

INFLUENCE OF ANTHROPOMETRIC CHARACTERISTICS ON
INSULIN RESPONSES TO UPHILL AND DOWNHILL
WALKING IN WOMEN

by

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ABSTRACT

Obesity is associated with insulin resistance as well as several leading causes of preventable death. Exercise is a lifestyle factor that decreases risk of these conditions. The type of exercise performed might play a role in the insulin response to exercise as well as a recommended post-exercise meal. Eccentric exercise is shown to increase insulin resistance while concentric is known to decrease insulin resistance. The purpose of this study was to determine if there are differences in post-exercise insulin levels between eccentric and concentric exercise conditions with a post-exercise meal, and the relationship with waist circumference (WC), a measure of central obesity. Participants were women ($n = 24$), stratified into low (LWC) and high (HWC) waist circumference groups. Participants completed three test conditions: a 45-minute walk at -10% grade at 3.0MPH, a 45-minute walk at +10% grade at a speed that maintained 60% of estimated VO_{2max} , and a control. A mixed meal was consumed thirty minutes after completion of the test. Finger stick measures of glucose were taken at 0, right before consumption, and then 15, 30, 45, 60, 90, 120 minutes following the meal. Insulin, C-peptide, and glucose were measured pre-test and immediately, 8 hours, and 24 hours after completion of the test. Muscle soreness was measured when blood was collected. The HWC group had higher ($p < 0.05$) insulin across time points compared to the LWC group in the control and uphill conditions. C-peptide was greater ($p < 0.05$) across time points for the HWC group in the control compared to the LWC group. The HWC group had greater ($p < 0.05$) insulin at 60-minutes in response to the meal during the control condition than the LWC group. The LWC had greater ($p < 0.05$) insulin at 60 minutes in the downhill compared to the control. Muscle soreness was increased ($p < 0.05$) the most in response to the downhill condition. We conclude that waist circumference influenced insulin levels following exercise, where uphill walking improved indices of glycemic control for those of greater waist circumference while downhill walking worsened indices of glycemic control for those of a lower waist circumference.

CHAPTER ONE

GENERAL INTRODUCTION

Introduction

Obesity is a focus of health professionals due to the many complications associated with increased body fat and body weight. According to the National Institutes of Health researchers (1), conditions that relate to obesity are among the leading causes of preventable death. These conditions include heart disease, stroke, type 2 diabetes, and various cancers (1). The researchers also found that one-third of adults in America are obese and even more are overweight (2). The above listed diseases are developed through lifestyle factors and are considered to be preventable, and in some cases, reversible.

Body composition, especially the percentage of body fat, is related to differences in sensitivity to insulin and glucose metabolism (3-5). Insulin is a regulatory hormone secreted by the pancreas in response to glucose in the blood and is responsible for the transport of glucose into skeletal muscle cells and adipose cells. An individual's fitness capacity is independently associated with insulin sensitivity (3). Conversely, those who are inactive experience an impaired insulin sensitivity, also defined as increased insulin resistance (6). Various other factors have been shown to increase insulin resistance (7-9). Lin et al. (8) found that waist circumference and body mass index, two anthropometric measures of body

composition and shape, were significantly associated with increased measures of insulin resistance.

The improvement of insulin sensitivity is desirable for those struggling with insulin resistance. Exercise has been shown to improve insulin sensitivity through the increase in glucose uptake by skeletal muscle (10-12). However, researchers have found that some forms of exercise increase insulin resistance (13-15). Depending on the type of muscle contraction, there are differences in how muscle fibers withstand imposed forces and are damaged during exercise. The damage induced in eccentric exercise alters the action of insulin, resulting in transient insulin resistance. Dietary factors have also been linked to changes in glucose metabolism following exercise (16, 17). Therefore, dietary as well as exercise prescription may depend on risk assessment of insulin status and the insulin responses to specific exercises.

Statement of Purpose

The purpose of this study was to determine if there are differences in post-exercise insulin levels between eccentric and concentric exercise conditions with a post-exercise meal. The relationship between insulin indices, waist circumference and body fat percentage was also determined.

Significance

Research conducted in the Montana State University Nutrition Research Laboratory found that women with higher BMI classifications experienced increased levels of insulin after inducing eccentric muscle damage (18). In general, insulin levels tends to decrease during repeated muscle contraction due to the insulin-like effect of exercise. The action of insulin, to stimulate the translocation of glucose transporter 4 (GLUT4) to the cell membrane, is bypassed when exercise is being performed and GLUT4 is signaled via muscle contraction. While this effect has been found in concentric contraction, eccentric contraction has been found to increase insulin response. Therefore, if insulin levels are elevated after eccentric exercise there might be altered effects on glucose metabolism during and after exercise. This change in insulin sensitivity in turn influences the glycemic response to a post-exercise meal and even metabolic health. While no studies were found to date that investigate the relationship between adiposity and eccentric exercise, the research at Montana State University did find a trend of increased insulin with increased BMI. Altered glucose metabolism is closely associated with obesity, and therefore it is important to further study the relationship between insulin resistance stimulating effects and body weight.

Delimitations

The study is delimited to

- Females between the ages of 18 and 40 years.
- The research upon which this study is expanding was done on a homogenous group of women, therefore the research sample included only women for the current investigation.
- The subjects were selected into groups based on BMI. The groups were normal weight and overweight/obese, classified by BMIs of 18.5-24.9 and 25-34.9 kg•m⁻², respectively.
- Subjects were not on any form of steroid hormone contraceptive (pills, injection, patches) or any anti-inflammatory drugs.
- Subjects also exhibited ≤1 cardiovascular risk factor as defined by the American College of Sports Medicine submaximal testing contraindications.

Limitations

The study is limited by:

- Subjects were at different training levels based on personal variation in activity status. Due to varying physical fitness status and body composition, the amount of muscle damage induced by the protocols was not matched as a criterion for test continuation or completion.

- Not all factors that influence glucose and insulin levels were controlled. Factors included sleep, stress levels, and menstrual cycle fluctuations.
- The sample size (N=24) was small and from the Bozeman, Montana, area only, leading to issues with generalization to the general population.

Assumptions

It was assumed for the purpose of this study that the qualifying individuals sustained similar muscle damage induced by the experimental eccentric exercise. The changes in insulin and inflammation were assumed to be in response to the condition. It is also assumed that individuals followed dietary guidelines during the duration of their study trials.

Hypotheses

Primary: It was hypothesized that the change in insulin in response to eccentric exercise would be greater than the control condition, which would in turn be greater than the response to concentric exercise.

$$H_0: \mu_{\Delta ECC} = \mu_{\Delta CON} = \mu_{\Delta CNC}$$

$$H_A: \mu_{\Delta ECC} > \mu_{\Delta CON} > \mu_{\Delta CNC}$$

Where μ_{Δ} was the sample mean of insulin measured post-exercise condition minus pre-exercise condition. The conditions of exercise are eccentric (ECC), concentric (CNC), and control (CON).

Secondary: It was hypothesized that individuals with higher body fat percentages would experience greater insulin responses across conditions.

$$H_0: \mu_{\Delta HBF} = \mu_{\Delta LBF}$$

$$H_A: \mu_{\Delta HBF} > \mu_{\Delta LBF}$$

Where μ_{Δ} is the sample mean of insulin measured post-exercise condition minus pre-exercise condition. The groups are high body fat (HBF) and low body fat (LBF), with a cutoff at 30% between groups.

Operational Definition of Terms

ATP: adenosine triphosphate, the source of energy used by the body

AUC: area under the curve, a method for a calculating insulin and glucose response

Body Mass Index (BMI): A calculated value based on mass and height that is used to categorize estimated body composition and weight status.

CK: creatine kinase, an enzyme found in the blood that is a clinical indicator of muscle damage

Concentric muscle contraction (CNC) condition: the shortening action of muscle while contracting, induced through uphill treadmill walking

Control (CON) condition: idle activity during the allotted exercise time

Eccentric muscle contraction (ECC) condition: the lengthening action of muscle while contracting, induced through downhill treadmill walking.

Normal weight: A BMI of 18.5-24.9 kg•m⁻²

Overweight: A BMI of 25-29.9 kg•m⁻²

Obese weight I: A BMI of 30-34.9 kg•m⁻²

VO_{2max}: maximal oxygen consumption as predicted by a submaximal graded treadmill test

Theoretical Definition of Terms

Insulin Resistance: the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population (19)

Insulin sensitivity: a range of ability for insulin to transport glucose across the cell membrane (20)

List of Abbreviations

BF%: body fat percentage

GLUT4: Glucose transporter 4

HBF: high body fat group

HWC: high waist circumference group

LBF: low body fat group

LWC: high waist circumference group

MMTT: Mixed meal tolerance test

MVC: maximal voluntary contraction

RPE: rating of perceived exertion

CHAPTER TWO

REVIEW OF LITERATURE

Introduction

Increased physical activity is a lifestyle factor that is associated with improved health status (21-24). Physical activity is defined, by the American College of Sports Medicine (25), as any type of bodily movement that results in substantial increased energy expenditure produced by skeletal muscle contraction. Muscle contractions are categorized into three types: eccentric, concentric and isometric (26). Eccentric contraction is classified as the action of skeletal muscle in which muscle develops force but lengthens due to a greater amount of external force opposing the contraction (26). Conversely, the muscle action of developing force and creating torque around a joint is concentric contraction, through shortening of the muscle belly (26). Isometric contraction is the contraction of skeletal muscle that results in no change in muscle length and therefore no movement around the joint (27).

Insulin

The control of blood glucose levels is largely regulated by the hormones insulin and glucagon. Insulin is secreted by the pancreatic beta cells located in the islets of Langerhans. This hormone stimulates the disposal of glucose from the blood

into cells, especially adipose and skeletal muscle cells which are insulin-dependent (27). Uptake of glucose in insulin-dependent tissues relies on insulin stimulation of the glucose transporter 4 (GLUT4). This protein remains in the cytosol until its translocation is activated by insulin binding to the receptors at the membrane.

Insulin is released in response to increased glucose in the blood as well as counter-regulatory hormones (28). The source of the glucose can be exogenous or endogenous, being released by the liver. While a sensitive insulin response leads to proper glycemic control, a decrease in sensitivity can lead to problems. Insulin resistance is described as abnormalities in insulin action and glucose metabolism at tissue membranes. When tissues become resistant, the pancreas produces and secretes more insulin, leading to an elevated insulin levels in the blood. Over time, the pancreas loses the ability to produce insulin and an individual can become insulin deficient, and therefore type 2 diabetic (29).

Demands of Muscle Contraction

Skeletal muscle requires energy to contract and therefore plays a major role in substrate metabolism. Researchers found that insulin as well as exercise stimulated the translocation of GLUT4 to the plasma membrane of skeletal muscle cells (30-33). When GLUT4 activity was inhibited, there was an increase in blood glucose and blood insulin levels (34). Insulin levels decreased in the blood when glucose uptake was promoted, providing the cell with adequate energy to carry out physiological demands.

The demands of eccentric and concentric muscle contraction differ metabolically. Eccentric muscle contraction oxygen demand is lower than that of concentric muscle contraction. Less adenosine triphosphate (ATP) is required, most likely due to the lower motor unit activity seen in eccentric contraction (35). For a given work load, determined by the same tension and constant contraction velocity, there are fewer motor units recruited in eccentrically contracting muscles than concentrically contracting muscles. This leads to more strain on individual muscle fibers and promotes damage at a cellular level. The demands on the cardiovascular system are also different between contraction types. In weight-loaded downhill walking, Fallowfield and fellow researchers (36) found that for a given weight, downhill walking induced a lesser heart rate (HR) as well as oxygen consumption response than level walking. Although there were decreased responses in absolute values of HR and maximal oxygen consumption (VO_2), the cardiovascular drift was greater during downhill walking compared to level walking. Concentric and eccentric muscle contractions require different energy requirements and fiber recruitment, so the effects of exercise differ between conditions.

Eccentrically-induced Muscle Damage

Eccentric muscle contraction has been found to induce structural damage to individual myofibril cells (35, 37). Because eccentric contraction recruitment is preferentially glycolytic fibers, the damage occurs to these fibers more so than oxidative fibers. Glycolytic fibers are situated for optimal tension development at

high angular velocities. Friden et al. (35), using an electron microscope, found that the contractile materials of these myofibrils were more extensively damaged than that of the oxidative fibers following eccentrically-biased cycling. This structural damage led to leakage of cell contents due to rupture of the cell membrane. Newham and colleagues (38) determined the extent of muscle fiber damage through direct measurement of muscle biopsy samples using light and electron microscopy. They found that morphological abnormalities were seen in z-line structures of sarcomeres following eccentric muscle contraction. Gibala and associates (37), upon analysis of muscle biopsies, identified significant damage in eccentrically exercised muscles, as evidenced by disrupted fibers. In contrast, concentric muscle contraction did not result in disrupted fibers over baseline in any of these studies (35, 37, 38).

The enzyme, creatine kinase (CK), has been shown to increase in the blood following structural damage to muscle fibers (14, 39, 40). Following maximal voluntary contraction of the thigh, Asp et al. (14) found that blood CK was increased from 123 U l^{-1} to 5266 U l^{-1} two days (48 hours) after completing the exercise. The controls for this study experienced no significant change. Ahmadi, Sinclair, and Davis (41) measured creatine kinase levels for many days after eccentric exercise and determined that levels remained significantly higher than baseline for five days, peaking at 72 hours post-exercise. Other researchers (42) found similar results for eccentric exercise only. In contrast, concentric exercise did not increase levels of CK at any point following exercise (13). Many of the protocols used were downhill

walking (41) or running (42-46), which requires the eccentric contraction of large muscle groups.

In multiple studies, downhill walking induced delayed onset muscle soreness, which is associated with eccentric muscle damage (14, 41). Asp et al. (14) developed an arbitrary and subjective scale for evaluating muscle soreness. This scale was used only per individual and was not analyzed as a mean. On this scale, there was a significant increase in perceived soreness^{q1} from baseline following eccentric exercise for each individual. Green et al. (15) developed a test for muscle soreness in an attempt to standardize the values. Subjects were asked to step up onto a platform that required 90 degree flexion of both the hip and knee, determining the pain required to complete this task. McNulty (47) used an algometer to determine muscle soreness. This device measured the depth of depression on the target area that first elicited pain. Ahmadi and associates (41) determined that muscle soreness peaked 72 hours post exercise, similar to the peak of creatine kinase. Just as creatine kinase did not increase, muscle soreness was also not experienced following concentric exercise (38, 46).

There are multiple tests to demonstrate damage in the target muscles that have been used in several studies (13, 41, 43). Ahmadi and colleagues (41) tested maximal voluntary contraction (MVC) and found there was a 25% decrease 24 hours post-eccentric exercise bout. Creatine kinase (CK) peaked three days post exercise and myoglobin was highest 24 hours post exercise. Although these are clinical determinants of muscle damage, the methods of direct measure of muscle

damage are limited to muscle biopsy and magnetic resonance imaging (MRI). All of the aforementioned indirect measures can be combined to estimate the muscle damage induced by repeated eccentric muscle contraction.

Concentric versus Eccentric Treadmill Walking

During the phases of downhill or negative grade locomotion, there are biases in concentric and eccentric muscle fiber recruitment (44). When evaluated, the concentric phase provided mostly propulsion force while the eccentric phase involved braking against ground reaction force (GRF) as well as parallel GRF (44). Therefore, the muscles of the leg were eccentrically contracting against the forces of forward propulsion as well as gravity. The extensor muscles of the leg were most responsible for the act of decelerating the center of mass once the forward foot contacted the ground before the swing phase of the contralateral leg began (42).

The major differences in muscle damage and action between concentric and eccentric muscle contractions elicit a need to match conditions when testing the differences in response. One strategy has been to complete a submaximal or maximal graded exercise test to determine maximal oxygen consumption (VO_{2max}) and complete exercises at different percentages of this value. Kirwan et al. (46) matched both downhill running and concentric cycle ergometry to 60% of VO_{2max} , with the intensity of the exercise bout being altered to maintain this level throughout the exercise. Newham and colleagues (38) matched external work output to ensure that internal work was equal between both conditions.

Muscle Damage and Glycemic Response

Glucose uptake into skeletal muscle cells can be altered by stimulation or inhibition of GLUT4. Eccentrically-induced damage has been shown to decrease the GLUT4 protein content by 39% two days after exercising (14). When the different fiber types were examined for GLUT4 levels, Asp et al. (14) determined that there was a 65% decrease in GLUT4 in fast-twitch, or glycolytic, muscle fibers. This adds to the evidence that eccentric exercise preferentially recruits, and therefore damages, glycolytic fibers.

With decreased GLUT4 in skeletal muscle following eccentric muscle damage, it is assumed that glucose metabolism is altered. Green et al. (15) conducted an oral glucose tolerance test (OGTT) 48 hours after eccentric exercise. They found that glucose area under the curve (AUC) was significantly greater following a novel bout of eccentric exercise. A similar effect was found for insulin. King and colleagues (13) determined that the peak insulin concentration following eccentric bouts of exercise was 28% higher than the control. By tracing C-peptide concentrations levels in relation to insulin levels, they were able to calculate the secretion of insulin from the pancreas. They found that insulin secretion was significantly increased in the eccentric condition (13). This conflicts somewhat with the expected insulin levels in the blood throughout exercise, which are known to decrease as exercise by-products stimulate GLUT4 independent of insulin (27). In order to quantify the insulin secretion and resistance, researchers administered a variable infusion of

glucose to maintain an elevated blood glucose level and the rate of glucose disposal is measured, a method known as a hyperglycemic clamp (48). Within the initial ten minutes of a hyperglycemic clamp, insulin levels increased dramatically, however, after the initial phase, insulin levels were no longer elevated (13). The researchers suggested this was due to insulin being fractionally extracted from the blood after 10 minutes, in response to increased levels. In the same study, concentric exercise did not elicit a significant change in insulin or glucose above control levels. Similarly, other researchers (49) found a biphasic insulin response. In the early phase, insulin levels were increased due to more secretion of insulin, while the late phase saw less insulin due to increased uptake.

The studied effects of exercise on insulin sensitivity have been generally positive. Researchers have found that as training status of an individual progresses, there is an increase in insulin sensitivity (10, 11, 50). This is due to a decrease in insulin released from the pancreas, resulting in a lower plasma insulin response. Insulin levels fluctuate in response to changes in blood glucose as well as other hormones. The liver secretes glucose once exercise has begun, in order to meet the energy demands of the exercising muscles (27). Exercise increases the secretion of epinephrine in the blood which in turn inhibits the secretion of insulin from the pancreas. Both the lack of stimulation from glucose and the inhibition from epinephrine result in a drop in insulin levels after exercise has begun and for a transient period following exercise. Once insulin levels have dropped, insulin sensitivity is then improved (27). For those who are at risk for developing, or

already have, glycemic control issues, increasing insulin sensitivity is desired. Therefore, it has been common practice to suggest increases in exercise and physical activity in order to improve glycemic control.

Prolonged Effects of Eccentrically-induced Muscle Damage

The consequences of eccentric muscle damage last for many days after exercise. Creatine kinase levels were found to be elevated five days after exercise, in a test that only tracked CK for five days after the exercise bout (42). Muscle soreness has been reported to last for a similar amount of time. Several researchers required two weeks between bouts of exercise to attempt at ensuring blood levels of markers of muscle damage were returned to baseline (37, 43).

A difficulty when studying eccentric exercise is the repeated bout effect. Researchers (15) have found that a novel bout of eccentric work induced muscle damage and subsequently increased insulin resistance. However, when eccentric exercise was repeated within a few months (15, 51) of a novel bout, the response is lessened. Measures of soreness and pain are decreased from the first exercise as well as markers of damage such as CK. Green et al. (15) provided evidence that MVC was significantly decreased following the initial eccentric bout but did not significantly change from baseline upon completion of a repeated bout. McHugh and colleagues (51) found that the repeated bout effect was not due to neural adaptation but rather to the muscle's structural abilities to generate force. Researchers (15, 51) suggested that the repeated bout effect was a protective measure against further

muscle damage. This adaptation poses a challenge when studying eccentric muscle damage and must be addressed in study methods.

Anthropometric Characteristics and Insulin Resistance

Obesity, as defined by BMI, is associated with greater risk of developing type II diabetes (5) and being insulin resistant (52). Kolterman and colleagues (1980) studied the response to insulin in obese individuals in order to determine glucose disposal rate. They found that obese individuals experienced significantly less uptake of glucose despite increases in insulin compared to control subjects. They suggested that decreased insulin receptor concentration in individuals with higher body mass indexes was responsible for the increased insulin resistance, but the mechanism is not yet fully known (53). Other researchers went further, comparing percent body fat to glucose disposal rate (3). They found a significant negative relationship, where those with greater percent body fat experience less glucose disposal, and therefore greater insulin resistance. One proposed mechanism is the inhibition of GLUT4 translocation due to increased fatty acids in the blood (54). Obesity is associated with an increased serum level of fatty acids (55). Increased plasma fatty acid concentration in the blood increases the levels of insulin receptor synthase, which in turn decreases the action of insulin at the plasma membrane. GLUT4 translocation is not stimulated and glucose is not taken up into the cell (54).

Waist circumference (WC) is another measure that has been associated with insulin metabolism (8, 56) and general mortality (57). This specific measure of a

circumference is an attempt to identify differences in body fat distribution. The anthropometric measurement of WC is simple to measure and has been found to independently predict visceral fatness (58). Visceral adiposity, considered fat accumulation in the abdomen, has been associated with altered glucose metabolism. Decreased glucose tolerance, greater fasting hyperinsulinemia, and increased insulin during a tolerance test were all found in subjects with greater upper body adiposity (4). Lin and colleagues (8) also found that there was association with waist circumference and increased measures insulin as measured by the homeostatic model assessment.

Increased waist circumference is a factor in metabolic syndrome (MetS), along with obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension. The cutoff for metabolic syndrome as defined by the International Diabetes Federation and the European Group for the Study of Insulin Resistance (EGIR) for waist circumference is >94cm in men and >80cm in women (59-61). The EGIR describes this cutoff as action level 1, where there is increased risk for health complications and further weight gain is discouraged. Above 102cm for men and 88cm for women, there is an action level 2, where medical consultation and weight loss should be urged (62). The Adult Treatment Panel III (ATP III) defines the level of waist circumference for clinical identification of metabolic syndrome as >102cm for men and >88cm for women, and this is the cutoff most commonly found (63).

Fitness Level and Insulin Sensitivity

There are differences in insulin sensitivity due to fitness level and activity. In fit adults, researchers found maximal oxygen consumption (VO_{2max}) was negatively associated with serum insulin and positively associated with glucose uptake (64). For individuals with at least one risk factor for developing type 2 diabetes, cardiorespiratory fitness, as measured by a step test, predicted insulin resistance (56). Physical activity, like physical fitness, is related to insulin resistance. Hamburg and colleagues (6) found that when healthy individuals were physically inactive for five days there was an increase in serum insulin.

Dietary Factors

Post-exercise meals are often suggested in order to provide needed nutrients to a body that is in the process of adapting to the bout performed. The Academy of Nutrition and Dietetics (65) emphasized increased intake of carbohydrates in order to replenish glycogen. However, researchers are finding that not all responses are the same depending on the meal or the individual. Carbohydrates alone, following an eccentric bout of exercise, were shown to increase insulin significantly during a day of high-carbohydrate meals (16). Miles et al. (16) evaluated the insulin response to the BMI of the subjects and found an association between diet and body composition. For those with a greater BMI values, insulin increased to a greater extent following a carbohydrate meal than those of lower BMI values. Conversely,

for a meal comprised of fat and protein, the individuals with lower BMI values experienced a greater insulin response. The differences in response were suggested to be due to variations in inflammation response to the eccentric damage and its interaction with body composition.

Sherman et al. (17) conducted research on the insulin response to an oral glucose tolerance test 48-hours following eccentric exercise. The researchers measured the response to an ingestion of pure glucose as opposed to a mixed meal test as seen in previously mentioned research. They found that individuals experienced greater insulin levels as well as insulin area under the curve 48-hours after completing eccentric exercise.

Tolerance Tests for Evaluating Glucose Metabolism

While fasting glucose and insulin can be used to evaluate glucose at one time point, the use of tolerance tests can demonstrate the response to a given load. The standard for measuring glucose tolerance is the oral glucose tolerance test (OGTT). A 75g load of glucose is ingested, then glucose and insulin are measured at regular time points for up to 3 hours (66). Researchers (67) tested the validity of using the OGTT as a measure of insulin and glucose homeostasis through comparison with direct measurements, determining that the OGTT provides values to accurately predict an individual's insulin resistance and β -cell function.

Another method for evaluating response to loads is a mixed meal tolerance test (MMTT), which introduces food items rather than glucose alone. Introducing

the macronutrients fat and protein changes gastric emptying and absorption. However, the composition of the meal is more similar to a typical meal than the OGTT (68). Mixed meal tolerance test values can be transformed to determine the area-under-the-curve (AUC). Marena et al. (68), determined that the AUC of a MMTT is significantly correlated with that of a OGTT and can be used to evaluate glucose intolerance.

Measuring Insulin Sensitivity and Resistance

The most direct way to measure glucose and insulin homeostasis is through utilization of the hyperinsulinemic-euglycemic clamp technique (48). This technique maintains a level of elevated insulin in the blood while measuring whole body glucose disposal rate by measuring levels needed to prevent hypoglycemia. The hyperinsulinemic-euglycemic clamp is considered the gold standard method for measuring insulin resistance but not practical in all settings. The clamp protocol requires extensive time and resources not available to all populations.

Indirect methods of measuring insulin resistance include calculating indices from values relevant to glucose metabolism such as blood glucose, c-peptide, and insulin. Matthews, et al., (69) relate fasting basal glucose and insulin concentrations based on their roles in a feedback loop. Through computer modeling of World Health Organization data, Matthews and colleagues were able to take direct measurements from the hyperinsulinemic-euglycemic clamp method and create a predictive model for insulin resistance as well as β -cell function. With this model,

called the homeostatic model assessment (HOMA), values for plasma glucose and insulin can be plotted and predictive values produced.

Plasma insulin values represent the systemic level of insulin at the point of blood draw and do not distinguish between insulin secretion and uptake. The liver is responsible for the removal of insulin from circulation (70, 71), so the peripheral levels of insulin may not be inclusive of the overall secretion of insulin from the pancreas. When insulin is secreted from the pancreas, it is co-released with an amino acid segment, C-peptide. Researchers have determined that C-peptide and insulin are secreted in equimolar concentrations from the β -cells in the pancreas (72). While insulin is extracted from the blood through the portal circulation as it passes through the liver, C-peptide does not get extracted at the same rate. Therefore, plasma insulin values represent the sum of secretion and extraction whereas C-peptide levels indicate the only secretion from the pancreas. Due to these kinetics, C-peptide can be used to estimate insulin secretion.

Summary

Insulin and glucose metabolism are altered acutely and chronically, through various mechanisms. Increased insulin sensitivity is associated with increased muscle contraction, when there is not eccentric damage. Through testing of positive and negative grade treadmill walking, transient effects of exercise on insulin can be evaluated. There are current recommendations for a post-exercise meal that may elicit differing responses in those of differing body composition. There is no

published research to date characterizing the differences in insulin response to uphill and downhill walking with a post-exercise meal between those with varying anthropometrics.

CHAPTER THREE

INFLUENCE OF ANTHROPOMETRIC CHARACTERISTICS ON
INSULIN RESPONSES TO UPHILL AND DOWNHILL
WALKING IN WOMEN

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Conceived and implemented study design. Collected and analyzed data. Helped run statistical analyses. Wrote first draft of manuscript.

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Contributions: Helped conceive and implement the study design. Provided expertise and funding. Ran statistical analyses. Provided feedback on drafts of the manuscript.

Co-Author: Karen Brown

Contributions: Helped conceive and implement the study design. Collected data.

Co-Author: Sara Jay

Contributions: Helped conceive and implement the study design. Collected data.

Co-Author: Jay Porter

Contributions: Ran blood analyses and collected data.

Co-Author: Andrea Steward

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INFLUENCE OF ANTHROPOMETRIC CHARACTERISTICS ON
INSULIN RESPONSES TO UPHILL AND DOWNHILL
WALKING IN WOMEN

Introduction

Obesity, a condition affecting over a third of American adults, is associated with increased risk for cardiovascular and metabolic diseases (1, 2). According to the National Institutes of Health (NIH), conditions related to obesity, such as type 2 diabetes, heart disease, and stroke, are among the leading causes of preventable death (1). These diseases are preventable and reversible through diet and physical activity (22, 24). Physical activity has been shown to decrease risk and improve health status (22, 23).

Physical activity is broadly defined as bodily movement produced by skeletal muscle contraction that results in increased energy expenditure (25). Skeletal muscle contraction can be isometric, concentric, or eccentric. Exercise, in general, has been shown to increase peripheral uptake of glucose without the need for insulin (10-12). However, eccentrically-biased exercise induces damage to the muscle fibers, leading to a transient increase in measures of insulin resistance (13-15). An increase in physical activity to improve insulin sensitivity may not be effective if the type of exercise increases insulin resistance.

Anthropometric measures can be used to predict body composition. The use of body mass index (BMI) as a measure of general obesity is common, but other

measures are considered more accurate in identifying risk. Waist circumference (WC) is considered a measure of central obesity, localized fat accumulation in the abdomen, which has been associated with altered glucose metabolism (58). Waist circumference has been identified as the best predictor of increased risk for elevated fasting insulin and glucose in women (73).

The elevated insulin response to downhill walking, a predominantly eccentric exercise, has been evaluated in normal, healthy individuals. The influence of anthropometric characteristics on eccentric exercise insulin responses has not yet been explored, despite the known differences in metabolism of those with greater measures of central obesity. Therefore, the purpose of this study was to determine the differences in insulin response between uphill, downhill, and resting exercise conditions in individuals who vary by waist circumference.

Participants

Participants were women aged 18-40 years of age. Thirty subjects who qualified for our study requirements were scheduled for an intake (Figure 3.1). Six dropped out for unrelated reasons. A total of 24 females, twelve of whom were normal and twelve of whom were overweight/obese I, (aged 28.5 ± 5.6 years, $25.6 \pm 5.0 \text{ kg}\cdot\text{m}^{-2}$) completed the study. One participant was excluded from analysis for not following protocol.

To qualify for the study, participants were limited to zero or one risk factors for cardiovascular disease based on the American College of Sports Medicine

Guidelines for Exercise Testing and Prescription (25). It should be noted that a BMI classified as overweight or obese is itself a risk factor. Therefore, individuals who qualified for the higher BMI groups displayed no other risk factors. To avoid the repeated bout effect of eccentric exercise, individuals accustomed to downhill walking or running, or who had completed a weight-training program in the past two months were excluded. Subjects were also excluded if they were currently taking hormone contraceptives (oral, injection, or patch) or other medications that may interact with the response to exercise such as lipid-lowering medications. Presence of any condition that would alter insulin or inflammation, or musculoskeletal limitations, were reasons for exclusion. All subjects were informed of the purpose, nature, and potential risks of participation and all gave written informed consent acknowledging their understanding of the risks and discomforts involved with participation in the study. This study was approved by the Institutional Review Board for protection of human subjects at Montana State University (APPENDIX A).

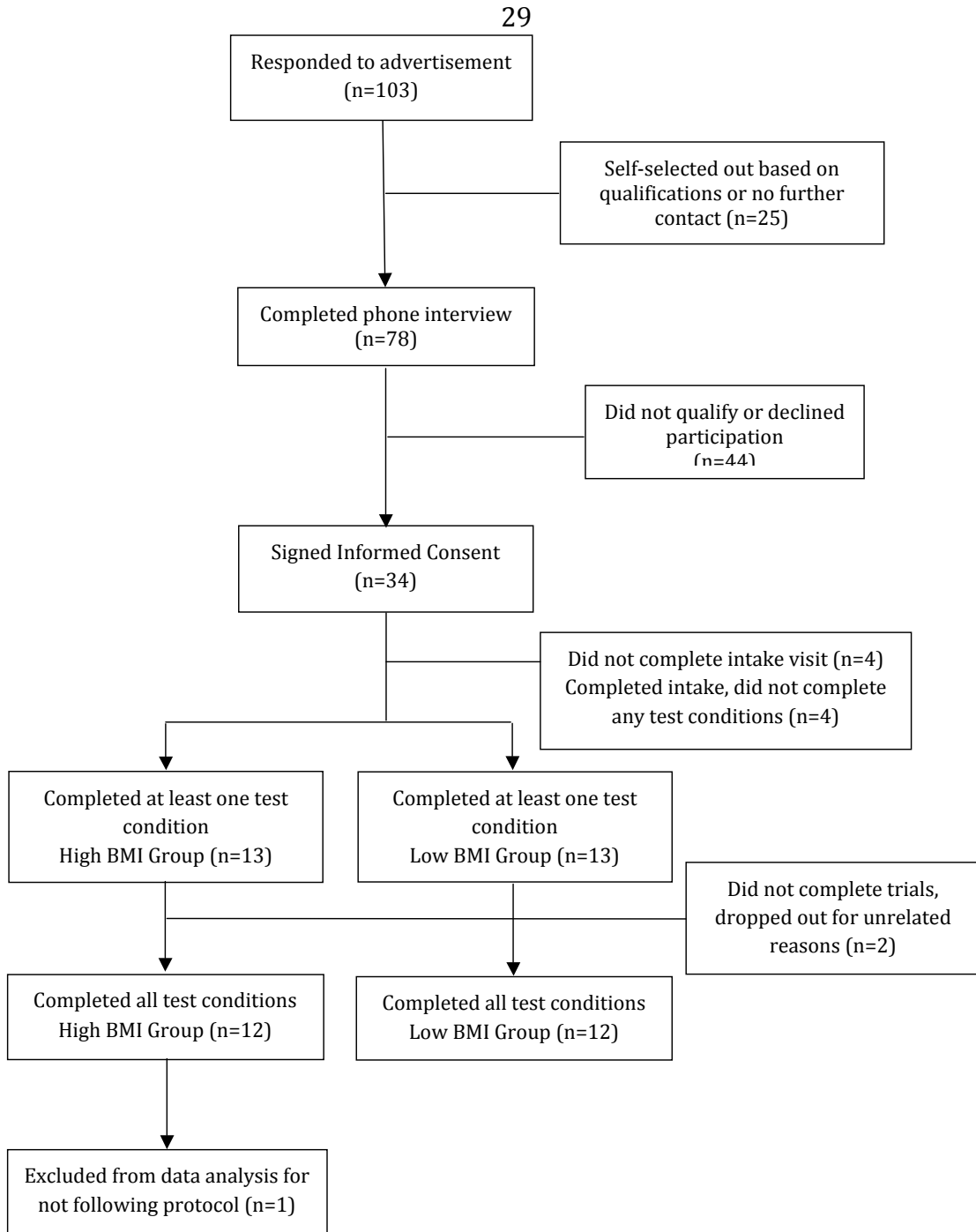


Figure 0.1 Consort diagram of research participation

Research Design

This study relied on a convenience sample of volunteers. The study was a within-subjects, randomized, counter-balanced, crossover design. Visit 1 consisted of a review of informed consent, a risk assessment using a health screening questionnaire (APPENDIX B) (74), completion of the Physical Activity Questionnaire (APPENDIX C) (75), the gathering of anthropometric and resting data, familiarization protocols, and the completion of a modified Bruce protocol submaximal fitness test (76). For visits 2, 5, and 8, participants reported to the Nutrition Research Laboratory (NRL) at ~7:00AM and followed the protocol described in Figure 3.2. After 15 minutes of rest, blood was drawn from an antecubital vein. Then one of the following conditions was completed: 1) uphill (U), 2) downhill (D), and control (C). Immediately following the condition, more blood was drawn. The participants were then allowed to cool down for 2 minutes and then rest for 30 minutes. After 30 minutes post-exercise, a mixed meal tolerance test was completed, with 7 finger stick blood collections over the course of 2 hours. The participants returned to the NRL after 8 hours for another blood draw and then again at 24 hours. With at least two weeks between visits, the other conditions were completed.

Prior to data analysis, participants were stratified into low waist circumference (LWC) and high waist groups (HWC). The International Diabetes Federation and the European Group for the Study of Insulin Resistance both identify

80cm as the cutoff above which metabolic risk is increased (61, 77). Therefore, the value for cutoff in this study is $\leq 80\text{cm}$ for the LWC group and $>80\text{cm}$ for the HWC group.

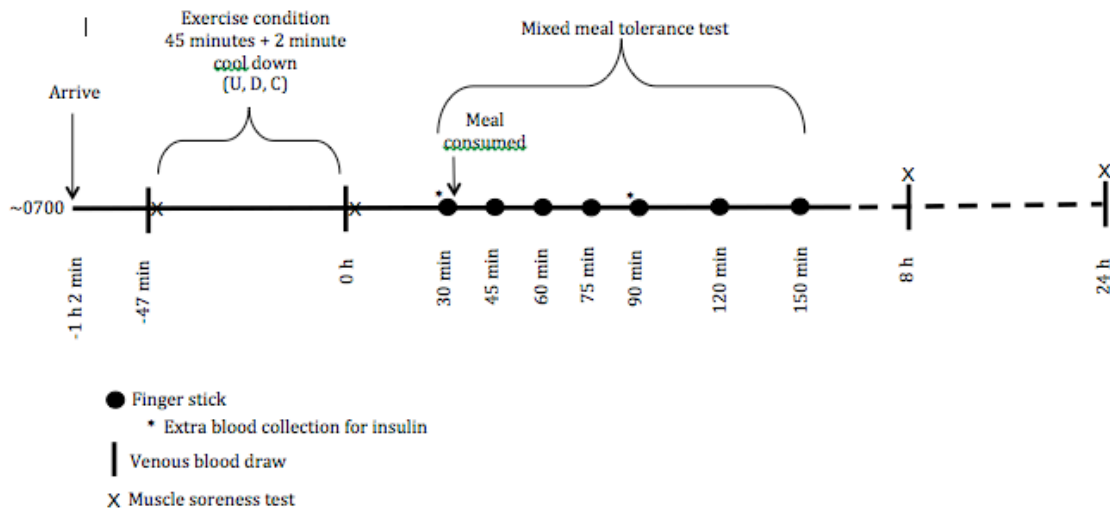


Figure 0.2 Design of exercise visit. Visits 2, 5, and 8 were randomized between uphill (U), downhill (D), and control (C).

Procedures

Informed Consent

After confirming eligibility with a phone interview (APPENDIX D), the participants were sent a copy of the informed consent form via email, which they were instructed to read through prior to first visit. At the beginning of Visit 1, the informed consent form was reviewed again and signed. The participants were provided with details of each visit as well as instructions for the duration of the

study. Discussion of risks and procedures was reviewed with participants at the beginning of each condition.

Anthropometrics

Body fat percentage was measured using air displacement plethysmography (BodPod GS, Cosmed, Rome, Italy) (78). Waist circumference was measured at the narrowest part of the torso between the xiphoid process and umbilicus (25) and hip circumference was taken at the widest circumference of the buttocks at the level of the greater trochanters (25); three measurements were taken of each circumference and repeated if measurements were more than 2cm apart. The values were then averaged.

Resting Measures

After sitting for at least 15 minutes, a brachial cuff (Omron Digital Blood Pressure Monitor, Omron Healthcare, Inc., Bannockburn, IL) was used to measure blood pressure and heart rate, displaying an average of two measures taken one minute apart.

Submaximal Testing

A modified Bruce protocol was conducted on a treadmill (PPS55, Woodway, Waukesha, WI). This test included stages of increasing intensity. The intensity was altered via grade and speed every 3 minutes until 85% of age-predicted maximal heart rate (APMHR) was reached or subject requested stopping the test. APMHR

was calculated using the equation $220 - \text{age}$ in years. Data from this test were used to plot heart rate and VO_2 values in order to determine HR at 65% of predicted $\text{VO}_{2\text{max}}$. Throughout the test, participants wore a heart rate monitor (WearLink + Coded transmitter 31, Polar, Kempele, Finland), ventilated through a mouthpiece, and wore a nose clip. Metabolic data were collected through a gas measurement system (TrueOne 2400, ParvoMedics, Sandy, UT).

Muscle Soreness

Perceived muscle soreness was measured using an algometer at the same marked locations on the anterior thigh. Four sites were labeled on the right anterior thigh and each site tested for soreness of the quadriceps femoris muscles. Participants were instructed to inform the researcher when the pressure applied by the algometer elicited pain or discomfort, at which point the algometer was removed and measures recorded. Participants also completed a 12-inch step-up and rated their soreness on a 100mm visual analog scale. Each leg was used twice, for a total of four step-ups, and participants were prompted to use leg muscles to achieve the step. These tests were repeated before exercise and immediately, 8-hours, and 24-hours post-exercise.

Exercise Conditions

Participants completed a 45-minute condition prior to completing the mixed meal tolerance test. Rating of perceived exertion was evaluated on the Borg scale (79).

Downhill Condition. Participants warmed up on the treadmill for 5 minutes at 2.0MPH and 0% grade. They walked downhill (-10%) with a vest (MiR Women's Weighted Vest, MiR Vest, Inc., San Jose, CA) equal to 10% of their body weight for 45 minutes at 3.0MPH. Water was consumed *ad libitum* and recorded for volume consumed. At 5-minute intervals, participants were asked to rate their perceived exertion. During the test, a heart rate monitor was worn and during the last minute of every other interval, a mouthpiece was worn to measure oxygen consumption. Subjects cooled down at 2.0MPH for 2 minutes at 0% grade.

Uphill Condition. Participants warmed up on the treadmill for 5 minutes at 2.0MPH and 0% grade. They walked uphill (+10%) for 45 minutes at a speed where heart rate reaches the value of 60% of estimated VO_{2max} , based on the test completed during visit 1. Water was consumed *ad libitum* and recorded for volume consumed. At 5-minute intervals, participants were asked to rate their perceived exertion. During the test, a heart rate monitor was worn and during the last minute of every other interval, a mouthpiece was worn to measure oxygen consumption. Subjects cooled down at 2.0MPH for 2 minutes at 0% grade.

Control Condition. No exercise was performed during the 45-minutes and subjects sat quietly.

Blood

Approximately 10-15 milliliters of blood was drawn from an antecubital vein pre-exercise as well as immediately, 8 hours, and 24 hours after completion of exercise (SST, BD Vacutainer, Franklin Lakes, NJ; K2 EDTA 7.2mg, BD Vacutainer, Franklin Lakes, NJ). Blood was gathered in a capillary tube (ClearCrit) and spun for five minutes in a micro-capillary centrifuge (model MB, International Equipment Company, Boston, MA). Hematocrit was then measured using a Critocaps capillary tube reader. Blood was collected in a microcuvette (Hb 201, HemoCue, Angelholm, Sweden) and analyzed for hemoglobin (Hb 201+, HemoCue, Angelholm, Sweden). Blood was immediately analyzed in a glucose analyzer (Glucose 201 with Plasma Conversion, HemoCue, Angelholm, Sweden) using microcuvettes (Glucose 201 Microcuvettes, HemoCue, Angelholm, Sweden).

Blood samples were centrifuged (Fisher 21000R, International Equipment Company, Needham Heights, MA), then serum was collected and stored at -80°C until assays were performed. Samples were analyzed for insulin, C-peptide, and creatine kinase (CK).

Extra blood was drawn during the first condition and analyzed for lipids. Immediately following the draw, approximately 100µL were pipetted into a reagent disc and then analyzed (Lipid Panel, Abaxis, Inc., Union City, CA; Piccolo Xpress Chemistry Analyzer, Abaxis, Inc., Union City, CA). Samples were analyzed for triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and non-HDL cholesterol.

Insulin and C-peptide Assays

Insulin and C-peptide concentrations were determined using an enzyme-linked immunosorbant assay (ELISA) according to the specifications of the manufacturer (MP Biomedicals, Solon, OH). Serum samples were thawed to room temperature. Then 50 μ L of controls, calibrators, and samples were pipetted into wells followed by 100 μ L of Insulin Enzyme Reagent. The plate was swirled and then left for 120 minutes. It was then decanted, washed with a buffer (300 μ L), and decanted again (repeated this for a total of three washes). Working substrate solution (100 μ L) was added to each well, which then sat at room temperature for 15 more minutes. Then stop solution (50 μ L) was added and mixed for 15-20 seconds. The samples were analyzed using KC Junior software and data were downloaded and calculated.

Creatine Kinase

Serum samples were thawed to room temperature. KC Junior software was used to analyze samples. Serum samples were pipetted into wells (15 μ L), in duplicate. After incubating the reagent (CK-NAC, Sigma Aldrich, St. Louis) to 37°C, 300 μ L was pipetted into each well. Immediately, the plate was placed in the spectrophotometer and read for a two-minute period to determine change in absorbance due to the creatine kinase enzyme activity. Data were downloaded for calculation of creatine kinase enzyme activity.

Mixed Meal Tolerance Test

Thirty minutes after exercise was completed, blood was drawn from a finger stick. Then a meal consisting of mostly carbohydrates and proteins, in similar composition to the recommended post-exercise meal by the Academy of Nutrition and Dietetics, was consumed (65). The composition of the mixed meal is presented in Table 3.1. Participants had 10 minutes to consume all of the food provided. Finger stick draws were repeated at minutes 15, 30, 45, 60, 90, and 120 from when eating commenced. For draws 0 and 60, additional blood was collected in Microtainers (BD, Franklin Lakes, NJ) and placed in a centrifuge (model 16K, Bio Rad, Hercules, CA) before being stored at -80°C. The blood gathered at all time points was immediately analyzed in a glucose analyzer (Glucose 201 with Plasma Conversion, HemoCue, Angelholm, Sweden) using microcuvettes (Glucose 201 Microcuvettes, HemoCue, Angelholm, Sweden).

Table 0.1 Macronutrient and energy breakdown of mixed meal

Food	Amount	CHO (g)	Pro (g)	Fat (g)	Kilocalories
Graham Crackers	31g	24	2	3	130
Chobani Nonfat Strawberry Yogurt	227g	27	19	0	190
Raisins	30g	23.25	0.75	0	97.5
Gatorade	8oz	14	0	0	50
TOTAL grams		88.25	21.75	3	467.5
Total Kcals		353	87	27	467.5
% of Kcals		75.6%	18.6%	5.8%	

Diet Analysis

The day leading up to the exercise as well as the day of the exercise, subjects were instructed to eat a similar diet for each trial. Subject diet records were input into NutritionistPro (Axxya Systems, Stafford, TX) based on recorded items and amounts. Entries were then analyzed for relevant energy and nutrient content.

Calculations

All blood values from immediately, 8 hours, and 24 hours after exercise completion were corrected for changes in blood volume using hemoglobin and hematocrit values from their pre-exercise blood draw (80). Glucose, insulin, and C-peptide values were used to calculate HOMA-IR values using the HOMA2 computer model (81). Fasting levels of glucose, insulin, and HOMA-IR were averaged for the three conditions as metabolic characteristics. Glucose area under the curve (AUC) was calculated using the trapezoid method.

Statistical Analysis

For each group, means were calculated. A normal distribution of variables was confirmed using the Kolmogorov-Smirnov test. Creatine kinase was non-normally distributed and was log transformed for analysis. Baseline and single point measures were tested for significance by an independent samples test, including Levene's test for equality of variances and 2-tailed t-test for equality of means. A multivariate analysis of variance (MANOVA) was performed for insulin, glucose, C-peptide, and HOMA, all values with two main time points, for within-subjects,

repeated measures significance. Bonferroni post-hoc tests were used to identify significant differences within multiple comparisons. Significance was set at an alpha of 0.05.

Results

Participants

Anthropometric and baseline characteristics divided by waist circumference groups are presented in Table 3.2. The HWC group had greater ($p<0.001$) body mass, BMI, BF%, and fat mass compared to the LWC group. The HWC group also had higher ($p<0.05$) lean mass, systolic blood pressure, and diastolic blood pressure while the LWC had higher ($p<0.05$) estimated VO_{2max} values. Physical activity days per week of 30 minutes or more and muscular exercises were similar, but days per week of stretching was higher ($p<0.05$) in the HWC group.

Table 0.2 Characteristics of subjects in low and high waist circumference groups

Variable	LWC (M ± SD) N = 13	HWC (M ± SD) N = 10	p-value
Age (years)	26.4 ± 5.1	30.9 ± 5.5	0.055
Body mass (kg)	60.0 ± 6.8	83.5 ± 12.6	<0.001*
Height (cm)	164.7 ± 4.4	166.1 ± 5.6	0.525
BMI (kg m ⁻²)	22.1 ± 2.3	30.2 ± 3.6	<0.001*
Body fat %	24.8 ± 6.8	38.7 ± 8.6	<0.001*
Lean mass (kg)	44.8 ± 4.5	50.5 ± 4.9	0.009*
Fat mass (kg)	15.1 ± 5.1	32.9 ± 11.2	0.001*
WC (cm)	71.5 ± 4.6	92.6 ± 9.0	<0.001*
PAQ			
d/wk >30 minutes PA	2.9 ± 1.7	4.0 ± 2.4	0.217
d/wk of muscular exercises	1.1 ± 1.3	0.7 ± 0.8	0.421
d/wk of stretching exercises	1.1 ± 1.3	3.2 ± 2.7	0.043*
Est VO _{2max}	46.9 ± 7.7	38.0 ± 8.9	0.017*
Systolic BP (mmHg)	98.2 ± 6.5	108.8 ± 8.2	0.002*
Diastolic BP (mmHg)	65.8 ± 4.4	71.6 ± 6.0	0.007*
RHR (bpm)	74.2 ± 11.8	72.5 ± 8.7	0.701

* $p < 0.05$ between groups

Metabolic characteristics are presented in Table 3.3. There were no significant differences found in metabolic characteristics between the two groups, and all values fell within metabolically healthy ranges. There was a trend ($p=0.074$) for the HWC group to have greater fasting insulin levels.

Table 0.3 Metabolic characteristics in low and high waist circumference groups

Variable	LWC (M ± SD) N = 13	HWC (M ± SD) N = 10	p-value
Triglycerides (mg/dL)	75.0 ± 31.7	83.5 ± 30.9	0.526
Total Cholesterol (mg/dL)	163.9 ± 33.4	164.9 ± 27.3	0.914
HDL (mg/dL)	63.2 ± 11.3	55.5 ± 8.0	0.083
LDL (mg/dL)	86.3 ± 27.1	93.2 ± 28.1	0.556
nHDLc (mg/dL)	102.3 ± 35.8	109.4 ± 29.2	0.616
VLDL (mg/dL)	14.9 ± 6.2	16.6 ± 6.2	0.525
Average FPG (mg/dL)	95.1 ± 7.7	98.5 ± 8.1	0.328
Average fasting insulin (μU/ml)	7.5 ± 3.3	10.8 ± 4.9	0.074
Average HOMA-IR	0.87 ± 0.38	1.21 ± 0.56	0.093

Diet Analysis

All conditions were similar for reported diet and are presented in Table 3.4. All subjects submitted at least one condition worth of records and one record was omitted due to lack of information. Each condition consisted of the day prior to exercise (day 1) and the day of exercise (day 2).

Table 0.4 Group means of diet records for the day prior to and day of exercise conditions

	Control		Uphill		Downhill	
	Day 1 (M ± SD)	Day 2 (M ± SD)	Day 1 (M ± SD)	Day 2 (M ± SD)	Day 1 (M ± SD)	Day 2 (M ± SD)
LWC (N= 12)						
Kcals	1547±250	1931±551	1689±422	2029±628	1898±269	2202±470
CHO (g)	184 ± 49	278 ± 74	209 ± 37	296 ± 37	227 ± 67	293 ± 70
Pro (g)	68 ± 28	81 ± 25	75 ± 23	79 ± 23	77 ± 18	92 ± 24
Fat (g)	62 ± 20	58 ± 32	65 ± 30	62 ± 25	79 ± 22	76 ± 25
Sugar (g)	68 ± 33	138 ± 30	75 ± 32	156 ± 50	86 ± 34	144 ± 38
Fiber (g)	18 ± 7	21 ± 7	23 ± 6	21 ± 6	22 ± 6	22 ± 8
HWC (N= 7)						
Kcals	1956±244	2103±666	1878±533	2129±515	1634±695	2015±314
CHO (g)	249 ± 40	267 ± 77	215 ± 76	271 ± 43	194 ± 87	252 ± 45
Pro (g)	70 ± 23	95 ± 30	76 ± 36	102 ± 30	71 ± 47	94 ± 25
Fat (g)	78 ± 18	76 ± 29	82 ± 29	74 ± 27	65 ± 34	73 ± 14
Sugar (g)	86 ± 47	138 ± 60	64 ± 40	124 ± 33	60 ± 41	126 ± 22
Fiber (g)	15 ± 6	17 ± 7	17 ± 4	16 ± 5	15 ± 7	14 ± 3

Exercise Protocols

The uphill walk was performed at 10% grade at an average of 56.8 ± 6.2 percent of estimated VO_{2max} . The downhill walk was performed at a set speed of 3.0MPH at -10% grade. Heart rate and RPE averages were not different between groups. The LWC group had higher ($p<0.05$) average relative VO_2 values but walked

at a similar percentage of estimated VO_{2max} . Average values for the treadmill conditions are presented in Table 3.5.

Table 0.5 Exercise responses during exercise conditions across WC groups

Variable	LWC (M \pm SD) N = 13	HWC (M \pm SD) N = 10	p-value
Eccentric Downhill Walking			
Average HR (bpm)	89 \pm 26	95 \pm 11	0.501
Average VO_2 (ml/kg/min)	11.3 \pm 1.8	10.0 \pm 2.1	0.130
Average % of Est. VO_{2max}	24.7 \pm 5.6	27.4 \pm 7.3	0.325
Average RPE	9.4 \pm 1.4	10.4 \pm 1.6	0.135
Concentric Uphill Walking			
Average HR (bpm)	135 \pm 14	136 \pm 11	0.882
Average VO_2 (ml/kg/min)	26.2 \pm 3.2	21.7 \pm 5.3	0.019*
Average % of Est. VO_{2max}	56.5 \pm 6.3	57.2 \pm 6.4	0.804
Average RPE	11.1 \pm 1.0	11.6 \pm 0.8	0.273

* $p < 0.05$ between groups

Muscle Damage Indices

Indirect measures of muscle damage were elevated in the uphill walking and downhill walking compared to the control condition. The step test measures of muscle soreness were increased threefold ($p < 0.05$) a day after the uphill walk and eightfold ($p < 0.05$) a day after the downhill walk (Figure 3.3). The pre-condition soreness ratings were similar. Two participants with high (greater than two standard deviations from the mean) baseline values in one or more condition were excluded from the analysis. One participant was not included in analysis. During the uphill condition, creatine kinase was increased ($p < 0.05$) from pre-exercise at immediately, 8 hours, and 24 hours post-exercise. In the downhill condition,

creatine kinase was increased ($p < 0.05$) at 8 hours compared to the pre-exercise value.

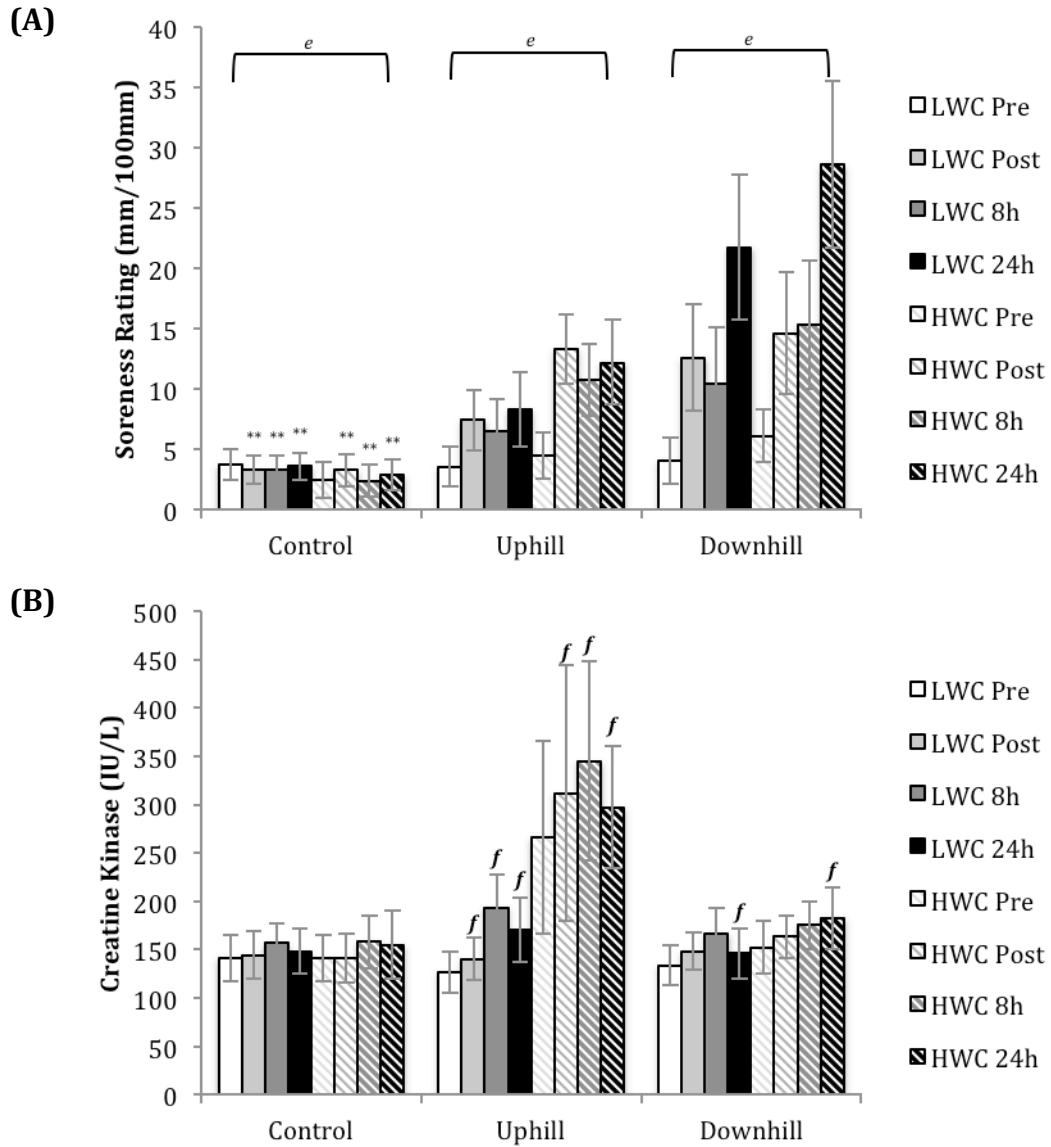


Figure 0.3 Muscle soreness (A) and creatine kinase (B) as indirect measures of muscle damage
 e $p < 0.05$ across conditions f $p < 0.05$ between time points compared to pre within that condition $**$ $p < 0.05$ compared to that same time point in other conditions

Insulin and Glucose Responses to Exercise

The intra-assay coefficient of variation for insulin was 13.5% and for C-peptide was 12.9%. Insulin measures were lower immediately following all conditions (Table 3.4). For both groups, there was a decrease ($p<0.05$) in insulin, C-peptide, and glucose from pre- to immediately post-exercise. For the control and uphill conditions, the HWC insulin values across pre-post time points were significantly higher ($p<0.05$) than those of the LWC group. The HWC group also had greater ($p<0.05$) C-peptide values across pre-post time points compared to the LWC group in the control condition.

Insulin and Glucose Responses to MMTT

Insulin increased in response to the mixed meal tolerance test. Compared to the LWC group, the HWC group had greater insulin ($p<0.05$) at 60 minutes during the control condition. Insulin at 60 minutes was also greater ($P<0.05$) following the downhill walk for the LWC group, compared to their control condition. For all conditions, there was a trend ($P=0.055$) for those of greater WC to have elevated glucose areas under the curve (AUC). There was a trend that the glucose AUC for the control was greater than that for the uphill condition for all participants ($P=0.058$). The downhill glucose AUC was greater than the AUC for the uphill walking ($P<0.05$).

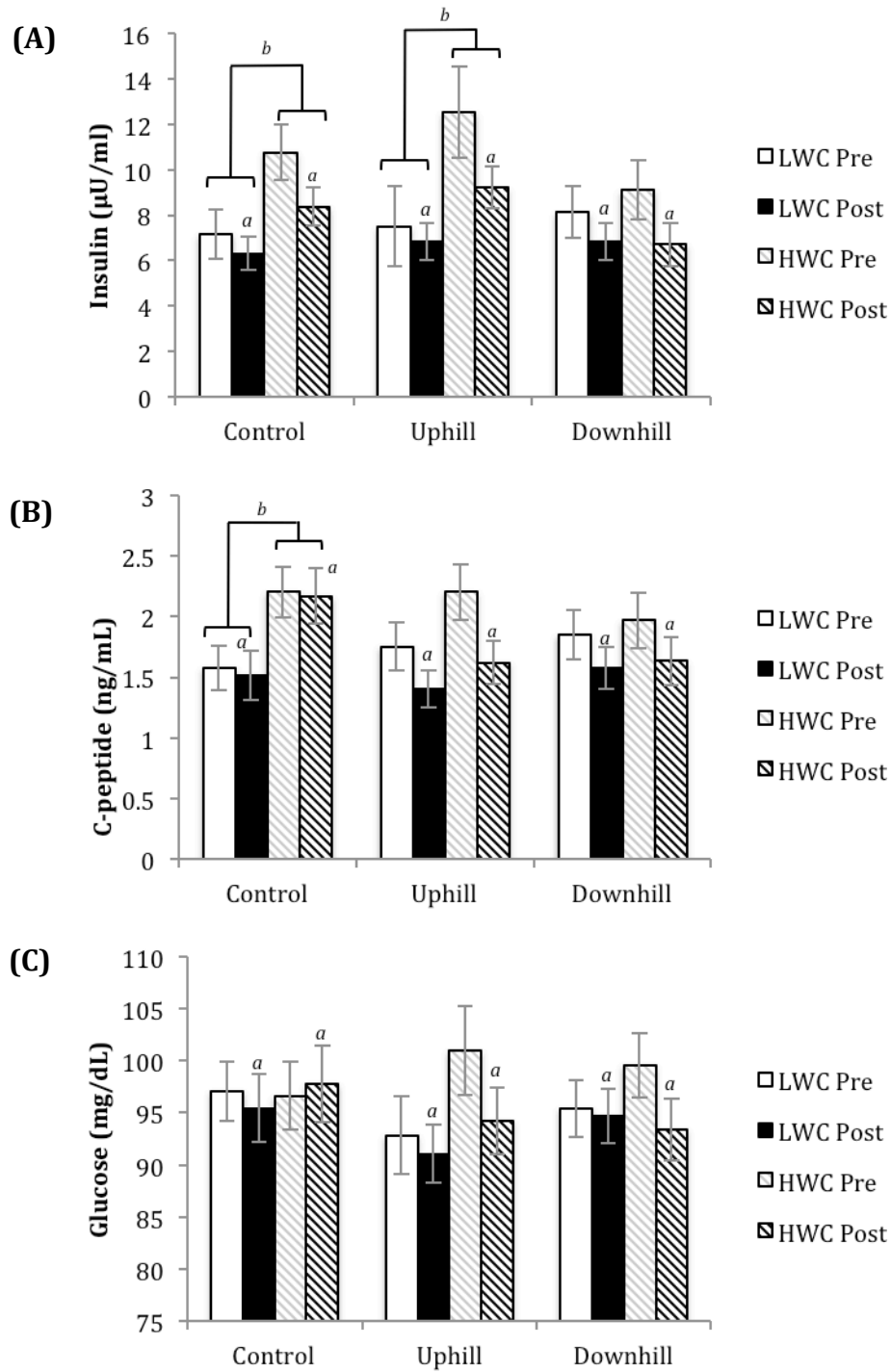


Figure 0.4 Insulin (A), C-peptide (B), and (C) glucose responses to exercise condition. ^a $p < 0.05$ for main effect of pre- compared to post- values, ^b $p < 0.05$ between groups for condition across pre-post time points

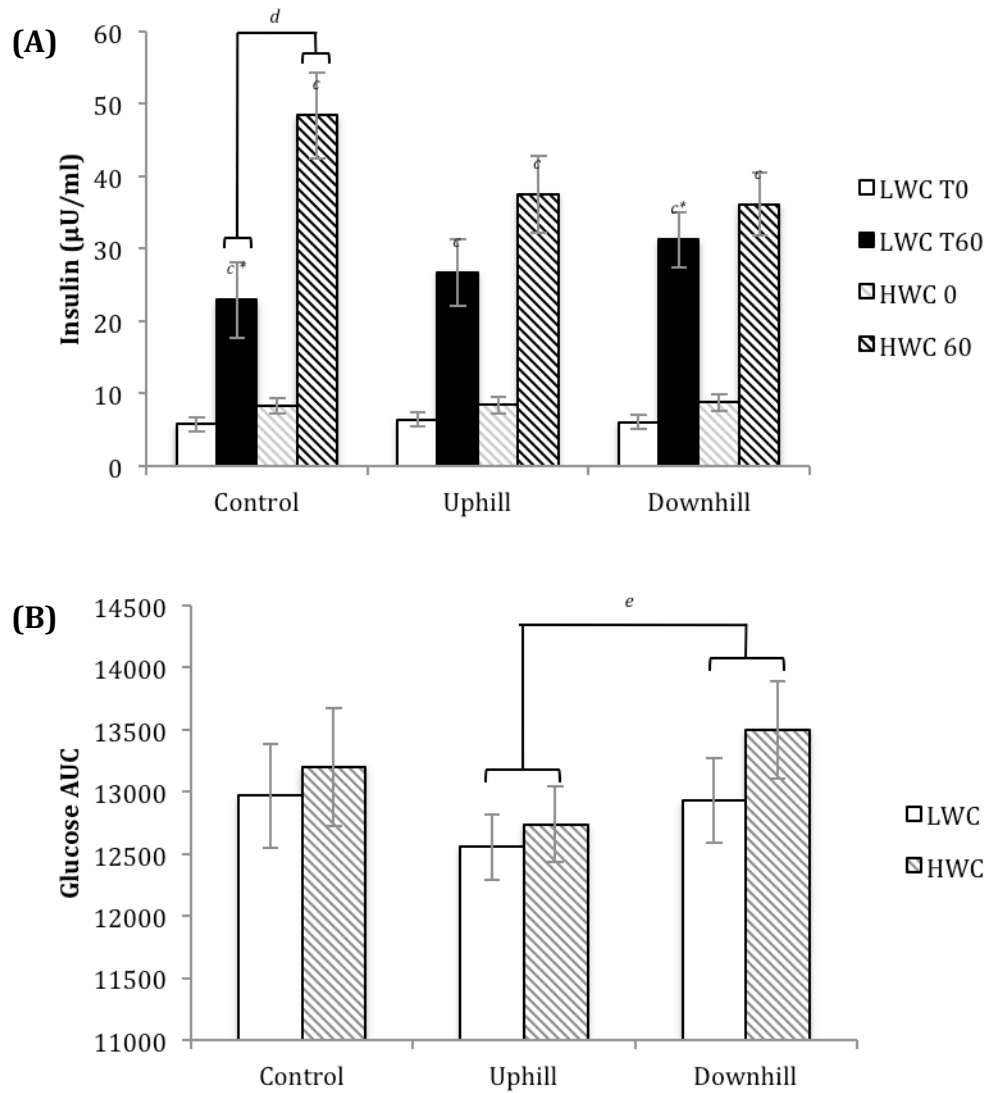


Figure 0.5 Insulin (A) and glucose (B) responses to mixed meal tolerance test. ^c $p < 0.05$ for main effect of 0 minute to 60 minute values ^d $p < 0.05$ between groups at that time point with condition * $p < 0.05$ within group differences between conditions ^e $p < 0.05$ between conditions.

Discussion

Post-exercise insulin measures were not different between uphill and downhill walking in women. There was an influence of waist circumference, where the HWC group had higher insulin across conditions. Waist circumference also influenced response to a mixed meal tolerance test completed following exercise conditions. In the control condition, a HWC was found to increase insulin in response to the meal at 60 minutes compared to a LWC, indicating differences in baseline metabolism. Exercise conditions differed in ratings of muscle soreness and creatine kinase.

We hypothesized that there would be a difference in insulin response to uphill walking, downhill walking, and resting. The mechanical damage induced by eccentric muscle contraction has been shown to transiently increase insulin in healthy subjects (13, 45). Despite increased measures of muscle damage, there was no difference in insulin response to these exercises for both groups. Based on preliminary research (18, 46), we expected an increase in insulin following the eccentric exercise. Our study did not confirm the previous findings. Other researchers have found decreased insulin following uphill walking, due to the insulin-like effect of exercise (45). However, we did not find the insulin response to be different during the uphill condition compared to the other conditions. The control condition provided a baseline measure of insulin levels during a fasted resting state, with which to compare the uphill and downhill walking conditions.

Waist circumference did not predict metabolically unhealthy resting values in young, healthy women. The International Diabetes Federation defines the cutoff for unhealthy WC at >80cm for women (59). A WC above 80cm is known to be associated with greater upper body fat distribution and increased risk for cardiovascular or metabolic risk factors (61). Decreased glucose tolerance, greater fasting insulin, and increased insulin during a tolerance test were all previously found in subjects with greater upper body adiposity (4). Willis and colleagues (73) found that minimal waist circumference was more highly and positively correlated with cardiovascular risk factors, such as fasting insulin and glucose compared with other anthropometric measurements in women. Despite being classified as having what the IDF would consider greater risk, for other metabolic values the HWC women did not exhibit further risk factors.

Waist circumference did influence insulin response to exercise. Resting metabolic values were similar between groups, but the HWC group had greater insulin across time points in the control and uphill walking. C-peptide values were used to determine insulin secretion based on the equimolar release of both C-peptide and insulin from the pancreas (72). C-peptide was higher during the control for the HWC group across time points compared the LWC group. The higher C-peptide corresponds with the elevated insulin found during this condition. The glucose AUCs were similar between groups in the control condition, so the increased insulin values indicate there was more insulin needed in the HWC group individuals, for the disposal of glucose into the peripheral tissues. During the uphill condition, C-

peptide levels were not higher than that of the LWC group, while the insulin levels were. Therefore, for the uphill walk, the HWC group did not require elevated insulin secretion to promote uptake of glucose into peripheral tissues, decreasing insulin disposal. This is considered an improvement in insulin sensitivity. Waist circumference did not influence response to downhill walking, possibly due to the fact that eccentric muscle damage caused similar insulin resistance in both groups. A WC over 80cm has been linked to increased fasting insulin values (73), but not to insulin responses to exercise. While the HWC group only demonstrated a trend for higher fasting insulin, the increased values in response to the control condition might be related to or associated with processes that lead to altered metabolism.

A mixed meal tolerance test following exercise was used to identify alterations in glycemic control. For the control condition, the insulin at 60 minutes in the HWC group was increased sixfold while the LWC group increased only fourfold. The meal compositions were identical, so the HWC group secreted more insulin than the LWC group in response to the same load. Elevated insulin values at 60 minutes have been associated with decreased insulin sensitivity (82). Therefore, the HWC group, while having healthy fasting insulin, presented as less insulin sensitive in response to the mixed meal. Transient effects of exercise can alter the response to a tolerance test (15, 83). Other researchers found that insulin was 50% greater at 60 minutes of an OGTT in a healthy population two days after completing eccentrically-biased exercise (15, 83). The LWC group had an increased insulin response at 60 minutes following downhill walking, compared to the control. This agrees with the

primary hypothesis that eccentric exercise increases insulin levels over control. The thirty minutes between termination of exercise and the MMTT may also contribute to the capturing of post-exercise increases in insulin in response to eccentric muscle damage.

Creatine kinase and soreness ratings of the quadriceps femoris muscles were used to indirectly measure muscle damage. The pre-exercise soreness tests were similar across conditions and groups, indicating that the subsequent increases were due to the exercise conditions. Eccentric exercise, in the form of downhill walking, is known to induce mechanical damage to muscle fibers, increasing measures of damage for up to five days following exercise (35, 37). Therefore, it was expected that measures of CK and soreness would be increased following the downhill walking condition. Creatine kinase differed across conditions. Levels of CK were elevated following the uphill walk, indicating there may have been oxidative damage as a result of the exercise. Creatine kinase was higher at 8 hours from pre-exercise levels following the downhill walk, indicating there was some damage from the exercise. Soreness was rated as 8 times higher at 24 hours in the downhill walking and 3 times higher in the uphill walking, compared to the control. Others researchers found the peak of soreness following muscle damage to be at least 24 hours, and up to 72 hours, post-exercise (41). In this study, the soreness was highest for the eccentrically-biased downhill walk at 24 hours, the last measure for the condition.

The focus of this study was the acute effect of an exercise bout on insulin measures and a mixed meal tolerance test, and there were several limitations to the design. In order to capture the effects of exercise, measures were gathered immediately following exercise. There were differences identified from this time point between groups and conditions. However, it is possible that the effects of the exercise bouts peaked at other times. For feasibility reasons, only four blood draws were included in the protocol but more time points could have revealed more information about the post-exercise differences. The intensity of the exercise bouts was chosen to reflect a real-world bout of physical activity. A more intense protocol could have elicited greater magnitudes of response but could be considered less applicable. The carbohydrate, specifically sugar, composition of the diet the day of exercise was greater than that of the background diet, most likely due to the mixed meal provided. Subjects ingested 49 grams of carbohydrates and 58 grams of sugar more on the test day than the day prior. It is possible that the subjects were unaccustomed to such high levels of carbohydrates, influencing their response to the MMTT.

In summary, uphill and downhill walking did not differ in insulin levels in a range of normal to obese women. When accounting for anthropometric differences, the differences between exercises were identifiable. Waist circumference does influence the insulin response to exercise and a mixed meal test, showing unhealthy trends towards altered metabolism in those of greater than 80cm WC.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

The primary hypothesis was not confirmed because the exercise conditions did not differ in insulin response. Exercise alone did not explain differences in responses that were found. Differences were found between HWC and LWC groups and it is possible that the differences in groups made differences between conditions alone less significant.

While the secondary hypothesis related body fat percentage and insulin response, another measure of body composition, waist circumference was used to identify metabolic differences. The use of BMI as a measure of general obesity is common, but WC is considered more accurate in identifying risk. Willis and colleagues (73) found that minimal waist circumference was more highly correlated with cardiovascular risk factors than other circumferences in women. Similarly, Ho et al. (84) found, that for women, BMI was not as good of a predictor of metabolic abnormalities as it was for men. Waist circumference is a more direct measure of central adiposity, increased levels of which have been associated with insulin resistance, inflammation, and poor metabolic health (58, 73). In most cases, women with a greater waist circumference had higher body fat percentages as well. It is possible, due to gender differences in fat distribution (4), that body fat was not allocated in unhealthy locations in some of our subjects, and measurements of central adiposity are more important in identifying alterations in metabolism (73).

Therefore, the subjects were grouped by WC to best identify metabolic differences.

Table 4.1 shows the three anthropometric classifications and the individuals who switched between groups while Figure 4.1 demonstrates the relationship between waist circumference and body fat percentage in the subjects.

Table 0.1 Grouping of subjects that did not consistently fall into high and low groups

Group for WC	Group for BMI	Group for BF%
80cm	Normal vs OW/Obese	30%
High	High	Low
High	Low	Low
Low	High	Low
Low	High	Low
Low	Low	High
Low	Low	High
spacLow	Low	High

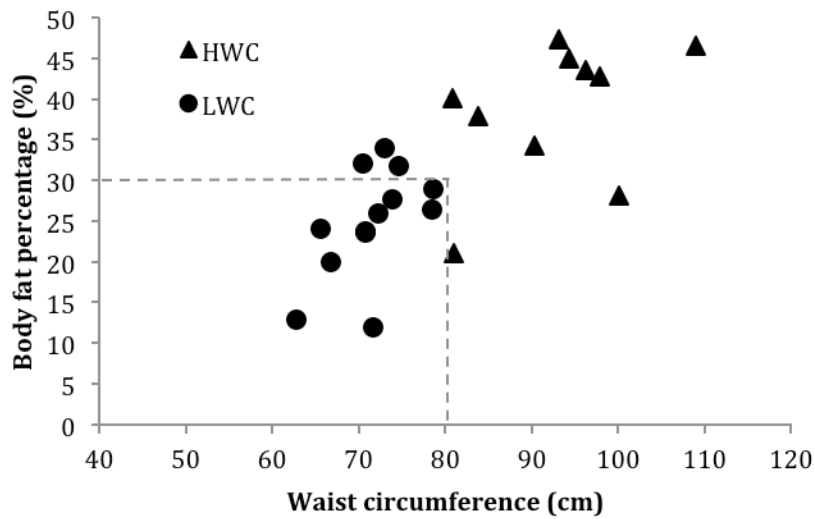


Figure 0.1 Scatterplot of HWC and LWC groups based on BF% and WC with cutoff points for the grouping identified with dotted lines.

Some baseline differences between the two groups were expected due to the association with increased waist circumference and other factors of cardiovascular or metabolic health. Researchers (84) found that increased WC was associated with elevated fasting insulin, elevated blood pressure and decreased HDL levels. While the differences between the two groups were not significant for HDL or fasting insulin, there was a trend for those in the HWC to have less healthy values. Both systolic and diastolic blood pressure measures were significantly higher in the HWC, in agreement with the literature. Levels of cardiorespiratory fitness did differ between groups. Higher levels of fitness are associated with improved glycemic control. Those in the LWC group did have a trend for lower fasting insulin while also having significantly higher estimate maximal oxygen uptakes. While there were cardiorespiratory differences between groups, there were not statistical differences in the metabolic values at rest. Both groups were metabolically healthy, with resting values falling within normal ranges.

Insulin indices were evaluated in the context of this study but ultimately not included to compare exercise conditions. The homeostatic model of assessment (HOMA-IR) values calculated from fasting concentrations were averaged and included to characterize the metabolic health of the groups. HOMA-IR was evaluated at the four major time points, but did not demonstrate statistical significance or interaction when included in the multivariate analysis. Instead, measures of C-peptide were included in the analysis to add to the levels of insulin, to better demonstrate the insulin dynamics throughout the conditions. C-peptide is released

in equimolar concentrations as insulin from the pancreas, but does not clear from the blood as quickly as insulin (72). An increased C-peptide value without an elevated insulin value is indicative of increased insulin secretion as well as insulin disposal. An elevated insulin without similar increases in C-peptide is indicative of normal secretion but decreased disposal of insulin. These dynamics helped identify the levels of insulin secretion without having to directly measure secretion or disposal.

The function soreness test did provide evidence for increased soreness following the eccentric condition. The studies relating insulin resistance to a bout of eccentric exercise found up to twenty fold increases in creatine kinase (14, 41), indicating elevated levels of muscle damage. While we did not find those elevations, the downhill walk protocol was a realistic bout of exercise for individuals unaccustomed to eccentric leg exercises. The low intensity of the walk could be identified as a limitation to eliciting responses of a detectable magnitude. However, we feel as though an increased intensity would not be as applicable as the intensity we chose.

Anthropometrics characteristics influence the insulin response to exercise and a mixed meal tolerance test. The exercise conditions were at applicable intensities and elicited significant response differences. Women of increased waist circumference who incorporate a novel bout of eccentrically-biased exercise into real life scenarios might experience similar alterations in insulin.

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APPENDICES

APPENDIX A

INSTITUTIONAL REVIEW BOARD CONSENT FORM

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

Study Title: Using body composition and metabolic differences to identify nutrition and exercise combinations that maximize health benefits.

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Summary:

You are being asked to volunteer as a participant in a research study where the researchers are trying to learn whether the health benefits of exercise are influenced by what you eat after exercise and how much fat you have in your body. Each person who participates will be asked to complete three study conditions: 1) a 45-minute downhill walk wearing a backpack with 10% of your body weight, 2) a 45-minute uphill walk at a moderate exercise pace, and 3) a control condition in which you will not exercise but all other experimental procedures will be performed. 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 from an arm vein twelve 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. We will also see how your blood glucose and insulin levels change after a meal eaten about 30 minutes after you exercise or rest (depending on the condition), in which we collect blood by sticking your finger before you eat and then six more times during the two hours after you eat a meal.

The purpose of this study is to determine how body composition influences glucose, insulin, and inflammation responses following two different types of exercise that differ in the way the muscles are used during exercise and in whether inflammation and soreness are induced. Specifically, we are asking the following questions: 1) How does the type of exercise influence insulin responses during exercise and to a post-exercise meal? 2) How does body composition influences inflammation after two types of exercise? 3) How does body composition influences glucose and insulin responses after two types of exercise?

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, with a body mass index 18.5 to 35 kg/m², and are not currently involved in an exercise program that could alter the muscular response to downhill walking, for example, activities that made your legs sore for a few days. You may not be a participant if you have an allergy to wheat, gluten, dairy, or peanuts, if you are currently using steroid hormone

APPROVED
MSU IRB
07/08/2013
Date approved

contraceptives (pills, injection, or patches), if you are taking blood pressure, cholesterol lowering or anti-inflammatory medications, if you are pregnant, if you have hypertension (high blood pressure), diabetes or 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 visit the Nutrition Research laboratory at Montana State University on 10 different occasions to complete baseline measures and three separate experimental conditions. In total, there will be 12 blood draws (4 draws in each of 3 conditions) and 21 finger sticks (7 finger sticks in each of 3 conditions) over the course of the entire study. During these visits you will be asked to do the following things:

You will need to refrain from eating or exercising for 3 hours prior to this visit. Visit 1 will take approximately 1 hour and include the following activities:

- 1) **Informed consent.** Read and provide written informed consent (this form). We also will provide you with a copy of this form and discuss it with you prior to Visit 1.
- 2) **Health screening questionnaire.** Complete a health history questionnaire that asks questions about your health and the health of your family, particularly regarding heart disease.
- 3) **Resting blood pressure measurement.** Two measures of blood pressure will be taken with a standard blood pressure cuff.
- 4) **Body size measurements.** The researcher will take baseline measurements of height, 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.
- 5) **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, and 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.
- 6) **Submaximal exercise test.** You will complete a sub-maximal treadmill test that will begin with you walking for 5 minutes to warm up the exercise test. The test involves a series of stages that get increasingly more difficult every 3 minutes. You will wear a heart rate monitor, breathe through a mouthpiece that is connected to an analyzer, and wear a nose clip. This allows researchers to measure how much you are breathing and the amount of oxygen you are using during the test. The test begins with walking, and as you move to more difficult stages, intensity is increased by increasing grade and speed, meaning that you may 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

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will take less than 20 minutes. You will not run at maximum intensity ever during the test but you will feel fatigued.

Dietary control during conditions 1, 2, and 3 will be needed so that the food and drinks that you consume will not interfere with the measurements of the study or be different from one condition to the next. The investigators will work with you to develop a diet of foods that you typically eat that will be approximately 60% carbohydrate, 20% protein, and 20% fat for the day prior to and the day of laboratory testing for each condition. A written copy of this 2-day diet plan will be given to you so that you can follow the same diet for these two days during each of the three conditions of the study.

Condition 1 (visits 2 to 4): Visit 2 will occur at least 4 days after visit 1, and you will be asked to not consume alcohol 24 h prior to, not to consume caffeine 18 h prior to, and not to eat anything 10 hours prior to your visit 2 appointment. You should drink plenty of water so that you are well hydrated throughout the study. This visit will take approximately 4 hours and include the following activities:

- 1) **Blood Collection.** After arriving at the appointment, you will sit for 15 minutes before having your blood drawn.
- 2) **Muscle soreness** will be determined using an algometer and using a questionnaire. 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. This will be done in 6 locations on one leg. The questionnaire will involve stepping up and then down on a step and then rating the level of soreness you feel by marking a line on the questionnaire. Four separate ratings of soreness will be made.
- 3) **One of the following protocols for 45 minutes: 1) downhill walking exercise, 2) uphill walking exercise, 3) no exercise (rest).**

Downhill walking: You will warm up on the treadmill for 5 minutes at a speed of 2.0 MPH and 0% grade. You will then walk downhill (-10%) with a backpack equal in weight to 10% of your body weight for 45 minutes. You may stop the test at any point. During this time, you may drink as much water as you would like. You will rate your perceived exertion at 5-minute intervals throughout the test. You will be wearing a heart rate monitor around your chest throughout the test. Towards the end of the test, you will breathe through a mouthpiece to measure how much oxygen you are consuming. After 45 minutes, you will have 2 minutes to cool down at 0% grade and a comfortable speed for you.

or

Uphill walking: You will warm up on the treadmill for 5 minutes at a speed of 2.0 MPH and 0% grade. You will then walk uphill (+10%) for 45 minutes at the speed where your heart rate reaches the value at 60% of estimated VO_{2max} , based on the test you did during visit 1. You may stop the test at any point. During this time, you may drink as much water as you would like. You will assess your rate of perceived exertion at 5-minute intervals throughout the test. You will be wearing a heart rate monitor around your chest throughout the test. Towards the end of the test, you will breathe through a mouthpiece to measure how much oxygen you are

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consuming. After 45 minutes, you will have 2 minutes to cool down at 0% grade and a comfortable speed for you.

or

No exercise: No treadmill exercise will be completed during this visit. You will just sit in the lab during the allotted time.

- 4) **Blood collection.** After a 2-minute cool down or continued rest, you will go directly from the treadmill or where you are seated to have your blood drawn. After this blood is drawn, you will get the chance to cool down as long as you desire.
- 5) **Mixed meal tolerance test.** This test is called a mixed meal tolerance test because it is a mixture of carbohydrate, protein, and fat in a meal followed by measurement of your blood glucose over a period of two hours. The test will begin 20 minutes after the exercise or no exercise protocol is completed. You will sit for at least 10 minutes and then blood will be collected from a finger stick. You will eat a meal consisting of graham crackers, peanut butter, and yogurt in 10 minutes or less. You will stay in the lab and additional blood samples will be collected from finger sticks 15, 30, 45, 60, 90, and 120 minutes after starting to eat the meal.

Visit 3 and visit 4 will occur 8 and 24 hours after you complete the exercise or no exercise protocol in visit 2. Again, you will need to not eat anything 10 hours before the 24 h appointment. These visits will take about 30 minutes, and you will do the following things at these visits:

- 1) **Blood Collection.** After arriving at the appointment, you will sit for 15 minutes before having your blood drawn.
- 2) **Muscle soreness.** The procedures described above using the algometer and questionnaire will be repeated.

Condition 2 (visits 5 to 7): At least 2 weeks after the completion of condition 1, you will repeat the procedures of visits 2 to 5, but with one of the protocols (downhill, uphill, or no exercise) not yet performed.

Condition 3 (visits 8 to 10): At least 2 weeks after the completion of condition 2, you will repeat the procedures of visits 2 to 5, but with the protocol (downhill, uphill, or no exercise) not yet performed.

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.

Risks: 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 12 occasions (4 draws in each of 3 conditions). This is a standard medical procedure, and will be performed by a

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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. You can stop at any time.
- 4) The uphill walking protocol will be performed at a moderate intensity of exertion. However, the exercise is likely to produce fatigue during and for up to a few hours following the exercise. People rarely have adverse side effects, but some have occurred before. 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.
- 5) The downhill walking 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.
- 6) Approximately 5 drops of blood will be removed with each finger stick (7 for each condition of the study). This is a standard method used to obtain blood for routine hospital laboratory tests. You will experience pain when the lancet goes into your finger. Other than this momentary pain, the discomfort of finger stick should be minimal. However, in about 10% of the cases a small amount of bleeding under the skin will produce a bruise (hematoma). A small scar may persist for several weeks. The risk of local infection is less than 1 in 1,000. You can stop at any time.

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

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- 1) You will get information about your body composition, 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 \$300 upon completion of your testing, \$100 for each of the three conditions completed. You may withdraw from the study at any time. If you choose not to complete a condition, then the amount of money paid to you will be prorated depending on how much of the condition is completed.

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 (to Mary Miles at 994-6678 or Sara Jay, Laura Horrigan, or Karen Brown at 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: This study is funded by the Montana IDeA Networks of Biomedical Research Excellence (INBRE).

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 data will be kept locked in a file cabinet in the Nutrition Research Laboratory. This information 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.

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STATEMENT OF AUTHORIZATION

Study Title: Using body composition and metabolic differences to identify nutrition and exercise combinations that maximize health benefits.

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: _____

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APPENDIX B

HEALTH SCREENING QUESTIONNAIRE

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health status by marking all *true* statements

History

You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac
- defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.

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 concerns about the safety of exercise.
- You take prescription medication(s).
- You are pregnant.

Cardiovascular risk factors

- You are a man older than 45 years.
- You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal.
- You smoke, or quit smoking within the previous 6 months.
- Your blood pressure is >140/90 mm Hg.
- You do not know your blood pressure.
- You take blood pressure medication.
- Your blood cholesterol level is > 200 mg/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 <30 minutes of physical activity on at least 3 days per week).
- You are > 20 pounds overweight.

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.

-
- None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

APPENDIX C

PHYSICAL ACTIVITY QUESTIONNAIRE

PHYSICAL ACTIVITY QUESTIONNAIRE

1) On how many of the past 7 days did you participate in physical activity for a total of 30-60 minutes or more over the course of a day? This includes moderate activities (walking, slow bicycling, or outdoor play) as well as vigorous activities (jogging, active games, or active sports such as basketball, tennis, or soccer)

_____ days during the past 7 days

2) On how many of the past 7 days did you do exercises to strengthen your muscles? This includes exercises such as push-ups, sit-ups, or weight lifting.

_____ days during the past 7 days

3) On how many of the past 7 days did you do stretching exercises to loosen up or relax your muscles? This includes exercises such as toe touches, knee bends, or leg stretching.

_____ days during the past 7 days

Taken from: Physical Best Physical Activity Questionnaire, developed from the YRBSS

CITATION: Cooper Institute for Aerobics Research. Fitnessgram Test Administration Manual. (2nd ed). Champaign, IL: Human Kinetics, 1999.

APPENDIX D

PHONE QUESTIONNAIRE

Phone Questionnaire	Body Composition and Metabolism Data	Tech. Initials _____
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Date: _____

Are you a woman between the ages of 18 and 40 years?

Name: _____

Gender: Male Female

Email: _____

Phone: _____

You are being asked to volunteer as a participant in a study where the researchers are trying to learn whether the health benefits of exercise are influenced by what you eat after exercise and your body composition. The study is comprised of a submaximal treadmill test as well as three test conditions: a downhill walk, an uphill walk and a control non-exercise trial. The downhill walk will make your leg muscles sore for a few days after the exercise. There will be 4 blood draws and 7 finger sticks with each of the 3 conditions. Body composition will also be measured by having you sit in chamber to determine your body volume.

There are a total of ten visits. Three of these visits are four hours long and take place in the morning in the Montana State University Nutrition Research Lab in 18 Herrick Hall. The other visits will not last as long but will be follow ups to the longer visits. Food will be provided for post-exercise meals but other components of the diet will not be provided but will be tracked.

Some benefits of participation in the study include body composition information as well as fasting glucose and blood lipid levels. Compensation for each condition will be \$100, up to three completed conditions, for a maximum of \$300.

Are you still interested?

 YES NO

Exclusion Criteria

In order to determine your eligibility in the study, I have a few questions to ask.

Are you currently participating in an exercise program with focus on leg muscles (plyometrics, downhill running)?

 YES NO

If yes, please describe: _____

Have you experienced any muscle soreness in the past 2 months?

 YES NO

If yes, please describe: _____

Do you have any musculoskeletal limitations/injuries?

 YES NO

If yes, please describe: _____

Do you take any anti-inflammatory medications regularly (ie aspirin, ibuprofen)

 YES NO

If yes, please describe: _____

Do you take lipid-lowering or cholesterol-lowering medications, or other types of medications including contraceptives?

YES NO

If yes, please describe: _____

If yes, does your contraceptive contain estrogen and/or progesterone? YES NO

Are you pregnant? YES NO

Are you allergic to peanuts, wheat or gluten? YES NO

Body weight: _____ (pounds) Height: _____ (inches)

Body Mass Index

kg/m² = _____ Normal Overweight/Obese Class I

The first visit to the lab consists of the following: informed consent, gathering body size measurements and body composition as well as a submaximal fitness test. For the best body composition accuracy, it is required that you wear tight fitting clothing made of lycra or a similar fabric. A swim suit or sports bra and compression shorts are suggested. Do not perform exercise within the 4 hours prior to this test and do not eat for 3 hours. Water may be consumed sparingly. For the performance of the submaximal treadmill test, running attire should be worn including your own shoes. Prior to this visit, I will be sending you an email with all of this information as well as the informed consent. Please read this before you come to the lab. You will be provided a printed copy at this first visit.

At the end of this appointment we can schedule further visits.

Do you have any questions?

Scheduled Date of Visit One: _____ **Time:** _____

Added to schedule