NM_012588 | Igfbp3 | -1.51 | -1.54 | -1.49 | -1.71 |
| XM_223124 | Col9a1_predicted | -2.09 | -2.55 | -1.95 | -2.22 |
| XM_220752 | Rnf135_predicted | -1.67 | -1.43 | -1.49 | -1.52 |
| NM_031347 | Ppargc1a | 2.12 | 1.80 | 2.08 | 2.46 |
| NM_053356 | Col1a2 | -2.11 | -1.98 | -2.62 | -2.67 |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| XM_213440 | | -2.52 | -2.70 | -2.03 | -3.12 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| XM_346228 | | 1.62 | 1.57 | 1.47 | 1.33 |
| TC495193 | | 1.43 | 1.52 | 1.28 | 1.36 |
| XM_225173 | Pfn3_predicted | -1.81 | -1.72 | -2.11 | -2.39 |
| NM_012912 | Atf3 | 4.95 | 5.23 | 2.89 | 6.41 |
| NM_022501 | RGD:1302959 | -1.34 | -1.53 | -1.46 | -1.65 |
| XM_346881 | LOC360478 | -1.71 | -1.93 | -2.33 | -2.44 |
| NM_053356 | Col1a2 | -1.96 | -2.68 | -1.79 | -2.13 |
| AF139830 | Igfbp5 | -1.91 | -1.64 | -1.88 | -1.46 |
| AA964296 | | 2.17 | 2.07 | 3.23 | 2.22 |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| TC483914 | LOC310395 | 2.65 | 1.71 | 2.09 | 2.16 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| XM_344122 | LOC365949 | -1.49 | -1.28 | -1.28 | -1.30 |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| XM_214443 | Ecm2_predicted | -1.45 | -1.51 | -1.45 | -1.24 |
| BI282748 | | -1.38 | -1.80 | -1.89 | -1.73 |
| M12199 | | -1.96 | -1.95 | -2.10 | -3.06 |
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| NM_199093 | Serping1 | -1.23 | -1.51 | -1.40 | -1.31 |
| XM_343169 | Adn | -2.76 | -3.28 | -6.80 | -5.17 |
| NM_013086 | Crem | 2.25 | 2.15 | 1.89 | 3.34 |
| XM_226041 | | 1.59 | 1.33 | 1.27 | 1.50 |

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|---|---|---|---|---|---|
| NM_134399 | RGD:621165 | -1.50 | -1.31 | -1.25 | -1.27 |
| BF544403 | | 3.32 | 4.07 | 3.53 | 8.78 |
| NM_031688 | Sncg | -1.66 | -1.43 | -1.52 | -1.26 |
| AA924335 | | 1.57 | 1.29 | 1.70 | 1.75 |
| XM_218977 | Rhog_predicted | -1.74 | -1.49 | -1.44 | -1.30 |
| AA858639 | | -1.76 | -1.84 | -2.20 | -3.02 |
| BE117007 | | -1.38 | -1.88 | -1.71 | -1.42 |
| AI013671 | | 4.01 | 2.34 | 1.92 | 3.11 |
| XM_341859 | Tulp2_predicted | -1.30 | -1.19 | -1.47 | -1.27 |
| NM_145669 | Fhl1 | -1.30 | -1.58 | -1.82 | -1.77 |
| TC487987 | | 3.29 | 3.05 | 1.69 | 3.40 |
| NM_033351 | Fcgrt | -1.73 | -1.61 | -1.49 | -1.27 |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| NM_053326 | Enh | -1.47 | -1.49 | -1.29 | -1.21 |
| AW920693 | | -1.56 | -1.68 | -1.25 | -1.43 |
| NM_017151 | Rps15 | -1.89 | -1.54 | -2.73 | -2.24 |
| XM_346193 | LOC363397 | 1.48 | 1.25 | 1.28 | 1.20 |
| XM_345940 | | -1.36 | -1.83 | -2.19 | -1.74 |
| BC079462 | | -1.80 | -1.34 | -1.56 | -1.34 |
| BF549650 | | 3.36 | 2.30 | 1.67 | 3.28 |
| M34136 | Tpm1 | -1.32 | -1.25 | -1.54 | -1.64 |
| AW917764 | | -1.25 | -1.60 | -1.67 | -1.37 |
| BQ200408 | | -1.21 | -1.54 | -1.55 | -1.33 |
| BC079165 | | -1.25 | -1.50 | -1.48 | -1.79 |
| NM_017240 | Myh7 | -1.66 | -1.86 | -2.74 | -3.16 |
| U87983 | | 1.68 | 1.31 | 1.65 | 2.08 |
| XM_345834 | | 1.34 | 1.81 | 1.33 | 1.71 |
| NM_001002850 | Prm1 | -1.74 | -1.59 | -2.81 | -2.72 |
| XM_216335 | | -1.24 | -1.62 | -1.87 | -1.54 |
| CA512073 | Igfbp4 | -1.23 | -1.81 | -1.64 | -1.49 |
| XM_237971 | Mast3_predicted | -1.31 | -1.34 | -1.83 | -1.71 |
| XM_236348 | Cilp_predicted | -1.26 | -1.59 | -1.21 | -1.35 |
| ENSRNOT00000030063 | | -1.30 | -1.51 | -1.81 | -1.31 |
| BI275716 | | -1.81 | -1.72 | -1.74 | -1.20 |
| AW917911 | | -1.33 | -1.58 | -1.60 | -1.19 |
| NM_145775 | Nr1d1 | -1.26 | -1.42 | -1.80 | -1.81 |
| NM_017259 | Btg2 | 2.82 | 1.91 | 1.69 | 3.72 |
| NM_138887 | Hspb6 | -2.24 | -1.29 | -2.23 | -2.32 |
| XM_213911 | LOC289155 | -1.50 | -1.26 | -1.96 | -1.53 |
| XM_235164 | Frs2_predicted | 1.60 | 1.36 | 1.19 | 1.27 |
| NM_012595 | Ldhb | -1.42 | -1.54 | -2.15 | -1.42 |
| AW918768 | | -1.47 | -1.98 | -2.45 | -1.48 |
| AI409900 | | 1.47 | 1.30 | 1.21 | 1.15 |
| NM_012893 | Actg2 | -1.49 | -1.23 | -1.55 | -1.20 |
| AY075025 | Car11 | -1.66 | -1.44 | -1.19 | -1.32 |
| NM_181368 | Mustn1 | 2.64 | 2.08 | 4.22 | 7.25 |
| XM_221952 | Ftsj2_predicted | -1.93 | -1.39 | -2.71 | -1.76 |
| TC493257 | | 1.47 | 1.53 | 1.34 | 1.14 |

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|---|---|---|---|---|---|
| XM_216062 | Smarcd3_predicted | -1.61 | -1.56 | -3.00 | -2.16 |
| NM_013069 | Cd74 | -1.32 | -1.31 | -1.89 | -1.81 |
| NM_017239 | Myh6 | -1.50 | -2.04 | -1.72 | -3.06 |
| XM_227366 | LOC310585 | 1.19 | 1.25 | 1.47 | 1.16 |
| NM_138549 | Gpsn2 | -1.33 | -1.21 | -1.40 | -1.74 |
| AI574673 | | -1.60 | -1.34 | -2.28 | -1.55 |
| AW918430 | Lpin1_predicted | 2.57 | 1.59 | 3.41 | 4.96 |
| NM_001000262 | Olr383_predicted | 1.62 | 1.24 | 1.19 | 1.48 |
| BG673253 | | -1.27 | -1.65 | -1.30 | -1.21 |
| AW917657 | | -1.11 | -1.48 | -1.34 | -1.27 |
| NM_021261 | Tmsb10 | -1.65 | -1.61 | -1.45 | -1.14 |
| NM_012649 | Sdc4 | 1.97 | 1.29 | 1.34 | 1.58 |
| NM_031675 | Actn4 | -1.40 | -1.11 | -1.55 | -1.47 |
| XM_231617 | | 1.70 | 1.19 | 1.40 | 1.80 |
| XM_217539 | | 1.60 | 1.43 | 1.32 | 1.14 |
| NM_024156 | Anxa6 | -1.73 | -1.72 | -1.31 | -1.21 |
| A_44_P708381 | | 1.20 | 1.92 | 1.70 | 2.18 |
| BF408982 | Zfpm2_predicted | -1.39 | -1.96 | -1.76 | -2.96 |
| XM_216546 | RGD1305453_predicted | -1.14 | -1.55 | -1.49 | -1.27 |
| XM_216399 | Col15a1_predicted | -1.70 | -2.25 | -1.47 | -1.29 |
| XM_221348 | LOC288019 | -1.38 | -1.33 | -2.10 | -1.49 |
| NM_053933 | Pcdha4 | 1.84 | 1.80 | 1.16 | 1.52 |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| XM_347128 | | 1.53 | 1.50 | 1.20 | 1.17 |
| NM_053638 | Idh3a | 1.42 | 1.16 | 1.35 | 1.70 |
| XM_346252 | | -1.64 | -1.41 | -2.95 | -2.30 |
| NM_057208 | Tpm3 | -1.33 | -1.60 | -1.72 | -2.54 |
| ENSRNOT00000034797 | | -1.22 | -1.56 | -1.91 | -1.35 |
| TC504821 | | -1.29 | -1.40 | -1.17 | -1.69 |
| XM_219345 | LOC308991 | -1.45 | -1.45 | -1.85 | -1.18 |
| NM_031509 | Gsta5 | -1.43 | -1.85 | -2.12 | -1.26 |
| TC489660 | | -1.45 | -1.75 | -1.32 | -1.17 |
| AI008316 | | 2.09 | 1.55 | 2.53 | 4.39 |
| XM_216850 | LOC299625 | -1.50 | -1.56 | -1.15 | -1.23 |
| XM_345948 | LOC367102 | -1.17 | -1.48 | -1.39 | -1.15 |
| NM_212505 | RGD:1303321 | -1.55 | -2.20 | -1.78 | -3.70 |
| XM_343196 | Mybpc1 | -1.60 | -1.54 | -1.29 | -1.14 |
| NM_173111 | RGD:708368 | -1.40 | -1.53 | -2.76 | -1.96 |
| XM_225097 | | -1.19 | -1.75 | -1.86 | -1.37 |
| BG664147 | | -1.12 | -1.58 | -1.30 | -1.37 |
| XM_213440 | | -4.68 | -1.89 | -2.40 | -1.72 |
| XM_230278 | RGD1311701_predicted | -1.48 | -1.33 | -1.23 | -1.10 |
| AI031053 | | 2.72 | 1.27 | 3.07 | 3.49 |
| NM_134349 | Mgst1 | -1.57 | -1.67 | -2.62 | -2.07 |
| NM_031841 | Scd2 | -1.30 | -1.85 | -1.73 | -2.77 |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| XM_215285 | Rbp4 | -1.49 | -1.28 | -1.80 | -1.20 |

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|---|---|---|---|---|---|
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| NM_012604 | Myh3 | -1.55 | -1.62 | -3.25 | -3.31 |
| BE116572 | | -1.19 | -1.50 | -1.28 | -1.79 |
| NM_080903 | Trim63 | 2.08 | 1.46 | 2.80 | 4.37 |
| NM_017100 | Plk1 | 1.31 | 1.16 | 1.75 | 1.54 |
| XM_341145 | RGD1310442_predicted | 1.67 | 1.19 | 1.24 | 1.31 |
| XM_237998 | RGD1306643_predicted | -1.46 | -1.61 | -1.19 | -1.19 |
| NM_001007678 | Mss4 | 1.49 | 1.19 | 1.63 | 2.08 |
| XM_341875 | | -1.63 | -1.71 | -1.77 | -1.10 |
| NM_022597 | Ctsb | -1.64 | -1.48 | -1.38 | -1.10 |
| S43941 | | -1.83 | -1.44 | -2.67 | -1.40 |
| XM_341642 | Dok4_predicted | -1.52 | -1.56 | -2.90 | -3.30 |
| NM_175844 | Stars | 2.04 | 1.86 | 2.84 | 6.20 |
| XM_340759 | RGD1307032_predicted | -1.29 | -1.44 | -2.05 | -1.34 |
| NM_017184 | Tnni1 | -1.67 | -1.64 | -3.78 | -3.84 |
| AI638986 | | 2.07 | 1.75 | 1.18 | 1.47 |
| XM_224849 | | -1.10 | -1.50 | -1.28 | -1.24 |
| AI548750 | | -1.86 | -2.43 | -1.51 | -1.25 |
| XM_213943 | Mgst3_predicted | -1.07 | -1.33 | -1.42 | -1.47 |
| XM_216109 | Ube2h_predicted | 1.50 | 1.20 | 1.24 | 1.13 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_001000739 | Olr150_predicted | -1.51 | -1.30 | -1.09 | -1.51 |
| NM_130431 | Hspb2 | -1.43 | -1.08 | -1.57 | -1.32 |
| XM_233606 | LOC313641 | -1.21 | -1.48 | -1.85 | -2.26 |
| NM_022401 | Plec1 | -1.42 | -1.49 | -1.14 | -1.16 |
| NM_033539 | Eef1a2 | -1.09 | -1.72 | -1.81 | -1.60 |
| BC083725 | | 1.58 | 1.08 | 1.37 | 1.56 |
| AF540887 | | -1.12 | -1.29 | -1.68 | -1.43 |
| XM_237718 | Tuba6_predicted | -1.66 | -1.21 | -1.78 | -1.22 |
| TC466730 | | -1.51 | -1.52 | -1.65 | -1.07 |
| AW914778 | | 1.47 | 1.10 | 1.62 | 1.81 |
| AW919109 | | -1.10 | -1.19 | -1.47 | -1.35 |
| NM_080698 | Fmod | -1.16 | -1.21 | -1.62 | -1.28 |
| BI286389 | | 1.52 | 1.47 | 1.07 | 1.32 |
| NM_022596 | Golga2 | 1.66 | 1.18 | 1.55 | 1.18 |
| NM_017265 | | 1.62 | 1.13 | 1.66 | 2.16 |
| TC478102 | | -1.11 | -1.77 | -1.36 | -1.49 |
| NM_080903 | Trim63 | 2.70 | 1.22 | 3.93 | 4.03 |
| BC083721 | | -1.44 | -1.85 | -1.11 | -1.50 |
| TC504962 | Eif4g3_predicted | -1.24 | -1.19 | -1.14 | -1.55 |
| NM_053502 | Abcg1 | -1.63 | -1.98 | -1.79 | -1.09 |
| NM_024129 | Dcn | -1.18 | -1.70 | -1.83 | -1.26 |
| NM_031825 | Fbn1 | -1.23 | -1.55 | -2.49 | -1.66 |
| AI232147 | | 1.62 | 1.15 | 1.20 | 1.31 |
| AI317880 | | 1.11 | 1.47 | 1.20 | 1.20 |
| NM_001004080 | RGD:1303089 | -1.89 | -2.15 | -1.33 | -1.23 |
| NM_031054 | Mmp2 | -1.09 | -1.34 | -1.69 | -1.54 |
| NM_022634 | Lst1 | -1.43 | -1.18 | -2.20 | -1.81 |

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|---|---|---|---|---|---|
| NM_134388 | Tnnt1 | -1.36 | -1.58 | -3.09 | -3.06 |
| XM_226680 | | -1.19 | -1.90 | -1.69 | -1.26 |
| NM_031582 | Aoc3 | -1.19 | -1.39 | -1.93 | -2.17 |
| A_44_P470892 | | -1.41 | -1.08 | -1.67 | -1.34 |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| NM_139324 | Ehd4 | -1.11 | -1.37 | -1.76 | -1.36 |
| NM_024349 | Ak1 | 1.54 | 1.47 | 2.02 | 3.56 |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| NM_031140 | Vim | -1.25 | -1.92 | -1.69 | -1.20 |
| NM_017131 | Casq2 | -1.34 | -1.10 | -1.30 | -1.65 |
| AW919066 | | -1.36 | -1.27 | -1.13 | -1.74 |
| XM_231103 | | -1.26 | -1.18 | -1.49 | -1.10 |
| AABR03001498 | | 1.38 | 1.09 | 1.65 | 1.81 |
| XM_228553 | LOC317258 | -1.67 | -1.32 | -1.48 | -1.08 |
| XM_340817 | Myh4 | 1.48 | 1.91 | 4.48 | 5.28 |
| XM_347347 | LOC301596 | -1.56 | -1.42 | -1.05 | -1.60 |
| XM_342599 | Col9a3_predicted | -2.54 | -1.68 | -2.44 | -1.15 |
| AW141699 | Tgfbi | -1.29 | -1.56 | -1.79 | -1.12 |
| TC476390 | | 2.00 | 1.17 | 1.45 | 1.35 |
| BF411421 | | -1.37 | -1.49 | -1.22 | -1.07 |
| XM_345482 | LOC311710 | -1.21 | -1.89 | -1.63 | -2.84 |
| NM_017147 | Cfl1 | -1.28 | -1.54 | -1.15 | -1.14 |
| BF548976 | | -1.19 | -1.66 | -1.53 | -1.13 |
| AA859053 | | -1.20 | -1.08 | -1.48 | -1.51 |
| NM_030826 | Gpx1 | -1.08 | -1.57 | -2.08 | -1.88 |
| XM_347332 | Lamc3_predicted | -1.13 | -1.71 | -2.04 | -2.60 |
| NM_181090 | Slc38a2 | 2.06 | 1.14 | 1.99 | 3.04 |
| XM_219933 | Cyp2c65_predicted | 1.62 | 1.23 | 1.30 | 1.11 |
| XM_342245 | Postn_predicted | -1.63 | -1.46 | -3.20 | -1.52 |
| NM_175758 | Slc1a5 | -1.13 | -1.51 | -1.21 | -1.69 |
| XM_238167 | LOC293860 | -1.10 | -1.57 | -1.26 | -1.23 |
| XM_341958 | Eps8l2_predicted | -1.37 | -1.19 | -2.23 | -2.06 |
| ENSRNOT00000028943 | | -1.17 | -1.12 | -1.35 | -1.57 |
| ENSRNOT00000027259 | | -1.76 | -1.90 | -1.05 | -1.64 |
| AA997829 | | -1.72 | -2.09 | -1.35 | -1.15 |
| XM_340760 | RGD1309385_predicted | -1.78 | -1.30 | -3.22 | -1.67 |
| XM_343647 | LOC363306 | 1.43 | 1.51 | 1.07 | 1.20 |
| XM_233107 | LOC298169 | -1.16 | -1.24 | -1.84 | -1.44 |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| AW917238 | | 1.94 | 1.51 | 1.28 | 1.16 |
| XM_222302 | | -1.22 | -1.70 | -1.22 | -2.02 |
| NM_001009639 | RGD1305061_predicted | -1.58 | -1.23 | -2.84 | -1.76 |
| NM_199087 | Spint2 | -1.61 | -2.22 | -1.79 | -1.09 |
| AW434670 | | 2.56 | 2.12 | 1.29 | 1.32 |
| AW142344 | | 1.13 | 1.23 | 1.69 | 1.30 |
| XM_213835 | LOC288925 | -1.16 | -1.71 | -1.21 | -1.28 |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| NM_173111 | RGD:708368 | -1.21 | -1.09 | -1.52 | -1.59 |

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| NM_031832 | Lgals3 | -1.06 | -1.35 | -1.54 | -1.24 |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| XM_223715 | | 1.62 | 1.65 | 1.43 | 1.03 |
| XM_345415 | LOC366153 | 1.49 | 1.38 | 1.54 | 1.02 |
| NM_019143 | Fn1 | -1.49 | -1.79 | -3.75 | -1.53 |
| NM_139115 | Coro6 | -1.27 | -1.16 | -1.83 | -2.01 |
| TC489044 | | -1.38 | -1.32 | -1.52 | -1.03 |
| NM_031796 | Galnt5 | -1.53 | -1.24 | -1.74 | -1.10 |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| XM_220874 | LOC303471 | -1.42 | -1.74 | -1.29 | -1.08 |
| XM_228900 | LOC317454 | -1.61 | -1.89 | -5.68 | -7.71 |
| AI103276 | | -1.57 | -1.14 | -1.12 | -1.32 |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| TC503900 | | -2.13 | -1.28 | -1.34 | -1.28 |
| BE103095 | | 1.86 | 1.03 | 1.57 | 1.63 |
| AW143724 | | -1.34 | -1.69 | -4.54 | -3.11 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| XM_229609 | | 1.47 | 1.22 | 1.19 | 1.06 |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | |
| AW916908 | | -1.53 | -1.75 | -1.53 | -1.02 |
| NM_001007678 | Mss4 | 1.30 | 1.26 | 1.64 | 2.43 |
| NM_022514 | Rpl27 | -1.37 | -1.57 | -1.35 | -1.03 |
| CA338648 | | -1.43 | -2.09 | -1.09 | -1.65 |
| XM_216577 | RGD1306651_predicted | 1.67 | 1.40 | 1.97 | 4.23 |
| CB547639 | Cpeb4_predicted | 1.73 | 1.33 | 1.28 | 2.65 |
| NM_199390 | Flcn | 1.85 | 1.05 | 1.46 | 1.97 |
| NM_177927 | Serpinf1 | -1.25 | -1.14 | -1.93 | -1.85 |
| AW913985 | | -1.10 | -1.71 | -1.66 | -1.23 |
| NM_033234 | Hbb | 1.58 | 1.08 | 1.93 | 2.38 |
| NM_017149 | Meox2 | -1.14 | -1.86 | -1.64 | -1.21 |
| XM_341824 | | -1.65 | -1.68 | -1.28 | -1.06 |
| XM_344015 | LOC363767 | -1.17 | -1.19 | -1.77 | -1.32 |
| BF550246 | | -1.31 | -1.45 | -1.74 | -1.06 |
| BE128566 | | -1.17 | -1.07 | -1.54 | -1.37 |
| NM_023972 | Arl6ip5 | -1.57 | -1.30 | -1.17 | -1.09 |
| NM_030826 | Gpx1 | -1.13 | -1.34 | -2.16 | -1.92 |
| TC473838 | | 1.55 | 1.11 | 1.12 | 1.41 |
| NM_031531 | Spin2c | -1.45 | -1.01 | -1.54 | -1.60 |
| AA996757 | | -1.23 | -1.55 | -1.09 | -1.75 |
| AW918186 | | 1.59 | 1.11 | 1.59 | 2.44 |
| X52757 | | 1.19 | 1.11 | 1.53 | 1.16 |
| NM_138900 | C1s | -1.20 | -1.57 | -1.67 | -1.09 |
| AI232783 | | 1.17 | 1.38 | 2.00 | 2.56 |
| XM_215403 | Marcks | -1.44 | -1.33 | -2.13 | -1.16 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| AW917756 | | -1.09 | -2.03 | -2.00 | -1.38 |
| BF290649 | | 1.38 | 1.12 | 1.66 | 2.23 |
| NM_022265 | Pdcd4 | -2.00 | -1.38 | -1.70 | -1.07 |

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| NM_134402 | Bzw2 | -1.08 | -1.26 | -1.68 | -1.29 |
| AF053360 | | -1.42 | -1.39 | -3.96 | -3.29 |
| NM_013057 | F3 | -1.18 | -1.69 | -1.97 | -1.18 |
| XM_216288 | Gabarapl1_predicted | 1.60 | 1.02 | 1.48 | 1.85 |
| XM_341671 | Tradd | -1.92 | -1.37 | -4.22 | -1.65 |
| NM_022582 | Lgals7 | -1.49 | -1.15 | -1.09 | -1.21 |
| AABR03073563 | | 1.88 | 1.10 | 1.31 | 1.39 |
| XM_341462 | LOC361179 | 1.83 | 1.12 | 1.20 | 1.59 |
| XM_229147 | LOC302827 | -1.26 | -1.08 | -1.69 | -1.30 |
| NM_012848 | Fth1 | -1.65 | -1.42 | -1.22 | -1.06 |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| XM_344113 | LOC363925 | -1.21 | -1.86 | -2.81 | -4.70 |
| NM_022531 | Des | -1.57 | -1.40 | -1.15 | -1.08 |
| NM_017113 | Grn | -1.17 | -1.17 | -1.50 | -1.10 |
| XM_236238 | LOC315661 | -1.01 | -1.65 | -1.75 | -1.46 |
| XM_341158 | LOC360883 | -1.08 | -1.37 | -2.01 | -1.50 |
| TC486677 | | 1.55 | 1.36 | 1.09 | 1.13 |
| XM_214014 | Igfbp7_predicted | -1.80 | -1.43 | -1.85 | -1.03 |
| BF291173 | | -1.31 | -1.25 | -1.14 | -1.86 |
| XM_341238 | Hmx1_predicted | -1.28 | -1.23 | -1.17 | -1.86 |
| AW918626 | | -1.97 | -1.82 | -1.33 | -1.07 |
| BF567631 | | -1.07 | -1.49 | -1.71 | -1.22 |
| TC494356 | | -1.07 | -1.50 | -1.68 | -1.21 |
| TC478611 | | -1.21 | -1.40 | -2.05 | -1.20 |
| NM_019292 | Ca3 | -1.20 | -2.06 | -4.67 | -5.85 |
| NM_019904 | Lgals1 | -1.25 | -1.50 | -1.07 | -1.15 |
| NM_013036 | Sstr4 | -1.53 | -1.05 | -1.26 | -1.22 |
| AW142568 | | -1.19 | -1.21 | -1.22 | -1.82 |
| TC482103 | | 1.56 | 1.08 | 1.16 | 1.57 |
| XM_343674 | LOC363336 | 1.51 | 1.17 | 1.44 | 1.05 |
| XM_341957 | Ifitm3 | -1.02 | -1.23 | -1.50 | -1.42 |
| NM_012618 | S100a4 | -1.10 | -1.93 | -2.38 | -1.39 |
| L00382 | | -1.51 | -1.03 | -1.50 | -1.23 |
| NM_198776 | MGC72973 | 1.80 | 1.04 | 2.27 | 3.09 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| ENSRNOT00000005403 | | -1.44 | -1.13 | -1.54 | -2.43 |
| NM_001004238 | RGD:1303150 | 1.36 | 1.08 | 1.25 | 1.77 |
| TC486532 | | -1.22 | -1.50 | -2.35 | -1.26 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| TC484553 | | 10.88 | 6.24 | 1.27 | 2.41 |
| CB548368 | | 1.16 | 1.30 | 2.51 | 1.95 |
| NM_019230 | Slc22a3 | 1.66 | 1.58 | 1.03 | 1.26 |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| AI045171 | | -1.14 | -1.43 | -2.92 | -2.13 |
| ENSRNOT00000030063 | | -1.55 | -1.46 | -2.35 | -1.09 |
| CB547615 | | 1.45 | 1.36 | 1.90 | 3.93 |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| XM_343643 | | -1.19 | -1.30 | -2.50 | -2.61 |

| | | | | | |
|---|---|---|---|---|---|
| XM_213823 | | -2.22 | -2.61 | -1.16 | -1.31 |
| AW917988 | | -1.11 | -1.28 | -1.49 | -1.07 |
| AA945152 | | 1.17 | 1.07 | 1.36 | 1.64 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| XM_214108 | | 1.75 | 1.16 | 1.15 | 2.00 |
| XM_341989 | | 1.99 | 1.09 | 1.44 | 1.27 |
| XM_226390 | Arhgap10_predicted | 1.63 | 1.15 | 1.28 | 1.09 |
| XM_344825 | LOC365122 | 1.54 | 1.50 | 1.05 | 1.15 |
| AF329828 | | -1.31 | -1.27 | -1.84 | -1.08 |
| AW507352 | | 1.57 | 1.24 | 1.02 | 1.29 |
| XM_217332 | | 1.60 | 1.37 | 1.19 | 1.04 |
| AW914778 | | 1.18 | 1.09 | 1.64 | 1.81 |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| XM_213688 | Pex11c_predicted | 1.47 | 1.06 | 1.14 | 1.21 |
| TC483382 | | -1.19 | -1.10 | -1.68 | -1.23 |
| AI548201 | | 1.22 | 1.09 | 1.62 | 1.17 |
| AW914778 | | 1.05 | 1.24 | 1.51 | 1.83 |
| NM_134399 | RGD:621165 | -1.05 | -1.64 | -1.21 | -1.28 |
| AI171966 | | -1.01 | -1.22 | -1.53 | -1.47 |
| XM_342274 | Hapln2 | -1.05 | -1.18 | -1.63 | -1.63 |
| TC496068 | | 1.36 | 1.04 | 1.88 | 1.35 |
| XM_343661 | | 1.82 | 1.25 | 1.22 | 1.12 |
| L33722 | | -1.39 | -1.61 | -3.07 | -1.24 |
| NM_031345 | Dsipi | 1.47 | 1.20 | 1.28 | 1.01 |
| XM_345156 | Itga2 | -1.32 | -1.70 | -1.01 | -1.87 |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| TC513661 | | 1.27 | 1.04 | 1.49 | 1.89 |
| XM_344814 | Qki | -1.33 | -1.00 | -1.58 | -1.26 |
| XM_214977 | | -1.20 | -1.61 | -1.39 | -1.02 |
| NM_031001 | Ube2d2 | -1.57 | -1.13 | -1.31 | -1.07 |
| U42627 | Dusp6 | -1.28 | -1.79 | -1.84 | -1.03 |
| BQ204109 | | 1.10 | 1.53 | 1.11 | 1.19 |
| AI236635 | | 1.49 | 1.01 | 1.19 | 1.28 |
| XM_229650 | | 1.49 | 1.21 | 1.11 | 1.07 |
| TC474582 | | -1.30 | -1.66 | -1.82 | -1.00 |
| XM_342225 | RGD1307747_predicted | -1.01 | -1.56 | -1.25 | -1.74 |
| XM_236348 | Cilp_predicted | -1.61 | -1.32 | -2.98 | -1.24 |
| BQ780215 | | -1.23 | -1.07 | -2.06 | -1.84 |
| BM386565 | | 1.04 | 1.47 | 1.21 | 1.80 |
| XM_224969 | Slc25a15_predicted | 1.55 | 1.09 | 1.24 | 1.10 |
| XM_217130 | LOC300684 | 1.03 | 1.53 | 1.20 | 1.22 |
| XM_217218 | RGD1311381_predicted | -1.49 | -1.20 | -1.70 | -1.03 |
| AA817799 | | 1.50 | 1.05 | 1.49 | 1.11 |
| NM_145677 | Slc25a25 | 1.37 | 1.15 | 1.49 | 2.65 |
| TC464530 | | -1.76 | -2.38 | -2.29 | 1.08 |
| XM_213925 | Dpt_predicted | -1.28 | -1.39 | -1.56 | 1.02 |
| M57728 | Pmpca | 1.49 | 1.11 | 1.42 | 1.04 |
| BF555415 | | -1.11 | -1.15 | -1.08 | -1.47 |

| | | | | | |
|---|---|---|---|---|---|
| NM_019243 | Ptgfrn | -1.22 | -2.06 | -1.45 | -1.09 |
| XM_343174 | Ilvbl_predicted | -1.59 | -1.04 | -1.48 | -1.13 |
| AW915860 | | -1.10 | -1.89 | -1.66 | -1.12 |
| XM_228594 | LOC317270 | 1.47 | 1.00 | 1.54 | 1.19 |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| NM_012606 | Myl3 | -1.04 | -2.07 | -1.93 | -4.37 |
| XM_232202 | Adamts9_predicted | 1.46 | 1.15 | 1.82 | 3.53 |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| XM_232671 | Chd7_predicted | 1.03 | 1.23 | 1.54 | 1.90 |
| XM_216882 | Mrpl42_predicted | 1.68 | 1.07 | 1.13 | 1.36 |
| XM_216768 | Asb2_predicted | 1.34 | 1.07 | 1.55 | 2.39 |
| XM_343946 | LOC363657 | 1.09 | 1.09 | 1.16 | 1.47 |
| XM_217446 | | 1.17 | 1.76 | 1.33 | 1.06 |
| XM_344837 | LOC365141 | 1.63 | 1.31 | 1.09 | 1.09 |
| XM_214266 | Tncc_predicted | -1.01 | -1.66 | -3.28 | -4.05 |
| XM_346178 | | 1.54 | 1.16 | 1.20 | 1.04 |
| AI104924 | | 1.36 | 1.62 | 1.03 | 1.16 |
| XM_236776 | | -1.38 | -1.01 | -2.51 | -2.12 |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| ENSRNOT00000016811 | | -1.21 | -1.00 | -1.23 | -1.52 |
| BQ200332 | | 1.53 | 1.13 | 1.28 | 1.03 |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| XM_215692 | Myoz2_predicted | -1.04 | -1.38 | -2.89 | -2.39 |
| AA866409 | | 1.04 | 1.54 | 1.10 | 1.53 |
| TC473591 | | 1.24 | 1.02 | 1.38 | 1.79 |
| XM_345677 | | -1.49 | -1.00 | -1.16 | -1.50 |
| XM_342556 | LOC362250 | 1.10 | 1.08 | 1.47 | 1.13 |
| XM_217334 | Apobec2_predicted | -1.00 | -1.32 | -1.62 | -1.21 |
| XM_221690 | Krtap15-1_predicted | -3.01 | -2.12 | -1.00 | -1.50 |
| AA891242 | | -1.02 | -1.36 | -2.55 | -2.40 |
| XM_221937 | | 1.78 | 1.30 | 1.09 | 1.14 |
| NM_031677 | Fhl2 | -1.25 | -1.04 | -1.92 | -1.37 |
| XM_345970 | Chst2_predicted | 1.52 | 1.03 | 1.47 | 1.11 |
| NM_013082 | Sdc2 | -1.16 | -1.43 | -1.73 | -1.03 |

# APPENDIX B

## DNA MICORARRY RESULTS LISTED BY FAMILY

| Function/GenBank | Symbol | Array 1 | Array 2 | Array 3 | Array 4 |
|---|---|---|---|---|---|
| mitosis | | | | | |
| Accession | Gene | | | | |
| NM_017100 | Plk1 | 1.31 | 1.16 | 1.75 | 1.54 |
| immune response | | | | | |
| Accession | Gene | | | | |
| NM_013069 | Cd74 | -1.32 | -1.31 | -1.89 | -1.81 |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| NM_013057 | F3 | -1.18 | -1.69 | -1.97 | -1.18 |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| NM_033351 | Fcgrt | -1.73 | -1.61 | -1.49 | -1.27 |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| dopamine catabolism | | | | | |
| Accession | Gene | | | | |
| AA858639 | Comt | -1.76 | -1.84 | -2.20 | -3.02 |
| mRNA splice site selection | | | | | |
| Accession | Gene | | | | |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| locomotory behavior | | | | | |
| Accession | Gene | | | | |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| positive regulation of transcription | | | | | |
| Accession | Gene | | | | |
| NM_031347 | Ppargc1a | 2.12 | 1.80 | 2.08 | 2.46 |
| amino acid metabolism | | | | | |
| Accession | Gene | | | | |
| XM_224969 | Slc25a15_predicted | 1.55 | 1.09 | 1.24 | 1.10 |
| actin filament bundle formation | | | | | |
| Accession | Gene | | | | |
| NM_031675 | Actn4 | -1.40 | -1.11 | -1.55 | -1.47 |
| positive regulation of fatty acid metabolism | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| negative regulation of transport | | | | | |
| Accession | Gene | | | | |
| NM_023972 | Arl6ip5 | -1.57 | -1.30 | -1.17 | -1.09 |
| mitochondrial ornithine transport | | | | | |
| Accession | Gene | | | | |
| XM_224969 | Slc25a15_predicted | 1.55 | 1.09 | 1.24 | 1.10 |
| positive regulation of apoptosis | | | | | |
| Accession | Gene | | | | |

| | | | | | |
|---|---|---|---|---|---|
| NM_012588 | Igfbp3 | -1.61 | -1.74 | -1.48 | -1.59 |
| cell death | | | | | |
| Accession | Gene | | | | |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| N-acetylglucosamine metabolism | | | | | |
| Accession | Gene | | | | |
| XM_345970 | Chst2_predicted | 1.52 | 1.03 | 1.47 | 1.11 |
| lamellipodium biogenesis | | | | | |
| Accession | Gene | | | | |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| intracellular sequestering of iron ion | | | | | |
| Accession | Gene | | | | |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| peroxisome division | | | | | |
| Accession | Gene | | | | |
| XM_213688 | Pex11c_predicted | 1.47 | 1.06 | 1.14 | 1.21 |
| acetylcholine receptor signaling, muscarinic pathway | | | | | |
| Accession | Gene | | | | |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| glutathione metabolism | | | | | |
| Accession | Gene | | | | |
| NM_031509 | Gsta5 | -1.43 | -1.85 | -2.12 | -1.26 |
| NM_134349 | Mgst1 | -1.57 | -1.67 | -2.62 | -2.07 |
| metabolism | | | | | |
| Accession | Gene | | | | |
| NM_053638 | Idh3a | 1.42 | 1.16 | 1.35 | 1.70 |
| XM_214108 | Ugp2_predicted | 1.75 | 1.16 | 1.15 | 2.00 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| BF567631 | Sulf1 | -1.07 | -1.49 | -1.71 | -1.22 |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| response to wounding | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| nucleobase, nucleoside, nucleotide and nucleic acid metabolism | | | | | |
| Accession | Gene | | | | |
| XM_236348 | Cilp_predicted | -1.26 | -1.59 | -1.21 | -1.35 |
| protein-nucleus import | | | | | |
| Accession | Gene | | | | |
| NM_017147 | Cfl1 | -1.28 | -1.54 | -1.15 | -1.14 |
| focal adhesion formation | | | | | |
| Accession | Gene | | | | |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| protein amino acid phosphorylation | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| NM_017100 | Plk1 | 1.31 | 1.16 | 1.75 | 1.54 |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |

| | | | | | |
|---|---|---|---|---|---|
| transcription initiation from RNA polymerase I promoter | | | | | |
| Accession | Gene | | | | |
| BG664147 | Ptrf_predicted | -1.12 | -1.58 | -1.30 | -1.37 |
| regulation of transcription from RNA polymerase II promoter | | | | | |
| Accession | Gene | | | | |
| NM_031677 | Fhl2 | -1.25 | -1.04 | -1.92 | -1.37 |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| antigen presentation, endogenous antigen | | | | | |
| Accession | Gene | | | | |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| activation of MAPK | | | | | |
| Accession | Gene | | | | |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| circulation | | | | | |
| Accession | Gene | | | | |
| NM_017149 | Meox2 | -1.14 | -1.86 | -1.64 | -1.21 |
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| BI275716 | Col3a1 | -1.81 | -1.72 | -1.74 | -1.20 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| ER-nuclear signaling pathway | | | | | |
| Accession | Gene | | | | |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| Wnt receptor signaling pathway | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| response to oxidative stress | | | | | |
| Accession | Gene | | | | |
| NM_030826 | Gpx1 | -1.08 | -1.57 | -2.08 | -1.88 |
| NM_019292 | Ca3 | -1.20 | -2.06 | -4.67 | -5.85 |
| sodium ion transport | | | | | |
| Accession | Gene | | | | |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | 0.00 |
| smooth muscle contraction | | | | | |
| Accession | Gene | | | | |
| AA891242 | Myl7_predicted | -1.02 | -1.36 | -2.55 | -2.40 |
| negative regulation of complement activation | | | | | |
| Accession | Gene | | | | |
| NM_199093 | RGD:735225 | -1.23 | -1.51 | -1.40 | -1.31 |
| intermediate filament-based process | | | | | |
| Accession | Gene | | | | |
| NM_031140 | Vim | -1.25 | -1.92 | -1.69 | -1.20 |
| apoptosis | | | | | |
| Accession | Gene | | | | |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| BF567631 | Sulf1 | -1.07 | -1.49 | -1.71 | -1.22 |
| XM_341671 | Tradd | -1.92 | -1.37 | -4.22 | -1.65 |
| NM_022265 | Pdcd4 | -2.00 | -1.38 | -1.70 | -1.07 |

| | | | | | |
|---|---|---|---|---|---|
| NM_022582 | Lgals7 | -1.49 | -1.15 | -1.09 | -1.21 |
| unknownP | | | | | |
| Accession | Gene | | | | |
| NM_080903 | Trim63 | 2.08 | 1.46 | 2.80 | 4.37 |
| XM_227366 | LOC310585 | 1.19 | 1.25 | 1.47 | 1.16 |
| XM_233606 | LOC313641 | -1.21 | -1.48 | -1.85 | -2.26 |
| NM_053933 | Pcdha11 | 1.84 | 1.80 | 1.16 | 1.52 |
| NM_013082 | Sdc2 | -1.16 | -1.43 | -1.73 | -1.03 |
| XM_346228 | LOC367620 | 1.62 | 1.57 | 1.47 | 1.33 |
| XM_237998 | RGD1306643_predicted | -1.46 | -1.61 | -1.19 | -1.19 |
| NM_139324 | Ehd4 | -1.11 | -1.37 | -1.76 | -1.36 |
| XM_344015 | LOC363767 | -1.17 | -1.19 | -1.77 | -1.32 |
| XM_219345 | LOC308991 | -1.45 | -1.45 | -1.85 | -1.18 |
| NM_031531 | Spin2c | -1.45 | -1.01 | -1.54 | -1.60 |
| XM_343674 | LOC367576 | 1.51 | 1.17 | 1.44 | 1.05 |
| NM_177927 | Serpinf1 | -1.25 | -1.14 | -1.93 | -1.85 |
| AY075025 | RGD:735155 | -1.66 | -1.44 | -1.19 | -1.32 |
| XM_344825 | LOC308198 | 1.54 | 1.50 | 1.05 | 1.15 |
| AI103276 | LOC499691 | -1.57 | -1.14 | -1.12 | -1.32 |
| AW142568 | LOC500067 | -1.19 | -1.21 | -1.22 | -1.82 |
| CA338648 | MGC109519 | -1.43 | -2.09 | -1.09 | -1.65 |
| NM_134399 | RGD:621165 | -1.50 | -1.31 | -1.25 | -1.27 |
| BC079165 | LOC362934 | -1.25 | -1.50 | -1.48 | -1.79 |
| NM_199390 | RGD:735088 | 1.85 | 1.05 | 1.46 | 1.97 |
| XM_346193 | LOC367523 | 1.48 | 1.25 | 1.28 | 1.20 |
| NM_022634 | Lst1 | -1.43 | -1.18 | -2.20 | -1.81 |
| XM_343647 | LOC363306 | 1.43 | 1.51 | 1.07 | 1.20 |
| XM_217218 | RGD1311381_predicted | -1.49 | -1.20 | -1.70 | -1.03 |
| AI548750 | Ttyh3_predicted | -1.86 | -2.43 | -1.51 | -1.25 |
| AW917238 | Hint2_predicted | 1.94 | 1.51 | 1.28 | 1.16 |
| XM_215375 | LOC294337 | -1.96 | -1.82 | -1.95 | -2.05 |
| XM_341957 | Ifitm2 | -1.02 | -1.23 | -1.50 | -1.42 |
| XM_345948 | LOC367102 | -1.17 | -1.48 | -1.39 | -1.15 |
| NM_022596 | Golga2 | 1.66 | 1.18 | 1.55 | 1.18 |
| XM_217332 | LOC301215 | 1.60 | 1.37 | 1.19 | 1.04 |
| AW918626 | LOC361340 | -1.97 | -1.82 | -1.33 | -1.07 |
| XM_218977 | Rhog | -1.74 | -1.49 | -1.44 | -1.30 |
| NM_033539 | Eef1a2 | -1.09 | -1.72 | -1.81 | -1.60 |
| NM_199087 | RGD:735123 | -1.61 | -2.22 | -1.79 | -1.09 |
| XM_228594 | LOC317270 | 1.47 | 1.00 | 1.54 | 1.19 |
| XM_213835 | LOC288925 | -1.16 | -1.71 | -1.21 | -1.28 |
| BQ204109 | RGD1306947_predicted | 1.10 | 1.53 | 1.11 | 1.19 |
| NM_017265 | Hsd3b1 | 1.62 | 1.13 | 1.66 | 2.16 |
| XM_228553 | LOC317258 | -1.67 | -1.32 | -1.48 | -1.08 |
| XM_215692 | Myoz2_predicted | -1.04 | -1.38 | -2.89 | -2.39 |
| NM_031796 | Galnt5 | -1.53 | -1.24 | -1.74 | -1.10 |
| AI232147 | Ggps1 | 1.62 | 1.15 | 1.20 | 1.31 |
| NM_017259 | Btg2 | 2.82 | 1.91 | 1.69 | 3.72 |

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|---|---|---|---|---|---|
| XM_341958 | Eps8l2_predicted | -1.37 | -1.19 | -2.23 | -2.06 |
| AF329828 | RGD:620649 | -1.31 | -1.27 | -1.84 | -1.08 |
| NM_001009639 | RGD1305061_predicted | -1.58 | -1.23 | -2.84 | -1.76 |
| NM_145677 | Slc25a25 | 1.37 | 1.15 | 1.49 | 2.65 |
| XM_216850 | LOC299625 | -1.50 | -1.56 | -1.15 | -1.23 |
| AI574673 | LOC317232 | -1.60 | -1.34 | -2.28 | -1.55 |
| NM_012618 | S100a4 | -1.10 | -1.93 | -2.38 | -1.39 |
| XM_231617 | RGD1310722_predicted | 1.70 | 1.19 | 1.40 | 1.80 |
| XM_345677 | LOC366631 | -1.49 | -1.00 | -1.16 | -1.50 |
| XM_238167 | LOC293860 | -1.10 | -1.57 | -1.26 | -1.23 |
| NM_175844 | RGD:708493 | 2.04 | 1.86 | 2.84 | 6.20 |
| NM_139115 | RGD:708560 | -1.27 | -1.16 | -1.83 | -2.01 |
| AW434670 | Cmya1_predicted | 2.56 | 2.12 | 1.29 | 1.32 |
| XM_344122 | LOC363942 | -1.49 | -1.28 | -1.28 | -1.30 |
| XM_340760 | RGD1309385_predicted | -1.78 | -1.30 | -3.22 | -1.67 |
| NM_138549 | Gpsn2 | -1.33 | -1.21 | -1.40 | -1.74 |
| XM_346881 | LOC360478 | -1.71 | -1.93 | -2.33 | -2.44 |
| BI286389 | RGD:1303181 | 1.52 | 1.47 | 1.07 | 1.32 |
| XM_227446 | LOC310670 | -1.50 | -1.72 | -1.53 | -1.65 |
| AW141699 | RGD:620017 | -1.29 | -1.56 | -1.79 | -1.12 |
| AI236635 | Skp1a | 1.49 | 1.01 | 1.19 | 1.28 |
| XM_217130 | LOC300684 | 1.03 | 1.53 | 1.20 | 1.22 |
| BQ200408 | Cst3 | -1.21 | -1.54 | -1.55 | -1.33 |
| BC079462 | Pon2_predicted | -1.80 | -1.34 | -1.56 | -1.34 |
| NM_053326 | Enh | -1.47 | -1.49 | -1.29 | -1.21 |
| XM_345415 | LOC366153 | 1.49 | 1.38 | 1.54 | 1.02 |
| XM_217539 | LOC302192 | 1.60 | 1.43 | 1.32 | 1.14 |
| XM_220752 | | -1.67 | -1.43 | -1.49 | -1.52 |
| TC495193 | | 1.43 | 1.52 | 1.28 | 1.36 |
| XM_225173 | | -1.81 | -1.72 | -2.11 | -2.39 |
| AA964296 | | 2.17 | 2.07 | 3.23 | 2.22 |
| TC483914 | | 2.65 | 1.71 | 2.09 | 2.16 |
| M12199 | | -1.96 | -1.95 | -2.10 | -3.06 |
| XM_226041 | | 1.59 | 1.33 | 1.27 | 1.50 |
| BF544403 | | 3.32 | 4.07 | 3.53 | 8.78 |
| AA924335 | | 1.57 | 1.29 | 1.70 | 1.75 |
| AI013671 | | 4.01 | 2.34 | 1.92 | 3.11 |
| XM_341859 | | -1.30 | -1.19 | -1.47 | -1.27 |
| TC487987 | | 3.29 | 3.05 | 1.69 | 3.40 |
| AW920693 | | -1.56 | -1.68 | -1.25 | -1.43 |
| XM_345940 | | -1.36 | -1.83 | -2.19 | -1.74 |
| BF549650 | | 3.36 | 2.30 | 1.67 | 3.28 |
| U87983 | | 1.68 | 1.31 | 1.65 | 2.08 |
| XM_345834 | | 1.34 | 1.81 | 1.33 | 1.71 |
| NM_001002850 | | -1.74 | -1.59 | -2.81 | -2.72 |
| XM_216335 | | -1.24 | -1.62 | -1.87 | -1.54 |
| CA512073 | | -1.23 | -1.81 | -1.64 | -1.49 |
| XM_237971 | | -1.31 | -1.34 | -1.83 | -1.71 |

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|---|---|---|---|---|---|
| ENSRNOT00000030063 | | -1.30 | -1.51 | -1.81 | -1.31 |
| XM_213911 | | -1.50 | -1.26 | -1.96 | -1.53 |
| AI409900 | | 1.47 | 1.30 | 1.21 | 1.15 |
| XM_221952 | | -1.93 | -1.39 | -2.71 | -1.76 |
| TC493257 | | 1.47 | 1.53 | 1.34 | 1.14 |
| XM_216062 | | -1.61 | -1.56 | -3.00 | -2.16 |
| AW918430 | | 2.57 | 1.59 | 3.41 | 4.96 |
| AW917657 | | -1.11 | -1.48 | -1.34 | -1.27 |
| A_44_P708381 | | 1.20 | 1.92 | 1.70 | 2.18 |
| BF408982 | | -1.39 | -1.96 | -1.76 | -2.96 |
| XM_216546 | | -1.14 | -1.55 | -1.49 | -1.27 |
| XM_221348 | | -1.38 | -1.33 | -2.10 | -1.49 |
| XM_347128 | | 1.53 | 1.50 | 1.20 | 1.17 |
| XM_346252 | | -1.64 | -1.41 | -2.95 | -2.30 |
| ENSRNOT00000034797 | | -1.22 | -1.56 | -1.91 | -1.35 |
| TC504821 | | -1.29 | -1.40 | -1.17 | -1.69 |
| TC489660 | | -1.45 | -1.75 | -1.32 | -1.17 |
| AI008316 | | 2.09 | 1.55 | 2.53 | 4.39 |
| XM_225097 | | -1.19 | -1.75 | -1.86 | -1.37 |
| XM_230278 | | -1.48 | -1.33 | -1.23 | -1.10 |
| AI031053 | | 2.72 | 1.27 | 3.07 | 3.49 |
| BE116572 | | -1.19 | -1.50 | -1.28 | -1.79 |
| XM_341145 | | 1.67 | 1.19 | 1.24 | 1.31 |
| NM_001007678 | | 1.49 | 1.19 | 1.63 | 2.08 |
| XM_341875 | | -1.63 | -1.71 | -1.77 | -1.10 |
| S43941 | | -1.83 | -1.44 | -2.67 | -1.40 |
| XM_340759 | | -1.29 | -1.44 | -2.05 | -1.34 |
| AI638986 | | 2.07 | 1.75 | 1.18 | 1.47 |
| XM_224849 | | -1.10 | -1.50 | -1.28 | -1.24 |
| XM_216109 | | 1.50 | 1.20 | 1.24 | 1.13 |
| XM_237718 | | -1.66 | -1.21 | -1.78 | -1.22 |
| TC466730 | | -1.51 | -1.52 | -1.65 | -1.07 |
| AW914778 | | 1.47 | 1.10 | 1.62 | 1.81 |
| TC478102 | | -1.11 | -1.77 | -1.36 | -1.49 |
| BC083721 | | -1.44 | -1.85 | -1.11 | -1.50 |
| TC504962 | | -1.24 | -1.19 | -1.14 | -1.55 |
| AI317880 | | 1.11 | 1.47 | 1.20 | 1.20 |
| XM_226680 | | -1.19 | -1.90 | -1.69 | -1.26 |
| A_44_P470892 | | -1.41 | -1.08 | -1.67 | -1.34 |
| AW919066 | | -1.36 | -1.27 | -1.13 | -1.74 |
| XM_231103 | | -1.26 | -1.18 | -1.49 | -1.10 |
| AABR03001498 | | 1.38 | 1.09 | 1.65 | 1.81 |
| XM_340817 | | 1.48 | 1.91 | 4.48 | 5.28 |
| XM_347347 | | -1.56 | -1.42 | -1.05 | -1.60 |
| TC476390 | | 2.00 | 1.17 | 1.45 | 1.35 |
| XM_345482 | | -1.21 | -1.89 | -1.63 | -2.84 |
| BF548976 | | -1.19 | -1.66 | -1.53 | -1.13 |
| AA859053 | | -1.20 | -1.08 | -1.48 | -1.51 |

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|---|---|---|---|---|---|
| XM_347332 | | -1.13 | -1.71 | -2.04 | -2.60 |
| ENSRNOT00000028943 | | -1.17 | -1.12 | -1.35 | -1.57 |
| ENSRNOT00000027259 | | -1.76 | -1.90 | -1.05 | -1.64 |
| XM_233107 | | -1.16 | -1.24 | -1.84 | -1.44 |
| XM_222302 | | -1.22 | -1.70 | -1.22 | -2.02 |
| AW142344 | | 1.13 | 1.23 | 1.69 | 1.30 |
| XM_223715 | | 1.62 | 1.65 | 1.43 | 1.03 |
| TC489044 | | -1.38 | -1.32 | -1.52 | -1.03 |
| XM_220874 | | -1.42 | -1.74 | -1.29 | -1.08 |
| XM_228900 | | -1.61 | -1.89 | -5.68 | -7.71 |
| TC503900 | | -2.13 | -1.28 | -1.34 | -1.28 |
| BE103095 | | 1.86 | 1.03 | 1.57 | 1.63 |
| AW143724 | | -1.34 | -1.69 | -4.54 | -3.11 |
| XM_229609 | | 1.47 | 1.22 | 1.19 | 1.06 |
| AW916908 | | -1.53 | -1.75 | -1.53 | -1.02 |
| XM_216577 | | 1.67 | 1.40 | 1.97 | 4.23 |
| BF550246 | | -1.31 | -1.45 | -1.74 | -1.06 |
| TC473838 | | 1.55 | 1.11 | 1.12 | 1.41 |
| AW918186 | | 1.59 | 1.11 | 1.59 | 2.44 |
| X52757 | | 1.19 | 1.11 | 1.53 | 1.16 |
| AI232783 | | 1.17 | 1.38 | 2.00 | 2.56 |
| BF290649 | | 1.38 | 1.12 | 1.66 | 2.23 |
| XM_216288 | | 1.60 | 1.02 | 1.48 | 1.85 |
| AABR03073563 | | 1.88 | 1.10 | 1.31 | 1.39 |
| XM_341462 | | 1.83 | 1.12 | 1.20 | 1.59 |
| XM_229147 | | -1.26 | -1.08 | -1.69 | -1.30 |
| XM_344113 | | -1.21 | -1.86 | -2.81 | -4.70 |
| XM_236238 | | -1.01 | -1.65 | -1.75 | -1.46 |
| XM_341158 | | -1.08 | -1.37 | -2.01 | -1.50 |
| TC486677 | | 1.55 | 1.36 | 1.09 | 1.13 |
| XM_214014 | | -1.80 | -1.43 | -1.85 | -1.03 |
| BF291173 | | -1.31 | -1.25 | -1.14 | -1.86 |
| TC494356 | | -1.07 | -1.50 | -1.68 | -1.21 |
| TC478611 | | -1.21 | -1.40 | -2.05 | -1.20 |
| TC482103 | | 1.56 | 1.08 | 1.16 | 1.57 |
| L00382 | | -1.51 | -1.03 | -1.50 | -1.23 |
| ENSRNOT00000005403 | | -1.44 | -1.13 | -1.54 | -2.43 |
| TC486532 | | -1.22 | -1.50 | -2.35 | -1.26 |
| TC484553 | | 10.88 | 6.24 | 1.27 | 2.41 |
| CB548368 | | 1.16 | 1.30 | 2.51 | 1.95 |
| CB547615 | | 1.45 | 1.36 | 1.90 | 3.93 |
| AA945152 | | 1.17 | 1.07 | 1.36 | 1.64 |
| XM_226390 | | 1.63 | 1.15 | 1.28 | 1.09 |
| TC483382 | | -1.19 | -1.10 | -1.68 | -1.23 |
| AI548201 | | 1.22 | 1.09 | 1.62 | 1.17 |
| XM_342274 | | -1.05 | -1.18 | -1.63 | -1.63 |
| TC496068 | | 1.36 | 1.04 | 1.88 | 1.35 |
| XM_343661 | | 1.82 | 1.25 | 1.22 | 1.12 |

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|---|---|---|---|---|---|
| L33722 | | -1.39 | -1.61 | -3.07 | -1.24 |
| TC513661 | | 1.27 | 1.04 | 1.49 | 1.89 |
| XM_344814 | | -1.33 | -1.00 | -1.58 | -1.26 |
| XM_214977 | | -1.20 | -1.61 | -1.39 | -1.02 |
| XM_229650 | | 1.49 | 1.21 | 1.11 | 1.07 |
| TC474582 | | -1.30 | -1.66 | -1.82 | -1.00 |
| BQ780215 | | -1.23 | -1.07 | -2.06 | -1.84 |
| AA817799 | | 1.50 | 1.05 | 1.49 | 1.11 |
| TC464530 | | -1.76 | -2.38 | -2.29 | 1.08 |
| XM_232202 | | 1.46 | 1.15 | 1.82 | 3.53 |
| XM_216768 | | 1.34 | 1.07 | 1.55 | 2.39 |
| XM_343946 | | 1.09 | 1.09 | 1.16 | 1.47 |
| XM_217446 | | 1.17 | 1.76 | 1.33 | 1.06 |
| XM_344837 | | 1.63 | 1.31 | 1.09 | 1.09 |
| XM_346178 | | 1.54 | 1.16 | 1.20 | 1.04 |
| XM_236776 | | -1.38 | -1.01 | -2.51 | -2.12 |
| ENSRNOT00000016811 | | -1.21 | -1.00 | -1.23 | -1.52 |
| AA866409 | | 1.04 | 1.54 | 1.10 | 1.53 |
| TC473591 | | 1.24 | 1.02 | 1.38 | 1.79 |
| XM_342556 | | 1.10 | 1.08 | 1.47 | 1.13 |
| XM_221690 | | -3.01 | -2.12 | -1.00 | -1.50 |
| response to stress | | | | | |
| Accession | Gene | | | | |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| establishment and/or maintenance of cell polarity | | | | | |
| Accession | Gene | | | | |
| AW919109 | Cap2 | -1.10 | -1.19 | -1.47 | -1.35 |
| synaptic vesicle endocytosis | | | | | |
| Accession | Gene | | | | |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| inactivation of MAPK | | | | | |
| Accession | Gene | | | | |
| U42627 | Dusp6 | -1.28 | -1.79 | -1.84 | -1.03 |
| negative regulation of adenylate cyclase activity | | | | | |
| Accession | Gene | | | | |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| positive regulation of I-kappaB kinase/NF-kappaB cascade | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| circadian rhythm | | | | | |
| Accession | Gene | | | | |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| NM_145775 | Nr1d1 | -1.26 | -1.42 | -1.80 | -1.81 |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| inner cell mass cell proliferation | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |

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|---|---|---|---|---|---|
| intracellular signaling cascade | | | | | |
| Accession | Gene | | | | |
| XM_341989 | RGD1304930_predicted | 1.99 | 1.09 | 1.44 | 1.27 |
| lipid catabolism | | | | | |
| Accession | Gene | | | | |
| NM_017113 | Grn | -1.17 | -1.17 | -1.50 | -1.10 |
| visceral muscle development | | | | | |
| Accession | Gene | | | | |
| NM_017239 | Myh6 | -1.50 | -2.04 | -1.72 | -3.06 |
| AI104924 | Myh6 | 1.36 | 1.62 | 1.03 | 1.16 |
| regulation of heart contraction rate | | | | | |
| Accession | Gene | | | | |
| M34136 | Tpm1 | -1.32 | -1.25 | -1.54 | -1.64 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| G-protein signaling, adenylate cyclase inhibiting pathway | | | | | |
| Accession | Gene | | | | |
| NM_013036 | Sstr4 | -1.53 | -1.05 | -1.26 | -1.22 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| striated muscle contraction | | | | | |
| Accession | Gene | | | | |
| XM_343196 | RGD:735102 | -1.60 | -1.54 | -1.29 | -1.14 |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| NM_017131 | Casq2 | -1.34 | -1.10 | -1.30 | -1.65 |
| AI045171 | Casq2 | -1.14 | -1.43 | -2.92 | -2.13 |
| NM_012604 | Myh3 | -1.55 | -1.62 | -3.25 | -3.31 |
| NM_017240 | Myh7 | -1.66 | -1.86 | -2.74 | -3.16 |
| NM_017239 | Myh6 | -1.50 | -2.04 | -1.72 | -3.06 |
| AI104924 | Myh6 | 1.36 | 1.62 | 1.03 | 1.16 |
| prostaglandin biosynthesis | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| anti-apoptosis | | | | | |
| Accession | Gene | | | | |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| NM_031345 | Dsipi | 1.47 | 1.20 | 1.28 | 1.01 |
| skeletal development | | | | | |
| Accession | Gene | | | | |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| XM_213440 | Col1a1 | -2.52 | -2.70 | -2.03 | -3.12 |
| NM_031825 | Fbn1 | -1.23 | -1.55 | -2.49 | -1.66 |
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| BI275716 | Col3a1 | -1.81 | -1.72 | -1.74 | -1.20 |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| NM_053356 | Col1a2 | -2.11 | -1.98 | -2.62 | -2.67 |
| BI282748 | Col1a2 | -1.38 | -1.80 | -1.89 | -1.73 |
| XM_342245 | Postn_predicted | -1.63 | -1.46 | -3.20 | -1.52 |

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|---|---|---|---|---|---|
| ossification | | | | | |
| Accession | Gene | | | | |
| NM_012656 | Sparc | -1.67 | -1.85 | -1.71 | -1.87 |
| cell communication | | | | | |
| Accession | Gene | | | | |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| positive regulation of epithelial cell differentiation | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| embryonic heart tube development | | | | | |
| Accession | Gene | | | | |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| glycosaminoglycan metabolism | | | | | |
| Accession | Gene | | | | |
| BF567631 | Sulf1 | -1.07 | -1.49 | -1.71 | -1.22 |
| phospholipase C activation | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| perception of sound | | | | | |
| Accession | Gene | | | | |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| XM_213440 | Col1a1 | -2.52 | -2.70 | -2.03 | -3.12 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_053356 | Col1a2 | -2.11 | -1.98 | -2.62 | -2.67 |
| BI282748 | Col1a2 | -1.38 | -1.80 | -1.89 | -1.73 |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| cell cycle arrest | | | | | |
| Accession | Gene | | | | |
| NM_024349 | Ak1 | 1.54 | 1.47 | 2.02 | 3.56 |
| mRNA editing | | | | | |
| Accession | Gene | | | | |
| XM_217334 | Apobec2_predicted | -1.00 | -1.32 | -1.62 | -1.21 |
| cell proliferation | | | | | |
| Accession | Gene | | | | |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| neurotransmitter catabolism | | | | | |
| Accession | Gene | | | | |
| AA858639 | Comt | -1.76 | -1.84 | -2.20 | -3.02 |
| positive regulation of myoblast differentiation | | | | | |
| Accession | Gene | | | | |
| NM_012588 | Igfbp3 | -1.61 | -1.74 | -1.48 | -1.59 |
| cholesterol transport | | | | | |
| Accession | Gene | | | | |
| NM_053502 | Abcg1 | -1.63 | -1.98 | -1.79 | -1.09 |
| innate immune response | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| negative regulation of gluconeogenesis | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| endocytosis | | | | | |
| Accession | Gene | | | | |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| regulation of body size | | | | | |
| Accession | Gene | | | | |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| perception of smell, sensory transduction of chemical stimulus | | | | | |
| Accession | Gene | | | | |
| NM_001000739 | Olr150_predicted | -1.51 | -1.30 | -1.09 | -1.51 |
| NM_001000262 | Olr383_predicted | 1.62 | 1.24 | 1.19 | 1.48 |
| myogenesis | | | | | |
| Accession | Gene | | | | |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| cytoskeleton organization and biogenesis | | | | | |
| Accession | Gene | | | | |
| AW919109 | Cap2 | -1.10 | -1.19 | -1.47 | -1.35 |
| AA891242 | Myl7_predicted | -1.02 | -1.36 | -2.55 | -2.40 |
| XM_343643 | Mrlcb | -1.19 | -1.30 | -2.50 | -2.61 |
| NM_021261 | Tmsb10 | -1.65 | -1.61 | -1.45 | -1.14 |
| NM_012893 | Actg2 | -1.49 | -1.23 | -1.55 | -1.20 |
| NM_012606 | Myl3 | -1.04 | -2.07 | -1.93 | -4.37 |
| NM_012604 | Myh3 | -1.55 | -1.62 | -3.25 | -3.31 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| NM_017239 | Myh6 | -1.50 | -2.04 | -1.72 | -3.06 |
| AI104924 | Myh6 | 1.36 | 1.62 | 1.03 | 1.16 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| regulation of muscle contraction | | | | | |
| Accession | Gene | | | | |
| NM_138887 | Hspb6 | -2.24 | -1.29 | -2.23 | -2.32 |
| NM_057208 | RGD:621546 | -1.33 | -1.60 | -1.72 | -2.54 |
| NM_173111 | RGD:621546 | -1.40 | -1.53 | -2.76 | -1.96 |
| AF053360 | RGD:621546 | -1.42 | -1.39 | -3.96 | -3.29 |
| M34136 | Tpm1 | -1.32 | -1.25 | -1.54 | -1.64 |
| NM_017184 | Tnni1 | -1.67 | -1.64 | -3.78 | -3.84 |
| XM_214266 | Tncc_predicted | -1.01 | -1.66 | -3.28 | -4.05 |
| NM_024156 | Anxa6 | -1.73 | -1.72 | -1.31 | -1.21 |
| NM_134388 | Tnnt1 | -1.36 | -1.58 | -3.09 | -3.06 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| NM_017131 | Casq2 | -1.34 | -1.10 | -1.30 | -1.65 |
| AI045171 | Casq2 | -1.14 | -1.43 | -2.92 | -2.13 |
| cell aging | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |

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|---|---|---|---|---|---|
| phosphoinositide-mediated signaling | | | | | |
| Accession | Gene | | | | |
| NM_001004080 | RGD:1303089 | -1.89 | -2.15 | -1.33 | -1.23 |
| fatty acid oxidation | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| angiogenesis | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| chromatin assembly or disassembly | | | | | |
| Accession | Gene | | | | |
| XM_232671 | Chd7_predicted | 1.03 | 1.23 | 1.54 | 1.90 |
| regulated secretory pathway | | | | | |
| Accession | Gene | | | | |
| AW507352 | Gars_predicted | 1.57 | 1.24 | 1.02 | 1.29 |
| glucose homeostasis | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| determination of left/right symmetry | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| transcription | | | | | |
| Accession | Gene | | | | |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| NM_031628 | Nr4a3 | 15.16 | 18.09 | 11.99 | 24.66 |
| XM_221937 | Papolb_predicted | 1.78 | 1.30 | 1.09 | 1.14 |
| NM_145775 | Nr1d1 | -1.26 | -1.42 | -1.80 | -1.81 |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| BG664147 | Ptrf_predicted | -1.12 | -1.58 | -1.30 | -1.37 |
| adenine metabolism | | | | | |
| Accession | Gene | | | | |
| NM_024349 | Ak1 | 1.54 | 1.47 | 2.02 | 3.56 |
| muscle cell differentiation | | | | | |
| Accession | Gene | | | | |
| NM_022531 | Des | -1.57 | -1.40 | -1.15 | -1.08 |
| response to unfolded protein | | | | | |
| Accession | Gene | | | | |
| NM_130431 | Hspb2 | -1.43 | -1.08 | -1.57 | -1.32 |
| entrainment of circadian clock | | | | | |
| Accession | Gene | | | | |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| plasma membrane repair | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| sensory organ development | | | | | |
| Accession | Gene | | | | |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| antimicrobial humoral response (sensu Vertebrata) | | | | | |

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|---|---|---|---|---|---|
| Accession | Gene | | | | |
| XM_213943 | Mgst3_predicted | -1.07 | -1.33 | -1.42 | -1.47 |
| transmembrane receptor protein serine/threonine kinase signaling pathway | | | | | |
| Accession | Gene | | | | |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| NAD biosynthesis | | | | | |
| Accession | Gene | | | | |
| BC083725 | Nmnat3_predicted | 1.58 | 1.08 | 1.37 | 1.56 |
| regulation of cell differentiation | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| Rho protein signal transduction | | | | | |
| Accession | Gene | | | | |
| NM_017147 | Cfl1 | -1.28 | -1.54 | -1.15 | -1.14 |
| negative regulation of axon extension | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| development | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| NM_031825 | Fbn1 | -1.23 | -1.55 | -2.49 | -1.66 |
| NM_017149 | Meox2 | -1.14 | -1.86 | -1.64 | -1.21 |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| NM_019237 | Pcolce | -1.44 | -1.45 | -1.50 | -1.57 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| acute-phase response | | | | | |
| Accession | Gene | | | | |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| heterophilic cell adhesion | | | | | |
| Accession | Gene | | | | |
| NM_019904 | Lgals1 | -1.25 | -1.50 | -1.07 | -1.15 |
| NM_031832 | Lgals3 | -1.06 | -1.35 | -1.54 | -1.24 |
| NM_022582 | Lgals7 | -1.49 | -1.15 | -1.09 | -1.21 |
| protein transport | | | | | |
| Accession | Gene | | | | |
| BE128566 | Copz2_predicted | -1.17 | -1.07 | -1.54 | -1.37 |
| integrin-mediated signaling pathway | | | | | |
| Accession | Gene | | | | |
| XM_345156 | Itga2 | -1.32 | -1.70 | -1.01 | -1.87 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| proton transport | | | | | |
| Accession | Gene | | | | |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| regulation of cell cycle | | | | | |

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|---|---|---|---|---|---|
| Accession | Gene | | | | |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| NM_017100 | Plk1 | 1.31 | 1.16 | 1.75 | 1.54 |
| U42627 | Dusp6 | -1.28 | -1.79 | -1.84 | -1.03 |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| regulation of endocytosis | | | | | |
| Accession | Gene | | | | |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| negative regulation of caspase activation | | | | | |
| Accession | Gene | | | | |
| NM_021740 | Ptma | -1.25 | -1.69 | -1.26 | -1.10 |
| nuclear mRNA splicing, via spliceosome | | | | | |
| Accession | Gene | | | | |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| BQ200332 | Snrpa_predicted | 1.53 | 1.13 | 1.28 | 1.03 |
| CB547639 | Cpeb4_predicted | 1.73 | 1.33 | 1.28 | 2.65 |
| cellular morphogenesis | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| chemotaxis | | | | | |
| Accession | Gene | | | | |
| NM_080698 | Fmod | -1.16 | -1.21 | -1.62 | -1.28 |
| positive regulation of cell proliferation | | | | | |
| Accession | Gene | | | | |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| NM_022501 | RGD:1302959 | -1.34 | -1.53 | -1.46 | -1.65 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| XM_341824 | Capns1 | -1.65 | -1.68 | -1.28 | -1.06 |
| cell surface receptor linked signal transduction | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| O-linked glycosylation | | | | | |
| Accession | Gene | | | | |
| BG673253 | Ogt | -1.27 | -1.65 | -1.30 | -1.21 |
| pyridine nucleotide biosynthesis | | | | | |
| Accession | Gene | | | | |
| BC083725 | Nmnat3_predicted | 1.58 | 1.08 | 1.37 | 1.56 |
| L-glutamate transport | | | | | |
| Accession | Gene | | | | |
| NM_023972 | Arl6ip5 | -1.57 | -1.30 | -1.17 | -1.09 |
| induction of apoptosis | | | | | |
| Accession | Gene | | | | |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| urea cycle | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| XM_224969 | Slc25a15_predicted | 1.55 | 1.09 | 1.24 | 1.10 |
| lipid biosynthesis | | | | | |
| Accession | Gene | | | | |
| NM_031841 | Scd2 | -1.30 | -1.85 | -1.73 | -2.77 |
| signal complex formation | | | | | |
| Accession | Gene | | | | |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| somite specification | | | | | |
| Accession | Gene | | | | |
| NM_017149 | Meox2 | -1.14 | -1.86 | -1.64 | -1.21 |
| inflammatory response | | | | | |
| Accession | Gene | | | | |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| spermatogenesis | | | | | |
| Accession | Gene | | | | |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| NM_013086 | Crem | 2.25 | 2.15 | 1.89 | 3.34 |
| NM_021989 | Timp2 | -1.52 | -1.59 | -1.47 | -1.52 |
| AW918612 | Timp2 | -1.79 | -1.73 | -1.95 | -1.81 |
| response to nutrients | | | | | |
| Accession | Gene | | | | |
| BG673253 | Ogt | -1.27 | -1.65 | -1.30 | -1.21 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| regulation of nuclear mRNA splicing, via spliceosome | | | | | |
| Accession | Gene | | | | |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| blood coagulation | | | | | |
| Accession | Gene | | | | |
| NM_013057 | F3 | -1.18 | -1.69 | -1.97 | -1.18 |
| antigen presentation | | | | | |
| Accession | Gene | | | | |
| AI171966 | RGD:735096 | -1.01 | -1.22 | -1.53 | -1.47 |
| NM_033351 | Fcgrt | -1.73 | -1.61 | -1.49 | -1.27 |
| neuron differentiation | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| eye morphogenesis (sensu Mammalia) | | | | | |
| Accession | Gene | | | | |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| protein biosynthesis | | | | | |
| Accession | Gene | | | | |
| NM_022514 | Rpl27 | -1.37 | -1.57 | -1.35 | -1.03 |
| AW507352 | Gars_predicted | 1.57 | 1.24 | 1.02 | 1.29 |
| XM_216882 | Mrpl42_predicted | 1.68 | 1.07 | 1.13 | 1.36 |
| NM_017151 | Rps15 | -1.89 | -1.54 | -2.73 | -2.24 |
| lung development | | | | | |

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|---|---|---|---|---|---|
| Accession | Gene | | | | |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| G-protein signaling, coupled to cAMP nucleotide second messenger | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| extracellular amino acid transport | | | | | |
| Accession | Gene | | | | |
| NM_175758 | Slc1a5 | -1.13 | -1.51 | -1.21 | -1.69 |
| neuron migration | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| regulation of blood pressure | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| defense response | | | | | |
| Accession | Gene | | | | |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| regulation of cell migration | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| rhythmic behavior | | | | | |
| Accession | Gene | | | | |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| protein kinase cascade | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| cell cycle | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| NM_017100 | Plk1 | 1.31 | 1.16 | 1.75 | 1.54 |
| AA997829 | Calm1 | -1.72 | -2.09 | -1.35 | -1.15 |
| negative regulation of cell proliferation | | | | | |
| Accession | Gene | | | | |
| NM_013036 | Sstr4 | -1.53 | -1.05 | -1.26 | -1.22 |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| negative regulation of transcription | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| cell-substrate junction assembly | | | | | |
| Accession | Gene | | | | |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| vascular endothelial growth factor receptor signaling pathway | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| lipoprotein metabolism | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |

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|---|---|---|---|---|---|
| cell-cell signaling | | | | | |
| Accession | Gene | | | | |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_012649 | Sdc4 | 1.97 | 1.29 | 1.34 | 1.58 |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| detection of abiotic stimulus | | | | | |
| Accession | Gene | | | | |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| ion transport | | | | | |
| Accession | Gene | | | | |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | 0.00 |
| NM_019230 | Slc22a3 | 1.66 | 1.58 | 1.03 | 1.26 |
| fluid secretion | | | | | |
| Accession | Gene | | | | |
| NM_031345 | Dsipi | 1.47 | 1.20 | 1.28 | 1.01 |
| tricarboxylic acid cycle intermediate metabolism | | | | | |
| Accession | Gene | | | | |
| NM_012595 | Ldhb | -1.42 | -1.54 | -2.15 | -1.42 |
| patterning of blood vessels | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| electron transport | | | | | |
| Accession | Gene | | | | |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| XM_219933 | Cyp2c65_predicted | 1.62 | 1.23 | 1.30 | 1.11 |
| AW917988 | RGD1305146_predicted | -1.11 | -1.28 | -1.49 | -1.07 |
| NM_031347 | Ppargc1a | 2.12 | 1.80 | 2.08 | 2.46 |
| positive regulation of cytosolic calcium ion concentration | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| carbohydrate metabolism | | | | | |
| Accession | Gene | | | | |
| AW913985 | Slc3a1 | -1.10 | -1.71 | -1.66 | -1.23 |
| NM_053638 | Idh3a | 1.42 | 1.16 | 1.35 | 1.70 |
| substrate-bound cell migration, cell extension | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| cytoskeletal anchoring | | | | | |
| Accession | Gene | | | | |
| NM_022401 | Plec1 | -1.42 | -1.49 | -1.14 | -1.16 |
| regulation of neuronal synaptic plasticity | | | | | |
| Accession | Gene | | | | |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| G-protein signaling, coupled to cyclic nucleotide second messenger | | | | | |

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|---|---|---|---|---|---|
| Accession | Gene | | | | |
| NM_013036 | Sstr4 | -1.53 | -1.05 | -1.26 | -1.22 |
| somatic muscle development | | | | | |
| Accession | Gene | | | | |
| NM_130431 | Hspb2 | -1.43 | -1.08 | -1.57 | -1.32 |
| humoral immune response | | | | | |
| Accession | Gene | | | | |
| NM_033351 | Fcgrt | -1.73 | -1.61 | -1.49 | -1.27 |
| proteolysis and peptidolysis | | | | | |
| Accession | Gene | | | | |
| XM_343169 | Adn | -2.76 | -3.28 | -6.80 | -5.17 |
| NM_022597 | Ctsb | -1.64 | -1.48 | -1.38 | -1.10 |
| M57728 | Pmpca | 1.49 | 1.11 | 1.42 | 1.04 |
| NM_031054 | Mmp2 | -1.09 | -1.34 | -1.69 | -1.54 |
| NM_019237 | Pcolce | -1.44 | -1.45 | -1.50 | -1.57 |
| XM_341824 | Capns1 | -1.65 | -1.68 | -1.28 | -1.06 |
| one-carbon compound metabolism | | | | | |
| Accession | Gene | | | | |
| NM_019292 | Ca3 | -1.20 | -2.06 | -4.67 | -5.85 |
| branched chain family amino acid biosynthesis | | | | | |
| Accession | Gene | | | | |
| XM_343174 | Ilvbl_predicted | -1.59 | -1.04 | -1.48 | -1.13 |
| actin filament-based movement | | | | | |
| Accession | Gene | | | | |
| AA891242 | Myl7_predicted | -1.02 | -1.36 | -2.55 | -2.40 |
| NM_012604 | Myh3 | -1.55 | -1.62 | -3.25 | -3.31 |
| tRNA aminoacylation for protein translation | | | | | |
| Accession | Gene | | | | |
| AW507352 | Gars_predicted | 1.57 | 1.24 | 1.02 | 1.29 |
| regulation of transcription, DNA-dependent | | | | | |
| Accession | Gene | | | | |
| NM_013086 | Crem | 2.25 | 2.15 | 1.89 | 3.34 |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| XM_214108 | Ugp2_predicted | 1.75 | 1.16 | 1.15 | 2.00 |
| NM_031628 | Nr4a3 | 15.16 | 18.09 | 11.99 | 24.66 |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| XM_341238 | Hmx1_predicted | -1.28 | -1.23 | -1.17 | -1.86 |
| NM_017149 | Meox2 | -1.14 | -1.86 | -1.64 | -1.21 |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| NM_145775 | Nr1d1 | -1.26 | -1.42 | -1.80 | -1.81 |
| NM_012912 | Atf3 | 4.95 | 5.23 | 2.89 | 6.41 |
| BG664147 | Ptrf_predicted | -1.12 | -1.58 | -1.30 | -1.37 |
| XM_232671 | Chd7_predicted | 1.03 | 1.23 | 1.54 | 1.90 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |

| | | | | | |
|---|---|---|---|---|---|
| NM_031347 | Ppargc1a | 2.12 | 1.80 | 2.08 | 2.46 |
| NM_031345 | Dsipi | 1.47 | 1.20 | 1.28 | 1.01 |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| transport | | | | | |
| Accession | Gene | | | | |
| AW913985 | Slc3a1 | -1.10 | -1.71 | -1.66 | -1.23 |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| NM_033234 | Hbb | 1.58 | 1.08 | 1.93 | 2.38 |
| NM_198776 | Hbb | 1.80 | 1.04 | 2.27 | 3.09 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_053502 | Abcg1 | -1.63 | -1.98 | -1.79 | -1.09 |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| BE128566 | Copz2_predicted | -1.17 | -1.07 | -1.54 | -1.37 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | 0.00 |
| XM_224969 | Slc25a15_predicted | 1.55 | 1.09 | 1.24 | 1.10 |
| AW918768 | Sln_predicted | -1.47 | -1.98 | -2.45 | -1.48 |
| NM_181090 | Slc38a2 | 2.06 | 1.14 | 1.99 | 3.04 |
| XM_215285 | Rbp4 | -1.49 | -1.28 | -1.80 | -1.20 |
| NM_019230 | Slc22a3 | 1.66 | 1.58 | 1.03 | 1.26 |
| inhibition of caspase activation | | | | | |
| Accession | Gene | | | | |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| G-protein coupled receptor protein signaling pathway | | | | | |
| Accession | Gene | | | | |
| NM_013036 | Sstr4 | -1.53 | -1.05 | -1.26 | -1.22 |
| NM_001000739 | Olr150_predicted | -1.51 | -1.30 | -1.09 | -1.51 |
| AA997829 | Calm1 | -1.72 | -2.09 | -1.35 | -1.15 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| NM_001000262 | Olr383_predicted | 1.62 | 1.24 | 1.19 | 1.48 |
| XM_235164 | Frs2_predicted | 1.60 | 1.36 | 1.19 | 1.27 |
| chromatin modification | | | | | |
| Accession | Gene | | | | |
| XM_232671 | Chd7_predicted | 1.03 | 1.23 | 1.54 | 1.90 |
| biosynthesis | | | | | |
| Accession | Gene | | | | |
| BC083725 | Nmnat3_predicted | 1.58 | 1.08 | 1.37 | 1.56 |
| oxygen transport | | | | | |
| Accession | Gene | | | | |
| NM_033234 | Hbb | 1.58 | 1.08 | 1.93 | 2.38 |
| NM_198776 | Hbb | 1.80 | 1.04 | 2.27 | 3.09 |
| cellular defense response | | | | | |
| Accession | Gene | | | | |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| transmembrane receptor protein tyrosine kinase signaling pathway | | | | | |
| Accession | Gene | | | | |
| XM_341642 | Dok4_predicted | -1.52 | -1.56 | -2.90 | -3.30 |

| | | | | | |
|---|---|---|---|---|---|
| XM_235164 | Frs2_predicted | 1.60 | 1.36 | 1.19 | 1.27 |
| regulation of transcription | | | | | |
| Accession | Gene | | | | |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| regulation of axon extension | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| hemopoiesis | | | | | |
| Accession | Gene | | | | |
| NM_022501 | RGD:1302959 | -1.34 | -1.53 | -1.46 | -1.65 |
| response to hypoxia | | | | | |
| Accession | Gene | | | | |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| catecholamine metabolism | | | | | |
| Accession | Gene | | | | |
| AA858639 | Comt | -1.76 | -1.84 | -2.20 | -3.02 |
| spermatid development | | | | | |
| Accession | Gene | | | | |
| NM_021261 | Tmsb10 | -1.65 | -1.61 | -1.45 | -1.14 |
| secretion | | | | | |
| Accession | Gene | | | | |
| NM_024156 | Anxa6 | -1.73 | -1.72 | -1.31 | -1.21 |
| DNA replication | | | | | |
| Accession | Gene | | | | |
| NM_031975 | Ptms | -2.00 | -1.25 | -4.33 | -1.41 |
| tissue regeneration | | | | | |
| Accession | Gene | | | | |
| NM_181368 | Mustn1 | 2.64 | 2.08 | 4.22 | 7.25 |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| heart development | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_017131 | Casq2 | -1.34 | -1.10 | -1.30 | -1.65 |
| AI045171 | Casq2 | -1.14 | -1.43 | -2.92 | -2.13 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| fatty acid beta-oxidation | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| regulation of striated muscle contraction | | | | | |
| Accession | Gene | | | | |
| NM_017184 | Tnni1 | -1.67 | -1.64 | -3.78 | -3.84 |
| morphogenesis | | | | | |
| Accession | Gene | | | | |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| iron ion transport | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| axon guidance | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| regulation of macrophage activation | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| induction of bacterial agglutination | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| glucose metabolism | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| regulation of cell proliferation | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| regulation of translational initiation | | | | | |
| Accession | Gene | | | | |
| NM_134402 | Bzw2 | -1.08 | -1.26 | -1.68 | -1.29 |
| glycyl-tRNA aminoacylation | | | | | |
| Accession | Gene | | | | |
| AW507352 | Gars_predicted | 1.57 | 1.24 | 1.02 | 1.29 |
| positive regulation of glucose import | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| cell adhesion | | | | | |
| Accession | Gene | | | | |
| NM_031582 | Aoc3 | -1.19 | -1.39 | -1.93 | -2.17 |
| XM_342599 | Col9a3_predicted | -2.54 | -1.68 | -2.44 | -1.15 |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| XM_213440 | Col1a1 | -2.52 | -2.70 | -2.03 | -3.12 |
| NM_012649 | Sdc4 | 1.97 | 1.29 | 1.34 | 1.58 |
| XM_223124 | Col9a1_predicted | -2.09 | -2.55 | -1.95 | -2.22 |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| AA996757 | Cldn10_predicted | -1.23 | -1.55 | -1.09 | -1.75 |
| XM_343196 | RGD:735102 | -1.60 | -1.54 | -1.29 | -1.14 |
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| BI275716 | Col3a1 | -1.81 | -1.72 | -1.74 | -1.20 |
| XM_345156 | Itga2 | -1.32 | -1.70 | -1.01 | -1.87 |
| XM_216399 | Col15a1_predicted | -1.70 | -2.25 | -1.47 | -1.29 |
| NM_053356 | Col1a2 | -2.11 | -1.98 | -2.62 | -2.67 |
| BI282748 | Col1a2 | -1.38 | -1.80 | -1.89 | -1.73 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |

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|---|---|---|---|---|---|
| XM_213925 | Dpt_predicted | -1.28 | -1.39 | -1.56 | 1.02 |
| XM_342245 | Postn_predicted | -1.63 | -1.46 | -3.20 | -1.52 |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| amino acid transport | | | | | |
| Accession | Gene | | | | |
| AW913985 | Slc3a1 | -1.10 | -1.71 | -1.66 | -1.23 |
| NM_181090 | Slc38a2 | 2.06 | 1.14 | 1.99 | 3.04 |
| blastocyst development | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| regulation of translation | | | | | |
| Accession | Gene | | | | |
| XM_213823 | Rbms2_predicted | -2.22 | -2.61 | -1.16 | -1.31 |
| embryonic limb morphogenesis | | | | | |
| Accession | Gene | | | | |
| NM_181368 | Mustn1 | 2.64 | 2.08 | 4.22 | 7.25 |
| synaptic transmission | | | | | |
| Accession | Gene | | | | |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| pregnancy | | | | | |
| Accession | Gene | | | | |
| NM_033351 | Fcgrt | -1.73 | -1.61 | -1.49 | -1.27 |
| negative regulation of neuron differentiation | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| transmembrane receptor protein tyrosine phosphatase signaling pathway | | | | | |
| Accession | Gene | | | | |
| XM_235164 | Frs2_predicted | 1.60 | 1.36 | 1.19 | 1.27 |
| epithelial cell differentiation | | | | | |
| Accession | Gene | | | | |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| epidermis development | | | | | |
| Accession | Gene | | | | |
| XM_213440 | Col1a1 | -2.52 | -2.70 | -2.03 | -3.12 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| cell migration | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| ubiquitin cycle | | | | | |
| Accession | Gene | | | | |
| BF411421 | Herc4_predicted | -1.37 | -1.49 | -1.22 | -1.07 |
| NM_031001 | Ube2d2 | -1.57 | -1.13 | -1.31 | -1.07 |
| negative regulation of smooth muscle contraction | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| organic cation transport | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| NM_019230 | Slc22a3 | 1.66 | 1.58 | 1.03 | 1.26 |
| cell fate commitment | | | | | |
| Accession | Gene | | | | |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| learning and/or memory | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| glial cell differentiation | | | | | |
| Accession | Gene | | | | |
| NM_138900 | C1s | -1.20 | -1.57 | -1.67 | -1.09 |
| positive regulation of signal transduction | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| RNA processing | | | | | |
| Accession | Gene | | | | |
| XM_213823 | Rbms2_predicted | -2.22 | -2.61 | -1.16 | -1.31 |
| neurogenesis | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| XM_341642 | Dok4_predicted | -1.52 | -1.56 | -2.90 | -3.30 |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| negative regulation of signal transduction | | | | | |
| Accession | Gene | | | | |
| NM_012588 | Igfbp3 | -1.61 | -1.74 | -1.48 | -1.59 |
| protein amino acid dephosphorylation | | | | | |
| Accession | Gene | | | | |
| BM386565 | Ptpn18_predicted | 1.04 | 1.47 | 1.21 | 1.80 |
| U42627 | Dusp6 | -1.28 | -1.79 | -1.84 | -1.03 |
| negative regulation of cell cycle | | | | | |
| Accession | Gene | | | | |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| intracellular transport | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| visual perception | | | | | |
| Accession | Gene | | | | |
| NM_031825 | Fbn1 | -1.23 | -1.55 | -2.49 | -1.66 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| XM_215285 | Rbp4 | -1.49 | -1.28 | -1.80 | -1.20 |
| antigen processing, endogenous antigen via MHC class I | | | | | |
| Accession | Gene | | | | |
| NM_012512 | B2m | -1.06 | -1.39 | -1.88 | -1.21 |
| negative regulation of transcription from RNA polymerase II promoter | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |

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|---|---|---|---|---|---|
| branching morphogenesis | | | | | |
| Accession | Gene | | | | |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| NM_031340 | Timeless | -1.95 | -1.27 | -1.32 | -1.58 |
| regulation of cell growth | | | | | |
| Accession | Gene | | | | |
| NM_012588 | Igfbp3 | -1.61 | -1.74 | -1.48 | -1.59 |
| AF139830 | Igfbp5 | -1.91 | -1.64 | -1.88 | -1.46 |
| AW917764 | Igfbp5 | -1.25 | -1.60 | -1.67 | -1.37 |
| transforming growth factor beta receptor signaling pathway | | | | | |
| Accession | Gene | | | | |
| AW917911 | Tgfbr2 | -1.33 | -1.58 | -1.60 | -1.19 |
| potassium ion transport | | | | | |
| Accession | Gene | | | | |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| pattern specification | | | | | |
| Accession | Gene | | | | |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| negative regulation of apoptosis | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| protein modification | | | | | |
| Accession | Gene | | | | |
| NM_031001 | Ube2d2 | -1.57 | -1.13 | -1.31 | -1.07 |
| fatty acid alpha-oxidation | | | | | |
| Accession | Gene | | | | |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| protein homooligomerization | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| actin cytoskeleton organization and biogenesis | | | | | |
| Accession | Gene | | | | |
| AW919109 | Cap2 | -1.10 | -1.19 | -1.47 | -1.35 |
| NM_017147 | Cfl1 | -1.28 | -1.54 | -1.15 | -1.14 |
| NM_021261 | Tmsb10 | -1.65 | -1.61 | -1.45 | -1.14 |
| induction of apoptosis by oxidative stress | | | | | |
| Accession | Gene | | | | |
| NM_030826 | Gpx1 | -1.08 | -1.57 | -2.08 | -1.88 |
| intracellular protein transport | | | | | |
| Accession | Gene | | | | |
| BE128566 | Copz2_predicted | -1.17 | -1.07 | -1.54 | -1.37 |
| regulation of mitotic cell cycle | | | | | |
| Accession | Gene | | | | |
| NM_021693 | Snf1lk | 5.24 | 3.38 | 2.39 | 3.66 |
| vesicle-mediated transport | | | | | |
| Accession | Gene | | | | |
| NM_001004238 | RGD:1303150 | 1.36 | 1.08 | 1.25 | 1.77 |
| response to heat | | | | | |

| Accession | Gene | | | | |
|---|---|---|---|---|---|
| NM_130431 | Hspb2 | -1.43 | -1.08 | -1.57 | -1.32 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| synaptic transmission, cholinergic | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| mRNA processing | | | | | |
| Accession | Gene | | | | |
| XM_221937 | Papolb_predicted | 1.78 | 1.30 | 1.09 | 1.14 |
| XM_217334 | Apobec2_predicted | -1.00 | -1.32 | -1.62 | -1.21 |
| NM_019257 | Sfrs5 | -1.11 | -1.79 | -1.16 | -1.53 |
| BQ200332 | Snrpa_predicted | 1.53 | 1.13 | 1.28 | 1.03 |
| CB547639 | Cpeb4_predicted | 1.73 | 1.33 | 1.28 | 2.65 |
| muscle development | | | | | |
| Accession | Gene | | | | |
| NM_057208 | RGD:621546 | -1.33 | -1.60 | -1.72 | -2.54 |
| NM_173111 | RGD:621546 | -1.40 | -1.53 | -2.76 | -1.96 |
| AF053360 | RGD:621546 | -1.42 | -1.39 | -3.96 | -3.29 |
| M34136 | Tpm1 | -1.32 | -1.25 | -1.54 | -1.64 |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| NM_017184 | Tnni1 | -1.67 | -1.64 | -3.78 | -3.84 |
| XM_214266 | Tncc_predicted | -1.01 | -1.66 | -3.28 | -4.05 |
| AA891242 | Myl7_predicted | -1.02 | -1.36 | -2.55 | -2.40 |
| NM_134388 | Tnnt1 | -1.36 | -1.58 | -3.09 | -3.06 |
| XM_343196 | RGD:735102 | -1.60 | -1.54 | -1.29 | -1.14 |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| XM_343643 | Mrlcb | -1.19 | -1.30 | -2.50 | -2.61 |
| NM_017131 | Casq2 | -1.34 | -1.10 | -1.30 | -1.65 |
| AI045171 | Casq2 | -1.14 | -1.43 | -2.92 | -2.13 |
| NM_012893 | Actg2 | -1.49 | -1.23 | -1.55 | -1.20 |
| NM_012606 | Myl3 | -1.04 | -2.07 | -1.93 | -4.37 |
| NM_012604 | Myh3 | -1.55 | -1.62 | -3.25 | -3.31 |
| NM_017240 | Myh7 | -1.66 | -1.86 | -2.74 | -3.16 |
| NM_017239 | Myh6 | -1.50 | -2.04 | -1.72 | -3.06 |
| AI104924 | Myh6 | 1.36 | 1.62 | 1.03 | 1.16 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| NM_022531 | Des | -1.57 | -1.40 | -1.15 | -1.08 |
| ubiquitin-dependent protein catabolism | | | | | |
| Accession | Gene | | | | |
| NM_031001 | Ube2d2 | -1.57 | -1.13 | -1.31 | -1.07 |
| signal transduction | | | | | |
| Accession | Gene | | | | |
| NM_013086 | Crem | 2.25 | 2.15 | 1.89 | 3.34 |
| NM_024388 | Nr4a1 | 4.48 | 3.96 | 4.65 | 7.93 |
| BG673253 | Ogt | -1.27 | -1.65 | -1.30 | -1.21 |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |

| | | | | | |
|---|---|---|---|---|---|
| AW919109 | Cap2 | -1.10 | -1.19 | -1.47 | -1.35 |
| BF555415 | Gfra3 | -1.11 | -1.15 | -1.08 | -1.47 |
| NM_031035 | Gnai2 | -1.42 | -1.66 | -1.23 | -1.08 |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| XM_213943 | Mgst3_predicted | -1.07 | -1.33 | -1.42 | -1.47 |
| AF139830 | Igfbp5 | -1.91 | -1.64 | -1.88 | -1.46 |
| AW917764 | Igfbp5 | -1.25 | -1.60 | -1.67 | -1.37 |
| protein folding | | | | | |
| Accession | Gene | | | | |
| NM_013069 | Cd74 | -1.32 | -1.31 | -1.89 | -1.81 |
| NM_130431 | Hspb2 | -1.43 | -1.08 | -1.57 | -1.32 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| cell growth | | | | | |
| Accession | Gene | | | | |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| myoblast differentiation | | | | | |
| Accession | Gene | | | | |
| NM_019904 | Lgals1 | -1.25 | -1.50 | -1.07 | -1.15 |
| complement activation, alternative pathway | | | | | |
| Accession | Gene | | | | |
| XM_343169 | Adn | -2.76 | -3.28 | -6.80 | -5.17 |
| cell maturation | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| wound healing | | | | | |
| Accession | Gene | | | | |
| NM_181368 | Mustn1 | 2.64 | 2.08 | 4.22 | 7.25 |
| NM_019143 | Fn1 | -1.50 | -1.88 | -4.12 | -2.81 |
| cell differentiation | | | | | |
| Accession | Gene | | | | |
| BC061782 | Fhl1 | -1.29 | -1.56 | -1.51 | -1.38 |
| NM_145669 | Fhl1 | -1.42 | -1.65 | -1.76 | -1.93 |
| U42627 | Dusp6 | -1.28 | -1.79 | -1.84 | -1.03 |
| NM_053959 | Bin1 | -1.79 | -1.64 | -1.21 | -1.26 |
| AF540887 | Dmbt1 | -1.12 | -1.29 | -1.68 | -1.43 |
| NM_013172 | Myf6 | 1.78 | 1.15 | 2.26 | 4.26 |
| dendrite morphogenesis | | | | | |
| Accession | Gene | | | | |
| AW917756 | Nrp1 | -1.09 | -2.03 | -2.00 | -1.38 |
| peripheral nervous system development | | | | | |
| Accession | Gene | | | | |
| BF555415 | Gfra3 | -1.11 | -1.15 | -1.08 | -1.47 |
| NM_031688 | Sncg | -1.66 | -1.43 | -1.52 | -1.26 |
| rhythmic process | | | | | |
| Accession | Gene | | | | |
| NM_012543 | Dbp | -1.66 | -1.65 | -1.71 | -1.60 |

| | | | | | |
|---|---|---|---|---|---|
| transcription from RNA polymerase II promoter | | | | | |
| Accession | Gene | | | | |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| muscle contraction | | | | | |
| Accession | Gene | | | | |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| M34136 | Tpm1 | -1.32 | -1.25 | -1.54 | -1.64 |
| NM_012567 | Gja1 | -1.42 | -1.99 | -1.24 | -1.35 |
| NM_053981 | Kcnj12 | 1.30 | 1.03 | 1.42 | 1.55 |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | |
| XM_343196 | RGD:735102 | -1.60 | -1.54 | -1.29 | -1.14 |
| NM_012935 | Cryab | -1.16 | -1.50 | -2.46 | -3.28 |
| NM_012606 | Myl3 | -1.04 | -2.07 | -1.93 | -4.37 |
| collagen catabolism | | | | | |
| Accession | Gene | | | | |
| NM_031054 | Mmp2 | -1.09 | -1.34 | -1.69 | -1.54 |
| UDP-glucose metabolism | | | | | |
| Accession | Gene | | | | |
| XM_214108 | Ugp2_predicted | 1.75 | 1.16 | 1.15 | 2.00 |
| actin filament severing | | | | | |
| Accession | Gene | | | | |
| NM_001004080 | RGD:1303089 | -1.89 | -2.15 | -1.33 | -1.23 |
| extracellular matrix organization and biogenesis | | | | | |
| Accession | Gene | | | | |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| XM_345156 | Itga2 | -1.32 | -1.70 | -1.01 | -1.87 |
| NM_024129 | Dcn | -1.18 | -1.70 | -1.83 | -1.26 |
| protein amino acid alkylation | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| DNA damage response, signal transduction by p53 class mediator | | | | | |
| Accession | Gene | | | | |
| NM_031051 | Mif | -1.32 | -1.27 | -1.00 | -1.53 |
| exocytosis | | | | | |
| Accession | Gene | | | | |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| fibroblast growth factor receptor signaling pathway | | | | | |
| Accession | Gene | | | | |
| XM_235164 | Frs2_predicted | 1.60 | 1.36 | 1.19 | 1.27 |
| cell-matrix adhesion | | | | | |
| Accession | Gene | | | | |
| XM_214443 | Ecm2_predicted | -1.45 | -1.51 | -1.45 | -1.24 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| regulation of T cell proliferation | | | | | |
| Accession | Gene | | | | |
| NM_212505 | RGD:1303321 | -1.55 | -2.20 | -1.78 | -3.70 |
| chromatin remodeling | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| XM_342225 | RGD1307747_predicted | -1.01 | -1.56 | -1.25 | -1.74 |
| heme biosynthesis | | | | | |
| Accession | Gene | | | | |
| BF567803 | Nfe2l1_predicted | -1.47 | -1.60 | -1.26 | -1.07 |
| negative regulation of transcription, DNA-dependent | | | | | |
| Accession | Gene | | | | |
| NM_024383 | Hes5 | -1.29 | -1.15 | -2.36 | -2.23 |
| NM_053328 | Bhlhb2 | -1.24 | -1.38 | -1.92 | -1.24 |
| feeding behavior | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| MAPKKK cascade | | | | | |
| Accession | Gene | | | | |
| XM_341642 | Dok4_predicted | -1.52 | -1.56 | -2.90 | -3.30 |
| response to drug | | | | | |
| Accession | Gene | | | | |
| NM_031509 | Gsta5 | -1.43 | -1.85 | -2.12 | -1.26 |
| ATP metabolism | | | | | |
| Accession | Gene | | | | |
| NM_024349 | Ak1 | 1.54 | 1.47 | 2.02 | 3.56 |
| lipid transport | | | | | |
| Accession | Gene | | | | |
| NM_053502 | Abcg1 | -1.63 | -1.98 | -1.79 | -1.09 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| cholesterol homeostasis | | | | | |
| Accession | Gene | | | | |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| lipid metabolism | | | | | |
| Accession | Gene | | | | |
| NM_053674 | Phyh | -1.08 | -1.49 | -1.21 | -1.22 |
| XM_213943 | Mgst3_predicted | -1.07 | -1.33 | -1.42 | -1.47 |
| transcription termination | | | | | |
| Accession | Gene | | | | |
| BG664147 | Ptrf_predicted | -1.12 | -1.58 | -1.30 | -1.37 |
| regulation of bone mineralization | | | | | |
| Accession | Gene | | | | |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| response to reactive oxygen species | | | | | |
| Accession | Gene | | | | |
| NM_030826 | Gpx1 | -1.08 | -1.57 | -2.08 | -1.88 |
| NM_138828 | Apoe | -2.37 | -2.38 | -2.00 | -1.74 |
| phosphate transport | | | | | |
| Accession | Gene | | | | |
| NM_053714 | Ank | -1.14 | -1.08 | -1.57 | -1.23 |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| XM_342599 | Col9a3_predicted | -2.54 | -1.68 | -2.44 | -1.15 |
| XM_213440 | Col1a1 | -2.52 | -2.70 | -2.03 | -3.12 |
| XM_223124 | Col9a1_predicted | -2.09 | -2.55 | -1.95 | -2.22 |

| | | | | | |
|---|---|---|---|---|---|
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| BI275716 | Col3a1 | -1.81 | -1.72 | -1.74 | -1.20 |
| XM_216399 | Col15a1_predicted | -1.70 | -2.25 | -1.47 | -1.29 |
| NM_053356 | Col1a2 | -2.11 | -1.98 | -2.62 | -2.67 |
| BI282748 | Col1a2 | -1.38 | -1.80 | -1.89 | -1.73 |
| neuropeptide signaling pathway | | | | | |
| Accession | Gene | | | | |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| cation transport | | | | | |
| Accession | Gene | | | | |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| NM_031686 | RGD:61922 | -1.40 | -1.07 | -1.50 | |
| NM_019230 | Slc22a3 | 1.66 | 1.58 | 1.03 | 1.26 |
| copper ion homeostasis | | | | | |
| Accession | Gene | | | | |
| NM_019288 | App | -1.75 | -1.65 | -1.98 | -1.08 |
| negative regulation of protein biosynthesis | | | | | |
| Accession | Gene | | | | |
| NM_019243 | Ptgfrn | -1.22 | -2.06 | -1.45 | -1.09 |
| adenylate cyclase activation | | | | | |
| Accession | Gene | | | | |
| AW919109 | Cap2 | -1.10 | -1.19 | -1.47 | -1.35 |
| NM_017338 | Calca | 1.55 | 1.26 | 1.11 | 1.39 |
| gluconeogenesis | | | | | |
| Accession | Gene | | | | |
| NM_012912 | Atf3 | 4.95 | 5.23 | 2.89 | 6.41 |
| glycolysis | | | | | |
| Accession | Gene | | | | |
| NM_012595 | Ldhb | -1.42 | -1.54 | -2.15 | -1.42 |
| regulation of cell adhesion | | | | | |
| Accession | Gene | | | | |
| NM_001004080 | RGD:1303089 | -1.89 | -2.15 | -1.33 | -1.23 |
| calcium ion transport | | | | | |
| Accession | Gene | | | | |
| NM_024156 | Anxa6 | -1.73 | -1.72 | -1.31 | -1.21 |
| NM_017290 | Atp2a2 | -1.06 | -1.86 | -2.72 | -2.88 |
| organogenesis | | | | | |
| Accession | Gene | | | | |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| NM_019334 | Pitx2 | -1.61 | -1.77 | -3.77 | -1.46 |
| XM_223124 | Col9a1_predicted | -2.09 | -2.55 | -1.95 | -2.22 |
| NM_032085 | Col3a1 | -1.78 | -2.12 | -1.98 | -1.45 |
| BI275716 | Col3a1 | -1.81 | -1.72 | -1.74 | -1.20 |
| iron ion homeostasis | | | | | |
| Accession | Gene | | | | |
| NM_012848 | Fth1 | -1.71 | -1.39 | -1.22 | -1.11 |
| tricarboxylic acid cycle | | | | | |
| Accession | Gene | | | | |

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|---|---|---|---|---|---|
| NM_053638 | Idh3a | 1.42 | 1.16 | 1.35 | 1.70 |
| fatty acid biosynthesis | | | | | |
| Accession | Gene | | | | |
| NM_031841 | Scd2 | -1.30 | -1.85 | -1.73 | -2.77 |
| creatine biosynthesis | | | | | |
| Accession | Gene | | | | |
| BQ200807 | Gamt | 1.03 | 2.71 | 1.40 | 1.91 |
| response to glucose stimulus | | | | | |
| Accession | Gene | | | | |
| NM_144744 | Acdc | -1.87 | -2.17 | -3.18 | -3.66 |
| mesoderm formation | | | | | |
| Accession | Gene | | | | |
| NM_031628 | Nr4a3 | 15.16 | 18.09 | 11.99 | 24.66 |
| cell motility | | | | | |
| Accession | Gene | | | | |
| BE117007 | Marcks | -1.38 | -1.88 | -1.71 | -1.42 |
| XM_215403 | Marcks | -1.44 | -1.33 | -2.13 | -1.16 |
| AW915860 | Marcks | -1.10 | -1.89 | -1.66 | -1.12 |
| NM_031675 | Actn4 | -1.40 | -1.11 | -1.55 | -1.47 |
| NM_031520 | Myh10 | -1.18 | -1.37 | -3.16 | -1.67 |
| BC081984 | Pxn_predicted | 1.25 | 1.07 | 1.15 | 1.51 |
| heparan sulfate proteoglycan metabolism | | | | | |
| Accession | Gene | | | | |
| BF567631 | Sulf1 | -1.07 | -1.49 | -1.71 | -1.22 |
| ribosome biogenesis | | | | | |
| Accession | Gene | | | | |
| NM_022514 | Rpl27 | -1.37 | -1.57 | -1.35 | -1.03 |
| NM_017151 | Rps15 | -1.89 | -1.54 | -2.73 | -2.24 |