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The Inflammatory Response to Acute Maximal Sprint Exercise

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By

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Abstract

The purpose of this study was to determine whether acute maximal sprint exercise would elicit an inflammatory response in trained and untrained runners marked by increased systemic immune proteins and byproducts of oxidation, as well as to determine a time course for the presence of these markers. In procedures approved by the Eastern Michigan University College of Health and Human Services Human Subjects Review Board, subjects participated in a total of five study visits consisting of both exercise and blood draws. Blood analysis seems to indicate that there was a secondary tissue injury, which was responsible for the greatest changes in the measured parameters, although a larger population must be examined to confirm these results. It was also noted that increased cytokine concentrations preceded an increase in oxidative byproducts, indicating the existence of a cytokine dependant cascade, in addition to that created by the initial mechanical insult.

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Chapter I

Introduction

The muscular system is one of the largest organ systems in the human body, and its proper function is essential for the completion of activities of daily living as well as for athletic performance. This system is highly adaptive to repeated physical activity and inactivity which may result in modifications of muscular strength and endurance as well as musculotendinous integrity. It has been thought until recently that the skeletal muscle system was highly dependent on endocrine and paracrine stimulation during physical activity to effectively maintain tissue homeostasis. However, there has been a great deal of research conducted in recent years suggesting that muscle tissue plays a role in the release of cytokines during exercise, which have been found to be involved in cell signaling and metabolism. This indicates that skeletal muscle may, to an extent, be somewhat capable of auto regulation of its homeostatic mechanisms during exercise, as well as affecting systemic cytokine concentrations. Studies conducted by Steensberg et al. (Steensberg, Keller, Hillig, Frøsig, Wojtaszewski, Pedersen, Pilegaard and Sander, 2007) have shown that muscle tissue is capable of releasing cytokines (aptly named “myokines” by Pedersen (2007) in response to contraction. These acute myokine responses due to exercise have been shown to elicit several beneficial effects such as a reduction in systemic inflammation (Petersen and Pedersen, 2005) and increased glucose sensitivity in the muscle (Glund S, Deshmukh, Long, Moller, Koistinen, Caidahl, Zierath and Krook, 2007) and may also play a vital role in several inflammatory cascades and tissue regeneration/adaptation after muscle injury.

Tumor necrosis factor-alpha (TNF-α), Interleukin-1 (IL-1), interleukin-8 (IL-8), and Interleukin-15 (IL-15) are associated with bouts of inflammation and have shown the greatest systemic increases due to eccentric-coupled exercise. While the presence of these cytokines in systemic circulation have most commonly been correlated to negative health outcomes such as fatigue, muscle wasting, and cachexia, it has recently suggested that these cytokines may play a role in eliciting a beneficial inflammatory response after muscle damaging exercise, which may lead to healing that might not otherwise occur.

Reactive oxygen species (ROS) have long been thought of as detrimental to health and performance, resulting in the degradation of cellular material and leading to cell necrosis. ROS production during exercise is most commonly attributed to the immense proton gradient at the end of the electron transport chain remaining at the conclusion of activity. ROS are also created by the immune system within neutrophils and macrophages in response to tissue damage or foreign bodies such as bacteria. Recent research in the area of exercise-induced ROS production has began to uncover evidence that ROS may also be involved in cell signaling pathways for protein synthesis and gene transcription and are part of a sensitive balance that is essential for proper adaptation to exercise and tissue repair after injury (Powers and Jackson, 2008). It is plausible that the ROS production due to the immune response (e.g. neutrophils and macrophages) to muscle tissue damage, as suggested by Close et al. (2004) may provide the signal to induce cytokine activity in the area.

Current research in this area has focused primarily on the creation of exercise-induced myokines and ROS through long duration endurance exercise (Pedersen and Febbraio, 2008) and eccentric resistance training. This is the first study exploring the effect of anaerobic sprinting on

plasma myokine and ROS concentrations. It has been shown through the use of high resolution accelerometry (HRA) that accelerations during sprint performance are far greater than those associated with extremely high intensity running near $VO2_{max}$ (McGregor, In Review) indicating that the amount of eccentric loading of the muscles is also much greater. This increase in eccentric stress could elicit further tissue injury and result in a greater ROS and cytokine response.

McGregor et al. have recently exhibited that concentric-only lower extremity exercise of similar intensity and duration (McGregor, In Review) elicited no significant Cytokine or ROS response in direct response to the exercise bout. Therefore, if any systemic change in the previously mentioned variables is noted following high intensity exercise with substantial eccentric muscle loading, it will provide insight into the mechanism in which these proteins and radicals are produced.

Exercise-induced myokines released into systemic circulation have the ability to carry out their function on all tissues of the body. This could be indicated by the overall improvements in health status that have been observed due to chronic exercise programs. If an eccentric component to physical activity shows an acute increase in systemic levels of cytokines and ROS, it may in turn provide greater health benefits of exercise such as improved muscular restructuring, increased bone density, and greater angiogenesis. Additionally, the inhibition of these responses with drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) may inhibit the establishment of this potentially beneficial environment.

Statement of Purpose

The purpose of this study is to determine the effect of acute eccentric anaerobic sprinting in trained and untrained participants on 1) systemic levels of the cytokines IL-1, IL-8, IL-15, and TNF-α; 2) systemic levels of ROS markers including protein carbonyls, oxidized prostaglandins, malonyl dialdehyde (MDA) and hydroxy nonenal (4-HNE); 3) and the presence of an inflammatory response marked by C-reactive protein (CRP) and muscle tissue damage marked by creatine kinase (CK), as well as to compare these results to those found in a concentric-only exercise of a similar duration and intensity.

Research Null Hypotheses

This investigation evaluated the following null hypotheses:

1. There will be no significant difference in systemic ROS concentration after an acute bout of anaerobic sprinting.
2. There will be no significant difference in systemic cytokine concentrations after an acute bout of anaerobic sprinting.
3. There will be no significant difference in systemic CRP or CK concentrations after an acute bout of anaerobic sprinting.
4. There will be no significant difference in systemic ROS, cytokine, CRP, or CK concentrations between trained and untrained subjects.
5. There will be no significant difference in systemic ROS, cytokine, CRP, or CK concentrations between concentric exercise and exercise with an eccentric component of a similar intensity and duration.

Assumptions

For this investigation the following assumptions were made:

1. Participants would not have significant changes in blood marker concentration due to changes in training or heath status.
2. Participants provided a maximal volitional effort during all trials.
3. Trained populations were NCAA Division I track and field athletes.

Limitations

1. All subjects were college-aged and may not be representative of populations outside of this age range.
2. Untrained populations may have had several mechanical differences in gait while running, which may have influenced the amount of trauma induced to the muscle tissue. This may have affected the cytokine, ROS, CRP, and CK responses.
3. Moderately trained populations may have shown very different responses in cytokine, ROS, CRP, and CK responses due to reduced adaptation to this type of exercise.

Significance of the Study

This study examined the effect of acute anaerobic exercise with an eccentric component on muscle damage, inflammation, and systemic cytokine and ROS concentrations in trained and untrained populations. These data were then compared to that of concentric-only exercise of similar duration and intensity.

The assessment of these data may provide some insight into the benefits and drawbacks of exercise involving an eccentric component. It is important to understand the potential benefits

of increased eccentric loading on muscle tissue such as greater signaling for protein production, muscle anabolism, and angiogenesis for new tissue. It is also important to understand the potential drawbacks that this mode of exercise may elicit, such as severe muscle soreness, fatigue, or a greater potential for injury.

Related Definitions

Angiogenesis: the formation of new blood vessels

***Cytokine*:** Any class of immunoregulatory proteins that are secreted by cells especially of the immune system.

***C-Reactive Protein*:** A protein produced by the liver that is normally present in trace amounts in the blood serum but is elevated during episodes of acute inflammation.

***Creatine Kinase*:** Any of three isoenzymes found especially in vertebrate skeletal and myocardial muscle and the brain that catalyze the transfer of a high-energy phosphate group from phosphocreatine to ADP with the formation of ATP and creatine and typically occur in elevated levels in the blood following injury to brain or muscle tissue.

***Eccentric-coupled exercise*:** Exercise involving both and eccentric and concentric compontent.

***Reactive Oxygen Species*:** Atoms or molecules with highly reactive unpaired electrons.

Chapter II

Review Of Literature

Introduction

The purpose of this study was to examine the effect of an acute bout of anaerobic sprinting on the plasma levels of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α), Interleukin-1 (IL-1), Interleukin-8 (IL-8), and Interleukin-15 (IL-15), as well as the systemic accumulation of byproducts of reactive oxygen species (ROS) activity. There are currently no studies exploring the effect of high intensity sprinting on the plasma levels of these factors. This chapter will explore existing literature on cytokine and ROS expression resulting from eccentric exercise and their possible influences within the body.

The accumulation of cytokines after eccentric exercise

While chronic systemic inflammation is commonly associated with the presence of illness, the acute inflammatory response resulting from intense exercise has been deemed necessary for proper muscular remodeling and growth after muscular injury (reviewed by Tidball (2005). The amplification of the inflammatory response created by an influx of neutrophils and macrophages into damaged tissue upon injury regulates muscular tissue repair by initiating the production of several inflammatory cytokines, which may lead to the clearance of tissue debris and the subsequent initiation of new tissue growth and remodeling (Vassilakopoulos, Karatza, Katsaounou, Kollintza, Zakynthinos and Roussos, 2003).TNF-α, IL-1, IL-8, and IL-15 are some cytokines that are commonly associated with inflammation and tissue injury. Their potential effects will be detailed in this chapter.

Tumor Necrosis Factor-α

Tumor Necrosis Factor-α (TNF) is one of the most widely studied inflammatory cytokines due to its relationship to chronic illness. Consistently elevated serum TNF has been associated with degenerative environments that may lead to complications such as heart failure (Kaur, Sharma, Dhingra and Singal, 2006) and rheumatoid arthritis (Voulgari, Kolios, Padopoulos, Katsaraki, Seferiadis and Drosos, 1999), in addition to the cachexia that coincides with chronic wasting diseases including several types of cancer and HIV/AIDS (Roubenoff, Grinspoon, Skolnik, Tchetgen, Abad, Spiegelman, Knox and Gorbach, 2002). Despite these negative linkages, recent research has provided evidence that TNF may also have several beneficial effects and be an essential part in the regenerative process of muscle tissue injury. TNF plays several roles in the signaling processes of the innate immune system. A study by Peterson et al. showed that the upregulation of this cytokine can result in the accumulation of neutrophils and macrophages to clear debris and facilitate apoptosis of cells damaged as a result of strenuous exercise, while also signaling the proliferation and differentiation of new cells to rebuild the damaged areas of the muscle and connective tissue in a murine model (Peterson, Feeback, Baas and Pizza, 2006). Mice were treated with 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of murine TNF-α for seven days via osmotic minipumps. After the treatment period, dissections of the extensor digitorum longus (EDL) and soleus muscles of treatment and control mice were analyzed. The treatment group showed seven- and five-fold elevations in neutrophil concentration and three- and two-fold elevations in macrophage concentration in the EDL and soleus muscles, respectively, compared to the control group. Despite the accumulation of these leukocytes, there were no signs of muscle injury, atrophy, or regeneration, indicating that the presence of other factors may be necessary in order to see changes in these areas. This agrees with a previous study

in a murine model conducted by Pizza et al. that indicated that macrophages may require significant signs of muscular injury to become present in large concentrations, while neutrophil concentrations may become slightly elevated through muscular contraction regardless of the presence of significant tissue damage (Pizza, Koh, McGregor and Brooks, 2002). While TNF is commonly associated with inflammation, a study by Smith et al. (Smith, Anwar, Fragen, Rananto, Johnson and Holbert, 2000) has shown that there was no significant increase in systemic TNF concentration after intense unaccustomed resistance training in young men. Six male subjects completed eccentric bench press and leg curl exercises at 100% of their predetermined 1 repetition-maximum. Blood samples were taken at several time points after the exercise session and analyzed for cytokine concentrations, including TNF-α. This study showed no change in TNF-α from baseline values after the exercise protocol. This trend has also been shown in exhaustive endurance exercise despite the presence of muscular injury in nine well-trained male Iron Man Triathlon athletes (Suzuki, Peake, Nosaka, Okutsu, Abbiss, Surriano, Bishop, Quod, Lee, Martin and Laursen, 2006). There was no increase in TNF-α concentrations after exercise despite decreases in muscular strength and increases in soreness, C reactive protein, and other cytokines typically marking an inflammatory response.

Interleukin-1

Interleukin-1 is highly responsible for inflammation and the body's innate immune response (O'Neill, 2008) to foreign invaders such as bacteria and in the occurrence of injury. IL-1 promotes the infiltration of inflammatory cells (neutrophils and macrophages) into the affected area by increasing the expression of cell adhesion molecules (CAM; Dinarello, 2009) and has been shown to promote angiogenesis in human liver cells, (Ma, Sawai, Matsuo, Ochi, Yasuda,

Takahashi, Wakasugi, Funahashi, Sato, Okada, Takeyama and Manabe, 2008), which may promote tissue remodeling after injury. Although studies involving muscle damaging eccentric exercise (through resistance training) have shown little or no increase in plasma IL-1 concentration, there is strong evidence of an increase in IL-1 receptor ($IL\text{-}1_r$; Chen, Hubal, Hoffman, Thompson and Clarkson, 2003). Three men in their early twenties underwent a series of several eccentric and eccentric/concentric coupled contractions, which induced muscular injury. Although there was no measured increase in IL-1 levels, it was found that within one hour post-exercise, $IL\text{-}1_R$ mRNA had increased in response to the eccentric exercise, indicating a possible upregulation of $IL\text{-}1_r$ and an increase in IL-1 sensitivity. The previously mentioned study by Smith et al. (Smith, Anwar et al., 2000) actually showed a significant decrease in IL-1 after intense eccentric-only strength training despite signs of muscle damage; however, it was hypothesized that this may be attributed to the speed in which IL-1 is cleared from the body, or that IL-1 acted only locally. Suzuki et al. (2006) investigated serum IL-1 concentrations after the completion of an iron-man triathlon. Despite signs of muscle damage, there were no significant increases in IL-1 systemically (P=0.143), while there was a significant increase in plasma Inerleukin-1 receptor antagonist ($IL\text{-}1_{ra)}$ within 30 minutes following the race, which remained non-significantly elevated the following day. However, this may not be representative of the current study, because although there was muscle injury, this was an ultra-endurance event rather than one of anaerobic nature. Another study by Suzuki et al. involving ten men who completed a marathon race found that IL-1 concentration in the urine increased 4.2-fold immediately post-race, while plasma levels remained unchanged (Suzuki, Nakaji, Yamada, Liu, Kurakake, Okamura, Kumae, Umeda and Sugawara, 2003). This agrees with the previous theory of Smith et al. that IL-1 may have a relatively short half life or may experience very rapid clearance from the

plasma. Significant increases in IL-1 have been measured via muscle biopsy both immediately and 6 hours following 30 minutes of near maximal eccentric cycling in young men (age 19-32) by Malm et al. (2000). These increases in IL-1 protein were negatively correlated to relative VO2 ($P=0.006$), signifying peak potential for IL-1 expression during times of less oxidative stress. This study also indicated a negative relationship between IL-1 and muscle CD56+ (satellite cell activation), indicating that a relatively short time of activation for IL-1 (in agreement with aforementioned studies) may be necessary to allow tissue regeneration to begin. The findings of these studies suggest that the level of IL-1 activation (and other cytokines) may be highly dependent not only on the extent of tissue injury, but also the circumstances under which such injury is endured (e.g., ultra endurance vs marathon vs anaerobic). A protocol involving longer duration, high intensity eccentric loading of large muscle groups (e.g., high intensity sprinting) may be warranted to better understand the circumstances in which upregulation of IL-1 and/or its related receptors may occur.

Interleukin-8

Interleukin-8 (IL-8) also plays a role in eliciting an inflammatory response. Frydelund-Larsen et al. have provided evidence that IL-8 may also be an angiogenic factor leading to adaptation and restructuring after injury (Frydelund-Larsen, Penkowa, Akerstrom, Zankari, Nielsen and Pedersen, 2007). The plasma concentrations of IL-8 have been shown to rise in response to intense exercise with an eccentric component, such as marathon running (Ostrowski, Rohde, Asp, Schjerling and Pedersen, 2001). Eight male subjects in their late 20s to early 30s completed a marathon race with blood samples being taken prior to, and every 30 minutes for four hours post-race. IL-8 levels where shown to peak at 30 min post exercise (6.7-fold increase),

at which point they began steadily declining towards baseline levels. In another study ten male subjects completed a marathon race with blood samples being collected pre- and immediately post-race (Suzuki, Nakaji et al., 2003). This study found a ten-fold increase in plasma IL-8 concentrations. Conversely Chan et al. found that non-damaging concentric exercise elicited a significant increase in IL-8 mRNA; however, there was no identified effect on the amount of translation taking place (Chan, Carey, Watt M and Febbraio, 2004). A study conducted by Nieman et al. (2006) has also shown that there was no significant change in IL-8 levels after two hours of intensive resistance exercise in trained male subjects, but there was a significant difference in intramuscular levels of IL-8 mRNA, also indicating that IL-8 may be produced locally even in non-damaging exercise and that the greater injury encountered by untrained subjects may in fact have elicited a systemic increase of IL-8.

Interleukin-15

Interleukin-15 (IL-15) is an anabolic factor for skeletal muscle that primarily acts on fully differentiated mytubes and myofibers (Furmanczyk and Quinn, 2003). When active in skeletal muscle tissue, IL-15 causes an increase in the accumulation of myosin heavy chain proteins, leading to muscle anabolism. Prolonged aerobic efforts, such as a three-hour run in teen marathon runners, have not been shown to increase levels of IL-15 protein systemically, or levels of IL-15 mRNA locally in the muscle tissue (Nieman, Davis, Henson, Walberg-Rankin, Shute, Dumke, Utter, Vinci, Carson, Brown, Lee, McAnulty and McAnulty, 2003). This study, however, only explored muscle biopsies immediately pre- and post- exercise. Similarly, Nielsen et al. (2007) showed no increase in plasma IL-15 concentration but did show in increase in IL-15 mRNA 24 hours after an intense resistance exercise. Muscle biopsies taken at 6, 24, and 48 hours

post exercise revealed a two-fold up-regulation of IL-15 mRNA at the 24 hour time point which had returned to pre-exercise levels by the 48 hour time point. There were no significant differences in IL-15 protein expression, however, at any point. In a contradicting study by Riechman et al., there were increases in plasma IL-15 protein immediately after intense resistance exercise. This study followed 153 subjects through 10 weeks of a full body resistance training program. Blood samples from immediately before and after the first and final workout session were analyzed for IL-15 content. It was found that plasma IL-15 content increased after both bouts of exercise (Riechman, Balasekaran, Roth and Ferrell, 2004). This also indicates that there is no IL-15 response to a prolonged training program. Collectively, these studies may indicate that intense full body, muscle damaging exercise may be required to elicit increased IL-15 protein expression.

Byproducts of Reactive Oxygen Species as a result of exercise induced muscle damage

Reactive oxygen species (ROS) are highly reactive molecules with unpaired valence shell electrons. Due to the volatility of ROS, their presence cannot be quantified directly, and must be measured by concentration of byproducts in the plasma. ROS are produced as a result of natural metabolism, and our body's natural antioxidant defenses typically prevent any damage that may be caused by these molecules. However, in the case of tissue injury such as muscle damaging eccentric exercise, ROS production may become greatly increased due to the influx of leukocytes, therefore creating the potential to overwhelm our antioxidant defenses and create further damage to surrounding tissue (Tidball, 2005). Despite these acute negative effects of ROS, it has recently been found that they may play an essential role in gene transcription and

protein synthesis. A study conducted by Steensberg et al. in young male athletes found that the inhibition of nitric oxide (NO) resulted in the attenuation of some cytokine mRNA after exercise, specifically interleukin-6 and interleukin-8, and that the infusion of NO lead to an upregulation of these cytokines (Steensberg, Keller et al., 2007). Another study by Vassilakopoulos et al. showed a decrease in plasma cytokine concentrations in young men after cycle exercise following supplementation of antioxidants (vitamins A, C, and E; 2003). These studies provide evidence that some cytokine regulation may utilize ROS dependent pathways. McGregor et al. found that short repeated bouts of maximal anaerobic concentric cycling exercise (30 second wingate protocols) failed to elicit a significant increase in the byproducts of ROS activity (MDA and 4-HNE) in 16 untrained subjects (10 male/6 female; 22±3.5 yr; In Review). Since exercise involving only concentric contractions failed to elicit a marked change in lipid peroxidation, if muscle-damaging exercise of a similar nature with a significant eccentric component (30 second sprinting bouts) elicits an alteration in ROS production, it may be deduced that this response was due to an influx of leukocytes. This could be manifested through secondary injury and respiratory burst to the damaged region, as exhibited by Frenette et al. (2000) and not as a result of increased oxidative activity in the mitochondria.

Accelerometry

Typically measurements of workload such as VO_2, power output, or force production have been determined in a laboratory setting using metabolic carts, power meters, or force plates. These methods are not practical in analyzing "real-life" scenarios, however, and there have been many efforts made throughout the last several years to identify a consistent way to quantify the work done by athletes in the field. Low resolution accelerometers have been heavily studied as

activity monitors (Chen and Bassett, 2005) to estimate energy expenditure during activities of daily living. High Resolution Accelerometry (HRA) allows for much greater resolution (e.g. real-time streaming/data logging) as opposed to "cut points" used by low resolution activity monitors, providing much more accurate estimations of energy expenditure, and the advent of microelctromechanical systems (MEMS) provides sufficient storage capacity to enable users to spend larger amounts of time completing activities away from a lab or base station. A recent study by McGregor et al. used MEMS HRA to estimate energy expenditure of NCAA division I distance runners and untrained college students as well as to compare running mechanics between the two groups over a variety of walking and running speeds (McGregor, Busa, Yaggie and Bollt, 2009). This study found HRA to be an accurate and reliable predictor of VO2 at several exercise intensities and found significant differences between trained and untrained acceleration parameters. Another study examined the acceleration parameters of NCAA division I sprint althetes (McGregor and Muth, Preliminary Data) and found that the accelerations encountered by trained sprint athletes were markedly higher than their endurance trained counterparts. This indicates that there is much greater eccentric muscle loading during an all-out sprint activity than in endurance running, and therefore a greater potential for tissue damage to occur. The use of HRA may prove invaluable in estimating the extent of eccentric loading during locomotion and, with blood analysis, may provide insight into the extent and mechanism of tissue injury.

High intensity anaerobic exercise and inflammation

It is understood that intense muscle injuring exercise elicits both a cytokine and ROS response, but the specific mechanisms that cause inflammation are not currently well understood.

Resistance training and running have eccentric components, therefore providing a strong possibility of muscular injury and inflammation. Therefore, sprint exercise through greater eccentric loading than endurance running, and greater exercise volume than resistance training exercise, may provide a greater stimulus for greater tissue injury and inflammation. The timing of the introduction of ROS and cytokines to systemic circulation in this study may provide some insight into the role of these groups as signaling and repair models.

CHAPTER III

Research Design and Methodology

Study Design

Five untrained (Group 1; 2 male/3 female; Table 1) and five sprint-trained runners (Group 2; 4 male, 1 female; Table 2) participated in this study. Subjects for this experiment were recruited by word-of-mouth from the Eastern Michigan University Track and Field team and the Eastern Michigan University student body. The College of Health and Human Services Human Subjects Review Board approved this study prior to implementation. Health history and written informed consent were obtained from all subjects prior to exercise testing. All subjects met the inclusion and exclusion criteria set for trained (pole vaulters and long jumpers regularly participating in Eastern Michigan University Track and Field practice for at least three months) and untrained (less than three days of exercise per week and no participation in a formal training regimen) individuals.

Subjects participated in a total of five study visits: an initial $VO2_{max}$ test, a maximal sprint exercise test (MSE; including three blood draws), and three subsequent visits where blood samples were recovered (for a total of six draws). The initial $VO2_{max}$ protocol was separated from the MSE protocol by one week, and blood draws were taken immediately pre- and post-MSE, as well as 24, 48, and 72 hours post MSE.

Table 1

Subject Characteristics

Parameter	Value	Parameter	Value
Age (yrs)		**$VO2_{max}$ (L/min)**	
Group 1	19.4	**Group 1**	3.07
	0.89		8.34
Group 2	26.2	**Group 2**	3.77
	5.93		0.73
Mass (kg)		**$VO2_{max}$ (ml/kg/min)**	
Group 1	72.27	**Group 1**	38.46
	9.01		0.96
Group 2	85.45	**Group 2**	51.73
	17.72		8.63
Height (cm)			
Group 1	170.18		
	13.56		
Group 2	168.66		
	6.12		

Mean values represented in first row with standard deviation below.

$VO2_{max}$ protocols for both groups were completed on a treadmill (True ZX-9, St. Louis, MO) until volitional exhaustion was reached. Metabolic gasses where collected continuously using portable open circuit spirometry (Jeager Oxycon Mobile, CA) and averaged over 5-second intervals for the determination of oxygen consumption (VO2), carbon dioxide expiration (VCO2), and respiratory exchange ratio (RER). The metabolic cart was calibrated to ambient conditions, known volume flow, and known gasses prior to each test. Subjects were also fitted with a MEMS (microelectromechanical system) high-resolution triaxial accelerometer (HRA; model ADXL210, G-link Wireless Accelerometer Node ±10g Microstrain, Inc, Williston, VT) which determined acceleration in three axes at 617 Hz continuous sampling rate. The accelerometer was attached at the subjects estimated center of mass on the posterior side of the body in line with the top of the illiac crest and secured with a semi-rigid strap and athletic tape to ensure no measurement of extraneous movement.

MSE tests were conducted on a 200m track located in Bowen Fieldhouse at Eastern Michigan University. Subjects were again fitted with a MEMS high-resolution triaxial accelerometer at their estimated center of mass as previously described. The HRA was set to datalog accelerations in three axes at 128Hz for the duration of the MSE test. An individual trained in phlebotomy collected Blood draws from an antecubital vein in 10ml samples. Serum was then extracted and stored at –80°c until analysis.

Methods

Pre-Visit Requirements

To avoid confounding effects of physical activity or nutrition on this study (e.g. adaptations to exercise, changes in metabolic status, etc.), subjects were asked to refrain from any unaccustomed high intensity or duration exercise 24 hours prior to their testing dates and to avoid physical activity for the 72 hours following MSE testing. Subjects were also asked to fast for two hours prior to each exercise session as well as refrain from alcohol or caffeine use 24 hours before testing. All subjects completed health history forms in an attempt to eliminate any effect of pre-existing illness or medication on the results of the study.

Visit 1

Upon their first visit, all subjects provided health history information and written informed consent to participate in the study. Subjects were also briefed with study protocol, testing procedures, and possible risks and benefits associated with participation in the study. Participant height and weight were recorded before being fitted with any testing equipment. A backpack containing a portable open circuit spirometry system was attached comfortably to the subject and connected to a mask designed to collect expired gasses. A MEMS HRA was also affixed to the subjects' estimated center of mass and secured with an elastic strap and athletic tape to minimize the measurement of extraneous movement. Both groups began the test with a one-minute standing stage while baseline metabolic and accelerometry data were collected. Following determination of baseline values, subjects were instructed to begin walking on the treadmill at the initial pace of the test (4km/hr for Group 1; 6km/hr for Group 2). The treadmill was set to a constant 1% grade throughout the test to best simulate flat ground running. Pace was increased every three minutes by 2km/hr until the subjects reached volitional exhaustion and

ended the test. At this point all equipment was removed from the subjects and they were allowed to cool down in a manner of their choosing.

Visit 2

One week following Visit 1, subjects returned to the lab for Visit 2. Upon arrival, the first blood draw was taken and the subjects proceeded to the track where they were allowed to warm up by completing approximately five minutes of low intensity walking. No hard sprints were allowed during this time. After the warm-up subjects, were again fitted with a MEMS HRA as described above and given final instructions of study protocol. At the signal of the investigator, subjects performed the first of three maximal 30-second sprints from an upright starting position (timed with a stop-watch). Verbal encouragement was provided throughout the exertion, and upon completion, subjects completed a four-minute passive rest period in the nearest area provided (no more than 50m apart). It was required that subjects remained seated during the entirety of each rest period, with no stretching, walking, or running between bouts. During the last 10-15 seconds of the rest period, subjects returned to the track in preparation for the next bout. This protocol continued for a total of three 30-second maximal sprints and two four-minute rest periods. After completion of the final sprint bout, a second blood draw was taken and the subjects were allowed to cool down in a manner of their choosing. All subjects were required to stay in the laboratory for one hour after the second blood draw for a third blood draw to be taken (1hr post MSE).

Visits 3, 4, and 5

All subjects were required to arrive at the exercise physiology lab at the same time for the next three successive days (24, 48, and 72 hours post MSE) for additional blood draws. The draws followed the same protocol as described for Visit 2.

Upon collection, blood samples were allowed to coagulate for 15 minutes under refrigeration and were then separated into red cells and serum via at least 15 minutes of centrifugation. Serum was aliquoted into 800μl samples and frozen at -80°C until analysis.

Statistical Analysis

An ANOVA was completed to analyze metabolic data for VO2, VCO2, and RER. These values were taken over five second averages throughout the duration of the V_{O2max} protocol and compared between groups (trained vs. untrained) at each running speed as an evaluation of overall aerobic fitness and running economy.

Accelerometer data from MSE were analyzed and gait patterns between trained and untrained runners were assessed. Vectors were calculated from individual axes, and the resultant Euclidean scalar will be calculated using Perl Scripting software. The equation for the resultant scalar is as follows:

$$R_{xyz}^2 = A_x^2 + B_y^2 + C_z^2$$

Where x, y, and z are equivalent to the magnitude of acceleration in the vertical, lateral, and anterior/posterior axes respectively. Root mean square (RMS) of acceleration will be calculated for each second in all three axes and their resultant scalar for each bout of MSE and for a three second deceleration period following each bout. RMS values will be compared both between and within groups for each MSE trial and its associated deceleration phase, using an MANOVA to

assess the degree of eccentric loading experienced by subjects in relation to training status and fatigue.

Blood samples were analyzed for blood cytokine concentration (IL-1, IL-8 ; Thermo Scientific, IL), oxidized substrates (malonyl dialdehyde (MDA), hydroxynonenal (4-HNE; Cell Biolabs, MA) using the Enzyme-linked immunosorbent assays (ELISA). Total Creatine Kinase was measured using a Vitros 250 auto analyzer (Johnson and Johnson, U.S.A.) as a marker of muscle cell damage, and C-reactive protein was measured qualitatively using latex agglutination (Remel, KS) to determine the presence of an inflammatory response. ANOVA was performed for each identified blood marker to compare responses at each time point both within and between groups resulting from MSE and prior training status. After initial analysis was completed, an ANCOVA was performed to normalize all data to each subject's baseline measurement of each marker in order to reduce the amount of statistical variance introduced. A simple contrast of each group by time was then completed to uncover significant differences between time points in each group. TNF-α and IL-15 concentrations were not analyzed due to resource limitations.

SPSS version 16.0 (SPSS Inc., IL; α=0.05) was used for all statistical analysis in the present study.

CHAPTER IV

Results

This study investigated the following null hypothesis:

1. *There will be no significant difference in systemic ROS concentration after an acute bout of anaerobic sprinting.*
2. *There will be no significant difference in systemic cytokine concentrations after an acute bout of anaerobic sprinting.*
3. *There will be no significant difference in systemic CRP or CK concentrations after an acute bout of anaerobic sprinting.*
4. *There will be no significant difference in systemic ROS, cytokine, CRP, or CK concentrations between trained and untrained subjects.*
5. *There will be no significant difference in systemic ROS, cytokine, CRP, or CK concentrations between concentric exercise and exercise with an eccentric component of a similar intensity and duration.*

For purposes of cohesion, the results of this study will be reported and discussed in a slightly different context. The results for each blood marker will be reported as separate groups, and then all aspects will be discussed as a whole.

$VO2_{max}$ protocol

Metabolic data collected during the $VO2_{max}$ protocol were analyzed for significant differences over five-second averages (Table 2). There was no significant difference in overall aerobic fitness between groups measured by absolute VO2 (p=.163), but the trained group (group 2) recorded significantly higher relative $VO2_{max}$ values (p=.039; Tables 1 and 2), and VO2 values at each given stage (p<.001; Figure 1). There was no significant difference in RER or VCO2 as a result of prior training status (P=.233; P=.281). There was, however, a significant speed by training interaction in all measured variables (P<.001).

Table 2

Speed by training MANOVA output for metabolic data

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.
Speed	V'O2 ml/min	2.575E8	4	6.438E7	177.784	*.000
	VO2/kg/ml/min	50547.223	4	12636.806	315.486	*.000
	RER	1.181	4	.295	66.554	*.000
	V'CO2	3.076E8	4	7.689E7	257.166	*.000
Training	V'O2 ml/min	706081.596	1	706081.596	1.950	.163
	VO2/kg/ml/min	1671.967	1	1671.967	41.742	*.000
	RER	.006	1	.006	1.427	.233
	V'CO2	348062.673	1	348062.673	1.164	.281
Speed * training	V'O2 ml/min	6863487.376	4	1715871.844	4.738	*.001
	VO2/kg/ml/min	1586.166	4	396.541	9.900	*.000
	RER	.140	4	.035	7.866	*.000
	V'CO2	6569542.818	4	1642385.705	5.493	*.000

*= $P \leq 0.05$

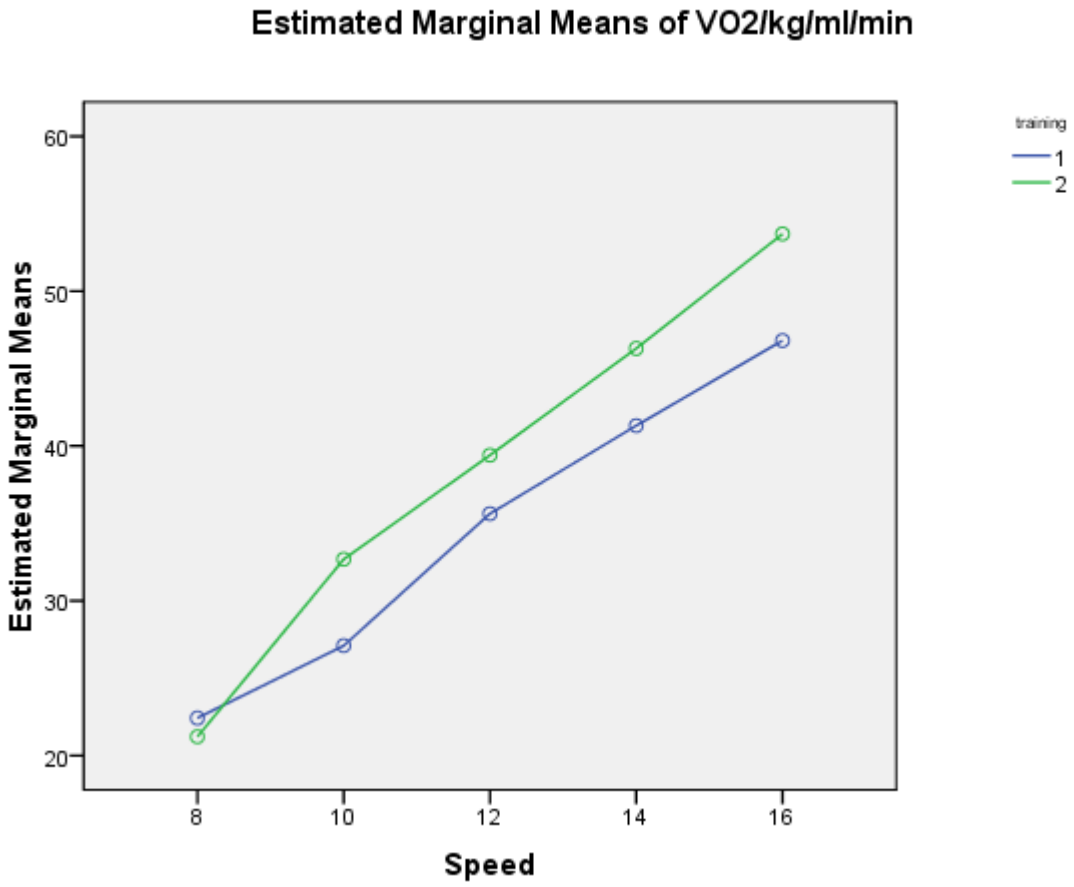


Figure 1. Relative oxygen consumption from 8-16 km/hr.

Accelerometry

Accelerations (Figure 2) in the vertical axis remained constant within groups throughout all three MSE efforts (efforts 1, 2, and 3; $p=.979$) while all other axes experienced significant reductions in magnitude ($p<.001$; Table 3). Group 2 experienced significantly greater acceleration in all axis during all three bouts of MSE (VERT: $p=.003$; ML, AP, RES: $p<.001$). Although Group 2 experienced slightly larger accelerations during the MSE bouts, there was no effort by training effect within the data set indicating that the reductions in accelerations experienced by both groups in later trials occurred in a similar fashion.

Both groups experienced a drop in overall magnitude of acceleration (Figure 3) from the first deceleration period (effort 4) to the latter two (efforts 5 and 6) in all axis ($p<.001$; Table 4). Post Hoc testing revealed that this was due to a large decline from effort 4 to effort 5, while the differences between efforts 5 and 6 were non-significant in all axes. Unlike the MSE efforts, there was no significant difference between groups during the deceleration periods in any axis, nor was there a significant effort by training interaction.

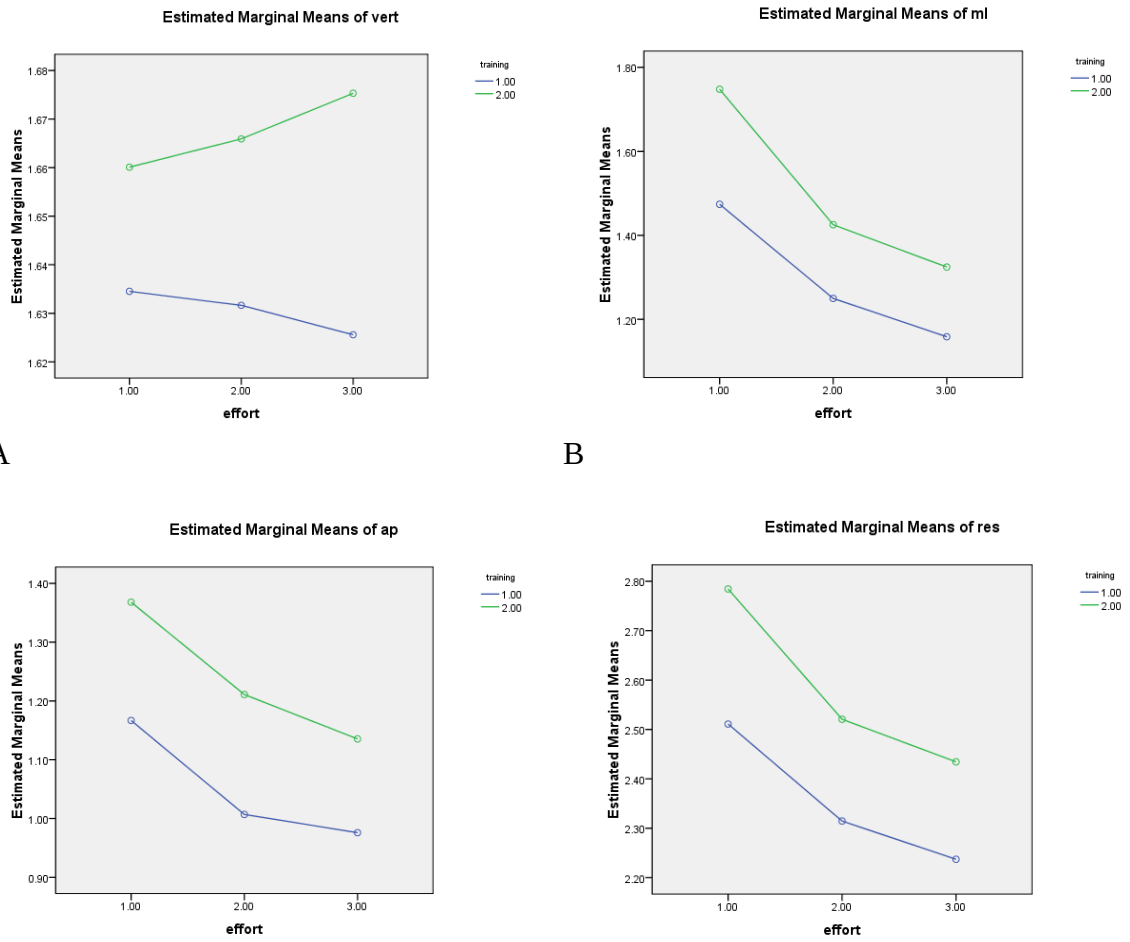


Figure 2. Accelerations during MSE efforts 1, 2, and 3; Marginal means of RMS of accelerometry by training status during the effort phase of MSE; A: Vertical, B: Medial-Lateral, C: Anterior-Posterior, D: Resultant Scalar

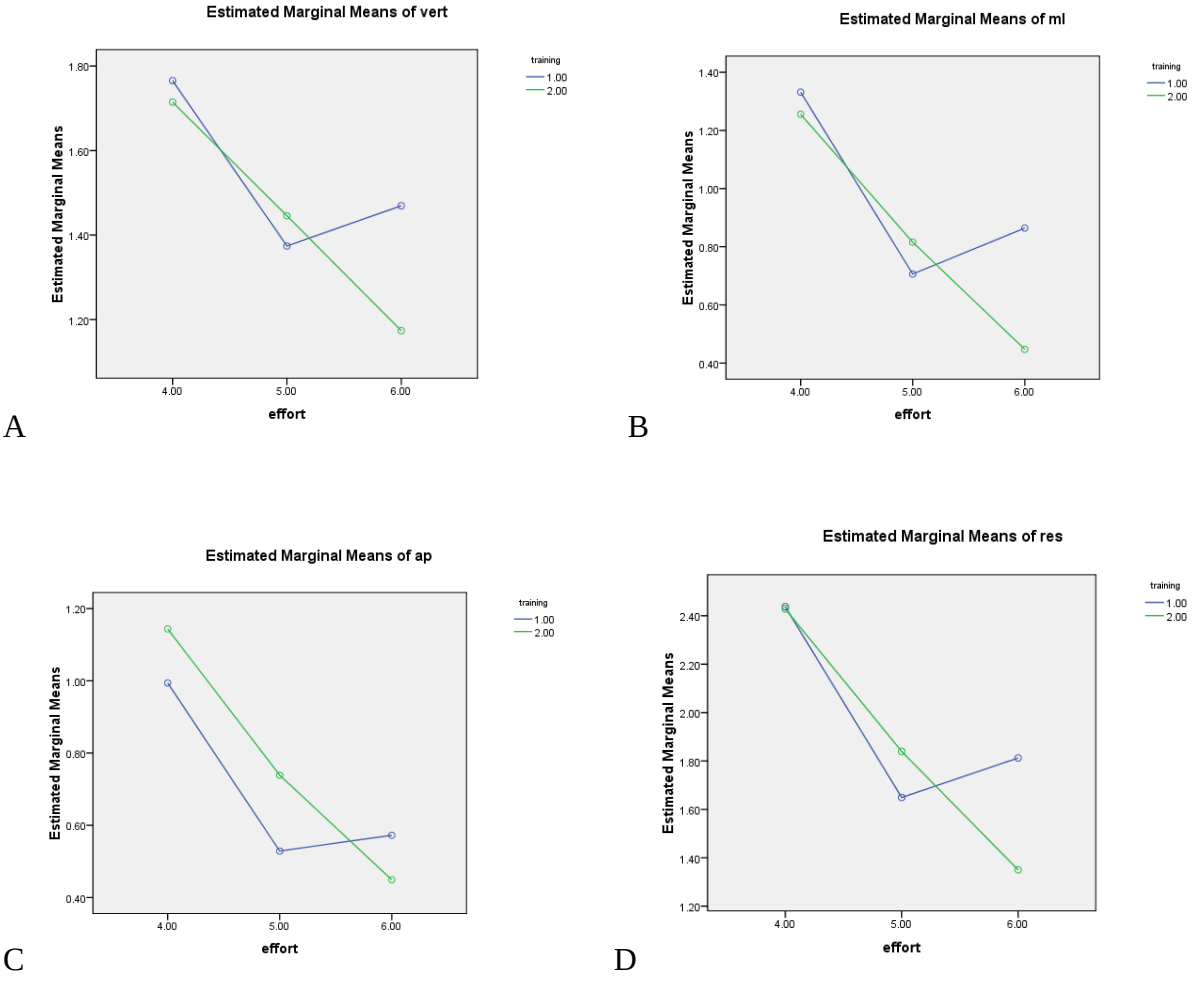


Figure 3. Accelerations during MSE efforts 4, 5, and 6; Marginal means of RMS of accelerometry by training status during the deceleration phase of MSE; A: Vertical, B: Medial-Lateral, C: Anterior-Posterior, D: Resultant Scalar

Table 3

Effort by Training MANOVA output for MSE efforts 1, 2, and 3

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
effort	vert	.001	2	.001	.021	.979
	ml	15.122	2	7.561	61.054	*.000
	ap	4.993	2	2.497	51.909	*.000
	res	10.763	2	5.381	48.778	*.000
training	vert	.206	1	.206	8.674	*.003
	ml	6.497	1	6.497	52.466	*.000
	ap	5.470	1	5.470	113.737	*.000
	res	7.853	1	7.853	71.181	*.000
effort * training	vert	.015	2	.008	.324	.723
	ml	.367	2	.184	1.482	.228
	ap	.064	2	.032	.667	.513
	res	.179	2	.089	.809	.446

*= $P \leq 0.05$

Table 4

Effort by Training MANOVA output for MSE efforts 4, 5, and 6

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Effort	vert	2.002	2	1.001	11.595	*.000
	ml	4.812	2	2.406	20.367	*.000
	ap	3.538	2	1.769	18.342	*.000
	res	8.401	2	4.201	18.028	*.000
Training	vert	.130	1	.130	1.501	.226
	ml	.252	1	.252	2.136	.149
	ap	.095	1	.095	.990	.324
	res	.135	1	.135	.581	.449
effort * training	vert	.360	2	.180	2.084	.134
	ml	.734	2	.367	3.107	.052
	ap	.325	2	.162	1.683	.195
	res	1.151	2	.575	2.469	.094

*= $P \leq 0.05$

Creatine Kinase and C-Reactive Protein

Initial analysis revealed no evidence of tissue damage marked by increased blood concentrations of CK (Table 5) in either group at any of the time points following MSE (p=.923). There was also no evidence of a systemic inflammatory response, as the only subjects to test positive for the presence of CRP (Table 6) also did so pre MSE. There was, however, a discernible difference between groups overall, showing elevated serum levels of CK in the trained population (p=.031). Due to the large amount of variance expressed in baseline levels of CK between groups, an ANCOVA was utilized to normalize the data between groups (Table 7) with each subject's baseline CK value used as the covariate. ANCOVA showed that a training effect still existed between groups; however. in this case the untrained population experienced greater alteration in CK levels as a result of MSE (p=.004; Figure 4). ANCOVA also uncovered a significant time (p=.001) and time by training (p=.007) effect following MSE. The trained population peaked immediately post exercise (p=.006) and returned to near-baseline values within one hour post-exercise. The untrained population had a trend for increased CK immediately post-exercise (p=.144), which also began to decline within one hour post-exercise. The untrained group also experienced a significant secondary increase in CK, peaking 24 hours post-MSE (p<.001) and remaining elevated at 48 hours post (p=.037). CK remained non-significantly elevated in this group throughout the remainder of the trial period (72 hrs; p=.129).

There was a significant difference in blood lactate concentrations between groups (p=.034) as well as a significant training by time interaction (p=.013). This group difference was only noted immediately following MSE, with the trained group peaking at 14.6mmol/L and the

untrained group peaking at 12.1mmol/L (Figure 5). Within one hour of MSE, blood lactate levels returned to near resting conditions in both groups.

Table 5

CK Time by Training ANOVA outputs

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	CK	74311.130	5	14862.226	.277	.923
training	CK	264386.274	1	264386.274	4.931	*.031
Time * training	CK	50928.643	5	10185.729	.190	.965

*= $P \leq 0.05$

Table 6

Qualitative CRP Testing

subject number	POS test	NEG test	subject number	POS test	NEG test
1	x		6		x
1	x		6		x
1		x	6		x
1		x	6		x
1		x	6		x
1	x		6		x
2	x		7		x
2	x		7		x
2	x		7		x
2	x		7		x
2	x		7		x
2	x		7		x
3		x	8		x
3		x	8		x
3		x	8		x
3		x	8		x
3		x	8		x
3		x	9		x
4		x	9		x
4	x		9		x
4		x	9		x
4	x		9		x
4		x	9		x
4		x	10		x
5		x	10		x
5		x	10		x
5		x	10		x
5		x	10		x
5		x			
5		x			

Qualitative analysis for presence of CRP; Left column: Group 1, Right column: Group 2

Table 7

CK Time by Training ANCOVA outputs

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	CK	87470.660	5	17494.132	5.309	*.001
Training	CK	29594.403	1	29594.403	8.981	*.004
Time * training	CK	60378.129	5	12075.626	3.664	*.007

*= $P \leq 0.05$

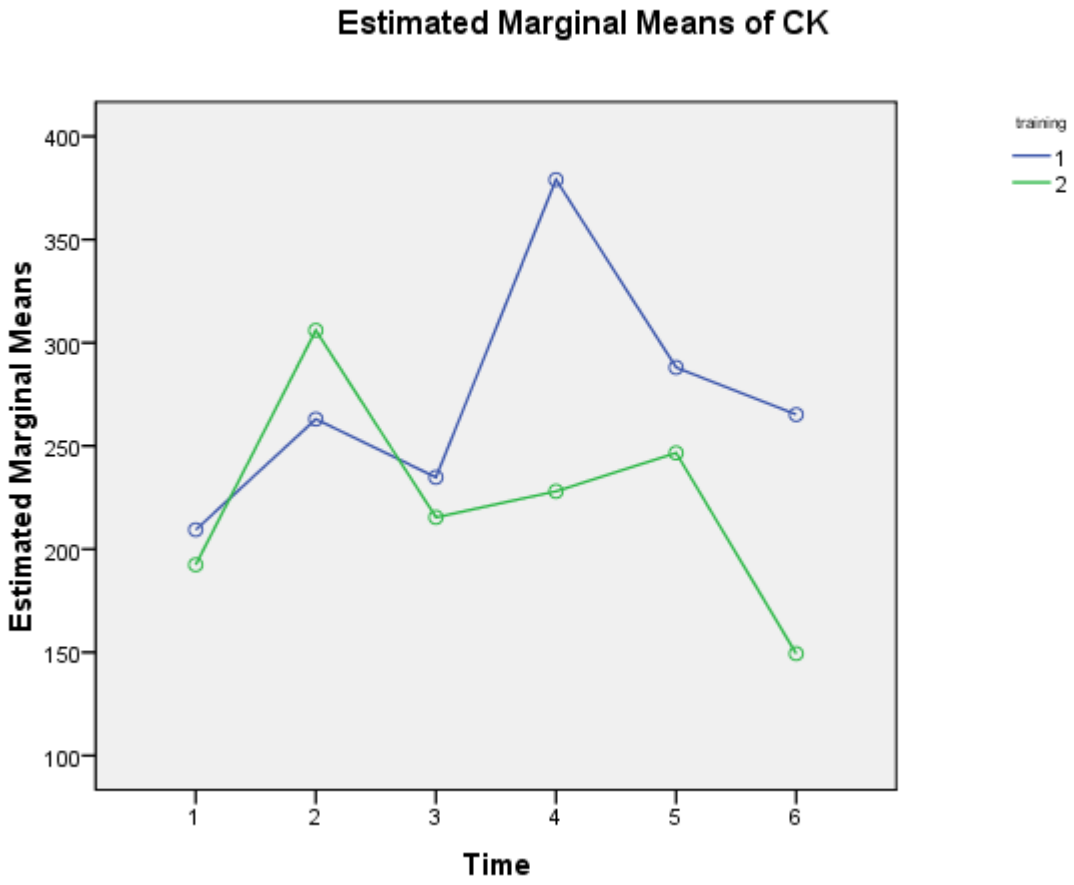


Figure 4. Group CK Concentration by time

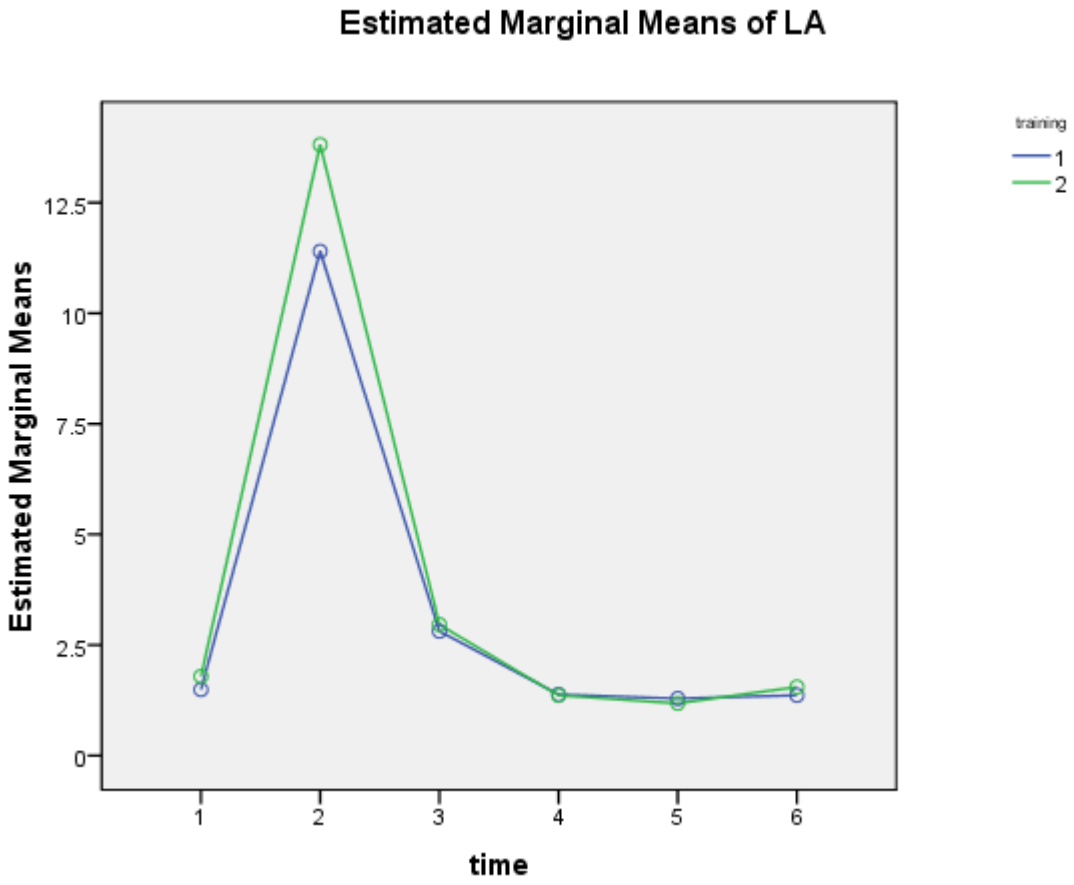


Figure 5. Group Lactate Concentration by time

Cytokines

Like the CK values, there was no significant difference in concentration of IL-1 or IL-8 (Table 8) at any point following MSE (p=.999 and p=.642 respectively) found with initial ANOVA testing. The untrained subjects did show elevated levels of IL-8 overall (p=.045), but there was no difference between groups for IL-1, nor was there any time by treatment interaction for either cytokine. Again, ANCOVA was utilized to normalize data with subjects resting cytokine concentrations. Although this analysis did not expose significant changes in cytokine quantity, several possible trends were uncovered (Table 9). There appears to be trend for decreased IL-1 and increased IL-8 expression (p=.321;p=.155 respectively) over time as a result of MSE. Group 2 nearly expressed a significant reduction of IL-1 24 hours post MSE (p=.054), which began to gradually move towards baseline from 24-72 hours post MSE (Figure 6). As shown in Figure 6, both groups expressed non-significant increased of IL-8 immediately post-exercise (Group 1: p=.185, Group 2: p=.121), which began to decline within 1 hour of the exercise bout; however, the trained subjects IL-8 concentration remained higher longer, remaining near significance at 1 hour post-exercise (p=.204).

IL-8 increases arose in both groups immediately post-exercise and returned to near baseline within one hour following activity. The ANCVOA also pointed to a potential training effect for IL-1 (p=.151) with the untrained subjects experiencing a spike in concentration at the 72-hour post-time point.

Table 8

Cytokine Time by Training ANOVA outputs

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	2.385	5	.477	.037	.999
Training	1.444	1	1.444	.111	.740
Time * training	.601	5	.120	.009	1.000

A

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	24.601	5	4.920	.678	.642
Training	30.944	1	30.944	4.265	*.045
Time * training	2.075	5	.415	.057	.998

B

Time by Training ANOVA output: A) IL-1, B) IL-8

*= $P \leq 0.05$

Table 9

Cytokine Time by Training ANCOVA outputs

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	1.610	5	.322	1.207	.321
Training	.569	1	.569	2.135	.151
Time * training	.169	5	.034	.127	.986
Corrected Total	600.351	57			

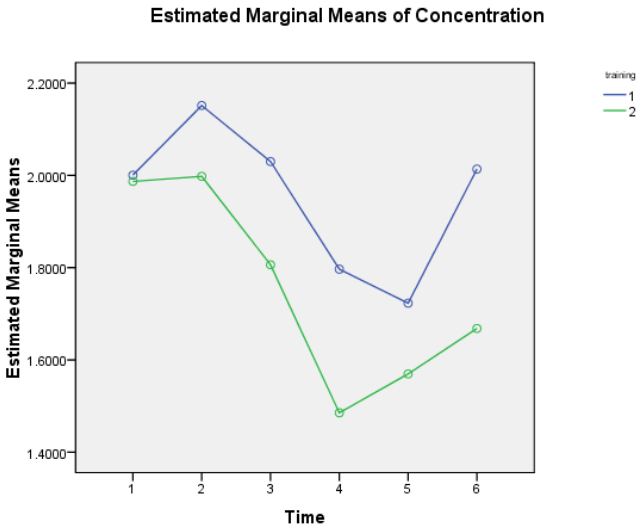
A

Tests of Between-Subjects Effects

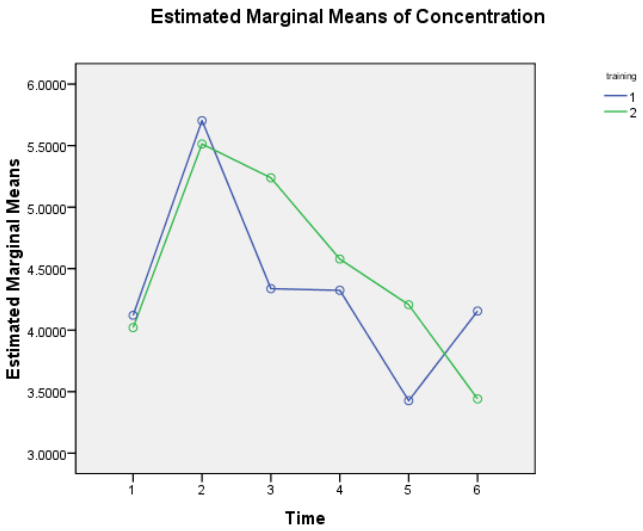
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	23.194	5	4.639	1.698	.155
Training	.290	1	.290	.106	.746
Time * training	4.229	5	.846	.310	.905

B

Time by Training ANCOVA output: A) IL-1, B) IL-8
*= $P \leq 0.05$



A



B

Figure 6. Group Cytokine Concentration by Time*;* Normalized group cytokine concentration at pre, post, 1hr, 24hrs, 48hrs, and 72 hrs post MSE: A) IL-1, B) IL-8

Markers of ROS

The ANOVA of ROS byproducts (Table 10) also revealed no significant changes as a result of MSE (HNE p=.610; MDA p=.161). Group 2 expressed significantly higher levels of MDA overall (p<.001) than Group 1, while Group 1 expressed significantly higher levels of HNE (p<.001) than Group 2. There was a trend for increased MDA concentration between the immediate post and one hour post-MSE time points, but this did not reach statistical significance (p=.146). There was no time by training interaction in either of these markers. A secondary analysis utilizing an ANCOVA showed a stronger trend for a time interaction in both MDA and HNE with MDA nearly reaching statistical significance (p=.059; Table 11). Both parameters saw concentrations dropping below baseline conditions immediately post-MSE, primarily for MDA, (Group 1: p=.051; Group 2: p=.218 and recovering to normality within one hour (Figure 7). Group 1 also expressed a trend for increased HNE levels at 48 and 72 hours post-MSE, reaching its highest point at the 72-hour time point (p=.171)

Table 10

ROS byproducts Time by Training ANOVA outputs

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	.991	5	.198	.723	.610
training	4.307	1	4.307	15.705	*.000
Time * training	.879	5	.176	.641	.669

A

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	38.703	5	7.741	1.669	.161
training	75.440	1	75.440	16.266	*.000
Time * training	15.201	5	3.040	.655	.659

B

Time by Training ANOVA output: A) HNE, B) MDA

*= $P \leq 0.05$

Table 11

ROS byproducts Time by Training ANCOVA outputs

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	.856	5	.171	.977	.442
training	1.620	1	1.620	9.242	*.004
Time * training	1.088	5	.218	1.242	.306

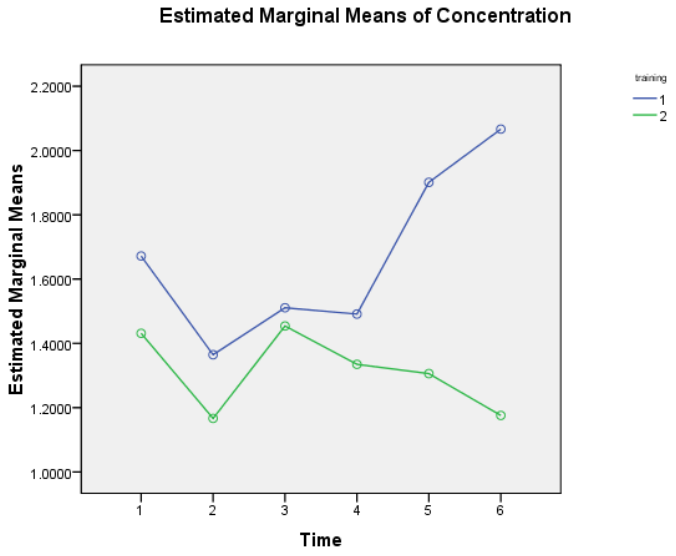
A

Tests of Between-Subjects Effects

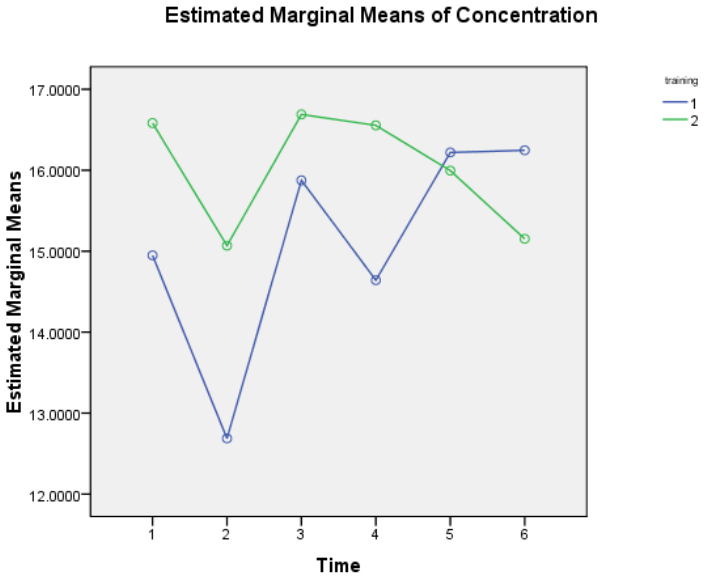
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	37.088	5	7.418	2.318	.059
training	8.288	1	8.288	2.590	.115
Time * training	19.802	5	3.960	1.237	.308

B

Time by Training ANCOVA output: A) HNE, B) MDA
*= $P \leq 0.05$



A



B

*Figure 7.*Group ROS Byproduct Concentration by Time; Normalized group ROS Byproduct concentration at pre, post, 1 hr, 24 hrs, 48 hrs, and 72 hrs post MSE: A) IL-1, B) IL-8

CHAPTER V

Discussion

Summary

This study was designed to assess the effect of acute, high-intensity, anaerobic exercise on blood serum levels of the cytokines IL-1 and IL-8, the byproducts of ROS activity MDA and 4-HNE, and markers of muscle tissue disruption and inflammation (CK and CRP). It was also the aim of this study to investigate the different responses between populations accustomed to such activity and those that are not, in addition to establishing a timeline in which these responses occur.

Metabolic analysis

As expected, the trained population had a higher relative $VO2_{max}$ related to the untrained population. However, it was unexpected that the overall fitness level as shown by absolute $VO2_{max}$ was not different between groups and that absolute oxygen consumption, RER, and V_{CO2} at each stage of the test was not different between groups. This can most likely be explained by the nature of training completed by Group 2 (short intermittent anaerobic bursts). This lack of disparity between groups supports the design of the study, indicating that any differences in response to MSE resulted from musculo-skeletal or immunological adaptations to training rather than adaptations to aerobic metabolic systems between groups.

Accelerometry

HRA data from each effort revealed that there was a significant difference in magnitude of acceleration between groups, indicating that the trained population may have experienced a slightly larger stimulus for muscle tissue damage during each bout. This theme was not maintained during the deceleration periods, as magnitude was comparable between Group 1 and Group 2. These findings somewhat contradict previous research by McGregor et al. (In Review) in that, despite slightly higher accelerations in the trained group, accelerations were not analogous in magnitude to those witnessed in trained sprinters. In this study, the accelerations during the deceleration periods where notably smaller than those reported in the preliminary data collected by McGregor and Muth (McGregor and Muth, Preliminary Data). It should also be noted that accelerations in the vertical axis remained constant throughout each effort in both groups signifying a constant stimulus for muscle tissue damage between bouts of MSE. Again, contradicting previous research by McGregor et al., accelerations in the vertical axis during the deceleration phase were not significantly higher than those experienced during the exertion portion of each effort, and in the last two efforts VERT accelerations were actually lower than those elicited during the effort itself. Since the deceleration phase was originally hypothesized to be the source of most intense eccentric loading, and there was no difference between groups, one can make the inference that the amount of loading (and potential for muscle damage) may be more comparable between groups than initially understood. Although the magnitude of accelerations in the trained group were not as great as reported in the previously mentioned data, as expected, they did experience significantly higher accelerations than the untrained individuals. The reduction in overall acceleration in Group 2 may be explained by the type of training these athletes were accustomed to. The McGregor study examined specifically trained sprinters, while

this study assessed athletes trained for participation in field events (pole vault and long jump). Although both of these events involve short intermittent sprint exercise, they rarely partake in bouts with durations longer that a few seconds. There may be gait adaptations associated with longer duration sprint training that may not be present in the subjects in this study.

Both groups experienced substantial eccentric loading and increased blood lactate as a result of MSE. It can be concluded that the intensity of this exercise was high enough to potentially elicit muscle injury and a subsequent immunological response.

Markers of Injury and Inflammation

Initial investigation for the presence of markers of tissue damage (CK) and inflammation (CRP) revealed that, while there was no difference in CRP by time or group, Group 2 expressed higher levels of CK throughout the study period. It is supported that athletes have elevated CK levels compared to non-athletes. The CK differences in this study agree with Mougios (2007), whose study reported athletes having an upper reference limit twice that of non-athletes (728U/L vs. 1479U/L in men; 345U/L vs. 836U/L in women). Due to the large variance between populations, ANCOVA was utilized to normalize each subject according to his/her baseline CK value. The secondary analysis maintained the presence of a training effect between groups with the addition of a time ($p=.001$) effect and a time by training effect ($p=.007$). This response was expected and supported in the literature. Newton et al. reported significant differences in CK between resistance-trained and non-trained men after eccentric arm exercise, indicating a resistance to tissue damage in the trained population and greater susceptibility to secondary tissue damage in the untrained group (2008). However, Newton's subjects exhibited peak CK responses after five days following eccentric loading. In the current study, peak CK

responses occurred immediately post-exercise in Group 2 and 24 hours post-exercise in Group 1. It is likely that the disparity between studies can be explained by the previous training status of the subjects. Newton's study defined a trained subject to participate in resistance training 3 times per week, while the current study assumed the trained population to partake in regular activity similar to the testing protocol (including intensity) on a daily basis. Also, this study utilized an untrained population that was not sedentary, so it is plausible that they may have possessed some adaptations preventing extensive tissue injury.

Reactive Oxygen Species

ANOVA and ANCOVA of ROS byproducts held no time interaction for 4-HNE or MDA. There was a significant group effect for both, with Group 2 exhibiting higher levels of MDA and Group 1 showing higher levels of HNE. These data argue that found by Bloomer and Fisher-Wellman, which exhibited that trained men and women have higher baseline MDA than untrained men and women (Bloomer and Fisher-Wellman, 2008). There were no significant differences within groups by time, indicating no change in overall oxidative stress as a result of MSE. This finding is consistent with the work of Muth et al. (2010) in which there was also no change in HNE or MDA following a similar protocol involving Wingate exercise as opposed to sprint exercise. There were, however, trends for decreased MDA and HNE concentration immediately post MSE. This was unexpected because maximal intensity exercise is generally believed to cause increased ROS production within the mitochondria. Lovelin et al. (Lovlin, Cottle, Pyke, Kavanagh and Belcastro, 1987) found that while intermittent exercise below $VO2_{max}$ (<70% VO_{2max}) reduced MDA production, exercise nearing maximal exertion resulted in increased MDA production. However, it is unlikely that exercise intensity was below 70% of

$VO2_{max}$ in the current study, as blood lactate values where significantly higher following MSE than at baseline, and accelerometry profiles indicated little to no change in overall exercise intensity from the first bout to the last. Groussard et al. (2003) have also found a systemic decrease in MDA within 20 minutes of the Wingate cycling test. This decrease was associated with a decrease in Glutathione and Superoxide Dismutase, which may indicate that these antioxidants were being consumed to scavenge radicals, resulting in an overall decline in levels of all three in the serum. This study also revealed a trend for increased oxidative stress in untrained subjects in the days following MSE as measured by HNE. At 72 hours post exercise HNE was nearing statistical significance (p=.171) in the untrained group. Since this was not present in the trained population, this finding argues for the presence of a secondary insult to the tissue damaged by the initial MSE bout, most likely caused by an influx of leukocytes into the area. It is likely that the trained subjects possessed adaptations preventing significant muscular damage (and an associated immune response; Tidball, 2005) resulting from mechanical strain during exercise. It is also likely that this increase was in response to muscular injury, as this response was not present in untrained subjects completing concentric only Wingate bouts of similar duration and intensity (Muth, Ratz et al., 2010). This secondary increase in HNE coincides with the secondary increase of CK in Group 1, again supporting the notion of leukocyte invasion resulting in subsequent oxidative damage (and cell death) in the surrounding tissue.

Cytokines

ANOVA of IL-1 and IL-8 offered no evidence of alterations due to MSE; it was, however, noted that Group 2 expressed lower levels of IL-8 at all time points in relation to Group

1. When normalized using ANCOVA, it was discovered that both groups experienced a decline in IL-1 from immediately post-exercise, which progressed until 24 hours post-exercise in Group 2 and 48 hours-post exercise in Group 1. The decrease in the trained group nearly reached statistical significance (p=.054) at 24 hours in agreement with the findings of Smith and colleagues (2000), who also detected a decrease in IL-1 after intense eccentric exercise. As suggested by Smith, this decrease could be attributed to a localized accumulation of IL-1 at the site of injury, or there could be a dissociation between IL-1 production and IL-1 release making IL-1 changes undetectable by serum analysis. Also, according to Chen (2003) there is an upregulation of IL-1r mRNA within one hour post-exercise. It is plausible that if bound to a receptor, IL-1 may not be detectable in the serum and therefore may appear to be a decline in overall IL-1 population.

ANCOVA of IL-8 revealed a non-significant trend for increased concentration immediately post-exercise in both groups. Within one hour post-exercise, the untrained group had nearly returned to baseline levels, while the trained population maintained elevated serum IL-8. Since this alteration was detectable within 1 hour of the MSE bout, it seems likely this is in fact released as a myokine, as proposed by Pedersen (2008) and may be at least partially responsible for the accumulation of leukocytes further down the immunological chain. The relatively short window of activation expressed in this study also backs this hypothesis. In agreement with Ostowski (2001), IL-8 levels peaked at 1 hour post-exercise (IL-8 peak occurred at 30 min post according to Ostrowski; this time point was unmeasured in the current study) and began to steadily decline to baseline, again indicating a relatively short window of activation.

Limitations and Directions for Future Research

A primary limitation of this study is the subject pool with which testing was completed. A larger sample size (10+ in each group) consisting of only one gender would greatly reduce the amount of variance and provide vastly improved statistical power. Due to poor turnout for subject recruitment, this was not possible in the current study, but it would be warranted for any future research in this area. Training groups were also heterogeneous in regard to gender. It would be prudent to utilize homologous groups in future research to minimize variability created between gender (e.g. hormonal differences). Also, it may be advisable to utilize more defined training groups. The current study utilized field event athletes who were trained for maximal sprints of approximately 5 seconds or less, rather than sprinters who are specifically trained for bouts lasting upwards of 10 seconds. Also the moderately active individuals in the untrained population undoubtedly introduced variability to the study design. Although activity was restricted to less than 3 days per week, there was no control of the type, intensity, or duration of exercise they did partake in (if they were active at all). In order to see a more clear contrast between groups, it is suggested that the trained population consist of athletes specifically trained for short distance, intermittent sprinting of similar duration to that of the study design, and a more defined population of minimally active individuals. In addition to modifications in subject recruitment, future study design would also be well served from the addition of muscle biopsies. While serum sampling is relatively inexpensive and non-invasive, it is only useful to hypothesize what is changing in the muscular environment, as muscular levels cannot be directly measured. It is also plausible that the markers found in the serum originate from somewhere other than the muscle tissue (e.g. fat cells). Muscle biopsy would provide a direct window into the muscle tissue and is a much more direct and accurate way of measuring these markers' response,

specifically to muscular stress. It would also be of benefit to implement blood analysis at a 6-hour post-exercise time point, as it appears there may be significant activity between the 1- and 24- hour marks in this study. Although the aforementioned limitations may be viewed as potentially problematic, there were still several significant differences at the $p<.05$ level and several non-significant trend to be reported by the current study.

Conclusion

Despite a growing body of work, there has yet to be a general consensus on the role that ROS and cytokines play in the inflammatory/regeneration response created by intense exercise, or a time course in which these bodies are present and active. From this study it may be concluded that maximal sprint exercise may have a significant effect on cellular damage, ROS production, and cytokine expression. Since IL-8 was the first entity to be expressed, it is supported that it may in fact be released from the muscle tissue itself and act as an initiator inflammation in response to muscle activity and initial injury. Both groups IL-1 concentration began to decline at 1 hour post-MSE. If the decrease in measurable serum IL-1 is, in fact, indicative of localized activity or receptor binding, it is also plausible that IL-1 be released as a myokine, which may also initiate leukocyte invasion of the tissue. Increased serum CK measured at 24 hours post-exercise in the untrained group marks the occurrence of a secondary tissue insult (as this response was not present in the more resistant, trained population), most likely due to the influx of leukocytes to the area of assault. At 48 and 72 hours post-exercise, oxidative activity peaked in the untrained group, again marking the likelihood of leukocyte influx and activity which remained elevated until the conclusion of the study. Therefore it is proposed that the initial muscle activity and/or injury caused by MSE resulted in the release of

IL-1 and IL-8, which potentially initialized leukocyte invasion of the tissue, resulting in a secondary insult of the muscle tissue.

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Appendix A

Informed Consent

Eastern Michigan University
Applied Physiology Laboratory
Informed Consent for Research Involving Human Subjects

Title of Project:
The Inflammatory Response to Acute Maximal Sprint Exercise

Principal Investigator: Timothy A. Muth
318 Porter Building
(734) 487-7120 ext. 2731

Co-Principal Investigator: Stephen J McGregor
318 Porter Building
(734) 487-7120 ext. 2726

Introduction:

The purpose of this study is to analyze blood samples for evidence of an inflammatory response, muscular injury, and the presence of proteins that may direct the subsequent repair process of the injured tissue following repeated maximal sprint exercise. It is also a priority of this study to collect accelerometry data during real-world sprint exercise, and to determine the Control Entropy (CE) of the accelerometry signal in sprint trained runners using a triaxial accelerometer. Control entropy is a statistic that determines the randomness/regularity measurements and has been proposed as a means of assessing physiological parameters in a clinical setting.

Methods:

You will be asked to come to the track located inside Bowen Field House and the exercise physiology laboratory at Eastern Michigan University on five separate occasions, each separated by one week. On your first visit you will complete a graded maximal exercise test. On the second occasion you will perform three 30-second maximal sprints while wearing a small accelerometer which will be mounted to a heart rate monitor strap and attached to your lower back. This device will measure your accelerations in three dimensions. This information will then be processed by a mathematical software application to calculate your CE.

Your first visit will consist of a graded exercise test to maximal volitional exhaustion. During this test you will be asked to start walking at 4 or 6 km/hr (trained or untrained participants) at a 1% incline. This pace will be increased 2 km/hr every three minutes until you feel you can no longer continue. You may stop this test at any time.

A Jaeger Oxycon mobile metabolic cart will be utilized to collect expired gases. To do this, you will wear a face mask attached to a small unit worn on your body during exercise. This device measures inhaled oxygen and expired carbon dioxide. This information will give the physiologists the data needed to determine aerobic work capacity along with other information

that will be shared with you at the end of the study. Your heart rate will be monitored throughout the test with a Polar heart rate monitor.

You will also be asked to give intravenous blood samples immediately before the first effort, immediately after the third effort, and 1, 6, 24, 48, and 72 hours after the third effort. Each of the blood samples will be 10 ml in volume (approximately one tablespoon) and collected by a person trained in blood collection techniques. The blood that is collected will then be analyzed for the presence and levels of cytokines (chemicals that affect your immune response) and reactive oxygen species (also called free radicals).

You will be asked to adhere to several restrictions prior to testing sessions and blood draws. You will be asked to refrain from exercise throughout the testing period. You will also be asked to abstain from alcohol or caffeine use for 24 hours prior to testing.

Benefits:

You will be compensated $100.00 for completion of the entire study, paid in one sum at the conclusion of your participation. You will also benefit by learning your individual running mechanics and your Control Entropy. You may also benefit by learning the immune system's response to high intensity running sprints.

It is important for you to understand that at any time, you may withdraw from the study without prejudice or effect on your relationship to Eastern Michigan University.

All of the results from this study will be kept confidential. All participants will be assigned an ambiguous study number to maintain confidentiality. The identities of study participants will be known only by investigators directly working with the participants, and the coding of subject information will be known only by the primary investigator of this study. If publication occurs, only numbers, not names, will be used. Throughout the study, some of the data obtained from your participation will be made available to you. At the conclusion of the study, any additional data obtained from your participation will be made available to you.

Any concerns with regard to approval or research procedures should be directed to the Eastern Michigan University College of Health and Human Services Human Subjects Review Committee. The Chairperson of the committee George Liepa, PhD, may be contacted at (734) 487-0077

Risks:

The potential risks involved with this study are similar to those associated with exercise. The risk of cardiac event and even death is possible given the nature of the maximal physical effort required. These risks are minimal in a young, healthy population, and the individual being constantly monitored during testing will minimize any remaining risk. There is also the risk of infection due to blood draws. This will be minimized by the use of sterile, single-use syringes by persons trained in phlebotomy (blood collection).

I, _____, hereby give my consent to participate in the research study entitled, “The Inflammatory Response to Acute Maximal Sprint Exercise” The details of which have been provided to me, including anticipated benefits, risks, and potential complications.

I fully understand that I may withdraw from this research project at any time without prejudice or effect on my standing with Eastern Michigan University or Eastern Michigan athletics if I am a member of the track and field team. I also understand that I am free to ask questions about any techniques to be used or procedures to be undertaken.

I understand that in the unlikely event of physical injury resulting from research procedures that medical treatment will be arranged but the costs of treatment will be my responsibility since Eastern Michigan University will not provide financial compensation.

Finally, I understand that the information about me that is obtained during the course of this study will be kept confidential unless I consent to its release.

_____
Participant’s Signature

I hereby certify that I have given an explanation to the above individual of the contemplated study and its risks and potential complications.

_____
Principal Investigator

Appendix B

Human Subjects Approval Form

EASTERN
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www.emich.edu/chhs

October 26, 2010

Timothy Muth
c/o Stephen McGregor
Eastern Michigan University
School of Health Promotion and Human Performance
Ypsilanti, Michigan 48197

Dear Timothy Muth,

The CHHS Human Subjects Review Committee has reviewed the revisions to your proposal entitled: "The Inflammatory Response to Acute Maximal Sprint Exercise" (CHHS 09-053) and concluded that the risk to participants is minimal. Your study is approved by the committee.

Good luck in your research endeavors.

Sincerely,

George Liepa, Ph.D.
Chair, CHHS Human Subjects Review Committee

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