

INFLAMMATION, INSULIN, AND GLUCOSE DIFFERENCES BETWEEN HIGH
AND LOW GLYCEMIC INDEX DIETS FOLLOWING DOWNHILL
RUNNING IN OVERWEIGHT AND OBESE WOMEN

by

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ABSTRACT

Low-grade chronic inflammation is associated with excess adipose tissue, and often precedes chronic disease. Overweight and obese individuals lose the ability to control inflammation as percent body fat increases, likely linked to inefficient carbohydrate oxidation that amplifies inflammation. Little is known about how the quality of carbohydrates influences inflammation of muscle in this population. The purpose of this study was to determine whether there are differences in inflammation and glucose metabolism between low glycemic index (LGI) and high glycemic index (HGI) diets following downhill running in overweight and obese women. This study was a pre-post design in which overweight and obese women ($n = 20$) were placed in matched pairs for percentage body fat, each receiving an isomacronutrient LGI or HGI diet. Participants completed a downhill run at heart rates at 65% predicted maximal oxygen consumption ($\text{VO}_{2\text{max}}$) until 15% loss of isometric force of the hip and knee extensors, or volition fatigue, was achieved. Participants ate their prescribed diet for 24 h post exercise. Glucose, insulin, creatine kinase (CK), C-reactive protein, tumor necrosis factor- α , and interleukin-6 were measured pre-exercise, 0, 24, and 48 h post-exercise. Isometric force and muscle tenderness were measured when blood was collected. Serum CK changed significantly over time ($p < 0.001$), with the peak at 24 h in the HGI group, and at 48 h in the LGI group. Isometric force decreased most at 24 h for the HGI group, and at 48 h for the LGI group, with significant differences occurring over time ($p = 0.01$), and a significant time/diet interaction ($p = 0.02$). No significant changes were seen in any of the inflammatory variables for diet or time, with the exception of the expected increase in IL-6 immediately post-exercise. There was a significant ($p = 0.035$) difference in delta insulin between groups. Delta IR also changed significantly over time between groups ($p = 0.044$). We conclude that while inflammation was not different between groups, a LGI diet following downhill running results in an acute improvement in insulin and IR in overweight and obese women.

CHAPTER 1

INTRODUCTION

Obesity is becoming an issue of paramount importance as it is associated with the development of chronic diseases. In 2009, researchers from the Centers for Disease Control (CDC) published projections about the rise and cost of obesity in the United States. The report stated that the US spends around \$147 billion on obesity related health care costs alone every year, and this number is on the rise (CDC 2009). The CDC also noted that heart disease, stroke, diabetes, and cancer, which are on the list of the top ten causes of death in the US, have links to obesity (CDC 2009). These are diseases of lifestyle, and the association between adipose tissue and disease risk indicates the importance of lifestyle interventions for both disease prevention and treatment.

Adipose tissue is considered an endocrine organ and sends hormonal and inflammatory signals to the rest of the body (Pederson 2009). Inflammation is an important part of the immune response, however, chronic inflammation can promote insulin resistance (IR), atherosclerosis, and tumor growth (Handschin and Spiegelman 2008). While obesity has health consequences, abdominal obesity is of special concern as it elicits a clear, chronic low-grade level of inflammation (Pederson 2009). Shoelson supports this idea of chronic inflammation, stating that increased adiposity promotes an increase in lipids, cells deformation, and an increase in systemic inflammatory biomarkers, all increasing disease risk (Shoelson, Herrero, and Naaz 2007). While fat loss is a primary goal for overweight and obese individuals, it is important to find ways to

ameliorate this chronic inflammation to make disease prevention more attainable for the general population (Forsythe et al. 2008).

Abdominal obesity is associated with inefficient carbohydrate (CHO) oxidation and increased inflammation (Pederson 2009), a process that the glycemic index (GI) could potentially influence. Investigators have indicated that chronic inflammation is promoted by the combination of central obesity and high CHO, which can precede type II diabetes (T2DM), certain cancers, CVD, and stroke (Sears 2009; Mitrou et al. 2007). A high GI (HGI) diet is implicated as a mechanism for increases in the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF- α) in overweight and obese populations, promoting IR (Kirwan, Krishnan, Weaver, Del Aguila, and Evans 2001). A benefit of a low GI (LGI) diet is that it may lower TNF- α and other inflammatory biomarkers (Kirwan et al. 2001).

As inflammation is a generalized response ranging from injury to that elicited from excess adiposity, it can be triggered in multiple ways (Smith & Miles 2000). High-force eccentric exercise causes micro-tears in muscle, initiating a systemic inflammatory response (Miles et al. 2010), and thus can be used as a model to study the influence of diet on the inflammation process. Miles and colleagues (2010) noted increased IR and inflammation with a HGI diet following a high-force eccentric arm exercise, but less is known surrounding the LGI diet and inflammation. Furthermore, few researchers have utilized large muscle mass, such as that used in downhill running, in eccentric exercise models to elicit a greater inflammatory response. More research is necessary to elucidate the relationship between increases in systemic inflammation and LGI diets. Increased

knowledge on this issue could provide insight into preventative and treatment measures to decrease chronic disease.

Statement of the Purpose

The purpose of this study was to determine whether there are differences in inflammation and glucose metabolism between low and high GI diets after a downhill run in overweight and obese women. Inflammatory markers measured included TNF- α , interleukin-6 (IL-6), and C-reactive protein (CRP). Glucose metabolism measures included plasma glucose and insulin, and an estimation of IR using the homeostatic model of assessment for IR (HOMA-IR) (McMillan-Price et al. 2006).

Significance

Interventions may be better tailored to individual needs of disease prevention and/or treatment once relationships between inflammation and diet in the overweight population are further discovered based on the inflammation-inducing eccentric exercise model. Low-grade chronic inflammation is associated with numerous disease states, but CVD, stroke, certain cancers, and type II diabetes mellitus (T2DM) are diseases that have the potential to be altered through lifestyle interventions (CDC 2009). Evidence from this study could allow investigators to compare the inflammatory responses associated with a HGI versus a LGI diet to help direct individuals on proper diet for disease prevention. Downhill running has an eccentric phase, with lengthening of the hip and knee extensors, which leads to muscle damage as evidenced by modest increases in CK

and inflammatory markers (Eston, Mickleborough, and Baltzopolous 1995; Peake, Nosaka, and Suzuki 2005). Previous researchers demonstrated increases in inflammatory biomarkers following a high CHO diet compared to a low CHO diet after high-force eccentric exercise (Depner, Kirwan, Frederickson, and Miles 2010). The investigator in this study proposed that the use of downhill running could expand on the previous research by using a larger muscle mass to induce inflammation, and the use of the GI as opposed to total CHO amount could better reveal the relationship between CHO quality and inflammation following eccentric exercise.

Hypothesis

It was hypothesized that the biomarkers of inflammation and IR (TNF- α , IL-6, HOMA-IR, and CRP) would be lower in the LGI compared to HGI diet groups after downhill running.

$$\mathbf{H_0: \mu_{LGI} = \mu_{HGI}}$$

$$\mathbf{H_a: \mu_{LGI} < \mu_{HGI}}$$

Where μ_1 is the sample mean of biomarkers of inflammation and IR measured for the LGI intervention, and μ_2 is the sample mean of inflammatory biomarkers of the control, HGI diet.

Assumptions

For the purposes of this study, it was assumed that individuals would have similar muscle damage responses to the exercise. It was also assumed that the variability in muscle damage responses would be similar between groups.

Delimitations

- 1) The study was delimited to females between the ages of 18-40 years who were overweight and obese. There is some controversy as to whether there are sex-differences in the muscle damage and inflammation responses to exercise. Therefore, to increase homogeneity of our research sample, only women were used in the current investigation.
- 2) Subjects were selected with a BMI ≥ 25 and $\leq 35 \text{ kg}\cdot\text{m}^{-2}$
- 3) Subjects were free of diabetes, CVD, and were not taking anti-inflammatory medications.
- 4) Subjects were not on any form of hormonal contraceptive.

Limitations

- 1) Subjects were provided with the prescribed diet for the day of the downhill run, but were not monitored to determine if they consumed all the prescribed food or if they ate more than prescribed. They were asked to return any uneaten food and report any additional food they consumed on the next visit.

- 2) The lag time from the end of the downhill run to consumption of breakfast was not controlled for, which could have influenced the results.
- 3) Subjects ate their own chosen diet from 24 - 48 hrs. post-exercise, which could have influenced final inflammation and metabolic variables measured.
- 4) Based on the HGI and LGI groups consuming different foods, it was not possible to account for all nutritional differences such as matching foods for exact vitamin, mineral, and fiber contents, which could have influenced the results.
- 5) Subjects worked to a percentage of strength loss as measured by a dynamometer. Subjects ran downhill for 20 minutes, got off and had another strength test. The researchers did what was possible to attempt to make the muscle damage stimulus as similar as possible, but there was variability in the muscular response and inflammatory responses between subjects.

Operational Definitions

Body Mass Index (BMI) – A tool used to approximate body fat based on the weight and height of an individual.

Overweight – A BMI of $25 - 29.9 \text{ kg}\cdot\text{m}^{-2}$.

Class 1 Obesity – A BMI of $30 - 34.9 \text{ kg}\cdot\text{m}^{-2}$.

Isocaloric – The same percentage of kilocalories was determined as a percentage of each participant's predicted resting metabolic rate.

Isomacronutrient – The same percentage of kilocalories comes from proteins, carbohydrates, and fats.

Central/abdominal obesity – Obesity occurring around the abdomen.

Eccentric muscle contraction – The lengthening phase of a muscular contraction.

Exercise Induced Muscle Damage (EIMD) – Muscular damage resulting in increases in creatine kinase (CK) caused from mechanical or metabolic stresses during muscular activity.

CRP – An acute phase protein used as a measurement of systemic inflammation.

IL-6 – An inflammatory regulating cytokine used as an indication of systemic inflammation.

TNF- α – An inflammatory initiating cytokine used as an indication of systemic inflammation.

Glycemic Index (GI) - The rate of glucose entry into the blood.

High Glycemic Index (HGI) diet- The foods for this group were based on a GI of 70 or greater on the GI scale based on pure table sugar.

Low Glycemic Index (LGI) diet – The carbohydrates for this group were based on a GI of 55 or lower on the GI scale based on pure table sugar.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Introduction

With a greater understanding of inflammation and IR in overweight and obese women, dietary strategies to decrease disease risk could be more effectively developed for this target population. This review of related literature will delve into research surrounding this area, beginning with the significance of inflammation in disease status, as there is evidence supporting a relationship between inflammation and disease risk (Kelly et al. 2011; Pederson 2009). An overview of specific inflammatory cytokines, IL-1, IL-6, TNF- α , and the acute phase protein CRP will be discussed as they are important for understanding the way diet could influence inflammation. IR will be discussed, as IR and inflammation provide a better picture of what may occur metabolically following exercise and macronutrient consumption. The relationship between inflammation and obesity will be discussed, as it is through a decrease in inflammation that disease risk stratification could be improved in overweight and obese individuals. A discussion of CK as a measurement of muscular damage will provide reasoning for the use of the downhill running protocol. The GI will be discussed, indicating the importance of the quality of CHO, not only the quantity. Pilot results will also be provided, indicating the preliminary findings associated with a high GI diet and the relationship of this to inflammation and IR.

Inflammation and Disease Risk

The inflammatory response is a generalized immune response to tissue damage or illness (Smith & Miles 2000). It causes an influx of cytokines, or chemical regulatory messengers, to promote healing. Some commonly measured cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), and TNF- α . CRP, an acute phase protein, is also commonly measured to indicate inflammation levels. Inflammation is important acutely to promote healing. However, if this inflammation becomes chronic, it can increase disease risk. It is this chronic, low-grade inflammation associated with obesity that diet could positively influence, possibly serving as a preventative measure against disease.

Chronic inflammation is commonly implicated as a precursor for chronic disease, with CVD and T2DM making the top of the list of great concern due to their prevalence in American society. Due to the rising prevalence of these metabolic diseases, chronic, systemic inflammation has been studied at length, however; less is known about the relationship between adipose-induced inflammation, the effects of diet on said inflammation, and disease. Also of heightened interest regarding inflammation is IR, as it is thought that IR could be caused by increased levels of inflammation, and can also initiate inflammation, presenting a cyclic effect (Kelly et al. 2011).

Inflammatory Cytokines

The inflammatory process is an integration of plasma proteins, fluid, and leukocytes that are shuttled to an injured/affected area (Smith and Miles 2000). While people commonly think of inflammation as including symptoms such as edema, dolor,

calor, and pain, it is the asymptomatic, systemic inflammation that is of concern regarding disease (Smith and Miles 2000). The initiation of inflammation follows a specific, regulated pattern, resulting in delivery of neutrophils, monocytes, and macrophages through an increase in blood flow to the affected area (Smith and Miles 2000). Local production of inflammatory cytokines occurs following this initial response, as the cytokines are derived from monocytes and macrophages once inflammation is signaled (Smith and Miles 2000; Yudkin, Kumari, Humphries, & Mohamed-Ali 1998). Cytokines make up one part of the inflammatory factors that intensify the inflammatory response, with chemokines and cell adhesion molecules making up the rest; however, for the purposes of this proposal, only cytokines will be discussed.

A cytokine is a regulatory, signaling molecule (Smith and Miles 2000). Cytokines activate both local and systemic systems to prolong the inflammatory response. The acute phase response of inflammation causes rapid infiltration of TNF- α and IL-1 β first, both of which then stimulate creation of more TNF- α , IL-1, and IL-6 (Smith and Miles 2000).

The inflammatory cytokines are necessary for the release of acute phase proteins such as CRP (Yudkin et al. 1998; Xing et al. 1998). While IL-1, TNF- α , and IL-6 facilitate the release of CRP, IL-6 demonstrates the ability to activate the hypothalamic-pituitary-adrenal (HPA) axis, making its influence on CRP great (Xing et al. 1998; Yudkin et al. 1998). Animal knockout models were used to demonstrate this, where the knockout of IL-1 and TNF- α resulted in an unchanged inflammatory response, while IL-6

knockout significantly impaired the acute phase response (Yudkin et al. 1998). It is clear that IL-1, TNF- α , and IL-6 are important mediators of the inflammatory response.

While the pro-inflammatory response is necessary, of equal importance is the ability to decrease inflammation to maintain homeostasis. IL-6 exhibits both pro and anti-inflammatory effects, but this distinction remains elusive. Evidence for the anti-inflammatory effects of IL-6 comes from mice-models in which the absence of IL-6 results in a significant increase in systemic elevation of pro-inflammatory cytokines (Xing et al. 1998). Interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) are other anti-inflammatory cytokines, resulting in diminution of the pro-inflammatory cytokines. While IL-6, IL-1ra and IL-10 exhibit anti-inflammatory properties, they can't replace one another, as it appears that they act through separate mechanisms (Xing et al. 1998).

In summary, the inflammatory cytokines, IL-1, IL-6, and TNF- α play an important role in augmenting the acute phase response to promote tissue and cellular healing. These all stimulate the release of CRP, with IL-6 potentially playing a greater role in CRP release. IL-6 also serves dual purposes, acting as both a pro-inflammatory and anti-inflammatory cytokine given different circumstances. IL-10 and IL-1ra play integral roles in decreasing systemic inflammation, however, the effects of IL-6, IL-1ra and IL-10 appear independent of one another.

Obesity as a Cause of Inflammation and IR

The prevalence of obesity is on the rise across the country, and with this comes an increased risk for disease. Of great concern is increased abdominal obesity. Ectopic fat is associated with inefficient CHO oxidation and increased inflammation, indicating the relevance of central obesity in disease risk (Pederson 2009). Carvalho et al. (2010) measured increasing levels of IL-1, impaired fasting glucose, and IR in females that directly associated with increased waist-to-hip ratio (WHR). Another research group that sought to determine the relationship between age, abdominal obesity, and IR noted that abdominal obesity explained more than 40% of the variance in the action of insulin, whereas age only accounted for 10% of the variance, indicating that abdominal obesity is the better predictor of development of IR (Kohrt, Kirwan, Staten, Bourey, King, and Holloszy 1993). Carvalho also noted increases in IL-10, indicating the body's attempt to decrease pro-inflammatory cytokines (Carvalho et al. 2010). Infiltration of adipose tissue with macrophages and inflammatory cytokines causes "inflamed fat," and because fat is considered an endocrine organ, inflammation is distributed throughout the body (Handschin and Spiegelman 2008; Pederson 2009). This becomes a vicious cycle, allowing adipose to release inflammatory cytokines into the blood simultaneously with hormone secretion, resulting in chronic, low-grade systemic inflammation (Handschin and Spiegelman 2008). This elevation of inflammation over a period of time can result in adaptation of the immune system, constantly triggering the acute phase response to be active (Liu, Manson, Buring, Stampfer, Willett, and Ridker 2002). This research

suggests that WHR and abdominal obesity are stronger predictors of IR and systemic inflammation than BMI alone.

Researchers noted that the ability to decrease inflammation is lost as percent body fat increases, again indicating adipose infiltrated with inflammation as a potential cause (Kirwan and del Aguila 2003). This inflammation is believed to cause IR, and eventually the development of T2DM (Kelly et al. 2011; Frost, Leeds, Trew, Margara, and Dornhorst 1998). Strong evidence exists for this association, as ectopic fat loss in older men and women results in reversal of IR and onset of normal glucose metabolism (O’Leary, Marchetti, Krishnan, Stetzer, Gonzalez, and Kirwan 2005). A primary link exists between TNF- α and insulin sensitivity, as increases in TNF- α and IL-6 are noted with increases in abdominal obesity (Kelly et al. 2011). The increased, over-expression of TNF- α in individuals with obesity is thought to decrease the ability of insulin to bind to its receptors (Frost et al. 1998), possibly due to improper phosphorylation of the receptor itself (Xing et al. 1998). Inflammation is implicated as a cause of IR in overweight and obese populations, as the insulin response in normal weight, healthy individuals is normal, relating to the normal levels of circulating inflammatory cytokines (Sears 2009; Frost et al. 1998). A LGI diet could potentially help control TNF- α and decrease IR.

Glycemic Index

Food consumption and inflammation is a hot topic, as inflammation may be induced or decreased by food intake (O’Keefe, Gheewala, and O’Keefe 2008).

Macronutrient intake today consists of an excess of processed foods, stripped of nutrients only to be replaced with chemicals and non-nutrients. This style of diet leads to over-exaggeration of glucose and insulin responses, triggering inflammation (O'Keefe, Gheewala, & O'Keefe 2008). Evidence from researchers regarding carbohydrate ingestion spans from it not being harmful to it being a leading cause of inflammation in individuals who are overweight and obese. Advocates of low CHO diets insist that they result in decreased plasma glucose, a blunted insulin response, and decreases in CRP (Forsythe 2007). This could be beneficial in counteracting the exaggerated post-prandial glucose and insulin spikes seen with high-processed food consumption. However, a more realistic, sustainable approach appears to be through the use of the GI. Researchers discovered that in obese, older individuals matched for exercise, hyperinsulinemia only was reduced in the LGI diet group, not the HGI diet group (Solomon et al. 2010). They postulated that a primary cause for this was increased β -cell response to the increased entry of glucose into the blood following the HGI diet, even with exercise (Solomon et al. 2010). Furthermore, through use of a hyperglycemia clamp, acute hyperglycemia elicited an increase in inflammatory cytokines, IL-6, IL-18, and TNF- α (Esposito et al. 2002). This relationship appears to be related to increased abdominal obesity, as obese women lose the ability to control inflammation with hyperglycemia (Gonzalez, Rote, Minium, O'Leary, and Kirwan 2007). As the GI is based on glucose, it would make sense that HGI foods would elicit a similar, hyperglycemic response.

The GI accounts for how rapidly glucose enters the blood based on CHO quality (Foster-Powell, Holt, and Brand-Miller 2002). Lower GI foods result in a smaller

deviation in blood glucose, hence a lesser dependence on insulin to uptake glucose in the cells. It is hypothesized that the LGI diet works by decreasing glucose-insulin dynamics, resulting in less oxidative damage and decreased prevalence of IR-inducing inflammation (Frost et al. 1998). While the GI looks at quality of CHO, glycemic load (GL) may be a better way to measure glucose entry into the blood, as it accounts for CHO quality and quantity (Foster-Powell, Holt, and Brand-Miller 2002). Glycemic load (GL) is defined as the GI multiplied by the amount of carbohydrate consumed ($GL = GI \times \text{carbohydrate amount}$). High GL diets are linked to weight gain, IR, inflammation, improper insulin signaling and secretion, and the development of T2DM (Pittas et al. 2006). Pittas and colleagues (2006) studied the effects of GL on risk factors for T2DM, comparing high GL (HGL) to low GL (LGL) diets for six months. The composition of the HGL diet was 60% carbohydrate, 20% protein, and 20% fat, with a GI average of 86. The LGL diet was comprised of 40% carbohydrates, 30% protein, and 30% fat, the same composition that the creator of the Zone Diet, Dr. Barry Sears, recommends (Pittas et al. 2006; Sears 2009). They discovered that while subjects following both interventions lost weight and had decreases in inflammation, the group on the LGL diet saw a greater, although not significant, decline in CRP. They concluded that more research is needed in this area, possibly using an isomacronutrient intervention for both HGL and LGL, which this study attempted, using isomacronutrient composition of 55% CHO, 30% fat, and 15% protein. This diet is typical of the American diet, and because the goal was to account for differences in GI, not CHO amount, this seemed a reasonable diet composition.

To gain an understanding of how a LGI diet could improve inflammation, a look at the pro-inflammatory effects of a HGI diet is useful. A primary concern with HGI and HGL diet is the exacerbation of glucose spike that occurs immediately after eating (Pittas et al. 2006). Not only can this spike alter glucose and insulin dynamics, but increases in glucose in the blood can have a damaging effect on the blood vessels, increasing risk of CVD and stroke. Researchers have indicated that normal weight individuals have a normal response to HGI foods, however, the glucose-insulin dynamics are significantly altered with a rise in BMI (Sears 2009; Liu et al. 2002). The effects of a HGI diet results in increased levels of CRP, IL-6, and TNF- α ; issues that are simply not present in individuals of normal weight (Sears 2009; Liu et al. 2002; Kelly et al 2011). This relationship is likely elevated in individuals already at high risk for T2DM, who are obese, sedentary, and have a genetic predisposition (Pittas et. al, 2006). Essentially, a chronically elevated acute phase response could lead to increased incidence of IR, T2DM, and CVD, and this chronic inflammation is present with what appears to be a direct relationship to increasing levels of obesity (Sears 2009; Pittas et al., 2006; Liu et al. 2002). This elevated inflammation is exacerbated by HGI due to the drastic fluctuations in glucose and insulin, further promoting fat storage (Sears 2009), and adding to the state of inflamed adiposity (Liu et al. 2002). As previously noted, this response appears to increase linearly with increases with elevated levels of abdominal adiposity as measured by WHR (Kohrt et al. 1993).

The benefits of a LGI diet appear to lie in the ability of greater glucose control. While scientists have established a link between high glycemic CHO load and

inflammation, research is lacking in the study of complex, low glycemic CHO and their effects on the inflammatory response (Miles et al. 2010; Depner et al. 2010). It is suggested that complex CHOs, which are the foundation of the Mediterranean (MED) diet, may have a positive effect on inflammation through decreasing glucose and insulin spikes (Ordovas 2007; Mitrou et al. 2007). Additionally, researchers have indicated that chronic inflammation is the combination of central obesity and high CHO intake that induces inflammation, preceding T2DM, certain cancers, CVD, and stroke (Sears 2009; Mitrou et al. 2007). Sears stated that these diseases are based on an inactive lifestyle combined with poor nutrient intake, and that if people chose health enhancing foods, they could reverse many of their diseases (Sears 2009).

A common form of the LGI diet including unprocessed grains, a wide range of colorful, antioxidant rich fruits, vegetables, and legumes, as well as olive oil, nuts, and red wine, is the MED diet. The glucose-insulin dynamics associated with this style diet reflect little change in post-prandial blood glucose as a result of carbohydrates with minimal glucose amplifying effects. Mitrou and group described a negative association between a MED diet and all causes of mortality (Mitrou et al. 2007). In a longitudinal study at the National Institutes of Health, they noted that those subjects with the greatest adherence to the MED diet, as indicated by subject recall and journals, reaped the greatest health benefits. Investigators indicated a dose-response relationship between conformity and risk for all causes of death. For example, they found that those with a lower BMI and high diet conformity had a 56% and 61% decrease in risk of all causes of death as

compared to their obese counterparts, for men and women, respectively (Mitrou et al. 2007).

Mitrou and group proposed that the effects of a MED diet on mortality are mediated through reducing inflammation and oxidative stress. They postulated that the high antioxidant load eradicates oxidative stress which can lead to cellular deformation, which could lead to cancer growth and heart disease (Mitrou et al. 2007). Investigators that study the MED diet stated that this is the most cutting edge diet to prevent disease and aging to date, and postulate that a primary mechanism is through enhancement of the ratio of good Omega-3 fats to bad Omega-6 fats, which increases levels of protective antioxidants (Karlsson 1997). Antioxidants are thought to be the strongest inflammation quenchers in the human diet, and are found in large amounts in richly colored fruits, vegetables, red wine, and olive oil (Karlsson 1997). Antioxidants are largely missing from HGI diets, but are seen in higher amounts in LGI diets due to increased consumption of fruits and vegetables.

Eccentric Exercise Model

There is strong evidence that obesity elicits chronic, low-level inflammation, but is this inflammation the same that can be triggered from eccentric exercise? Eccentric exercise is defined as the lengthening phase of muscle contraction, and investigators have established that it elicits greater inflammation than concentric, or shortening contractions (Miles et al. 2010). Overstretching of sarcomeres with repeated eccentric contractions results in disrupted sarcomeres and excitation-contraction coupling damage, and

ultimately, muscle damage and inflammation, (Peake 2005; Proske and Morgan 2004). Much like with obesity-induced inflammation, IL-1, IL-6, and TNF- α have been studied regarding the inflammatory response to eccentric exercise (Smith and Miles 2000). Following eccentric exercise, increases in IL-1 and IL-6 indicate muscular damage, while anti-inflammatory cytokines appear to be downregulated and inhibited (Peake, Nosaka, and Suzuki 2005). High-force eccentric exercise produces sizeable increases in IL-6, with IL-1 and TNF- α showing little to no change; however, it is postulated that because IL-1 and TNF- α precede IL-6 production, that these cytokines are increased as well, but decrease post-exercise (Smith and Miles 2000). While immediate increases in inflammatory cytokines can be measured almost immediately following exercise, they can be elevated for up to four days following intense exercise (Smith and Miles 2000). Muscle damage is evidenced by elevated CK, which spikes within 48 hours of exercise, and can remain elevated for up to seven days (Smith and Miles 2000; Malm et al. 2004). While CK is thought to not be related to muscular inflammation, it is a good marker of muscle damage, which in itself triggers an inflammatory response (Malm et al. 2004).

The eccentric nature of downhill running in the hip and knee extensors makes this a good model to use to elicit muscle damage, as previously noted. Muscle damage can be triggered in many ways, and previous researchers have looked at muscle damage-induced inflammation involving marathon running, plyometric training, lunging, eccentric cycling, high-force eccentric contractions of the elbow flexors, and downhill running, among others that likely exist (Smith and Miles 2000; Peake, Nosaka, & Suzuki 2005; Bruunsgaard 2005; Smith et al. 2007; Malm et al. 2004). Inflammation is involved in

remodeling and adaptation, indicating the relevance of an eccentric exercise model in inducing a spike in inflammation (Malm et al. 2004).

While many forms of eccentric exercise exist, it was predicted that the larger muscle mass involved in downhill running would result in a greater inflammatory response. Greater inflammation could result in a larger response from the interventions, with the ultimate goal of being better able to tailor suitable programs to decrease inflammation and disease risk in overweight and obese individuals. Evidence exists for this prediction, as researchers have shown that high-intensity downhill running and eccentric cycling at 75% or greater of maximal oxygen consumption ($\text{VO}_{2\text{max}}$) results in increased expression of all the inflammatory markers previously discussed, far greater than lower-intensity downhill running or high-force eccentric elbow flexion (Hirose et al. 2004; Peake et al. 2005; Toft et al. 2002). Furthermore, these researchers noted that the elevation of the inflammatory cytokines is longer with high-intensity activity such as downhill running.

A goal in performing this type of muscle damage model via downhill running was to standardize the test amongst the participants. Previous researchers have used a percentage of $\text{VO}_{2\text{max}}$ or submaximal oxygen consumption ($\text{VO}_{2\text{submax}}$) to ensure that participants were working at the same relative intensity (Malm et al. 2004). Other studies had subjects run to a percentage of volitional fatigue before stopping the protocol (Simpson, Florida-James, Whyte, and Guy 2006). Heart rate monitoring at 65% of $\text{VO}_{2\text{submax}}$ has also been used to standardize the protocol in a study performed in the MSU Nutrition Research Lab in the summer of 2011 (Miller et al., abstract in review

2011). The researcher believed that changes in ground reaction forces (GRF) could be different between subjects based on differing running intensities, possibly resulting in different levels of muscle damage. Investigators have shown that as speed increases, the peak forces increased with a decrease in contact time (Nilsson and Thorstensson 2008). Anteroposterior peak force and mediolateral peak force is doubly increased from walking to running, and the individual has a shorter support phase with running conditions (Nilsson and Thorstensson 2008). These differences in force production result in vastly different strategies of motor recruitment, and ultimately, increases in force production (Nilsson and Thorstensson 2008). Therefore, it was predicted that a protocol based on aerobic capacity, such as those utilizing percentages of VO_2 , could cause enhanced muscle damage due to running versus slower jogging, and could result in high variability in muscle damage. As a purpose of this study was to quantify muscle damage, the use of a dynamometer to quantify losses in muscular strength pre and post downhill running complimented the established standard aerobically-based downhill protocol. Eston and colleagues (1999) used an isometric strength test to help quantify the muscular damage associated with downhill running (Eston, Lemmey, McHugh, Byrne, and Walsh 1999). They used an isokinetic dynamometer with the participants' legs extended to 110° for the test. They noted significant decreases in strength from pre-downhill running (Eston et al. 1999), serving as another way to measure individual muscle damage to compliment CK measurements.

Integration of GI and an Eccentric Exercise Model

Miles and colleagues researched the changes from baseline in inflammation associated with a high CHO vs. low CHO diet following eccentric arm exercise in a normal weight population (Miles et al. 2010). They found a linear increase in IL-1 and levels of circulating insulin, and impaired blood glucose following the high CHO treatment in individuals of normal weight, as well as increased levels of IR (Miles et al. 2010). Based on this evidence, it was postulated that downhill running would serve as a larger scale muscle damage model to initiate a more dramatic increase in systemic inflammatory biomarkers, as inflammation is directly proportional to levels of muscle damage (Miles et al. 2010). The group concluded that both WHR and a high CHO diet are associated with increased levels of inflammation, and the greater WHR a person has, the more likely he/she is to have IR. Furthermore, adiponectin, an anti-inflammatory hormone secreted from adipose tissue, is inversely related to obesity, and improvements in WHR can lead to increased adiponectin cellular protection (Xydakis et al. 2004), indicating the relevance of improved WHR. This research furthers the field by establishing associations between WHR, CHOs, and inflammation in prediction of disease development.

Previous researchers indicated that there is a greater inflammatory response with supplementation of a high CHO meal compared to a low CHO meal following high-force eccentric arm exercise (Depner et al. 2010). Depner further noted increases in CRP, however, noted that IL-6 did not increase significantly (Depner et al. 2010). In contrast, Mathieu et al. noted that ectopic adipose secretes sizeable amounts of IL-6, which is

what prompts the significant increase seen in CRP (Mathieu, Poirier, Pibarot, Lemieux, and Despres 2009). Ectopic fat is suggested in the portal theory of IR, stating that the free fatty acids from ectopic fat can enter portal blood and inhibit insulin effects, resulting in IR (Bergman et al. 2007). Furthermore, researchers have indicated that ectopic fat is more closely associated with CVD and certain cancers than BMI alone (Pederson 2009). The link between ectopic fat and inflammation, IR, and mortality provide evidence that WHR is a good tool to determine disease risk, indicating a possible continuum of inflammatory responses based on level of abdominal adiposity (Miles et al. 2010, Depner et al. 2010; Pederson 2009). The above researchers used non-obese subjects, but extrapolated the data, suggesting that in a population that already has low-grade inflammation, high CHO ingestion could exacerbate IR and disease risk.

Preliminary Findings

Preliminary, pilot findings indicated that women of normal weight had improvements in insulin sensitivity (IS) 24-hours after downhill running and consuming a HGI diet, but as BMI increased beyond $25 \text{ kg} \cdot \text{m}^{-2}$, IS decreased, indicating a likely association with increased body fat (Miller et al., abstract in review 2011). Furthermore, a HGI diet triggered an increased insulin response and increased β -cell response, which were both positively associated with increases in BMI (McNulty et al., abstract in review 2011). This initial work, in combination with previous research supporting potential anti-inflammatory effects of a LGI diet, provides evidence to support the hypothesis that a LGI diet could improve measurements of IR and inflammation.

In the current study, several modifications were made. The downhill running protocol was improved upon through inclusion of a strength measure, with a primary goal of standardizing the muscular inflammation model as much as possible between participants. Also, as BMI is not a good measure of body fat, an air displacement plethysmography technique was used to give a more accurate representation of body fat, allowing the researcher to better understand the relationship between inflammatory responses and IR resistance as they potentially relate to body fat. Furthermore, by using the actual target population of overweight and class 1 obese women as research participants, the researcher was able to gain a better understanding of the relationship between downhill running and interventions on these individuals, with no need to extrapolate and predict outcomes.

Summary

To the researcher's knowledge, there has been no research on the acute effects of a LGI compared to a HGI diet following a downhill running protocol in an obese population. The prediction made that a LGI diet would result in decreased inflammation compared with a HGI after the eccentric exercise for 24 hours was based on many assumptions, but particularly on the dynamics of the acute phase inflammatory response. Chronic, low-grade inflammation is present in individuals with abdominal obesity, and is essentially the continuation of the acute phase response. The acute phase response also occurs following high force eccentric exercise, and because inflammation is a generalized response, it was postulated that inflammation from both exercise and obesity could be

impacted through dietary mechanisms. A goal in using the eccentric exercise model was to exacerbate the already active inflammatory response in an obese population to better watch the dynamics of inflammation based on CHO quality. To the researcher's knowledge, previous investigators have not performed such a study with the target population of overweight and obese individuals in mind, but extrapolated data instead. It was hoped that findings from this study could help close this gap to better tailor efficacious programs to ameliorate disease risk as associated with chronic inflammation in at-risk populations.

CHAPTER THREE

INFLAMMATION, INSULIN, AND GLUCOSE DIFFERENCES BETWEEN HIGH
AND LOW GLYCEMIC INDEX DIETS FOLLOWING DOWNHILL RUNNING IN
OVERWEIGHT AND OBESE WOMEN

Contribution of authors and co-authors

Chapter 3

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Abstract

Low-grade chronic inflammation is associated with excess adipose tissue, and often precedes chronic disease. Overweight and obese individuals lose the ability to control inflammation as percent body fat increases, likely linked to inefficient carbohydrate oxidation that amplifies inflammation. Little is known about how the quality of carbohydrates influences inflammation of muscle in this population. The purpose of this study was to determine whether there are differences in inflammation and glucose metabolism between low glycemic index (LGI) and high glycemic index (HGI) diets following downhill running in overweight and obese women. This study was a pre-post design in which overweight and obese women ($n = 20$) were placed in matched pairs for percentage body fat, each receiving an isomacronutrient LGI or HGI diet. Participants completed a downhill run at heart rates at 65% predicted maximal oxygen consumption ($VO_2\text{max}$) until 15% loss of isometric force of the hip and knee extensors, or volition fatigue, was achieved. Participants ate their prescribed diet for 24 h post exercise. Glucose, insulin, creatine kinase (CK), C-reactive protein, tumor necrosis factor- α , and interleukin-6 were measured pre-exercise, 0, 24, and 48 h post-exercise. Isometric force and muscle tenderness were measured when blood was collected. Serum CK changed significantly over time ($p < 0.001$), with the peak at 24 h in the HGI group, and at 48 h in the LGI group. Isometric force decreased most at 24 h for the HGI group, and at 48 h for the LGI group, with significant differences occurring over time ($p = 0.01$), and a significant time/diet interaction ($p = 0.02$). No significant changes were seen in any of the inflammatory variables for diet or time, with the exception of the expected increase in IL-6 immediately post-exercise. There was a significant ($p = 0.035$) difference in delta insulin between groups. Delta IR also changed significantly over time between groups ($p = 0.044$). We conclude that while inflammation was not different between groups, a LGI diet following downhill running results in an acute improvement in insulin and IR in overweight and obese women.

Introduction

Obesity is associated with the development of chronic diseases, many of which are preventable through lifestyle changes. The endocrine function of adipose tissue is of particular relevance in the understanding, prevention, and treatment of these metabolic diseases. Adipose tissue has the ability to transmit inflammatory and hormonal signals throughout the body (Pederson 2009). While the inflammatory response is important for

tissue healing, when chronic, it can precede cardiovascular diseases (CVD), insulin resistance (IR), and tumor growth (Handschin and Spiegelman 2008). Inflammation is a generalized, systemic response, and can be triggered in a multitude of ways (Smith and Miles 2000). It is well accepted that eccentric exercise elicits an inflammatory response (Miles et al. 2010). As exercise induced muscle damage (EIMD) and adipose tissue both amplify the inflammatory response, researchers can use EIMD as a model to study the inflammatory response following differing interventions, including dietary interventions.

Macronutrient intake on the inflammatory process is of heightened interest, particularly because abdominal obesity is associated with inefficient carbohydrate (CHO) oxidation and increased inflammation (Pederson 2009). One way to measure the effects of CHO in the body is through the use of the glycemic index (GI), which is a measure of the rate of glucose entry into the blood. Researchers have noted that when all other conditions were held constant, the only variable that was associated with improved insulin control was a low GI (LGI) diet as opposed to a high GI (HGI) diet (Solomon et al. 2010). The GI accounts for how rapidly glucose enters the blood based on CHO quality (Foster-Powell, Holt, and Brand-Miller 2002). Lower GI foods result in a smaller deviation in blood glucose, hence a lesser dependence on insulin to uptake glucose in the cells. It is hypothesized that the LGI diet works by decreasing glucose-insulin dynamics, resulting in less oxidative damage and decreased prevalence of IR-inducing inflammation (Frost et al. 1998). This is evidenced by the effects of hyperinsulinemia and the GI in obese individuals.

Researchers discovered that in obese, older individuals matched for exercise, hyperinsulinemia was only reduced in the LGI diet group, not the HGI diet group (Solomon et al. 2010). These researchers postulated that a primary cause for this was increased β -cell response to the increased entry of glucose into the blood following the HGI diet, even with exercise. Consistent with Solomon et al., a study conducted in our lab indicated that a HGI diet triggered insulin and β -cell responses that were positively associated with increased BMI (McNulty et al., abstract in review 2011). Furthermore, through use of a hyperglycemia clamp, acute hyperglycemia elicited an increase in inflammatory cytokines, IL-6, IL-18, and TNF- α , indicating the association between glycemia and the inflammatory response (Esposito et al. 2002). This relationship appears to be related to increased abdominal obesity, as obese women lose the ability to control inflammation with hyperglycemia (Gonzalez, Rote, Minium, O'Leary, and Kirwan 2007). Similarly, IR, interleukin-1 (IL-1) and impaired glucose are thought to be directly proportional to increases in waist-to-hip ratio (WHR) (Carvalho et al. 2010). These findings indicate that there is a relationship between body fat, blood glucose, glucose metabolism, and the inflammatory process.

Higher levels of body fat and increased blood glucose are related to elevated CRP, IL-6 and TNF- α in overweight individuals, while normal weight individuals have normal insulin and inflammatory responses to HGI foods (Sears 2009; Liu et al. 2002; Kelly et al. 2011). This relationship between body fat, increased blood glucose, and elevated inflammatory molecules is likely present in individuals already at high risk for T2DM, who are obese, sedentary, and have a genetic predisposition (Pittas et al. 2006).

Essentially, a chronically elevated acute phase inflammatory response, associated with increased obesity, could lead to increased incidence of IR, T2DM, and CVD (Sears 2009; Pittas et al. 2006; Liu et al. 2002). This elevated inflammation is exacerbated by HGI due to the drastic fluctuations in glucose and insulin, further promoting fat storage (Sears 2009), and adding to the state of inflamed adiposity (Liu et al. 2002). Results from these various studies may indicate that elevated blood glucose, as seen with HGI diets, can exacerbate the already heightened inflammatory response present in obese individuals. While it is evident that obese individuals have heightened inflammation, it remains unclear how they respond to a HGI versus a LGI diet, as researchers have not used this target population to determine this relationship.

While there is strong evidence that obesity elicits chronic, low-level inflammation, less is known about eccentric exercise as it relates to obesity-induced inflammation. Eccentric exercise is defined as the lengthening phase of muscle contraction, and investigators have established that it elicits greater inflammation than concentric, or shortening contractions (Miles et al. 2010). Overstretching of sarcomeres with repeated eccentric contractions results in disrupted sarcomeres and excitation-contraction coupling damage, and ultimately, muscle damage and inflammation, (Peake 2005; Proske and Morgan 2004). Following eccentric exercise, increases in IL-1 and IL-6 indicate muscular damage, while anti-inflammatory cytokines appear to be downregulated and inhibited (Peake, Nosaka, and Suzuki 2005). High-force eccentric exercise produces sizeable increases in IL-6, with IL-1 and TNF- α showing little to no change (Smith and Miles 2000). It is postulated that because IL-1 and TNF- α precede

IL-6 production, that these cytokines are increased as well, but decrease post-exercise (Smith and Miles 2000). While inflammatory cytokines increase immediately following exercise, they can remain elevated for up to four days following intense exercise (Smith and Miles 2000). Although CK is not directly related to muscular inflammation, it is a good marker of muscle damage, which in itself triggers an inflammatory response (Malm et al. 2004).

Downhill running causes eccentric contractions of the hip and knee extensors, eliciting EIMD (Peake, Nosaka, and Suzuki, 2005). Muscle damage can be triggered in many ways, including marathon running, plyometric training, lunging, eccentric cycling, high-force eccentric contractions of the elbow flexors, and downhill running (Smith & Miles 2000; Peake, Nosaka, and Suzuki 2005; Bruunsgaard 2005; Smith et al. 2007; Malm et al. 2004). Inflammation is crucial for the remodeling and adaptation following EIMD (Malm et al. 2004), and it was predicted that the larger muscle mass involved in downhill running would result in a greater inflammatory response. Researchers have shown that high-intensity downhill running and eccentric cycling at 75% or greater of maximal oxygen consumption (VO_{2max}) results in increased expression of all the inflammatory markers previously discussed, far greater than lower-intensity downhill running or high-force eccentric elbow flexion (Hirose et al. 2004; Peake et al. 2005; Toft et al. 2002). Furthermore, these researchers noted that the elevation of the inflammatory cytokines is longer with high-intensity activity such as downhill running, making this a good model to study the inflammatory and metabolic effects of diet interventions.

Exercise induced muscle damage and the effects of macronutrient ingestion is not a novel concept. Miles and colleagues researched the changes from baseline in inflammation associated with a high CHO vs. low CHO diet following high-force eccentric arm exercise in a normal weight population (Miles et al. 2010). They found a linear increase in IL-1 and levels of circulating insulin, and impaired blood glucose following the high CHO treatment in individuals of normal weight, as well as increased levels of IR (Miles et al. 2010). Based on this evidence, it was postulated that downhill running would serve as a larger scale muscle damage model to initiate a more dramatic increase in systemic inflammatory biomarkers, as inflammation is directly proportional to levels of muscle damage (Miles et al. 2010). The group concluded that both WHR and a high CHO diet are associated with increased levels of inflammation, and the greater WHR a person has, the more likely he/she is to have IR.

The purpose of this study was to determine whether there are differences in inflammation and glucose metabolism between LGI and HGI diets after a downhill run in overweight and obese women. Measured variables included TNF- α , interleukin-6 (IL-6), and C-reactive protein (CRP), plasma glucose, insulin, and an estimation of IR using the homeostatic model of assessment for IR (HOMA-IR) (McMillan-Price et al. 2006). It was hypothesized that all dependent variables would be lower in the LGI compared to HGI diet groups after downhill running. More knowledge about inflammation and glucose metabolism in overweight and obese women could allow investigators to more effectively direct individuals towards proper diet for disease prevention and better overall health.

Methods

Participants

Overweight and obese women volunteered to participate in the study, but three either dropped out or did not meet the American College of Sports Medicine Guidelines for Exercise Testing and Prescription for safe participation in the study. This study was delimited to females, as there is controversy as to whether or not sex differences exist in the inflammatory response to exercise. Furthermore, this study was an expansion of a previous study performed in our lab utilizing only female participants. Participants were not familiar with downhill running and were not consistently involved in a strength training program. Inexperience with downhill running was important to avoid the repeated bout effect and ensured that participants had not adapted protective mechanisms against the EIMD used in this study (Smith et al. 2007, Schwane and Armstrong 1993). Potential participants were excluded if they had more than one CVD risk factor, diabetes, inflammatory conditions, joint health issues limiting their ability to walk and run, had hypertension, were pregnant, had certain food intolerances or allergies, or were taking oral contraceptives, anti-inflammatories, lipid lowering medications, or anti-hypertensives. These exclusion criteria were determined by a pre-screening conducted over the phone, as well as through a Pre-Participation Screening Questionnaire (Appendix B) modeled after the ACSM Guidelines (ACSM 2010). Participants were asked to refrain from the use of anti-inflammatory medications and refrain from activity other than their normal daily activity for the week prior to, and the days of the study. All participants read and signed an informed consent document (Appendix A) approved by

the Institutional Review Board (IRB) for protection of human subjects at Montana State University, acknowledging their understanding of the risks and discomforts of the study. Participants were encouraged to ask any questions throughout the entirety of the study.

Research Design

This study was a pre-test post-test quasi-experimental design that used a convenience sample. Crossover designs have been used in similar studies, however, researchers have indicated that there can be a learning curve associated with performing the same activity, resulting in protection from muscle damage via the repeated bout effect, resulting in a blunted inflammatory response (Schwane & Armstrong 1983; Smith et al. 2007). Therefore, participants were placed in matched pairs based on similar levels of body fat, where a member of each pair received the LGI diet, while the other received the HGI diet.

Participants came to the laboratory on four separate occasions. On the first visit, they completed the HHQ, informed consent, and had any questions answered prior to beginning any testing. Resting blood pressure and pulse measurements were taken, followed by anthropometric measurements of body mass and height, as well as waist and hip circumferences. Body composition was measured using air displacement plethysmography. Following body composition screening, participants completed a submaximal exercise test using the Bruce protocol treadmill test to predict rate of maximal oxygen consumption (VO_{2max}) and heart rate at 65% of VO_{2max} . Lastly, isometric strength of the knee extensor muscles was measured. This measure was later

used as the downhill run termination criteria, and was performed for the purposes of familiarization and to allow for neurological adaptation to occur.

Between four and seven days after the first visit, participants returned to the lab following a 12-hour fast. A baseline blood sample was taken, followed by a muscle soreness test. Participants performed the EIMD downhill run, which was immediately followed by a post-exercise blood draw and a muscle soreness test. Food was then provided to the participants for the day, with calories calculated from the average of the “sedentary” and “low activity” caloric recommendations from the body composition screening. The goal was to provide caloric needs based on muscle mass and fat mass, as opposed to providing calories based on percentage body mass (McArdle, Katch and Katch 2000). The macronutrient composition for the LGI diet was 55% carbohydrate, 15% protein, and 30% fat, while the HGI diet was 55% carbohydrate, 30% fat, and 15% protein. Foods for each group were based on the GI of table sugar, with the HGI diet CHO with a GI greater than 70, and the LGI carbohydrates lower than 55. Those on the HGI diet received Cheerios, 2% milk, and watermelon for breakfast, a peanut butter and jelly sandwich on white bread and lemonade for lunch, peanuts, fruit roll-ups and pretzels for snacks, pre-cooked frozen chicken breast, baked potato, butter, broccoli, and French bread for dinner. The LGI diet consisted of 100% whole grain oats, 2% milk, and low fat vanilla yogurt for breakfast, peanuts, apples and oranges for snacks, peanut butter and jelly on 100% whole wheat bread for lunch, and pre-cooked frozen chicken breast, broccoli, 100% whole wheat bread, butter, and pre-cooked pearled barley for dinner. Participants were instructed to consume the food in the order indicated above, with

breakfast first, and dinner last. They were allowed to consume the snacks and lunch as desired throughout the day. Current ServSafe instructions were followed for all food preparation and handling. Participants were instructed to drink at least 10 cups of water throughout the day. At 24 and 48 hours post-exercise, participants returned to the lab after a 12-hour fast and sat quietly for ten minutes prior to having blood drawn. Muscle soreness and isometric strength were measured at both post-exercise appointments.

A limitation of this study was the differences in micronutrient and fiber content between diets. A primary reason that many foods have a LGI is due to the effects of fiber slowing gastric emptying and decreasing the rate of entry of glucose in the blood. Furthermore, antioxidants and vitamins could influence the inflammatory response, possibly leading to a decrease in inflammation not associated with the differences in GI (Karlsson 1997). Some labs have the ability to develop foods that are balanced for isomacronutrients, isomicronutrients, and fiber, and only differ by their GI; however, these foods are likely not something the general population would eat. While this is a limitation of the current study, one goal of this study was to find interventions that individuals could easily incorporate into their lives, which includes foods which are readily available. In an attempt to avoid research bias, participants were told which diet they were receiving, but were not told predicted results.

For the duration of the study, several restrictions were enforced. Participants were instructed to not participate in exercise or activity that was novel to them in the week prior to, and the days during testing. They were allowed to continue any activities which they were habituated to, with the exception of strength training. Participants were

also instructed to not take anti-inflammatory medications, but to continue taking any medications or vitamins they took on a regular basis.

Baseline Measurements

Blood pressure and resting pulse measurements were taken using an Omron IntelliSense® Professional Blood Pressure Monitor (Model HEM-907XL, HealthCheck Systems, Inc., Brooklyn, NY) after participants sat quietly for ten minutes. Waist measurements were taken at the top of the right iliac crest at the end of a normal expiration using an anthropometric tape measure. Hip circumferences were taken using the anthropometric tape measure around the greater trochanter. Three measurements were taken for hip and waist circumferences, and the average for each was computed and recorded. Body height was recorded using a stadiometer, and body mass was measured using a two-beam scale. Excess clothing such as coats, sweatshirts, and shoes were removed, but no other clothing was removed.

Percent lean mass and fat mass were measured using air displacement plethysmography with a BodPod Gold Standard – Body Composition Tracking System (Concord, CA). Participants were instructed to not eat or drink anything and to not participate in any physical activity two hours prior to this visit. Participants changed into a swimsuit or lycra in a separate room, and were provided with a swim cap to reduce surface area that could alter the body composition measurement. Predicted lung volumes were used to determine body composition for each participant, and the Siri equation was used for all participants.

Submaximal Exercise Test

A modified Bruce protocol was used to predict downhill run target heart rates (Miller et al., abstract in review 2011). Participants wore a Polar WearLink + Coded Transmitter 31 (Kemple, Finland) heart rate monitor. Participants were allowed to walk for a few minutes on the treadmill (Woodway – User System WUS Treadmill, Waukesha, WI), to get familiarized with wearing the metabolic cart mouthpiece and nose clip (Parvo Medics True One® 2400 Metabolic Measurement System, Sandy, UT). The ramp-style test began at a 10% grade and 2.7 km/hr, and both speed and grade were increased every three minutes (Stage 2 = 4 km/hr, 12% grade; Stage 3 = 5.5 km/hr, 14% grade; Stage 4 = 6.8 km/hr, 15% grade; Stage 5 = 8 km/hr, 15% grade; Stage 6 = 8.9 km/hr, 15% grade). Heart rates were recorded at minutes two and three of each three minute stage. The test was terminated upon reaching 85% of predicted maximum heart rate ($220 - \text{age}$). Participants were disconnected from the metabolic cart and were allowed to cool down for as long as desired. Results from this test were used to predict maximal oxygen consumption ($\text{VO}_{2\text{max}}$) and a plot of HR versus VO_2 was used to identify the heart rate at 65% of predicted $\text{VO}_{2\text{max}}$.

Downhill Run and Muscular Strength

A heart rate monitor was worn for the entire downhill run. The protocol began with an eight minute warm-up, with gradually increasing speed and grade until the target heart rate was reached in the last three minutes of the warm-up. Baseline isometric strength was measured after the warm-up to allow for maximal force production capability to increase during the warm-up period.

Three maximal isometric strength measurements were taken against a handheld dynamometer (Nicholas Manual Muscle Tester Model 01160, Lafayette Instruments, Lafayette, IN). Participants were seated on a counter with their legs hanging over the edge and the non-dominant knee extended to 110° as measured by a goniometer in line with the greater trochanter. They rested against a cushion, and their arms were crossed against their abdomen to prevent them from pushing against the table or swinging their arms to aid in the strength measure. The dynamometer was held against the participants' shin five inches distal to the patella, and a mark was made for subsequent measurements. They were instructed to push against the dynamometer as forcefully as possible for roughly three seconds. There were five seconds between each measurement. The measurements were averaged to determine baseline strength. The same researcher performed all of the strength measures for consistency.

After the isometric strength measurement, participants immediately got back on the treadmill which was then set to a -10% grade. Participants began running at the speed they had achieved their target heart rate in the last three minutes of the warm-up. Speed was increased over the next five minutes until the target heart rate was reached. This speed remained the same through the duration of the protocol. This speed was maintained to apply a constant EIMD stimulus. Participants were assisted off the treadmill for a strength measurement after 20 minutes. The downhill run was terminated when participants reach at least a 15% decrement in muscular strength compared to the pre-run average. If the participant did not achieve this loss of strength after 20 minutes, she continued running in five minute increments up to 40 minutes until the strength loss

was reached. None of the participants ran for more than 30 minutes. Rate of perceived exertion (RPE) and heart rate were recorded every two minutes. RPE or indication of volitional fatigue were used as alternative criteria for test-termination if the participant did not reach the desired -15% strength loss. One participant stopped the protocol at minute eleven due to volitional fatigue.

Muscle Soreness

Muscle soreness was measured immediately pre and post-downhill run, 24 hours post, and 48 hours post using an algometer (Wagner Instruments Algometer-Pain Test FPK/FPN, Greenwich, CT). Three measurements were taken at a marked point halfway between the patella of the knee and the hip joint on the rectus femoris. Participants informed the researcher when the increasing pressure of the algometer turned to pain or tenderness, at which point the measurement was finished. The average of the three measurements was recorded for each of the four measurement periods.

Blood Analysis

Blood samples were collected by an experienced phlebotomist from a vein in the forearm using the standard venipuncture technique. Blood was drawn into a vacuum tube with clot activator and serum separator for collection of serum to analyze glucose, CK, and CRP. Due to the need of an anticoagulant, EDTA was used for collection of plasma for measurements of insulin, TNF- α , and IL-6. Blood sat for several minutes until it was clotted, at which point it was placed in a centrifuge (Fisher Scientific, Pittsburgh, PA) for

15 minutes. Blood was then pipetted into microtubes and stored at -80°C until it was analyzed.

Insulin was analyzed using a microplate enzyme-linked immunosorbent assay (ELISA) (MP, Diagnostic Division, Solon, OH) following manufacturer instructions. Creatine kinase was analyzed with an ultraviolet, kinetic assay kit (Thermo Electron Corporation, Lake Success, NY) by the addition of heat and the reagent, *n*-acetyl-L-cysteine (NAC) to drive the reaction forward (creatine phosphate + ADP (catalyzed by CK yields) creatine + ATP). C-reactive protein was analyzed using a High Sensitivity Enzyme Immunoassay for the Quantitative Determination of C-Reactive Protein Concentration in Human Serum (MP Biomedicals Diagnostics Division, Orangeburg, NY). An ELISA was used to analyze IL-6 and TNF- α (R&D Systems Inc., Minneapolis, MN). Duplicates of all samples were run. Glucose and lipids were analyzed at Deaconness Medical Lab in Bozeman, MT.

Statistics

Data were analyzed using the software program, Statistix 9.0. The researcher checked the statistical assumptions of normality and independence using a one-sample (Kolmogorov-Smirnov test prior to running the analyses. *A priori* criteria for inclusion in data analysis included successful completion of the downhill run as determined by at least a 100% increase in CK values. A t-test assuming independent means was used to compare the descriptive measures of the two groups. A two-way repeated measures analysis of variance (ANOVA) was used to identify differences between groups over time for the dependent variables of IL-6, TNF- α , CRP, CK, glucose, and HOM-IR, as

well as for magnitude of change at 24 and 48 hours for these variables. Main effects for time and for condition were analyzed along with interaction between time and condition. A Scheffe's post hoc test was used to determine location of significant differences. A Pearson product moment correlation was used to determine associations between magnitude of change for inflammatory markers, glucose, insulin, and HOMA-IR with waist-to-hip ratio, waist circumference and percent body fat. Magnitude of change was calculated as post-exercise measurement minus pre-exercise measurement. Significance was set at an alpha of 0.05.

Results

All baseline variables were similar for the LGI and HGI diet groups, as depicted in Table 1.

Table 1. Baseline characteristics and resting serum or plasma concentrations of blood lipid and inflammation variables for LGI and HGI diet groups.

	LGI	HGI
Age (years)	25.8 ± 6.0	30.0 ± 6.4
Height (cm)	165.5 ± 5.8	165.4 ± 7.7
Body Mass (kg)	80.7 ± 11.8	77.6 ± 10.4
BMI (kg/m ²)	29.4 ± 3.5	28.3 ± 2.2
Fat mass (kg)	30.4 ± 8.4	29.9 ± 6.6
Fat (%)	37.3 ± 6.4	38.4 ± 4.9
WHR	0.76 ± 0.04	0.77 ± 0.05
Insulin (μU·ml ⁻¹)	10.6 ± 8.4	8.4 ± 3.8

Table 1 Continued

CRP (mg·l ⁻¹)	2.1 ± 1.4	2.5 ± 2.6
IL-6 (pg·ml ⁻¹)	2.3 ± 1.6	3.0 ± 1.8
Glucose (mg·dl ⁻¹)	85.3 ± 4.4	80.0 ± 7.8
TNF-α (μg·dl ⁻¹)	1.1 ± 0.6	1.8 ± 1.3
CK activity (IU·l ⁻¹)	126.8 ± 100.9	127.8 ± 93.7
VO ₂ max (ml·kg ⁻¹ ·min ⁻¹)	46.7 ± 2.6	45.7 ± 6.3

Values = mean ± SD. BMI = body mass index, WHR = waist to hip ratio, fat = percent body fat, TNF-α = tumor necrosis factor alpha, IL = interleukin, CRP = C-reactive protein, CK = creatine kinase, VO₂max = predicted maximal oxygen consumption as measured by a standard Bruce protocol, *p<0.05 compared to LGI diet group.

Exercise Bouts

The exercise bouts were similar between groups in intensity and downhill run duration, as indicated in Table 2. The muscle damage stimulus was also similar between groups, with a difference over time in isometric force production ($p = 0.02$), CK ($p < 0.001$), and muscle tenderness ($p < 0.001$), as noted in Table 3. Of the 20 participants who completed all testing days, 18 successfully completed the downhill run according to the *a priori* criteria of a 100% increase in CK. The two non-responders were excluded from all data analysis. These data allow the researcher to compare markers of inflammation, insulin, and glucose across diet groups because the exercise bouts were similar.

Table 2. Exercise bout descriptive characteristics for LGI and HGI diet groups.

	LGI	HGI
Run time (mins.)	21.7 ± 0.8	23.3 ± 1.2
Treadmill speed (km/hr)	9.5 ± 0.37	9.7 ± 0.47
HR (bpm)	163 ± 3	160 ± 3
RPE	6.1 ± 0.5	5.9 ± 0.7

Values = mean ± SE. HR = average heart rate taken in two-minute intervals over the entire downhill run, RPE = average rate of perceived exertion on a scale of 1-10 taken every two minutes.

Markers of Muscle Damage

Markers of muscle damage were similar between the LGI and HGI diet groups, however, there was a significant difference in the percent change of isometric force between the HGI and LGI diet groups at 48 h, as noted in Table 3. The LGI diet group had a significantly greater decline in isometric strength at 48 h than the HGI diet group.

Inflammatory Markers

Interleukin-6 increased ($p = 0.008$) immediately post exercise, and was non-significant after post-hoc analysis (Table 4). C-reactive protein and TNF- α did not change over time, and there were no diet/time interactions, and no difference between the diet groups (Table 4).

Table 3. Comparisons of markers of muscle damage between LGI and HGI diet groups.

	LGI group	HGI group	ANOVA p-values <i>Group</i> <i>Time</i> <i>G x T</i>
Muscle tenderness (lbs.)			
Pre-exercise	5.2 ± 0.7	4.8 ± 0.9	0.33
Post-exercise*	4.8 ± 0.7	4.3 ± 0.8	0.00*
24 h	5.4 ± 1.0	4.3 ± 0.6	0.58
48 h	4.7 ± 0.8	5.5 ± 0.9	
Serum CK activity (IU/l)			
Pre-exercise	126.8 ± 33.6	127.8 ± 31.2	0.69
Post-exercise	159.9 ± 38.4	142.0 ± 28.7	0.00*
24 h*	400.7 ± 96.3	453.4 ± 130.2	0.28
48 h*	500.8 ± 183.9	301.9 ± 51.9	
Isometric Force (% change)			
Pre-exercise	-	-	0.20
Post-exercise	-31.2 ± 3.6	-29.5 ± 4.0	0.02*
24 h*	-12.1 ± 5.7	-23.4 ± 4.8	0.41
48 h*	-27.7 ± 6.0	-10.8 ± 5.7	

Values = mean ± SE. Muscle tenderness = tenderness measurement using a handheld algometer, CK = creatine kinase, isometric force = the percent change from each condition minus the baseline. The p-value column indicates the p-values from the two-way repeated measures analysis of variance (ANOVA). The order of p-values is group, time, and the group by time interaction. *p < 0.05, indicating significant differences over time. The asterisks indicate when the significant differences occurred.

Table 4. Mean inflammatory markers for all time points for both diet conditions.

	LGI group	HGI group	ANOVA p-values <i>Group</i> <i>Time</i> <i>G x T</i>
TNF (pg/mL)			
Pre-exercise	1.13 ± 0.18	1.79 ± 0.43	0.24
Post-exercise	1.06 ± 0.11	1.67 ± 0.26	0.08
24 h	0.98 ± 0.13	1.20 ± 0.13	0.29
48 h	1.12 ± 0.16	1.32 ± 0.18	
IL-6 (pg/ml)			
Pre-exercise	2.28 ± 0.52	3.05 ± 0.61	0.42
Post-exercise*	3.0 ± 0.52	3.96 ± 0.59	0.008*
24 h	2.59 ± 0.49	2.84 ± 0.50	0.08
48 h	2.23 ± 0.39	2.64 ± 0.51	
CRP (mg/l)			
Pre-exercise	2.06 ± 0.48	2.46 ± 0.86	0.59
Post-exercise	2.00 ± 0.51	2.87 ± 1.06	0.95
24 h	2.23 ± 0.36	2.81 ± 1.13	0.83
48 h	2.13 ± 0.38	2.40 ± 0.77	

Values = mean ± SE. The p-value column indicates the p-values from the two-way repeated measures analysis of variance (ANOVA). The order of p-values is group, time, and the group by time interaction. *p < 0.05, indicating a significant difference immediately post-exercise.

Blood Glucose and Insulin

Mean concentrations of blood glucose and insulin were similar between groups at baseline. Mean concentrations of blood glucose were similar between treatment groups (p > 0.05), and a main effect of time (p < 0.001) was measured, however, there were no significant changes over time after post-hoc analysis. There were no interactions (p >

0.05) between diet and time for blood glucose. Insulin concentrations were similar ($p > 0.05$) between treatment groups for all time points. There was a trend for both glucose and insulin to increase immediately post-exercise (Fig. 1 and Fig. 2), however, this was non-significant. After post-hoc analysis, the differences for both glucose and insulin were non-significant, and there were no diet/time interactions for either variable. Insulin decreased below baseline for the LGI group at 48 h post-exercise, however; insulin increased above baseline for the HGI group at this same time point (Fig. 2), although non-significant. The same time effect was seen for the variable of IR (Fig. 3), also non-significant. However, when magnitude of change was analyzed for insulin, there was a significant interaction between diet and time ($p = 0.035$) as seen in Fig. 4. When the magnitude of change at 24 and 48 hours post-exercise was analyzed for IR, there was a significant ($p = 0.044$) interaction between diet and time in IR (Fig. 5), occurring between 24 and 48 h.

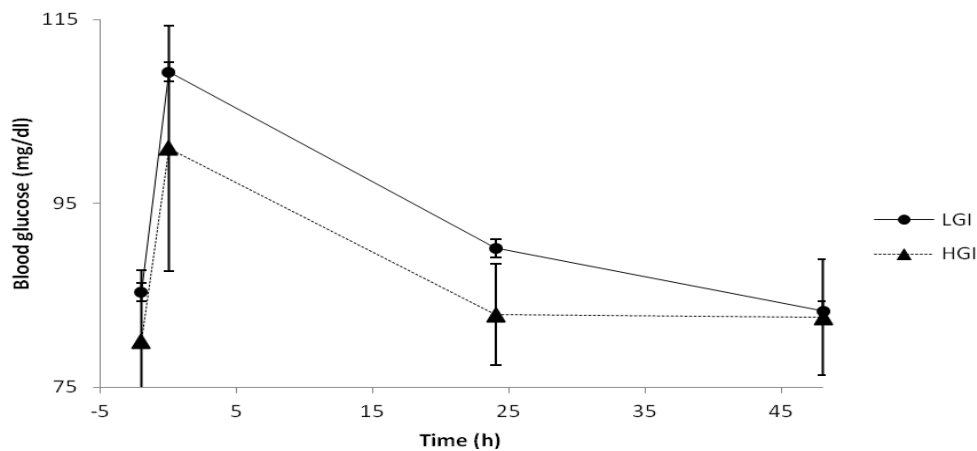


Fig. 1 Mean concentration of plasma glucose for LGI and HGI diet groups at pre-exercise, immediately post exercise, 24 h, and 48 h post-exercise. Values are mean $\pm$ SE. LGI = Low glycemic index, HGI = High glycemic index.

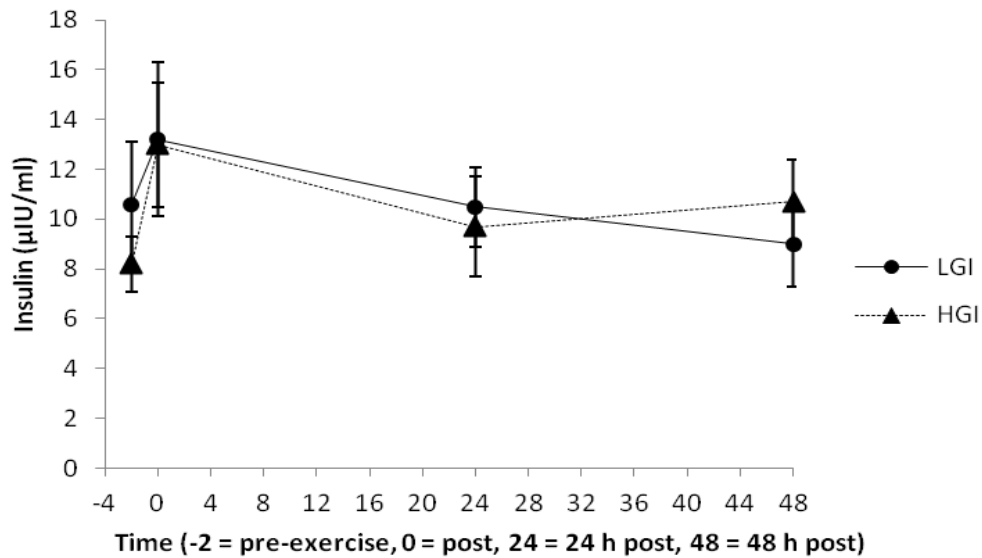


Fig. 2 Mean insulin concentrations for LGI and HGI diet groups at pre-exercise, immediately post-exercise, 24 h, and 48 h post-exercise. Values are mean $\pm$ SE. LGI = Low glycemic index, HGI = High glycemic index.

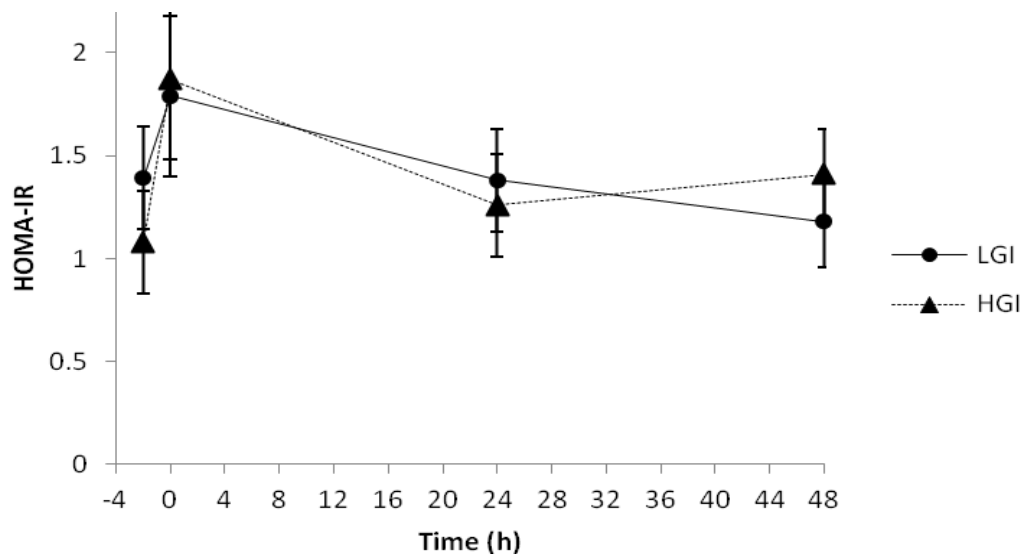


Fig. 3 Mean HOMA-IR for LGI and HGI diet groups at pre-exercise, immediately post-exercise, 24 h, and 48 h post-exercise. Values are mean $\pm$ SE. LGI = Low glycemic index, HGI = High glycemic index.

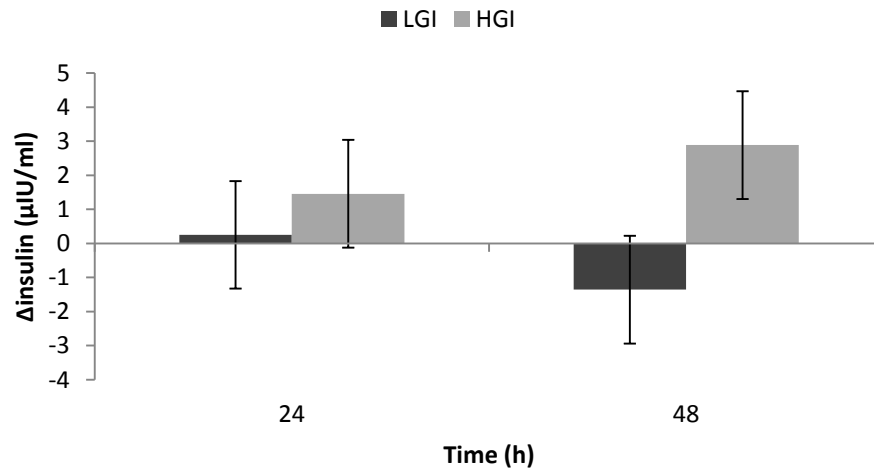


Fig. 4 Magnitude of change in insulin for the LGI and HGI groups at 24 and 48 hours post-exercise. Magnitude of change was determined as the value at the given post-exercise time minus the baseline value. Values are mean $\pm$ SE. LGI = Low glycemic index, HGI = High glycemic index.

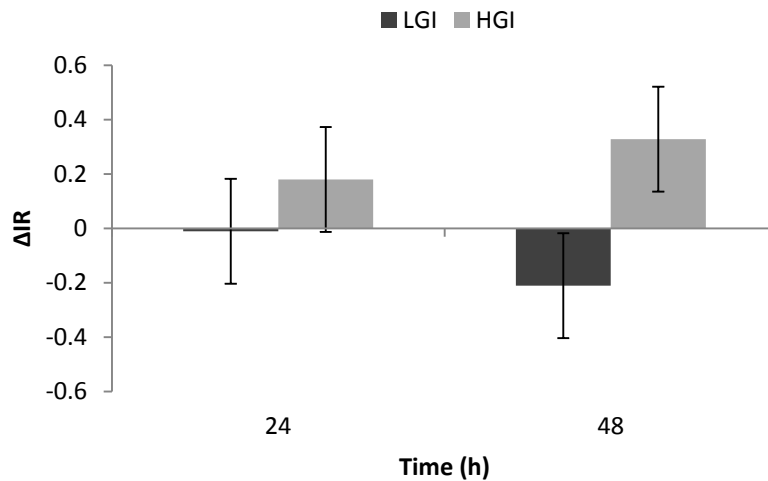


Fig. 5 Magnitude of change in IR for the LGI and HGI groups at 24 and 48 hours post-exercise. Magnitude of change was determined as the value at the given post-exercise time minus the baseline value. Values are mean $\pm$ SE. LGI = Low glycemic index, HGI = High glycemic index.

Correlations

Pearson product moment correlations were determined between WHR, waist circumference, and percent body fat for the magnitude of change for insulin, IR, TNF- α , IL-6, and CRP 24 and 48 h post-exercise. No significant correlations were found between any of the variables (Table 5).

Table 5. Correlations between waist circumference, WHR, and fat percentage for magnitude of change for dependent variables at 24 and 48 h.

	Waist (cm)	WHR	Fat (%)
Δ Insulin (2)(μ IU/ml)	-0.36	-0.14	-0.19
Δ Insulin-(3)(μ IU/ml)	-0.45	-0.37	-0.29
Δ IR(2)	-0.33	-0.07	-0.21
Δ IR(3)	-0.43	-0.32	-0.30
Δ IL-6 (2) (pg/ml)	0.09	0.04	-0.19
Δ IL-6 (3) (pg/ml)	0.03	0.14	-0.16
Δ TNF- α (2) (pg/ml)	0.39	0.42	0.35
Δ TNF- α (3) (pg/ml)	0.26	0.30	0.30
Δ CRP (2) (mg/l)	0.34	0.35	0.27
Δ CRP (3) (mg/l)	0.34	0.32	0.09

Values = correlation coefficient. (2) = 24 h – pre-exercise; (3) = 48 h – pre-exercise. $P > 0.05$ for all above correlations.

Discussion

Summary of Overall Findings

The purpose of this study was to determine whether there are differences in inflammation and glucose metabolism between low and high GI diets after a downhill run in overweight and obese women. The key findings of this study were that delta insulin and delta IR decreased for the LGI group, while they increased for the HGI group 24 and 48 hours post downhill running ($p = 0.035$ and $p = 0.044$, respectively). While all inflammatory molecules increased immediately post-exercise, there were no significant differences found for time, diet, or the interaction between time and diet.

Description of Participants

The eighteen participants included in data analysis had BMIs of $25 \text{ kg}\cdot\text{m}^2$ or greater, and had similar percentages of body fat (Table 1) for the LGI and HGI groups, respectively. This level of body fat for women is considered in excess, and puts them at higher risk for health complications associated therein (McArdle, Katch and Katch 2000). Fat is considered an endocrine organ, with the ability to secrete inflammatory molecules throughout the body (Pederson 2009), indicating that these participants likely had elevated baseline markers of systemic inflammation. Participants had similar metabolic and inflammatory baseline characteristics. Furthermore, three participants who were assigned by matched pairs to the LGI group were considered insulin resistant at baseline. HOMA-IR values of 1 or less are optimal, but increasing levels beyond this indicate varying levels of IR. Specifically, HOMA-IR values above 1.78 are considered

diagnostic criteria for metabolic syndrome, while HOMA-IR values at or greater than of 3.8 are considered diagnostic criteria for T2DM (Esteghamati et al. 2010). Insulin resistance is highly driven by insulin, indicating the higher the insulin level, the higher the IR. These three participants, with HOMA-IR values from 1.7 – 3.2 at baseline, could have swayed the results; however, they were included in all data analysis. While IR is noted immediately following eccentric exercise (Kirwan 2010), exercise could have decreased IR in these individuals over time, contributing to the improvement seen in IR (Petersen and Pedersen 2005).

Markers of Muscle Damage

The downhill running protocol elicited similar muscle damage responses between the groups, resulting in modest increases in CK and muscle soreness. The downhill running protocol used a larger muscle mass than previous studies, with the intention of eliciting a larger inflammatory response. While variability was seen in the muscle damage stimulus, 18 participants met the inclusion criteria of a 100% increase in CK. This increase in CK peaked at 24 hours post-exercise for the HGI group, which is consistent with previous research on downhill running (Eston, Mickleborough, and Baltzopolous 1995). The phenomena of peak CK at 48 hours post-exercise as seen in the LGI group is also not uncommon (Malm et al. 2004). Creatine kinase is indicative of loss of muscle integrity, and researchers have suggested that muscle integrity is most compromised in high force eccentric contractions, as opposed to downhill running (Eston, Mickleborough, and Baltzopolous 1995). A potential reason for the seemingly unlikely disparity between elevations in CK after maximal eccentric contractions versus

the larger muscle mass involved in downhill running could be that there are greater forces imposed over a greater range of strain during high force exercise (Eston, Mickleborough, and Baltzopolous 1995). This could help explain why we only saw increases of magnitude in the hundreds in CK, while studies utilizing high force maximal eccentric exercise resulted in CK increases in the thousands. Furthermore, the strength loss associated with eccentric exercise is suggested to be related to the length of the muscle during the eccentric contraction (Eston, Mickleborough, and Baltzopolous 1995). These researchers suggested that strength loss is typical in downhill running for up to ten days due to this muscle length strain, and is measureable immediately following exercise (Eston, Mickleborough, and Baltzopolous 1995). Muscle tenderness decreased immediately post-exercise and at 24 hours post-exercise, then peaked at 48 hours, which is consistent with previous downhill running studies (Eston, Mickleborough, and Baltzopolous 1995; Malm et al. 2004). Generally, eccentric exercise elicits an inflammatory response for repair and adaptation, leading to increases in IL-1 and IL-6, while anti-inflammatory cytokines appear to be downregulated and inhibited (Peake, Nosaka, and Suzuki 2005). Researchers have shown that high-intensity downhill running and eccentric cycling at 75% or greater of maximal oxygen consumption (VO_{2max}) results in increased expression of all the inflammatory markers previously discussed, far greater than lower-intensity downhill running or high-force eccentric elbow flexion (Hirose et al. 2004; Peake et al. 2005; Toft et al. 2002). Participants were included in the study based on having had no previous experience with downhill running, and who were not involved in a current strength training program. If they had performed strenuous

exercise in the weeks prior, this could have influenced their muscle damage response and explain some of the variability in muscle damage (Miles et al. 2010). Ultimately, because the groups were similar at baseline and had similar exercise responses, we were able to compare them for all variables.

The EIMD elicited was similar between groups, however, the responses to CK and muscle soreness were of interest. As seen in Table 1, the CK response was attenuated in the HGI group, but increased compared to the LGI group at 48 hours post-exercise. The LGI group had a greater increase in CK at 24 hours, with a decrease at 48 hours. CK can be elevated for several days in individuals (Miles et al. 2010). This difference could be a result of a greater increase in insulin following consumption of a HGI diet versus the LGI diet after exercise. Insulin is a powerful promoter of protein synthesis (Cockburn, Robson-Ansley, Hayes, and Stevenson 2010). The HGI diet could have resulted in a quicker glucose response, and therefore, a faster spike in insulin. Carbohydrate ingestion immediately following exercise is useful for the replenishment of muscle glycogen, resulting in limited breakdown and increased repair of the muscle protein structure (Cockburn et al. 2012). It would have been useful knowing the amount of stored glycogen each participant began with, as muscle glycogen in itself is a mediator of insulin action (Stevenson, Williams, and Biscoe 2005). The HGI group had greater decrements in strength at 24 hours, with improvements at 48 hours, while the opposite was true for the LGI group. Differences in baseline glycogen stores could have been a primary reason behind this, as glycogen depletion is a known cause of muscular fatigue (Cockburn et al. 2012; Stevenson et al. 2005). This should be researched more to

determine the relationship between CK levels and associated levels of muscular strength loss.

Glucose, Insulin, and Insulin Resistance

The key findings that delta IR and delta insulin decreased in the LGI group over time, while the HGI groups increased over time, were of primary interest. This represents an interaction between diet and time that is consistent with researchers who found that hyperinsulinemia was only decreased in a LGI group as compared to a HGI group over time (Solomon et al. 2010). Similar findings were noted in a previous study on a HGI diet following downhill running, with results indicating an increased insulin response 24 hours after downhill running in normal and overweight women (McNulty et al., abstract in review 2011). Exercise is known to have an insulin-like effect, aiding in translocation of the GLUT-4 transporter to uptake glucose into the cell; however, a state of transient IR occurs following eccentric exercise (Kirwan 2010). This could be due to impaired insulin signaling, in spite of the elevated insulin levels (Kirwan 2010). Due to this obvious increase in IR following exercise in both groups, we evaluated delta insulin and delta IR values at 24 and 48 hours post exercise, which reflected metabolism including the exercise and the diet intervention of either HGI or LGI. The increase in both of these variables seen in the HGI group is consistent with previous research that has shown that a high amount of carbohydrates with a high GI produce greater IR than low GI foods (Willett, Manson, and Liu 2002). Furthermore, adverse metabolic effects are seen in women who present with underlying IR (Willett, Manson, and Liu 2002). The decrease in insulin below baseline indicated in the LGI group could be due to the anti-

inflammatory effects of exercise, as well as the lesser demand for insulin from the pancreas. The findings of the current study are consistent with Solomon et al. (2010) who found that even with exercise, the only improvement in hyperinsulinemia and glucose control was seen in a LGI group compared to a HGI group. Furthermore, the decrease in IR measured in the individuals on the LGI diet may not only be a diet effect, but could be because the LGI group had participants with baseline IR, and exercise could have resulted in decreased IR (Petersen and Pedersen 2005). The increase in IR and insulin at both 24 and 48 hours could also simply indicate a greater demand from the pancreas to secrete insulin necessary to bring blood glucose back down to normal levels. Researchers have suggested that the long-term effect of a LGI diet are much more profound, attenuating the onset of T2DM. The data indicate no significant differences in inflammatory markers, which is similar to a study from Miles et al. (2007), which found no significant changes in IL-6 following CHO ingestion.

Inflammation

It is established that obesity results in chronic, low-grade inflammation. Obese individuals can have a two to three fold increase in TNF- α , CRP, IL-1 and IL-6 over their normal weight counterparts (Petersen and Pedersen 2004). It is believed that the mediator for this is TNF- α from the adipose, which is the first of the inflammatory mediators in the inflammatory cascade (Petersen and Pedersen 2005). While the participants in this study likely had elevated inflammation at baseline, the hypothesized increased in inflammation did not occur.

Tumor necrosis factor- α , IL-6, nor CRP increased significantly in this study. The nonsignificant elevation in TNF- α in both groups makes sense along with the nonsignificant elevations in IL-6 because TNF- α is the first part of the cascade. Interleukin-6 increased immediately after exercise significantly, which was expected, as exercising skeletal muscle is a powerful stimulator of IL-6 (Petersen and Pedersen 2005). Increases in IL-6 from exercising skeletal muscle are not linked to the same inflammatory pathway as tissue damage (Miles et al. 2010). The non-significant elevation in IL-6 in the current study is consistent with previous studies in which researchers noted that the elevation in IL-6 does not impair or promote immune or muscular response (Miles et al. 2010). Furthermore, exercise is considered a powerful anti-inflammatory agent (Petersen and Pedersen 2005), which in a population with elevated baseline inflammation, could possibly have mitigated the effects of pro-inflammatory pathways.

The absence of increased inflammation 24 and 48 hours post-exercise in this study is consistent with previous research on downhill running, where despite obvious delayed onset muscle soreness (DOMS), none of the inflammatory markers increased (Malm et al. 2004). This is consistent despite the changes measured in CK, muscle strength, and rectus femoris tenderness, which indicates that the muscle damage may not have been large enough to elicit a systemic inflammatory response (Malm et al. 2004). Researchers have suggested that elevations in CRP are often associated with CK elevation 24 – 72 hours post EIMD (Phillips et al. 2003). While CK was elevated in the current study, this elevation was modest. It did not result in significant increases in IL-6, thus no increases in systemic inflammation occurred, so CRP did not significantly

increase in either the LGI or HGI group. Inflammation follows a specific cascade of events, where TNF- α precedes IL-6, which is a primary trigger of CRP (Smith & Miles 2000). The results indicate no increases in these three variables, which makes sense based on this knowledge of an inflammatory cascade; if one inflammatory marker increased, they all would likely have increased.

Correlations

There were no significant correlations between any of the dependent delta values and waist circumference, WHR, and percent body fat. This is inconsistent with previous researchers who found that increasing levels of inflammatory markers and IR are highly correlated to increased abdominal obesity and WHR (Carvalho et al. 2010). Further, researchers established that abdominal obesity, which is considered a strong promoter of inflammation due to inefficient CHO oxidation (Pedersen 2009), explains more than 40% of the variance in insulin action in obese individuals (Kohrt et al. 1993). A potential reason for the lack of correlation could be the conservative exercise model used, not eliciting enough of an inflammatory response.

Conclusions

In conclusion, we emphasize that there were favorable, significant trends towards decreased insulin demand and IR following a LGI diet for one day after downhill running in overweight and obese women. While no significant trends were seen with the inflammatory markers, there appeared to be no deleterious effects from ingesting either a LGI or HGI diet following exercise. Future research on this topic should look at the

influence of muscle glycogen stores at the commencement of the study, as this could be an influencing factor in the metabolic variables. Furthermore, plasma volume should be measured, and hematocrit and hemoglobin concentrations determined to ensure that changes in inflammatory molecules are indicative of inflammation, and not just a drop in plasma volume. The EIMD model used in the current study was meant to produce a larger muscle damage stimulus than that used in previous studies, however, working with an overweight and obese, “at risk” population limited the intensity with which we were able to have the participants work for safety purposes. Had they worked at more similar work rates and intensities used in previous studies, inflammatory and metabolic variables may have been more definitive. More research is needed to determine if these results would be similar for increased levels of muscle damage and inflammation.

CHAPTER FOUR

CONCLUSIONS

Downhill running was used as a model to elicit an inflammatory response that could be measured in the blood of overweight and obese women to determine their inflammatory and metabolic responses to a LGI versus a HGI diet. Overall, there were no changes in the inflammatory markers for diet intervention group, time, or an interaction between diet and time. There were significant trends for improved delta insulin and improved delta IR over time after following the LGI diet condition.

The results from this study indicate that just one day of a LGI leads to favorable metabolic outcomes. The absence of an increase in inflammation could be due to the use of a relatively conservative EIMD stimulus created from downhill running. Researchers have determined that, although downhill running involves significant muscle mass, high force eccentric contractions lead to greater increases in CK which suggests more muscle damage. Eighteen of the 20 participants in this study met the *a priori* criteria of at least a 100% increase in CK, but perhaps future studies should use a different EIMD model to elicit greater inflammation. The less severe induction of muscle damage from this protocol compared to high force eccentric exercise is likely a primary reason for the non-significant changes in inflammation. This could be an indication that the inflammation that occurred at the muscle, which resulted in DOMS, was not enough to cause an increase in blood cytokine levels.

While the results from this study indicated promising metabolic outcomes associated with a LGI diet, limitations were present. Of the nine participants included in data analysis who followed the LGI diet, three commenced the study with IR, also having higher levels of fasting insulin consistent with IR. Improvements in insulin and IR could have been altered by these participants, as exercise is a known mediator of increased insulin sensitivity. Even with this limitation, these results are promising and are consistent with researchers who have indicated improved metabolic profiles in individuals on a LGI diet, and not in those following a HGI diet. This acute improvement is also consistent with researchers who have established that the benefits of a LGI diet are most noticeable in individuals who follow this diet long-term. We conclude that a LGI diet can improve glucose metabolism acutely and does not appear to be harmful in any way.

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APPENDICES

APPENDIX A

SUBJECT CONSENT FORM

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

Study Title: Inflammation, insulin, and glucose differences between high and low glycemic index diets following downhill running in overweight and obese women.

Investigators: Katherine McNulty
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Summary:

You are being asked to volunteer as a participant in a study where the researcher will investigate how differences in carbohydrates (low and high glycemic index, which differ in how quickly glucose reaches your blood after you eat food) and anthropometric measurements (height, weight, waist and hip circumference) relate to inflammation and metabolism after a downhill running protocol that will lead to leg muscle soreness. The researcher is trying to gain an understanding of the factors that influence disease risk stratification for development of cardiovascular diseases and type 2 diabetes mellitus. This downhill exercise induces a small amount of damage and muscle soreness in leg muscles. You have probably experienced this type of muscle soreness before in your day to day life, as it occurs commonly with various activities. You will have blood drawn four times during the course of this study so that we can measure inflammation and metabolic variables including glucose, insulin, lipids, an enzyme called creatine kinase, different types of molecules related to inflammation, and other related variables.

The purpose of this study is to determine the differences in inflammation and glucose metabolism between low and high glycemic index diets after a downhill run in overweight and obese women.

Participants in the study:

You have been asked to participate in this study because you meet the following criteria: 18-40 years of age, female, are not currently involved in an exercise program that could alter the muscular response to downhill running, ie, activities that involve generating high force with the legs such as plyometrics or have ran downhill in the past 2 months. You may not be a participant if you are currently taking blood pressure, cholesterol lowering, or anti-inflammatory medications, if you are pregnant, have hypertension (high blood pressure), are diabetic, have known heart disease, or if you have other health concerns you feel may interfere with the study or that make it difficult for you to participate.

Procedures:

Upon your agreement to participate in this study, you will be asked to do the following:

You will be asked to visit the Nutrition Research laboratory at Montana State University on 4 different occasions.

Visit # 1 will include the following:

- 1) **Informed consent.** Read and provide written informed consent (this form).
- 2) **Health history.** Complete a health history questionnaire that asks questions about your health and the health of your family, particularly regarding heart disease.
- 3) **Body size measurements.** The researcher will take baseline measurements of height, weight, and circumferences of your waist and hips. You will remain clothed during these measurements, however, you will be asked to remove extra clothing items such as coats or sweatshirts.
- 4) **Body composition (%fat and %lean).** You will change into either a swimsuit or other tight fitting clothing such as lycra compression shorts and a sports bra or tank top. You will stand on a scale, then sit in an egg-shaped chamber for measurement of the volume of your body. To do this, we close the chamber door. There is a window for you to see the investigator at all times. The door will be closed for about one minute while the first measurement is taken and then opened again. The measurement will be repeated one or two times. A test will then be performed to determine your lung volume. To do this test, you will be inside the chamber with the door closed and breathe in and out according to instructions for about one minute. This measurement may be repeated.
- 5) **Submaximal exercise test.** You will complete a sub-maximal treadmill test that will begin with you warming up until you feel comfortable enough to begin an exercise test. The test involves a series of stages that get increasingly more difficult every 3 minutes. You will wear a heart rate monitor and will have your mouth around a mouthpiece that is connected to a metabolic cart, and your nose will be plugged with a plug. This allows researchers to measure how much you are breathing, the composition of what you exhale, and the amount of oxygen you are using at each part of the test. The test begins with walking, and as you move to more difficult stages, intensity is increased by increasing grade and speed, meaning you will likely have to run uphill during the final stages. The researcher will end the test when you have reached 85% of your age predicted maximal heart rate (220-age in years). The test will take less than 30 minutes. You will feel fatigued at the end of the test, but you will not run at maximum intensity ever during the test.

Visit #2 will occur at least 4 days after visit #1, and you will be asked to not eat anything 12 hours prior to your appointment as it will involve a fasting blood draw.

This visit will include:

- 1) **Blood Collection.** After arriving at the appointment, you will sit for 10 minutes before having your baseline blood draw.

- 2) **Muscle soreness** will be determined using an algometer. An algometer is a small instrument with a round, rubber tip that will be pressed onto your thigh (half the measured distance between your hip and knee) with increasing pressure until you say it is painful or tender. A mark will be made on your leg with a marker for the post-measurement to follow after the downhill protocol.
- 3) **Downhill running exercise.** Before the treadmill exercise begins, you will have a muscular strength test performed on your non-dominant leg. You will push your leg as forcefully as you can against a device called a dynamometer that measures strength. This will last roughly 5 seconds. You will then be asked to put on the heart rate monitor used on the first visit, and will wear this for the entire protocol. You will then perform the downhill walking/jogging protocol at a -10% grade with speed determined by your target heart rate at 65% of your estimated VO_2max that was determined on visit #1. This protocol will be of moderate to somewhat difficult intensity. You will have a 5 minute warm up period before the protocol begins, and then jog at the heart rate determined for you. After 20 minutes of downhill running, you will step off the treadmill briefly to again measure muscular strength and allow us to assess some variables related to your exercise. You may be asked to get back on the treadmill to continue running downhill for 5 minutes. You may do from 0 to 4 of these 5 minute bouts for a total downhill running time from 20 to 40 minutes.
- 4) **Blood collection.** You will go directly from the treadmill to have your blood drawn again. After this blood is drawn, you will get the chance to cool down as long as you desire.
- 5) **Muscle soreness** will again be determined using the same algometer procedure mentioned above.
- 6) **The food that you will eat for the rest of the day will be given to you.** You will be provided with food that is measured according to your caloric needs and is based upon the intervention that you have been randomly selected to receive. Foods you could be given include Cheerios, rolled oats, 2% milk, watermelon, oranges, apples, peanuts, yogurt, pretzels, fruit roll-ups, juice, lemonade, a peanut butter and jelly sandwich on either wheat or white bread, a baked potato, wild rice, barley, pasta, frozen, pre-cooked chicken breasts, broccoli, and bread. You will be given instructions for the food you receive and any preparation such as heating tips that are suggested. You will be asked to eat only the food provided to you until you return for your next appointment the next day.

Visit #3 and visit #4 will occur 24 and 48 hours after visit #2, and again, you will need to not eat anything 12 hours before the appointment. You will do the following things at these visits:

- 1) **Blood Collection.** After arriving at the appointment, you will sit for 10 minutes before having your baseline blood draw.
- 2) **Return any uneaten food** so the researcher can measure and account for it.
- 3) **Muscle soreness.** The algometer will be used to assess muscle soreness.

- 4) **Strength measurement.** The dynamometer will be used to determine muscle strength.

If you volunteer to participate in this study, you will be asked to refrain from vigorous physical activity or any extracurricular physical activity you usually would not partake in for 24 hours before the downhill testing, and for 24 hours after testing.

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

- 1) For the blood draws, approximately 10-15 ml of blood (2-3 teaspoons) will be taken from you by placing a needle into one of your veins on 4 occasions. This is a standard medical procedure, and will be performed by a certified phlebotomist (someone who is certified to draw blood). You will likely experience a small, momentary amount of pain when the needle is first inserted, but other pain should be minimal. Some people (about 10%) get a bruise from where blood was taken. Risk of infection is less than 1 in 1,000 people.
- 2) People who are claustrophobic may be uncomfortable in the chamber used to determine body composition. If needed, participants can open the chamber door at any time.
- 3) The sub-maximal exercise test will likely make you feel uncomfortable in the last stages, possibly leaving you a little bit fatigued. This sub-maximal test is commonly used in clinical procedures for clinical diagnostics and disease evaluation. People rarely have adverse side effects, but some have occurred before. The mortality rate of this test is approximately 1 in 10,000 tests, and serious complications such as abnormal heart rhythm or chest pain for prolonged periods of time present in about 4 out of every 10,000 tests.
- 4) The downhill protocol will result in muscle soreness and fatigue for about 5-6 days, but should subside after this. You will experience some loss in muscular strength during these days, but this should return to normal within the 5-6 days. Your daily activities should not be limited by the pain or the loss of strength. You will most likely feel the muscle soreness, pain, and fatigue during activities such as going up and down stairs, and when walking. It is recommended that you avoid strenuous activities for 3 days after the downhill protocol to allow time for your muscles to start healing. In a very small percentage (2-3%), strength losses can last for up to 2 months, but would only be noticeable in activities that would require high force generation. Also, a small percentage of people get slight swelling in the legs, but this is not serious and will diminish within 2 weeks. There is the risk for serious injury like a muscle pull or strain from the exercise, but this is minimal in healthy individuals who do not have cardiovascular, metabolic, or musculoskeletal problems and have not had recent surgery. You can stop at any time.

You may gain some benefits by participating in this study, such as:

- 1) You will get information about your fasting blood glucose, lipids, and inflammation.
- 2) You will receive information about your level of cardiovascular fitness.

No other benefits are promised to you.

Compensation: You will receive \$60 upon completion of your testing, \$15 for each time you come into the Nutrition Research Laboratory.

Freedom of Consent: You have the right to withdraw from participating in the study at any time with a no questions asked policy. You may withdraw in writing, over the phone (Katherine McNulty-994-5001), or in person. If you withdraw, you will not lose any benefits you incurred up to the time of withdrawal. Your participation in this study is completely voluntary.

Funding: The costs of this study will be covered in part by the McGown Endowment to Montana State University.

Please ask any questions: You are encouraged by the researcher to ask any and all questions you may have, as well as addressing any concerns about the study. The researcher will answer your questions as fully and as accurately as possible. Your peace of mind and comfort in the study is of utmost importance to the researcher.

Confidentiality: All data and information received from you for this study will be kept completely confidential. You will be given a subject identification number that will be used to describe all data. This information will be kept locked in a file cabinet in the Nutrition Research Laboratory. Data from this study could be published in scientific and/or medical journals, but your identity will remain confidential. If you withdraw from the study at any time, all of your information will be deleted from the study records, and you will not be contacted again regarding the study. There are absolutely no penalties for withdrawing.

In the unlikely event of injury to you due to participation in this study, medical treatments such as first aid and help getting to adequate health care providers (such as transport to Bozeman Deaconess Hospital) will be provided, however, there is no compensation for any of this provided by Montana State University. You can access further information involving this policy and treatment by contacting Mary Miles at 994-6678, or emailing her at mmiles@montana.edu.

Any other questions you may have regarding your rights as a participant may be answered by the chairman of the Human Subjects Committee, Mark Quinn. He can be reached at 406-994-4707.

STATEMENT OF AUTHORIZATION

Study Title: Inflammation, insulin, and glucose differences between high and low glycemic index diets following downhill running in overweight and obese women.

AUTHORIZATION: By signing this document, I acknowledge that I have read the above and I understand the discomforts, inconvenience, and risks associated with my participation in this study. I, _____ (name of subject), agree to participate in this study. I fully understand that I may later refuse participation at any time, and may withdraw from the study at that time. I have been given a copy of this consent form for my own records.

Signed: _____

Witness: _____ (optional)

Investigator: _____

Date: _____

APPENDIX B

PRE-PARTICIPATION SCREENING QUESTIONNAIRE

Pre-participation Screening Questionnaire

Please circle all that apply

History

You have had:

A heart attack

Heart surgery

Cardiac catheterization

Cardiac angioplasty (PTCA)

Pacemaker/implantable cardiac defibrillator/rhythm disturbance

Heart valve disease

Heart failure

Heart transplantation

Congenital heart disease

Symptoms

You experience chest discomfort with exertion

You experience unreasonable breathlessness

You experience dizziness, fainting, or blackouts

You take heart medications

Other health issues

You have diabetes

You have asthma or other lung disease

You have burning or cramping sensation in your lower legs when walking short distances

You have musculoskeletal problems that limit your physical activity

You have arthritis or known joint issues that limit your ability to walk or run

You have concerns about the safety of exercise

You take prescription medications

You are pregnant

Cardiovascular risk factors

You are a man older than 45

You are a woman older than 55, have had a hysterectomy, or are post-menopausal

You smoke or quit smoking within the previous 6 months

Your blood pressure is >140/90 mmHg

You do not know your blood pressure

You take blood pressure medication

Your blood cholesterol is >200mg/dl

You do not know your cholesterol level

You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)

You are physically inactive (i.e., you get less than 30 minutes of physical activity on at least 3 days per week)

You are >20 pounds overweight