

THE CONSISTENCY OF INFLAMMATORY RESPONSES AND MUSCLE
DAMAGE TO HIGH-FORCE ECCENTRIC EXERCISE

by

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ABSTRACT

High-force eccentric exercise causes muscle damage and leads to inflammation resulting in increased levels of C-reactive protein (CRP) and interleukin-6 (IL-6). Indicators of muscle damage are creatine kinase (CK), decreased maximal isometric strength, muscle soreness (SOR) and swelling. This study investigated rank ordering of eccentric exercise of the non-dominant (ND) and dominant (D) elbow flexors. Rank ordering is determined by the magnitude and duration of the inflammatory response indicated by CRP, IL-6 and IL-10. Twelve subjects enrolled in the study, nine subjects performed high-force eccentric exercise on each arm, 3 weeks apart, consisting of 3x15 reps in the ND and D elbow flexors. Blood samples were taken at pre-exercise (0h), 4h, 8h, 12h, 24h and 120h post-exercise. Two subjects were eliminated from the data analysis because of elevated baseline IL-6 levels and insufficient strength losses. A Spearman rank order correlation was used to determine the consistency of the inflammatory response. A repeated measures ANOVA was used to detect significant changes over time and between arms as well as to determine an arm by time interaction. For CRP, no significant rank order correlation was detected and a RM ANOVA detected no significant differences. For IL-6, no significant rank order correlation was detected, but a trend ($p = 0.071$) was found. Also, a RM ANOVA detected a trend ($p = 0.107$) for IL-6 with a peak increase at 8h post-exercise. A significant rank order correlation was detected for CK ($p < 0.05$). A RM ANOVA detected a trend ($p = 0.099$) for a higher response in the ND arm. A significant rank order correlation was detected for maximal isometric strength ($p < 0.05$). A RM ANOVA detected significant strength decreases ($p < 0.001$) immediately post-exercise for both the ND and D arms. No significant rank order correlation was found for muscle soreness. A significant increase ($p < 0.001$) in soreness was detected at 24h post-exercise. For swelling, no significant rank order correlation was detected and no significant changes occurred. The consistency of the inflammatory response after high-force eccentric exercise in the ND and D elbow flexors is undetermined at this time.

CHAPTER ONE

INTRODUCTION

Development of Problem

Coronary heart disease (CHD), defined as disease of the heart muscle and/or the blood vessels surrounding and supplying the heart with oxygen, is deadly and takes thousands of lives every year. In 2002, diseases of the heart were the leading cause of death for adults in the United States in all races, with the exception of Asian/Pacific Islander, in which diseases of the heart came in second (Anderson & Smith, 2005). The preliminary data for 2003 reveal a similar trend. Thus, a means of further understanding and preventing CHD is needed. Several risk factors predispose individuals to developing CHD including family history of CHD, hypertension, obesity, sedentary lifestyle, smoking, diabetes and hypercholesterolemia (Balady et al., 2000). Additionally, atherosclerosis, a condition in which plaque builds up on arterial walls and occludes blood flow to the heart, is a primary contributor to the development and progression of heart disease.

Inflammation is an underlying issue concerning atherosclerosis and risk of CHD. Associations between CHD and the biomarkers of inflammation have been reported in various research studies (Blake & Ridker, 2002; Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker et al., 2001). Chronic low levels of inflammation and improper regulation of the inflammatory response put an individual at increased risk for developing CHD (Blake & Ridker, 2002). The research gap lies in the reason for elevated

biomarkers of inflammation. It is not known if high levels of inflammation cause the elevation in inflammatory biomarkers or if there is some other process involved. Therefore, the purpose of this study was to develop a model to better understand inflammation and the control of inflammation, ultimately more clearly understanding this risk factor and influencing CHD risk.

Tissue damage is one of several causes of inflammation. The source of the tissue damage is irrelevant; whether the damage is caused by illness, laceration or muscle disruption, the response is similar throughout. One way of inducing tissue damage is to perform high-force eccentric exercise, an exercise in which the muscle lengthens as it generates tension. Once the damage occurs, the structure of muscle is altered, the inflammatory response is launched into action and other changes take place (Nosaka, Clarkson, McGuiggin, & Byrnes, 1991; Smith & Miles, 2000). For example, inflammatory biomarkers, such as C-reactive protein (CRP) and interleukin-6 (IL-6), increase after high-force eccentric exercise. Creatine kinase (CK), an enzyme indicative of muscle damage, increases but is not linked to CHD (Byrnes et al., 1985; Nosaka et al., 1991; Smith et al., 1998). Also, muscle soreness levels increase greatly with unaccustomed eccentric exercise (Byrnes et al., 1985; Connolly, Sayers, & McHugh, 2003; Miles & Clarkson, 1994). This is known as delayed onset muscle soreness (DOMS). Additionally, maximal isometric strength decreases immediately after high-force eccentric exercises (Nosaka et al., 1991; Stupka et al., 2000; Stupka, Tarnopolsky, Yardley, & Phillips, 2001; Willoughby, McFarlin & Bois, 2003).

The major function of the inflammatory response is to promote healing (Smith & Miles, 2000). Once the inflammatory response begins, synthesis and release of cytokines, leukocytes, adhesion molecules and proteins occur. Specific biomarkers of inflammation associated with CHD are CRP and IL-6 (Blake & Ridker, 2002; Willoughby et al., 2003). C-reactive protein has the strongest association to CHD relative to other inflammatory biomarkers (Ridker et al. 1997).

The current study served as a continuation of Dr. Mary Miles' research "Downregulation of inflammation: Are individuals at risk for CHD less capable of turning off the inflammatory process," which utilizes high-force eccentric exercise to induce muscle damage and inflammation. This research model is used to characterize the inflammatory response for individuals with different basal CRP levels. The reliability of this model has not been assessed for IL-6 and CRP responses. Dr. Miles' research is comprised of one exercise condition in the non-dominant (ND) elbow flexors and a control condition. This study added a second exercise condition in the dominant (D) elbow flexors. The variables measured, the time course used and the subjects involved in this study stemmed from Dr. Miles' research.

The purpose of this study was to investigate the consistency of muscle damage and inflammatory responses in untrained college males and females after performing two bouts of high-force eccentric exercise in the ND and D elbow flexors. It was hypothesized that the dependent variables would respond in a rank ordered fashion regarding the magnitude of the inflammatory response. That is, subjects with dramatic responses after the first bout of exercise would respond dramatically after the second bout

of high-force eccentric exercise and that all subjects would be in the same order regarding the magnitude of the inflammatory response. If the inflammatory responses within subjects were consistent, the results could provide important information as to why the biomarkers of inflammation, such as CRP and IL-6, are elevated. In addition to understanding the inflammatory response more completely, researchers may be better able to design interventions to influence CHD risk by lowering chronic inflammation.

Purpose

The aim of this study was to investigate the consistency of muscle damage and inflammatory responses within individuals in response to muscle damage in the non-dominant (ND) elbow flexors and dominant (D) elbow flexors.

Hypothesis

It was hypothesized that dependent variables of CRP, IL-6, IL-10, CK, maximal isometric strength, muscle soreness and swelling would respond consistently, in a rank ordered fashion, after performing two high-force eccentric exercise bouts in the ND and D elbow flexors.

$$\begin{aligned}H_o: \rho_s &= 0 \\H_a: \rho_s &\neq 0\end{aligned}$$

The notation ρ_s represents the Spearman rank order correlation coefficient.

Assumptions

It was assumed that participants abided by and were truthful about inclusion and exclusion criteria. Also, it was assumed that subjects gave a maximal effort during each repetition of exercise.

Limitations

A number of limitations are worth consideration for the current study. The sample population consisted of only non-athlete, college-aged males and females. The primary basis for this sample was convenience. The subjects were easily accessible and willing to volunteer. Participation in the study was purely voluntary; thus, the sample was not a true representation of the population because it was not random. The sample size was small which served as a limitation. As with any other research study, human error must not go unnoted. Possible errors could have occurred during exercise testing and data collection, but the measures were standardized and assays were run in duplicate to minimize the possibility of error.

Definitions

Acute-phase proteins: activated proteins released by the liver in response to the initiation of the inflammatory response that participate in the promotion of the healing process

Assay: the analysis of a substance or mixture to determine its constituents and the relative proportion of each

Atherosclerosis: plaque build-up in coronary arteries that slowly progresses through the lifespan

Concentric contraction: type of muscle contraction characterized by shortened muscle length

Coronary heart disease (CHD): disease of the cardiac muscle and/or arteries resulting in impaired function of the heart

C-reactive protein (CRP): an acute-phase protein detectable in the blood only during the active phase of inflammation

Creatine kinase (CK): an enzyme in skeletal muscle, cardiac muscle and brain catalyzing the reversible transfer of high-energy phosphate bonds

Cytokine: small polypeptide that facilitates an influx of lymphocytes, neutrophils, monocytes and other cells

Delayed onset muscle soreness (DOMS): pain, stiffness and tenderness of muscles that occurs 24 -72 hours after unaccustomed exercise

Eccentric contraction: muscle contraction in which the muscle lengthens as it generates tension

Hypercholesterolemia: cholesterol levels ≥ 240 mg/dL

Interleukin-6 (IL-6): a cytokine involved in the mediation of inflammation

Interleukin-10 (IL-10): a cytokine involved in the inhibition of the inflammatory response.

Maximal isometric strength: maximum force output with no change in muscle length

CHAPTER TWO

LITERATURE REVIEW

Overview of Acute Inflammation

Acute inflammation is a generalized process that occurs when muscle tissue is damaged or injured. The goal of the inflammatory response is to heal the damaged tissue. Common symptoms accompanying inflammation are swelling, pain, redness, heat production, reduced range of motion and decreased or loss of function (Smith & Miles, 2000).

After tissue damage occurs, cytokines are released in a general order and each cytokine plays an integral role in the acute-phase inflammatory response. First, tumor necrosis factor-alpha (TNF- α) and interleukin-1 β (IL-1 β) are released. The release of these cytokines initiates the inflammatory response and stimulates the release of interleukin-6 (IL-6) (Smith et al., 2000). Interleukin-6 provides negative feedback, which inhibits the production of TNF- α and IL-1 β . Additionally, IL-6 stimulates the downstream components of the inflammatory cascade such as increasing circulating leukocytes and permitting the migration of leukocytes into the damaged tissue, which are stimulated by the upregulation of cellular adhesion molecules (CAMs) and colony stimulating factors (CSFs) (Shephard, 2002; Smith et al., 2000). IL-6 also aids in the synthesis of acute phase proteins (APP), specifically CRP (Yudkin, Kumari, Humphries & Mohamed-Ali, 2000). The role of IL-6 in the inflammatory process is both pro-inflammatory and anti-inflammatory. Interleukin-6 is a pro-inflammatory cytokine

because IL-6 upregulates and promotes the inflammatory response (Smith et al., 2000) and IL-6 is an anti-inflammatory cytokine because IL-6 inhibits TNF- α and enhances interleukin-1 receptor agonist (IL-1ra) and interleukin-10 (IL-10) (Shephard, 2002; Steensberg, Fischer, Keller, Moller, & Pedersen, 2003). Interleukin-1 receptor antagonist and IL-10 are categorized as anti-inflammatory cytokines and control as well as down-regulate inflammation (Smith & Miles, 2000).

Once CSFs are released, leukocytes are released from the bone marrow. Leukocytes, more specifically neutrophils and monocytes, migrate from circulation to the injured tissue and are the first cells to arrive at the site of tissue damage. Adhesion molecules assist the vascular endothelium in directing the leukocytes (Smith & Miles, 2000). Macrophages and neutrophils act as the first line of defense and are responsible for eradicating damaged tissue. Once inside injured tissue, monocytes are activated and differentiate to become macrophages.

Following the release of cytokines (from activated inflammatory leukocytes), APP synthesis is induced. C-reactive protein, an APP that is produced in the liver in response to IL-6, is the main APP involved with CHD (Blake & Ridker, 2002). Acute-phase proteins are an integral component of the acute-phase inflammatory response, serving as the primary mechanism for maintaining control of inflammation and protecting tissue from unwarranted damage resulting from inflammation (Smith & Miles, 2000).

Termination is a crucial step of the inflammatory response. When the inflammatory response is not regulated, tissues and cells can be severely damaged because leukocytes break down the tissue prior to eradication of the damaged tissue.

Thus, improper regulation of inflammation can lead to excess tissue damage. Interleukin-10 is the primary anti-inflammatory cytokine that works to suppress the production of pro-inflammatory cytokines and shuts down the inflammatory process (Smith & Miles, 2000).

Inflammation and Coronary Heart Disease (CHD)

Inflammation plays a fundamental role in the progression of atherosclerosis, which can lead to the development of CHD (Blake & Ridker, 2002). The inflammatory response is essential and promotes healing. However, when improperly regulated and CRP and IL-6 levels are chronically elevated, the inflammatory response appears to play a role in the development of atherosclerosis, leading to an increased risk of CHD.

C-reactive protein is an APP indicative of the inflammatory response and is strongly linked to CHD when chronically elevated (Ridker et al., 1997). The relationship between CRP and CHD could be due to the possible atherogenic properties of CRP. Those properties involve cellular adhesion molecules (CAMs) that can increase the rate of plaque production. Leukocytes do not adhere to normal endothelium, but as the endothelium becomes diseased the expression of adhesion molecules increases and the CAMs selectively bind to circulating leukocytes. Once inflammatory leukocytes adhere to the endothelium, they infiltrate the subendothelium and contribute to the local inflammatory response. Next, macrophages, endothelial cells and smooth muscle cells produce TNF- α , which stimulates the production of IL-6, which serves as a stimulus for hepatic (liver) production of CRP. Additionally, arterial tissue produces CRP and

complement proteins resulting in an up-regulation of plaque build up which can lead to atherosclerosis (Blake & Ridker, 2002).

Inflammatory Biomarkers

The association of CRP and CHD has been the focus of a number of research studies. Ridker et al. (1997) investigated the increased risk of a coronary event and whether or not aspirin therapy would decrease this risk. C-reactive protein was measured in 543 healthy men who later suffered from a myocardial infarction (MI). The men in the highest quartile of CRP values had three times the risk of MI (relative risk = 2.9) compared to men in the lowest quartile. Researchers drew two conclusions from this study. The first was that elevated baseline levels of CRP predicted the risk of future MI. Secondly, aspirin therapy reduced risk of MI by lowering levels of CRP via anti-inflammatory mechanisms. Ischemia (lack of oxygen) is also associated with elevated CRP levels. A study comparing 118 subjects with known exercise-induced ischemia to 111 subjects without ischemia was conducted to determine the association between CRP and exercise-induced ischemia. Individuals in the highest quartile for CRP levels experienced ischemia 75% of the time compared to only 38% in the lowest quartile (Beattie et al., 2003). Researchers interpreted these studies as a possible positive relationship between CRP and CHD, in which elevated levels of CRP lead to an increased risk of developing CHD.

Additionally, the American Heart Association (AHA) and the Center for Disease Control (CDC) designed three categories according the basal CRP levels to determine the risk of developing CHD. Low risk individuals have less than 1.0 mg/L, average risk

individuals have 1.0 – 3.0 mg/L and high risk individuals have more than 3.0 mg/L of basal CRP levels. Individuals in the high risk category are more than twice as likely to develop CHD relative to individuals in the low risk category (Pearson et al. 2003).

C-reactive protein is not the only inflammatory biomarker associated with CHD. Interleukin-6 works to control inflammation and serves as an underlying factor relating the inflammatory response to CHD. In past studies, including the Physicians' Health Study (PHS) (Ridker, Rifai, Stampfer & Hennekens, 2000), these associations have been reported. In the PHS, men in the highest quartile of baseline IL-6 levels had a 2.3-fold increased risk of future coronary events versus the lowest quartile. Elevated IL-6 levels were associated to complications during in-patient care and increased morbidity with coronary heart disease (Blake & Ridker, 2002).

Exercise-Induced Muscle Damage

Skeletal muscle works in numerous ways. Two specific movements are concentric contractions and eccentric contractions. Concentric contractions occur when the muscle shortens while generating tension. Eccentric contractions occur when the muscle lengthens while generating tension. For example, in a bicep curl exercise the muscle contracts and shortens while lifting the weight (concentric contraction) and the muscle generates tension and lengthens as the weight is lowered (eccentric contraction). Eccentric contractions are widely used in muscle damage research because eccentric contractions cause extensive muscle damage relative to concentric contractions (Stupka et al., 2001; Willoughby et al., 2003). Previously, researchers concluded that eccentric exercise resulted in inflammation, increased serum creatine kinase (CK), reduced

strength, delayed onset muscle soreness (DOMS) and swelling of the muscle group (Nosaka et al., 1991; Stupka et al., 2001; Willoughby et al., 2003).

High-force eccentric exercise causes muscle damage and results in inflammation, which, leads to increased CRP (Akimoto et al., 2002), IL-6 (MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2001; Smith et al., 2000) and IL-10 levels (Hirose et al., 2004). Akimoto et al. (2002) investigated the effect of strenuous exercise on adhesion molecules and CRP levels. To induce muscle damage, subjects completed 30 minutes of downhill running, a form of eccentric exercise. Researchers reported that CRP levels increased significantly (pre = 78.6 ng/ml, 1d post = 270.3 ng/ml) one day after the downhill running. MacIntyre et al. (2001) and Smith et al. (2000) studied how eccentric exercise affected IL-6 levels. Both research teams observed that IL-6 increased significantly after the exercise was completed; however, results from the two studies differed in the time at which IL-6 peaked. MacIntyre et al. (2001) reported that IL-6 levels rose from 1.15 pg/ml at baseline and peaked at 5.0 pg/ml at 6 hours post-exercise. Smith et al. (2000) reported that IL-6 levels rose from 1.8 pg/ml at baseline and peaked at 4.5 pg/ml at 24 hours post-exercise. Interleukin-10 increased significantly (2.71 ± 1.26 pg/ml to 3.67 ± 1.53 pg/ml) 6 hours after an eccentric exercise bout (Hirose et al., 2004).

When evaluating muscle damage and CK levels, sex differences must be taken into account. In response to exercise-induced muscle damage, sex differences for CK levels, inflammation and structural disruptions were reported in rats. Stupka et al. (2000) postulated that female humans would have an attenuated effect on CK increases compared to their male counter-parts. They concluded that sex differences are

distinguishable in responses to exercise-induced muscle damage and females generally have an attenuated CK response after exercise-induced muscle damage. Although both sexes responded to muscle damage, female CK responses were significantly lower than males. Creatine kinase levels for males were 1,000 U/L at 48 hours post-exercise and 1,100 U/L at 6 days post-exercise while female CK levels were 250 U/L at 48 hours post-exercise and 500 U/L at 6 days post-exercise.

Researchers have also investigated inflammation in response to eccentric exercise in animals (St. Pierre Schneider, Correia & Cannon, 1999). St. Pierre Schneider et al. (1999) investigated the inflammatory response of male and female mice. Researchers discovered that female mice had fewer leukocyte infiltration and the time course was longer (5 days for males, 7 days for females) compared to males. Researchers suggested that female mice had an attenuated inflammatory response after exercise-induced muscle damage.

Strength loss is a further indicator of muscle damage. After eccentric exercise, the muscle undergoes physiological changes resulting in structural damage to the muscle and loss of strength (Miles & Clarkson, 1994; Stupka et al., 2000; Stupka et al., 2001). Investigators reported the greatest strength losses occurred immediately following eccentric exercise and lasted up to 24 hours post-exercise, then gradually returned to baseline (MacIntyre et al., 2001; Nosaka et al., 1991; Nosaka, Sakamoto, Newton, & Sacco, 2001). Nosaka et al. (1991, 2001) determined that eccentric exercise resulted in 53% strength loss in the elbow flexors and 60% strength loss in the forearm flexors in an immediate post-exercise isometric strength test.

In addition to increased CK levels, muscle soreness is indicative of muscle damage. Unaccustomed eccentric exercise results in DOMS (Miles & Clarkson, 1994; Nosaka et al., 1991; Stupka et al., 2000; Stupka et al., 2001). Delayed onset muscle soreness develops approximately 24 hours post-exercise, peaks 1 – 3 days post-exercise and fades by 7 – 10 days. Delayed onset muscle soreness is characterized by dull, aching pain felt with movement and/or palpation (Miles & Clarkson, 1994; Nosaka, Newton, & Sacco, 2002). Delayed onset muscle soreness coincides with the inflammatory response, as muscle damage is present. Delayed onset muscle soreness varies from individual to individual depending on training status and individual perception of pain. Untrained individuals experience more intense soreness compared to trained individuals (Armstrong, 1984).

Swelling also accompanies exercise-induced muscle damage. Nosaka et al. (2001) revealed that the mid-muscle circumference of the elbow flexors increased by 8 millimeters immediately after eccentric exercise and increased by an additional 25 millimeters 5 days post-exercise. These changes in circumference occurred after one bout of high-force eccentric exercise in the ND elbow flexors.

Eccentric exercise is a tool that induces muscle damage and is used in many research studies to investigate the inflammatory response. Muscle damage produces an inflammatory response leading to increased levels of inflammatory cytokines, therefore completing the relationship between exercise-induced muscle damage and inflammation.

Repeated Bout Effect

High-force eccentric exercise produces muscle damage, but repetition of the same exercises generates an adaptation effect, known as the repeated bout effect (RBE). The RBE causes direct and indirect muscle damage indicators to respond less dramatically when high-force eccentric exercises of the same muscle group are performed repeatedly (Nosaka et al., 2001; Stupka et al., 2001; Willoughby et al., 2003). This adaptation effect results in a less significant CK increase, less intense DOMS and swelling, faster recovery of strength and a blunted immune response after repeated bouts of eccentric exercise. Nosaka and Clarkson (1994) investigated the effects of repeated bouts of eccentric exercise. Two bouts of eccentric exercise of the elbow flexors were performed with either 3 or 5 days of rest between the two bouts. Creatine kinase levels did not increase as much in the second bout when compared to the first bout. Researchers concluded that the repeated bout effect occurred during the 3 or 5 days of rest (Nosaka & Clarkson, 1994).

Creatine kinase levels, an indirect marker of muscle damage, undergo significant attenuation when performing repeated bouts of eccentric exercise in the muscles previously injured (Nosaka et al., 1991; Smith et al., 1998; Stupka et al., 2001). Stupka et al. (2001) investigated the CK response, structural damage and inflammation with repeated bouts of eccentric exercise of the knee extensors. They found that CK levels decreased significantly in the second bout of exercise compared to the first bout. Ninety-six hours after the eccentric exercise took place both female and male subjects had significantly lower CK responses after the second bout when compared to the first bout of

exercise. Male CK levels ranged from 302 – 2,110 U/L for the first bout of exercise and 137 – 1,078 U/L for the second bout, while female CK levels ranged from 229 – 1,376 U/L for the first bout and 172 – 545 U/L for the second bout of exercise. Smith et al. (1998) and Nosaka et al. (2001) reported similar findings.

Variation exists regarding the duration of the RBE among variables. The duration of the adaptation effect on CK levels is inconsistent across research studies and does have a lasting effect. In 2001, Nosaka et al. conducted a study primarily focusing on the duration of the repeated bout effect and CK levels. Researchers revealed the duration of the adaptation effect lasted more than six months, but diminished between nine and twelve months.

Significant differences between DOMS and strength exist when comparing the two bouts of high-force eccentric exercise. The duration of DOMS does not last as long in bout 2 and strength is recovered more rapidly (Nosaka et al., 2001; Smith et al., 1998).

Willoughby et al. (2003) hypothesized that IL-6 would respond in a similar fashion as the muscle damage indicators do in the repeated bout effect (strength and soreness), thus facilitating an explanation for this adaptation effect. Subjects completed two eccentric exercise bouts of the knee extensors. Each exercise bout was separated by three weeks. Interleukin-6 levels were reported with no significant decrease after the second bout of eccentric exercise. Researchers concluded that strength and soreness endured a repeated bout effect while IL-6 did not.

The exact mechanism as to how this adaptation occurs is still undetermined. However, there are a few proposed explanations including neural, cellular or mechanical

adaptations. Regarding neural adaptations, there is some evidence that researchers suggest there may be a greater recruitment of slow twitch muscle fibers or the activation of a bigger motor unit pool. Cellular adaptations could be attributed to the addition of sarcomeres and adaptations in the inflammatory response after the initial bout of eccentric exercise. Concerning mechanical adaptations, after the disruption of muscle cells an adaptation in the cytoskeleton proteins responsible for maintaining the alignment and structure of sarcomeres could occur, which could serve as protection against future disruption and damage (McHugh, 2003).

Acute inflammation occurs when tissue is damaged and is followed by several steps involved to heal the injured tissue. Inflammation is associated with the development of CHD, specifically CRP and IL-6, both of which are inflammatory biomarkers. Tissue damage can occur from various sources, one is high-force eccentric exercise. When examining inflammation in response to exercise, it is important to take into account the adaptation effect caused by repeated bouts of exercise.

The purpose of this study was to investigate the consistency of muscle damage and the inflammatory response within individuals in response to muscle damage in the non-dominant (ND) elbow flexors and dominant (D) elbow flexors. The consistency of the inflammatory response was determined by using a Spearman rank order correlation for each individual subject. It was hypothesized that the dependent variables would respond consistently, in a rank ordered fashion, after performing two bouts of high-force eccentric exercise in the ND and D elbow flexors.

CHAPTER THREE

METHODS

Subject Sample

Participants were twelve college-aged males ($n = 6$) and females ($n = 6$). This age group was targeted because of accessibility and willingness to volunteer. Flyers and word of mouth were used as a means of recruitment of participants. Incentive for participating in the study included \$250 and a coupon for a free massage.

Researchers utilized phone-screening questionnaires to determine an individual's qualification for participation. Potential subjects were screened for any activities that would condition the upper body; including, but not limited to, weight lifting, cross-country skiing, rock climbing, mountain biking and push-ups. Potential subjects also were screened via self-report for known diseases such as anemia, latent anemia, heart disease and diabetes and conditions such as musculoskeletal limitations or injuries and inflammatory conditions. Further questions were asked about their history of injuries and surgeries to assess musculoskeletal limitations. Report of these diseases and conditions served as exclusion criteria. In addition, individuals taking anti-inflammatory medication, like aspirin or ibuprofen, were excluded from the study. Pregnant women were also excluded from the study. If an individual qualified for participation, next the individual completed appropriate forms and questionnaires and the first day of the first condition was scheduled.

The screening process was very important because untrained individuals have a more dramatic response to exercise-induced muscle damage when compared to trained individuals (Nosaka et al., 1991). An adaptation effect, known as a repeated bout effect, occurs in skeletal muscle when exposed to high-force eccentric exercise resulting in different responses to the exercise after a second bout (Nosaka et al., 2001; Smith & Miles, 2000). Consequently, subjects were queried on their involvement in activities such as weight lifting, cross-country skiing, rock climbing, mountain biking or other activities that condition the biceps brachii and biceps brachialis (elbow flexors).

Subjects were aware that invasive procedures, handling/sight of blood and needles, were involved. Subjects who were anxious about or uncomfortable with these factors were discouraged from participating in the study.

Prior to participation, subjects completed an informed consent (Appendix A), a Physical Activity Readiness Questionnaire (PAR-Q) (Appendix B) and a Health History Questionnaire (HHQ) (Appendix C). An informed consent was used to inform the subject of the procedures, purposes and risks associated with participation. A PAR-Q allowed researchers to identify individuals for whom physical activity might be deemed inappropriate (Balady et al., 2000). If subjects answered yes to any of the questions on the PAR-Q the individual was not allowed to participate in the study. A health history questionnaire was used to inform researchers of family history, current and past illnesses and medical history. The Human Subjects Committee approved the experimental protocol and informed consent. Subjects' personal information was kept confidential by using coding and files were kept in a cabinet within a locked office.

Experimental Design

The study consisted of three conditions, high-force eccentric exercise of the non-dominant elbow flexors (ND), high-force eccentric exercise of the dominant elbow flexors (D) and a control condition (which consists of only blood draws and no exercise). All subjects completed each condition and were originally randomly assigned to a specific order of completion using the Latin-square design. This design is a counterbalancing method ensuring random assignment such that each condition was equally accounted for. A key point was that subjects were not compared to other subjects; rather, subjects served as their own control. The Latin-square design would have enabled a group of subjects to complete the conditions in the order of ND elbow flexors, D elbow flexors, control; another group would have completed the conditions in the order of D elbow flexors, control, ND elbow flexors and the last group of subjects' order of completion would have been control, ND elbow flexors, D elbow flexors (Table 3.1). Due to equipment failure (equipment was out of order for 6 weeks) and attrition, the Latin-square design was not used. Each subject completed all three conditions, but the order of completion was altered. Researchers arranged the conditions according to which arm was exercise first and the placement of the control condition was inconsequential. Four of the twelve subjects were scheduled to exercise the D arm first and eight subjects were scheduled to exercise the ND arm first.

A series of steps were completed on the first day of each condition. First, subjects met at the Movement Science Lab (MSL) on the campus of Montana State University (Bozeman, MT) at 7:00 a.m. Subjects fasted for 12 hours and had not participated in any

exercise prior to reporting to the MSL. Anthropometric assessments including height, weight and upper arm circumference at the mid-biceps level were measured and recorded for the two exercise conditions.

Table 3.1 Latin-square design of conditions.

1 st Condition	2 nd Condition	3 rd Condition
ND	D	Ctrl
D	Ctrl	ND
Ctrl	ND	D

Subjects starting an exercise condition completed three exercise tests on the first day of the condition (Table 3.2). A pre-exercise isometric strength test was completed on both arms before the high-force eccentric exercise, which was performed on just one arm. A post-exercise isometric strength test was completed on both arms after the high-force eccentric exercise.

Table 3.2 Tests performed on day 1 of each exercise condition.

Exercised Arm	Non-exercised Arm
Pre-exercise Isometric Strength Test	Pre-exercise Isometric Strength Test
High-force Eccentric Exercise Test	
Post-exercise Isometric Strength Test	Post-exercise Isometric Strength Test

Six blood samples were taken from each subject, pre-exercise (0h), 4, 8, 12, 24 and 120 hours post-exercise for each condition resulting in 18 total samples taken (Table

3.3). In order to make a complete analysis, numerous samples must be taken at different times because the variables of interest peak at different times (Table 3.4). Data collection

Table 3.3 Time course for one exercise condition and the variables and analyses involved with each blood draw.

Time	0h	4h	8h	12h	24h	120h
Blood Collection	✓	✓	✓	✓	✓	✓
Eccentric Exercise	✓					
Maximal Isometric Strength	✓				✓	✓
Soreness	✓	✓	✓	✓	✓	✓
Swelling	✓	✓	✓	✓	✓	✓
CRP Analysis	✓	✓	✓	✓	✓	
IL-6 Analysis	✓	✓	✓	✓	✓	
CK Analysis	✓				✓	✓

Table 3.4 Schedule of peak responses for each variable.

Variable	Time to peak response
CRP	12 - 24h
IL-6	8 - 24h
CK	96 - 120h
Strength	Immediately post-ex
Soreness	24 - 48h
Swelling	Variable

at 0, 24 and 120 hours occurred at 7 a.m. for each condition to control for daily variation. Cortisol is an immuno-regulatory hormone that peaks early in the morning and decreases throughout the day, reaching the lowest levels at night (Vanheesen, 2005). The effects of physical activity, eating, as well as cortisol changes are controlled for by collecting data at 7 a.m.

Variables of interest were CRP, IL-6, CK, maximal isometric strength, muscle soreness and swelling. When muscle damage occurs after high-force eccentric exercise, levels of CRP, IL-6, CK, muscle soreness and swelling can increase significantly while isometric strength decreases (Blake & Ridker 2002; Nosaka et al., 2001). These variables were the dependent variables and were influenced by the independent variable of high-force eccentric exercise of the elbow flexor muscles. Interleukin-10 was proposed to be measured, however preliminary analysis from a larger study conducted in the same laboratory indicated no response after the high-force eccentric exercise. Furthermore, IL-10 was not measured and analyzed.

Time Course

Subjects were originally scheduled to complete one condition per month, but due to equipment failure the time course was extended. Each condition was completed within 6 days and was followed by at least a 3-week period of rest.

Severe hormonal fluctuations occur throughout the female menstrual cycle. At the onset of menstruation estrogen and progesterone are low and stable. There is evidence of estrogen having a protective affect on muscle damage (Vanheesen, 2005). Consequently, female subjects were scheduled for each condition at the onset of

menstruation to control for these hormonal fluctuations and to prevent any influence on the dependent variables.

High-Force Eccentric Exercise

During an eccentric exercise, the muscle lengthens as it contracts. High-force eccentric exercise causes more muscle damage, resulting in a greater inflammatory response, when compared to concentric exercise (Stupka et al., 2001; Willoughby et al., 2003).

A Kin-Com 125 E Plus dynamometer (Isokinetic International, Inc. Harrison, TN) was used for the eccentric exercises and the isometric strength tests. The Kin-Com is a dynamometer, which can perform isokinetic and isometric movements for testing, rehabilitation and measurement purposes. The Kin-Com consists of a padded table, a padded lever arm and a force transducer, which is all controlled by a computer (Appendix D, Figure 1). The padded table served as a support for the upper arm during testing. The arm of the subject was attached to the lever arm. The force transducer, which is located on the lever arm, measures force production and sends data to the computer to be recorded. The measurements of the padded table and the lever arm were recorded on the first day of each exercise condition and used throughout the study to eliminate variability between days.

Prior to the exercise, researchers adjusted the dynamometer for each subject. A reference angle of 90° was established (Appendix D, Figure 2), which was needed for determining the range of motion (ROM). A complete ROM for both muscle groups was crucial when inducing muscle damage (Appendix D, Figures 3 and 4). A wider ROM

results in more muscle damage while a narrower ROM does not (Nosaka & Sakamoto, 2001). The start and stop angles for the subjects' ROM were recorded and used for both exercise conditions to control for any differences.

Several steps were involved in positioning the subject and setting up the exercise protocol. The padded table was raised or lowered to ensure that the upper arm was parallel to the table and floor, reducing the risk of increased stress to the elbow joint. The pad of the lever arm was placed one inch below the wrist joint and the subjects' arm was strapped in tightly, but comfortably (Appendix D, Figure 2). The axis of rotation for the lever arm and the subjects' arm were lined up so that they rotated on the same plane to minimize stress on the elbow joint.

For the eccentric exercise bout, subjects completed a total of 45 repetitions divided into 3 sets of 15 repetitions. Subjects were instructed to resist maximally while the dynamometer pulled the muscle through the full ROM resulting in a high-force eccentric exercise. Each repetition was completed at a rate of 45 degrees per second with 10 seconds of recovery time between repetitions. Sets were separated by 3-5 minutes of rest. Researchers verbally encouraged the subjects throughout the eccentric exercise to maximize effort. Phrases used to encourage subjects were: 'Come on, pull as hard as you can, as hard as you can!', 'Keep pulling, keep pulling, keep pulling!' and 'Good job, pull, pull, pull!'. The graduate advisor was present at each session to ensure consistency of verbal encouragement.

Reliability and validity of the Kin-Com dynamometer was determined to be high. To test the reliability and validity of the Kin-Com, Farrell and Richards (1986)

simultaneously measured the lever arm position, force and lever arm velocity using the Kin-Com, an external force transducer and an independent spring drive system. Lever arm position was found to have no differences (ICC = 0.999), force measurements averaged a difference of 3.2% or less (RI = 0.948) and lever arm velocity averaged a difference of less than 1.5% (RI = 0.990) (Farrell & Richards, 1986).

Maximal Isometric Strength

Maximal isometric strength was used to determine the difference between the pre-exercise and post-exercise strength tests. Post-exercise strength tests were expressed as a percent change from the pre-exercise isometric strength test. Post-exercise isometric strength values had at least a 10% strength loss of the pre-exercise isometric strength test to ensure sufficient muscle damage. The force produced at the wrist attachment represents the general concept of 'strength'. Maximal isometric strength was another means of further evaluating the level of exercise-induced muscle damage.

Maximal isometric strength was measured using the Kin-Com. The subject was positioned in the exercise apparatus so the elbow was at a 90° angle and the upper arm was parallel to the table and floor. The axis of rotation of the lever arm of the Kin-Com and the axis of rotation for the subjects' elbow were lined up so that they rotated on the same plane. Researchers instructed the subject to exert maximal effort by pulling on the lever toward the shoulder for three seconds. Three maximal contractions were performed and 60 seconds of recovery time was allotted between each contraction. The strength test was completed pre- and post-exercise for the eccentric exercise conditions as well as 24 and 120 hours post-exercise (Table 3.3).

Muscle Soreness

Muscle soreness was a subjective perception as to how much pain the subject experienced. This assessment was used to further assess the subject's perception of soreness. Soreness was quantified using a 100-millimeter visual analog scale (VAS) that ranged from 'no soreness' to 'very, very sore' (Figure 3.1). Subjects rated soreness while palpating the muscle during flexion and extension while holding a 1-kilogram weight. Soreness was recorded before each blood draw for each exercise condition. A blank scale was used at each blood draw to eliminate bias from previous measures. Subjects also were given visual analog scales to take home and fill out during the three days that no blood draws were taken (48h, 72h and 96h post-exercise).

The use of a visual analog scale (VAS) to quantify acute pain is reported to be valid and reliable. An intraclass correlation coefficient (ICC) and a Bland-Altman analysis were used to determine the reliability of the VAS on acute pain. Bijur et al. (2001) concluded that the ICC for all paired VAS scores was 0.97 (95% CI) (Bijur, Silver & Gallagher, 2001).

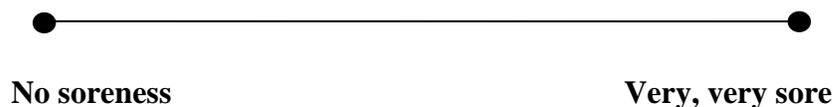


Figure 3.1 Visual Analog Scale for muscle soreness.

Swelling

Mid-muscle circumference of the upper arm was measured at each blood draw (0, 4, 8, 12, 24 and 120 hours) of each exercise condition (Table 3.3). The greatest circumference of the biceps was palpated during flexion and three ink dots (one on the medial, ventral and lateral sides) were placed on the upper arm. The dot on the medial side of the arm was measured from the medial epicondyle to ensure consistent measurements. These marks were maintained throughout the condition and the following measurements were made using the dots as landmarks.

Blood Collection

Blood samples were collected from the antecubital vein into chilled evacuated tubes (Vacutainer, Franklin Lakes, NJ) using a standard venipuncture technique. Subjects sat for 10 minutes before blood collection. One tube coated with tri-potassium EDTA, an anticoagulant, was used for plasma and whole blood samples. One tube with no coating was used for serum samples. The tube coated with EDTA was placed on a rocker for 2-3 minutes after blood collection to ensure thorough mixing of the anticoagulant and blood sample. The tube for the serum sample was left untouched for 10 minutes to allow clotting. Both tubes were then placed in a 21000R Marathon centrifuge (Fisher Scientific, Pittsburgh, PA) at 2900 repetitions per minute for 16 minutes to separate the red blood cells (RBC) from the plasma and serum. Plasma and serum were then aliquotted into several microcentrifuge tubes and stored at -80°C until analysis. All analyses for each variable were run at the same time to eliminate error.

Laboratory Assays

Assays were used to determine the magnitude and time to peak response for each subject. Levels of CRP, IL-6 and CK were measured to calculate the magnitude and the time to peak response of the inflammatory response. The magnitude of the peak response was calculated by subtracting the baseline value (0h of that exercise condition) from the exercise value. Time to peak response was calculated by pinpointing the hour at which the variable peaked.

C-Reactive Protein (CRP)

C-reactive protein was measured for purposes of determining the time to peak response and the magnitude of the inflammatory response resulting from high-force eccentric exercise. Akimoto et al. (2002) documented that levels of CRP increase with muscle damage caused by eccentric exercise.

A high sensitivity enzyme immunoassay (EIA) test kit (ICN Pharmaceuticals, Costa Mesa, CA) was used to determine the serum concentration of CRP. The sensitivity of this EIA kit is 0.1 mg/L and an intra-assay coefficient of variation equal to 7.5 to 4.1% within the range of expected values (0.068 to 8.2 mg/L). Absorbance of the EIA was measured at 450 nm using a μ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). Samples were run in duplicate.

Interleukin-6 (IL-6)

Interleukin-6 holds an integral role in the acute-phase response of inflammation, which is why plasma levels of IL-6 increase in response to high-force eccentric exercise

(Willoughby et al., 2003). Interleukin-6 also induces the synthesis of CRP (Blake & Ridker, 2002). Interleukin-6 is an indicator of magnitude and the time to peak response of the inflammatory response.

The serum concentration for IL-6 was determined using a high sensitivity EIA kit (R&D Systems, Minneapolis, MN). High sensitivity kits for IL-6 were used to increase accuracy for characterization of the inflammatory response and differentiation among subjects. Plates were read at 450 nm and corrected for imperfections in the plate by subtracting readings at 540 nm using a μ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). Samples were run in duplicate.

Creatine Kinase

Creatine kinase is an indirect marker of muscle damage. The amount of CK in the plasma is not directly proportional to the severity of muscle damage induced by high-force eccentric exercise, but CK is related to muscle damage and inflammation (Miles & Clarkson, 1994).

Serum CK levels were measured using an ultraviolet, kinetic assay (Thermo Electron Corporation, Waltham, MA). Modifications for microplate analysis were made and were read using a μ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). Samples were run in duplicate.

Data Reduction

The magnitude of the peak response of each variable of interest was calculated by subtracting the baseline value (0h) from the exercise condition for each variable (Table

3.5). Initially, the control condition was to be used in the data reduction; however baseline levels of dependent variables of the exercise conditions and control condition were not comparable. The 0h blood draw for each exercise condition was used as the control throughout each exercise condition and the control condition was not used.

Table 3.5 Layout of calculations for the magnitude of response.

Non-dominant Biceps	Dominant Biceps
0h EX _{ND} – 0h EX _{ND} = Magnitude	0h EX _D – 0h EX _D = Magnitude
4h EX _{ND} – 0h EX _{ND} = Magnitude	4h EX _D – 0h EX _D = Magnitude
8h EX _{ND} – 0h EX _{ND} = Magnitude	8h EX _D – 0h EX _D = Magnitude
12h EX _{ND} – 0h EX _{ND} = Magnitude	12h EX _D – 0h EX _D = Magnitude
24h EX _{ND} – 0h EX _{ND} = Magnitude	24h EX _D – 0h EX _D = Magnitude
120h EX _{ND} – 0h EX _{ND} = Magnitude	120h EX _D – 0h EX _D = Magnitude

Data Analysis

Data were analyzed by determining the response of the inflammatory system regarding the magnitude of and the time to peak response. The magnitude of the response was calculated by subtracting the baseline value from the exercise condition for each of the variables (CRP, IL-6 and CK) for each blood draw, respectively. Creatine kinase was calculated at 24h and 120h because CK peaks between 96 and 120 hours post-exercise (Nosaka et al., 1991; Stupka et al., 2001). Finally, subjects were ranked individually for CRP, IL-6, CK, maximal isometric strength losses, muscle soreness and swelling according to the responses, using a Spearman rank order correlation. Time to

peak response was calculated by pinpointing the hour at which the variable peaked. A repeated measures ANOVA was used to determine differences over time, between arms and also to detect significant arm by time interactions. The primary use of this statistical analysis was for interpretation of the results as well as future research.

Subjects with elevated baseline levels were omitted from analysis. Elevated CRP levels were defined as greater than 10.0 mg/L. Elevated IL-6 levels were defined as greater than 5.0 pg/mL. Also, subjects with variables that were greater than two units of measurement apart for the ND and D conditions at baseline were excluded from analysis. All subjects included in the statistical analysis experienced at least a 10% decrease in maximal isometric strength performed immediately post-exercise. No parameters were set for CK, muscle soreness or swelling.

CHAPTER FOUR

RESULTS

Subject Characteristics

Twelve healthy, college-aged subjects (six female and six male) participated in the study. Nine of the 12 subjects completed all three conditions. Three subjects chose to withdraw from the study due to equipment failure, time constraints or personal reasons. In addition, two subjects were eliminated from the statistical analysis due to failure of reaching sufficient strength loss and elevated IL-6 levels at baseline. Seven subjects were included in the statistical analysis (three female, four male). A 10% decrease in maximal isometric strength occurred with all but one subject who had an increase in strength and was excluded from the final analysis. Baseline IL-6 levels for the other subject, who was excluded from the final analysis, were higher than 5 pg/mL, which was the cut off point. During the D exercise condition, one subject had greater maximal isometric strength immediately following the high-force eccentric exercise in the D arm. Thus, researchers used the maximal isometric strength values for the D arm from the ND exercise condition. All subjects adhered to the inclusion and exclusion criteria while participating in the study.

The descriptive characteristics of the subjects included in the statistical analysis are reported in Table 4.1. Baseline values of all variables, except soreness, are reported in Table 4.2. The mean age of subjects was 19 ± 1.4 years and the mean BMI was 24.3 ± 3.4 kg/m². Five subjects completed the ND arm first and two subjects completed the D

arm first. As previously mentioned, exercise conditions were not balanced due to equipment failure and attrition.

Table 4.1 Descriptive characteristics of research subjects.

ID	Sex	Age	Height (m)	Weight (kg)	BMI (kg/m ²)	Order
1	M	19	1.80	95	29.2	ND, D
2	F	20	1.65	61	22.5	ND, D
3	M	18	1.83	96	28.5	ND, D
4	M	19	1.91	89	24.4	ND, D
5	F	20	1.73	62	20.9	ND, D
6	F	22	1.63	64	24.1	D, ND
7	M	18	1.75	64	20.7	D, ND
AVG		19.43	1.76	75.7	24.3	
SD		1.40	0.10	16.4	3.4	

M = male, F = female, BMI = body mass index, ND = non-dominant, D = dominant

Table 4.2 Baseline values for dependent variables.

	Avg ± SD	Min	Max
CRP (mg/L)			
D	1.14 ± 1.41	0.112	2.893
ND	1.15 ± 1.74	0.063	2.075
IL-6 (pg/ml)			
D	1.76 ± 1.23	0.45	3.36
ND	2.60 ± 2.48	0.59	3.34
CK (IU/L)			
D	233.84 ± 218.82	84.1	786.2
ND	179.76 ± 66.78	117.9	294.6
Strength (% decrease)			
D	28.46 ± 19.22	10.6	58.8
ND	33.52 ± 14.80	14.6	53.3
Circumference (cm)			
D	30.21 ± 4.02	25.3	36.1
ND	30.07 ± 4.56	24.5	36.5

D = dominant, ND = non-dominant

C-Reactive Protein

The time course for the average response for CRP is reported in Figure 4.1a. Individual responses for CRP in the ND arm and D arm for each subject are reported in Figure 4.1b and Figure 4.1c. Peak responses for CRP ranged from -0.155 mg/L to 0.137mg/L at 12h post-exercise for the ND elbow flexors. For the D elbow flexors, peak responses ranged from -0.37 mg/L to 8.332 mg/L. A repeated measures ANOVA did not detect differences over time ($F = 0.906$, $p = 0.382$) or between arms ($F = 1.044$, $p = 0.346$), and there was not a significant arm by time interaction ($F = 1.076$, $p = 0.347$). C-reactive protein responded to high-force eccentric exercise in the ND arm only, although not significant.

The peak response occurred at 12h post-exercise for the ND arm. There was no response in the D arm. The Spearman rank order correlation for the ND and D exercise conditions was not significant at 12h ($R = 0.393$, $p = 0.383$) (Figure 4.1d) or 24h ($R = -0.607$, $p = 0.148$) post-exercise (not shown).

Interleukin-6

A repeated measures ANOVA did not detect any differences between arms ($F = 2.066$, $p = 0.201$) and there was not a significant arm by time interaction ($F = 0.263$, $p = 0.745$). However, a trend was detected over time ($F = 3.045$, $p = 0.107$). Interleukin-6 peaked 8h post-exercise for both ND and D exercise conditions and returned to baseline at 12h (Figure 4.2a). Individual responses for IL-6 in the ND arm and D arm are reported in Figure 4.2b and Figure 4.2c. The peak IL-6 responses for the ND elbow flexors

ranged from -2.938pg/ml to 2.571 pg/ml at 8h post-exercise. For the D elbow flexors, peak responses ranged from -1.857 pg/ml to 4.447 pg/ml. The highest peak for both conditions occurred in the same subject.

The peak response occurred at 8h post-exercise for both arms. The Spearman rank order correlation detected a trend between the ND and D exercise conditions at 8h post-exercise ($R = 0.714$, $p = 0.071$) (Figure 4.2d).

Creatine Kinase

A repeated measures ANOVA detected a trend between arms ($F = 3.816$, $p = 0.099$), but did not detect any differences over time ($F = 2.282$, $p = 0.176$) and there was not a significant arm by time interaction ($F = 2.963$, $p = 0.133$). The time course for CK is shown in Figure 4.3a.

The Spearman rank order correlation found a significant correlation between the ND and D exercise conditions at 120h post-exercise ($R = 0.893$) ($p < 0.05$). The rank order correlation for CK is shown in Figure 4.3b.

Maximal Isometric Strength

A repeated measures ANOVA detected significant changes over time ($F = 18.670$, $p < 0.001$), but no significant changes occurred between arms ($F = 0.271$, $p = 0.621$) and there was not a significant arm by time interaction ($F = 0.780$, $p = 0.521$). For the ND and D exercise conditions, maximal isometric strength decreased significantly immediately post-exercise and returned to near baseline values by 120h (Figure 4.4a). There was a slightly greater strength loss with the ND arm, while not significant.

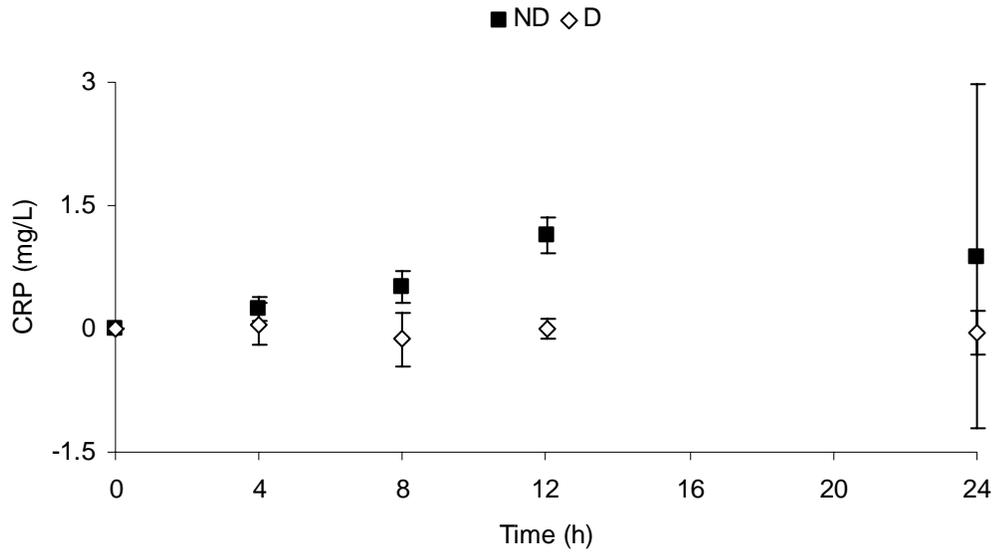


Figure 4.1a Time course for CRP following high-force eccentric exercise. Values are mean \pm SD.

ND = non-dominant arm trial, D = dominant arm trial.

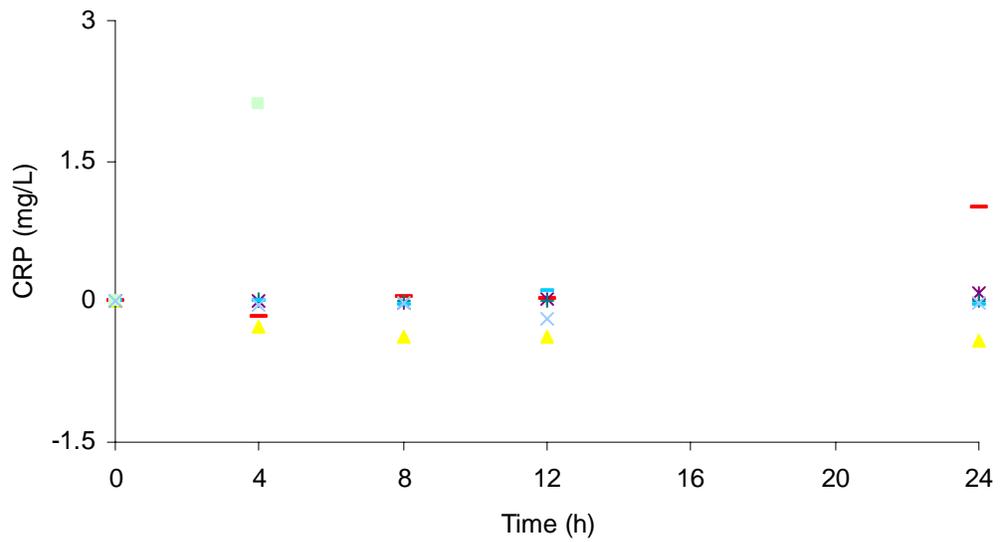


Figure 4.1b Time course for CRP for each subject in the ND arm following high-force eccentric exercise.

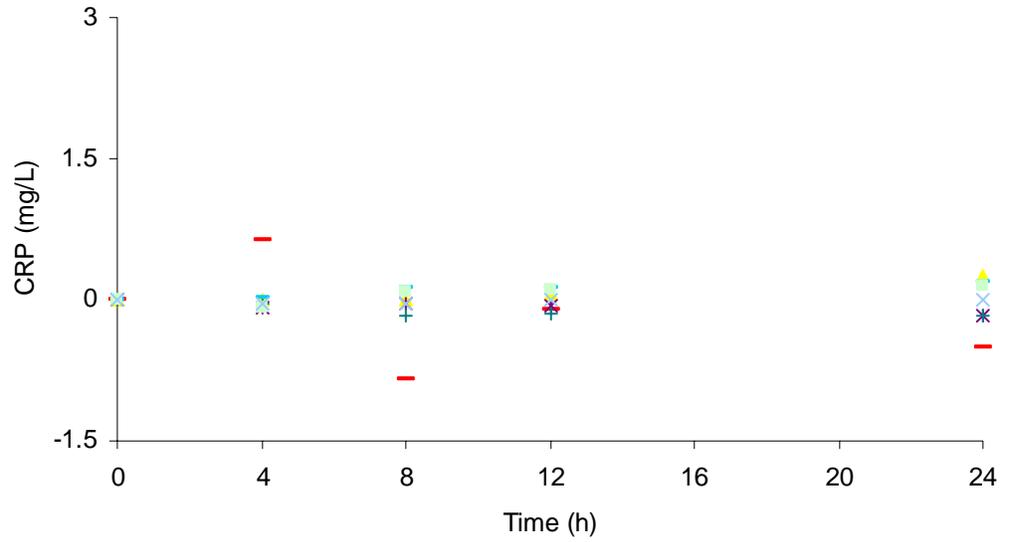


Figure 4.1c Time course for CRP for each subject in the D arm following high-force eccentric exercise.

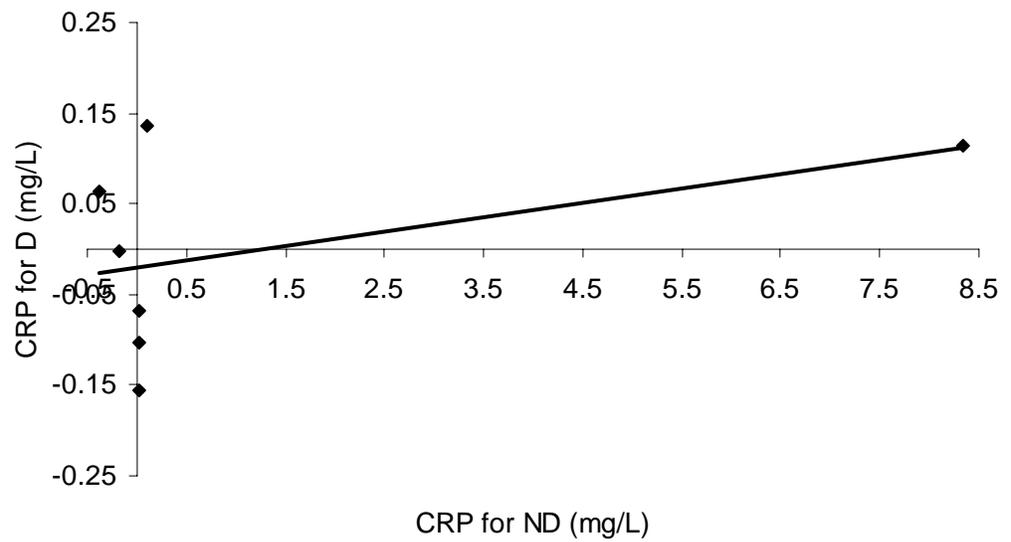


Figure 4.1d Peak responses for CRP (12h post-exercise) for the dominant (D) versus the non-dominant (ND) arms.

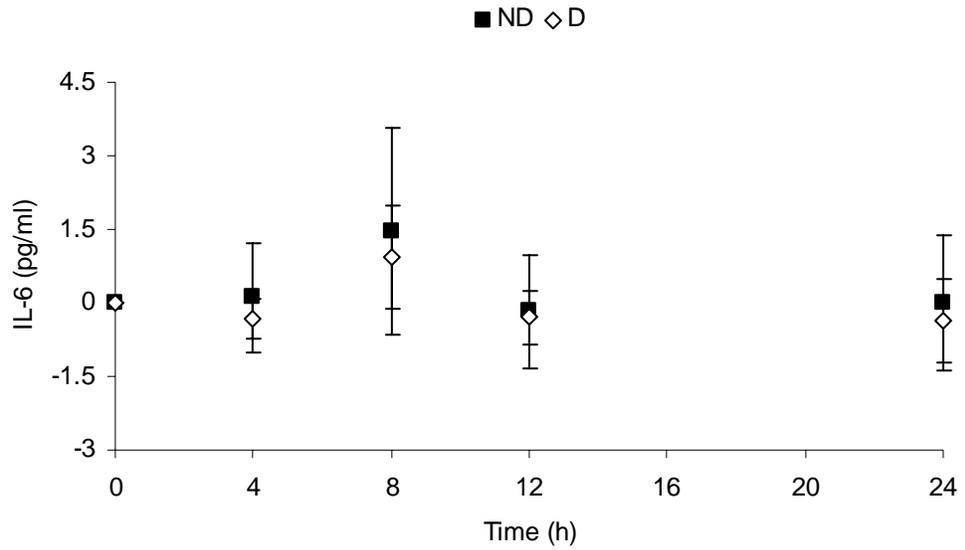


Figure 4.2a Time course for IL-6 following high-force eccentric exercise. Values are mean ± SD.

ND = non-dominant arm trial, D = dominant arm trial.

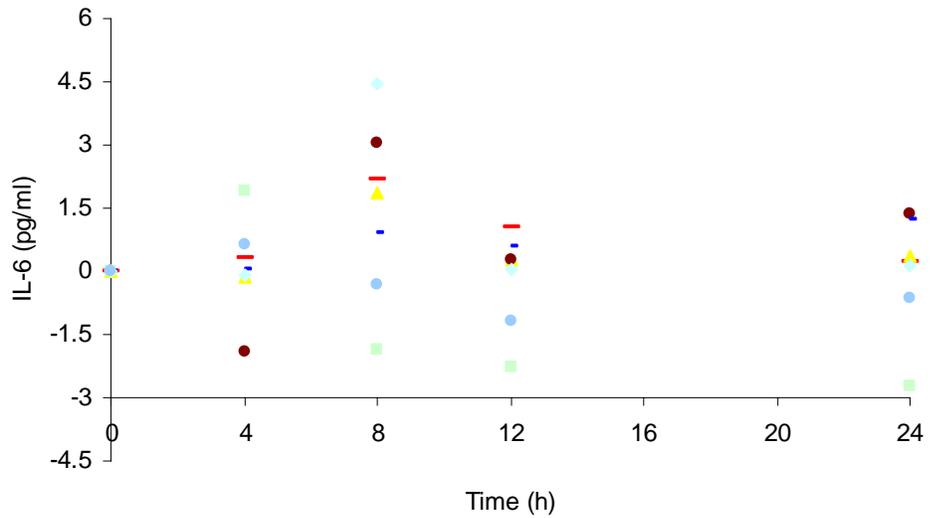


Figure 4.2b Time course for IL-6 for each subject following high-force eccentric exercise in the ND arm.

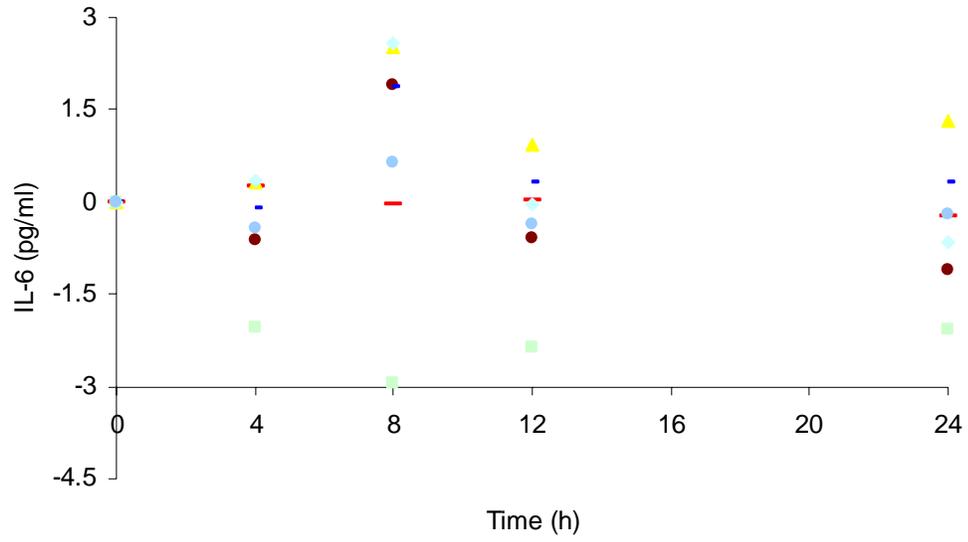


Figure 4.2c Time course for IL-6 for each subject following high-force eccentric exercise in the D arm.

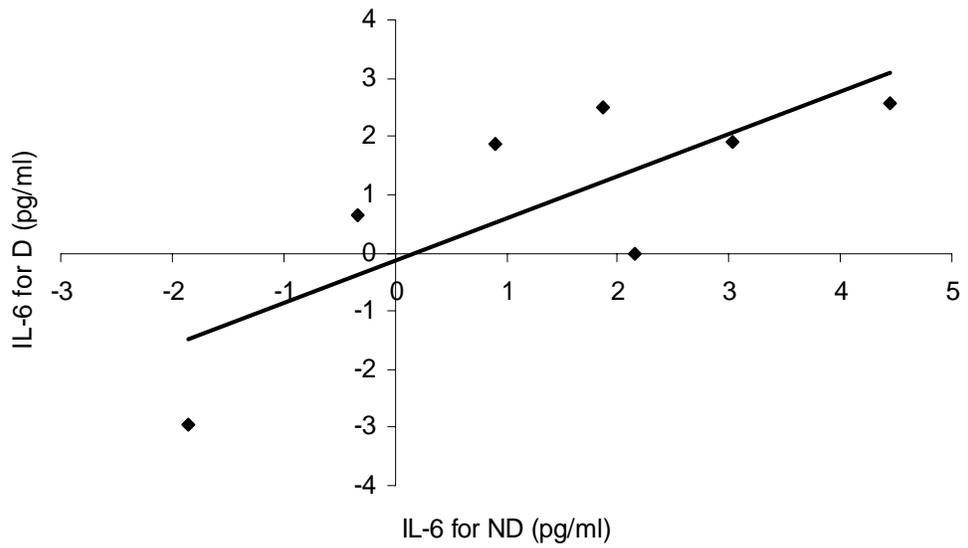


Figure 4.2d Peak responses for IL-6 (8h post-exercise) for the dominant (D) versus the non-dominant (ND) arms.

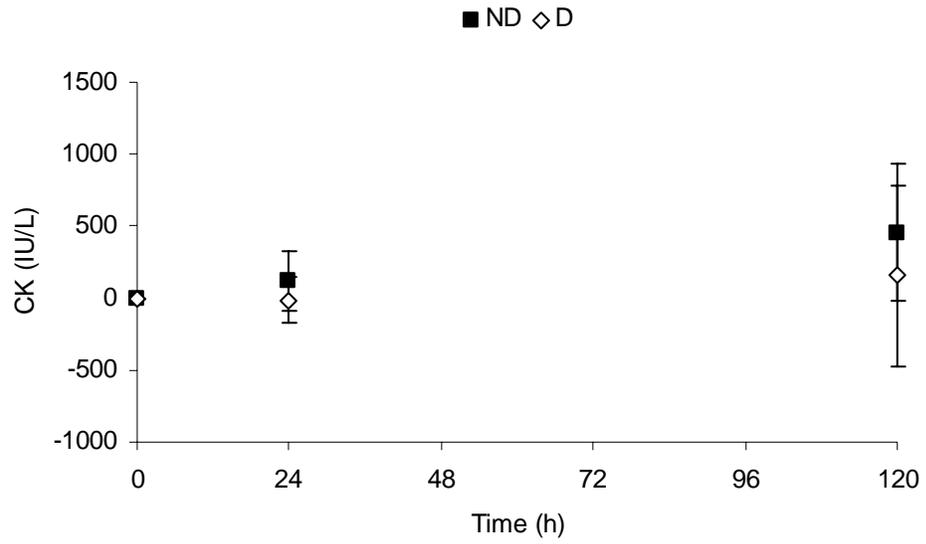


Figure 4.3a Time course for CK following high-force eccentric exercise. Values are mean \pm SD.

ND = non-dominant arm trial, D = dominant arm trial.

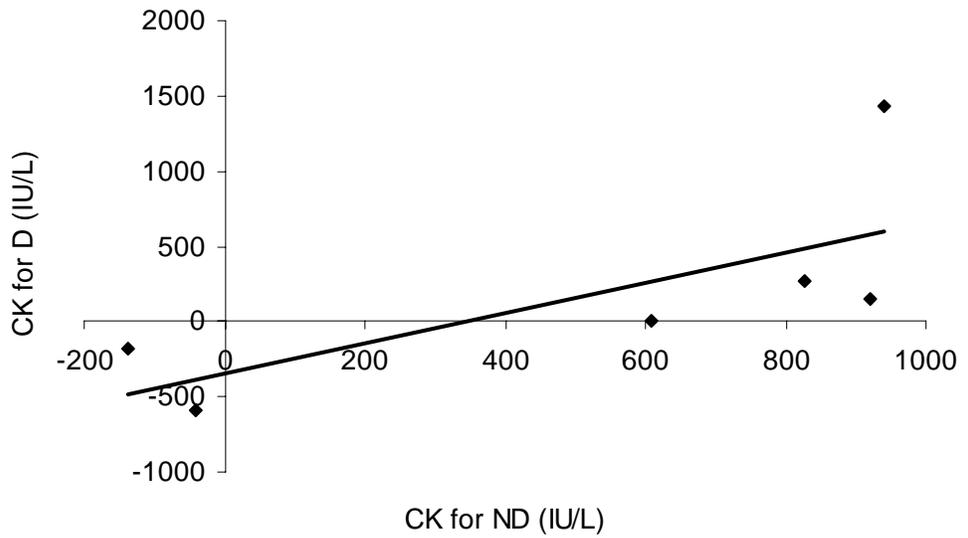


Figure 4.3b Peak responses for CK (120h post-exercise) for the dominant (D) versus non-dominant (ND) arms.

A significant rank order correlation ($R = 0.786$) ($p < 0.05$) was found for maximal isometric strength losses for the two exercise conditions immediately post-exercise. The rank order correlation is shown in Figure 4.4b.

Muscle Soreness

A repeated measures ANOVA detected a significant difference over time ($F = 11.742$, $p < 0.001$) but did not detect differences between arms ($F = 0.464$, $p = 0.521$) and there was not a significant arm by time interaction ($F = 0.923$, $p = 0.407$). Soreness increased for both conditions at 24h post-exercise, while the D arm was reported as having less soreness than that of the ND arm (Figure 4.5a). Five of the seven subjects completed take home soreness logs that included time points at 48h, 72h and 96h post-exercise (Figure 4.5b). Soreness returned to near baseline levels by 120h post-exercise.

A Spearman rank order correlation detected no significant correlation at 24h ($R = -0.143$, $p = 0.76$) (Figure 4.5c). Analyses were run at 48h as well, but no significance was found ($R = -0.200$, $p = 0.747$).

Swelling

A repeated measures ANOVA did not detect any significant changes between arms ($F = 0.441$, $p = 0.532$), over time ($F = 1.909$, $p = 0.122$) and there was not a significant arm by time interaction ($F = 0.968$, $p = 0.453$). The time course of swelling is reported in Figure 4.6a.

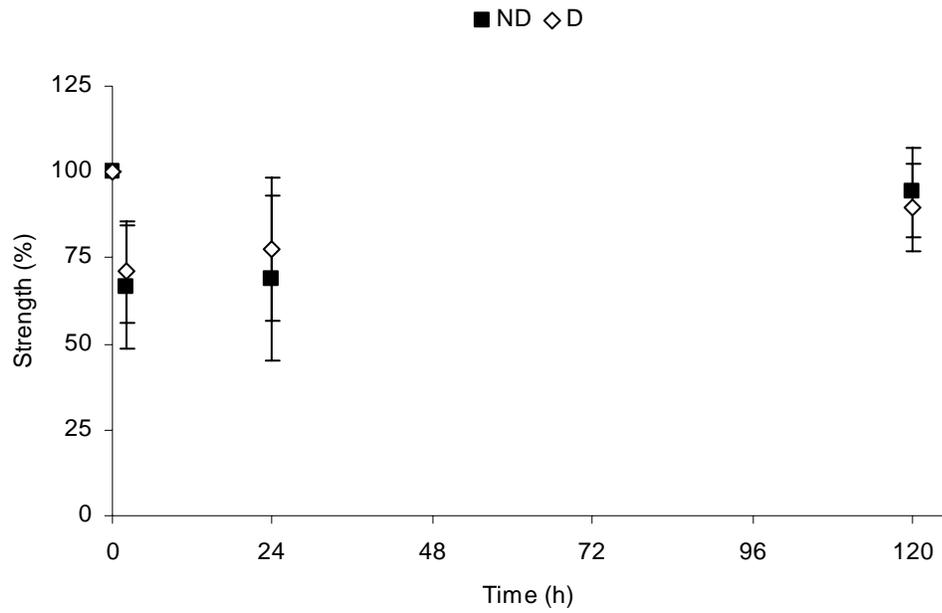


Figure 4.4a Time course for maximal isometric strength following high-force eccentric exercise.

Values are mean \pm SD. ND = non-dominant arm trial, D = dominant arm trial.

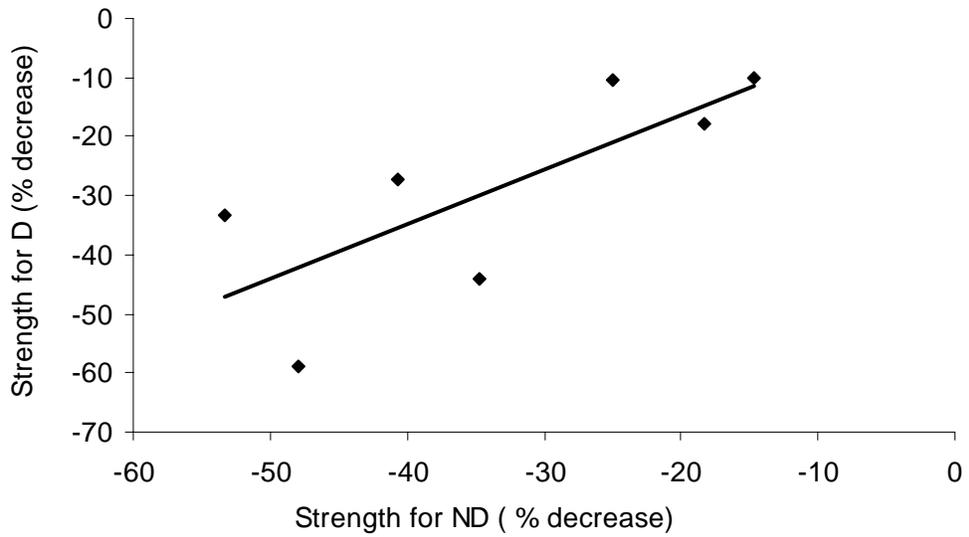


Figure 4.4b Peak responses for maximal isometric strength (immediately post-exercise) for the dominant (D) versus the non-dominant (ND) arms.

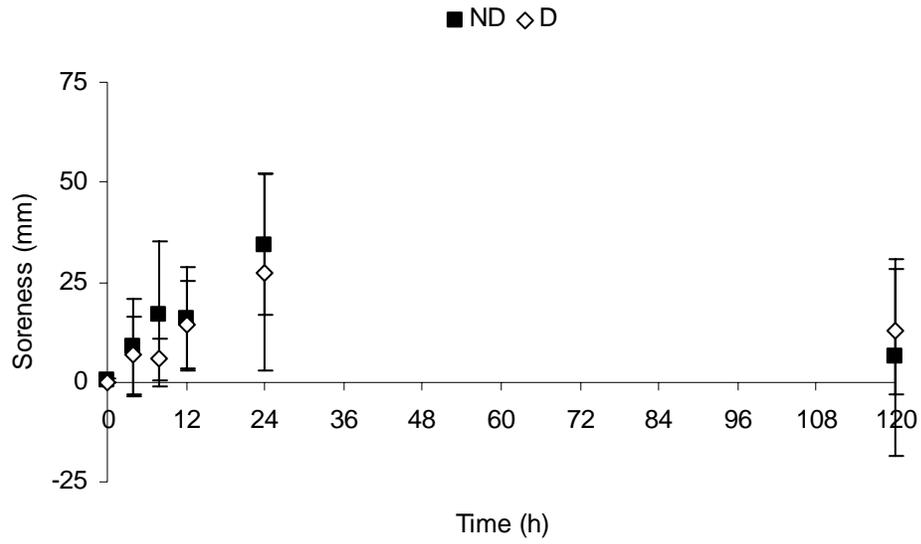


Figure 4.5a Time course for soreness following high-force eccentric exercise. Values are mean \pm SD. ND = non-dominant arm trial, D = dominant arm trial.

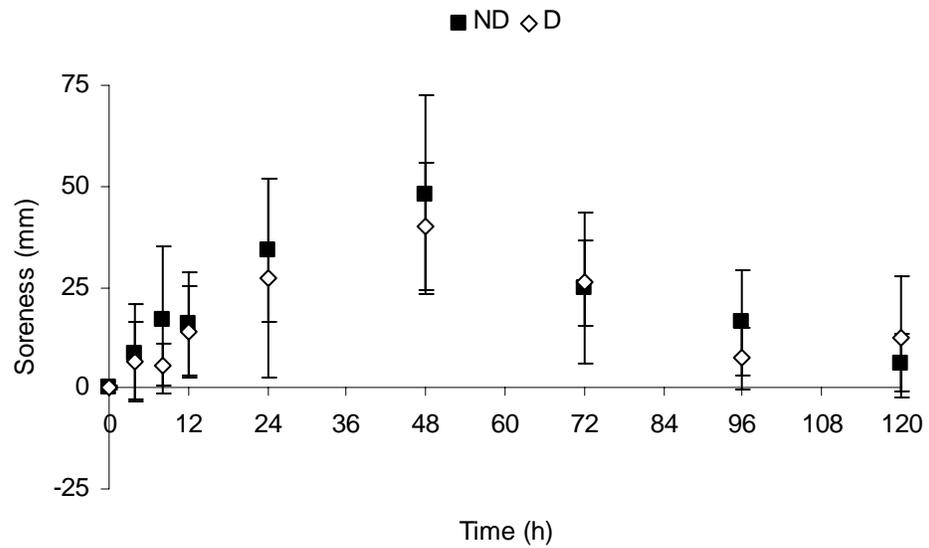


Figure 4.5b Time course for soreness following high-force eccentric exercise including the take home log at 48h, 72h and 96h. Values are mean \pm SD. ND = non-dominant arm trial, D = dominant arm trial.

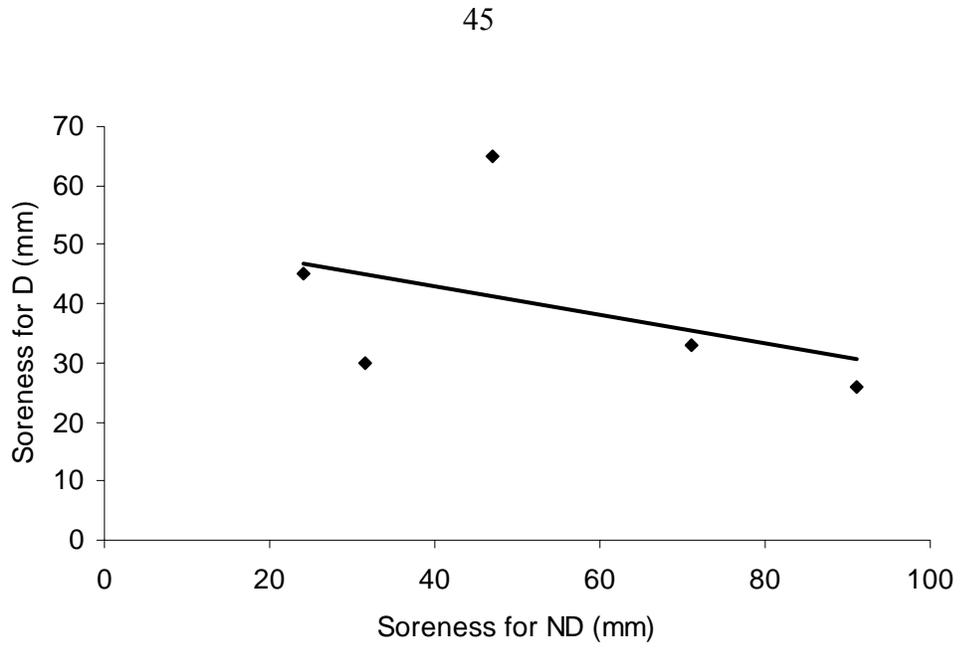


Figure 4.5c Peak responses for soreness (24h post-exercise) for the dominant (D) versus the non-dominant (ND) arms.

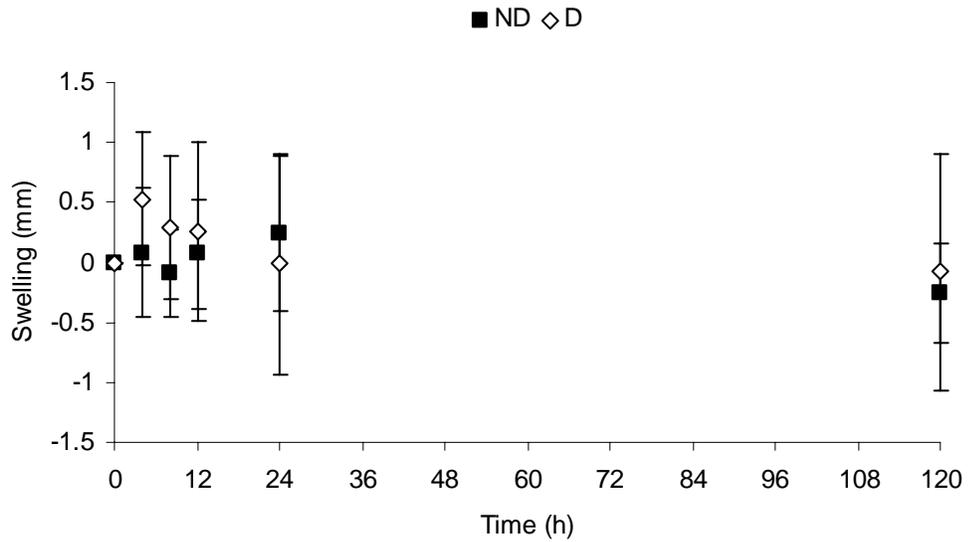


Figure 4.6a Time course for swelling following high-force eccentric exercise. Values are mean \pm SD. ND = non-dominant arm trial, D = dominant arm trial.

The Spearman rank order correlation was not significant for swelling ($R = -0.324$) ($p = 0.48$) at 24h post-exercise. The rank order correlation for swelling is shown in Figure 4.6b.

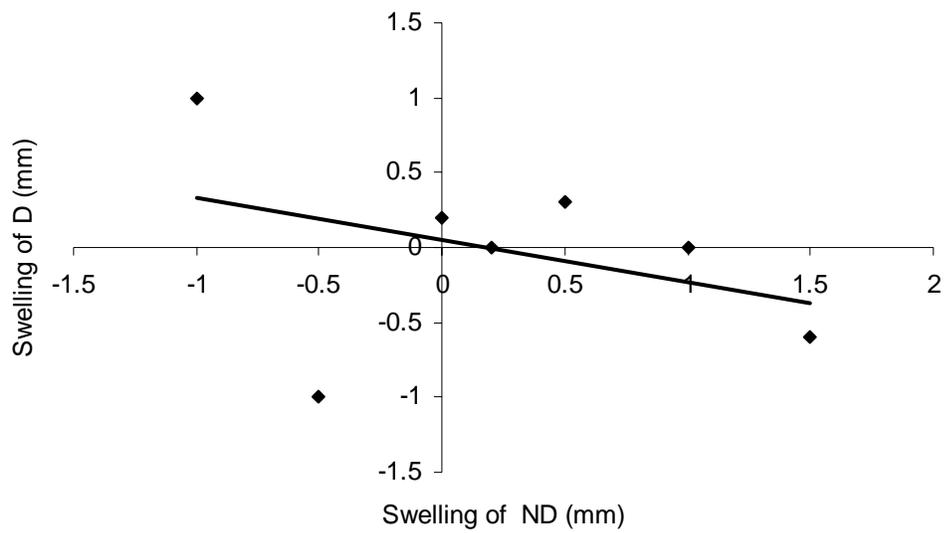


Figure 4.6b Peak responses for swelling (24h post-exercise) for the dominant (D) versus the non-dominant (ND) arms.

CHAPTER FIVE

DISCUSSION

Introduction

Inflammation is positively associated with the development of atherosclerosis and CRP is one of the best markers of this inflammation (Blake & Ridker, 2002). Other inflammatory markers are not used to assess CHD risk because they are not as stable as CRP, the assays are not readily available, or the costs to run them are high (Pearson et al., 2003). C-reactive protein can be used to assess the risk of developing CHD even in the absence of traditional risk factors. Pearson et al. (2003) conducted analyses of more than 40,000 subjects from more than 15 different populations to evaluate basal CRP levels. The American Heart Association (AHA) and the Center for Disease Control (CDC) collaborated and designed categories for CRP levels and the risk of developing CHD: low risk < 1.0 mg/L, average risk = 1.0 – 3.0 mg/L and high risk > 3.0 mg/L. Individuals categorized as high risk had a two-fold relative risk increase in developing CHD compared with the low risk group. The use of CRP as a risk marker for CHD is becoming common; however, more extensive research is needed before CRP may be categorized as an actual risk factor and causally linked to the development of the disease.

The present study was a continuation of Dr. Mary Miles' larger study, in which she is investigating the inflammatory response using high-force eccentric exercise of the elbow flexors. The aim of that study is to determine how individuals control inflammation and how basal CRP levels affect the magnitude of the CRP response. The

present study was used to evaluate the consistency of the inflammatory response within individuals as well as assess the model of Dr. Miles' current research.

The aim of this study was to investigate how consistent the inflammatory response is within individuals after high-force eccentric exercise of the ND and D elbow flexors. We hypothesized that each variable measured would respond consistently after each exercise condition; specifically, the two separate inflammatory responses, one for the D arm and one for the ND arm, would correlate significantly in a Spearman rank order correlation (Table 5.1). Due to the limited sample size, only CK and maximal isometric strength significantly rank order correlated while a trend was measured for IL-6. A larger sample size could clarify and strengthen the findings.

Table 5.1 Summary of variables and rank order correlation coefficients.

	R	p
CRP	0.393	0.383
IL-6	0.714	0.071 [†]
CK	0.893	< 0.05*
Isometric Strength Decrease	0.786	< 0.05*
Muscle Soreness	-0.143	0.76
Swelling	-0.324	0.48

[†] denotes a trend, * denotes significance

C-Reactive Protein

All subjects from the present study had low basal CRP levels and were categorized as low risk or average risk for developing CHD (Pearson et al., 2003). For both exercise conditions, CRP ranged from 0.06 – 2.9 mg/L with 2 subjects in the average risk group and 5 subjects in the low risk group.

C-reactive protein was analyzed to determine the magnitude of the inflammatory response after high-force eccentric exercise of the elbow flexors. It was hypothesized that CRP would respond in the same rank order for both exercise conditions. There was no rank order correlation between the CRP responses of the ND and D arms within individuals. However, CRP did not respond to either exercise condition, thus the lack of response was consistent. Generally, subjects did not respond to the high-force eccentric exercise. However, following the high-force eccentric exercise of the ND arm one subject approximately doubled the CRP levels at 4h post-exercise, but had no response for the D condition.

A possible explanation for the lack of response in CRP could be that high-force eccentric exercise only induces a response when basal CRP levels are elevated. This concept stems from the findings in Dr. Miles' current research. As shown in Figure 5.1, subjects with high basal CRP levels responded to high-force eccentric exercise in the ND elbow flexors and subjects who did not have elevated basal levels did not respond. All subjects in the present study had low basal CRP levels, which could explain why no response occurred. If the sample size was larger and included subjects in the high risk CRP category, it is speculated that those subjects would have responded to the high-force eccentric exercise. Once a wider range of responses is reported, the consistency of CRP following high-force eccentric exercise could be determined. The findings from Dr. Miles' research are preliminary, but are important to the interpretation of the present study.

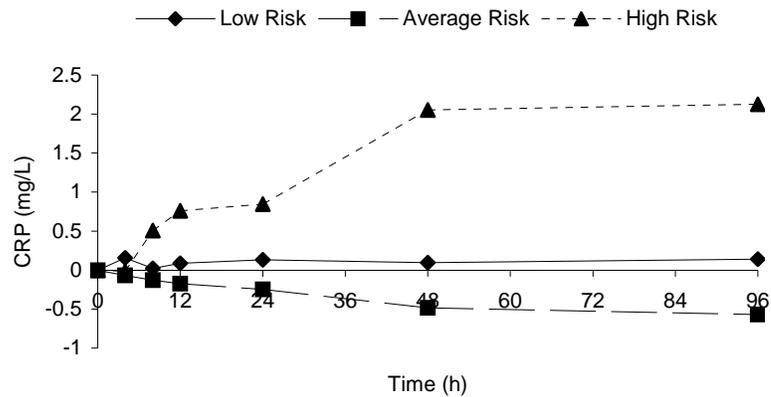


Figure 5.1 Peak responses of CRP after high-force eccentric exercise in the ND elbow flexors.

The CRP response to exercise differs depending on different types of muscle damage and which muscle group is targeted. For example, Akimoto et al. (2002) investigated inflammation in response to bicycle ergometer exercise (80% VO_2 max), endurance running (42 km) and downhill running (-5% grade for 30 min). C-reactive protein increased significantly ($p < 0.01$) after endurance and downhill running, however a much greater increase occurred with endurance running. The significant differences for both variables were calculated one day post-exercise. One possible explanation for the difference could be that different muscle groups were used. The amount of muscle mass in the leg is much greater than that of the arm, which may affect the response. Furthermore, two different muscle groups were exercised in Akimoto's study (knee flexors and knee extensors) and the current study focused on one muscle group (elbow flexors). As reviewed by Peak et al. (2005), the cytokine response is distinctly different between downhill running and high-force eccentric exercise of the elbow flexors; the

time course is altered and there may be a greater extent of muscle damage with downhill running. However, more research needs to be conducted in order to draw a conclusion.

In regards to the present study, CRP was of interest because it is not known as to why this inflammatory biomarker is elevated in some individuals and not others. Furthermore, it is not known as to why individuals with low basal CRP levels do not respond to high-force eccentric exercise with an increase in CRP. Thus, a model to investigate inflammation would be a valuable tool for determining the mechanisms differentiating individuals in low versus high risk categories for CHD. The aim of this study was to evaluate the consistency of inflammatory responses in one particular model.

Interleukin-6

Interleukin-6 was analyzed because it is an important regulator of the inflammatory response after high-force eccentric exercise in the ND and D elbow flexors. It was hypothesized that IL-6 would respond consistently and a significant rank order correlation would be detected between the ND and D arms within individuals. Although no significant rank order correlation was measured, a trend was detected. A larger sample size could clarify whether or not that trend could be significant. Thus, IL-6 has the potential to be consistent in response to two high-force eccentric exercise bouts in the ND and D elbow flexors if the sample size was increased.

The time course for peak IL-6 responses was similar between the ND and D elbow flexors. In general, IL-6 levels peaked at 8h post-exercise. Specifically, five subjects peaked at 8h post-exercise and two subjects peaked at 4h post-exercise for the ND condition and for the D condition four subjects peaked at 8h post-exercise, 2 subjects

peaked at 4h post-exercise and one subject peaked at 24h post-exercise. The sample size was limited so the individual responses were reported as well as the mean responses.

Another finding from Dr. Miles' current research is that high-force eccentric exercise might stimulate an increase in IL-6 only in the low risk CRP group (Figure 5.2). The study at hand was consistent with this suggestion because all subjects fell into either the low or average risk CRP categories while IL-6 increased at 8h post-exercise. However, subjects in all three CRP risk categories are needed before a conclusion can be made.

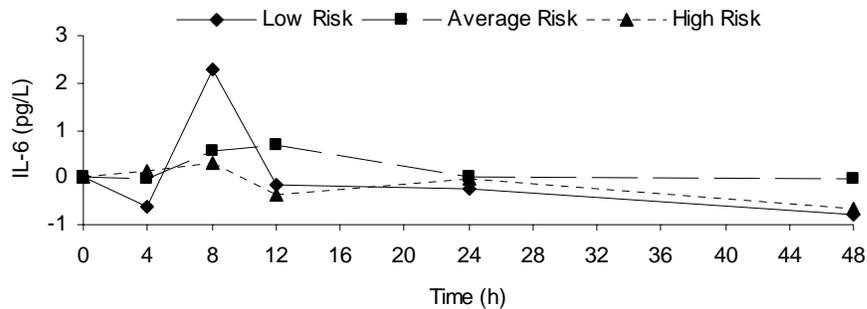


Figure 5.2 Peak responses of IL-6 after high-force eccentric exercise in the ND elbow flexors.

The outcomes from Dr. Miles' research and the present study conflict with previous research. The primary difference is that an increase in IL-6 was highly correlated to an increase in CRP (Ridker, Rifai, Stampfer & Hennekens, 2000; Yudkin, Kumari, Humphries & Mohamed-Ali, 2000). However, in the present study, IL-6 increased but did not lead to an increase in CRP. A plausible explanation for the difference could be that Ridker et al. (2000) and Yudkin et al. (2000) investigated chronic

inflammation and the present study utilized high-force eccentric exercise to induce acute inflammation.

The time course for IL-6 is quite variable. The mode, intensity and duration of the exercise as well as the muscle group being exercised all affect the response. Smith et al. (2000) investigated the cytokine response after the eccentric phases of a bench press and leg curl. Subjects completed 4 sets of 12 repetitions. Smith et al. reported a significant increase at 12h, 24h and 72h post-exercise of more than 4 times that of baseline values, while IL-6 increased only at 8h post-exercise and the magnitude was not as high in the present study. Studies involving larger muscle groups, such as the quadriceps, and different exercise protocols (70 – 300 repetitions) resulted in a greater IL-6 response (MacIntyre et al. 2001; Willoughby et al. 2003). Hirose et al. (2004) investigated repeated bouts of eccentric exercise in the elbow flexors. Subjects completed 2 bouts of eccentric exercise (30 repetitions) with 4 weeks of rest between bouts. No significant change was found for IL-6 after either exercise bout, which is consistent with results from the present study because a trend, but no significant change, was reported over time. These findings are important for future research because this exercise model and basal CRP levels could affect the magnitude and time course of IL-6 in response to high-force eccentric exercise.

Creatine Kinase

Creatine kinase was analyzed to assess muscle damage in response to high-force eccentric exercise in the ND and D elbow flexors. Creatine kinase is an intramuscular enzyme that increases in the blood after muscle damage (Nosaka et al., 1991). It was

hypothesized that a significant rank order correlation would be detected between the ND and D elbow flexors within individuals.

Creatine kinase was consistent ($p < 0.05$) within individuals in response to two bouts of high-force eccentric exercise. These results are consistent with the results from a study directed by Nosaka and Clarkson (1996), which assessed the variability in CK responses after eccentric exercise of the elbow flexors in the ND and D arms; a significant rank order correlation was detected in 3 out of 5 subjects. Although Nosaka and Clarkson (1996) and the present study had similar CK responses, both studies have a limited sample size. Thus, further research and larger sample sizes would provide a more clear understanding of CK responses in the ND and D arms.

In response to high-force eccentric exercise in the elbow flexors, CK increased at 24h post-exercise and peaked at 120h post-exercise. This time course is similar, but not identical to previous research. Hirose et al. (2004) and Nosaka et al. (2001) reported increases at 24h but the peak response occurred at 96h post-exercise, but no measurements of CK were taken at 120h post-exercise. If the two previous studies and the present study collected data at 96h and 120h post-exercise a more complete comparison could have been made.

The magnitude of the CK response was modest in the study at hand relative to previous research. Creatine kinase has been reported to increase greatly in response to high-force eccentric exercise. Hirose et al. (2004) and Nosaka et al. (2001) reported increases up to 7,000 – 10,000 IU/L after 24 and 30 eccentric contractions, respectively, in the elbow flexors. Mean increases of 153 IU/L for the D arm and 455 IU/L for the ND

arm were measured in the present study. A reason for this difference could be that not as much muscle damage occurred in the present study. This may be due to lack of maximal effort with each repetition or to different exercise protocols.

Researchers have reported attenuated CK responses in females relative to males (Stupka et al., 2000) after exercise-induced muscle damage. The findings from this study are not consistent with previous research. The average increase in CK at 120h post-exercise for males was 418.8 ± 534.1 IU/L and the average increase was 503.4 ± 499.3 IU/L for females at 120h. The findings from this study and previous research differ in that males had a lower response relative to females. If sex differences do occur in CK response to high-force eccentric exercise it is possible that sex differences occur for the inflammatory response as well. Regardless of sex differences, further research is needed to determine the consistency of CK response after high-force eccentric exercise. Due to the variability of the response and the limited sample size (three female, four male) a larger sample size is needed to verify the results from previous research.

Maximal Isometric Strength

A significant rank order correlation was measured for maximal isometric strength losses ($p < 0.05$) within individuals in response to the two exercise conditions. Very little research has been conducted to determine the rank order correlation of strength losses in response to high-force eccentric exercise in the ND and D elbow flexors.

Strength decreased significantly ($p < 0.001$) immediately post-exercise for both exercise conditions. These results are consistent with results from various other studies. After high-force eccentric exercise, strength decreased immediately and started returning

to baseline levels after that and reached or nearly reached baseline 5 – 10 days post-exercise (Hirose et al., 2004; Nosaka & Clarkson, 1996; Nosaka et al., 2001).

The magnitude of strength loss is dependent on the extent of eccentric exercise. Nosaka et al. (2001) reported a 20% strength loss after 2 high-force eccentric contractions, a 33% strength loss after 6 high-force eccentric contractions and a 56% strength loss after 24 high-force eccentric contractions. In response to the 45 repetitions completed in the current study, a 28% strength loss for the D arm and a 33% strength loss for the ND arm were reported immediately post-exercise. This difference could be due to the difference in exercise protocols or that not as much muscle damage occurred.

Muscle Soreness

Muscle soreness was measured to help determine the extent of muscle damage. There was no significant rank order correlation between the soreness responses of the ND and D arms within individuals. Muscle soreness is a subjective measure, which could explain the lack of rank order correlation.

Soreness increased significantly ($p < 0.001$) over time and peaked at 24h post-exercise. These results are not consistent with previous research, which reported that soreness peaked at 48h post-exercise (Hirose et al., 2004; Nosaka et al., 2001). Plausible reasoning behind this difference is that soreness was not measured at 48h in the current study. A take home soreness log (for 48h, 72h and 96h post-exercise) was used, but only five of the seven subjects completed and returned the log so a complete analysis could not be performed. However, the findings from the take home soreness log were that

soreness peaked at 48h post-exercise then diminished and nearly reached baseline at 120h post-exercise, which is consistent with Hirose et al. (2004) and Nosaka et al. (2001).

Although the exercise protocols differed (24 and 30 versus 45 repetitions) the magnitude of peak soreness of the current study (34 mm) was similar to that of peak soreness values documented in previous research (37 mm) (Hirose et al., 2004; Nosaka et al., 2001) for the ND arm. Muscle soreness did not increase as greatly for the D arm. It was assumed that the D arm was more accustomed to muscle damage because the elbow flexors of the D arm may have been used more often, which resulted in less muscle soreness.

Swelling

No significant rank order correlation was found within individuals in response to high-force eccentric exercise. However, no response occurred so it is worth mentioning that the response was consistently unresponsive.

Typically, swelling of the elbow flexors occurs immediately post-exercise and peaks 96h post-exercise (Hirose et al., 2004; Nosaka et al., 2001). Although no swelling was reported, no measurements were taken at 96h.

While the present study did not measure any significant changes in swelling, other studies have. Hirose et al. (2004) and Nosaka et al. (2001) both document significant increases in muscle circumference in response to high-force eccentric exercise. One possible explanation could be that these research studies measured circumference at 3, 5, 7, 9 and 11 cm from the elbow joint and the present study used only one point of

measurement at mid-muscle which ranged from 12.2 cm – 17.5 cm (average = 14.6 cm) from the elbow joint. Another possible explanation for the difference in the magnitude of swelling could be that Nosaka et al. (2001) may have used a wider ROM, whereas this study may have used a narrower ROM. A wider ROM does induce more muscle damage (Nosaka et al., 2001) and perhaps the location of where the swelling occurs.

Summary

Upon completion of this exploratory study, the consistency of the inflammatory response after high-force eccentric exercise in the ND and D elbow flexors is yet to be determined. Future research with larger sample sizes including all three CRP risk categories is necessary before conclusions can be made about the consistency of the inflammatory response. Although no distinct conclusion was made about the consistency of the inflammatory response, this study could contribute to the interpretation of future research in which the relationship between CRP, IL-6 and CHD is investigated. Inflammation does contribute to the development and progression of CHD, however the risk markers associated are poorly understood. If the inflammatory response is determined to be consistent, researchers can use this model to more thoroughly study those risk markers as well as inflammation and CHD.

While the conclusions of this study are limited and preliminary, the use of this model to induce muscle damage was effective. Muscle damage was greater in the ND arm in comparison to the D arm, which could be due to the notion that the D arm was already accustomed to some muscle damage from everyday activities that favor the D arm. Regarding CK, maximal isometric strength loss, muscle soreness and swelling, the

exercise protocol used in this study did not induce as much of muscle damage as previous research (Hirose et al., 2004; Nosaka et al., 2001). However, a larger sample size would clarify or strengthen the results of the muscle damage response.

CHAPTER SIX

CONCLUSION

The goal of this study was to investigate the consistency of the inflammatory response after high-force eccentric exercise of the ND and D elbow flexors. C-reactive protein and IL-6 were analyzed to assess the magnitude and time course of the inflammatory response and CK, maximal isometric strength, muscle soreness and swelling were measured to determine the magnitude and time course of the muscle damage.

Twelve untrained and healthy subjects participated in the study, nine completed the entire study and two of those were excluded from the statistical analysis due to elevated basal IL-6 levels and insufficient strength loss. The final seven subjects (three female, four male) were compliant with the study criteria. The sample size served as a limitation of the study. Originally, 15 subjects were to be used in the statistical analyses, but equipment failure, attrition and elevated baseline levels contributed to the limited sample size.

C-reactive protein did not increase after the high-force eccentric exercise for either arm. Therefore no significant rank order correlation was detected, although it was consistently unresponsive. Ultimately, researchers cannot determine the consistency of CRP at this time. Further research with a larger sample size with individuals in the low, average and high risk CRP categories is needed to make a conclusion regarding CRP in response to high-force eccentric exercise of the ND and D elbow flexors.

No significant rank order correlation was measured for IL-6. However, the sample size was small and a trend did occur, thus, IL-6 might respond consistently to high-force eccentric exercise in the elbow flexors. Both conditions induced peak responses occurring at 8h post-exercise with a non-significant, yet slightly higher increase for the ND arm.

A significant rank order correlation was found for CK between the ND and D arms within individuals. Creatine kinase peaked at 120h post-exercise, but was less remarkable than results from previous research with similar exercise protocols.

A significant rank order correlation was detected for maximal isometric strength. Immediately post-exercise, strength decreased then started to return to baseline at 24h and reached or nearly reached baseline at 120h post-exercise.

Muscle soreness was not consistent in response to high-force eccentric exercise in the ND and D elbow flexors, meaning no significant rank order correlation was detected. The time to peak response occurred at 24h post-exercise.

No significant changes in swelling occurred in response to either exercise condition, therefore, swelling was consistently unresponsive. The changes in muscle circumference may have been more precise if a different method of measuring muscle circumference was used.

Application of the results of this study are limited. A larger sample size is needed in order to make an accurate conclusion about the consistency of the inflammatory response after high-force eccentric exercise in the ND and D elbow flexors. In addition to a larger sample size, a balanced order of completion is needed to accurately assess the

inflammatory and muscle damage responses. The results from this study were consistent with the concept that CRP responds to high-force eccentric exercise in the ND elbow flexors when basal levels are elevated, which could aid in the interpretation of future research.

REFERENCES

- Akimoto, T., Furudate, M., Saitoh, M., Sugiura, K., Waku, T., Akama, T., et al. (2002). Increased plasma concentrations of intercellular adhesion molecule-1 after strenuous exercise associated with muscle damage. *European Journal of Applied Physiology*, *86*, 185 – 190.
- Anderson, R.N. & Smith, B.L. (2005). Deaths: leading causes for 2002. *National Vital Statistics Reports*, *53*, 1 –89.
- Armstrong, R.B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and Science in Sports and Exercise*, *16*, 529-538.
- Balady, G.J., Berra, K.A., Golding, L.A., Gordon, N.F., Mahler, D.A., Myers, J.N., et al. (2000). *ACSM's Guidelines for Exercise Testing and Prescription*. Baltimore, MD: Lippincott Williams & Wilkins.
- Beattie, M.S., Shlipak, M.G., Liu, H., Browner, W.S., Schiller, N.B., & Whooley, M.A. (2003). CRP: More evidence suggests a link between inflammatory protein and heart disease. *Geriatrics*, *58*, 56.
- Bijur, P.E., Silver, W., & Gallagher, E.J. (2001). Reliability of the visual analog scale for measurement of acute pain. *Academic Emergency Medicine*, *8*, 1153 – 1157.
- Blake, G.J., & Ridker, P.M. (2002). Inflammatory bio-markers and cardiovascular risk prediction. *Journal of Internal Medicine*, *252*, 283 – 294.
- Byrnes, W.C., Clarkson, P.M., White, J.S., Hsieh, S.S., Frykman, P.N., & Maughan, R.J. (1985). Delayed onset muscle soreness following repeated bouts of downhill running. *Journal of Applied Physiology*, *59*, 710-715.
- Connolly, D.A.J., Sayers, S.P., & McHugh, M.P. (2003). Treatment and prevention of delayed onset muscle soreness. *Journal of Strength and Conditioning Research*, *17*, 197-208.
- Farrell, M., & Richards, J.G. (1986). Analysis of the reliability and validity of the Kinetic Communicator exercise device. *Medicine and Science in Sports and Exercise*, *18*, 44-49.
- Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., et al. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exercise Immunology Review*, *10*, 75-90.

- MacIntyre, D., Sorichter, S., Mair, J., Berg, A., & McKenzie, D. (2001). Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *European Journal of Applied Physiology*, *84*, 180-186.
- McHugh, M. (2003). Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scandinavian Journal of Medicine and Science in Sports*, *13*, 88-97.
- Miles, M.P., & Clarkson, P.M. (1994). Exercise-induced muscle pain, soreness, and cramps. *Journal of Sports Medicine and Physical Fitness*, *34*, 203-216.
- Nosaka, K. & Clarkson, P.M. (1994). Effect of eccentric exercise on plasma enzyme activities previously elevated by eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology*, *69*, 492 – 497.
- Nosaka, K. & Clarkson, P.M. (1996). Variability in Serum Creatine Kinase Response After Eccentric Exercise of the Elbow Flexors. *International Journal of Sports Medicine*, *17*, 120 – 127.
- Nosaka, K., Clarkson, P.M., McGuiggin, M.E., & Byrnes, J.M. (1991). Time course of muscle adaptation after high force eccentric exercise. *European Journal of Applied Physiology*, *63*, 70-76.
- Nosaka, K., Newton, M., & Sacco, P. (2002). Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scandinavian Journal of Medicine and Science in Sports*, *12*, 337-346.
- Nosaka, K., & Sakamoto, K. (2001). Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors. *Medicine and Science in Sports and Exercise*, *33*, 22-29.
- Nosaka, K., Sakamoto, K., Newton, M., & Sacco, P. (2001). How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine and Science in Sports and Exercise*, *33*, 1490-1495.
- Peak, J., Nosaka, K., & Suzuki, K. (2005). Characterization of inflammatory responses to eccentric exercise in humans. *Exercise Immunology Review*, *11*, 64 – 85.
- Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., et al. (2003). Markers of Inflammation and Cardiovascular Disease Application to Clinical and Public Health Practice. *Circulation*, *107*, 499 – 511.

- Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P., & Hennekens, C.H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *The New England Journal of Medicine*, 336, 973 – 979.
- Ridker, P.M., Rifai, N., Clearfield, M., Downs, J.R., Weis, S.E., Miles, S., et al. (2001). Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *The New England Journal of Medicine*, 344, 1959-1965.
- Ridker, P.M., Rifai, N., Stampfer, M.J., Hennekens, C.H. (2000). Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*, 101, 1762 – 1772.
- Shephard, R.J. (2002). Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. *Critical Reviews in Immunology* 22, 165-182.
- 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. *European Journal of Applied Physiology*, 82, 61-67.
- Smith, L.L., Bond, J.A., Holbert, D., Houmard J.A, Israel, R.G., McCammon, M.R., et al. (1998). Differential white blood cell count after two bouts of downhill running. *International Journal of Sports Medicine*, 19, 432-437.
- Smith, L.L., & Miles, M.P. (2000). Exercise-induced muscle injury and inflammation. *Exercise and Sport Science*, 410-411.
- Steensberg, A., Fischer, C., Keller, C., Moller, K., & Pedersen, B. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Endocrinology and Metabolism*, 285, E433-E437.
- St. Pierre Schneider, B., Correia, L.A., & Cannon, J.G. (1999) *Research in Nursing and Health*, 22, 243 – 250.
- Stupka, N., Lowther, S., Chorneyko, K., Bourgeois, J.M., Hogben, C., & Tarnopolsky, M.A. (2000). Gender differences in muscle inflammation after eccentric exercise. *Journal of Applied Physiology*, 89, 2325-2332.
- Stupka, N., Tarnopolsky, M.A., Yardley, N.J., & Phillips, S.M. (2001). Cellular adaptation to repeated eccentric exercise-induced muscle damage. *Journal of Applied Physiology*, 91, 1669-1678.

- Vanheest, J.L., Mahoney, C.E., & Rodgers, C.D. (2005). Oral Contraceptive Use and Physical Performance. In W.J. Kraemer & A.D. Rogol (Eds.), *The Endocrine System in Sports and Exercise* (pp. 250 – 260). Malden, MA: Blackwell Publishing.
- Willoughby, D.S., McFarlin, B., & Bois, C. (2003). Interleukin-6 expression after repeated bouts of eccentric exercise. *International Journal of Sports Medicine*, *24*, 15-21.
- Yudkin, J.S., Kumari, M., Humphries, S.E., & Mohamed-Ali, V. (2000) Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*, *148*, 209 – 214.

APPENDICES

APPENDIX A

INFORMED CONSENT

SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH
MONTANA STATE UNIVERSITY

Study Title: Is the inflammatory response consistent within individuals in two different muscle groups after high-force eccentric exercise?

Funding: This study is funded in part by the American Heart Association

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Bozeman, MT 59717-3540
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You are being asked to participate in a study investigating the response of several markers of inflammation that can be measured in the blood to a resistance exercise that will make the biceps muscles of both arms sore on separate occasions. This type of exercise is designed to induce a small bit of damage to the muscles used. It is likely that you have experienced this type of muscle damage in your daily life, as it is very common. When you do a physical activity that you are not accustomed to and experience soreness in muscles starting a day or so after the activity, that soreness is the result of the same type of muscle damage being studied in this investigation.

Risk of heart disease is often associated with the presence of a low-level of inflammation over a long period of time, perhaps many years. It is not known whether individuals who have this chronic, low-level inflammation are experiencing a chronic stimulus to keep the inflammation active or whether they simply cannot shut the inflammatory process down. We will measure some of the inflammatory markers associated with heart disease, have you perform the resistance exercise, and measure the same markers over several days following the exercise. The levels of inflammatory markers will be compared to levels measured in a control condition in which you go through all of the same procedures but do not perform the exercise. The inflammatory markers that we will measure are found in the blood, thus you will have your blood drawn on several occasions during this investigation. You will be tested in the Montana State University Movement Science Laboratory in Romney Gymnasium and the Nutrition Research Laboratory in Herrick Hall.

The purpose of this study is to determine whether the magnitude and duration of the inflammatory response to muscle damage varies between individuals with higher and lower resting inflammatory levels in the blood.

If you agree to participate in this study you will do the following things:

- 1) Read and sign this document, and you will fill out a physical activity readiness questionnaire, a 7-day physical activity questionnaire and a health history
- 2) questionnaire that includes questions regarding the presence of heart disease and diabetes in your family.
- 3) Report to the Movement Science Laboratory or Nutrition Research Laboratory on the MSU campus for measurements for baseline and follow up measurements (including pre-exercise blood collection and maximum force resistance exercise and 4, 8, 12, 24, and 120 hours post-exercise).
- 4) You will perform an exercise using a machine that controls speed of movement and amount of force. The machine consists of a padded chair with a padded lever system. You will place your wrist between the pads of the lever and the exercise apparatus will exert resistance for the exercise. Forty-five (45) contractions will be performed. You will begin each contraction from a fully flexed position of the arm and flex your arm against the resistance of the exercise apparatus to a fully extended position. Three sets of 15 contractions will be performed with five minutes rest between each set. Each contraction is performed at a rate of 45 degrees per second with a 10-second rest between contractions. As you fatigue, the resistance exerted by the machine will decrease, but it will always keep the resistance maximum for you. This exercise is called an eccentric exercise because your muscle is lengthening as it is producing force. This type of exercise will be done twice, once focusing on the biceps muscle of your non-dominant arm and once on the biceps of your dominant arm. The third condition is a control in which no exercise takes place.
- 5) You will perform three maximal contractions with the arm flexed at 90 degrees to determine your maximum force production for both exercise conditions. You will be seated on the exercise machine described above. Three maximal contractions will be performed with 60 seconds of rest between trials.
- 6) Muscle soreness will be assessed using a 100-millimeter scale with the left end indicating 'no soreness' and the right end indicating 'very, very sore'. You will be asked to rate your perception of soreness when attempting to flex your arm with little resistance and then place a vertical mark on the scale to indicate your level of soreness.
- 7) You will complete a physical activity questionnaire asking you to describe the frequency and intensity of physical activity that you typically perform in a week's time.
- 8) Subjects participating in the AHA Study (Downregulation of inflammation: Are individuals at risk for CHD less capable of turning off the inflammatory process?) will receive \$200 for the non-dominant biceps exercise condition and the control condition. An additional \$50 and a coupon for a free massage will be given to subject who complete the dominant biceps exercise condition, resulting in a total of \$250 plus a coupon for a free massage if all three

conditions are completed. If all visits are not completed, due to withdrawal from the study, you will receive \$10.50 for each blood draw that was completed.

Sometimes there are side effects from having blood drawn or doing certain activities. These side effects are often called risks, and for this project, the risks are:

- 1) Approximately 10-15 ml of blood (2-3 teaspoons) will be removed by putting a needle in your vein on 18 occasions (6 each for exercise and control conditions). This is the standard medical method used to obtain blood for tests. There is momentary pain at the time the needle is inserted into the vein, but other discomfort should be minimal. In about 10% of the cases there is a small amount of bleeding under the skin, which will produce a bruise. The risk of infection is less than 1 in 1,000.
- 2) After performing the resistance exercise, you will experience fatigue and soreness but this feeling should subside within 5 to 6 days. The extent of the soreness will be such that there is some loss of strength. However, this strength loss should not be enough to prevent daily activities such as brushing your teeth, or driving your car. On the two days following the exercise, the strength loss may affect the lifting of heavy objects. We recommend that you not perform strenuous exercise for 3 days following the exercise. In a small percentage of subjects (about 2-3%), strength loss can last for up to 2 months after the exercise, but you will not notice it, unless you perform an activity that requires maximal effort. In a small percentage of subjects, there will be delayed swelling of the upper arm and forearm. This is not serious and will disappear within 2 weeks. The risk of serious injury (such as a muscle pull or strain) from the exercise is small in healthy subjects who have no cardiovascular or musculoskeletal problems or have not had surgery to the arm or shoulder.
- 3) The Valsalva's (breath-holding) maneuver is sometimes performed by subjects during the resistance exercise. This maneuver has been shown to increase heart rate and blood pressure. To minimize this effect, you will exhale while exerting maximal forces.

There may be benefits from your participation in this study. These are:

- 1) Information about your personal blood lipid profile (i.e., LDL-cholesterol levels). However, you should be aware that these are research values and that you should contact your personal physician if you would like interpretation or actual clinical analysis of your blood lipids.
- 2) Exposure to a protocol for studying inflammation, along with an increased awareness of the possible factors linked to cardiovascular disease.

No other benefits are promised to you.

Confidentiality: The data and personal information obtained from this study will be regarded as privileged and confidential. A code number will identify the data that we collect from you, and all data will be kept in locked offices in the Nutrition Research Laboratory or in Dr. Miles' office. The information obtained in this study may be

published in scientific journals, but your identity will not be revealed. They will not be released except upon your written request/consent. If during the study you decide to cease your participation, your name will be removed from our study records, and we will not contact you again regarding this study. You will not be penalized in any way.

Freedom of Consent: You may withdraw consent in writing, by telephone, or in person with the investigator (Jan Andring at 406-994-5001) and discontinue participation in the study at any time and without prejudice or loss of benefits (as described above). Participation is completely voluntary.

In the event your participation in this research supported by the American Heart Association results in injury to you, medical treatment consisting of basic first aid and assistance in getting to Bozeman Deaconess Hospital or Student Health Services will be available, but there is no compensation for such injury available. Further information about this treatment may be obtained by calling Jan Andring at 994-5001.

You are encouraged to express any questions, doubts or concerns regarding this study. The investigator will attempt to answer all questions to the best of her or his ability. The investigator fully intends to conduct the study with your best interest, safety and comfort in mind. *The Chairman of the Human Subjects Committee, Mark Quinn can answer additional questions about the rights of human subjects at 406-994-5721.*

STATEMENT OF AUTHORIZATION

Study Title: Downregulation of inflammation: Are individuals at risk for CHD less capable of turning off the inflammatory process?

AUTHORIZATION: I have read the above and understand the discomforts, inconvenience and risk of this study. I, _____
 (PRINT YOUR NAME), agree to participate in this research. I understand that I may later refuse to participate, and that I may withdraw from the study at any time. I have received a copy of this consent from for my own records.

Signed: _____
Subject's Signature

Date: _____

Witness: _____
(if other than the investigator)

Date: _____

Investigator: _____
 Mary Miles, Ph.D.

Date: _____

APPENDIX B

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

PAR-Q and YOU

Physical Activity Readiness Questionnaire

Regular Physical activity is fun and healthy, however, some people should check with their doctors before they start becoming much more physically active. If you are planning on participating in the Arm Muscle Exercise/Inflammation Study please answer the following seven questions. These questions will help us determine whether you can safely and actively participate in this study without physician approval. Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly.

Yes	No	
___	___	Has your doctor ever said that you have a heart condition and that you should do only physical activity recommended by your doctor?
___	___	Do you feel pain in your chest when you do physical activity?
___	___	In the past month, have you had chest pain when you were not doing physical activity?
___	___	Do you lose your balance because of dizziness or do you ever lose consciousness?
___	___	Do you have a bone or joint problem that could be made worse by your participation in this study?
___	___	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
___	___	Do you know of any other reason that you should not do physical activity?

If you answered YES to one or more questions:

Talk with your doctor by phone or in person BEFORE you agree to participate in this study or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which question you answered YES to. You may be able to safely participate in this study.

If you answered NO to all questions:

You can be reasonably sure that you can safely participate in this study.

I have read, understood, and completed this questionnaire. Any questions I had were answered to my full satisfaction.

Signature: _____

Date: _____

Witness: _____

Date: _____

Signature of parent or guardian if under 18: _____

APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Health History Form

Gender: Male Female

Date of Birth: _____ Age _____

Address: _____
 City: _____ State: _____ Zip: _____

Telephone Numbers: Home: _____ Work: _____
 Cell: _____

Email Address: _____

Ethnic Heritage (optional):

	Caucasian	African American	Asian	American Indian	Hispanic	Other (please Specify)
Mother						
Father						
Maternal Grandmother						
Maternal Grandfather						
Paternal Grandmother						
Paternal Grandfather						

Physical Activity History

During the last 6 months, on average how many times per week did you exercise vigorously? _____ times per week

Smoking History

Do you smoke cigarettes? Yes No (Please circle)

How many cigarettes per day? _____

Did you ever smoke? Yes No (Please circle)

If yes, how many cigarettes did you smoke per day? _____

When did you quit smoking? _____

Do you currently smoke cigars? Yes No (Please circle)

How many cigars per day? _____

Do you currently smoke a pipe? Yes No (Please circle)

How often do you smoke a pipe? _____

Do you chew tobacco? Yes No (Please circle)

How often do you chew tobacco?

Alcohol Intake

Do you currently consume alcohol? Yes _____ No (Please circle)
 How many regular beers do you drink per week? _____
 How many light beers do you drink per week? _____
 How many glasses of wine do you drink per week? _____
 How many shots of liquor do you drink on average per week? _____
 Have you ever been treated for alcoholism? Yes _____ No (Please circle)

Medical History

Please circle if **you** have any of the following?

Cardiovascular: Yes _____ No (Please circle)
 Diabetes Mellitus Yes _____ No (Please circle)
 Anemia or Latent Anemia Yes _____ No (Please circle)
 Pulmonary disease: Yes _____ No (Please circle)
 Metabolic disease: Yes _____ No (Please circle)
 Kidney problems: Yes _____ No (Please circle)
 Cancer: Yes _____ No (Please circle)

Family History of Heart Disease and Diabetes Mellitus

	Heart Disease (if yes, brief description)	Diabetes Mellitus (if yes, brief description)
Mother		
Father		
Maternal Grandmother		
Maternal Grandfather		
Paternal Grandmother		
Paternal Grandfather		
Aunt(s)		
Uncle(s)		

APPENDIX D

THE KIN-COM



Figure 1. The Kin-Com

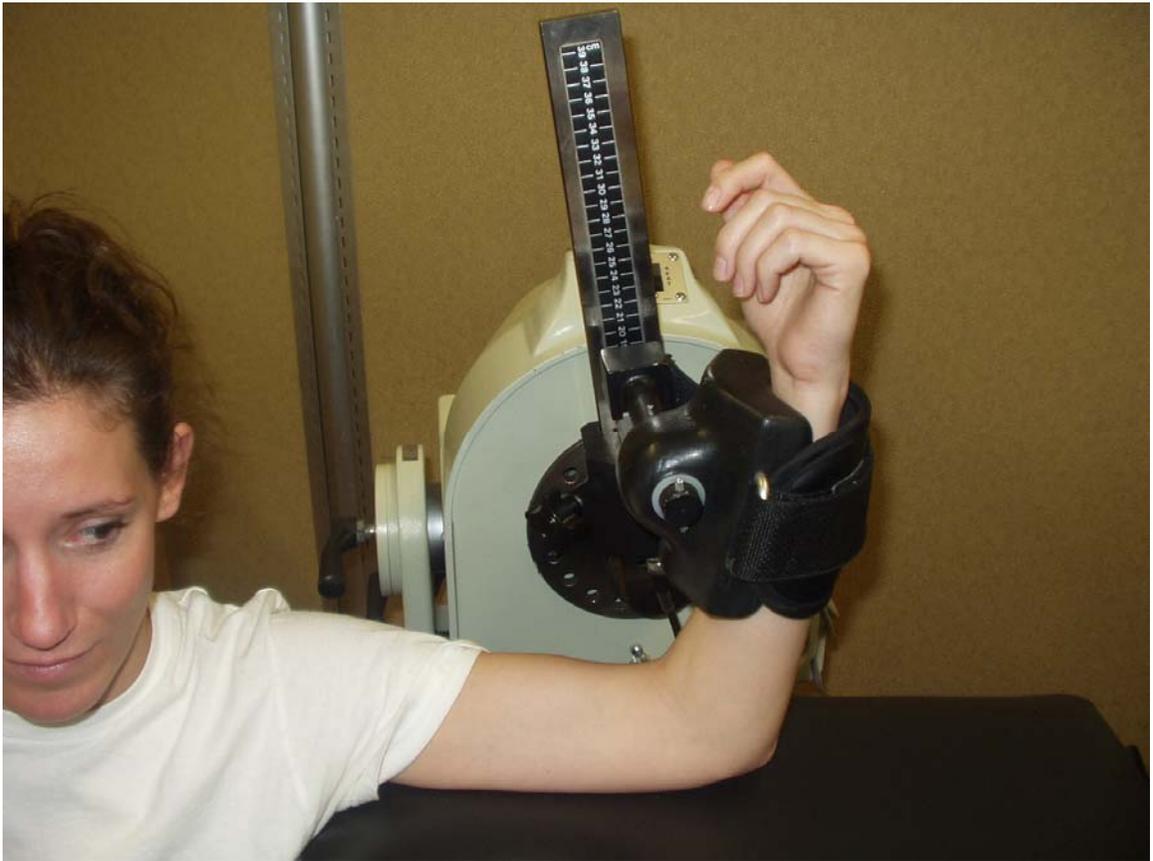


Figure 2. The elbow at 90°.

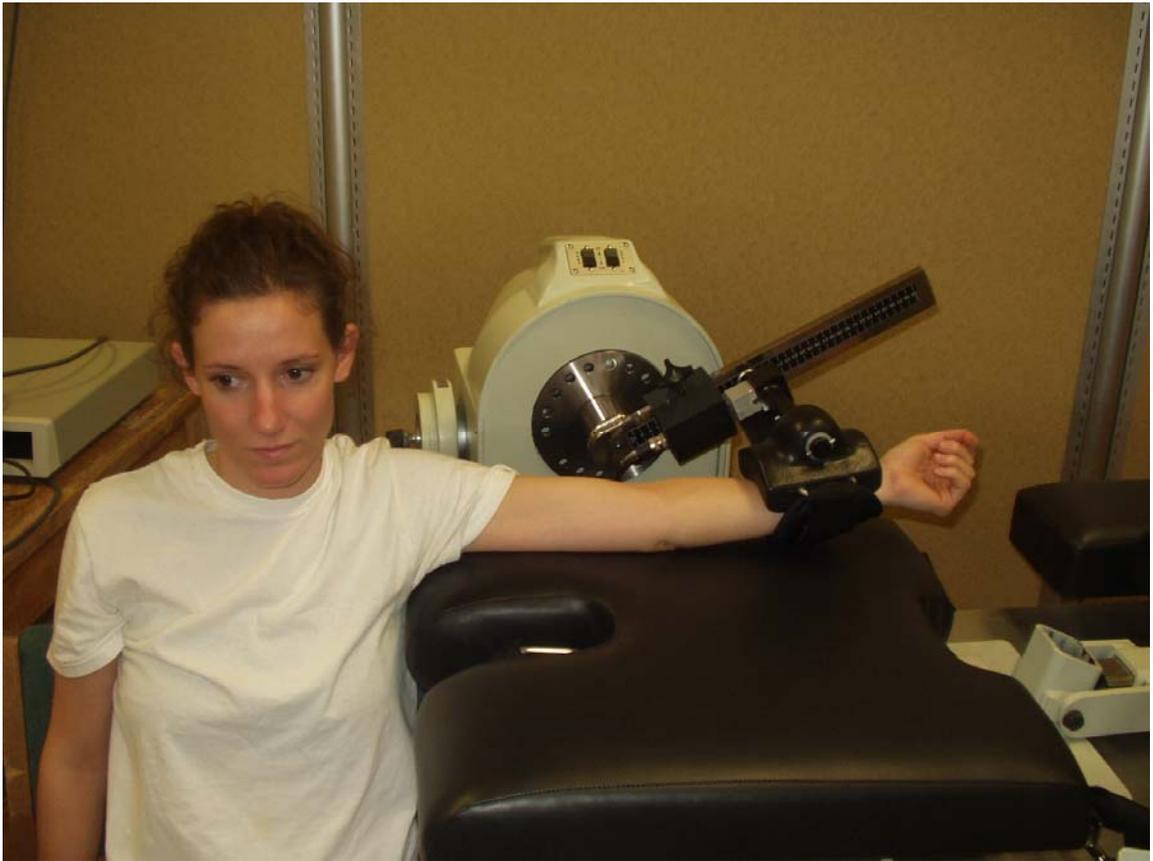


Figure 3. Fully extended position.



Figure 4. Fully flexed position.