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Research article

Gene response of the gastrocnemius and soleus muscles to an acute aerobic run in rats

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Abstract
Genes can be activated or inhibited by signals within the tissues in response to an acute bout of exercise. It is unclear how a particular aerobic exercise bout may influence two muscles with similar actions to the activity. Therefore, the purposes of this investigation was to determine the gene response of selected genes involved in the "stress" response of the gastrocnemius (fast-twitch) and soleus (slow-twitch) muscles to a single two hour aerobic exercise bout in female Sprague-Dawley Rats at the 1 hour time point after the exercise. Exercised rats were run (n=8) for 2 hours at 20 m·min^{-1} and one hour after the completion of the bout had their soleus (S) and gastrocnemius (G) muscles removed. Age and timed matched sedentary control rats had both S and G muscles removed also. RNA was isolated from all muscles. Real-time PCR analysis was performed on the following genes: NFκB, TNFα, and Atf3. GAPDH was used as the housekeeping gene for both muscles. S muscle showed more genes altered (n = 52) vs G (n = 26). NFκB gene expression was 0.83 ± 0.14 in the exercised S but was + 1.36 ± 0.58 in the exercised G and was not significantly different between the muscles. TNFα was altered 1.30 ± 0.34 in the exercised S and 1.36 ± 0.71 in the exercised G and was not significantly different between the muscles. The gene Atf3 was significantly altered at 4.97 ± 1.01 in the exercised S, while it was not significantly altered in the exercised G (0.70 ± 0.55). This study demonstrates that an acute bout of aerobic exercise can alter gene expression to a different extent in both the S and G muscles. It is highly likely that muscle recruitment was a factor which influenced the gene expression in theses muscles. It is interesting to note that some genes were similarly activated in these two muscles but other genes may demonstrate a varied response to the same exercise bout depending on the type of muscle.

Key words: Aerobic exercise, skeletal muscle, gene response.

Introduction

The alterations within skeletal muscles to a single bout of aerobic exercise are a topic of importance to understand how skeletal muscle may adapt. The genetic responses within skeletal muscle to exercise have been shown to be altered by chronic inputs such as endurance training to manifest genetic adaptations and changes in protein concentration. It has been clearly documented that an overloaded muscle will respond so that future bouts of stress are less damaging (Selye, 1956) if the stress can be accommodated. It has also been well established that skeletal muscle fiber types respond differently to the same stressors (Powers et al., 1994; Rockl, Witczak et al., 2008). The response of the muscle to stress is at least partially dictated by the gene response of the muscle. By investigating this phenomenon one could further our understanding as to why muscles may adapt to a different extent, as well as help to further clarify fiber type adjustments.

A brief review of fiber type differences is warranted. Type I fibers are slow-twitch fibers, and they are built for fatigue resistance. They have more capillaries and mitochondria per fiber than fast-twitch fibers (type II), yet have a slower speed of shortening and a produce less specific tension than type II fibers (Baldwin et al., 1972; Booth and Thomason, 1991; Gunning and Hardeman, 1991; Pette and Staron 1990). Fast fibers, specifically type IIx or IIb fibers produce more force and can produce greater power than type I fibers. They have the fastest speed of shortening and highest specific tension, yet are more fatigable as they have fewer capillaries and mitochondria per fiber (Baldwin et al., 1972; Booth and Thomason, 1991; Gunning and Hardeman, 1991; Pette and Staron 1990).

Recently, several investigators have reported differences in the genetic fiber type response to various stressors. It was reported that there are genetic differences between a paretic limb and non-paretic limb (McKenzie et al., 2008; 2009) following stroke. Additionally, exercise differences have been reported (Norman et al., 2009; Pogozelski et al., 2009), as has cancer (Krupa et al., 2011), and diabetes (Zadravec et al., 2010). Clearly these differences can start to explain some of the fiber type differences previously mentioned.

We have previously identified the gene response of the soleus muscle one hour following a single two hour run in female Sprague-Dawley (SD) rats (McKenzie and Goldfarb, 2007). Most of the genetic changes following exercise in the soleus muscles were from the following gene families; stress genes (Knight, 1999; McKenzie and Goldfarb, 2007; Sen, 1994), cell cycle genes (Mahoney et al., 2005; McKenzie and Goldfarb, 2007), muscle contraction (Adhihetty et al., 2003; Bonen et al., 1999; McKenzie and Goldfarb, 2007), homeostasis (Mahoney et al., 2005; McKenzie and Goldfarb, 2007), and mitochondrial biogenesis (McKenzie and Goldfarb, 2007; Pilegaard et al., 2003). Several of the genes were activated (n= 29) and some were inhibited (n= 23).

However, the previous experiment only examined the soleus muscle which is primarily a slow-twitch type I muscle and clearly may not reflect what may be going on in another skeletal muscle. It is currently unknown what

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the genetic responses would be in a fast-twitch predominant muscle which performs similar actions as the soleus and how they compare to the soleus genetic response. In order to differentiate the fiber type response to a single bout of aerobic exercise, gene microarrays were performed on both the soleus (S, a slow-twitch muscle) and the gastrocnemius (G, a fast-twitch muscle) (Castro et al., 1998; 1999; Powers et al., 1999) one hour after a 2 hour aerobic exercise bout. The exercise response changes were first compared to the same muscles from sedentary control animals. Then the changes that were manifested were compared from S to G muscles.

Since there are far too may genes to adequately study each and every gene in a single investigation, we focused on several well known stress-response genes. Three genes were chosen for investigation based on their previously identified stress roles: Nuclear Factor Kappa β (NFκB), Tumor Necrosis Factor α (TNFα), and Activating transcription factor 3 (Atf3). NFκB is a well known transcription factor involved with stress-induced , immune and inflammatory gene regulation (Ji et al., 2004). TNFα is also a well studied inflammatory marker (Andreakos et al., 2002) and can activate NFκB. Atf3 has been reported to be elevated following various stressors including exercise (Abe et al., 2003; Lindwall and Kanje, 2005; McKenzie and Goldfarb, 2007).

The purpose of this study was therefore twofold. The first purpose was to examine gene changes in the entire rat transcriptome in a muscle that is classified as slow compared to a muscle that is classified as fast which performs a similar action. It was hypothesized that although similar families of genes may be altered, the number of genes altered, as well as the types of genes altered would be dramatically different between the two muscles probably based on the recruitment of the muscles. In addition, our second purpose was the identification of the subset of inflammatory genes. We again hypothesized that these genes would be expressed to a different extent one hour following the exercise bout.

Methods

The protocol used was approved by the UNCG institutional animal care and use committee.

Animals

In order to limit genetic variation, sixteen 10-wk-old Sprague–Dawley female rats (Charles River Laboratories, Wilmington, MA) were used in this experiment. All rats were individually housed in a standard plastic cage using a 12:12-h reverse light cycle in a temperature (21 ± 1°C) and humidity-controlled (30–40%) room and were provided food and water ad libitum. Additionally, rats were housed for 2 wk before any intervention, to eliminate the stress response of transport and a new housing environment, and to become habituated before experimentation. All rats were handled daily and walked at a very mild intensity (10 m·min^{-1}) for 10 min to limit their apprehension attributable to these stressors during the 2-week habituation. This intensity of walking 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 h before the experiment, at which time the rats began fasting.

On day 15, after habituation, rats were randomly assigned to an exercise (E) group (n = 8) or a control (C) group (n = 8). The muscles from exercised soleus muscles are designated as ES, exercised gastrocnemius muscles are designated as EG, while control soleus muscles are CS and control gastrocnemius muscles are CG. Both groups were fasted for 12 h, with the E group then running for 2 h on the motorized treadmill. The 2-h duration was selected on the basis of work by Hildebrandt et al. (2003), who reported that transcriptional activation after exercise is intensity- and duration dependent. The 2-h run at 22 m·min^{-1} was of sufficient intensity and duration according to these authors to activate numerous gene changes at times ranging from 45 min to 3 h (15). The rats ran at 22 m/min at a 0% grade, which is considered a mild exercise intensity of about 65% VO_{2max} (Lawler et al., 1993), and which has been shown to increase protein carbonyls in both soleus muscle and blood from rats (You et al., 2005). All exercised animals were able to complete the 2-h run.

The 1-h post-exercise time point was selected on the basis of results of previously published exercise data that reported changes in gene expression at this time (Hildebrandt et al., 2003). Rats were sacrificed by decapitation 1 h after exercise for the E group, and at a matched time for rats in the C group. Decapitation was used to ensure that an anesthetic did not alter gene expression. On sacrifice, the S and G muscles from both legs were rapidly removed from each animal and placed in RNALater (Sigma-Aldrich R0901), immediately frozen in liquid nitrogen between cooled aluminum blocks, and stored at -80°C until assayed.

Homogenization and isolation

Whole S and G muscle samples were homogenized in an RNA isolation buffer from the RNA Isolation Kit from Fluka, (#83913 Sigma-Aldrich, St. Louis, MO. RNA was isolated from the entire S and G muscles using the RNA isolation kit from Fluka, and carried out per manufacturer's instructions. To determine the quality of the RNA isolated, all samples had their concentration of nucleic acids to amino acids measured in optical density buffer by an optical density ratio (A260/A280) via an Eppendorf Biophotometer (Eppendorf-Brinkman; Westbury, NY). A ratio of 1.8 or better was used as the threshold for acceptability, and the integrity gels and PCR were used as confirmation. All samples were checked for RNA quality using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), which checked each RNA sample as an RNA integrity gel. Group means of RNA were analyzed using an independent t-test. Significance was set a priori at $P \leq 0.05$.

Microarray

For aminoallyl labeling, 10 µgof DNase I-treated and purified total RNA was used with the Superscript II RT Transcriptase Kit (#18064-022 Invitrogen, Carlsbad, California) per manufacturer`s instructions. For reaction purification (removal of unincorporated aa-dUTP and free amines), the Qiagen QIAquick PCR purification kit was utilized (catalog # 28104) per manufacturer`s instructions.

Table 1. Primers and probes used for real-time PCR analysis

Gene	Genbank No.	Primer	Probe
NFκB	NM_199267	5'-GTCTCAAACCAAACAGCCTCAC-3' 5'-CAGTGTCTCTTCCTCGACATGGAT-3'	CCCCAAGCCAGCACCCCAGCCCTAT
TNFα	NM_012675	5'-GTTTCAGTTCTCAGGGTCCTA-3' 5'-CAGGATTCTGTGGCAATCTGG-3'	AAAGGGATGAGAAGTTCCCAAATGG
ATF3	NM_012912	5'-CATCAGACCTGATTTCCGAGAGT-3' 5'-AACAGTTTGTAGCCAAGGACAGC-3'	ACGCGTGCGAAAGAGCCCACCTGAAT

The first primer listed is the forward primer, whereas the second primer listed in the reverse primer.

cDNA was hybridized to the Agilent Rat Genome Array (rat microarrays catalog # G4130A from Agilent, Palo Alto, CA), and the arrays were analyzed using an Axon GenePix Pro 5.1 scanner (Union City, CA).

In the current experiment, the samples were split in half as samples were pooled; samples SE1–SE4 were run against SC1–SC4 on arrays 1 and 2, and samples SE5–SE8 were run against SC5–SC8 on arrays 3 and 4. This exact process was repeated using the G muscles. To ensure that dye incorporation was not a factor in this experiment, dye swapping was performed. On array 1 and array 3, the exercised muscles were incorporated with Cy3 dye, and on array 2 and array 4, exercised muscles were incorporated with Cy5 dye. For a gene to be classified as significantly different between the two groups, the gene dye intensity had to be at least 1.5-fold different on all four arrays.

Real-time PCR

Real-time PCR was used to validate the microarray because it allows for cycle-by-cycle comparisons of RNA levels. The Qiagen Omniscript Reverse Transcription kit (#205111, Valencia, CA) was used for this step according to the kit instructions. For the PCR step, Taqman probe analysis was used as a specific probe for each gene to be tested; it also helped eliminate nonspecific binding. All samples were loaded onto an ABI 96 well plate (#436767, Foster City, CA), and the procedure followed the manufacturer`s instructions. Known primer and probe sequences were used as listed in Table 1. All samples were performed in duplicate. GAPDH was the control gene for PCR, and it was unchanged across conditions.

Group differences were analyzed by an independent t-test using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). 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 (glyceraldehyde-3-phosphate dehydrogenase) was used as the housekeeping gene because GAPDH was reported unchanged through 48 h after exercise (Mahoney, Carey et al. 2004).

The differences between E and C for each muscle group were compared between the S and the G using a one way ANOVA with significance set at $p \leq 0.05$ a priori. This analysis allowed us to determine if the EG responded differently than the ES, as well as to the C condition for each muscle.

Results

There were no differences in the body weights of the animals at the time of the experiment (E = 175.3 ± 6.6 g, C = 170.9 ± 6.7 g) or the weights of the S muscle (E = 86 ±3 mg, C = 84 ± 3 mg), or the G muscle (E = 928 ± 30 mg, C = 917 ± 42 mg). 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 two groups regarding RNA quality as measured by the 260/280 ratio (E = 2.01 ± 0.045, C = 1.94 ± 0.029) or RNA concentration (E = 1524 ±177 $ng \cdot uL^{-1}$, C = 1617 ±160 $ng \cdot uL^{-1}$).

Array

In the G, 26 genes met the criteria on all arrays to be considered significantly altered and all were upregulated compared to the control animals (Table 2). In the G, the most common family type of genes altered was signal transduction genes. In the S, 52 genes met the criteria and were significantly altered on all arrays (Table 3). In the S, twenty genes were upregulated compared to the control and 32 were down regulated. The most common types of genes altered were cell signaling, tissue generation, inflammation, metabolism, muscle contraction, and cell cycle genes.

PCR

NFkB gene expression was 0.83 ± 0.14 in the ES, and was 1.36 ± 0.58 in the EG (P = 0.19). TNFα was altered 1.30 ± 0.34 in the ES and 1.36 ± 0.71 (p = 0.84) in the EG. The gene Atf3 was significantly upregulated to a level of 4.97 ± 1.01 in the ES, while it was not significantly activated in the EG (0.70 ± 0.55) ($p < 0.001$). Based on the 3 genes tested using PCR, the Atf3 gene was the only gene to be statistically different between the ES and the EG.

Additionally, Atf3 was the only gene tested using PCR which was statistically different between E and C in the soleus. There were no significant differences between the EG and the CG in the other three genes analyzed.

Discussion

The main findings of the current experiment were as follows:

1) According to the array data, 52 genes were significantly altered due to exercise in the S, while only 23 were altered in the G.

2) The PCR results confirmed the array results, as only atf3 was significantly altered in the S.

3) It appears that the same exercise bout can differentially alter not only the number of genes but also specific dependent genes in the muscle(s) tested.

Array

These data suggest that in the S and G muscles 1 h post a

Table 2. DNA Microarray results for the Gastrocnemius.

Gene Symbol/Bank #	Mean Delta	Function
Cyr61	1.88	Cell Growth/Adhesion Morphogensis, Chemotaxis, Cell Migration, Cell Cycle, Cell Proliferation, Angiogenesis
Olr383_predicted	2.04	G Protein Receptor Signaling
Atf3	2.12	Gluconeogenesis, Metabolism, Transcription
XM_345302	1.76	Membrane Function-Signaling
Nr4a3	5.87	Mesoderm formation, Transcription
Mymom2	-1.52	Muscle Contraction, Muscle Development
BG663460	1.88	Neurogensis
Hnmt	1.79	Neurotransmitter Levels, Respiratory Gas Exchange
Urod	1.62	Porphyrin Biosynthesis, Heme Biosynthesis
Adck1_predicted	2.33	Protein Phosphorylation Signaling
Brip1_predicted	2.12	Transcription
Rg9mtd1_predicted	2.35	Transcription
Snf1lk	5.87	Transcription, Cell Cycle, Protein Kinase Regulation, Cell Differentiation, Protein Phosphorylation
AI103842	1.79	Unknown
TC490471	1.82	Unknown
TC47591	2.18	Unknown
XM_345834	1.70	Unknown
AA893485	2.01	Unknown
TC472749	1.84	Unknown
XM_344052	1.97	Unknown
XM_344641	2.03	Unknown
AI008316	1.89	Unknown
TC487987	3.46	Unknown

Positive numbers indicate increases attributable to exercise, whereas negative numbers indicate decreases attributable to exercise. All numbers are in reference to control gastrocnemius levels.

2-h run at 22 m·min^{-1}, a variety of genes are significantly altered to the aerobic exercise stress. According to the array, the single session of exercise significantly altered 52 genes on all four arrays in the S, and 23 in the G. The functions of the genes most frequently altered were metabolism (~10% of all genes altered in S, 5% in G), apoptosis/cell cycle (~8% of all genes altered in S, 17% in G), muscle contraction (~10% of all genes altered in S, 4% in G), transcription/cell signaling (~17% of all genes altered in S, 35% in G), tissue generation (~15% of all genes altered in S, 9% in G), and inflammatory genes (~10% of all genes altered in S, 4% in G). Some genes are reported to play a role in several different functions and therefore may be counted more than once in the percentages.

These array results highlight a major difference in the response of these 2 muscles. Whether these differences in these 2 muscles are due to recruitment patterns or differences due strictly to fiber type will need further investigation. Several investigators have measured recruitment patterns of the S and G muscles in rodent models. It appears that at lower exercise intensities (below lactate threshold), the S is activated to a greater extent than the G. Gardiner previously reported that at slower running speeds, gastrocnemius EMG activity was only about 83% of soleus activity (Gardiner et al., 1982). Additionally, it has been previously reported that adaptations in muscle fibers of muscles comprised of primarily slow-twitch fibers (such as the soleus) occur at lower exercise intensities, whereas adaptations in fast-twitch muscle fibers increase dramatically at higher intensities (Dudley et al., 1982). Clearly, differences in recruitment patterns exist for these muscles. Additionally, since it is widely reported that slow and fast twitch fibers have very different functional and structural characteristics (Baldwin et al., 1972; Booth and Thomason, 1991; Gunning and Hardeman, 1991; Pette and Staron, 1990), the current data could begin to explain why these differences exist. While lactate levels were not measured in the current investigation, the fact that animals were able to run for 2 consecutive hours suggests that they remained fairly aerobic during the exercise bout.

Several investigators have studied how exercise affects global gene expression changes within a muscle, or examined factors affecting fiber type within a given muscle following exercise. However, this is the first investigation to examine the response of the entire genome in these two distinct muscles following a single bout of aerobic exercise that have similar muscle actions. There are several published studies examining the peroxisome proliferator-activated receptor gamma coactivator (PGC-1-α) response to exercise, which appears to be redox sensitive. PGC-1-α is a mitochondrial biogenesis marker and has been reported to increase as muscles become more aerobic. Several studies have shown an increase in this individual marker following exercise in working skeletal muscle (Chinsomboon et al., 2009; Little et al., 2010). Baar reported that a single bout of endurance exercise can upregulate PGC-1 α and subsequently stimulate mitochondrial protein synthesis (Baar et al., 2002). Recently, it was shown that an acute bout of sprint exercise in rats can activate the PGC-1 α pathway and stimulate mitochondrial biosynthesis (Kang et al., 2009). However few have compared differences between two different muscles with similar actions to the same bout. You et al. (2005) (among others) have previously shown a difference in markers of oxidative stress following exercise is a muscle dependent manner. You et al. measured stress markers in the soleus, vastus intermedius, and vastus lateralis and found

Table 3. DNA Microarray results for the Soleus.

Gene Symbol/Bank #	Mean Delta	Family/Function
Snf1lk	3.67	Cell Cycle
Myf6	2.46	Cell Cycle
Ak1	2.16	Cell Cycle
Prm1	-2.21	Cell Cycle
RGD:1303321	-2.31	Cell Cycle
Tradd	-2.34	Cell Cycle
Dbp	-1.91	Cell Cycle
Gpx1	-1.76	Cell Cycle/ Stress Response
Apoe	-2.12	Cell Cycle/Metabolism
Nr4a1	5.26	Cell Cycle/Signal Transduction
Ak1	2.16	Metabolism
Ppargc1a	2.11	Metabolism
Comt	-2.21	Metabolism
Acdc	-2.72	Metabolism/Signal Transduction
Fn1	-2.14	Metabolism/Stress Response
Atf3	4.87	Metabolism/Transcription
Myh7	-2.35	Muscle Contraction
Myh3	-2.43	Muscle Contraction
Tnni1	-2.73	Muscle Contraction/ Tissue Generation
Cryab	-2.1	Muscle Contraction/Stress, Response/Translation
Sparc	-1.78	Ossification/Signal Transduction
Crem	2.41	Signal Transduction/Transcription
Timp2	-1.54	Signal Transduction
AW918612	-1.82	Signal Transduction
Col1a2	-2.35	Signal Transduction
Col1a1	-2.59	Signal Transduction
Adn	-4.5	Signal Transduction
Mgst1	-1.91	Stress Response
Mustn1	4.05	Stress Response/Tissue Generation
Myh4	3.29	Tissue Generation
Myf6	2.46	Tissue Generation
Myh3	-2.43	Tissue Generation
Myh6	-2.08	Tissue Generation
XM_223124	-2.2	Tissue Generation
Myh7	-2.35	Tissue Generation
Nr4a3	17.47	Transcription
Myf7	2.46	Transcription
Stars	3.24	Transcription
Ppargc1a	2.11	Transcription
Rps15	-2.15	Translation
LOC310670	2.15	Unknown
BF544403	4.92	Unknown
Lpin1_predicted	3.13	Unknown
TC487987	2.86	Unknown
AI013671	2.84	Unknown
BF549650	2.65	Unknown
AI008316	2.64	Unknown
Btg2	2.53	Unknown
LOC310395	2.42	Unknown
LOC360478	-2.1	Unknown
LOC310670	-1.95	Unknown
LOC294337	-2.01	Unknown
Smarcd3_predicted	-2.08	Unknown
LOC317454	-4.22	Unknown
M12199	-2.27	Unknown

Positive numbers indicate increases attributable to exercise, whereas negative numbers indicate decreases attributable to exercise. All numbers are in reference to control soleus levels.

differences in a muscle dependent manner. This clearly suggests that aerobic exercise recruits and causes adaptations in muscles differently.

While the array data provided a snapshot of global gene expression, several unique genes of interest were further investigated to examine if a muscle dependent difference existed. Based on the previously mentioned PGC data, as well the research by You et al, a comparison of a primarily slow S muscle and a primarily fast G muscle was completed using real time PCR analysis. We selected three known "stress genes" to consider whether these muscles are likely exposed to differing amounts of stress, and therefore differences in their adaptations, as measured by gene expression.

NFκB

NFκB gene expression was not significantly different between muscles, or altered by exercise (ES vs EG 0.83 ± 0.14 vs + 1.36 ± 0.58). There is no shortage of investigations showing the role of NFκB in the stress response with exercise. NFκB has been reported elevated in everything from apoptosis (Ji et al., 2004) to inflammation (Viedt et al., 2002). However, most studies reported differences in NFκB p50 subunit protein activity, and not in gene expression. Ji et al. (2004) reported that it may take a full 24 hours to observe measurable NFκB concentration response changes after exercise. Recently, a repeated bout effect was reported to occur with exercise on NFκB protein level in gastrocnemius muscle after eight weeks of aerobic training (Brooks et al., 2008). This study reported that contraction induced activation of NFκB was down regulated in the exercised gastrocnemius and may have been related to less ROS generation. It remains unclear whether NFκB gene expression is unchanged, or if changes occurred after the 1 hour post exercise time following an acute exercise bout. Further study is needed to establish a time line for adaptation of NFκB gene expression and activity in both of these muscles in response to a single bout of exercise.

TNFα

TNFα has been shown to play a major role in the inflammatory process (Andreakos et al., 2002). However, TNFα gene expression levels were not significantly affected by this exercise in either muscle group examined (ES vs EG 1.30 ± 0.34 vs 1.36 ± 0.71). Liao et al. (2010) recently reported elevated TNFα expression through 24 hours following eccentric muscle actions. However, it is well established that eccentric muscle actions cause much greater damage than the muscle actions seen during a normal aerobic run (Goldfarb et al, 2002; Bloomer et al., 2004). A species dependent response for TNFα may occur because TNFα has been reported to be elevated post exercise in several human studies (Nieman et al., 2003, 2004) It was also reported that TNFα gene expression increased at various times up to 24 hours post exercise (Louis et al., 2007; Paton et al., 2006). In contrast, TNFα has been reported to not be significantly altered by exercise (Colbert et al., 2001). It is important to note that although the TNFα gene expression was not significantly altered, it is possible that TNFα gene level could be considered clinically elevated. How much of an increase in gene expression is needed to see an altered response is unknown and it is possible that these levels could cause alterations in the muscle. Regardless, there appears to be no significant muscle dependent gene response to TNFα following an acute exercise in these rats for either S or G muscle 1 hour after exercise. Clearly this gene should be examined over a longer time point to establish if this gene would be significantly activated within the 24 h following the acute exercise bout.

Atf3

The atf3 gene is a known stress response gene that is upregulated to a variety of stressors, but is mostly studied in cancer (Abe et al., 2003; Lindwall and Kanje, 2005). Recent evidence also suggests that atf3 may play an important role in the stress response and in the muscle growth stimuli (Weidenfeld-Baranboim et al., 2009), although that investigation used cell culture. While atf3 was upregulated almost 5 fold higher in the ES compared to CS, there was little change in the atf3 gene in EG compared to the CG, as the EG levels were roughly 70% of the control condition. Clearly, in regards to this particular gene, the muscle being measured can directly impact the atf3 differences. Whether the atf3 difference in the S was related to muscle activation responses or other factors remains to be elucidated. Again it would be important to ascertain the time course change of this gene in both muscles over the 24h after the acute exercise bout.

Conclusion

This study demonstrates that an acute exercise run will differentially influence the gene response in the S and G muscles. More genes were affected in the S compared to the G. Several of the genes were similarly upregulated but the majority of the genes influenced were specific to the muscle examined. The results from this study may be beneficial to understanding why muscles may adapt differently to the same type of exercise.

It is highly likely that recruitment of the muscles was a factor which influenced the gene expression in theses muscles as the S is typically activated more than the G (Roy et al., 1991). Additionally; it is also possible that different fiber types have a different time course for gene activation processes. Measuring samples at various time points following the acute exercise up through 24 h could help elucidate if the time course response to a single exercise differs both within and between muscles.

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These experiments comply with the current laws of the country in which they were performed.

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Key points

- The soleus (primarily slow twitch) and the gastrocnemius (primarily fast type) do not respond the same to a given exercise bout.
- There are gene transcription differences in stress genes between the 2 muscles.
- The results of exercise studies should be carefully viewed as the muscle used in measurements may not provide an adequate representation of all skeletal muscles.

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