USING MICRODIALYSIS TO INVESTIGATE THE LOCAL SKELETAL MUSCLE INFLAMMATORY RESPONSE TO RESISTANCE EXERCISE.

A Thesis
by
BRIAN A. PRATT

Submitted to the Graduate School
Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2011
Department of Health, Leisure and Exercise Science

USING MICRODIALYSIS TO INVESTIGATE THE LOCAL SKELETAL MUSCLE INFLAMMATORY RESPONSE TO RESISTANCE EXERCISE.

A Thesis
by
BRIAN A. PRATT
May 2011

APPROVED BY:

_____
Dr. Kevin A. Zwetsloot
Chairperson, Thesis Committee

_____
Dr. Steven R. McAnulty
Member, Thesis Committee

_____
Dr. N. Travis Triplett
Member, Thesis Committee

_____
Dr. Paul L. Gaskill
Chairperson, Department of Health, Leisure & Exercise Science

_____
Dr. Edelma D. Huntley
Dean, Research and Graduate Studies

ABSTRACT

USING MICRODIALYSIS TO INVESTIGATE THE LOCAL SKELETAL MUSCLE INFLAMMATORY RESPONSE TO RESISTANCE EXERCISE (May 2011)

Brian A. Pratt, B.S., Slippery Rock University

M.S., Appalachian State University

Chairperson: Dr. Kevin Zwetsloot

Tissue trauma is associated with marked increases in inflammatory cytokines such as Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor necrosis factor-α (TNFα), and Monocyte chemotactic factor-1 (MCP-1). Increased inflammatory cytokines have been detected in the blood after high intensity eccentric exercise and long duration aerobic exercise. The source of the increased inflammatory cytokines is not entirely clear, but increased levels of inflammatory cytokine mRNA has been detected in the skeletal muscle tissue in response to high-intensity exercise, thus implicating skeletal muscle as a potential source for the production of inflammatory cytokines in response to exercise. Microdialysis is a powerful technique that uses a small tube with a semi-permeable membrane to allow for the collection of tissue interstitial fluid samples. Only one other study to our knowledge has used the microdialysis technique to measure inflammatory cytokines in skeletal muscle during muscular contractions. The purpose of this study was to determine whether an acute bout of high-intensity resistance exercise increases inflammatory cytokines in the skeletal muscle interstitium and to gain a better understanding as to the source of where the inflammatory cytokines are derived. Six subjects randomly performed a resistance exercise (RE) trial and a

no-exercise control (NEC) trial at least one week apart. For the RE trial, collection of skeletal muscle interstitial fluid samples was performed at rest and for six hours after an acute bout of high-intensity RE (3 sets of 10 leg extensions at 80% of 1RM). The NEC trial was performed the same way but without the RE. Microdialysis probes were inserted in the vastus lateralis and blood samples were collected via venipuncture. Inflammatory cytokines were measured in the skeletal muscle interstitial fluid and the plasma using multiplex ELISA assays. Increases in inflammatory cytokines were detected in the interstitial fluid prior to increases in the blood; however, in general there were no differences between the RE and the NEC trials. There were a few points that were significantly different between the RE and NEC trials. TNFα and MCP-1 were lower in the RE trial, compared to the NEC trial, suggesting that RE induces an anti-inflammatory effect. Taken together, these results suggest that inflammatory cytokines are derived in and released from skeletal muscle.

Dedication

I dedicate the work to my parents, Jay and Laura, my older brothers, Casey and Kevin, and our beloved pets, Cleo and Belle. I appreciate their support throughout school, but more importantly the times that had nothing to do with school. Knowing I can fall back on my family and pets to relax is a calming feeling in itself. So, thank you for everything up to this point and beyond this point.

Acknowledgements

This research could not have been done without the vision of my advisor, Dr. Kevin Zwetsloot. He did a great job of teaching as well as forcing me to produce quality work. I have learned a great deal about research and writing from him in a short two years. Also, my fellow graduate students, Justin Hardee and Marcus Lawrence, were of great importance throughout the process of initial data collection to reviewing my writing. I find it hard to believe that this process would have been completed in the time it was or at all without their help.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER 1

Introduction and Background

Sepsis and tissue trauma are accompanied by an acute inflammatory response, marked by increases in both inflammatory cells and inflammatory cytokines. Inflammatory cytokines are regulatory proteins that are secreted by cells to induce an immune response and facilitate tissue repair. Local tissue inflammatory responses are usually accompanied by a systemic response, known as the acute-phase response. Typical inflammatory cytokines that appear in the systemic circulation in response to either sepsis or trauma are similar and include, but are not limited to, tumor necrosis factor α (TNFα), Monocyte chemotactic factor-1 (MCP-1), Interleukin-8 (IL-8), and Interleukin-6 (IL-6).

With the onset of tissue damage, typically a coordinated immune system response ensues to initiate stabilization and repair of the tissue. First, neutrophils (phagocytic granulocytes) invade the injured area within two hours of damage. Neutrophils are then followed by M1 macrophages (phagocytic monocytes located in the blood that can focus at the point of muscle damage), which arrive about 24 hours post-damage and remain high until about 48 hours after damage. Phagocytic cells are capable of destroying the damaged cells. The neutrophils and macrophages are lured to the damaged area by Th1 pro-inflammatory cytokines, most notably IL-1, TNFα, MCP-1, and IL-6. TNFα and MCP-1 are chemoattractant proteins responsible for attracting neutrophils and M1 macrophages to the

area of damage. These phagocytic cells are important to break down the damaged cells so they can be removed from the area and repair processes can commence. The neutrophils and M1 macrophages cause damage over about a two-day period, then the Th1 pro-inflammatory response begins to subside and the Th2 anti-inflammatory response becomes dominant. M2 macrophages replace the M1 macrophages and will begin to promote tissue repair. Th2 anti-inflammatory cytokines play a large role in the activation of M2 macrophages, thus facilitating the repair process. M2 macrophages may help to remove debris along with M1 macrophages. Both TNFα and MCP-1 are thought to remain present during the recovery process to facilitate repair by stimulating cell growth. The Th1 transition to Th2 is very important. If both phases do not occur, muscle damage may not recovery properly (Mackinnon, 1992; Tidball, 2005; Tidball & Villalta, 2010).

Relationship Between Exercise and Inflammation

Over the past two decades it has been well documented that high-intensity exercise has a profound effect on inflammatory processes, likely due to damage occurring to the skeletal muscle tissue during high force-producing contractions. Resistance exercise (RE) is important for muscle growth and muscle regeneration/repair (American College of Sports Medicine position stand. Progression models in resistance training for healthy adults, 2009; Kraemer, Ratamess, & French, 2002). A critical component of muscle growth and muscle regeneration/repair is the inflammatory response to muscle damage induced by resistance exercise. The Th1 response of neutrophils and macrophages (inflammatory response) not only facilitates repair and regeneration, but also promotes the activation of satellite cells.

Satellite cells are stem cell-like cells in the skeletal muscle that are necessary for muscle growth and repair (Tidball, 2005).

The general consensus is that the more severe the muscle damage, the greater the inflammatory response. For example, eccentric exercise is associated with substantial increases in plasma IL-6 levels, whereas no changes are found with concentric exercise (Bruunsgaard et al., 1997). However, it is not entirely clear as to the source of the inflammatory cytokines response to exercise, i.e. what cells/tissues produce the inflammatory cytokines with exercise. It has been suggested that the elevated inflammatory cytokines in response to exercise is the result of both activated circulating inflammatory cells (e.g. monocytes and macrophages) and the skeletal muscle itself responding to the tissue damage (Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1998). In fact, skeletal muscle tissue was recently identified to possess endocrine functions, whereby cytokines are produced in the muscle cells, secreted into the systemic circulation, and thus can potentially mediate signaling in other tissues or cells. Pedersen et al. (2007) have classified these cytokines that are produced in skeletal muscle and secreted into the circulation as "myokines."

There has been much research investigating inflammatory cytokines and their response to different types of exercise; from high-intensity running (Brenner et al., 1999; Buford et al., 2009; Peake et al., 2005; Suzuki et al., 2003) to RE (Calle & Fernandez, 2010; Hirose et al., 2004; Izquierdo et al., 2009; Nieman et al., 2004). The majority of these studies have measured the inflammatory cytokine response to exercise in the systemic circulation (blood), and a few other studies have demonstrated increased inflammatory cytokine gene expression in skeletal muscle tissue in response to high-intensity exercise (Nieman et al.,

2004; Ross et al., 2010). Taken together, these reports suggest that the skeletal muscle contributes to the inflammatory response to exercise.

Past Research

It has been well demonstrated that both high-intensity endurance exercise and eccentric resistance exercise cause large inflammatory responses. The majority of research investigating the inflammatory response to exercise has examined the systemic circulation for inflammatory cytokines. Many studies have reported that inflammatory cytokines increase in blood plasma after multiple types of exercise. The inflammatory response to high-intensity eccentric exercise is higher than either low-intensity eccentric exercise or high-intensity concentric exercise (Brenner et al., 1999; Bruunsgaard et al., 1997; Hirose et al., 2004; Nieman et al., 1998; Smith et al., 2000), thus supporting the idea that the level of the inflammatory response is dependent upon the magnitude of the muscle damage incurred during the exercise protocol. Peake et al. (2005) demonstrated that increasing intensities of eccentric (downhill) running lead to proportionate increases in inflammatory cytokines. Untrained individuals may display a more notable inflammatory response to resistance exercise than trained subjects (Calle & Fernandez, 2010). Furthermore, prolonged exercise, such as marathon running, causes severe muscle tissue damage and thus induces a significant inflammatory response (Ostrowski et al., 1998). These reports thoroughly demonstrate that high-intensity exercise induces a substantial inflammatory response; however, these results do not indicate the source of the inflammatory cytokines.

Messenger RNA or mRNA is created within a cell nucleus and is the beginning of the production of proteins, such as the cytokines. Circulating blood does contain inflammatory cytokines, but the production begins in the nuclei of cells. IL-6 has been a specific

inflammatory cytokine that is well studied with exercise. Many studies have shown that IL-6 mRNA increases in muscle cells in response to high intensity running exercise, indicating that muscle does in fact produce IL-6 (Steensburg et al. 2002; Pedersen et al. 2001; Brenner et al., 1999). Buford et al. (2009), Ross et al. (2010), and Nieman et al. (2004) provided more evidence of increased IL-6 mRNA in skeletal muscle after resistance exercise. Steensburg et al. (2000) demonstrated that contracting skeletal muscle is responsible for the increased inflammatory cytokine concentrations in the blood in response to exercise. This was accomplished by measuring the appearance of IL-6 in the femoral vein of exercising thigh muscles with prolonged (5 hours), light-intensity leg extensor exercise. Furthermore, Starkie et al. (2001) demonstrated that circulating monocytes are not the source of increased inflammatory cytokines after prolonged exercise. Taken together, these results suggest that skeletal muscle is predominantly responsible for the acute-phase inflammatory response to high-intensity exercise.

Microdialysis as a Tool to Measure Inflammatory Cytokines in Skeletal Muscle

Microdialysis is a technique used to determine interstitial concentrations of molecules in various tissues throughout the body. Originally developed for the investigation of brain tissue, the use of microdialysis has proven effective for use in skeletal muscle, even when performing exercise (Hickner, 2000). It is an important tool because it can measure interstitial fluids over a time period rather than at individual time points. Microdialysis is performed using a very small tube with a semi-permeable membrane (microdialysis probe; Figure 1) that acts as an artificial capillary that dialyzes, or exchanges fluid with, the tissue interstitial space. It is important to make sure the recovery is accurate relative to the cells

actually in the interstitium. When perfused at a very slow flow rate with a precisely calibrated pump, the microdialysis probe comes into equilibrium with the interstitial space and the dialysate is collected at essentially the same concentration of molecules as is in the tissue interstitial fluid (Amer, 1999; Hickner, 2000). The collected interstitial fluid can then be analyzed for a variety of components, including inflammatory cytokines.

To date, only one study has analyzed the human skeletal muscle interstitial fluid for inflammatory cytokines in response to exercise (Rosendal et al., 2005). In this study, subjects performed "repeated, low-force exercise" that consisted of 20 minutes of continuous overhead movements of the dominant hand. This exercise routine resulted in substantial increases in interstitial IL-6 concentrations. It is unknown if high-intensity resistance exercise will elicit similar increases in inflammatory cytokine concentrations in the skeletal muscle interstitial fluid. Therefore, the purpose of this study was to determine whether high-intensity resistance exercise increases inflammatory cytokines in the skeletal muscle interstitium and to gain a better understanding as to the source of where the inflammatory cytokines are derived.

Project Justification

Although several studies have investigated the inflammatory response to exercise, the exact source of the inflammatory cytokines is still unclear. Microdialysis is a useful technique for analyzing the contents of the tissue interstitium. The utilization of microdialysis for the purpose of detecting cytokine production in muscle interstitium/extracellular fluid has only been done on one occasion to our knowledge (Rosendal et al., 2005). It is important to determine the source of where inflammatory

cytokines are produced because this information can be used to determine which cytokines are being produced, and potentially released, by the skeletal muscle and what effect high-intensity resistance exercise has on the production and distribution of the various inflammatory cytokines. Several cytokines have been observed in both the blood and the muscle within six hours of exercise. Increases in IL-6 (Izquierdo et al., 2009) and TNFα (Petersen & Pedersen, 2005; Starkie et al., 2001; Steensberg et al., 2002) have been shown early after exercise. The same has been reported for IL-8 post-exercise (Buford et al., 2009; Nieman et al., 2004; Pedersen et al., 2007). Ross et al. (2010) demonstrated an increase of MCP-1 at three hours post-exercise.

Furthermore, chronic inflammation is associated with several different pathologies, such as atherosclerosis, type II diabetes, and some cancers (Brandt & Pedersen, 2010). Exercise is thought to provide anti-inflammatory benefits; therefore, determining exactly where inflammatory cytokines are produced can provide further information as to the health-related benefits associated with exercise.

I hypothesized that an acute bout of high-intensity resistance exercise will increase the concentration of inflammatory cytokines in the skeletal muscle interstitial fluid.

CHAPTER 2

Methods and Procedures

Subjects

Ten lean (BMI ≤ 25), recreationally-active, young males between the ages of 20 and 27 years participated in this study. Exclusion criteria included: resistance training experience or lower limb injuries within the six months prior to participating in the study, known musculoskeletal and cardiovascular diseases, recent anti-inflammatory medication use, and smoking. Appalachian State University Institutional Review Board (IRB) approval was received for this study (Protocol #10-0140).

Initial Visit

Subjects were asked to report to the Neuromuscular Laboratory in the Department of Health, Leisure, and Exercise Science at Appalachian State University for all visits during this study. At the initial visit subjects completed the informed consent document and a health history questionnaire. Height (cm) and weight (kg) were measured on a standard physician's scale and then subjects completed a DEXA scan to assess body composition. Next, subjects performed a one-repetition maximum (1 RM) on the bilateral leg extension machine. The subjects estimated their 1RM and we began with 50% of their estimate performed 8 times (repetitions). After a 3 minute recovery, subjects performed 5 reps at 70%, then 2 reps at

90%. Lastly, subjects performed 1RM on their estimated weight and increased 5 pounds each sequential set until they could not perform 1 repetition.

Randomized Sample Collection Visits

At least five days after the initial visit, subjects reported to the Neuromuscular Lab for the first of two sample collection visits. Rosendal et al. (2005) reported that insertion of the microdialysis probes induces an inflammatory response, indicated by a significant increase in skeletal muscle interstitial IL-6. The authors claim that this response is likely due to minor tissue damage caused by inserting the microdialysis probe into the tissue. Hence, this experimental design included both resistance exercise and resting, no-exercise control trials, whereby the process of inserting the microdialysis probes was duplicated with each separate trial.

Subjects were randomized to perform either the Resistance Exercise trial (RE) or the No Exercise Control trial (NEC) first, then, in the subsequent visit, subjects performed the opposite trial. At least five days after the completion of the first sample collection visit, subjects reported to the Neuromuscular Lab again to complete the second sample collection visit. Each of the sample collection visits lasted approximately nine hours long. Subjects ate a regular breakfast and were also allowed to eat a meal halfway through the visits. Subjects were instructed to maintain their daily diet on both of the sample collection visits.

Sample Collection Visits

At the start of both trials, subjects arrived at the lab in the morning and were asked to void their bladder before the 9-hour sample collection period. First, microdialysis probes

were inserted into the vastus lateralis muscle of the right leg and after a 30-minute equilibration period, a resting skeletal muscle interstitial fluid sample was collected via microdialysis (see Microdialysis Technique for details). All microdialysis samples were collected for 1 hour. Blood samples were taken at rest and at one-hour intervals as well (see Protocol Schematic in Figure 2).

For the RE trial, subjects completed a single, acute resistance exercise bout consisting of bilateral leg extension exercise (3 sets of 10 repetitions/each at 80% of pre-determined 1 RM) after collection of the resting sample. During the resistance exercise bout, subjects were encouraged to perform the exercise at maximal effort. If more than 10 repetitions could be performed in any one set, subjects were instructed to perform additional repetitions to exhaustion and 5% of 1 RM was added to the next set. Microdialysis samples were collected every hour for 6 hours after the completion of the resistance exercise bout and blood samples were collected via venipuncture after equilibration and exercise (see Protocol Schematic in Figure 2).

For the NEC trial, subjects did not perform the resistance exercise bout, but simply remained resting for the entire sample collection period and microdialyis samples were collected every hour for 7 hours continuously. To control for trial-to-trial variability in inflammatory cytokines, a resting (basal) blood sample was collected via venipuncture after the equilibration time for the NEC trial and compared to the basal sample from the RE trial. As stated earlier, Rosendal et al. (2005) reported that insertion of the microdialysis probe causes a minute amount of muscle damage, but enough to induce an inflammatory response,

thus this portion of the study was intended to compare the inflammatory response to insertion of the microdialysis probes only to the inflammatory response to an acute bout of resistance exercise.

Microdialysis Technique

Originally developed for the investigation of brain tissue, microdialysis has emerged as a powerful technique to determine the concentration of a variety of molecules in the skeletal muscle interstitium (Hickner 2000). Microdialysis is performed using very small tubing with a small section that contains a permeable membrane (aka. probe) that acts as an artificial capillary (Figure 3). The microdialysis probe, when perfused at a very slow flow rate, exchanges fluid across the membrane between the tubing and the interstitial fluid. In this study, microdialysis was utilized to examine inflammatory cytokines in the skeletal muscle interstitial fluid at rest and in response to an acute bout of resistance exercise. First, an area of skin on the subject's thigh was prepared for the insertion of the microdialysis probes. Using sterile techniques, 2 microdialysis probes (CMA 20, 100 kDa pore size; CMA Microdialysis – Stockholm, Sweden) were inserted into the distal third of the vastus lateralis muscle. Once the probes were secured to the surface of the skin with adhesive strips, the microdialysis probe tubing was connected to a calibrated syringe pump (CMA 402; CMA Microdialysis). The probes were perfused with a sterile saline solution (aka. perfusate) containing 37 g/L of Dextran 70 and 10 µM of ethanol. Initially, the probes were flushed at a rate of 10 µL/min for approximately 10 minutes. Then, the pump was reset to 2 µL/min and the probes were allowed to equilibrate for 30 minutes. After the equilibration period, the probes were continuously perfused at a rate of 2 µL/min for the remainder of the sample

collection period. Microdialysis samples were collected for 1 hour. The collected interstitial fluid samples were stored at -80 °C until all samples were collected and analyzed for inflammatory cytokine concentration (see next section).

Determination of Inflammatory Cytokine Concentrations

All skeletal muscle interstitial fluid and plasma samples were analyzed for the inflammatory cytokines IL-6, IL-8, TNFα, and MCP-1 using multiplex ELISA assays (Custom Human 4-plex Panel; MesoScale Discovery, Gaithersburg, MD). These assays were performed at the Immunoassay Laboratory in the Proteonomics Core Facility of the David H. Murdock Research Institute at the North Carolina Research Campus in Kannapolis, NC.

Multiplex ELISA assays function similar to traditional singleplex ELISA's, except that multiple target cytokines can be analyzed in the same sample well with the multiplex ELISA. Much like a traditional ELISA, multiplex ELISA assays utilize standard immunodetection antibodies; however, as opposed to traditional ELISA assays, the chemiluminescent signal in a multiplex ELISA is generated by applying an electrical charge to each individual well on the plate. This electro-chemiluminescent signal is measured and quantified by a SECTOR® Imager 6000 and software system (MesoScale Discovery).

Statistical Analysis

Repeated measures two-way ANOVAs (exercise x time) were performed to determine if there were changes in inflammatory cytokines in the skeletal muscle interstitial fluid. Following a significant F-ratio (interaction), Bonferroni post-hoc analyses were performed to determine differences between resistance exercise and no-exercise trials over

the time course studied. Repeated measures one-way ANOVAs were performed to determine if there were changes in inflammatory cytokines in the plasma. Following a significant F-ratio, Bonferroni post-hoc analyses were performed to determine differences over the time course studied during the resistance exercise trial. Paired *t*-tests were performed to determine if there were differences in inflammatory cytokines in resting (basal) plasma samples between the RE and NEC trials. Significance was set at $\alpha = P \leq 0.05$. Statistical analyses were performed using SigmaStat 2.0 statistical software (SPSS; Chicago, IL).

CHAPTER 3

Results

Subjects

Of the ten subjects participating in this study, only six subjects were included in the final statistical analyses; three subjects were unable to return for their second trial and one subject who completed both trials displayed abnormally high inflammatory cytokine levels upon analysis (determined by +2 SD from the mean) and therefore was not included in the final analyses. Subject characteristics for the six subjects included in the final statistical analyses of the inflammatory cytokines are described in Table 1.

Inflammatory cytokines in Skeletal Muscle Interstitial Fluid and Plasma

Interleukin-6 (IL-6)

Skeletal muscle interstitial IL-6 increased nearly 3.5-fold 3 hours post-exercise in RE, but returned toward baseline by 4 hours. In the NEC trial, skeletal muscle interstitial IL-6 increased approximately 6-fold at hours 5 and 6 of sampling (Figure 4). However, there were no differences between RE and NEC at any time point. There was no significant difference in plasma IL-6 concentrations over the sampling period in the RE trial (Figure 5).

Interleukin-8 (IL-8)

Skeletal muscle interstitial IL-8 concentrations increased approximately 5- and 7-fold at the 2- and 3-hour time points, respectively (main effect of time), but no interaction existed between the RE and NEC trials (Figure 6). Plasma IL-8 concentrations were higher than basal at 2 and 3 hours after RE (Figure 7).

Tumor Necrosis Factor-α (TNF-α)

Skeletal muscle interstitial TNFα increased at 3 hours and remained elevated through 6 hours in the NEC trial. In the RE trial, interstitial TNFα was significantly higher than basal at only the 6-hour time point. Furthermore, TNFα was significantly higher in NEC than RE at the 4- and 5-hour time points (Figure 8). Plasma TNFα concentrations were significantly lower at the 6-hour time point, compared to the 4- and 5-hour time points in the RE trial (Figure 9).

Monocyte Chemotactic Protein (MCP-1)

Skeletal muscle interstitial MCP-1 increased approximately 5-fold at 2 hours and remained elevated at 6 hours in the NEC trial. Similarly, in the RE trial interstitial MCP-1 increased at 2 hours and remained elevated until 5 hours, but returned to baseline at 6 hours. Subsequently, MCP-1 was significantly higher in NEC than RE at the 6-hour time point (Figure 10). Analysis of plasma MCP-1 revealed a significant F-ratio; however, post hoc analyses did not indicate any significant differences between the individual time points (Figure 11).

Basal Plasma Inflammatory Cytokines

To control for trial-to-trial variability of inflammatory cytokines within subjects, basal plasma was compared for inflammatory cytokine levels in resting plasma samples between the RE and NEC trials for each subject. There were no significant differences in any inflammatory cytokine in basal plasma samples between the RE and NEC trials (Table 2). TNFα tended to be higher ($p=0.058$) in the basal plasma of subjects on the day of the RE trial, compared to the NEC trial. Basal plasma IL-6 concentrations in both the RE and NEC trials were below detectable levels of the assay.

CHAPTER 4

Discussion

Skeletal muscle damage results in the activation of immune cells and cytokines, known as an acute inflammatory response. These cells infiltrate the damaged area to facilitate tissue breakdown and eventual repair of the tissue (Mackinnon, 1992; Tidball, 2005; Tidball & Villalta, 2010). The inflammatory response is critical for the repair and regrowth of skeletal muscle tissue. Skeletal muscle damage can occur through different modes of exercise. Previous research has demonstrated that the inflammatory response in skeletal muscle, as indicated by increased circulating inflammatory cytokines (e.g IL-6 and TNFα), is proportional to the damage sustained during exercise (Buford et al., 2009; Izquierdo et al., 2009; Nieman et al., 2004; Pedersen et al., 2007; Petersen & Pedersen, 2005; Ross et al., 2010; Steensberg et al., 2002). However, it is still unclear as to the source of the inflammatory cytokines in response to exercise-induced skeletal muscle damage. Therefore, the purpose of this study was to determine whether increases in inflammatory cytokines could be measured in the skeletal muscle interstitium in response to high-intensity resistance exercise and to gain a better understanding as to the source of where the inflammatory cytokines are derived.

Skeletal muscle as a potential source for the production of inflammatory cytokines

It is well known that skeletal muscle tissue damage, such that occurs with high-intensity exercise, induces an acute inflammatory response. Several studies have quantified this response by reporting increased inflammatory cytokines, such as IL-6, TNFα, and IL-1, in the blood hours after exercise (Izquierdo et al., 2009; Pedersen et al., 2007; Petersen & Pedersen, 2005; Starkie et al., 2001; Steensberg et al., 2002); however, these results do not indicate the source of the inflammatory cytokines, only that the concentrations increase in the blood. It seems logical that the tissue sustaining the damage would play a substantial role in initiating the processes responsible for its repair. There are a few studies that have demonstrated that skeletal muscle tissue displays increased inflammatory cytokine mRNA levels in response to high-intensity exercise (Buford et al., 2009; Nieman et al., 2004; Ross et al., 2010). Furthermore, it is generally presumed that if an mRNA is transcribed, that it will likely be translated and result in a functional protein. In the case of these inflammatory cytokines, data from these previous studies suggest that skeletal muscle tissue is a probable source of the elevated inflammatory cytokines observed in the systemic circulation after exercise.

The current study aimed to determine if increased inflammatory cytokines could be detected in the skeletal muscle interstitial fluid after an acute bout of resistance exercise. Microdialysis is a powerful technique to determine the concentration of a variety of molecules in the skeletal muscle interstitium. Using microdialysis, this study demonstrates that increases in the inflammatory cytokines IL-6, IL-8, TNFα, and MCP-1 can be detected in the skeletal muscle interstitium. While there is one previous study that used microdialysis to measure skeletal muscle interstitial IL-6 (Rosendal et al., 2005), this is the first study to

report that increases in IL-8, TNFα, and MCP-1 can be detected in the skeletal muscle interstitial fluid using the microdialysis technique. These novel findings suggest that skeletal muscle tissue is likely a potential source in the production of inflammatory cytokines. With the exception of IL-8, the other inflammatory cytokines, IL-6, TNFα, and MCP-1, were not increased in plasma samples during the same time course of the study; thus further supporting the notion that skeletal muscle is a potential source of producing inflammatory cytokines. In the instance where plasma IL-8 increased in accordance (at similar time points) with increased IL-8 in the interstitial fluid, this might suggest that IL-8 could possibly be entering the interstitial fluid from the circulation and not from the skeletal muscle tissue itself. Plasma concentrations of TNFα decreased at the 6-hour time point and increased in the skeletal muscle interstitium. This may indicate TNFα transfer from circulating blood to the interstitial space. Further time points may help unveil exactly what is happening. Analyzing the skeletal muscle tissue for inflammatory cytokine mRNA and protein content would further elucidate the source of the increased inflammatory cytokines in the skeletal muscle interstitial fluid.

While this study does not definitively implicate skeletal muscle as the sole source for inflammatory cytokine production, these novel findings support previous research and further suggest that skeletal muscle tissue potentially produces inflammatory cytokines in response to muscle damage. The evidence from this study supports the hypothesis that skeletal muscle is likely a source of inflammatory cytokines, as measured by increases in interstitial (or extracellular) inflammatory cytokines via microdialysis before their appearance in the systemic circulation.

The inflammatory response between resistance exercise and no-exercise control trials

Previous research indicates that the inflammatory response in skeletal muscle is proportional to the degree of damage caused by exercise. Only one previous study has utilized microdialysis to investigate the inflammatory response to damage in skeletal muscle. Upon the insertion of the microdialysis probe into the muscle, an unavoidable amount of trauma is likely to occur. Another aim of this study was to assess the difference in the inflammatory responses between simply inserting the microdialysis probe into the muscle with rest and inserting the probe into the muscle with subsequent high-intensity resistance exercise. Rosendal et al. (2005) reported that skeletal muscle interstitial IL-6 concentrations increased after inserting the microdialysis probes, but this response only accounted for 20-30% of the inflammatory response to repetitive, low-force contraction exercise.

Contrary to the original hypothesis that an acute bout of high-intensity resistance exercise would increase the concentration of inflammatory cytokines in the skeletal muscle interstitial fluid, this study revealed that, in general, there were no substantial differences in the skeletal muscle inflammatory responses between the resistance exercise and no-exercise control trials. While these results displayed large inter-subject variability, likely hindering the discovery of more statistical differences, power analyses between treatments (RE vs. NEC) revealed that analyzing a higher number of subjects would not have yielded greater statistical power. However, significant differences between the RE and NEC trials were detected at two time points for intersitial TNFα (4- and 5-hour; Figure 8) and one time point for interstitial MCP-1 (6-hour; Figure 10). Interestingly, at each of these three time points, the inflammatory cytokines in the NEC trial were significantly higher than the RE trial. The lack of significant differences between the RE and NEC trials may be explained by two

related scenarios. The intensity and/or type of resistance exercise stimulus (concentric vs. eccentric) was not enough to induce significant inflammatory responses above that of insertion of the microdialysis probe for that time period (within the 6- to 7-hour time frame). Similarly, the insertion of the microdialysis probe itself induced such a large inflammatory response that it masked the exercise effect. It is unclear at this time as to which scenario is more accurate to explain the lack of difference in the inflammatory responses to RE and NEC, but future research should investigate the skeletal muscle tissue for inflammatory cytokine production (by means of mRNA and/or protein levels). These unexpected findings were contrary to the Rosendal et al. (2005) study, where they demonstrated significantly higher levels of IL-6 in the skeletal muscle interstitial fluid after repetitive, low-force contraction exercise, compared to the trauma caused by simply inserting the microdialysis probe in resting control samples. Major differences between the two studies are that in the Rosendal et al. (2005) study, subjects performed 20 minutes of overhead arm raises (assessed as 8-9% of maximal contractile force via electromyography) with the microdialysis samples acquired from the trapezius muscle. The exercise protocol used in the current study was an acute bout of resistance exercise consisting of concentric, bilateral leg extensions (3 sets of 10 reps at 80% of 1RM). While the exercise stimulus utilized in the current study, regardless of the concentric nature of the contractions, is undoubtedly a much higher intensity exercise stimulus, Rosendal et al. (2005) reported a significantly increased level of IL-6 that exceeded the increase due to insertion of the probe alone. The NEC in our study served as a control for the inflammatory response due to insertion of the probe; however, we were unable to detect differences between the RE and NEC trials.

Previous research consistently used more intense exercise stimuli to detect inflammatory cytokine changes than the two discussed above; however, results from the Rosendal et al. (2005) study suggest that a lower intensity may be a more appropriate protocol for microdialysis use. Findings from this study and Rosendal et al. (2005) advance our understanding of the damage created to the skeletal muscle by insertion of the microdialysis probe and further research is needed to determine the best exercise protocol to use with microdialysis.

Results from this study reveal that there are apparently different responses for each of the cytokines measured (IL-6, IL-8, TNF-α, and MCP-1) within the first six hours after exercise. Nonetheless, they all increased within 1-2 hours after insertion of the probe in both the NEC and RE trials. Many studies had subjects perform high-intensity eccentric exercise (Bruunsgaard et al., 1997; B. Pedersen, Steensberg, & Schjerling, 2001; Ross et al., 2010) which create higher levels of damage than the concentric exercise used in this study. Other studies used extended lengths of exercise to detect changes in plasma cytokines or skeletal muscle mRNA (Brenner et al., 1999; Nieman et al., 2004; Steensberg et al., 2002). Plasma levels of cytokines were only seen to change in these studies after very long or intense exercise, which may have induced a large amount of tissue damage. This is an important consideration when using microdialysis because the technique allows for a much more sensitive measurement from the interstitial fluid, as opposed to the diluted effect that occurs with the inflammatory cytokines in the plasma. Therefore, a lower intensity of exercise can be performed while still detecting cytokine changes in the muscle interstitium with no changes in the plasma, further elucidating the source of the inflammatory cytokines with exercise.

It is also important to consider the time frame when investigating the inflammatory response to damage. Our study examined the inflammatory response over a 6 to 7 hour window, whereas the Rosendal et al. (2005) study only sampled for 2 hours. The results from the current study indicated that there were no significant differences in any inflammatory cytokine between trials within 2 hours of basal samples; however, our statistical analysis was performed including all samples in the 7-hour time course. If the collection lasted just 2 hours, similar results may have been found to Rosendal et al. (2005) in regards to IL-6 between RE and NEC trials. Buford et al. (2009) and Ross et al. (2010) reported significant increases in skeletal muscle mRNA for IL-6, IL-8, and MCP-1 at 3 hours post-exercise. Similarly, the current study revealed significant increases in the skeletal muscle interstitium at 3 hours post-exercise for the same cytokines. Plasma values at the 3-hour time point for all three studies were not different from basal plasma samples. Taken together, these data support the notion that skeletal muscle is a likely source of inflammatory production in response to exercise.

While it is thought that the inflammatory response in skeletal muscle is proportionate to the amount of damage sustained, it appears from the divergence of these results in the current study that either: 1) the exercise stimulus was not intense enough to stimulate a significant inflammatory response or 2) insertion of the microdialysis probe induces such a large inflammatory response that it overshadows the exercise effect. Further research is needed to determine the optimal protocol for the use of skeletal muscle microdialysis to investigate the inflammatory response to exercise.

Potential anti-inflammatory effects of exercise

It is thought that exercise can induce an anti-inflammatory effect, such that exercise can be beneficial in lowering inflammation in pathologies associated with elevated levels of inflammation (e.g. atherosclerosis, Type II diabetes, and some cancers). As mentioned previously, TNFα and MCP-1 displayed significant differences in the later time points between the NEC and RE trials. Specifically, TNFα was higher in the NEC trial at hours 4 and 5 (Figure 8) and MCP-1 was higher in the NEC trial at 6 hours (Figure 10), compared to the RE trial. These data suggest that an acute bout of resistance exercise may induce an anti-inflammatory effect by potentially lowering the inflammatory response or increasing the anti-inflammatory response after exercise. This has been demonstrated previously by Petersen and Pedersen (2005). The authors state that acute bouts of regular exercise can protect against low-grade inflammation. However, longitudinal studies of this type have not yet been performed. Based on previous research supporting this claim (Helle Bruunsgaard, 2005; Mathur & Pedersen, 2008; Petersen & Pedersen, 2005) combined with our study, acute exercise may protect against diseases associated with low-grade inflammation. This is attributed to the potential of acute resistance training to decrease the inflammatory response or increase the anti-inflammatory response. Further studies using the microdialysis technique to determine which inflammatory cytokines are present in the skeletal muscle interstitium and how they change in response to exercise could provide further information as to the health-related benefits of exercise and narrow down the source of inflammatory cytokines.

Improvements on Research and Experimental Design

To improve upon our study specifically, we could extend the protocol duration or possibly determine a way to insert the microdialysis probe into the muscle and have it remain in the muscle overnight. Then we could have the subjects return to the laboratory the following morning to perform the resistance exercise after the acute inflammatory response to insertion of the probe had subsided. This could prove to be difficult as the microdialysis probes are very fragile and could easily break with hours of muscular activity. In the current study, multiple probes (up to four) were inserted into the muscle and on several occasions one or two of the microdialysis probes would break. The CMA Microdialysis company makes a different probe that may be more suitable for overnight protocols. Inserting the probe, then performing exercise several hours later could provide more definitive results. Constant movement of the probe inside the muscle may also increase the inflammatory response with time.

Practical Application and Future Research

The purpose of this study was to determine whether high-intensity resistance exercise increases inflammatory cytokines in the skeletal muscle interstitium and to gain a better understanding as to the source of where the inflammatory cytokines are derived. The findings from this study may have larger implications for future research investigating the inflammatory response in skeletal muscle associated with pathological conditions. Exercise is thought to provide anti-inflammatory benefits and chronic inflammation is associated with several different pathologies, such as atherosclerosis, type II diabetes, and some cancers (Brandt & Pedersen, 2010). Sarcopenia is the loss of skeletal muscle strength and mass with

aging. A state of chronic, low-grade inflammation and the inability to recover from injury both contribute to sarcopenia (Burton & Sumukadas, 2010). People often use non-steroidal anti-inflammatory drugs (NSAIDs) to relieve pain and discomfort resulting from exercise-induced skeletal muscle damage. Tidball & Villalta (2010) and Tidball (2005) demonstrated the inflammatory process begins with neutrophil infiltration into the tissue and continues through satellite cell regeneration of damaged skeletal muscle cells. This is the natural process by which the body recovers from injury or damage. Disruption of the natural recovery process by NSAIDs may inhibit muscle growth and repair, resulting in prolonged damage. Trappe et al. (2002) demonstrated that ingestion of the common NSAIDs, Ibuprofen and Acetaminophen, inhibits protein synthesis that normally occurs after eccentric resistance exercise. Determining the potential positive effect of acute resistance exercise could reduce the use of NSAIDs. In addition to studying acute resistance training, continued use of microdialysis for cytokine studies could help diminish NSAID use. Rather than taking NSAIDs, individuals could be treated with specific cytokines to replicate the "normal" process the body uses for muscle repair and regeneration that is insufficient for that person.

The practical application of this research is probably not in the near future as more preliminary work is necessary at this point. First, effective resistance exercises and recovery time from the microdialysis probe insertion must be determined. Then, males and females, young and old, trained and untrained should be studied to determine differences among the different groups. Long-term studies also need to be conducted to see if regular acute resistance exercise presents the same anti-inflammatory effects as an acute bout of exercise. There are many possibilities for this research to take the next step because the inflammatory

response can relate to such a wide range of people for different reasons from athletes to people with chronic disease.

Conclusion

The inflammatory response is critical for the repair and regrowth of skeletal muscle tissue. It is thought that the inflammatory response is proportional to the damage sustained to skeletal muscle during exercise. This study sought to determine whether increases in inflammatory cytokines could be measured in the skeletal muscle interstitium in response to high-intensity resistance exercise. Findings from this study demonstrate that increases in the inflammatory cytokines IL-6, IL-8, TNFα, and MCP-1 can be detected in the skeletal muscle interstitium; however, there were few significant differences in the skeletal muscle inflammatory responses between the resistance exercise and no-exercise control trials. More specifically, TNFα was higher in the NEC trial at hours 4 and 5 and MCP-1 was higher in the NEC trial at 6 hours, compared to the RE trial. These data suggest that an acute bout of resistance exercise may induce an anti-inflammatory effect by potentially lowering the inflammatory response or increasing the anti-inflammatory response after exercise. Nonetheless, further research is needed to determine the optimal protocol for the use of skeletal muscle microdialysis to investigate the inflammatory response to exercise.

Table 1: Subject Characteristics (N=6). Data are represented as Mean ± SEM.

Age (yrs)	Height (cm)	Weight (kg)	Body Fat %	1-RM/kg bw
21.8 ± 1.3	183.0 ± 2.5	83.0 ± 2.5	15.4 ± 1.4	1.6 ± 0.1

Table 2: Basal Plasma Inflammatory Cytokine Concentrations (pg/mL). Data are represented as mean ± SEM. N=6 for each trial. ‡ IL-6 was lower than detectable levels for both trials (Not Detectable).

	IL-6	IL-8	TNFα	MCP-1
RE	ND ‡	2.19 ± 0.73	3.10 ± 0.96	226.58 ± 20.55
NEC	ND ‡	1.81 ± 1.22	2.22 ± 0.82	210.23 ± 42.01

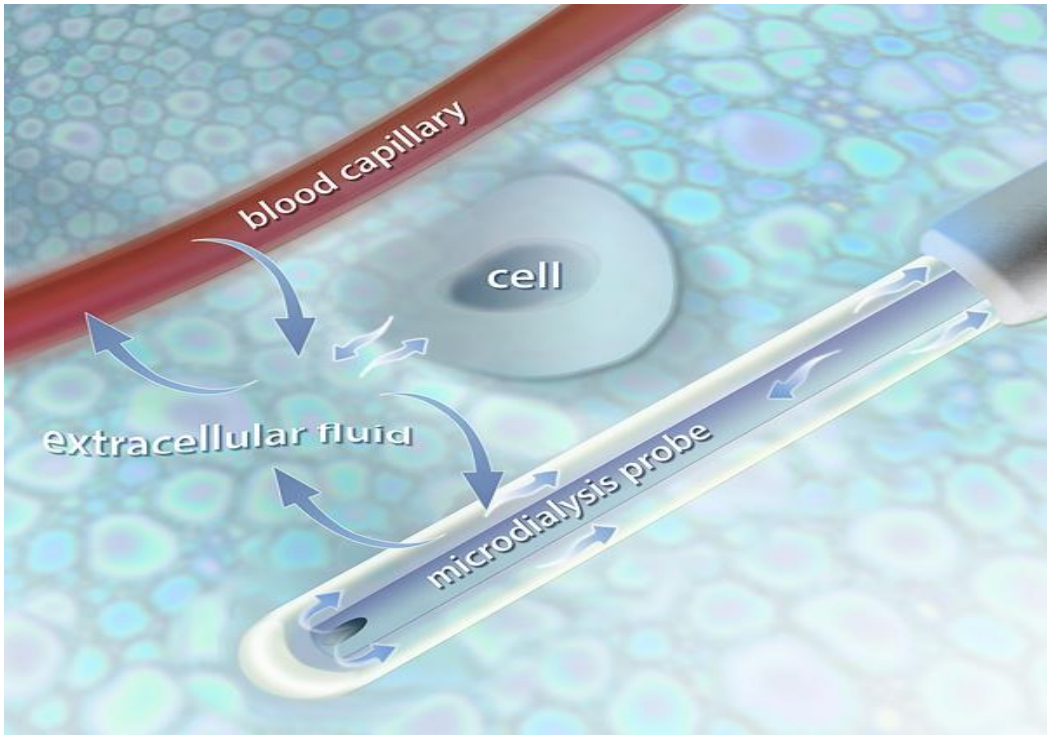

Figure 1. Diagram of a microdialysis probe membrane in the tissue interstitial space acting like an artificial capillary by exchanging perfused saline solution with extracellular fluid. (Courtesy of CMA Microdialysis; Stockholm, Sweden)

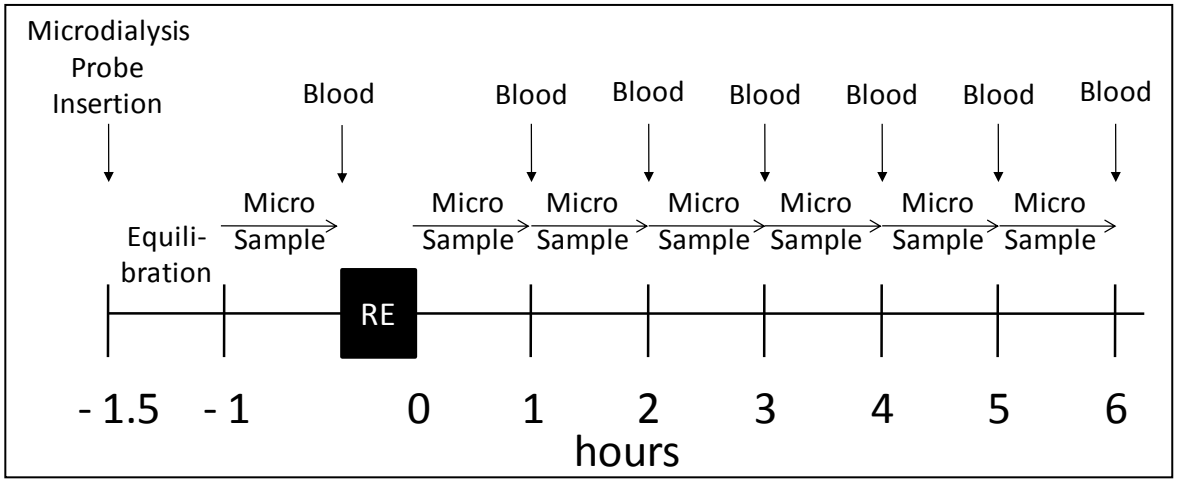

Figure 2. Protocol schematic. RE = resistance exercise; Blood = collection of venous blood sample; Micro Sample = collection of microdialysis sample

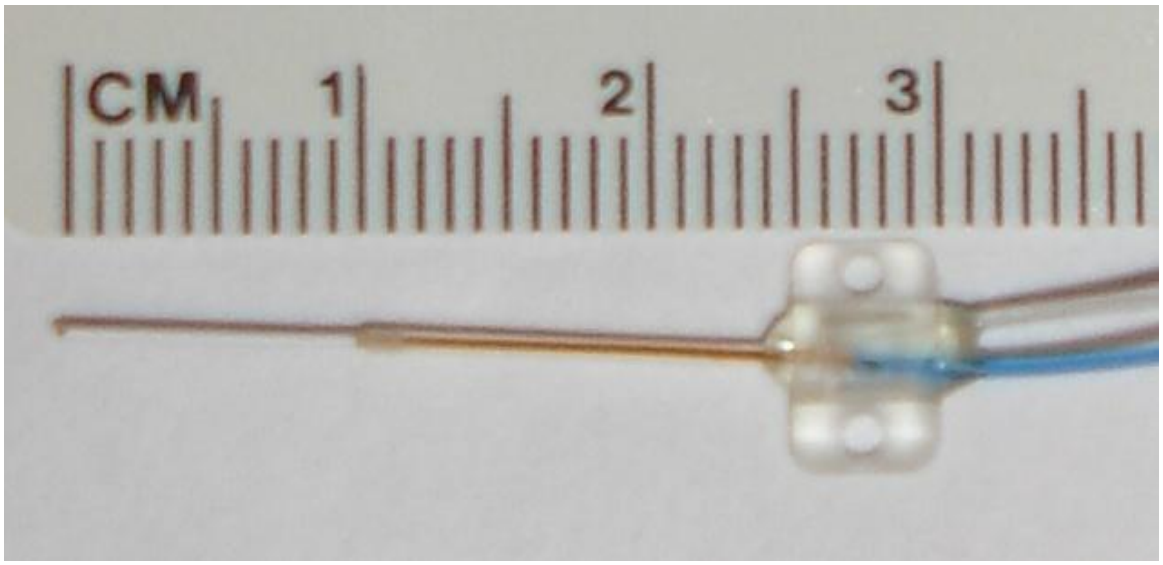

Figure 3. The microdialysis probe (CMA Microdialysis; Stockholm, Sweden) has two tubes: inflow (blue) and outflow (clear) that function to exchange components with the skeletal muscle interstitial fluid.

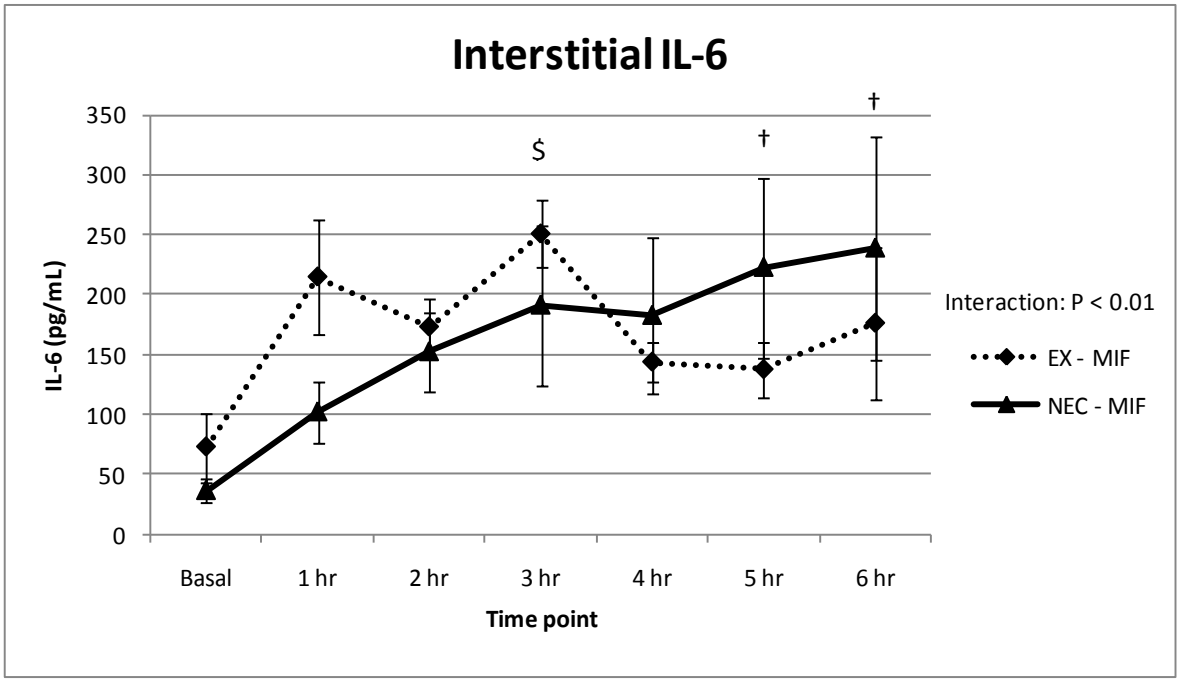

Figure 4. Skeletal muscle interstitial IL-6 increased at 3 hours for RE trial and at 5 and 6 hours for NEC trial. There were no differences between the trials at any time point. †-Significant difference within the NEC trial at that time point from basal. $-Significant difference within the RE trial at that time point from basal. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

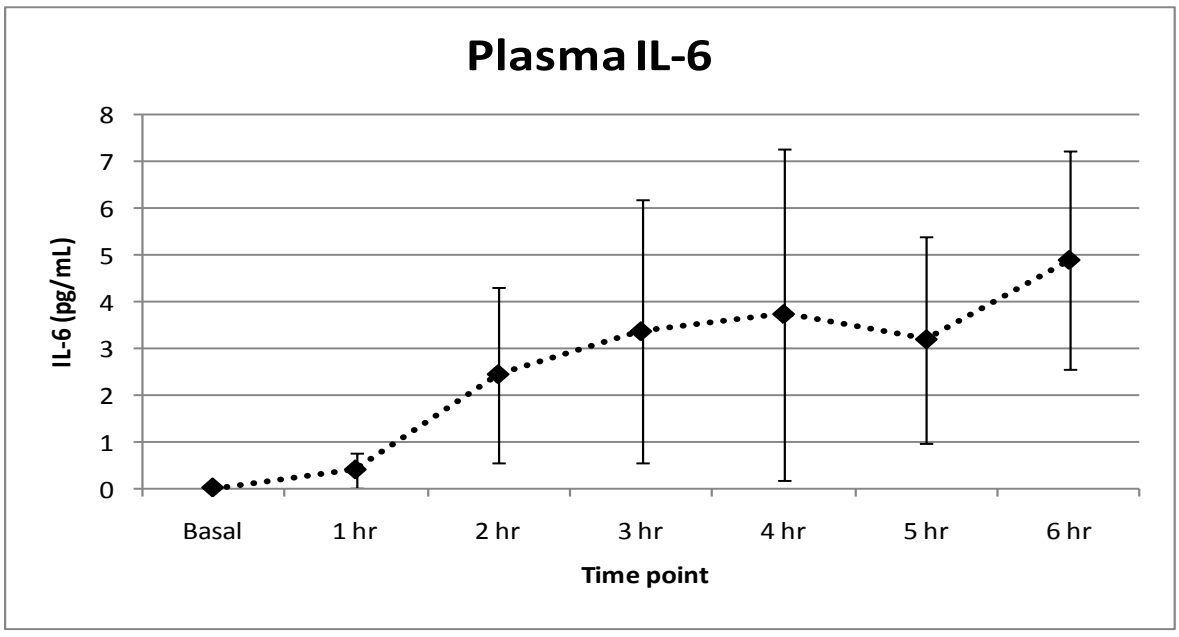

Figure 5. There was no significant difference in plasma IL-6 concentrations in the RE trial. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

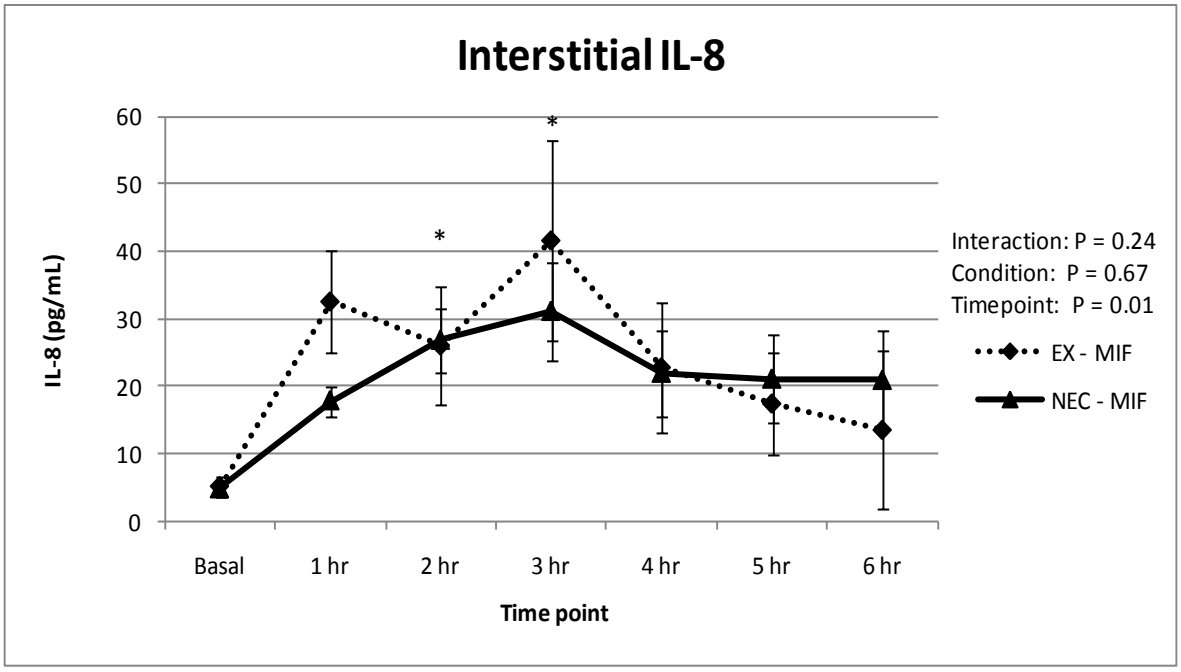

Figure 6. Skeletal muscle interstitial IL-8 showed no interaction between the NEC and RE trials. Significant differences from basal occurred at 2 and 3 hours. *-Significantly different from basal. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

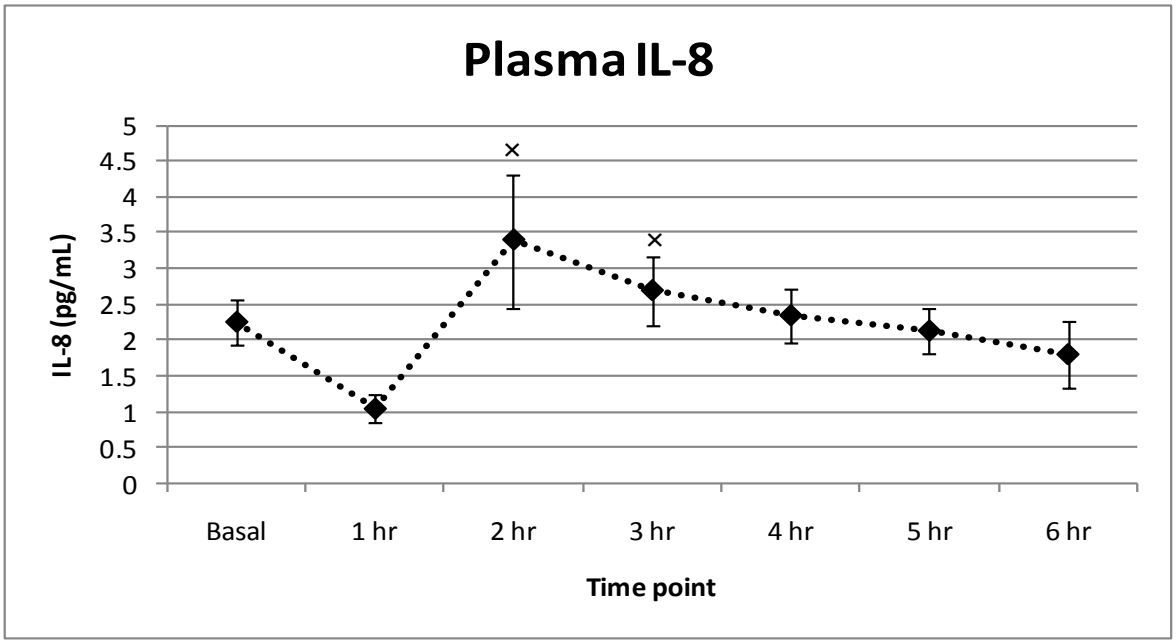

Figure 7. There was a significant difference in plasma IL-8 concentrations from basal at 2 and 3 hours. × - Significantly different from basal. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

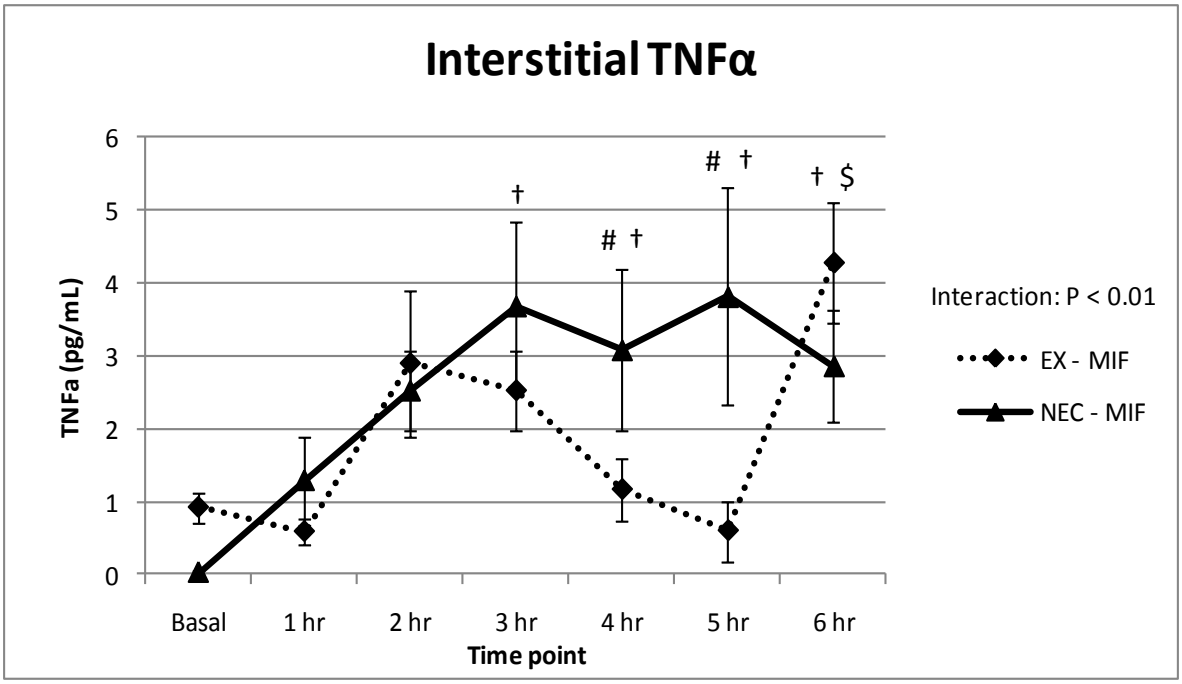

Figure 8. Skeletal muscle interstitial TNFα was significantly higher than basal for the NEC from hours 3 to 6. RE concentrations were significantly different than basal at 6 hours. NEC trial was significantly higher than RE trial at hours 4 and 5. #-Significant difference between the RE trial and NEC trial at that time point. †-Significant difference within the NEC trial at that time point from basal. $-Significant difference within the RE trial at that time point from basal. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

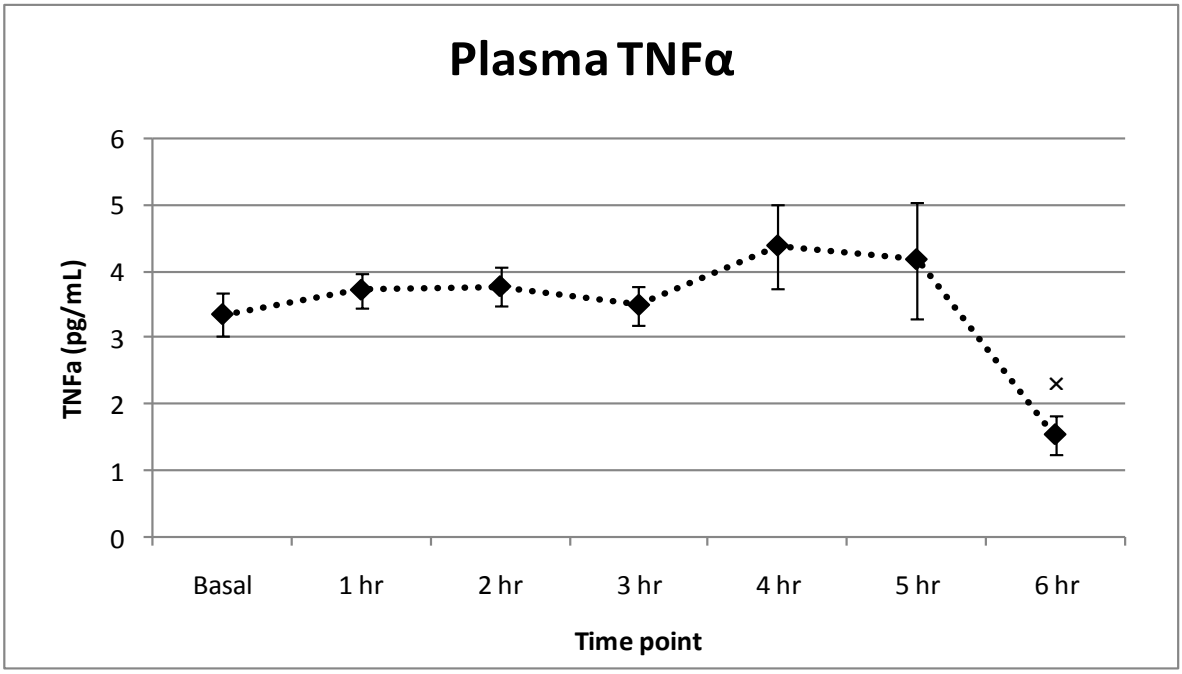

Figure 9. Plasma concentrations of TNFα were different between hours 5 and 6 and hours 4 and 6. ×-Significantly different from hours 4 and 5. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

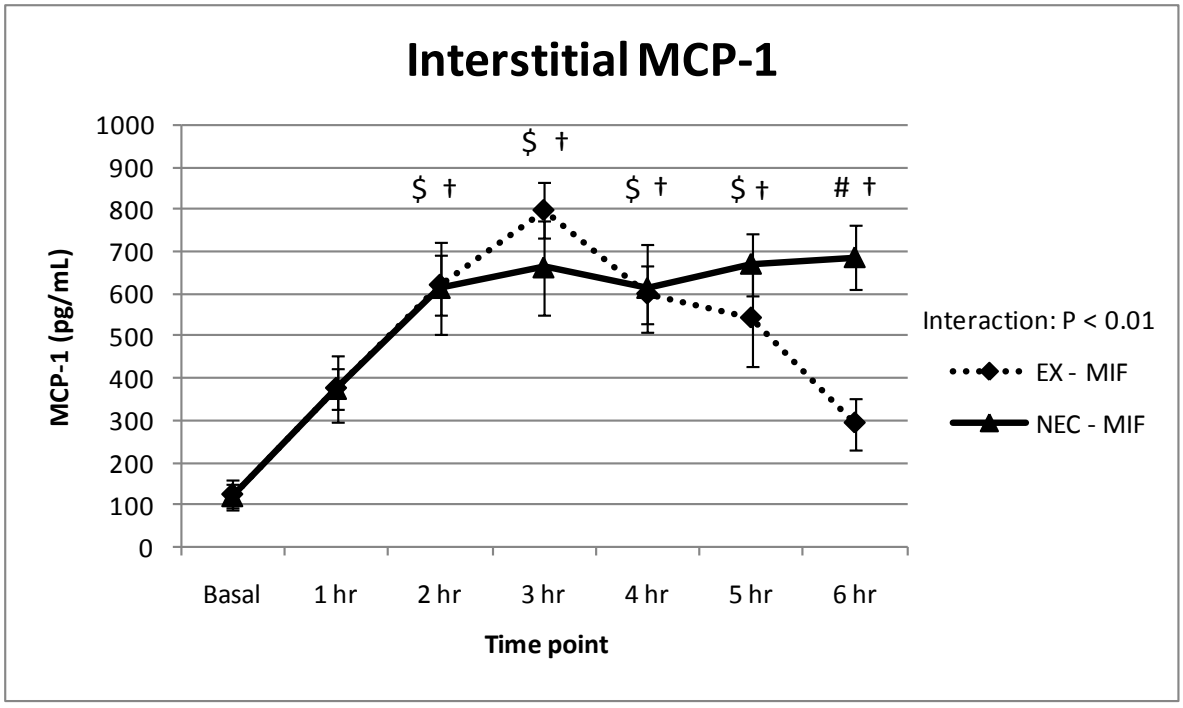

Figure 10. Skeletal muscle interstitial MCP-1 during RE trial were different from basal at hours 2 through 5. NEC trial concentrations were different from basal at hours 2 through 6. MCP-1 concentrations were significantly higher in NEC trial than RE trial at 6 hours. #-Significant difference between the RE trial and NEC trial at that time point. †-Significant difference within the NEC trial at that time point from basal. $-Significant difference within the RE trial at that time point from basal. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

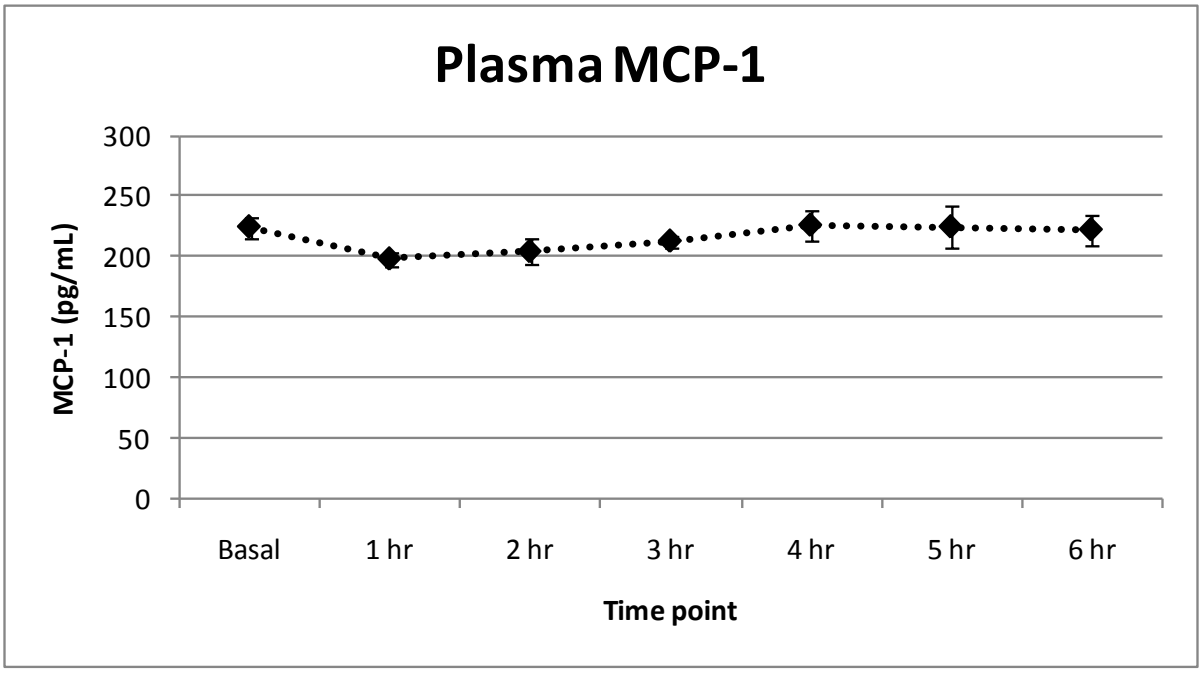

Figure 11. There were no significant differences between individual time points in MCP-1 plasma concentrations. Data are represented as mean ± SEM. N = 6 for each trial. Significance was set at α $p \leq 0.05$.

References

Amer, P. (1999). Microdialysis: use in human exercise studies. *Proc Nutr Soc, 58*(4), 913-917.

American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. (2009). *Med Sci Sports Exerc, 41*(3), 687-708. doi: 10.1249/MSS.0b013e3181915670 [doi]

Brant, C., & Pedersen, B. (2010). The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. *J Biomed Biotechnol. 2010, 520-528,* doi: 10.1155/2010/520528J

Brenner, I. K. M., Natale, V. M., Vasiliou, P., Moldoveanu, A. I., Shek, P. N., & Shephard, R. J. (1999). Impact of three different types of exercise on components of the inflammatory response. *European Journal of Applied Physiology & Occupational Physiology, 80*(5), 452-460.

Bruunsgaard, H. (2005). Physical activity and modulation of systemic low-level inflammation. *Journal of Leukocyte Biology, 78*(4), 819-835. doi: 10.1189/jlb.0505247

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T., MacLean, D., & Pedersen, B. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol, 499 (Pt 3)*, 833-841.

Buford, T. W., Cooke, M. B., Shelmadine, B. D., Hudson, G. M., Redd, L., & Willoughby, D. S. (2009). Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. *Applied Physiology, Nutrition & Metabolism, 34*(4), 745-753.

Burton, L., & Sumukadas, D. (2010). Optimal management of sarcopenia. *Clin Interv Aging, 5*, 217-228.

Calle, M., & Fernandez, M. (2010). Effects of resistance training on the inflammatory response. *Nutr Res Pract, 4*(4), 259-269. doi: 10.4162/nrp.2010.4.4.259

Hickner, R. (2000). Applications of microdialysis in studies of exercise. *Exerc Sport Sci Rev, 28*(3), 117-122.

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., & Suzuki, K. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exercise immunology review, 10*, 75-90.

Izquierdo, M., Ibanez, J., Calbet, J. A., Navarro-Amezqueta, I., Gonzalez-Izal, M., Idoate, F., . . . Gorostiaga, E. M. (2009). Cytokine and hormone responses to resistance training. *Eur J Appl Physiol, 107*(4), 397-409. doi: 10.1007/s00421-009-1139-x [doi]

Kraemer, W. J., Ratamess, N. A., & French, D. N. (2002). Resistance training for health and performance. *Curr Sports Med Rep, 1*(3), 165-171.

Mackinnon, L. T. (Ed.). (1992). *Current Issues in Exercise Science Series* (Vol. 2). Champaign, IL: Human Kinetics Publishers, Inc.

Nieman, D., Nehlsen-Cannarella, S., Fagoaga, O., Henson, D., Utter, A., Davis, J., . . . Butterworth, D. (1998). Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc, 30*(5), 671-678.

Nieman, D. C., Davis, J. M., Brown, V. A., Henson, D. A., Dumke, C. L., Utter, A. C., . . . McAnulty, L. S. (2004). Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. *Journal of Applied Physiology, 96*(4), 1292-1298.

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol, 508 (Pt 3)*, 949-953.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., & Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur J Appl Physiol, 95*(5-6), 514-521. doi: 10.1007/s00421-005-0035-2 [doi]

Pedersen, B., Steensberg, A., & Schjerling, P. (2001). Muscle-derived interleukin-6: possible biological effects. *J Physiol, 536*(Pt 2), 329-337. doi: PHY_12855 [pii]

Pedersen, B. K., Akerstrom, T. C., Nielsen, A. R., & Fischer, C. P. (2007). Role of myokines in exercise and metabolism. *J Appl Physiol, 103*(3), 1093-1098. doi: 00080.2007 [pii] 10.1152/japplphysiol.00080.2007

Pedersen, B. K., Ostrowski, K., Rohde, T., & Bruunsgaard, H. (1998). The cytokine response to strenuous exercise. *Can J Physiol Pharmacol, 76*(5), 505-511.

Petersen, A. M. W., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *J Appl Physiol, 98*(4), 1154-1162. doi: 10.1152/japplphysiol.00164.2004

Rosendal, L., Sogaard, K., Kjaer, M., Sjogaard, G., Langberg, H., & Kristiansen, J. (2005). Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. *J Appl Physiol, 98*(2), 477-481. doi: 10.1152/japplphysiol.00130.2004

Ross, M., Halson, S., Suzuki, K., Garnham, A., Hawley, J., Cameron-Smith, D., & Peake, J. (2010). Cytokine responses to carbohydrate ingestion during recovery from exercise-induced muscle injury. *J Interferon Cytokine Res, 30*(5), 329-337. doi: 10.1089/jir.2009.0079

Smith, L. L., Anwar, A., Fragen, M., Rananto, C., Johnson, R., & Holbert, D. (2000). Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. *Eur J Appl Physiol, 82*(1-2), 61-67.

Starkie, R. L., Rolland, J., Angus, D. J., Anderson, M. J., & Febbraio, M. A. (2001). Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Physiol Cell Physiol, 280*(4), C769-774.

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A., & Pedersen, B. K. (2002). IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab, 283*(6), E1272-1278. doi: 10.1152/ajpendo.00255.2002 [doi] 00255.2002 [pii]

Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., & Klarlund Pedersen, B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the

exercise-induced increase in plasma interleukin-6. *J Physiol, 529 Pt 1*, 237-242. doi: PHY_11483 [pii]

Suzuki, K., Nakaji, S., Kurakake, S., Totsuka, M., Sato, K., Kuriyama, T., . . . Sugawara, K. (2003). Exhaustive exercise and type-1/type-2 cytokine balance with special focus on interleukin-12 p40/p70. *Exerc Immunol Rev, 9*, 48-57.

Tidball, J. G. (2005). Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol, 288*(2), R345-353. doi: 10.1152/ajpregu.00454.2004

Tidball, J. G., & Villalta, S. A. (2010). Regulatory interactions between muscle and the immune system during muscle regeneration. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 298*(5), R1173-R1187. doi: 10.1152/ajpregu.00735.2009

Trappe, T. A., White, F., Lambert, C. P., Cesar, D., Hellerstein, M., & Evans, W. J. (2002). Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *American Journal of Physiology - Endocrinology And Metabolism, 282*(3), E551-E556. doi: 10.1152/ajpendo.00352.2001

Vita

Brian Pratt was born in Erie, PA. He is the youngest son of Jay and Laura Pratt. Brian earned his Bachelor's of Science degree in Exercise Science May 2009 from Slippery Rock University. Following a two year program of study, Brian graduated from Appalachian State University in May 2011 with a Masters of Science degree in Exercise Science.