DO CARBOHYDRATES INCREASE THE MAGNITUDE OF THE

INFLAMMATORY RESPONSE?

by

Chris Michael Depner

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Health and Human Development

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2008

APPROVAL

of a thesis submitted by

Chris Michael Depner

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. Mary P. Miles

Approved for the Department of Health and Human Development

Dr. Tim Dunnagan

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

Chris Michael Depner

July 2008

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ABSTRACT

Inflammation is the body's response to tissue damage and infection and is correlated with several chronic diseases like type II diabetes. Cytokines are cell signaling proteins with multiple functions including control of inflammation. Cytokines are influenced by several factors such as carbohydrate intake and exercise. Thus, carbohydrate intake and exercise can influence inflammation. Purpose: To determine the influence of high carbohydrate intake on the inflammatory response to exercise that induces muscle damage and inflammation. Methods: The study was a cross-over design. Each subject completed a high carbohydrate condition and a high fat and protein condition. Each condition consisted of 6 sets of 10 maximal high-force eccentric contractions of the elbow flexors and extensors. The exercise was followed by a controlled diet for the first 8 hours post-exercise based on the condition. The cytokines interleukin-6 (IL-6) and interleukin-1β (IL-β) were measured as indicators of local inflammation. C-reactive protein (CRP) was measured as an indicator of systemic inflammation. Creatine-kinase (CK), muscle soreness, upper arm circumference, and strength loss were measured as indicators of muscle damage. Blood glucose and insulin were measured to identify differences between diets in the conditions. Results: Insulin was significantly increased in the high carbohydrate condition compared to the high fat and protein condition at 1.5, 4, and 8 hours post-exercise. Perceived soreness was elevated at all time points post-exercise in both conditions and was significantly elevated in the high carbohydrate condition compared to the high fat and protein condition. There was a main effect trend for IL-6 to be greater in the high carbohydrate condition compared to the high fat and protein condition. Il-1β was significantly increased 24 hours post-exercise in the high carbohydrate condition compared to the high fat and protein condition. Conclusion: Elevated carbohydrate intake post-exercise augmented the local inflammatory response to the exercise observed by elevated IL-1β and IL-6. The augmented inflammatory response contributed to greater perceived muscle soreness post-exercise. Further research is required to investigate this mechanism further to provide better prevention and treatment methods for chronic diseases related to inflammation.

CHAPTER ONE

INTRODUCTION

Development of the Problem

The leading cause of death in the world today is chronic disease. Cardiovascular disease causes the most deaths followed by cancer, chronic lung disease, and diabetes mellitus. Regular exercise is correlated with a decreased risk for some of these diseases, primarily cardiovascular disease and type 2 diabetes (Blair, Cheng, & Holder, 2001). Also, physical training has been shown to be effective for treatment of patients with cardiovascular disease and type 2 diabetes (Boule, Haddad, Kenny, Wells, Sigal, 2001).

Low-grade systemic inflammation is present in patients with obesity, insulin resistance, type 2 diabetes, and atherosclerosis (Petersen & Pedersen, 2005). One of the major initiators of inflammation is the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) (Ostrowski, Rhode, Asp, Schjerling, & Pedersen, 1999). Patients with type 2 diabetes have high TNF-α expression in their skeletal muscle and plasma (Feingold & Grunfeld, 1992). TNF-α is correlated with impaired insulin mediated glucose uptake. Obese mice with a TNF-α gene knockout are protected from insulin resistance (Uysal, Weisbrock, Marino, & Hotamisligil, 1997). These studies and many others have demonstrated the correlation between chronic diseases, inflammation, TNF-α, and other pro-inflammatory cytokines. Therefore, the inflammatory mechanism is commonly studied in trying to better understand its role in chronic diseases.

Many studies try to define the mechanism that offers protection and treatment of

these diseases through exercise (Braun & von Duvillard, 2004; Gleeson & Bishop, 2000; Nehlsen-Cannarella et al., 1997; Nosaka & Clarkson, 1995; Ostrowski et al., 1999). One possible way exercise may offer protection against these diseases is through an increased anti-inflammatory response that will turn off production of the pro-inflammatory cytokine TNF-α (Petersen & Pedersen, 2005). However, this mechanism is not fully understood and there is still more research required in this area.

The majority of the research concerning exercise and its effect on inflammation deals with endurance type exercise. There is some research but a smaller amount investigating high intensity resistance training and its influence on inflammation. More research is required for both resistance and endurance training to better understand the mechanisms of how and why exercise can help prevent chronic diseases like Type 2 diabetes and heart disease. With a better understanding of the mechanism behind this response, better recommendations can be made for prevention and treatment of these diseases.

Purpose

The aim of the study was to use high-force eccentric exercise in combination with high and low carbohydrate diets to better understand the inflammatory mechanism. More specifically, with a better understanding of how exercise and carbohydrate levels in the diet are correlated with the inflammatory process, better prevention and treatment recommendations can be made about chronic low-level inflammation. Chronic low-level inflammation is correlated with several chronic diseases such as type II diabetes,

atherosclerosis, and Alzheimer's disease. Having better recommendations for prevention and treatment of chronic low-level inflammation will potentially help decrease the number of people with chronic diseases associated with chronic low-level inflammation. Specifically, this study will determine if a high carbohydrate diet over several hours at the start of acute inflammation will change the inflammatory response compared to a low carbohydrate diet over the same time period. Exercise in this study is simply used to induce inflammation so it can be studied. Previous studies have looked at the effect of carbohydrates on inflammation in non-healthy subjects, but this study will use healthy subjects to determine if there are any differences in the mechanisms at work.

Implications

The most significant long-term implication in this study is the potential to produce better prevention and treatment recommendations for inflammatory diseases based on nutrition and exercise. This category of diseases is the leading cause of death in the world and is still increasing. Therefore, it is highly significant to study the mechanisms of these diseases in order to provide better advice and information on prevention and treatment. This will require synthesizing the results of several studies to get to the point where improved recommendations could make a difference. This study has the potential to provide a more clear understanding of the mechanism of inflammation in relation to diet and exercise. The first step in being able to make better recommendations is to have a more clear understanding of the mechanisms at work.

Another possible implication of the present study will be to help athletes in

recovery from resistance-training. Overall, during recovery an increase in inflammation can initially cause an increase in tissue damage and actually result in a longer recovery period. One goal of many athletes is to shorten the recovery period as much as possible. So, one way to reduce the recovery period is to reduce inflammation. The results of this study will help better understand the mechanism of inflammation in response to resistance exercise and may help lead to better recovery methods that reduce inflammation and recovery time.

Future research possibilities after this study could lead to more mechanistic research based on inflammation. Eventually, when the mechanism is understood well enough, future studies could look at how to apply the mechanism to treatment and prevention techniques.

Hypothesis

It is hypothesized that the markers of inflammation (IL-1β, IL-6, and hs-CRP) will increase when post-exercise blood glucose levels are elevated:

H_o: $\mu_1 = \mu_2$
H_a: $\mu_1 < \mu_2$

Where, the notations of μ_1 and μ_2 are the sample means of blood glucose in the low and high carbohydrate conditions, respectively.

Delimitations

1. The study was restricted to males and females between the ages of 18 and 39 years.

2. The study was restricted to subjects whose arm muscles were not heavily exercised and naïve to the eccentric exercise.

Limitations

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

2. The results of the study cannot be generalized to unhealthy persons with chronic diseases or high risk factors for chronic diseases.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Inflammation is the body's general response to tissue damage and infection and is associated with several disease states. Therefore, inflammation has been widely studied. The inflammatory mechanism in adipose tissue has been well studied and is associated with chronic disease risk. Less is known about the inflammatory mechanism in skeletal muscle, and how closely it is associated with chronic disease risk. The inflammatory mechanism in skeletal muscle may be different from that in adipose tissue. Inflammation is very complex and not fully understood and more research is required on the topic. A common way of studying inflammation in skeletal muscle is to naturally induce muscle damage with exercise. Inflammatory responses are controlled by several different mechanisms. Cytokines are cell signaling proteins and one mechanism that contributes to control over inflammation and are often studied to gain a better understanding of the inflammatory process. Blood glucose is a potential external mediator of inflammation but is not fully understood and needs more research. The following review of literature will cover these topics in the order of muscle damage, cytokines, cytokines and endurance exercise, cytokines and resistance exercise, and glucose intake, hyperglycemia and inflammation.

Muscle Damage

There are three types of muscle contractions. One is isometric, one is eccentric and the other is concentric (Proske & Morgan, 2001). Isometric muscle contractions are defined by the muscle neither lengthening nor shortening during the contraction (Proske & Morgan, 2001). Concentric muscle contractions are defined by the muscle shortening as it contracts (Proske & Morgan, 2001). Eccentric muscle contractions are defined by the muscle being forcibly lengthened as it contracts (Proske & Morgan, 2001). It has been well documented that eccentric muscle exercise in untrained skeletal muscle will cause some muscle damage (Brown, Child, Day, Donnelly, 1996; Evans & Cannon, 1991; Lavender & Nosaka, 2006; Proske & Morgan, 2001; Smith & Miles, 2000). This type of muscle damage is best described as micro-trauma that causes disruptions to the muscle architecture. Concentric and isometric muscle contractions do not cause muscle damage. For this reason many studies use eccentric muscle exercise to induce experimental muscle damage (Evans & Cannon, 1991).

Currently there are multiple theories as to why eccentric muscle contractions cause muscle damage and concentric and isometric muscle contractions do not. One theory deals with reduced numbers of motor units being activated during eccentric contractions (Armstrong, 1990). A motor unit consists of a single motor neuron and all the fibers it controls. It is thought that one-third to one-fifth the numbers of motor units are activated during an eccentric contraction compared to a concentric or isometric contraction (Armstrong, 1990; Evans & Cannon, 1991; Smith & Miles, 2000). Therefore, fewer fibers will be activated during an eccentric contraction. This will increase the load

per fiber and will cause the mechanical damage to the muscle (Armstrong, 1990; Evans & Cannon, 1991; Smith & Miles, 2000).

In a different theory, the initial unequal resting length of the sarcomeres causes more tension on the shorter sarcomeres (Morgan, 1990). In turn, there is more stress on the shorter sarcomeres during an eccentric contraction causing them to elongate relatively further than the longer sarcomeres. So, this increased stress on the shorter sarcomeres causes them to "pop". This popping is what actually causes the muscle damage in the second theory (Morgan, 1990).

Immediately post-exercise with eccentric contractions there is no pain or soreness. However, pain and soreness will set in several hours later and will peak at about 48 hours post-exercise (Proske & Morgan, 2001). This muscle soreness is referred to as delayed-onset muscle soreness (DOMS) (Smith & Miles, 2000). The believed cause of the DOMS is the damage induced to the skeletal muscle tissue (Smith & Miles, 2000). It is unknown exactly why DOMS peaks several hours post-exercise, it is possibly connected with delayed chemical reactions involved in the breakdown of muscle resulting from the exercise but this topic warrants further investigation.

Maximal isometric strength is decreased post-exercise in response to eccentric contractions (Brown et al., 1996; Proske & Morgan, 2001). With all types of muscle contractions some loss of maximal isometric strength due to metabolic fatigue is expected. However, the loss associated with eccentric muscle contractions typically is greater. There can be up to a 60% decline in maximal isometric strength due to high force eccentric muscle contractions (Proske & Morgan, 2001). Prolonged force loss has often

been used as an indicator of muscle damage **(**Brown et al., 1996).

Another sign of muscle damage is the increase or appearance of enzymes in circulation that are normally only found in muscle tissue (Brown et al., 1996; Evans & Cannon, 1991). One possible mechanism for this occurrence is that physical tearing of the plasma membrane of the muscle cells occurs. This tearing or shearing is the result of excessive loads experienced during eccentric exercise and allows for the escape of specific muscle enzymes into circulation (Evans & Cannon, 1991).

It has also been suggested that the escape of specific muscle enzymes could be the result of disturbances in cell volume or energy state or both (Evans & Cannon, 1991). One way this could happen is that a reduced metabolic state inhibits the sarcolemmal Na-K-ATPase. This inhibition will then lead to increased intracellular accumulation of sodium. This increase in intracellular sodium will cause an increase in intracellular water content. The increase in water and sodium content will cause swelling that will lead to the rupture of the plasma membrane and allow escape of muscle enzymes (Evans & Cannon, 1991).

One of the main enzymes usually released into circulation is creatine kinase (CK) (Brown et al., 1996; Evans & Cannon, 1991; Lavender & Nosaka, 2006; Proske & Morgan, 2001; Smith & Miles, 2000). The level of increase of CK in circulation can vary from person to person and also depends on the intensity of the exercise and level of muscle damage inflicted (Evans & Cannon, 1991). Some other common enzymes released into circulation are glutamic oxaloacetic transaminase, aspartic amino transferase, myoglobin and lactic dehydrogenase (Evans & Cannon, 1991; Lavender &

Nosaka, 2006; Nosaka & Clarkson, 1996)

A training response or effect to multiple bouts of eccentric exercise has been demonstrated in some research studies (Lavender & Nosaka, 2006; Proske & Morgan, 2001). After the initial bout of eccentric exercise, subsequent bouts result in less damage and considerably less pain and soreness (Lavender & Nosaka, 2006). This adaptation is referred to as the repeated bout effect (Lavender & Nosaka, 2006). The repeated bout effect can last for several weeks up to several months depending on the individual (Lavender & Nosaka, 2006).

Cytokines

Cytokines are polypeptides that were originally discovered in the immune system. Cytokines general purpose is cell to cell signaling. Cytokines are produced in many cell types and have many functions other than the immune system. Virtually all nucleated cells can produce cytokines and have cytokine receptors on their cell membranes. Many cytokines released into circulation will induce the production and release of other cytokines leading to a cytokine cascade.

Cytokines are the signaling molecules that coordinate the local and systemic responses during inflammation (Smith & Miles, 2000). Cytokines exert their effects through specific cell receptors on the plasma membrane of target cells (Smith & Miles, 2000). Cytokine signaling as a whole is very complex; multiple cytokines can produce the same or close to the same effect on a single cell. Also, the effects of one cytokine can be increased or decreased by the presence of other cytokines and inhibitors (Smith &

Miles, 2000).

One local response to tissue injury or infection is production and release of cytokines (Ostrowski, et al., 1999). The first cytokines to be released are tumor necrosis factor α (TNF-α) and interleukin -1β (IL-1β). TNF-α has a very short half-life making it difficult to measure. Thus, it is common to measure soluble tumor necrosis factor receptor 1 (sTNF-R1), a protein with a longer half-life. sTNF-R1 is easier to measure and an indicator of TNF-α levels. TNF-α and IL-1β induce lymphocytes, neutrophils, monocytes, and other cells to clear antigens and heal tissue (Ostrowski et al., 1999). TNF-α and IL-1β also promote the liver to produce acute phase response proteins that act as a systemic inflammatory response. Injection of TNF-α, IL-1β, and interleukin 6 (IL-6) into laboratory animals or humans will produce the aspects of the acute phase response (Dinarello, 1992; Richards & Gauldie, 1998). One of the proteins produced in the acute phase response is C-reactive protein (CRP). Elevated levels of plasma IL-6 have been shown to increase plasma CRP (Nehlsen-Cannarella et al., 1997; Ostrowski et al., 1999). Therefore, TNF-α and IL-1β are considered the pro-inflammatory cytokines since they induce an overall inflammatory response (Ostrowski et al., 1999).

Inflammation is the general response of the body to tissue injury, regardless of the cause of injury. The overall goal of inflammation is healing (Smith & Miles, 2000). The local response to tissue injury consists of the production and release of cytokines at the site of inflammation. The local inflammatory response is followed by a systemic inflammatory response. The systemic inflammatory response is called the acute phase response.

The inflammatory response is highly controlled at each step to maximize its effects. Inflammation consists of movement of fluid, plasma protein, and leukocytes to the injured tissue (Smith & Miles, 2000). In the first few hours after injury the majority of processes involve local recruitment of white blood cells (WBC). Neutrophils are the first WBC's to arrive followed by monocytes. Neutrophils are the cells most important for the initial clean-up process of the damaged tissue. Then the monocytes and macrophages synthesize most of the inflammatory factors. These inflammatory factors are cytokines, chemokines, and cell adhesion molecules. The inflammatory factors control local and systemic amplification of the inflammatory response and are also crucial for the termination of inflammation (Smith & Miles, 2000)

TNF-α and IL-1β induce the production and release of IL-6. IL-6 promotes some of the proteins in the acute phase response (pro-inflammatory) while also inducing the production and release of anti-inflammatory cytokines (Nehlsen-Cannarella et al., 1997). So, IL-6 is considered both a pro-inflammatory and anti-inflammatory cytokine. TNF-α and IL-1β also activate the HPA axis and the sympathoadrenergic system that produces a strong anti-inflammatory effect (Nehlsen-Cannarella et al., 1997). This is part of the body's natural negative feedback system to control inflammation along with the increased production of anti-inflammatory cytokines.

The two main anti-inflammatory cytokines produced and released are interleukin 1 receptor antagonist (IL-1ra) and interleukin 10 (IL-10). These cytokines turn off the inflammatory cytokines TNF-α and IL-1β via a negative feedback mechanism. IL-10 specifically inhibits the release of TNF-α and IL-1β and induces production the anti-

inflammatory cytokine IL-1ra (Ostrowski et al., 1999). IL-1ra binds to IL-1 membrane receptors blocking binding and inhibiting the affects of IL-1. IL-1ra and IL-10 also decrease the concentration of immune system cells. As IL-1ra and IL-10 increase in concentration the immune response is turned off (Petersen & Pedersen, 2005).

The general cytokine cascade in sequential order is TNF-α, IL-1β, IL-6, IL-1ra, and IL-10 (Petersen & Pedersen, 2005). This is the order cytokine concentration will increase in circulation in response to tissue injury or infection. However, this is not the only way for cytokines to increase in circulation. It has been well demonstrated that contracting skeletal muscle increases production and release of IL-6 (Nehlsen-Cannarella et al., 1997; Nieman et al., 2003; Ostrowski et al.,1999). But, in this case, IL-6 is not being released due to an inflammatory response but for its role in glucose metabolism (Petersen & Pedersen, 2005).

<center>Cytokine Production and Endurance Exercise</center>

Multiple studies have examined how exercise affects the production and release of cytokines (Evans & Cannon, 1991; Nosaka & Clarkson, 1996; Starkie et al., 2000). It has been well documented that endurance exercise will cause a significant increase in plasma IL-6 levels and the subsequent cytokines in the cytokine cascade (Petersen & Pedersen, 2005). But, resistance exercise that is highly eccentric does not induce the same cytokine response (Nosaka & Clarkson, 1996). In highly eccentric exercise the rise in plasma IL-6 is much lower resulting in lower rises in the anti-inflammatory cytokines.

During endurance exercise our fuel comes from fat metabolism, blood glucose,

and skeletal muscle glycogen stores. As skeletal muscle glycogen stores are depleted the energy demand is met through blood glucose. IL-6 has been shown to promote lipolysis in adipose tissue and glycogen breakdown in the liver (Petersen & Pedersen, 2005). Lipolysis is the breakdown of stored fat in your body for energy. Glycogen is the storage form of carbohydrates in your body and is stored in high concentrations in the liver for when it is needed. These processes will help increase blood glucose levels. It is hypothesized that IL-6 is released from contracting skeletal muscle to help increase blood glucose by promoting lipolysis and glycogen breakdown.

When IL-6 is circulating in the blood it will promote the production and release of the anti-inflammatory cytokines IL-1ra and IL-10 regardless of the original reason IL-6 is circulating (Petersen & Pedersen, 2005). So, endurance exercise will induce an increase in circulating IL-6 and then IL-1ra and IL-10 in sequential order, resulting in an overall anti-inflammatory response. It has been hypothesized that this anti-inflammatory response is one of the ways that exercise can help reduce the risk of inflammatory related diseases like atherosclerosis, type II diabetes, and Alzheimer's disease (Petersen & Pedersen, 2005).

Multiple studies have examined the effects of carbohydrate loading on IL-6 production and release from skeletal muscle in response to endurance exercise (Nieman et al., 2003; Nehlsen-Cannarella et al., 1997). Nehlsen-Cannarella et al. (1997) compared the influence of 6% carbohydrate versus placebo ingestion on the cytokine response after 2.5 hours of running. IL-6 concentrations were significantly higher in the placebo condition immediately post-run and 1.5 hours post-run. Also, post-run levels of IL-6

correlated positively with IL-1ra levels. Nehlsen-Cannarella et al. (1997) concluded that

carbohydrate ingestion attenuated cytokine levels in the inflammatory cytokine cascade

in response to endurance exercise. Neither carbohydrate nor placebo ingestion produced a

significant increase in the pro-inflammatory cytokine IL-1β.

In a similar study with the same results, Nieman et al. (2003) hypothesized the

decrease in post-run IL-6 in the carbohydrate condition was due to increased blood

glucose levels. The increase in blood glucose from carbohydrate ingestion meant IL-6

was not required for its role in glucose metabolism. Since there was no or very little

tissue damage inflicted and IL-6 was not required to increase blood glucose, the

carbohydrate group had attenuation in post-exercise cytokine levels. These results have

been duplicated in many studies with similar protocols (Petersen & Pedersen, 2005).

Cytokine Production and Resistance Exercise

A few studies have looked at the effects of resistance training on circulating

cytokine levels (Brown et al., 1996; Lavender & Nosaka, 2006; Miles et al., 2008; Proske

& Morgan, 2001). Nosaka & Clarkson (1996) hypothesized eccentric exercise in

untrained muscle will induce damage to the muscle. In response, a series of local acute

inflammatory responses will follow to heal the damaged tissue. This damage and repair

process will also induce a systemic inflammatory response. The systemic inflammatory

response is called the acute-phase response.

Cytokines are the controlling molecules of the local and systemic inflammatory

responses (Nosaka & Clarkson, 1996).   So, after eccentric exercise that damages skeletal

muscle tissue there should be an increase in local cytokine production (Petersen &

Pedersen, 2005). First, there is expected to be an increase in the pro-inflammatory

cytokines TNF-α and IL-1β. Then, an increase in IL-6, and lastly an increase in the anti-

inflammatory cytokines IL-1ra and IL-10 (Petersen & Pedersen, 2005). Also, an increase

in the acute phase protein CRP in circulation is expected (Petersen & Pedersen, 2005).

In a study by Nosaka & Clarkson (1996) eccentric exercise was used to induce

muscle damage in the elbow flexors. While some signs of inflammation were present

such as soreness and swelling, there was no significant increase in any of the cytokines

measured. It is possible that this study missed the increase in cytokines based on the

timing of their blood draws. Each subject's blood was only analyzed pre-exercise, post-

exercise and 5 days post-exercise. If the concentration of cytokines in the plasma

increased and then returned to baseline after the post-exercise blood analysis but before

the 5 day post-exercise blood analysis, there would have appeared to be no change in the

concentration of the circulating cytokines.

A study by Miles et al. (2008) used similar eccentric exercise of the elbow flexors

and compared the cytokine response to a control condition. The exercise condition in this

study had significant indicators of muscle damage such as increased soreness and

detection of significantly elevated CK in circulation 24, 48 and 96 hours post-exercise.

There was an increase in IL-6 at 4, 8, and 12 hours post-exercise with a clear peak at 8

hours post-exercise. This study demonstrates the presence of an inflammatory response

post muscle damaging exercise. It also supports the possibility that Nosaka & Clarkson

could have missed increases in cytokines in circulation due to timing of their blood

draws.

Overall there are fewer studies on muscle damage and the inflammatory response than on endurance exercise and the cytokine response. While Nosaka & Clarkson (1996) did not find an increase in cytokine concentration in the plasma post-exercise, a few studies have (Brown et al., 1996; Evans & Cannon, 1991; Miles et al., 2008). But, since less has been researched on the topic, less is known about the mechanism and exactly what roles cytokines and inflammation play in healing damaged skeletal muscle tissue. For this reason more research is required in the area of exercise induced skeletal muscle tissue damage and the recovery and healing process.

Glucose Intake, Hyperglycemia, and Inflammation

According to the American Diabetics Association, hyperglycemia is a term used to describe high blood glucose (sugar). Chronic hyperglycemia that persists even in the fasting state is a central characteristic of diabetes mellitus. In general, normal fasting blood glucose levels for an adult range from 80-120 mg/dL. An oral glucose tolerance test (OGTT) is commonly used to measure one's ability to remove excess glucose from the blood. An OGTT consists of ingesting a 75g glucose solution and measuring blood glucose levels 2 hours later. If blood glucose is equal to or above 140 mg/dL after 2 hours it is considered impaired glucose tolerance and if blood glucose levels are equal to or above 200 mg/dL it is diagnosed as diabetes mellitus.

The glycemic index is a scale devised to indicate how rapidly different foods will influence blood glucose levels. The glycemic index was first measured by feeding test

subjects various foods and measuring their blood glucose levels over the following 2 hours. Blood glucose values were then expressed as a percentage of the area under the glucose response curve when the same amount of carbohydrate was ingested as straight glucose (Jenkins et al., 1981). This was usually done with 50g portions of carbohydrate. Foods that are higher on the glycemic index will cause a greater and more rapid peak in postprandial blood glucose per gram of carbohydrate compared to foods that are lower on the glycemic index (Foster-Powell, Holt, & Brand-Miller, 2002). Therefore, foods that are higher on the glycemic index are more likely to induce acute hyperglycemia when ingested in a short time frame.

Acute hyperglycemia has been suggested to induce an increase in inflammation. Esposito et al. (2002) injected three consecutive boluses of intravenous glucose into a large antecubital vein separated by 2 hours each and inducing acute hyperglycemia with each injection. Test subjects were made up of impaired glucose tolerance and normal individuals. In both sets of test subjects, acute hyperglycemia induced a significant increase in the cytokines TNF-α, IL-6, and IL-18. All three cytokines are considered to have pro-inflammatory effects. While both normal and impaired glucose tolerance individuals had a significant increase in the three cytokines, the impaired glucose tolerance subjects had a greater increase in the cytokines.

There is evidence that an oxidative mechanism controls the inflammatory effect of acute hyperglycemia (Esposito et al., 2002). Esposito et al. (2002) conducted the same protocol as above but added the antioxidant glutathione to the injections. When glutathione was added there was no significant increase in the three cytokines measured

for both normal and impaired glucose tolerance test subjects. However, the exact mechanism is unknown at this time.

In another study by Gonzalez et al. (2006), acute hyperglycemia in obese reproductive aged women caused an increase in TNF- α production. Obese individuals are in a chronic pro-inflammatory state and have elevated levels of inflammatory markers. This means the inflammatory process was already initiated in obese test subjects and hyperglycemia further increased this inflammation as seen by an increase in production of the pro-inflammatory cytokine TNF- α (Gonzalez et al., 2006).

The results of Esposito et al. (2002) and Gonzalez et al. (2006) suggest that acute hyperglycemia has the ability to induce an inflammatory response and or increase an inflammatory response that has already been initiated by another source such as obesity. This is the basis for the hypothesis in the present study. It is thought that acute hyperglycemia post-exercise will increase the inflammatory response to the muscle damage induced by the exercise. A chronic increase in the cytokine TNF- α has been associated with insulin resistance and diabetes mellitus. Also, chronically elevated IL-6 is considered a risk factor for cardiovascular disease. Thus, it is very important to control these cytokines as much as possible. While hyperglycemia is known to affect these cytokines, the exact mechanism is still unknown and more research is required.

Summary

Inflammation is the body's response to tissue damage and infection. The goal of inflammation is healing. Cytokines are cell signaling proteins with multiple functions,

including coordination and control of inflammatory responses. The cytokine cascade in sequential order is TNF-α, IL-1β, IL-6, IL-1ra, and IL-10. Exercise has been shown to influence cytokines and inflammation in different ways depending on the mode of exercise. Exercise can consist of isometric, concentric, or eccentric muscle contractions. Eccentric muscle contractions have been shown to cause muscle damage and result in an inflammatory response characterized by a rise in pro-inflammatory cytokines, most notably IL-6. Endurance exercise causes an increase in the pro- and anti-inflammatory cytokine IL-6 and the following anti-inflammatory cytokines in the cytokine cascade. Hyperglycemia has the ability to induce and or enhance inflammation once it has been initiated by an outside source such as obesity or eccentric exercise. Thus, it was hypothesized that the markers of inflammation, IL-1β, IL-6, and CRP would be increased when post-exercise blood glucose levels were elevated.

CHAPTER THREE

METHODS

<u>Participants and Informed Consent</u>

There were 14 subjects enrolled in the study but 2 did not finish due to illness, so 12 subjects were included in the final data analysis. There were 7 male and 5 female participants aged 19 to 36 years. Subjects were recruited for the study by posting flyers throughout Montana State University's campus. This method of recruiting subjects is defined as convenience sampling. Convenience sampling is not considered as good as a simple random sample but given the available funds and time frame for the study it was unrealistic to use a true simple random sample. Incentive for the study was payment of $100 upon completion.

All test subjects were informed of the risks and discomforts associated with partaking in the study prior to their participation. Each subject was required to sign an informed consent document approved by the Montana State University Human Subjects Committee prior to participation (Appendix A). Subjects were allowed to withdraw from the study at any time with no repercussions. All subject information was strictly confidential. Researchers associated with the study were required to pass the Human Subjects Committee qualification exam to work with human subjects.

All participants were required to have refrained from regular heavy lifting or lowering with the arms in the 6 months prior to the study. Participants also needed to have refrained from any activity that results in soreness of the arms in the 6 months prior

to the study. The aim of these requirements was to avoid the possible effects of the repeated bout effect and induce the maximal level of muscle damage possible (Brown et al., 1996).

Potential test subjects were excluded from the study if they had a known history or condition of anemia, muskuloskeletal limitations, inflammatory conditions, diabetes mellitus, heart disease, and known kidney problems (excluding kidney stones). Other restrictions on participation were smoking, alcohol use greater than 1 drink per day or 4 drinks on any one occasion, chronic use of anti-inflammatory medications, use of lipid lowering medications, use of oral contraceptives, pregnancy, and regular physical activity that results in bruising or muscle soreness. All of these conditions and or habits are known to affect inflammation. Since inflammation was studied, it was important to reduce the chance that subjects had confounding factors influencing inflammation.

Research Design

The general research design used was a pre-test post-test crossover design where each subject was their own control. There were two conditions or treatments implemented for each subject. For each condition there was a pre-test of the variables, then a treatment, and then a post-test of the same variables measured in the pre-test. Each condition measured the same variables but had a different treatment. At the conclusion of both conditions the results were analyzed for significant differences between the pre- and post-test measurements within each condition.

Prior to any testing each subject was assessed for anthropometric measurements.

Anthropometric measurements consisted of body height and weight, upper arm circumference, and waist to hip ratio. For the waist to hip ratio a circumference measure was taken at the waist and hip using an anthropometric tape measure. This measure was made by an investigator of the same sex as the subject and also was made over the clothing of the subject (no disrobing occurred).

Each subject participated in a high carbohydrate and high fat and protein condition. The order of conditions for each participant was balanced and separated by three to six weeks. The reason for separating the conditions by three to six weeks was to allow sufficient time for inflammation to return to normal baseline levels. The conditions were carried out in an overall balanced order. For each condition the participant reported to the lab at 7:00 am on the first day to perform a high-force eccentric exercise in the elbow flexor and extensor muscles in one arm. Subjects used one arm for the first condition and used their other arm for the second condition. Subjects did not use the same arm for both conditions because the repeated bout effect would cause a reduction in muscle damage and inflammation if the subjects exercised with the same arm within 3-6 weeks of the initial exercise (Brown et al., 1996). This reduction in muscle damage and inflammation would skew the results when comparing the two conditions. The number of subjects exercising their dominant and non-dominant arms was balanced within and between each condition to balance any possible differences resulting from different muscle strength in the dominant versus non-dominant arm.

During the high carbohydrate condition the participants ingested high carbohydrate content and high glycemic index food for the first 8 hours of recovery to

increase blood glucose levels to the point of hyperglycemia. The reason for inducing

hyperglycemia in the high carbohydrate condition was that hyperglycemia can enhance

the level of inflammation after it has been initiated by an outside source (Gonzalez,

Minium, Rote, Kirwan, 2005). For the high fat and protein condition, each participant

ingested an equal amount of food as in the high carbohydrate condition in terms of

kilocalories for the first 8 hours of recovery. But, the food was significantly lower in

carbohydrate content and glycemic index. Three meals were consumed per condition at

.5, 3, and 7 hours post-exercise. For a complete description of food consumed in each

condition refer to appendix B.

Blood was drawn from each participant at 7:00 am prior to exercising on the first

day of each condition and at 1.5,4, 8, 24, and 120 hours of recovery. The main purpose of

having the first blood draw at 7:00 am was that blood needs to be drawn on a 12 hour fast

for optimal baseline measurements. A 7:00 am blood draw was the most convenient time

for subjects to plan a 12 hour fast around without altering their normal routine too much.

Blood draws were analyzed for glucose, creatine kinase (CK), interleukin-1β (IL-1β),

interleukin-6 (IL-6), and C-reactive protein (hs-CRP). Also, pre-exercise, immediately

post-exercise, and 24, 48, 72, 96, and 120 hours post-exercise the subjects were assessed

for maximal force production. Subjects were analyzed for perceived muscle soreness and

mid-brachial arm circumference pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 hours

post-exercise.

Participants were required to abide by some restrictions during the time of each

condition to minimize variability between subjects. Prior to the first day of each

condition, each participant had to keep physical activity to a minimum, and fast after

7pm. This allowed for optimal baseline analysis of blood for the pre-exercise blood draw

at 7:00 am and helped to minimize variability in blood glucose levels. Also, during the

length of the experiment participants were required to refrain from strenuous exercise and

exercise that lasted longer than 60 minutes. The reason for this limitation on exercise was

that it can affect levels of inflammation. The goal was to have only the eccentric exercise

affect levels of inflammation and to reduce the potential for confounding factors that

could affect inflammation. Women began each condition within 4 days of the onset of

menstrual bleeding to minimize the effect of cyclical hormone variations. To reduce the

influence of illness on inflammatory factors, subjects were only tested if they had been

free from known infection for at least 1 week prior to testing.

Eccentric Exercise and Maximal Force Production

High-force eccentric exercise was used to induce muscle damage and the resulting

inflammatory response. A computer controlled isokinetic dynamometer (Kin Com

125E+, Chattecx Corporation, Chattanooga, TN) was used to perform the exercises. The

elbow flexor and extensor muscles were exercised. These sets of muscles were exercised

because they are the most readily exercised with the equipment available to carry out the

study. For both sets of muscles the isokinetic dynamometer was adjusted to the specific

dimensions of each test subject. The point of rotation in the dynamometer was aligned

with the axis of rotation in the elbow. The height of the dynamometer was adjusted so the

subject's torso and upper arm make a 90 degree angle and the upper arm was parallel

with the floor. The dynamometer was connected to the arm just below the wrist via a padded support and Velcro strap. The settings of the dynamometer were recorded for each participant so it could be set up exactly the same way each time the participant used it.

For high-force eccentric exercise of the elbow flexor and extensor muscles the subject started with their elbow in a flexed position at an approximate elbow angle of .79 radians (rad). Then, the dynamometer moved the elbow to a fully extended position. The subject was instructed to maximally resist the movement of the elbow being extended by attempting to keep the elbow in a fully flexed position. Then, from the fully extended position of approximately 3.14 rad, the dynamometer moved the elbow to a fully flexed position. The subject also maximally resisted this movement from the fully extended position to the flexed position. The movement of the elbow from flexed to extended and back to flexed will consisted of 1 repetition. The angular velocity of the dynamometer was set at 0.79 rad per second. There was a 10 second pause in between each repetition. There were 10 repetitions per set with a total of 6 sets. There was a 5 minute rest period between sets.

The same dynamometer was used to evaluate maximal isometric strength of each subject. Maximal isometric strength was measured pre-exercise, immediately post-exercise, 24, 48, 72, 96, and 120 hours post-exercise. The dynamometer was adjusted for each subject the same way it was adjusted during the high-force eccentric exercise. To test maximal isometric strength the dynamometer was set so the elbow is at an angle of 1.57 rad. The dynamometer was locked in that position and the subject was instructed to

flex their elbow with maximal effort for 3 seconds. For each measure of maximal

isometric strength the subject performed this procedure three times and the average of the

three trials was used as the maximal isometric strength for that specific time. There was a

30 second rest period in between the three trials for each measure of maximal isometric

strength. This method of measuring maximal isometric strength was based on the method

used by Lavender & Nosaka (2006).

<center>Markers of Muscle Damage</center>

High-force eccentric exercise in untrained muscle will result in the sensation

known as delayed onset muscle soreness (DOMS). Test subjects evaluated perceived

DOMS with the aid of a visual analog scale pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and

120 hours post-exercise. The scale consisted of a 100 mm line with one end representing

no soreness and the other end representing extreme soreness. To measure DOMS the

subjects were asked to flex and extend their arm several times while holding a 1 kg

weight in their hand. With their other hand the subject put pressure on both their flexor

and extensor muscles while flexing and extending their arm. After this procedure the

subject placed a vertical line on the scale representing their perceived level of DOMS.

The subjects were instructed to place a line on the scale in terms of soreness only, not in

comparison to other types of pain like a severe burn.

High-force eccentric exercise may also cause some swelling in the arm. The

amount of swelling was assessed on a time-matched schedule with DOMS evaluation.

The amount of swelling was measured by measuring the circumference of the mid-

brachium at the point half-way between the mid-biceps and the axis of rotation of the elbow. The arm was in a flexed position at the time of measurement. A spring-loaded anthropometric tape measure was used for measurement. The exact line of circumference to be measured was marked with three dots at the beginning of the experiment and maintained throughout the experiment. This method of measuring swelling of the upper arm was derived from Lavender & Nosaka (2006).

Blood Analysis

Blood was collected pre-exercise, 1.5, 4, 8, 24, and 120 hours post-exercise. The timing of blood draws was derived from preliminary studies. Blood was collected from an antecubital vein into evacuated tubes using a standard venipuncture technique. Blood was allowed to clot in the evacuated tubes. Then the serum was separated from cells using a refrigerated 21000R Marathon centrifuge (Fisher Scientific, Pittsburgh, PA). Samples were stored at -80 degrees Celsius until analysis.

Blood from the pre-exercise blood draw was analyzed for blood glucose, insulin, CK, IL-1β, IL-6, and hs-CRP. The purpose of the 1.5 hour post-exercise blood draw was for an early indication of pro-inflammatory cytokine concentrations and blood glucose concentrations. Blood glucose, insulin, IL-6, and IL-1β were analyzed at the 1.5 hour post-exercise blood draw. Blood from the 4 and 8 hour post-exercise blood draws were analyzed for the same components as the pre-exercise blood, except there was no analysis for CK or hs-CRP concentrations. Preliminary tests indicate that CK and hs-CRP do not increase significantly until around 120 hours post-exercise. So there was no need to use

resources to analyze CK or hs-CRP at these early blood draws. Blood from the 24 hour

post-exercise blood draw was analyzed for the same components as the pre-exercise

blood draw. The 120 hour post-exercise blood draw was analyzed for CK and hs-CRP.

Statistics

Data was analyzed using Statistical Program for Social Sciences (SPSS) for

Windows (version 13.0, SPSS Inc., Chicago, IL). Baseline measurements between

conditions were analyzed using a paired samples t-test for continuous variables. To

compare conditions over time a two-way repeated measures analysis of variance

(ANOVA) was used. All variables were normally distributed, as determined using the

Kolmogorov-Smirnov test. If there were significant main effects or interactions found,

paired t-tests analyses were run to determine the location of the significant differences.

Statistical significance was set at the alpha = 0.05 level.

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CHAPTER FOUR

RESULTS

<u>Subject Characteristics</u>

Twelve subjects, 5 female and 7 male, successfully completed both conditions and were included in the data analysis. Six subjects started with the high carbohydrate condition and 6 subjects started with the high fat and protein condition. Participants were 19 to 36 years of age with a mean ± SD age of 24.75 ± 6.02 years), height of 1.76 ± 0.05 m, weight of 72.73 ± 9.66 kg, waist to hip ratio 0.86 ± 0.08, and body mass index (BMI) of 23.74 ± 2.89 kg·m^{-2}. Baseline values of IL-6, IL-1, CRP, CK, glucose and insulin are reported in table 4.1. There were no significant differences between conditions for any baseline measures.

Table 4.1. Basal values of IL-6, IL-1, CRP, CK, glucose, and insulin for both conditions.

	Mean	SD	P Value
IL-6 (pg/ml)			
High Carbohydrate	1.89	1.44	0.213
Low Carbohydrate	1.43	0.98	
IL-1β (pg/ml)			
High Carbohydrate	0.19	0.14	0.464
Low Carbohydrate	0.21	0.18	
CRP (mg/L)			
High Carbohydrate	1.81	3.14	0.853
Low Carbohydrate	1.60	2.75	
CK (IU/L)			
High Carbohydrate	279.60	241.23	0.213
Low Carbohydrate	191.36	103.33	
Glucose (mg/dl)			
High Carbohydrate	104.73	9.02	0.286
Low Carbohydrate	99.88	13.83	
Insulin (μIU/ml)			
High Carbohydrate	15.35	3.40	0.623
Low Carbohydrate	14.58	6.80	

pg = picograms, mg = milligrams, ml = milliliter, dl = deciliter, L = liter, IU = international units, μIU = micro-international units

Blood Glucose and Insulin

Blood glucose and insulin concentrations were measured to identify differences in

the diets between conditions. Glucose and insulin analysis includes 10 subjects. There

was no significant difference (p = 0.521) for glucose between conditions, no changes

over time (p = 0.065), and no condition by time interaction (p = 0.159). Glucose data are
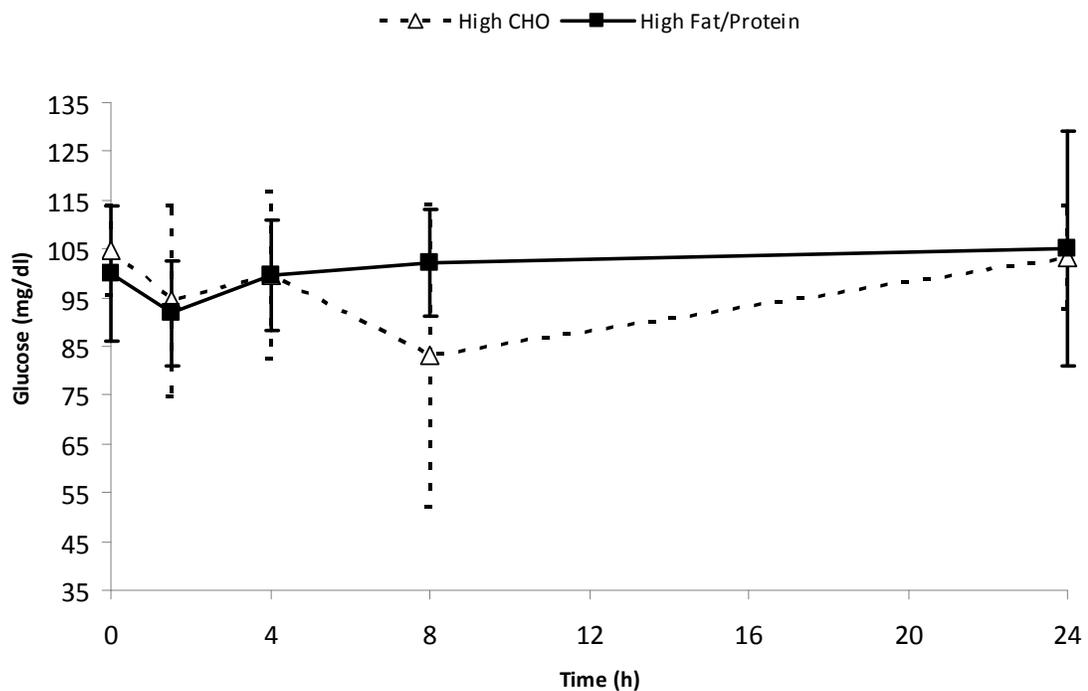
presented in figure 4.1.

Figure 4.1. Blood glucose concentrations in both conditions at pre-exercise, 1.5, 4, 8, and
24 hours post-exercise. Values are mean ± SD, CHO = carbohydrate, n = 10.

There was a significant condition effect (<0.01) for insulin with the high carbohydrate

condition higher than the high fat and protein condition. Additionally, there was a

significant time effect (p<0.01) with insulin greater than baseline at 1.5, 4, and 8 hours

post-exercise, and a condition by time interaction (p<0.001) where the high carbohydrate

condition was greater than the high fat and protein condition at 1.5, 4, and 8 hours post-exercise. Insulin data are presented in figure 4.2.
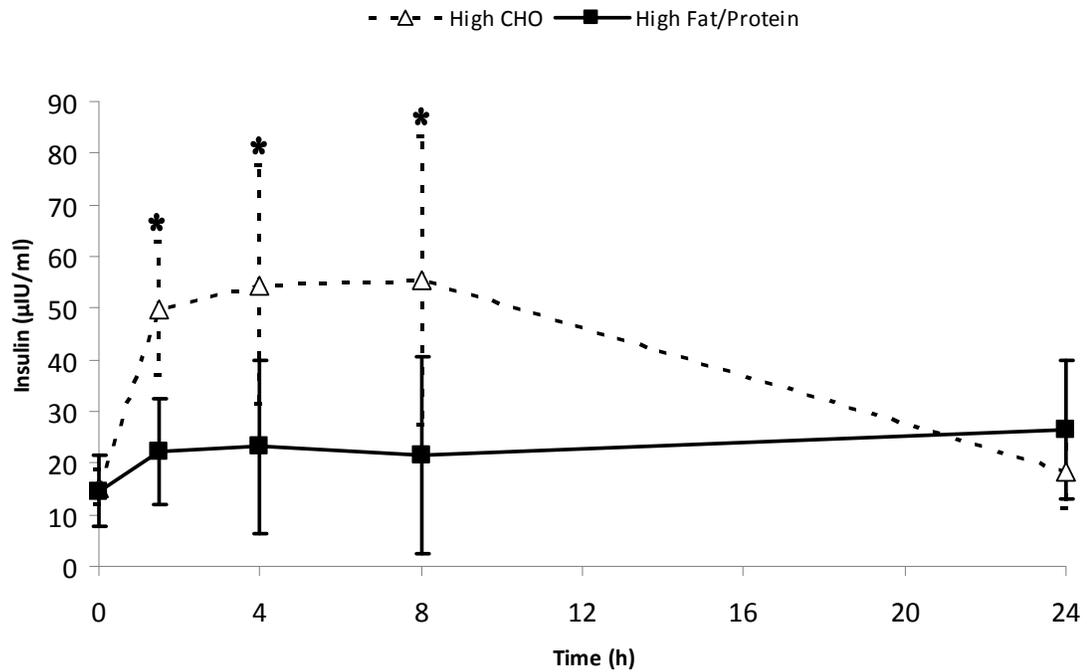

Figure 4.2. Insulin concentrations in both conditions at pre-exercise, 1.5, 4, 8, and 24 hours post-exercise. Values are mean ± SD, CHO = carbohydrate, n = 10. *P<0.001 compared to the high fat and protein condition.

Muscle Damage

Four indicators of muscle damage consisting of CK, soreness, mid-brachial arm circumference, and strength loss were assessed. Strength loss includes 11 subjects. Muscle damage was necessary to induce an inflammatory response. There was no significant difference (p = 0.655) for CK between conditions, changes over time (p =

0.655), and no condition by time interaction ($p = 0.772$). The CK data are presented in

figure 4.3.
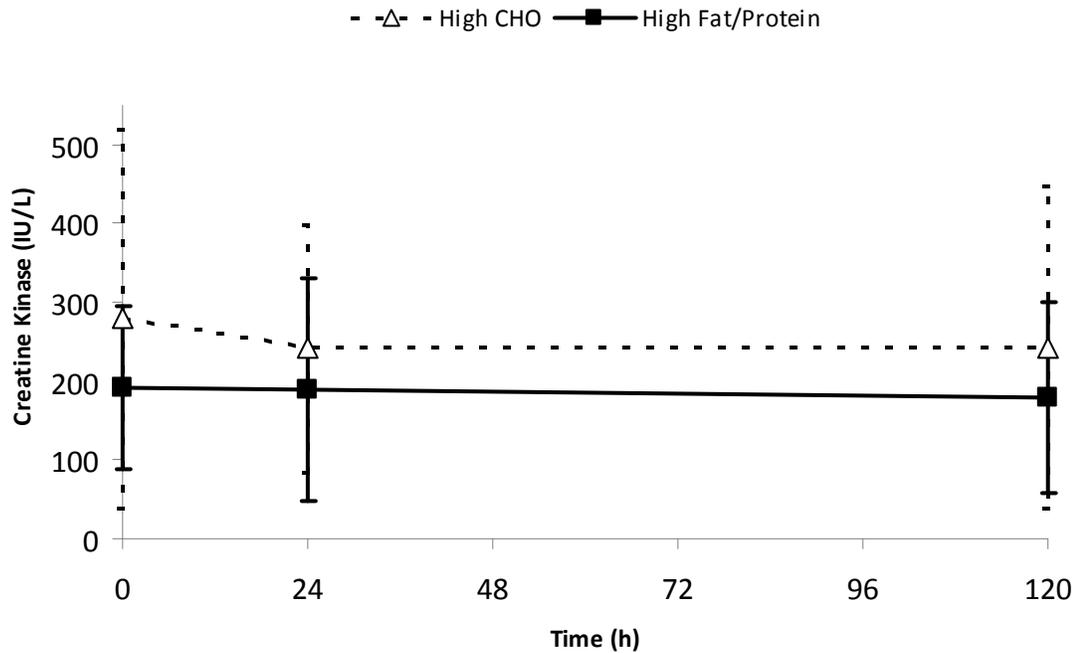
Figure 4.3. Creatine kinase concentrations in the both conditions at pre-exercise, 24, and 120 hours post-exercise. Values are mean ± SD, CHO = carbohydrate.

There was a significant difference ($p<0.05$) for soreness between conditions with the high

carbohydrate condition higher, and a significant time effect ($p<0.001$) where all time

points post-exercise were greater than baseline, but no condition by time interaction ($p = 0.145$). Soreness data are presented in figure 4.4.
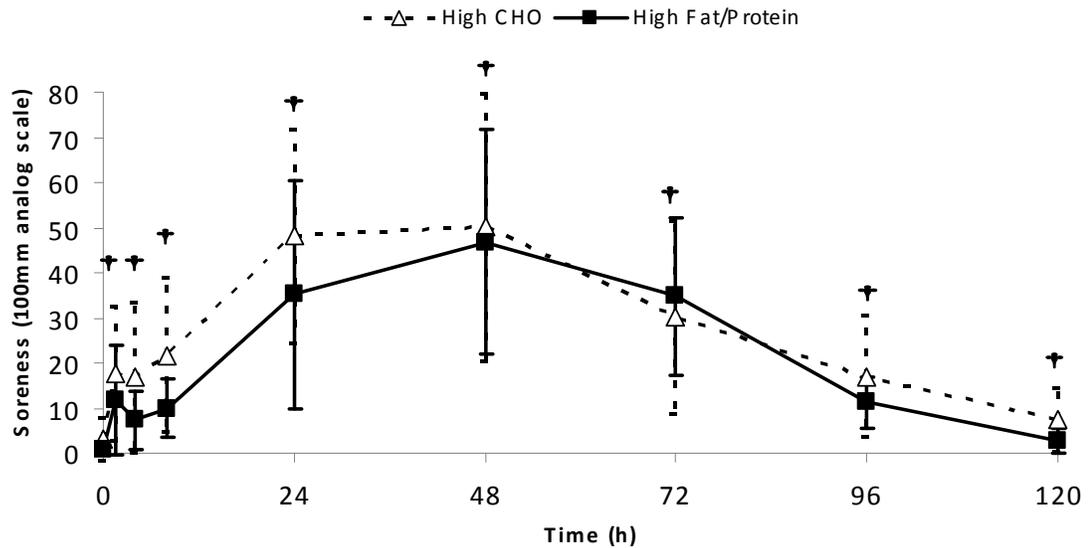
Figure 4.4. Soreness data for both conditions at pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 hours post-exercise. Subjects indicated their perceived soreness on a 100mm analog scale with 0 being no soreness and 100 being extremely sore. There was a main condition effect where the high carbohydrate condition was higher than the high fat and protein condition. Values are mean ± SD, CHO = carbohydrate. †P<0.001 compared to pre-exercise.

There was not a significant difference (p = 0.903) for mid-brachial arm circumference between conditions, and no condition by time interaction (p = 0.176), but there was a time effect (p<0.05), however post-hoc analysis did not identify significant differences relative to baseline. Mid-brachial arm circumference data are presented in figure 4.5.
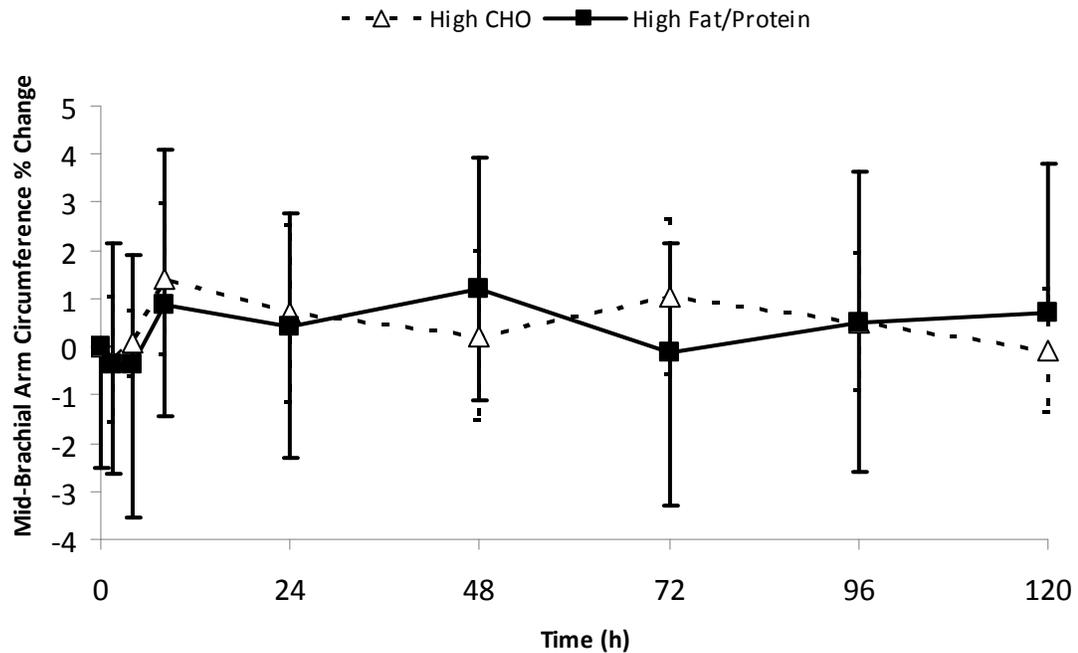
Figure 4.5. Mid-brachial arm circumference measured as percent change from pre-exercise for both conditions at pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 hours post-exercise. The main effect for time was significant but post-hoc analysis did not identify significant differences relative to baseline. Values are mean ± SD, CHO = carbohydrate.

There was not a main effect ($p = 0.534$) between conditions for percent strength loss for biceps, a condition by time interaction ($p = 0.331$), but there was a time effect ($p<0.001$). Biceps percent strength loss data are presented in figure 4.6. There was not a main effect ($p = 0.968$) between conditions for percent strength loss for triceps, a condition by time interaction ($p = 0.613$), but there was a time effect ($p<0.05$). Triceps percent strength loss data are presented in figure 4.7.

Figure 4.6. Biceps maximal isometric strength loss for both conditions measured as percent change from pre-exercise values. Measurements were made pre-exercise, immediately post-exercise, 24, 48, 72, 96, and 120 hours post-exercise. The 0 time-point corresponds to pre-exercise. Values are mean ± SD, CHO = carbohydrate, n = 11. †$P<0.001$ compared to pre-exercise.
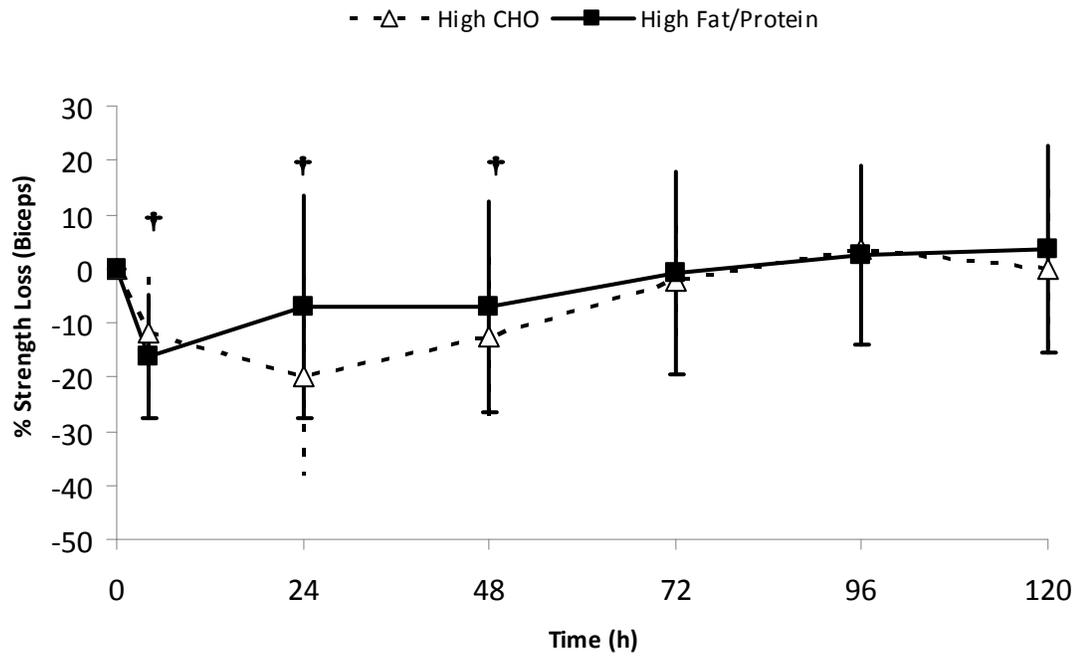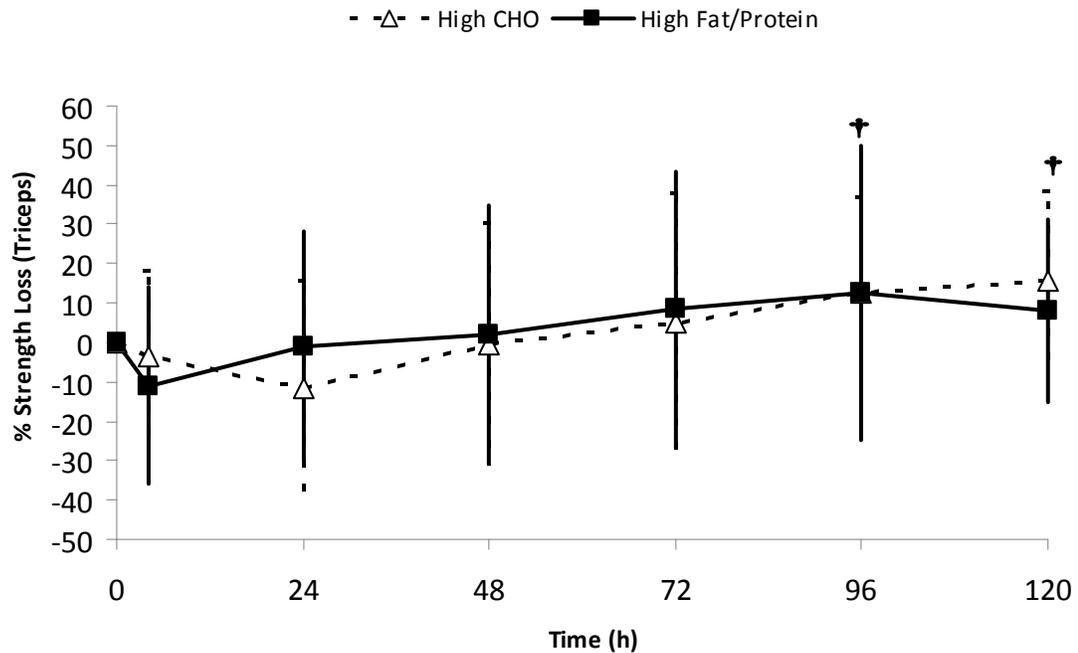
Figure 4.7. Triceps maximal isometric strength loss for both conditions measured as percent change from pre-exercise values. Measurements were made pre-exercise, immediately post-exercise, 24, 48, 72, 96, and 120 hours post-exercise. The 0 time-point corresponds to pre-exercise. Values are mean ± SD, CHO = carbohydrate, n = 11. †P<0.05 compared to pre-exercise.

Markers of Inflammation

Two cytokines, IL-6 and IL-1β, and one acute phase protein, CRP, were measured as indicators of inflammation as a result of muscle damage. CRP analysis only includes 11 subjects at this time. There was a trend (p = 0.064) for a main effect between conditions for IL-6 with the high carbohydrate condition higher, a time effect (p<0.05) with significant decreases below baseline at 1.5 and 4 hours post-exercise and a trend (p = 0.088) for an increase 8 hours post-exercise, and no condition by time interaction (p = 0.175). IL-6 data are presented in figure 4.8.
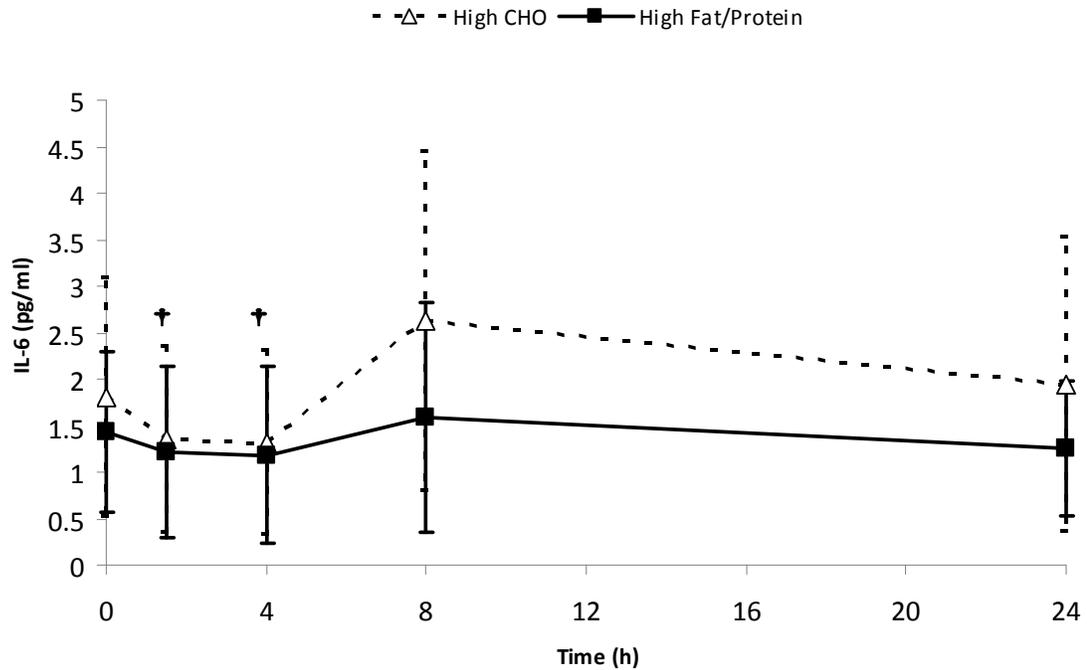
Figure 4.8. IL-6 data for both conditions at pre-exercise, 1.5, 4, 8, and 24 hours post-exercise. There was a time effect where IL-6 was decreased at 1.5 and 4 hours post-exercise and a trend for IL-6 to be increased 8 hours post-exercise. Values are mean ± SD, CHO = carbohydrate. †P< 0.05 compared to pre-exercise.

There was no main effect (p = 0.422) for IL-1β between conditions, or a time effect (p = 0.405), but there was a condition by time interaction (p = 0.05) with IL-1β higher in the high carbohydrate condition compared to the high fat and protein condition at 24 hours post-exercise. IL-1β data are presented in figure 4.9. There was not a significant main effect (p = 0.563) in CRP between conditions, a time effect (p = 0.434), or a condition by time interaction (p = 0.574). CRP data are presented in figure 4.10.
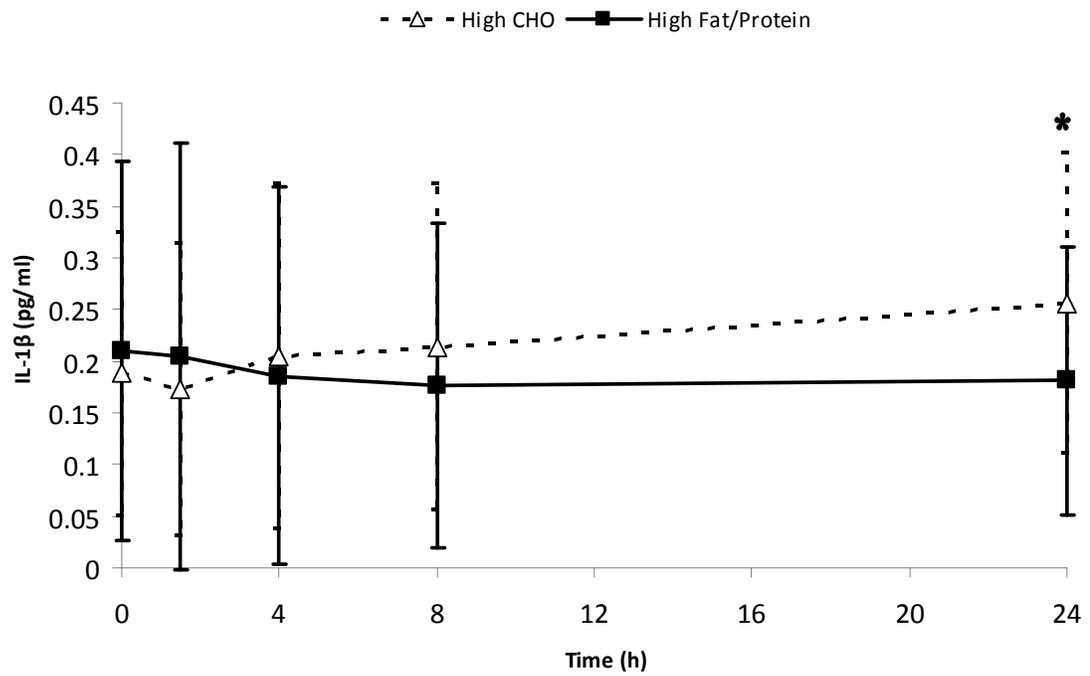
Figure 4.9. IL-1β concentrations in both conditions at pre-exercise, 1.5, 4, 8, and 24 hours post-exercise. Values are mean ± SD, CHO = carbohydrate. *P = 0.05 compared to the high fat and protein condition at 24 hours post-exercise.
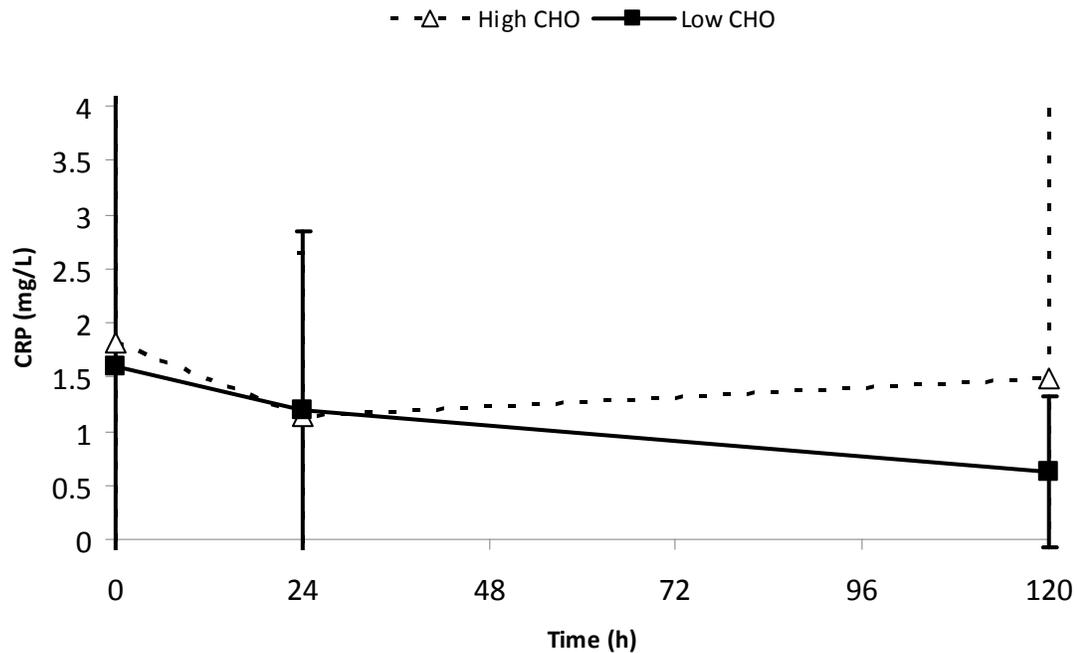
Figure 4.10. CRP concentrations in both conditions at pre-exercise, 24 and 120 hours post-exercise. Values are mean ± SD, CHO = carbohydrate, n = 11.

It was hypothesized that the markers of inflammation, IL-6, IL-1β, and CRP, would increase when levels of post-exercise blood glucose were elevated. To properly test the hypothesis it was crucial to induce muscle damage so there would be an inflammatory response to measure. Perceived muscle soreness with significant increases from baseline at all time points post-exercise in both conditions, and loss of maximal isometric strength immediately post-exercise, 24 and 48 hours post-exercise in the biceps in both conditions are positive indicators of muscle damage. While blood glucose was not significantly different between conditions, the insulin response was significantly higher in the high carbohydrate condition at 1.5, 4 and 8 hours post-exercise demonstrating higher carbohydrate intake. Evidence supporting the hypothesis is IL-1β significantly

higher in the high carbohydrate condition compared to the high fat and protein condition at 24 hours post-exercise and a trend for a main effect between conditions for IL-6 to be higher in the high carbohydrate condition.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

Introduction

The purpose of this study was to investigate how high carbohydrate versus high fat and protein diets influences the inflammatory response to high force eccentric exercise. This study utilized a model of high-force eccentric exercise to induce muscle damage and study the resulting inflammatory response. Two indicators of muscle damage, strength loss and perceived soreness, were indicative of a moderate level of muscle damage whereas the other two indicators of muscle damage, CK and mid-brachial arm circumference, were not indicative of a high level of muscle damage. There was not enough muscle damage to induce a systemic inflammatory response indicated by no changes in CRP. There was a local inflammatory response indicated by a significant increase in IL-1β in the high carbohydrate condition compared to the high fat and protein condition at 24 hours post-exercise and a trend for IL-6 to be higher in the high carbohydrate condition compared to the high fat and protein condition. While there were not significant differences in blood glucose between conditions, there was a much greater insulin response in the high carbohydrate condition, supporting the fact that much greater boluses of carbohydrate were consumed in the high carbohydrate condition.

Subject Characteristics

Overall, the subjects who completed the study were apparently healthy. A healthy BMI is in the range 18.5 - 25 kg·m^{-2}, an overweight BMI is in the range 25 - 30 kg·m^{-2} and is associated with an increased risk for disease, an obese BMI is above 30 kg·m^{-2} and is associated with further increased risk for disease. An underweight BMI is below 18.5 kg·m^{-2} and a BMI below 16 suggests a possible eating disorder (U.S. Department of Health and Human Services, 2000). The subjects' mean BMI fell in the healthy range. One subject had a BMI just below 18.5 and 4 subjects had BMI's in the overweight range. The remaining 7 subjects all had BMI's in the healthy range.

Waist to hip ratio is another simple measure to help determine one's relative risk for chronic diseases. Individuals who carry more weight around their hips are said to be more pear shaped whereas individuals who carry more weight in their abdomen are considered more apple shaped. The more apple shaped an individual is the higher risk they have for developing chronic diseases (Alberti & Zimmet, 1998). The waist to hip ratio measures this characteristic. Males with a ratio above 0.9 and females with a ratio above 0.85 are at higher risk for chronic diseases (Alberti & Zimmet, 1998). The mean waist to hip ratio of the male and female subjects was 0.87 and 0.83, respectively. This places the subjects in the low to moderate risk category. Two male and 2 female subjects were in the high risk group. Overall, based on waist to hip ratio most subjects were healthy and not at high risk for chronic disease.

Fasting blood glucose level is another measure used to determine one's relative risk for chronic disease, especially diabetes mellitus. Fasting blood glucose is measured

after at least an 8 hour fast. Individuals with fasting blood glucose above 110 mg/dL are at higher risk for developing chronic diseases (U.S. Department of Health and Human Services, 2001). Fasting blood glucose was taken the morning of the first day of both conditions. The mean fasting blood glucose was 105 mg/dL for the high carbohydrate condition and 100 mg/dL for the high fat and protein condition placing the mean for both conditions below the high risk point. Two subjects had fasting blood glucose above 110 mg/dL in both conditions. As a group most subjects had healthy fasting blood glucose and were not at high risk for chronic disease based on fasting blood glucose. One subject in the higher risk group for fasting blood glucose was also in the higher risk group for BMI and waist to hip ratio. These are all signs of the metabolic syndrome and it is likely this subject was the least healthy.

Blood Glucose and Insulin

The goal of the high carbohydrate condition versus the high fat and protein condition was to significantly elevate post-exercise blood glucose levels to the point of hyperglycemia (meals outlined in Appendix B). The meals for both conditions had equal amounts of calories within each subject and had different proportions of fat, carbohydrate, and protein between conditions. For the high carbohydrate condition, subjects consumed 1.45g carbohydrate per kg body weight for each meal. For the high fat and protein condition subjects consumed 0.46g carbohydrate per kg body weight for each meal.

The glycemic index is a scale that indicates how rapidly different foods influence blood glucose levels. The higher a food is on the glycemic index the greater and more rapidly it will increase blood glucose. However, when foods with high glycemic index values are consumed, blood glucose will return to fasting levels more rapidly than foods that have lower glycemic index values. Therefore, the food selected for the high carbohydrate condition ranged from moderate to high on the glycemic index. The goal was to quickly elevate blood glucose levels and sustain elevated blood glucose levels for a maximum amount of time. Conversely, for the high fat and protein condition foods that were extremely low on the glycemic index were selected. The goal of this was to have extremely low increases in blood glucose for this condition compared to the high carbohydrate condition.

The purpose of elevating blood glucose to the point of hyperglycemia in the high carbohydrate condition was because previous studies indicate that hyperglycemia can induce and or augment inflammation (Esposito et al., 2002; Gonzales et al., 2006). Hyperglycemia as defined by the American Diabetics Association is elevated blood glucose significantly above fasting blood glucose levels. Typically, blood glucose above 140 mg/dL is considered hyperglycemic. In non-diabetic people under normal eating conditions blood glucose will rarely be greater than 140 mg/dL. In order to properly test the hypothesis that elevated blood glucose levels will augment the inflammatory response it was crucial to have elevated blood glucose levels in the high carbohydrate condition compared to the high fat and protein condition.

There were no significant differences between the conditions with respect to blood glucose concentrations. Since the subjects consumed their meals under the supervision of investigators it is known that significantly more carbohydrates were consumed in the high carbohydrate condition versus the high fat and protein condition. However, this difference was not reflected in the blood glucose concentrations.

There are two possible reasons for the lack of difference in blood glucose between conditions. First, it is possible the food selected for the high carbohydrate condition was too high on the glycemic index. This could explain the lack of difference between conditions because high glycemic index foods will cause a sharp increase in blood glucose followed by a sharp decline back to baseline levels (Foster-Powell, Holt, & Brand-Miller, 2002). It appears that the food in the high carbohydrate condition caused a very rapid increase in blood glucose followed by a rapid drop back to baseline before blood was sampled. The 1.5, 4, and 8 hour post-exercise blood draws were taken about 1 hour after each meal was consumed. It is possible a rapid rise and decline in blood glucose took place in this 1 hour period in the high carbohydrate condition. If this did happen, the rise in blood glucose in the high carbohydrate condition would have been missed by these blood draws and no difference in blood glucose between the conditions would be detected.

Another possible reason for the lack of difference in blood glucose between the conditions could be due to the overall healthy status of the subjects. Based on mean BMI and waist to hip ratio the subjects were overall in low risk categories for chronic diseases including diabetes mellitus. Diabetes mellitus is a disease characterized by poor control

over blood glucose. Insulin is a hormone released by the pancreas that induces uptake of glucose out of the blood mainly by the liver and skeletal muscle. The glucose is then stored in the form of glycogen. People with diabetes either lack sensitivity to insulin or are not capable of producing enough insulin to decrease blood glucose in an efficient manner. Individuals also can be classified as having impaired glucose tolerance if they cannot bring their blood glucose levels down as quickly as a healthy individual, but quicker than someone with diabetes. Impaired glucose tolerance is on the pathway to developing diabetes and is a major warning flag.

It is possible that the subjects were capable of handling the high carbohydrate load in the high carbohydrate condition with only minimal increases in blood glucose. Based on the subjects low risk categorization for chronic diseases, it is likely they have very good control over their blood glucose. Insulin concentrations were measured on a time-match schedule with blood glucose. Insulin concentrations were significantly greater in the high carbohydrate condition compared to the high fat and protein condition at 1.5, 4, and 8 hours post-exercise.

This significant rise in insulin in the high carbohydrate condition supports the fact that the subjects received a much greater load of carbohydrates during this condition. It also supports the idea that the subjects are capable of producing proper amounts of insulin. The fact that the subjects' blood glucose levels were not significantly greater in the high carbohydrate condition suggests their cells were sensitive to the insulin and removed the excess glucose from the blood. This data supports that the subjects were healthy and had no signs of impaired glucose tolerance or diabetes mellitus. So, it is

possible that even though the subjects received a significant amount of carbohydrate during the high carbohydrate condition, they did not have large rises in blood glucose at the times measured. Given this, any significant differences between conditions could possibly be linked to the insulin response in addition to the higher carbohydrate intake in the high carbohydrate condition because the only differences detected between conditions with respect to diet was insulin and carbohydrate intake.

Additionally, the lack of significant increase in blood glucose in the high carbohydrate condition may have been the combination of the subjects being healthy with good control of their blood glucose and the food selected being too high on the glycemic index. In combination these explanations result in a rapid rise in blood glucose followed by a very rapid decline back to baseline. The high glycemic index foods only cause a short peak in blood glucose and the very healthy state of the subjects likely decreased this already short peak causing us to see no significant changes in blood glucose.

<div align="center">Muscle Damage</div>

High-force eccentric exercise was utilized to induce skeletal muscle damage and the resulting inflammatory response. Creatine kinase (CK), perceived muscle soreness, mid-brachial arm circumference, and isometric strength loss were assessed as markers of muscle damage.

Creatine kinase is an enzyme normally found only in skeletal muscle. So, appearance of this enzyme in circulation is an indicator of muscle damage (Brown et al., 1996). CK was measured pre-exercise, 24 and 120 hours post-exercise in both conditions.

There were no significant changes in CK between conditions or over time. This is evidence that a high level of muscle damage did not occur. This is consistent with the CK measurements of Miles, Pearson, Andring, Kidd & Volpe (2007), in which a similar exercise protocol and time frame was used. Miles et al. (2007) had 2 subjects out of 11 who had CK levels indicative of a high level of muscle damage, over 1000 IU·l^{-1}. Miles et al. (2008) conducted another study with a very similar exercise protocol and observed a mean CK of 2133±5082 IU·l^{-1} at 96 hours post-exercise. For clarity, Miles et al. (2007) will be referred to as the low muscle damage study and Miles et al. (2008) will be referred to as the high muscle damage study for the remainder of the discussion chapter. Contrary to the low muscle damage study and the current study, the high muscle damage study demonstrated that the current exercise protocol is capable of inducing high levels of muscle damage. While some of the subjects in this study showed small increases in CK, none of the subjects had CK increases that would indicate a high level of muscle damage. The exercise in all 3 studies requires the subjects to voluntarily maximally resist the motion of the dynamometer. It is possible that the subjects of the high muscle damage study put forth more effort in resisting the motion and thus more muscle damage was inflicted.

Another possible reason for the lack of CK response to the exercise for some subjects may be the repeated bout effect. The repeated bout effect is a training adaptation resulting from multiple bouts of eccentric exercise. It is believed this training effect will cause successive bouts of eccentric exercise to result in significantly less muscle damage and soreness (Lavender & Nosaka, 2006). A prerequisite for this study was for subjects to

have refrained from strenuous exercise that could result in soreness of their arms. In addition, subjects were required to be non weight trained or perform work or regular activities that are strenuous with the arms. While all subjects stated they met these criteria, it is possible that some may have done some strenuous action with their arms in the recent past without remembering. Since the repeated bout effect can last up to several months it is possible this training effect was present in some subjects without their knowledge and thus reduced their CK response to the exercise.

Perceived muscle soreness was another measure used as an indicator of muscle damage. With high-force eccentric exercise that causes muscle damage, subjects experience soreness in the muscle, this soreness generally peaks at 48 hours post-exercise (Proske & Morgan, 2001). This post-exercise muscle soreness is referred to as delayed onset muscle soreness (DOMS). DOMS was measured via a 100mm analog scale pre-exercise, 1.5, 4, 8, 24, 48, 72, 96, and 120 hours post-exercise.

For perceived muscle soreness, there was a main effect between conditions where the high carbohydrate condition was greater than the high fat and protein condition. There was also a time effect where every time point post-exercise was significantly more sore than pre-exercise for both conditions. Perceived muscle soreness peaked at 48 hours post-exercise in both conditions in agreement with the low muscle damage study and Proske & Morgan (2001). These results are indicative of muscle damage, contrary to the results of the CK analysis. Also, these results are very similar to those of the low muscle damage study and what was anticipated with this exercise protocol. The main effect between

conditions indicates that the carbohydrate condition may have led to greater perceived muscle soreness than the high fat and protein condition in general.

Mid-brachial arm circumference was used as a marker of muscle damage and inflammation. The idea is that if there is enough muscle damage inflicted by the exercise, there will be an inflammatory response to repair the damage. This inflammatory response will recruit several types of cells such as macrophages and lymphocytes to the area. This recruitment of cells will result in some swelling of the arm and cause an increase in the mid-brachial arm circumference.

There was a time effect for mid-brachial arm circumference. However, post-hoc analysis did not identify any significant differences between means. These are the same results the low muscle damage study obtained with a similar exercise protocol. This is indicative of some swelling of the arm but no significant differences between conditions.

The fourth measure of muscle damage was isometric strength loss. All types of exercise are expected to result in some strength loss due to metabolic fatigue. However, the strength loss associated with eccentric exercise is greater and lasts longer. Isometric strength loss resulting from eccentric exercise can be as much as 60% (Proske & Morgan, 2001).

Isometric strength was measured separately in the triceps and biceps for both conditions. For both triceps and biceps there was a significant main time effect with no differences between conditions. Biceps had significant strength loss immediately post-exercise, and 24, and 48 hours post-exercise for both conditions. Maximum strength loss for the biceps was 24 hours post-exercise at close to 20%. This is only considered a

moderate amount of strength loss resulting from eccentric contractions considering strength loss can reach as high as 60%. The low muscle damage study only observed strength loss immediately post-exercise. However, the high muscle damage study observed a strength loss of 31.4% immediately post-exercise and 27.5% at 24 hours post-exercise. So the present study indicates strength loss in the biceps somewhat greater than the low muscle damage study and less than the high muscle damage study observed with a similar exercise protocol. As with CK, this could be explained by the actual effort put forth by the subjects and decreases due to the repeated bout effect.

Triceps had significant strength gains at 96 and 120 hours post-exercise for both conditions. The movement of testing the triceps is much less normal than the movement of testing the biceps. It is likely the strength gains in the triceps can be explained by the subjects becoming more accustomed to the movement of the dynamometer as opposed to actual strength gains.

A review paper by Warren, Lowe, & Armstrong (1999) evaluated several different markers of muscle damage including isometric strength loss, appearance of myofiber proteins such as CK and subjective ratings of soreness. This review concluded that isometric strength loss was the most consistent and reliable indicator of muscle damage resulting from eccentric contractions. The major issue with isometric strength loss is that it is measured via a maximal voluntary contraction (MVC). With MVC even highly motivated individuals may not recruit all of their motor units resulting in false measures of strength loss (Warren, Lowe, & Armstrong, 1999). Warren, Lowe, & Armstrong (1999) concluded that appearance of myofiber proteins in circulation, such as

CK, correlates poorly with muscle damage. Two major issues with this measure are the repeated bout effect that drastically reduces the appearance of these proteins in circulation. Secondly, similar levels of muscle damage often result in highly variable levels of increases of these proteins in circulation between individuals. Warren, Lowe, & Armstrong (1999) looked at several studies utilizing soreness as an indicator of muscle damage and determined that isometric strength loss was a superior measure.

Taken together, appearance of CK in circulation and mid-brachial arm circumference showed no signs of significant muscle damage whereas isometric strength loss and perceived muscle soreness showed signs of moderate muscle damage. Thus, it is concluded that a moderate amount of muscle damage was inflicted from the exercise in both conditions. This is supported by the fact that this study inflicted about the same or slightly more muscle damage than the low muscle damage study but induced less muscle damage than the high muscle damage study. Also, it does not seem that either condition compared to the other resulted in significantly larger amounts of muscle damage. But, overall the high carbohydrate condition did have greater perceived soreness.

<center>Markers of Inflammation</center>

The purpose of this study was to investigate the influence of a high carbohydrate diet on the inflammatory response to high force eccentric exercise. The markers of inflammation measured were IL-6, IL-1β, and CRP. IL-6 and IL-1β are cytokines produced locally at the site of inflammation. IL-6 is considered to have both pro- and anti-inflammatory effects whereas IL-1β is considered to be a pro-inflammatory cytokine.

CRP is an acute phase protein produced in the liver and is indicative of a systemic

inflammatory response known as the acute phase response.

C-Reactive Protein (CRP)

C-reactive protein is an acute phase protein produced in the liver and is indicative

of a systemic inflammatory response (Ostrowski et al., 1999). There were no significant

changes in CRP post-exercise in either condition or over time in the current study. These

are the same results the low muscle damage study observed with a similar protocol. This

is indicative that not enough muscle damage was induced to develop a systemic

inflammatory response. The high muscle damage study had an exercise and control

condition, CRP increased in the exercise compared to control condition but differences

did not reach significance after adjustment. In the high muscle damage study there was

significantly more muscle damage than in the current study or in the low muscle damage

study. This does indicate that if enough muscle damage is inflicted there may be a

systemic inflammatory response as measured by CRP. It is possible that high

carbohydrate supplementation would have an effect on the CRP response if enough

muscle damage was inflicted to elicit a CRP response. Unfortunately, the current study

did not induce enough muscle damage and it was not determined if carbohydrate

supplementation could influence a systemic inflammatory response.

Interleukin-1β (IL-1β)

Interleukin-1β is a cytokine produced locally at the site of infection or tissue

damage. Cytokines are some of the molecules responsible for controlling the magnitude

of the local inflammatory response. IL-1β is considered a pro-inflammatory cytokine because it induces lymphocytes, neutrophils, monocytes, and other cells to clear antigens and heal tissue (Ostrowski et al., 1999). IL-1β also promotes the liver to produce the acute phase protein CRP.

Interleukin-1β was significantly greater in the high carbohydrate condition compared to the high fat and protein condition at 24 hours post-exercise. This is evidence that the inflammatory response was greater and more prolonged in the high carbohydrate condition versus the high fat and protein condition. This is in agreement with the hypothesis that a high carbohydrate diet post-exercise would augment the inflammatory response.

It is possible the elevated IL-1β 24 hours post-exercise in the high carbohydrate condition is associated with the trend for higher muscle soreness in the high carbohydrate condition. Flores et al. (1989) investigated the effects of TNF- α and IL-1β on protein breakdown in the rat model. They found that TNF- α and IL-1β worked in a synergistic manner to enhance muscle proteolysis. Nawabi, Block, Chakrabarti, & Buse (1990) found that human recombinant TNF- α and or IL-1β increased muscle protein breakdown in vivo in rats via increased branched chain α-keto acid dehydrogenase (rate limiting enzyme for amino acid breakdown in skeletal muscle). It is possible the elevated IL-1β 24 hours post-exercise in the high carbohydrate condition could have increased protein breakdown enhancing the muscle damage. It is also possible the higher intake of protein in the high fat and protein condition helped diminish protein breakdown. A study by Rhode, MacLean, Richter, Keins, & Pedersen (1997) demonstrated that branched chain

amino acid supplementation reduced net protein breakdown in response to eccentric exercise. The only indication of greater muscle damage in the high carbohydrate condition compared to the high fat and protein condition was a main effect for perceived soreness where the high carbohydrate condition was higher. The increased IL-1β in the high carbohydrate condition may not have been enough of an increase to cause a statistically significant change in the other indicators of muscle damage. Direct causation of this relationship cannot be verified in the current experiment. It is a possible relationship to investigate more thoroughly in future experiments.

Another possible reason for the elevated perceived soreness in the high carbohydrate condition could be enhanced pain sensitivity due to the elevated IL-1β 24 hours post-exercise. A study by Wolf et al. (2003) demonstrated impaired IL-1 signaling reduced pain sensitivity in mice. This suggests that IL-1 is linked with and likely enhances pain sensitivity. Moldawer, Svaninger, Gelin, & Lundholm (1987) demonstrated that IL-1β and IL-6 can induce prostaglandin synthesis in skeletal muscle. Elevated levels of the prostaglandin PGE_2 and muscle soreness after eccentric exercise have been found to coincide suggesting a relationship between PGE_2 and muscle soreness (Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1997). Inhibition of PGE_2 synthesis has been shown to be the target of aspirin-like drugs that reduce inflammation and pain (Vane, 1971). This implies that PGE_2 is an important mediator of pain sensation and also has a role in inflammation. However, this link is complex and not fully understood at this time. The elevated IL-1β 24 hours post-exercise in the high carbohydrate condition could have augmented PGE_2 synthesis in the skeletal muscle. This elevated PGE_2 could have

increased the subjects' sensitivity to pain and led to increased perceived muscle soreness post-exercise.

Interleukin-6 (IL-6)

Interleukin-6 was measured pre-exercise, and 1.5, 4, 8, and 24 hours post-exercise in this study. There was a trend for a main effect between conditions for IL-6 to be higher in the high carbohydrate condition compared to the high fat and protein condition. This trend shows support for the hypothesis that the inflammatory response will be elevated when post-exercise blood glucose is elevated. There were no differences in IL-6 between the high carbohydrate and placebo conditions in the low muscle damage study. Carbohydrate was consumed at 1.45g/kg body weight in three boluses in the current study whereas carbohydrate was consumed at 0.25g/kg weight per hour for 12 hours each on the day of and the day following exercise in the low muscle damage study. It is possible the larger boluses of carbohydrate in the current study elevated blood glucose more than the smaller boluses in the low muscle damage study. This possible difference in blood glucose cannot be proven because no significant differences in blood glucose were detected in the current study. However, if this was the case, the higher blood glucose could have lead to the trend for higher IL-6 in the current study

There was a time effect where IL-6 was significantly decreased 1.5 and 4 hours post-exercise in both conditions. This decrease is the normal diurnal variation observed with IL-6. Similar diurnal variations were observed in IL-6 in the control condition of the high muscle damage study with significant decreases at 4 and 8 hours relative to the early morning baseline. The high muscle damage study did not observe these diurnal variations

in the exercise condition, but observed a significant increase at 4, 8 and 12 hours post-exercise. While there was not a significant increase in IL-6 in the current study, there was a trend ($p = 0.088$) for IL-6 to be increased 8 hours post-exercise, showing no diurnal decrease at 8 hours post-exercise. This indicates a small IL-6 response in both conditions at 8 hours post-exercise. The smaller IL-6 response in the current study compared to the high muscle damage study is on par with less muscle damage inflicted in the current study. Together, these results indicate that the normal, daily IL-6 diurnal fluctuations can be removed with levels of muscle damage high enough to induce a significant inflammatory response.

These diurnal variations were not observed in the low muscle damage study where a similar protocol was used. Additionally, the low muscle damage study observed a significant time effect where IL-6 was elevated 8 hours post-exercise in both conditions, similarly to the increase in IL-6 in the exercise condition of the high muscle damage study. The fact that IL-6 was not significantly increased and diurnal variations were detected in the current study, is evidence that a higher level of muscle damage and more inflammation was inflicted in the high and low muscle damage studies than in the current study.

Presently there are conflicting opinions on the stimulus and origin of increases in IL-6 after muscle damaging eccentric exercise. One argument is that the IL-6 response from eccentric exercise is linked to the magnitude of muscle damage and inflammation. The other argument is that the IL-6 response is independent of muscle damage and inflammation. The results of Jonsdottir et al. (2000), Croisier et al. (1999), and Nosaka &

Clarkson (1996) all suggest that elevated IL-6 in response to muscle damaging exercise is not related to an inflammatory response from muscle damage. These three studies used longer exercise protocols than the current study and only detected IL-6 immediately post-exercise or very close to it. It is likely the IL-6 from these studies was produced in the skeletal muscle for its role in glucose metabolism like what is observed in endurance exercise with no eccentric component. None of these studies measured IL-6 near the 8 hour time-point where both the high and low muscle damage studies observed increases in IL-6. It is therefore conceivable that IL-6 was elevated in these studies at or near 8 hours post-exercise but it was not detected due to timing of blood collection. A study by Willoughby, McFarlin, & Bois (2003) implemented 2 eccentric exercise bouts separated by 3 weeks. IL-6 was detected at 4 and 6 hours post-exercise in both bouts with no difference between bouts. The first bout had significantly more muscle damage than the second due to the repeated bout effect. The authors concluded that the increases in IL-6 after eccentric exercise were not associated with the magnitude of muscle damage. However, even though more muscle damage was inflicted in the first bout, the second bout did have some signs of significant muscle damage and there may have been enough muscle damage in the second bout to elicit an equal IL-6 response. Smith et al. (2000) and Bruunsgaard et al. (1997) reported that the IL-6 response from eccentric exercise is related to the inflammatory response and magnitude of muscle damage.

The results of the current study in combination with both the high and low muscle damage studies support linking the IL-6 response to the level of muscle damage and inflammation. The high muscle damage study induced greater levels of muscle damage

and observed significant increases in IL-6 8 hours post-exercise relative to the early
morning baseline. The low muscle damage study also observed significant increases in
IL-6. The current study did not alleviate the diurnal variation at 1.5 or 4 hours post-
exercise but did at 8 hours post-exercise. Additionally, there was no increase in IL-6. The
current study had significantly less muscle damage than the high muscle damage study.
This suggests that this IL-6 response is closely linked to muscle damage and
inflammation and not related to duration of exercise. In all, the inflammatory response is
complex and not fully understood at this point. It is likely there are more than just one or
two factors influencing the IL-6 response such as duration, type, and intensity of exercise.

<u>Inflammatory Response</u>

The indications of a significant increase in inflammation in this study were the 24
hour post-exercise increase in IL-1β and the trend for IL-6 to be higher in the high
carbohydrate condition when the typical, non-exercised response is to decrease at this
time point. Overall, with no significant increases in IL-6 at any given time-point, the
amount of inflammation induced was lower compared to both the high and low muscle
damage studies. Even though the amount of inflammation was small, the high
carbohydrate condition appeared to have elevated the inflammatory response. Gonzalez
et al. (2005) showed that hyperglycemia can augment inflammation in mononuclear cells
via increased TNF- α. The current study shows further evidence that carbohydrate intake
has the ability to augment inflammation even when the initial inflammatory response is
small. Also, Gonzalez et al. (2005) found elevated inflammation in mononuclear cells

whereas the current study found evidence that inflammation derived from skeletal muscle can be enhance via carbohydrates.

The purpose of the current study was to investigate the influence of carbohydrates on the inflammatory response. However, it is important to consider there were high carbohydrate and high fat and protein conditions. The high carbohydrate condition was compared to the high fat and protein condition. The high fat and protein condition, while low in carbohydrates, was not a control and it is likely the high fat and protein did play a role in the results. While the specific influences of high fat and protein on inflammation were not specifically investigated in this study, a diet higher in fat and protein does not augment the acute inflammatory response like a diet higher in carbohydrates. It is not to be concluded that high fat and protein diets will decrease or increase acute inflammation, or that high fat and protein diets do not influence inflammation.

Several other studies have used models of eccentric exercise and found no significant changes in IL-1β 24 hours post-exercise (Bruunsgaard et al., 1997; Hirose etal., 2004; Smith et al., 2000). Smith et al. (2000) actually observed significant decreases in IL-1β 24 hours post-exercise. The current study showed increased IL-1β in the high carbohydrate condition 24 hours post-exercise and there is a lack of evidence for this response in other studies. This further strengthens the idea that carbohydrates were elevating the inflammatory response because none of these other studies elevated post-exercise carbohydrate intake.

If the current study was repeated, but significantly more muscle damage was inflicted such as in the high muscle damage study, there may be even more drastic

elevations in inflammation in the high carbohydrate condition. If enough muscle damage was inflicted to completely abolish the diurnal variation in IL-6, and cause a significant increase in IL-6 8 hours post-exercise, it is possible there could be a condition by time interaction between conditions for IL-6 at this time point. The trend in IL-6 in the current study supports the idea that IL-6 can be influenced by carbohydrates and if the IL-6 response was greater like in the high muscle damage study this trend could become statistically significant.

Conclusion

The goal of this study was to gain a better understanding of the inflammatory process in skeletal muscle. It was hypothesized that high carbohydrate supplementation would result in an elevated inflammatory response to muscle damage resulting from eccentric exercise. Several chronic diseases such as type II diabetes, atherosclerosis, and Alzheimer's disease are correlated with chronic low-level inflammation. With better prevention and treatment techniques the prevalence of these diseases could be reduced.

There were three indications that the high carbohydrate diet resulted in a greater inflammatory response. First, the significantly greater IL-1β in high carbohydrate condition 24 hours post-exercise compared to the high fat and protein condition. Secondly, there was a trend for IL-6 to be greater in the high carbohydrate condition. Thirdly, there was a main effect where perceived soreness was greater in the high carbohydrate condition. These results demonstrate that high carbohydrate intake will augment inflammation in skeletal muscle when already initiated.

The current study helped to further the knowledge of the timing, magnitude, and purpose of the presence of cytokines in circulation following skeletal muscle damage. This is very important because skeletal muscle has a large impact over the whole body. Potentially, chronically elevated inflammation in skeletal muscle could lead to chronic low level inflammation that is correlated with chronic diseases. As the inflammatory process in skeletal muscle is better understood, improved prevention and treatment recommendations can be made.

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APPENDICES

APPENDIX A:

INFORMED CONSENT

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

Study Title: Do carbohydrates increase the magnitude of the inflammatory rsponse?

Funding: This study is funded by The Department of Health and Human Development and ADVANCE
Investigator: Chris Depner
Dept. of Health and Human Development
Herrick Hall, Room 6, Montana State University
Bozeman, MT 59717-3540
Phone: (406) 994-5001, chris.depner@myportal.montana.edu

You are being asked to participate in a study investigating how carbohydrates affect the response of several markers of inflammation that can be measured in the blood in response to a resistance exercise that will make the muscles of both arms sore, one at a time in separate phases of the study. 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 and diabetes is often associated with the presence of a low-level of inflammation over a long period of time, perhaps many years. There are several factors that can influence the levels of this inflammation. One possible factor is blood glucose levels. Previous studies indicate that hyperglycemia can increase markers of inflammation once the inflammatory process is initiated. However, the mechanism is poorly understood and it is not known if hyperglycemia will increase inflammation initiated from different origins. We will measure some of the inflammatory markers associated with heart disease, have you perform the resistance exercise, eat a given amount of carbohydrates, and measure the same markers over several days following the exercise. The levels of inflammatory markers will be compared to levels measured in a high and low carbohydrate condition. The inflammatory markers that we will measure are found in the blood, thus you will have your blood drawn on 12 occasions over a 5 to 8 week period during this investigation. You will be tested in the Montana State University Movement Science Laboratory in Romney Gymnasium and the Nutrition Research Laboratory in Herrick Hall.

The purpose of this study is to determine whether the magnitude and duration of the inflammatory response to muscle damage varies between high and low carbohydrate diets.

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 form that includes questions regarding the presence of heart disease and diabetes in your family.

2) Report to the Movement Science Laboratory or Nutrition Research Laboratory on the MSU campus for measurements and feeding (including pre-exercise blood collection, maximum force resistance exercise, feeding, and 1.5, 4, 8, 24, 48,   and 120 hours post-exercise measurements).

3) Anthropometric measurements will that will be collected will consist of body height, weight, upper arm circumference, and waist to hip ratio. (A circumference measure will be taken at the waist and at the hip for measurement of the waist to hip ratio. This latter measure will be made by an investigator of the same sex as the participant and will be made over the clothing of the participant, i.e. no disrobing will occur.

4) You will perform an exercise using a machine that controls speed of movement and amount of force. The machine consists of a padded chair with a padded lever system. You will place your wrist between the pads of the lever and the investigator will exert resistance for the exercise. Sixty (60) 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, your arm will then be moved to a fully flexed position and you will resist this force too. Six sets of 10 contractions will be performed with five minutes rest between each set. Each contraction will last approximately 3 seconds with a 10-second rest between contractions. As you fatigue, the resistance exerted by the machine will decrease, but it will always keep the resistance maximum for you. This exercise is called an eccentric exercise because your muscle is lengthening as it is producing force.

5) You will eat a given amount of food following the exercise for each condition. This will be the only food you are allowed to eat for the first 6 hours post-exercise.

6) You will perform three maximal contractions with the arm flexed at 90 degrees to determine your maximum force production. You will be seated on a the exercise machine described above. Three maximal contractions will be performed with 60 seconds of rest between trials.

7) 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.

8) You will complete an International Physical Activity Questionnaire asking you to describe the frequency and intensity of physical activity that you typically perform in a week's time.

9) You will receive $8.33 compensation for each blood sample collected ($100 total for all 12 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 12 occasions (6 blood draws for each condition separated by 3 to 6 weeks). 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.
2) You will receive $8.33 compensation for each blood sample collected ($100 total for all 12 blood collection/visits involved).

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 (Mary Miles and or Chris Depner 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 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: Do carbohydrates increase the magnitude of the inflammatory response?

AUTHORIZATION: I have read the above and understand the discomforts,

inconvenience and risk of this study. I, _____

(PRINT YOUR NAME), agree to participate in this research. I understand that I may

later refuse to participate, and that I may withdraw from the study at any time. I have

received a copy of this consent from for my own records.

Signed: _____   Date: _____
              Subject's Signature

Witness: _____   Date: _____
              (if other than the investigator)

Investigator:_____   Date: _____
              Chris Depner

APPENDIX B

FOOD CONSUMED FOR EACH CONDITION

Food Calculation

Subject #____.

Weight in lbs.____/ 2.2 = _____kg

Weight in kg____/ 77 x 100 = _____% of base food

High CHO Condition (15% Fat, 75% CHO, 10% Protein)

Clif Bar:
        ___% x 68g = ___g of Clif Bar

Apple Juice:
        ___% x 240g = ___g of Apple Juice

2% Milk
        ___% x 244g = ___g of 2% Milk

Corn Flakes
        ___% x 28g = ___g of Corn Flakes

High Fat and Protein Condition (70% Fat, 6% CHO, 24% Protein)

Turkey:
        ___% x 51g = ___g of Turkey

Cheese:
        ___% x 56g = ___g of Cheese

Peanuts:
        ___% x 53g = ___g of Peanuts

Calories Per Meal Per Condition:

        ___% x 596 = _____Calories