

THE EFFECTS OF CARBOHYDRATE ON INFLAMMATION FOLLOWING  
AN ACUTE BOUT OF RESISTANCE EXERCISE

by

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## ABSTRACT

The immune response to inflammation involves the release of cytokines, which determine the intensity and duration of the immune response (Kuby, 1997). The cytokine interleukin-6 (IL-6), functions as a negative feedback signal that turns off pro-inflammatory mediators during the immune response. IL-6 also initiates the release of CRP, which induces inflammation. Therefore, IL-6 is known as both a pro and anti-inflammatory mediator of the immune response.

IL-6 is released during the immune response to inflammation. IL-6 peaks about 8 hours after an eccentric exercise session that induces muscle damage. Carbohydrate ingestion during endurance exercise attenuates the rise in IL-6 immediately post-exercise during recovery. IL-6 along with the acute phase protein C-reactive protein (CRP) (a marker of the systemic inflammatory response), and creatine kinase (CK) (a semi-quantitative marker of muscle damage), will be used to determine the affects of eccentric exercise on muscle damage and the inflammatory response. Purpose: To determine whether IL-6, CRP and CK, following eccentric exercise differ with a carbohydrate supplement versus a placebo. Methods: The study was a double-blind, cross-over design. Male and female subjects consumed a carbohydrate or placebo beverage the day of and day after the eccentric exercise. Subjects also consumed a controlled diet the day before, day of and the day after the exercise session. The diet consisted of 50% carbohydrate, 30% fat and 20% protein. The exercise session was two bouts of an eccentric exercise using the bicep muscle, three weeks apart, to induce muscle damage and initiate an inflammatory response. A repeated-measures ANOVA was used to determine whether carbohydrate ingestion influenced IL-6, CRP and CK. Results: Carbohydrate increased the rise in IL-6 8 hours post-exercise compared to placebo. An increase in arm circumference at 8, 12 and 120 hours and subjective soreness at 12, 24, and 48 hours post-exercise was indicative of muscle damage. Conclusion: Carbohydrate increased the local inflammatory response following resistance exercise, but had no effects on CRP and CK. This study is the first to show that carbohydrate following eccentric exercise has an effect on the local inflammatory response.

## CHAPTER 1

## INTRODUCTION

Inflammation is an immune response to tissue damage or trauma and is accompanied by redness, heat, swelling and pain in the affected area (Kuby, 1997). The purpose of inflammation is to remove debris due to infection or tissue damage, followed by the repair and regeneration of new tissue. There are two inflammatory responses, a local acute inflammatory response at the site of injury and a systemic response known as the acute phase response (Pedersen et al., 2001).

Localized acute inflammation results in the activation of various inflammatory cells within the immune response (Kuby, 1997) including lymphocytes, neutrophils, and monocytes (Pedersen et al., 2001). These cells generate cytokine signals to attract other inflammatory cells to an injured area. These signals also stimulate the phagocytosis (cellular ingestion) of antigens and/or debris (Kuby, 1997). Cytokines control the intensity and duration of an immune response by acting on various immune cells (Kuby, 1997). Cytokines can be pro-inflammatory molecules that up-regulate the immune response, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ), (Kuby, 1997), or they can be anti-inflammatory and down-regulate the immune response (Smith & Miles, 2000).

TNF- $\alpha$  and IL-1 $\beta$  are known as pro-inflammatory cytokines because of their ability to induce the synthesis of acute phase proteins, such as C-reactive protein (CRP) (Pedersen et al. 2001) and hence induce the acute phase immune response. The induction

of pro-inflammatory molecules results in the signaling and initiation of more cytokines, in particular IL-6.

IL-6 is both an anti-inflammatory and pro-inflammatory cytokine (Papanicolau et al., 1998). Once IL-6 is 'on' it acts as a negative feedback signal to turn off TNF- $\alpha$  and IL-1 $\beta$ . It also activates anti-inflammatory hormones such as epinephrine, norepinephrine and cortisol, which then turn off the pro-inflammatory cytokines (Smith & Miles, 2000). IL-6 activation of acute phase proteins, such as C-reactive protein (CRP), classifies IL-6 as a pro-inflammatory cytokine as well. With the IL-6 response, injury and inflammation of damaged tissue is controlled with the activation and de-activation of various protein molecules.

IL-6 also has roles beyond that of the immune system and appears to be a regulation of energy metabolism. The production and release of IL-6 during non-muscle damaging exercise is affected by the intensity, duration and type of exercise (Febbraio & Pedersen, 2000) and is independent of and different from the response of IL-6 to injury and inflammation (Petersen & Pedersen, 2005). As an example, plasma IL-6 has been found to be affected by intra-muscular glycogen content prior to bicycling and is implicated in the regulation of energy metabolism (Febbraio et al., 2003). In addition, plasma IL-6 has been found to be downregulated roughly 10 times from pre to post-exercise levels with the ingestion of carbohydrate during exercise (Starkie et al., 2001).

The dual role of IL-6 in mediating both inflammation and energy metabolism suggests that energy intake may influence the immune response. We hypothesize that the

downregulation of IL-6 by carbohydrate ingestion may result in a prolonged immune response to inflammation and injury due to a lack of the negative feedback signal necessary to turn off pro-inflammatory cytokines. As a result, the immune response to inflammation may not be turned off and pro-inflammatory cytokines may continue to initiate the inflammatory response. Continued suppression of IL-6 with carbohydrate may result in chronic inflammation due to an inability to properly repair damaged muscle tissue. This not only affects intense exercise training programs, but may also be a possible mechanism for future risk of chronic inflammatory diseases such as Type 2 diabetes, atherosclerosis, insulin resistance and obesity (Petersen & Pedersen, 2005).

Eccentric exercise is associated with an increase in IL-6 (Steensberg et al., 2002) and can be used as a model to induce muscle damage and inflammation. During eccentric exercise, the muscle is lengthened while weight is placed upon it, creating high forces and muscle tension (Jones & Round, 1990). Examples of eccentric exercise include downhill running or the downward portion of a bicep curl with weights. It is eccentric exercise that has been shown to cause muscle damage and is associated with delayed onset muscle soreness (DOMS) (Miles & Clarkson, 1994). DOMS occurs from the onset of exercise to 8-24 hours following exercise with the intensity peaking 24-96 hours post-exercise and is associated with muscle damage and the release of protein molecules (Miles & Clarkson, 1994). The protein creatine kinase (CK) is associated with DOMS and peaks 4 to 5 days after eccentric exercise and is, therefore, used as a

marker of muscle damage and inflammation (Jones & Round, 1990). In addition, C-reactive protein (CRP), an acute phase protein released by the liver with the initiation of inflammation, is used to measure the intensity of the immune response to tissue damage and inflammation (Kuby, 1997). Blood measurements of CK and CRP after eccentric exercise can be used as markers to determine the degree of muscle damage and intensity of the inflammatory response following eccentric exercise. The production of IL-6 due to eccentric exercise and the effects of carbohydrate on IL-6 following eccentric exercise can be used to assess how IL-6 affects inflammation and glucose metabolism. The difference in measured variables from post-exercise to pre-exercise will be used for data analysis. This will be done to eliminate the influence of changes in pre-exercise measures within subjects so that the magnitude of responses between trials can be compared.

#### Statement of the Problem

The objective of this study was to determine if a carbohydrate supplement following an eccentric bout of exercise would reduce the IL-6 response to muscle damage compared to placebo.

#### Research Hypotheses

Hypothesis #1: IL-6 will be downregulated with a carbohydrate supplement during the first 48 hours following an eccentric bout of exercise.

$$H_0: \mu_{\text{CHO}} = \mu_{\text{PLA}}$$

$$H_a: \mu_{\text{CHO}} \neq \mu_{\text{PLA}}$$

Hypothesis #2: C-reactive protein and creatine kinase will be higher with a carbohydrate supplement versus placebo.

$$H_0: \mu_{\text{CHO}} = \mu_{\text{PLA}}$$

$$H_a: \mu_{\text{CHO}} \neq \mu_{\text{PLA}}$$

Where:  $\mu_{\text{CHO}}$  = the mean of IL-6 during carbohydrate supplementation

$\mu_{\text{PLA}}$  = the mean of IL-6 during placebo

### Delimitations

1. The study was restricted to males and females between the ages of 18- and 39 years of age.
2. The study was restricted to subjects living in Montana throughout the duration of the study.
3. The study was restricted to subjects whose arm muscles were naïve to eccentric exercise.

### Limitations

1. The results of the study cannot be generalized to persons over the age of 40 and under the age of 18.

### Operational Definitions

*Acute-phase proteins* are protein molecules that are released from the liver with the onset of localized tissue damage. This results in an initiation of the inflammatory response with the development of a fever because the increase in body temperature helps to fight off pathogens in the affected area. C-reactive protein is one of the major acute-phase proteins released by the liver.

*Acute-phase response* is associated with acute inflammation and the systemic effects as a result of inflammation. This response includes the production of hepatocyte derived serum protein molecules (such as CRP), an increase in leukocytes and a fever.

*C-reactive protein (CRP)* is an acute-phase protein that increases dramatically with localized tissue damage and inflammation. It is a reliable blood marker to measure the intensity of the immune response to inflammation and damage.

*Carbohydrates* are molecules made-up of carbon, oxygen and hydrogen atoms. The simplest unit of a carbohydrate is chemically termed a monosaccharide, but is more commonly known as sugar or glucose. More complex carbohydrates are multiple monosaccharides in a chain known as disaccharides or polysaccharides.

*Creatine Kinase (CK)* is a protein found primarily in muscle tissue. CK increases with muscle damage and/or inflammation 4-5 days following a bout of exercise, therefore can be used as an assessment of damage and inflammation to muscle tissue with exercise.

*Cytokines* are molecules that are released from cells during the immune response to up-regulate various proteins and hormones involved in cellular and tissue damage

repair. They also regulate the intensity and duration of the immune response due to their various effects on immune system cells. Examples include IL-6, TNF- $\alpha$  and IL-1 $\beta$ .

*Eccentric exercise* is forced lengthening of the muscle while tension is concurrently being placed upon the muscle. An example of an eccentric exercise within the arm is the lowering of a barbell on the downward portion of a bicep curl with weight.

*Glycogen* is the storage supply of glucose within muscle and liver. Glucose is stored as glycogen when carbohydrate intake exceeds the body's current physiological needs. Low levels of glycogen have been implicated in an increase of IL-6 during exercise sessions.

*Interleukin-1  $\beta$  (IL-1 $\beta$ )* is a cytokine released during the onset of tissue damage with the primary inflammatory response to induce the synthesis of acute phase proteins from the liver.

*Interleukin-6 (IL-6)* is a cytokine released during the second phase of the immune response. It is a negative feedback signal to turn off TNF- $\alpha$  and IL-1 $\beta$  in addition to the regulation of anti-inflammatory hormones, epinephrine, cortisol and norepinephrine as well as acute phase proteins C-reactive protein and serum amyloid A (SAA). IL-6 is also released from muscle independent of the immune response.

*IL-6 gene transcription* occurs when a helical strand of deoxyribonucleic acid (DNA) copies itself to form a single ribonucleic acid (RNA) strand. The RNA can then be read and 'translated' into a protein molecule within the body. The protein is then recognized and functions within the body according to the information that was held within the helical DNA.

*IL-6 mRNA expression* occurs when IL-6 mRNA is expressed and seen within muscle biopsy samples. However, the complete IL-6 protein has not been released into circulation.

*Inflammation* is the response of the immune system to localized tissue injury and damage. Signs of inflammation within an injured area include redness, swelling, heat and pain.

*messengerRNA (mRNA)* is the molecule that contains the amino acid sequence for a protein.

*Tumor necrosis factor-  $\alpha$  (TNF- $\alpha$ )* is a cytokine released with the primary inflammatory response, during the onset of tissue damage, to induce the secretion of various cytokines.

## CHAPTER 2

## LITERATURE REVIEW

Muscle Activity

Daily activities and exercise programs require the contraction of muscle for actions to occur. The two types of muscular contractions related to exercise and movement are known as concentric and eccentric contractions. A concentric muscle action occurs during the lifting portion of a muscular movement, (Foss & Keteyian, 1998) with the muscle shortening in length. An example of a concentric exercise is the lifting of weights, such as a bicep curl. An eccentric muscle contraction occurs with the lengthening of the muscle fibers, while at the same time muscle fibers are contracted (Foss & Keteyian, 1998) resisting the muscular movement and creating tension within the muscle. Examples of eccentric exercises include downhill running or walking and the contraction of an extended bicep muscle against force or gravity, such as the lowering of weights (Foss & Keteyian, 1998).

Of the two contraction types, unaccustomed eccentric exercise is most likely to cause pain, injury and/or inflammation due to muscle damage. The muscle damage that occurs during eccentric exercise can result in injury, muscle soreness, loss of muscular force, stiffness and tenderness (Jones & Round, 1990). Damage sustained by muscle during strenuous exercise results in the initiation of the inflammatory response for healing to occur (Smith & Miles, 2000).

### Acute Inflammation

The onset of acute inflammation is a result of injury or infection to any body tissue, including muscle. Signs of inflammation include swelling, redness, heat, pain and reduced function (Kuby, 1997). Onset of acute inflammation results in vascular changes within the immediate area of injury, followed by the infiltration of a variety of cell types that work to mediate, reduce and repair inflamed tissue. Neutrophils are the first cell type to enter an inflamed area, shortly followed by macrophages (Kuby, 1997). A function of neutrophils and macrophages includes the production of cytokines, chemokines and cell adhesion molecules, which work to direct inflammation (Smith & Miles, 2000).

Cytokines are molecules that signal the activation of various cells within the immune response such as macrophages, B and T cells (Kuby, 1997). All cells that contain nuclei are capable of synthesizing cytokines and also have cytokine receptors on their cell membranes (MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2000). In addition, cytokines are made as they are needed and can act locally or systemically (Smith & Miles, 2000). They are grouped into families of interleukins (IL), tumor necrosis factors (TNF), interferons, colony-stimulating factors and growth factors (Smith & Miles, 2000).

Cytokines, in particular, are important for the cascade response of acute inflammation (Kuby, 1997). Cytokine production appears shortly after the beginning of strenuous exercise with a variable time to appearance in circulation (Smith & Miles, 2000). The pro-inflammatory cytokines, TNF-  $\alpha$  and IL-1 $\beta$  function as alarm cytokines

to increase the adhesion of molecules to endothelial tissue, synthesize acute-phase proteins from the liver, increase vascular permeability and induce the production of the cytokine IL-6 (Kuby, 1997). The stimulation of TNF-  $\alpha$  and IL-1 $\beta$  expression is often referred to as the primary inflammatory response, while the expression of IL-6 as a result of TNF-  $\alpha$  and IL-1 $\beta$  is referred to as the secondary inflammatory response (Nieman et al., 2003). IL-6 is produced from macrophages and neutrophils, but is also released from contracting skeletal muscle (Smith, & Miles, 2000).

#### Roles of IL-6

Some of the actions of IL-6 are the induction of anti-inflammatory hormones and acute phase proteins, and a negative feedback signal to down-regulate the alarm cytokines, TNF- $\alpha$  (Steensberg et al., 2002) and IL-1 $\beta$ . The various functions of the anti-inflammatory hormones such as epinephrine, norepinephrine and cortisol, act to reduce muscle injury and damage while also turning off IL-6. The activation of acute phase proteins from the liver, including C-reactive protein (CRP) and serum amyloid A (SAA) (Smith & Miles, 2000) results in a large phagocytic response to an inflamed site, killing pathogens in the affected area (Papanicolaou, Wilder, Manolagas & Chrousos, 1998). Lastly, the negative feedback signal produced by IL-6 to turn-off TNF-  $\alpha$  and IL-1 $\beta$  is an integral part of the inflammatory cascade to shut-off the primary inflammatory response, allowing injured tissue to repair and regenerate itself. As a result, IL-6 acts as both a pro-

inflammatory and anti-inflammatory mediator of the inflammation process (Papanicolaou et al., 1998).

IL-6 is also involved within other control mechanisms of the body, independent of inflammation. IL-6 is secreted due to the contraction of muscle during exercise, and Febbraio and Pedersen, (2002) concluded that the appearance of IL-6 in circulation is affected by the intensity, duration and type of exercise. Carbohydrate also affects the appearance of IL-6 during exercise. In addition, Nieman et al. (2001) studied cytokine changes after a marathon in several subjects and found that neither age, nor sex, had an effect on plasma cytokine levels.

IL-6 also has a glucoregulatory role during exercise. Henson et al. (2000) found that 2-hours of moderately intense rowing sessions performed on consecutive days with carbohydrate intake had no effect on the appearance of IL-6 from pre to post-exercise. However, Nieman et al. (2003) and Nehlsen-Cannerella et al. (1997) studied exercise of a higher intensity and duration and found that a three hour and 2 ½ -hour run respectively, along with the consumption of a carbohydrate containing beverage during exercise, attenuated the rise in IL-6 post-exercise. In addition, IL-6 release from contracting skeletal muscle is greater than the IL-6 released during the inflammatory response; however, it is still unknown which cells within muscle secrete IL-6 (Febbraio et al., 2002). According to various research reports, Petersen and Pedersen (2005) hypothesized that muscle derived IL-6 acts as a hormone to mobilize and increase energy availability during exercise. It has been shown that endurance exercise causes a decrease

in glycogen stores and it has been during these times that IL-6 release has increased due to its plausible glucoregulatory properties.

The roles of IL-6 are multi-faceted. It is involved in the inflammatory response due to inflammation and injury, but is also released during exercise. The role of IL-6 with inflammation is well understood, however, it is the release of IL-6 during exercise that requires further research.

#### Production of IL-6 by Skeletal Muscle During Exercise

Of the cytokines, IL-6 has the most marked increase with strenuous exercise (Febbraio et al., 2002). Circulating plasma IL-6 increases two to 100-fold independent of muscle damage and markers of inflammation during endurance exercise (Miles, 2004). In addition, MacIntyre et al. (2000) found increases in IL-6 to be positively correlated with labeled neutrophils during eccentric exercise. Thus, IL-6 has been shown to have a relationship as a marker of acute inflammation and muscle soreness after eccentric exercise (MacIntyre et al., 2000).

When the amount of carbohydrate stored inside muscle cells, glycogen, is lower, muscle produces more IL-6 during exercise. Decreases in glycogen content during a moderately intense 120-minute bicycle trial resulted in a roughly 20-fold increase in IL-6 mRNA expression during exercise (Febbraio et al., 2003), which indicates that muscle is producing IL-6. In addition, Fischer et al. (2004) determined that ten weeks of endurance exercise decreased the IL-6 mRNA release from skeletal muscle due to training effects that result in increased glycogen stores. The negative correlation observed between

glycogen stores and IL-6 mRNA in muscle showed that glycogen levels are a determining factor for IL-6 release during long exercise bouts (Fischer et al., 2004) and specifically effect IL-6 gene transcription (Petersen & Pedersen, 2005). Similar to Febbraio et al. (2003), Steensberg et al. (2002) showed that IL-6 mRNA expression also increased but with a much larger magnitude, roughly 100 times greater from pre-exercise to post-exercise levels with a concurrent decrease in glycogen stores during exercise. Therefore, Steensberg et al. (2002) proposed that IL-6 promotes glucose uptake by increasing insulin sensitivity to the muscles during exercise. In effect, an increase in muscle IL-6 along with a decrease in glycogen stores suggests that IL-6 has a function in maintaining glucose homeostasis and that skeletal muscle is capable of controlling metabolic pathways (Febbraio & Pedersen, 2005). It appears that low glycogen stores within muscle initiate an increased transcription rate of muscle IL-6 mRNA to maintain glucose levels.

Though the exact mechanism by which IL-6 maintains glucose levels during exercise are not fully understood, researchers have found evidence that IL-6 is clearly linked to glucose homeostasis during exercise. Febbraio, Hiscock, Sacchetti, Fischer, and Pedersen (2004) concluded that IL-6 has a role in endogenous glucose production (EGP). The researchers examined the effects of IL-6 on EGP during various exercise intensities, comparing high intensity exercise (HI), low intensity exercise (LO) and low intensity exercise with recombinant IL-6 injection (LO + IL-6) and rate of glucose appearance (GA) and glucose disappearance (GD) during and after exercise sessions. The researchers found that the LO + IL-6 trial had higher GA and

GD rates versus the LO trial, despite the same exercise intensities. This finding supports the theory that IL-6 is involved with glucose homeostasis during exercise. Because both LO groups had similar concentrations of glucoregulatory hormones, IL-6 appears to have a direct effect on glucose homeostasis (Febbraio et al., 2004). The role IL-6 has on glucose homeostasis and its response to glycogen levels, coincide with the affects carbohydrate are found to have on circulating levels of IL-6 during and after exercise sessions.

#### Production of IL-6 Following Exercise-Induced Muscle Damage

Eccentric exercise also produces IL-6 as evidenced by muscle cells that were positively stained for IL-6 before any damage or inflammatory cells were found within muscle (Tomiya, Aizawa, Nagatomi, Sensui, & Kokubun, 2004). IL-6 measurements were seven times greater 12 hours post-exercise, compared to pre-exercise concentrations, at which point IL-6 declined to pre-exercise levels over the course of several days. In addition, researchers found IL-6 within inflammatory and satellite cells (cells between muscle and plasma membrane that initiate regeneration) (Jones & Round, 1990) once muscle damage had occurred and the inflammatory process had begun, indicating non-muscle cells within the muscular compartment produce IL-6 (Tomiya et al., 2004).

Hirose et al. (2004) has suggested an adaptation to eccentric exercise via a decrease in inflammatory mediators with repeated exercise sessions. Various markers within the blood were analyzed to assess muscle damage and inflammation during the

first exercise bout and then re-assessed at the second exercise bout. Hirose et al. (2004) found that muscles adapted to exercises after the first session, with damage and many inflammatory mediators decreased following the second exercise session. However, with respect to cytokine levels, there were no significant changes found between the first and second exercise trials. Therefore, IL-6 and inflammation appear to occur independent of muscle damage.

The results of Hirose et al. (2004), however, are in contrast with Tomiya et al. (2004) in which cytokine levels were affected by eccentric exercise. Tomiya et al. (2004) determined that IL-6 increased 19.8% from immediately post-exercise to its peak, 12 hours later. There are conflicting reports regarding the time course of IL-6 increases following muscle damage, however this may be a function of differences in measurement timing. The timing of IL-6 measurements after eccentric exercise by Hirose et al. (2004) was at time points in which an increase in IL-6 is not expected. Therefore, any increases that may have occurred with IL-6 may have been missed due to poor timing of IL-6 measurements within the blood. In addition, it has been noted that endurance exercise has a greater effect on cytokine levels than single exercise sessions (Hirose et al., 2004).

#### Glucose Ingestion, Exercise and IL-6

Researchers examining the effects of carbohydrate ingestion on IL-6 during and after a 2.5 hour, high intensity run determined that IL-6 immediately post-run was roughly 20% higher in placebo participants than those who received carbohydrate during exercise (Nehlsen-Cannarella et al., 1997). In addition, participants receiving

carbohydrate during the run had lower levels of IL-1ra and cortisol, both anti-inflammatory molecules. Hence, a decrease in IL-6 appeared to result in a decreased anti-inflammatory response during exercise.

Nieman et al. (2003) found that carbohydrate ingestion decreased plasma IL-6 and other plasma cytokine levels after 3 hours of running compared to placebo. Carbohydrate also decreased the expression of IL-6 mRNA in muscle. Post-exercise measurements of muscle glycogen found higher glycogen levels in the carbohydrate group. This finding adds further support to the negative correlation found between glycogen content and IL-6 release (Fischer et al., 2004). However, carbohydrate had no effect on the expression of TNF- $\alpha$  and IL-1 $\beta$ . As a result, the researchers concluded that carbohydrate only has an effect on the secondary inflammatory response during exercise.

The previous two studies are in contrast to the findings of Starkie, Arkinstall, Koukoulas, Hawley, and Febbraio (2001). It was determined that carbohydrate ingestion, concentric and eccentric exercise had no effect on IL-6 mRNA expression in muscle. However, there was a two-hour difference in exercise times. In addition, a laboratory setting was used within one study, while the other was done during a marathon. Because the studies have different methods and different time-courses for measuring cytokines, an accurate comparison of the results cannot be made. Lastly, previous researchers have shown that endurance exercise (such as a marathon) has a greater effect on IL-6 levels.

Similarly, Nieman et al. (2003) found that carbohydrate ingestion had no effect on IL-6 expression compared to placebo in a 2-hour bout of intensive resistance training. However, as previously mentioned, high intensity, endurance exercise has been shown to

have a greater effect on IL-6 than low to moderate intensity exercises. In addition, IL-6 was measured 30 minutes pre-exercise, immediately and one-hour following exercise. While researchers have found plasma IL-6 to be increased when measured at these intervals (Nieman et al., 2002), Febbraio et al. (2003) found plasma IL-6 to be highest 120 minutes post-exercise. Furthermore, subjects had a minimum of 6 months resistance training prior to participating in the study and carbohydrate and placebo drinks were consumed only during the exercise session. Currently, there is no published research that has tested the affects of carbohydrate ingestion on IL-6 following resistance exercise at the stated time intervals.

Febbraio et al. (2003) determined the effects of glucose consumption on IL-6 during exercise compared to placebo. Plasma IL-6 increased steadily over a two-hour exercise period with carbohydrate ingestion, but to a lesser extent than in those without carbohydrate ingestion. At the same time, plasma glucose steadily decreased with carbohydrate but to a lesser extent than the non-carbohydrate group. Simultaneously, IL-6 release from muscle with carbohydrate is steady over a two-hour exercise period, while glucose uptake by muscle also increases over time with a greater magnitude than the non-carbohydrate group. The comparison of events between glucose and IL-6 during exercise illustrates how carbohydrate can downregulate the release of IL-6. Furthermore, the higher IL-6 values observed during the non-carbohydrate group indicate that IL-6 serves as a glucoregulatory hormone to maintain blood glucose levels when carbohydrate is unavailable. Whether the effects of glucose on IL-6 occur at the level of the brain or peritendon still remains unclear (Febbraio et al., 2003).

The expression of IL-6 during and after exercise has also been observed within adipose tissue cells (Keller, Keller, Marshal, & Pedersen, 2003) and is affected by carbohydrate ingestion. During a 3-hour bout of cycling, subjects either consumed a carbohydrate supplement or a placebo and then had fat biopsies and blood samples taken to determine IL-6 gene expression after exercise. IL-6 was blunted with carbohydrate ingestion during the exercise trial and one and a half hours post-exercise, while it increased about 3-fold without carbohydrate at the same times. It is possible that an increase in IL-6 without carbohydrate is due to decreased glycogen levels that results in an increased rate of lipolysis to meet energy needs during prolonged exercise.

## CHAPTER 3

## METHODS

Subjects

Study participants were volunteer men and women, 18-39 years of age, recruited from the university population and surrounding community. Participants were not active in lifting and lowering activities involving the arm muscles to allow for an accurate analysis of inflammatory markers during exercise trials of the study. In addition, participants did not have any known musculoskeletal limitations, inflammatory conditions, diabetes, or chronic use of anti-inflammatory medications, and they did not participate in activities that result in muscle soreness or bruising. Subjects were informed of the risks of the study beforehand and informed written consent was given prior to participation in the study (Appendix A). The study was approved by the Human Subjects Committee at Montana State University-Bozeman.

Experimental Design

The study was a double-blind, cross-over design in which participants underwent an exercise protocol to induce acute inflammation under conditions of either a carbohydrate supplement or a placebo (Figure 3.1). Carbohydrate intake was controlled the day prior to, the day of and the day after the exercise sessions. Participants consumed

a diet of 50% carbohydrate, 30% fat and 20% protein during the course of the controlled dietary intake. In addition to the prescribed diet, participants consumed either a 6% carbohydrate drink (Gatorade, Barrington, IL) or a placebo drink, the day of and the day after the exercise session. A month later, participants repeated the protocol with the beverage not consumed during the first trial. Half of the subjects within each carbohydrate condition used their dominant arm to perform the eccentric exercises while the other half used their non-dominant arm for the exercises. Subjects then used the arm that was not used within the first trial, for the second trial.

Subjects reported to the Movement Science Laboratory at 7:00 a.m. on the morning of the exercise trials. Blood samples were drawn pre-exercise and at 4, 8, 12, 24, 48 and 120 hours post-exercise for later assessment of C-reactive protein, interleukin-6, creatine kinase and blood glucose. IL-6 was analyzed pre-exercise and 4, 8, 12, 24 and 48 hours post-exercise. C-reactive protein was analyzed pre-exercise and 4, 8, 12 and 24 post-exercise. Creatine kinase was analyzed pre-exercise and 24, 48 and 120 hours post-exercise (Figure 3.2).

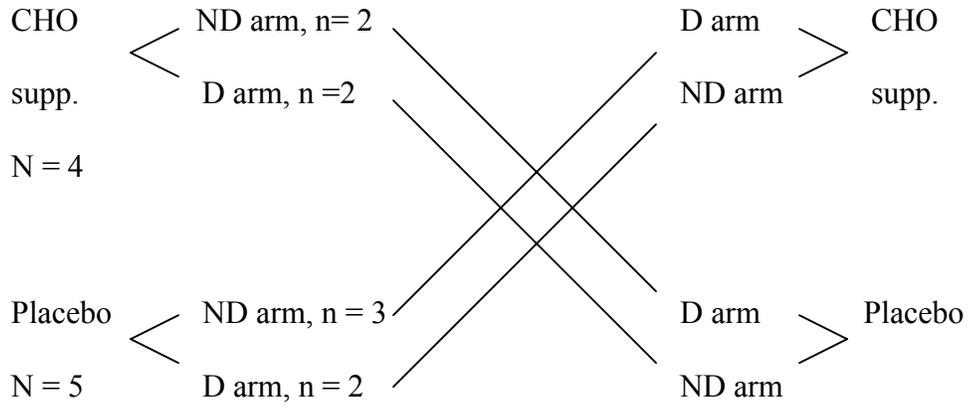


Figure 3.1: Cross-over design of study. Subjects were randomly assigned to either the carbohydrate supplemented group or the placebo group. Subjects were also randomly assigned to use either their non-dominant or dominant arm to perform the resistance exercises. There was three to four weeks between each condition, at which time, subjects began the condition, and used the arm, not used in the first trial.

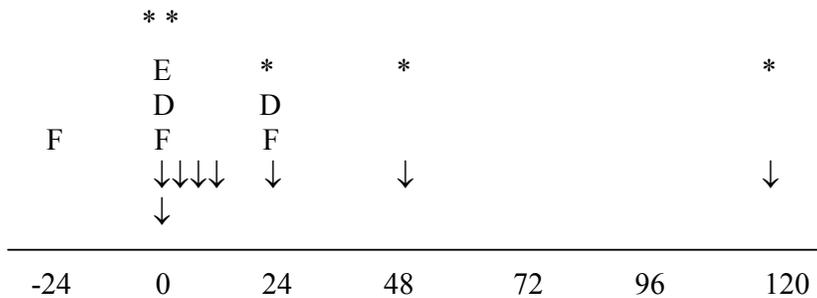


Figure 3.2. Schematic presentation of study design. \* = strength measures, E = eccentric exercise, D = drink (carbohydrate or placebo), F = food, ↓ = blood collection, soreness, and circumference measures.

### Controlled Diet

The day prior to, day of, and the day after the exercise session participants consumed a controlled diet. The diet consisted of 50% carbohydrate, 30% fat and 20% protein with either a carbohydrate or placebo drink as their sole source of kilocalories (kcal) throughout the day. The diet was planned using the Exchange List for Meal Planning (American Dietetic Association & American Diabetes Association, 2003). Caloric intake was determined by averaging the caloric results of a 24-hour recall and the Mifflin St.-Joer equation utilizing participant height, age and weight and self-reported activity level (Frankenfield, Rowe, Smith, & Cooney, 2003). An additional 10% of calories was added to the obtained average of caloric intake in case the participant desired more food and to address potential under-reporting inherent in self-reported dietary assessments. Once the average calories were obtained and the total calories were broken down into carbohydrate, protein and fat calories, the diet packs were assembled appropriately to match desired macronutrient percentages. Over the course of the three days meals were similar with minor variations to add variety to the subjects' diet and increase adherence. Modifications were made to individual diets to accommodate a participant if they had food allergies and/or restrictions. In order to make the conditions within each trial as similar as possible, food packs for the second trial were assembled to match exactly what the participants ate in the first trial.

Participants were given their food packs the day before they were to eat it and instructions were given before the diet was begun to assure that participants understood the requirements of the diet. Subjects were instructed to only eat and drink the food and beverage provided to them for the three day controlled diet period and were told to not deviate from the requirements of the study. All food packs contained a written log of food products and quantities in the bag. Using the written logs, subjects were instructed to write down how much of each food product they ate and at what time. Food was consumed *ad libitum*. Once the three days of each condition were over and the food logs were returned, individual food choices were entered into a nutrition analyses program to determine the exact macronutrient percentage of each participants diet. Dietary intake was analyzed using Nutritionist Pro (version 2.2.16, First Data Bank, Inc., San Bruno, CA.). Following the 48-hour blood draw, participants were allowed to resume their normal eating patterns.

#### Glucose Ingestion

A 6% carbohydrate or placebo drink was provided to subjects the day of and day after the exercise session. The placebo drink was matched to the carbohydrate beverage for color, flavor, and electrolyte content, but was sweetened with artificial sweetener, hence there were no carbohydrates in the drink. The beverages were consumed at a rate of 0.25 g/kg/hour, or 6-10 ounces per hour dependent on body weight. The 0.25 g/kg/hour was tested with pilot data to show a sustained increase in blood glucose throughout the day. The drink was consumed immediately following the exercise

session every hour for 12 hours and the following day beginning immediately after the 24-hour blood draw. Subjects were told to drink a small portion of the drink every 15 minutes so that the required hourly rate of consumption was done over a period of time, in an attempt to maintain blood glucose levels throughout the day, and not within one time point every hour.

The carbohydrate or placebo drink was consumed for 2 12-hour days until the evening before the 48-hour blood draw had been completed. Immediately following the 48-hour blood draw, subjects were able to resume their normal drinking patterns. Assignment to carbohydrate or placebo groups was randomized for the first trial with second trial group assignment dependent on the first trial. Administration of the drinks was double blind to prevent researcher and participant bias in results.

#### Eccentric Exercise to Induce Muscle Damage

High-force eccentric exercise with the arm muscles was used to induce muscle damage. During eccentric exercise, the muscle is contracting while trying to lengthen at the same time, thereby generating muscular resistance. Forty-five repetitions of eccentric elbow flexion were performed with maximal voluntary effort. Participants attempted to give maximal resistance even as fatigue decreased the actual force they were applying with each repetition. The exercises were done as three sets of 15 repetitions at a rate of one repetition per 15 seconds with 3 minutes rest between sets. Participants were seated at the Kin-Com exercise machine (Kin-Com 125 Et, Chattecx Corporation, Chattanooga, TN) with their chest and posterior brachium against padded rests with the

forearm secured on a padded lever. A repetition began with the elbow fully flexed and ended with the elbow fully extended. The machine moved the arm at a rate of 45 degrees/second. Participants exerted maximal resistance against the machine during the elbow extension portion of the exercise and relaxed during the elbow flexion portion of the exercise. Repetitions were repeated in this manner until 45 were completed.

Measurements of maximal isometric force production were done prior to and immediately following the eccentric exercise sessions to allow for comparison of effort between exercise sessions. The isometric exercises were done on each arm, regardless of which arm was used for the exercise session for strength comparison. The arm was placed on the Kin-Com as previously described for the eccentric exercise, except the forearm was held at a 90-degree angle from the elbow joint and upper arm. The isometric exercise was done three times for 10 seconds each on both arms for each session. Subjects exerted maximal resistance against the machine during this time and were verbally encouraged by researchers during the exercise to increase their effort. Isometric exercises were also done at 24, 48 and 120 hours post-exercise to determine when each individual's arm strength had returned.

### Blood Collection

Blood samples were obtained after participants rested for 10 minutes upon arrival to the lab. Blood was collected from an antecubital vein into evacuated tubes using a

standard venipuncture technique. Samples were obtained in a vacuum tube with EDTA for analysis of IL-6 and a tube without additive for analysis of CRP, CK and glucose. The EDTA tubes were immediately placed on ice and tubes without additive were allowed to clot. Blood was separated using a refrigerated 21000 Marathon centrifuge (Fisher Scientific, Pittsburgh, PA). Samples were then stored at  $-80^{\circ}\text{C}$  until analysis. All samples from a given subject were analyzed at the same time and within the same assay run for a given analysis to limit variability in tests.

#### Interleukin-6

IL-6 was measured as an indicator of acute inflammation and to determine the magnitude and duration of muscle damage induced by the high-force eccentric exercise. Measurements of IL-6 were assessed via a quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN). Assay plates were read at 650 nm using a  $\mu$ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) with correction for non-specific absorbance at 690 nm. A standard curve from standards of a known concentration was used to calculate the concentration of IL-6 in experimental samples. Samples were measured in duplicate.

#### C-Reactive Protein

CRP was measured as an indicator of the magnitude of the systemic inflammatory response to muscle damage and inflammation following eccentric exercise. CRP

measurements were made using an high sensitivity enzyme immunoassay (EIA) kit (MP Biomedicals, Orangeburg, NY). Assay plates were read at 450 nm using a  $\mu$ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were measured in duplicate.

#### Creatine Kinase

Creatine kinase was measured as an indirect marker of muscle damage following eccentric exercise. Serum CK was measured using an ultraviolet, kinetic assay (Sigma-Aldrich, St. Louis, MO). Modifications were made for microplate analysis and samples were read using a  $\mu$ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were measured in duplicate.

#### Blood Glucose

Blood glucose was measured as an assessment of whether subjects consumed the carbohydrate or placebo drinks as required during the study. Blood glucose was measured using a glucose hexokinase reagent set (Pointe Scientific, INC., Lincoln Park, Michigan). All samples were measured against a standard at an absorbance of 340 nm using a  $\mu$ Quant Universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were measured in duplicate.

### Muscle Soreness and Swelling

Muscle soreness was determined with subjective assessment by each participant using a 100-mm visual analog scale anchored at one end with ‘no soreness’ and ‘very, very, sore’ at the other end. The perception of soreness was assessed while participants flexed and fully extended the elbow using a one-kilogram weight. Measurement of the circumference of the mid-biceps was done to assess swelling. The peak of the biceps brachii muscle was palpated during flexion and three ink dots were placed on the arm perpendicular to the plane of axis. At this time, a measurement of ink-dot placement to the elbow was made for an accurate assessment of swelling should ink wash-off. These marks were used for assessment of swelling throughout the study protocol.

### Statistical Analysis

Data were analyzed using SPSS version of 11.5 for Windows (SPSS Inc., Chicago, IL). IL-6, CRP and CK data were analyzed by comparing the magnitude of the changes from baseline rather than the raw data. To calculate magnitude, the baseline (or pre-exercise) values of CRP, CK and IL-6 was subtracted from the values at a given time point. This was done so tested values could be compared between trials and subjects. Glucose measurements were done via raw data to assess blood glucose measures between the carbohydrate and placebo conditions throughout the duration of the testing phase. A repeated measures ANOVA test was done to detect differences in IL-6, CRP and CK concentrations across time intervals and carbohydrate intervention groups. A Mauchly’s

Test of Sphericity was done and, when the assumption of sphericity was violated, the Greenhouse Geisser correction factor was used to determine the p-value. If a significant p-value was found, Statistica 6.0 (Statsoft Inc., Tulsa, OK) was used to do a Tukey post-hoc analysis to determine where a main effect for time or condition by time was found. Statistical significance was set at an alpha level of 0.05.

## CHAPTER FOUR

## RESULTS

Subject Characteristics

Ten healthy subjects (six male, four female) participated in this study. Nine of the 10 subjects completed both conditions and data from these subjects was used in the data analysis. One subject dropped out due to health problems not related to the study.

The descriptive characteristics of the subjects are included in Table 4.1. Baseline values of all variables, except soreness, are reported in Table 4.2. Subjects were divided so that four began with the carbohydrate supplement and five began with the placebo. They were then assigned to use either their non-dominant or dominant arm for the exercise. The conditions were reversed for the second trial.

Table 4.1. Descriptive characteristics of subjects

ID	Sex	Age	Height (m)	Weight (kg)	BMI (kg/m <sup>2</sup> )	Order
1	M	24	1.88	78.2	22.1	ND-P, D-C
2	M	19	1.83	73.6	22.0	D-C, ND-P
3	M	29	1.78	74.1	23.4	D-P, ND-C
5	M	24	1.82	84.1	25.1	ND-P, D-C
6	M	19	1.88	88.6	26.5	D-C, ND-P
7	F	19	1.64	53.6	20.3	D-P, ND-C
8	F	31	1.68	93.2	33.1	ND-C, D-P
9	M	20	1.68	74.1	26.3	ND-P, D-C
10	F	39	1.74	70.0	23.1	ND-C, D-P
AVG		24.9	1.8	76.6	24.7	
SD		6.9	0.1	11.6	3.8	

M = male, F = female, BMI = body mass index, ND = non-dominant, D = dominant  
P = Placebo, C = Carbohydrate

Table 4.2. Baseline values of CRP, IL-6, CK and glucose for each condition.

	Avg	Min	Max
CRP (mg/L)			
Placebo	1.10	0.09	2.66
Carbohydrate	0.80	0.04	1.91
IL-6 (pg/ml)			
Placebo	1.17	0.22	4.06
Carbohydrate	0.62	0.20	1.36
CK (IU/L)			
Placebo	208.94	77.44	552.19
Carbohydrate	172.47	79.13	254.21
Glucose (mg/dl)			
Placebo	97.12	85.84	115.26
Carbohydrate	98.88	86.50	105.67
Strength (% decrease)			
Placebo	32.68	13.91	51.45
Carbohydrate	34.80	19.96	60.68
Circumference (cm)			
Placebo	0.00	0.00	0.00
Carbohydrate	0.33	0.00	3.00

### Controlled Diet

The food packs provided to subjects contained 50% carbohydrate, 20% protein and 30% fat. However, subjects frequently did not eat all of the provided food, therefore the diet macronutrient percentages were slightly different between individuals and between each trial (Table 4.3). However, within each individual, the diet percentages were similar between the two trials.

Table 4.3. Macronutrient diet percentages for each individual within the different conditions. CHO = carbohydrate, PRO = protein, P% = placebo percentage, C% = carbohydrate percentage.

Subject	CHO-Pavg	CHO-Cavg	PRO-Pavg	PRO-Cavg	FAT-Pavg	FAT-Cavg
1	50	50	16	16	34	34
2	55	55	16	16	29	28
3	55	54	15	15	30	30
5	55	56	16	16	29	28
6	57	56	14	14	30	30
7	55	54	18	18	27	28
8	49	55	18	18	33	28
9	52	49	19	19	30	30
10	56	51	16	16	30	34
Avg	54	53	16	16	30	30
SD	2.77	2.65	1.59	1.59	2.11	2.45

#### Interleukin-6

The ANOVA results are presented in Table 4.4. There was no significant time effect for IL-6 ( $p = 0.091$ ) and no significant difference for IL-6 between conditions ( $p = 0.146$ ). However, there was a significant condition by time interaction for IL-6 ( $p < 0.05$ ) as seen in Figure 4.1. There was a significant increase ( $P < 0.05$ ) in IL-6 from pre-exercise to 8 hours post-exercise within the carbohydrate condition. Confidence intervals for IL-6 were analyzed and it was found that the lower bound of the carbohydrate condition at the 8-hour time point overlapped with the mean of the placebo condition.

Table 4.4. IL-6 ANOVA results

Source		Type I Sum of Squares	df	Mean Square	F	Sig.
Condition	Greenhouse-Geisser	2.85	1.00	2.85	2.60	0.15
Time	Greenhouse-Geisser	11.57	1.30	8.88	3.30	0.09
Condition* Time	Sphericity Assumed	5.71	5.00	1.14	2.46	<0.05

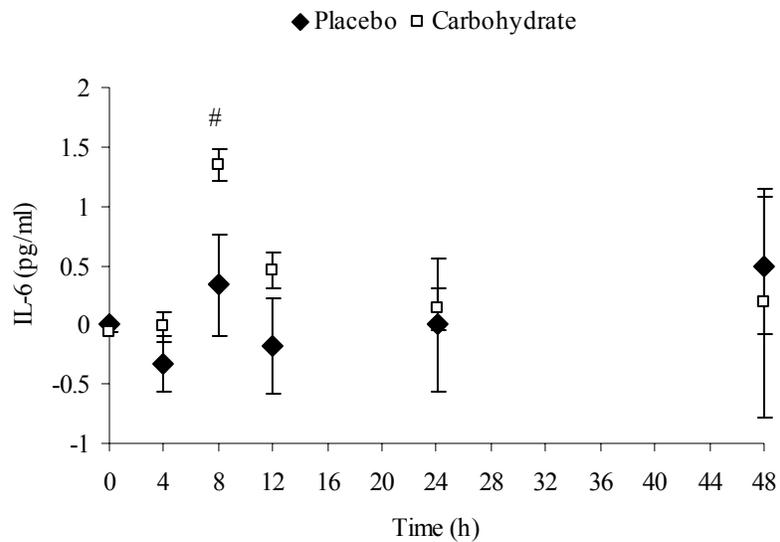


Figure 4.1. IL-6 responses for the carbohydrate and placebo conditions pre-exercise and at 4, 8, 12, 24 and 48 hours post-exercise. Values are mean  $\pm$  SD. # P < 0.05 compared to pre-exercise within carbohydrate condition.

### C-Reactive Protein

There was no significant difference for CRP between the carbohydrate and placebo conditions ( $p = 0.796$ ), a time effect ( $p = 0.112$ ) or a condition by time interaction ( $p = 0.576$ ). Data are presented in Figure 4.2.

Creatine Kinase

There was no significant effect for CK between the carbohydrate and placebo conditions ( $p = 0.415$ ), a time effect ( $p = 0.292$ ) or a condition by time interaction ( $p = 0.360$ ). Data are presented in Figure 4.3.

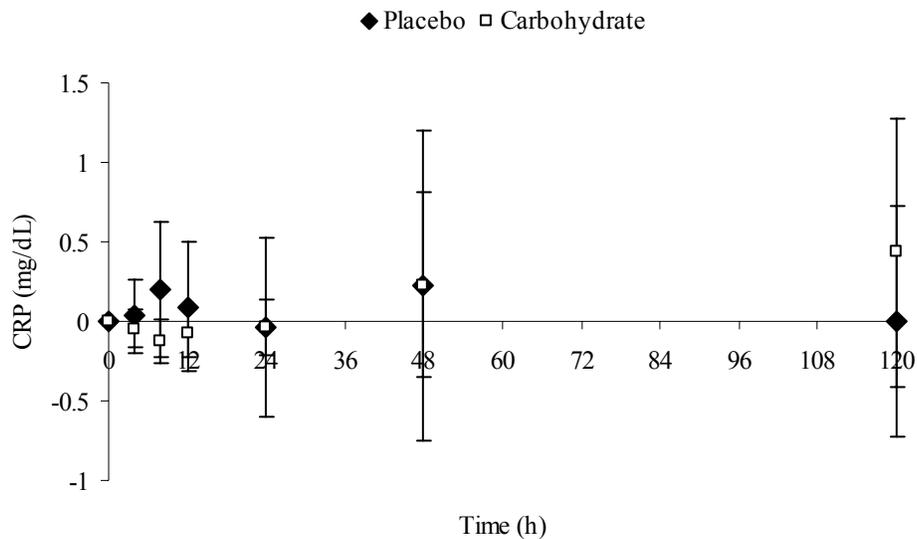


Figure 4.2. CRP response within the carbohydrate and placebo conditions, pre-exercise and 4, 8, 12, 24, 48 and 120 hours post exercise. Values are mean  $\pm$  SD.

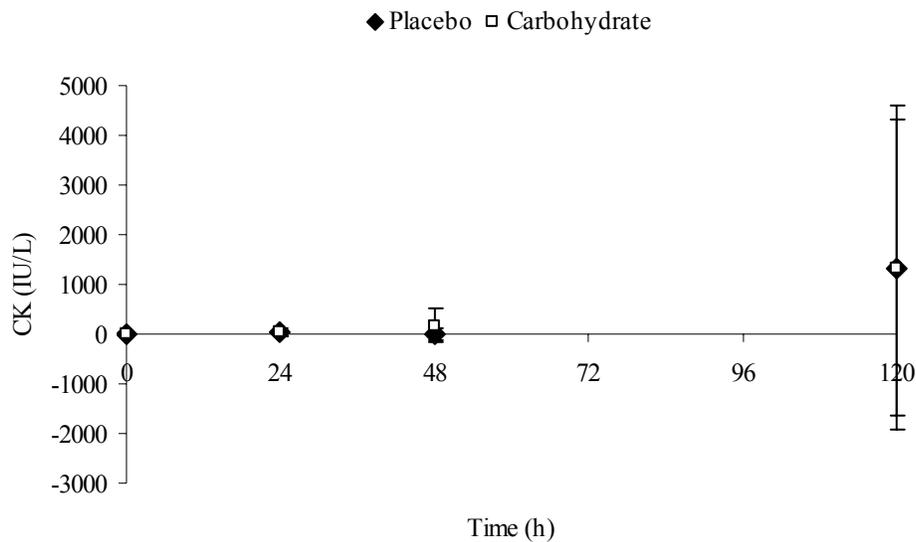


Figure 4.3. CK response within the carbohydrate and placebo conditions, pre-exercise, 24, 48 and 120 hours post-exercise. Values are  $\pm$  SD.

### Swelling

No differences ( $p = 0.903$ ) were detected in mid-brachial arm circumference between the carbohydrate and placebo conditions, and there was no significant condition by time effect ( $p = 0.886$ ). As an indicator of swelling, arm circumference increased over time ( $p = 0.001$ ). There was a significant increase in arm circumference from pre-exercise to 8 ( $p < 0.01$ ), 12 ( $p < 0.05$ ), and 120 ( $p < 0.05$ ) hours post-exercise. Data are presented in Figure 4.4.

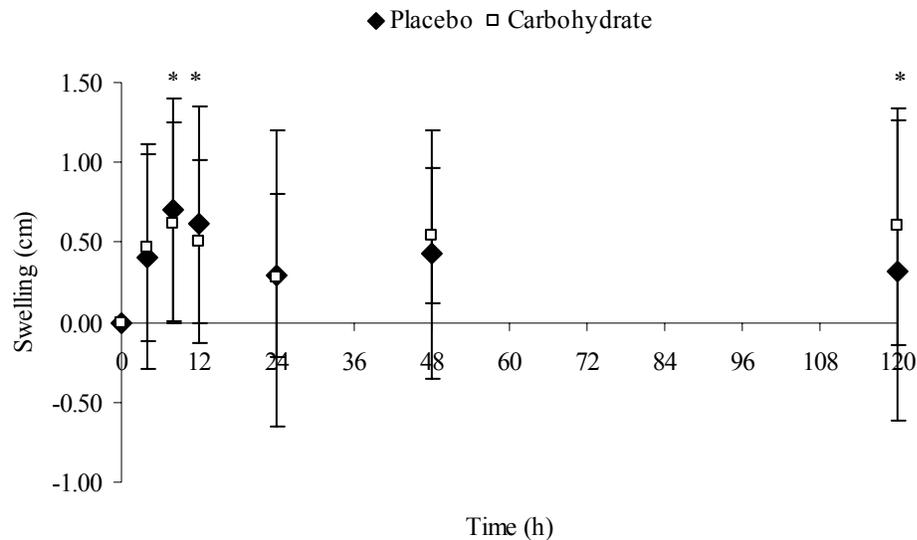


Figure 4.4. Change in arm circumference within the carbohydrate and placebo conditions pre-exercise and 4, 8, 12, 24, 48 and 120 hours post-exercise. Values are mean  $\pm$  SD. \*  $P < 0.05$  compared to pre-exercise.

### Soreness

No differences between the carbohydrate or placebo conditions ( $p = 0.965$ ) were detected for arm soreness, and there was no significant condition by time effect ( $p = 0.299$ ). A significant effect for time ( $p < 0.001$ ) was found for soreness. There was a significant increase in soreness from pre-exercise at 12 ( $p < 0.05$ ), 24 ( $p < 0.01$ ) and 48 ( $p < 0.01$ ) hours post-exercise. Data are presented in Figure 4.5.

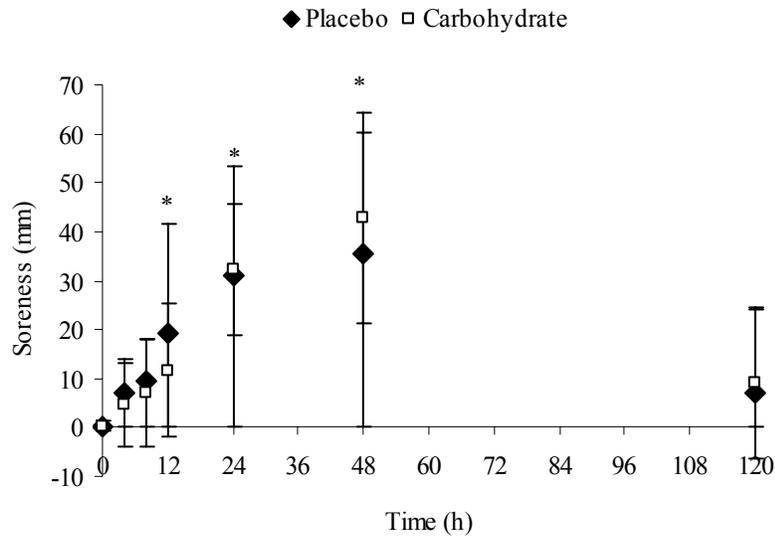


Figure 4.5. Soreness response within the carbohydrate and placebo conditions pre-exercise and 4, 8, 12, 24, 48 and 120 hours post-exercise. Values are mean  $\pm$  SD. \*  $P < 0.05$  compared to pre-exercise.

#### Maximal Isometric Force

There was no difference ( $p = 0.539$ ) in arm strength between the carbohydrate and placebo conditions and there was no significant condition by time effect ( $p = 0.797$ ). A significant effect for time was found for strength ( $p = 0.001$ ). There was a significant decrease in strength from pre-exercise to 0 hours post-exercise ( $p < 0.01$ ). Data are presented in Figure 4.6.

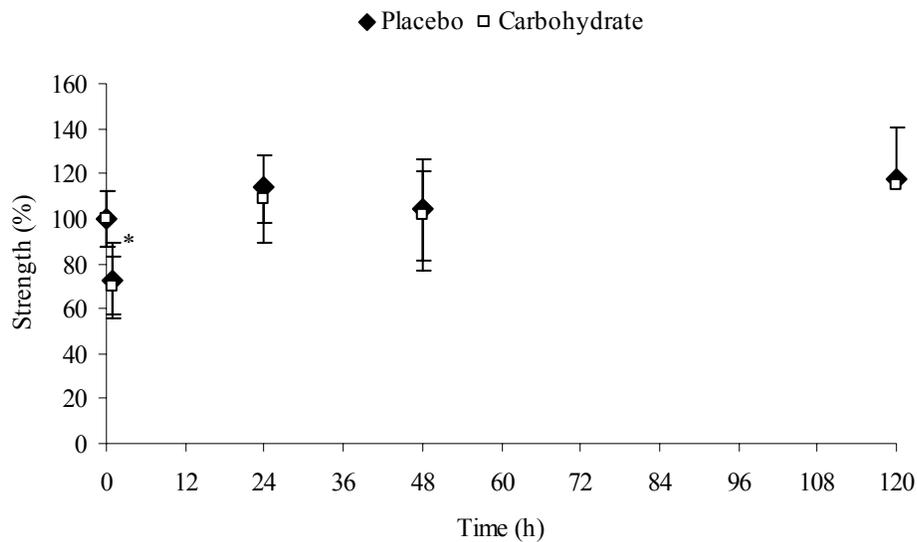


Figure 4.6. Strength response within the carbohydrate and placebo conditions pre-exercise and 24, 48 and 120 hours post-exercise. Values are mean  $\pm$  SD. \*  $P < 0.05$  compared to pre-exercise.

### Blood Glucose

There was no significant difference in blood glucose levels between the carbohydrate and placebo conditions, although a trend was detected ( $p = 0.076$ ). There was no significant condition by time effect ( $p = 0.960$ ) for blood glucose levels. There was a significant time effect for blood glucose levels ( $p = 0.034$ ). There was a significant increase in blood glucose levels from 4 to 12 hours post-exercise ( $p < 0.05$ ). Data are presented in Figure 4.7.

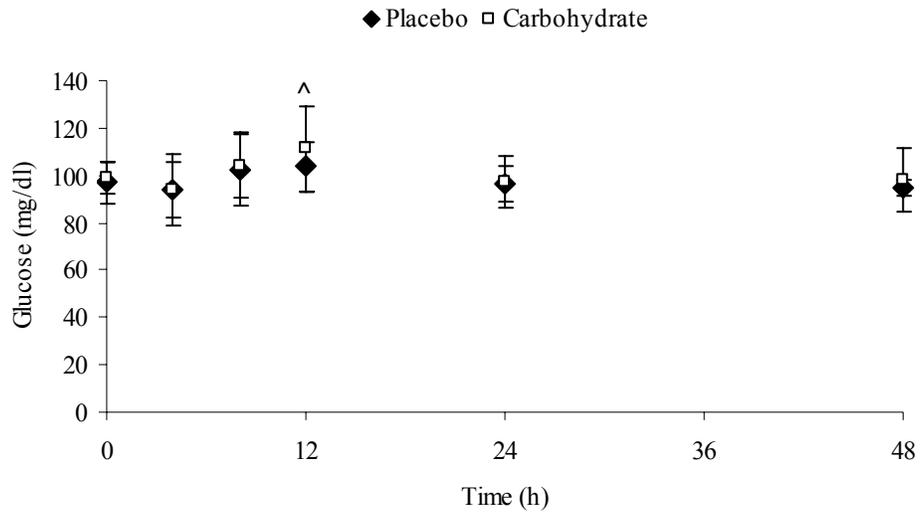


Figure 4.7. Glucose response within the carbohydrate and placebo conditions pre-exercise and at 4, 8, 12, 24, and 48 hours post-exercise. Values are  $\pm$  SD. <sup>^</sup>  $P < 0.05$  compared to 4 hours post-exercise.

## CHAPTER FIVE

### DISCUSSION

#### Introduction

This is the first known study to analyze the effect of carbohydrate on IL-6 4, 8, 12, 24 and 48 hours following resistance exercise. The main finding of this study was that carbohydrate increased the response of IL-6 8 hours post-exercise compared to placebo. This finding is in contrast with the first hypothesis of the study that states that IL-6 will be down regulated with a carbohydrate supplement versus a placebo during the first 48 hours following an eccentric bout of exercise.

The markers of muscle damage and the acute phase response to inflammation, creatine kinase and C-reactive protein, respectively, did not increase, and no difference was found between the carbohydrate and placebo groups for these measures. Carbohydrate had no effect on soreness or swelling between the conditions and the decrease in IL-6 with carbohydrate also had no effect on soreness or swelling.

#### Experimental Design

The goal of this study was to assess the affect of carbohydrate on IL-6, muscle damage, and inflammation following resistance exercise. Subjects consumed a controlled food plan with the addition of a carbohydrate or placebo drink within the conditions. It

was hypothesized that the condition with the carbohydrate drink would result in an attenuated response to IL-6 in comparison to placebo. The study was designed so that muscle damage would be induced via resistance exercise to the arm muscles. Muscle damage was measured with the indirect markers CK, swelling, soreness and decreased strength. Creatine kinase was measured at 0, 24, 48 and 120 hours post-exercise. Swelling was assessed by measuring the circumference of the mid-biceps in addition to a subjective measure of soreness by each subject. Strength was measured pre and immediately post-exercise, 24, 48 and 120 hours post-exercise. Inflammation was measured objectively by looking at C-reactive protein pre-exercise and at 4, 8, 12, 24, 48 and 120 hours post-exercise.

### Muscle Damage

Creatine kinase was used as an indirect marker of muscle damage in the present study. However, there was no increase in CK levels to indicate muscle damage. In addition to the objective assessment of muscle soreness and inflammation, this study also used arm circumference measurements of the mid-bicep brachii muscle to assess swelling and a subjective measurement of soreness by subjects as an indicator of muscle damage.

Despite no increase in CK, the perceived increase in soreness by subjects at 12, 24 and 48 hours post-exercise and the increase in arm circumference at 8, 12 and 120 hours post-exercise indicated that the exercise did induce muscle damage. The results of this study are consistent with those of Hirose et al. (2004) in which the same exercise

model was used. Researchers looked at the difference in the CK response between two exercise bouts. It was found that CK and muscle damage were higher in the first exercise bout versus the second bout indicating that the arm muscle adapted to the exercise. Stupka, Tarnopolosky, Yardley and Phillips (2000) had similar results and found that CK decreased about 20-30 percent in the second bout of exercise versus the first exercise session. In the present study, the criteria was that subjects arms were naïve to exercise so that the exercise model used in the study would induce soreness within each exercise trial. Since there was no detectable increase in CK, subjects may have been doing some more activity that involved frequent lifting with the arms than they were aware of resulting in the lack of a CK response due to muscular adaptations to the exercise.

Other possible explanations for the lack of an increase in CK could be the small amount of muscle mass or a maximal exercise session versus an endurance session. However, it has been shown that the amount of muscle mass involved in an exercise is not correlated to changes in markers of muscle damage (Nosaka & Clarkson, 1992). In fact, researchers have found that arm exercises versus leg exercises elicit a higher increase in CK (Jamurtas et al., 2005). Endurance versus maximal exercise have different affects on markers of muscle damage, but Nosaka, Newton & Sacco (2002) found that 12 maximal eccentric arm exercises had a larger increase in CK than 2-hours of continuous lifting with the arms. Hence, other studies support the use of the exercise model used in the present study in that arms were used rather than legs and the exercise consisted of a maximal eccentric exercise session. Future studies may need to use an exercise model

with the arms in which fewer repetitions and more force is applied for a detectable increase in muscle damage via CK.

### Diet and Glucose Ingestion

The goal of this study was to design two conditions in which carbohydrate intake and blood glucose levels were noticeably increased in one session versus the other. To accomplish this, a diet low in carbohydrates was supplied to each person over the course of three days. In addition to the diet plan, subjects were given either a carbohydrate or placebo drink to consume for two days. This was done so that consumption of the carbohydrate drink would result in a demonstrably increased blood glucose concentration. Subjects followed the pre-determined food plan and ate their food *ad libitum*. They were instructed to eat main meals following the blood draws and to eat in a consistent manner between the two trials. Blood glucose levels indicate that this was followed for the lunch time meal, but not the dinner meal, as blood glucose levels were significantly higher 12 hours post-exercise, opposed to 4 hours post-exercise due to meal consumption, not drink consumption. In the future it is suggested that subjects return to the lab to obtain and eat their food at a specified time following the blood draws. Controlling food intake would also help to keep the specified macronutrient content of the diet as required by the study design. However, the main goal of the study design was to keep a lower than normal carbohydrate intake during the three days of the controlled diet and to keep dietary conditions the same across both trials. Upon analyzing the macronutrient contents of all the subjects' diets, it was found that the carbohydrate, fat

and protein content were very similar for each individual within both trials (See Table 4.3) and the carbohydrate content was lower than normal according to the nutrition analyses from the 24 hour food re-call, hence our study goal was still met.

Specific instructions for beverage consumption were given to each subject in accordance with their body mass. A sustained increase in blood glucose was expected with the carbohydrate versus placebo drink, however, upon analysis of blood glucose this was not observed. The drink amount was small at .25 g/kg/hr and more g/kg/hr of carbohydrate may be required for a sustained increase in blood glucose throughout the day. In addition, similar to the diet, subjects were not monitored for drink consumption and there were no tracking methods in place to assure that subjects drank the required amount of the drink. Monitoring carbohydrate intake via drink consumption following exercise for up to 2 days presents itself with several challenges, one being scheduling. Drink consumption may be better analyzed if the study is designed for subjects to come into the lab to drink the provided beverages with a researcher present.

The sweetener used in the placebo drink was proprietary information, however, we believe it was likely Nutra-Sweet brand artificial sweetener. Nutra-Sweet in the placebo may have influenced our measurements because such a large quantity was ingested with the drink consumption. Large amounts of Nutra-Sweet do not affect blood glucose levels (Shigeta et al., 1985). However, amino acid levels are altered with large quantities of Nutra-Sweet. Nutra-Sweet is comprised of the amino acid phenylalanine. Phenylalanine levels are increased with large amounts of Nutra-Sweet, however, this was not a component of this research and is not known to affect the outcome of our results.

Thus, while substantial amounts of Nutra-Sweet were consumed this substance is not likely to have had a confounding effect on our findings.

### Interleukin-6

The present study sought to determine the affect of carbohydrate on the inflammatory response of IL-6 following high-force eccentric exercise. IL-6 was measured at 4, 8, 12, 24 and 48 hours post exercise and was significantly increased at 8 hours post-exercise in the carbohydrate versus placebo condition. IL-6 has multiple roles and is affected differently depending on the duration and type of exercise. In addition, it is affected by carbohydrate ingestion and current inflammatory conditions.

### Roles of IL-6

Interleukin-6 is involved in two different functional pathways related to exercise. In the first known IL-6 pathway, IL-6 has been found to increase in the acute-phase response to injury, inflammation and disease. With this response, IL-6 is produced by macrophages within the immune system and typically peaks several hours after exercise. In the second pathway muscle cells produce IL-6, likely due to exercise related changes in metabolism and independent of muscle damage or inflammation (Pedersen et al., 2001). It has been shown that due to the different time courses and magnitudes of response of IL-6 in inflammation versus exercise, the cytokine response to inflammation differs markedly from the exercise model (Bruunsgaard, 2005).

### Production of IL-6 Following Exercise-Induced Muscle Damage

The response of IL-6 to endurance exercise has been found to be of a larger magnitude and peaks immediately post-exercise in comparison to resistance exercise. Ostrowski et al. (1999) studied the cytokine response of ten participants in the Copenhagen marathon before and after more than three hours of running. IL-6 increased 128 fold immediately post-exercise from its pre-exercise levels. In contrast, exercise of a shorter duration lasting only 30 minutes using the elbow flexors found no significant difference in IL-6 from pre to immediately post-exercise and up to 4 days post-exercise (Hirose et al., 2004). The different responses of IL-6 appear to depend on the duration of the exercise session and in fact, exercise duration is closely linked to the magnitude of the increase in IL-6 (Pedersen et al., 2001).

IL-6 has been found to peak in shorter duration exercise sessions lasting less than thirty minutes, but at several hours, and even days post-exercise. Tomiya et al. (2004) found an increase in IL-6 at two different time points. The researchers used an eccentric contraction exercise model with the electrical stimulation of a mouse hind limb. IL-6 was found to increase up to 20% 12 hours post-exercise compared to pre-exercise levels. The initial increase in IL-6 at 12 hours post-exercise was attributed to its role in exercise and energy metabolism. IL-6 then began to decrease from its peak at 12-hours post-exercise, but was still higher 24 hours and 3 days later compared to the baseline levels. The sustained increase in IL-6 above baseline days following the exercise coincided with when muscle damage was found to be at its greatest and is attributed to IL-6's role with

inflammation and muscle damage. This indicates that the IL-6 response to muscle damage and inflammation is much more delayed and of a much smaller magnitude than the release of IL-6 due to exercise and metabolism.

In the present study, the increase in IL-6 observed 8 hours post-exercise is similar to the results of two studies in which IL-6 was found to peak 12 hours post-exercise (Tomoya et al., 2004) and 6 hours post-exercise (MacIntyre et al., 2001) with eccentric exercise to a single quadricep muscle. The results are different from those of Ostrowski et al. (1999) and Nehlsen-Cannarella et al. (1997) in which IL-6 was found to peak immediately following eccentric exercise of a longer duration, exceeding two hours. However, the two latter studies only measured IL-6 until 4 and 6 hours, respectively, post-exercise. In addition, because the exercise sessions lasted longer than two hours, the rise in IL-6 was likely due to its metabolic role as opposed to the inflammatory role. In an unpublished study, researchers have found that the time course for increases in IL-6 due to inflammation may be 8 hours post-exercise (M.P. Miles, personal communication, November 13, 2006). Because this time-point is not routinely analyzed, it is difficult to conclude if the results of the present study coincide or contradict currently published research.

#### Glucose Ingestion, Exercise and IL-6

IL-6 is released from muscle during exercise due to increases in IL-6 transcription factors, mRNA levels and protein levels within the working muscle fibers (Bruunsgaard, 2005). This increase is associated with exercise related metabolic changes. For example,

Steensberg et al. (2001) performed a study in which knee extensor exercises were done with one leg having a low glycogen content, while the other leg had 'normal' glycogen content. While IL-6 mRNA increased in both legs, indicating the release of IL-6 from muscle cells, the glycogen depleted leg had an almost double net release of IL-6 during the exercise session in comparison to the normal glycogen leg. The storage form of carbohydrate is glycogen, hence, Steensberg supported the hypothesis that IL-6 is involved in exercise metabolism via the production of endogenous glucose.

Longer duration, higher intensity exercise is known to deplete muscle glycogen stores more readily than exercise of a shorter duration and lower intensity (Manore & Thompson, 2000). Within longer duration exercise studies, subjects tend to exhibit decreased muscle glycogen content, and hence an immediate post-exercise increase in IL-6 to increase glucose availability. The shorter duration exercise sessions, as in this study, likely do not induce a large change in muscle glycogen content. The increased response of IL-6 several hours post-exercise suggests that IL-6 release with lower intensity exercise may be more closely linked to muscle damage and the inflammatory cascade as opposed to an energy metabolic state.

Carbohydrate ingestion prior to and during exercise has been found to attenuate the response of IL-6 during an exercise session. Febbraio et al. (2003) found that consumption of 250 ml of a carbohydrate-rich drink at the beginning and at 15-minute intervals during a 2-hour bicycling session decreased plasma IL-6 concentrations nearly two and a half times compared to a placebo. Studies done on running have had similar results. The IL-6 response was decreased by nearly half with carbohydrate versus placebo

ingestion immediately post-exercise and up to one and a half hours post-exercise during an intense two and a half hour running session (Nehlsen-Cannarella et al., 1997). IL-6 is increased in glycogen-depleted muscles, (Steensberg et al., 2001) and is a factor in endogenous glucose production and, hence controls glucose homeostasis (Febbraio et al., 2004). When glucose is ingested during the course of exercise, glycogen levels will not decrease as fast when compared to placebo ingestion and glucose production may, therefore, be minimal. IL-6 is not released as much with carbohydrate ingestion when compared to placebo during exercise sessions, indicative of its role in glucose homeostasis (Febbraio & Pedersen, 2002) and its link to exercise related metabolism.

#### Glucose Ingestion, Inflammation and IL-6

Carbohydrate ingestion also has an effect on the inflammatory response of IL-6 following exercise. In the present study, carbohydrate increased the response of IL-6 8-hours following a short duration resistance exercise session. While no other studies substantiate these findings, research has shown that a hyperglycemic state associated with increased carbohydrate intake increases inflammation. Hyperglycemia is a condition in which blood glucose levels are greater than 110 mg/dl during a fasted state or 140 mg/dl throughout the day with food and drink intake (Mahan & Escott-Stump, 2004). There are multiple physiological mechanisms that can induce hyperglycemia, however, it is typically a result of increased carbohydrate consumption. It has been found that inflammation is exacerbated with hyperglycemia due to the production of reactive oxygen species.

Reactive oxygen species (ROS) are a by-product of normal metabolism and are important in cell signaling. However, during environmental stress, ROS's dramatically increase and cause damage to cell structures (reactive oxygen species, n.d.). Lin et al. (2005) found that hyperglycemia in rats increased the production of ROS's in adipose, increasing inflammation and hence, increased adipose production of IL-6. However, both the over-expression of ROS inhibitors and the infusion of the anti-oxidant glutathione, attenuated the rise in IL-6 when coupled with hyperglycemia (Lin et al., 2005 & Esposito et al., 2002). This indicates that the increase in IL-6 associated with hyperglycemia is partially mediated by the production of reactive oxygen species.

Hyperglycemia in humans has also resulted in an increase in ROS and inflammatory parameters. Devaraj, Venugopal, Singh & Jialal (2005) found that hyperglycemia increased mRNA and intracellular IL-6, in addition to an increased secretion of IL-6 into the blood stream. Similarly, Esposito et al. (2002) found that acute hyperglycemia increased the production of IL-6 and TNF- $\alpha$  in control subjects and those with impaired glucose tolerance (IGT). In addition, those with IGT had higher increases in IL-6 and TNF- $\alpha$  in response to hyperglycemia. Impaired glucose tolerance is associated with hyperglycemia and is linked to increased ROS production (Mohanty, Hamouda, Garg, Aljada, Ghanim & Dandona, 2000). Therefore, an increased IL-6 in subjects with IGT compared to controls is expected. This is similar to the results of the present study, in that Mohanty et al. (2000) induced a hyperglycemic state in subjects, on top of the inflammatory state associated with IGT and found increases in IL-6. The

present study induced inflammation with muscle damage and then increased subject's carbohydrate intake for an increased rise in IL-6. However, the subjects did not exhibit a 'true' hyperglycemic state. This indicates that even a small increase in carbohydrate can increase the inflammatory response, despite the lack of a hyperglycemic state.

There are no known studies that specifically relate to increases in IL-6 with carbohydrate supplementation up to 48 hours post-exercise in normal subjects following resistance exercise. However, diabetes, a condition associated with poor carbohydrate metabolism and blood glucose control is considered an inflammatory disease. An active inflammatory state, such as that associated with diabetes, in addition to hyperglycemia and exercise increases IL-6. Galassetti et al. (2005) found a correlation between morning hyperglycemia levels and resting IL-6 levels. The researchers found a dose dependent increase in IL-6 during and following an exercise session in children with Type 1 diabetes mellitus. IL-6 increased in proportion to morning hyperglycemia and children with the highest blood glucose, greater than twice that of the lowest blood glucose group, had IL-6 levels that were roughly three times that of those with the lowest blood glucose level. Therefore, hyperglycemia with exercise does result in increased levels of IL-6, and those with higher blood glucose levels will have an even greater increase in IL-6. The researchers findings are supportive of the relationship between the hyperglycemic state and increased production of ROS and markers of inflammation, specifically IL-6. The finding of this study is unique in that it is the first known study to show that resistance exercise and increased carbohydrates in normal adults results in an increase in IL-6 post-exercise. This suggests that increased carbohydrate intake has an effect on inflammation

following exercise, even in healthy, non-diabetic adults with exercise-induced inflammation.

Increased adiposity in people has also been shown to increase inflammatory markers. Adipose tissue is a metabolically active tissue that produces several substances, including markers of inflammation. Body mass index (BMI) is a measure of adiposity in adults and a BMI greater than 25 indicates overweight or obesity. Hyperglycemia has been found to increase the inflammatory response in obese, reproductive age women (Gonzalez, Minium, Rote, & Kirwan, 2005). Hyperglycemia resulted in a 17% increase in TNF- $\alpha$  production from monocytes following hyperglycemia in obese women compared to non-obese controls who had a 69% decline in TNF- $\alpha$  production. In regards to the present study, TNF- $\alpha$  upregulates the expression of IL-6, therefore, it is presumed that an increase in TNF- $\alpha$  will result in an increase in IL-6. Researchers in the present study found increases in IL-6 with increased carbohydrate ingestion. However, in contrast, a majority of the subjects in the current study were not considered overweight or obese according to their BMI. This indicates that carbohydrate increases IL-6 regardless of BMI and obesity in healthy adults with exercise induced inflammation.

Thus far it has been established that the delayed increase in IL-6 several hours post-exercise is due to the inflammatory response of IL-6 as opposed to the metabolic role of IL-6 with exercise. With the present study, the increase in IL-6 several hours post-exercise indicates that carbohydrate has an effect on the response of IL-6 to inflammation. The result of an increased IL-6 response on the overall inflammatory cascade is still not known.

A limitation of the present study is the lack of a true hyperglycemic state according to blood glucose levels in all subjects. Fasting and casual blood glucose levels throughout the day were considered to be within normal ranges. However, the increased carbohydrate intake and associated increase in IL-6 following exercise, indicates that additional carbohydrates had an effect on the inflammatory response several hours post-exercise. In addition, the current study has shown that this effect appears to occur regardless of BMI or current inflammatory conditions. It may be concluded that, despite a true hyperglycemic state, additional carbohydrates increase the inflammatory response via increases in IL-6.

#### C-Reactive Protein

To assess the inflammatory response to eccentric exercise, CRP is considered a 'prototypic marker of inflammation' (Devaraj, O'Keefe, & Jialal, 2005) and was therefore used as an objective marker of systemic inflammation in this study. CRP was not found to increase over time, or across conditions. However, the detectable increase in arm circumference that was found indicates that there was swelling, likely induced by local inflammation of the arm muscle.

The intensity of exercise appears to be a much larger factor in the induction of a major inflammatory response and increase in CRP. Henson et al. (2000) conducted a moderately intense 2-hour exercise session of rowing that also did not elicit any change in CRP between a carbohydrate or placebo group. Not only was this exercise twice as long as the exercise session in the current study, but there was recruitment of major

muscle groups in the legs and back. Another difference between the studies was the timing of blood draws, which were done only up until 1.5 hours post-exercise in Henson's study whereas CRP was drawn at 4, 8, 12, 24, 28 and 120 hours post-exercise in this study. The focus of Henson et al. (2000) differed from the present study, in that Henson looked at the phagocytic and cytokine response to carbohydrate, whereas researchers in this study only analyzed the cytokine response to carbohydrate.

The results of this study are consistent with those of other studies in which CRP levels were not affected by carbohydrate intake. Researchers looking at the effects of a high protein or high carbohydrate diet in obese individuals found that neither diet type had an effect on CRP (Due, Toubro, Stender, Skov, & Astrup, 2004). However, a correlation was found between body fatness and CRP, similar to the results of Devaraj et al. (2005) in which individuals with a higher percentage of body fat and considered to be overweight or obese, had higher CRP levels. Most subjects in this study were not considered overweight or obese, hence this may partially explain the lack of an increase in CRP.

Glycemic load, referring to the type of carbohydrate eaten in the diet, however, has been found to effect CRP levels. Liu et al. (2002) found that dietary glycemic load did affect CRP levels in middle-aged women. Women who were in the highest quintile of dietary glycemic load, and hence consumed a higher refined carbohydrate intake and higher total carbohydrate intake had higher CRP levels. The researchers also found a significant correlation between BMI and CRP, similar to the previously mentioned studies, but the effect of dietary glycemic load was independent of BMI. In addition, the

average age of women in the study was 59 years old. It has been found that CRP is affected by increased age (Devaraj et al., 2005). Therefore it is plausible to speculate that age may have had a factor in the increased CRP levels within Liu et al.'s (2002) study, in addition to the dietary glycemic load and BMI. The increased CRP levels with carbohydrate are in contrast to the present study, in which carbohydrate versus placebo had no effect on CRP levels. However, the difference between the two studies is that all of the subjects in the current study were considered to be of a young age and most were considered to be within the healthy weight range according to their BMI, as opposed to the older and heavier women in Liu's study.

For the current study, it was concluded that without the confounding effects of age and BMI, dietary glycemic load had no effect on CRP levels. Though the diet protocol used in this study had a lower than normal carbohydrate content, the addition of a carbohydrate drink resulted in a high total dietary carbohydrate intake. If CRP was truly affected by dietary glycemic load, an increase in CRP partially due to diet would have been expected, without the confounding factors of age and weight. But, this was not found within this study. The response or lack of response of CRP may be due to several factors, including environmental, physical and metabolic variation between people, in addition to the timing of blood draws and laboratory errors (Liu et al., 2002).

The increase in arm circumference found in this study indicates a local inflammatory response did occur, despite no increase in systemic inflammation measured via CRP. The lack of an increase in CRP is consistent with the results of Milias, Nomikos, Fragopoulou, Athanasopoulos, & Antonopoulou (2005). The authors

conducted an eccentric exercise session with the arm muscles and found no increase in CRP at 2, 24, 48, 72 and 96 hours post-exercise compared to pre-exercise levels. Similar to this study, the authors concluded that the increase in arm circumference and muscle soreness was indicative of an inflammatory response to the exercise.

## CHAPTER 6

## CONCLUSION

In summary, carbohydrate increased the rise of IL-6 8 hours post-exercise in comparison to placebo. Carbohydrate did not have an effect on CRP, CK, swelling or soreness. A significant increase in soreness was found 12, 24 and 48 hours post-exercise. Circumference of the arm, indicative of swelling, was significantly increased at 8, 12 and 120 hours post-exercise. No significant difference was found for blood glucose levels between the carbohydrate or placebo condition, but there was a significant increase in blood glucose at 12 hours post-exercise in comparison to the 4-hour post-exercise blood draw.

One limitation of this study was the lack of a controlled environment for food and drink consumption. Though subjects were given a food pack with foods to represent a specific diet macronutrient percentage, they were allowed to eat the food *ad libitum*. They were asked to record the food that they did eat within the pack and that which they did not, however, inaccuracy in reporting and an over/underestimation of the food quantity eaten is possible. In addition, consumption of the carbohydrate versus placebo drink was expected to result in a significant difference in blood glucose levels. However, there was no difference in blood glucose levels between the carbohydrate and placebo conditions. Consumption of the drink was not controlled nor was it monitored. Subjects were only instructed on how much and when they were to drink.

Future studies should control the diet and drink consumption much more rigidly than done in the present study. This could be accomplished by having subjects come into the lab and eat their meals with a researcher present. In addition, it may be helpful if the drink is administered in such a way that the subject must consume the drink over several time intervals with a researcher present and, therefore, an accurate measurement of drink consumption can be made.

Another limitation of the study is the small sample size. It is difficult to extrapolate the results of a small study population to larger populations of people. However, this study has provided us with new insight into the effects of carbohydrate on inflammation following high-force eccentric exercise. In the future, a larger study population may help us to better understand the impact of carbohydrate on inflammation.

Regarding the exercise model used in this study, previous studies have used an exercise model similar to the one used in this study. Therefore, we know that the eccentric exercise model used is adequate to induce muscle damage and inflammation. The present study found no increases in systemic markers of inflammation and muscle damage, measured via CRP and CK respectively. However, the increase in IL-6 several hours post-exercise indicates a local inflammatory response occurred within the muscle cells. In addition, the increase in soreness at 12, 24 and 48 hours post-exercise and arm circumference at 8 and 120 hours post-exercise also suggest that muscle damage and inflammation did occur. To induce a detectable, systemic increase in muscle damage and inflammation, future studies may need to increase the number of repetitions during each exercise session and/or increase the resistance on the arm muscle.

Despite the limitations of the study, the results of this study are significant because this is the first study to show that carbohydrate ingestion following eccentric resistance exercise affects IL-6. Therefore, this is the first study to show that carbohydrate ingestion has an affect on the inflammatory response due to muscle damage following high-force eccentric exercise.

The functions of carbohydrate are multi-factorial. In general, carbohydrate is a primary source of energy for the body. However, this study has shown that carbohydrate also affects the inflammatory response. With the role of carbohydrate as an energy source, the recommendation is to increase carbohydrate intake, especially during long duration exercise sessions for sustained energy. However, this study shows that additional carbohydrate intake following exercise increases the body's inflammatory response. Though exercise itself produces muscle damage and inflammation, carbohydrate appears to exacerbate the inflammatory response. A possible mechanism for this is the hyperglycemic state that is a result of excess carbohydrate intake. However, we found that excess carbohydrate intake resulted in inflammation, despite the lack of a hyperglycemic state. The mechanism behind this is unknown. However, carbohydrate appears to play a 'good' role for energy, but a 'bad' role for inflammation. The conundrum then exists as to whether carbohydrate should be increased or decreased. The solution may be to consume carbohydrate during exercise for energy, but limit carbohydrate following exercise to prevent a rise in inflammation. The results of this study present with several questions related to carbohydrate intake, inflammation and

exercise. Much more research is required for a greater understanding of carbohydrate's role in inflammation.

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APPENDIX A:

INFORMED CONSENT

**SUBJECT CONSENT FORM FOR PARTICIPATION IN HUMAN RESEARCH  
MONTANA STATE UNIVERSITY**

**Study Title:** The effects of carbohydrate supplementation on IL-6 following an eccentric bout of exercise.

**Funding:** American Heart Association, Pacific Mountain Affiliate (In part)

**Investigator:** Sherri D. Pearson, B.S.

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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 muscles of one arm sore. 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. Carbohydrate intake may influence the control of inflammation. We will measure some of the inflammatory markers associated with heart disease, have you perform the resistance exercise, control your diet 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 have a low carbohydrate intake. 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. There may be a genetic component that influences how you respond to the resistance exercise. We also will determine whether you have variations in certain genes that may affect the response you have to the resistance exercise. 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 carbohydrate supplementation versus a placebo following exercise-induced muscle damage. 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 and health history questionnaire that includes questions regarding the presence of heart disease and diabetes in your family.
- 2) Report to the Nutrition Research Laboratory in 20 Herrick Hall for diet instructions prior to the initial exercise trial. A 24-hour recall will be done at this time to determine your daily caloric needs, in addition, your calorie needs will be assessed using an equation that takes into account your height, weight, age and activity level as determined from a physical activity questionnaire. An average of your daily caloric needs will be determined using the re-call and the equation. This average will then be used to determine the amount of foods you can eat within specific food groups. The diet will be proportioned as 50% carbohydrate, 30% fat and 20% protein.
- 3) Your diet will be controlled the day before, the day of and the day after the resistance exercises are performed. You will be provided a food pack for the three days of the controlled diet. The day before and day after the exercise trial you will eat the food packs on your own. The day of the exercise trial you will eat your meals in the laboratory after the 4, 8 and 12-hour blood draws. The diet will be repeated within each exercise trial with the addition of either a carbohydrate or placebo drink to be consumed every hour for 12 hours the day of and day after the resistance exercises are performed. The drink will be consumed at a rate of 6-10 ounces per hour dependent on your body weight. You will consume the drink from the time point immediately following the resistance exercises until the 48-hour blood draw has been completed. Once the 48-hour blood draw has been done, you will be allowed to resume your normal eating patterns.
- 4) Twenty-four hours after starting the controlled diet, you will report to the Movement Sciences Lab in the basement of the Romney gymnasium. At this time, you will perform a resistance 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 investigator 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 extend your arm against the resistance of the investigator to a fully extended position. Three sets of 15 contractions will be performed with five minutes rest between each set. Each contraction will last approximately 3 seconds with a 12-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. You will go through this protocol two times with about three weeks between each resistance exercise session.

- 5) Before the exercise and immediately, 24, 48, and 96 hours following the exercise, you will perform three maximal contractions with the arm flexed at 90 degrees to determine your maximum force production. You will be seated on the exercise machine described above. Three maximal contractions will be performed with 60 seconds of rest between trials.
- 6) Each time you come to the lab 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 and extend your arm holding a 1-pound weight 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) Report to the Movement Science Laboratory or Nutrition Research Laboratory on the MSU campus for measurements, including collection of a blood sample. Baseline and follow up measurements (including pre-exercise blood collection and maximum force, resistance exercise and 4, 8, 12, 24, 48, 96 hours post-exercise blood collection).
- 9) You will repeat numbers 3-8 two times, with three weeks between each diet and exercise session. The difference between each condition will be the consumption of either a carbohydrate or placebo beverage the day of and day after the exercise session.
- 10) We will determine which type of gene you have for inflammatory mediators e.g. interleukin-6. You will not be given any information regarding the types of genes that you have.
- 11) You will receive \$10.00 compensation for each blood sample collected (\$140 total for all 14 blood collection/visits involved).

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 14 occasions (7 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) 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 Sherri Pearson at 406-994-5001 or Mary Miles at 406-994-6678 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 Mary Miles at 994-6678.

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: The effects of carbohydrate supplementation on IL-6 during an eccentric bout of exercise.

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 form for my own records.

Signed: \_\_\_\_\_  
*Subject's Signature*

Date: \_\_\_\_\_

Witness: \_\_\_\_\_  
*(if other than the investigator)*

Date: \_\_\_\_\_

Investigator: \_\_\_\_\_  
Sherri D. Pearson, B.S.

Date: \_\_\_\_\_