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The Effect of Post-Exercise Caffeine and Chlorogenic Acid Supplementation on Blood Glucose Disposal and Insulin Sensitivity

Jason Beam

Jason R. Beam
Candidate

Health, Exercise & Sports Sciences
Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Christine M. Mermier, Ph.D. , Chairperson

Ann L. Gibson, Ph.D.

Chad M. Kerksick, Ph.D.

Carole A. Conn, Ph.D.

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by

JASON R. BEAM

B.S., Mathematics, Louisiana Tech University, 2006
M.S., Exercise Science, Louisiana Tech University, 2007

DISSERTATION

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By

Jason R. Beam

B.S., MATHEMATICS, LOUISIANA TECH UNIVERSITY, 2006

M.S., EXERCISE SCIENCE, LOUISIANA TECH UNIVERSITY, 2007

Ph.D., PHYSICAL EDUCATION, SPORTS & EXERCISE SCIENCE, UNIVERSITY OF NEW MEXICO, 2013

ABSTRACT

Caffeine and chlorogenic acid are two compounds in green coffee beans that alter blood glucose disposal and insulin sensitivity. Caffeine has been shown to decrease glucose disposal and insulin sensitivity when taken 60 minutes prior to an oral glucose tolerance test in humans, whereas chlorogenic acid has been shown to increase glucose disposal and insulin sensitivity in humans. The purpose of this study was to investigate the effect of ingesting caffeine with dextrose or chlorogenic acid with dextrose immediately after an exhaustive bout of cycling on blood glucose and insulin disposal when compared to ingesting dextrose alone. Ten men (age: 26 ± 5 years; height: 179.9 ± 5.4 cm; weight: 77.6 ± 13.3 kg; BMI: 24.0 ± 4.3; VO_2 peak: 55.9 ± 8.4 $ml \cdot kg^{-1} \cdot min^{-1}$) who were moderately to highly trained cyclists participated in this study. Each

participant completed three experimental trials in random order the morning after abstaining from food, caffeine, and chlorogenic acid supplements for 12 hours. Each trial consisted of a 30-minute high intensity bout of cycling at 60% of peak power output (~90% HR max). Immediately after the exercise, each participant consumed 5 mg/kg body weight of caffeine plus 75 g of dextrose (CAF), 5 mg/kg body weight of chlorogenic acid plus 75 g of dextrose (CGA), or 5 mg/kg body weight of dextrose plus 75 g dextrose (PLA). Blood was drawn to measure glucose and insulin immediately before exercise, immediately after exercise, every 15 minutes during the first hour of recovery, and every 30 minutes during the second hour of recovery. The blood glucose and insulin area under the curve (AUC) and Matsuda insulin sensitivity index (ISI) were calculated for each trial. There were no significant time-by-treatment effects for blood glucose and insulin. The two-hour glucose and insulin AUCs, respectively, for the CAF (658 ± 74 mmol/L and 30,005 ± 13,304 pmol/L), CGA (637 ± 100 mmol/L and 31,965 ± 23,586 pmol/L), and PLA (661 ± 77 mmol/L and 27,020 ± 12,339 pmol/L) trials were not significantly different ($p > .05$). The ISI for the CAF (9.7 ± 5.2), CGA (12.1 ±7.9), and PLA (10.0 ± 7.3) trials were also not significantly different ($p > .05$). There was substantial inter-subject variability in glucose and insulin responses during the three trials that likely contributed to the non-significant findings. Pearson correlation analyses were conducted to investigate variables that contributed to this variability. Body mass index was highly related to insulin AUC for the CAF ($r = .71$), CGA ($r = .80$), and PLA ($r = .73$) trials. Relative VO_2 peak was moderately-to-highly related to insulin AUC for the CAF ($r = -.82$), CGA ($r = -.63$), and PLA ($r = -.63$) trials. In conclusion, caffeine and chlorogenic acid may affect the body's ability to regulate post-exercise insulin-mediated

glucose transport into the exercised skeletal muscle through different mechanisms; however more research is warranted to verify this hypothesis. Additionally, body composition and training status should be similar to lessen the variability between subjects for investigations of glucose tolerance and insulin sensitivity.

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SYMBOLS/ABBREVIATIONS

&: and

~: approximately

=: equals

≥: greater than or equal to

>: greater than

<: less than

· multiplication

%: percent

±: plus or minus

®: registered trademark

™: trademark

bpm: beats per minute

cm: centimeter

°C: degrees Celcius

g: gram

kg: kilogram

L: liter

max: maximum

m: meter

ml: milliliter

mg: milligram

mmol: millimole

µg: microgram

µL: microliter

µM: micromolar

µmol: micromole

µU: micro units

ml: milliliter

mmHg: millimeters of mercury

mM: millimolar

min: minute

nM: nanomolar

nmol: nanomole

oz: ounces

pg: picogram

pM: picomolar

rpm: revolutions per minute

W: watts

AMPK: 5' adenosine monophosphate-activated protein kinase

ANOVA: analysis of variance

AUC: area under the curve

BMI: body mass index

CAF: caffeine experimental trial

cAMP: cyclic adenosine monophosphate

CGA: chlorogenic acid experimental trial

CHO: carbohydrate

CO_2: carbon dioxide

DECAF: decaffeinated black coffee

ET: endurance trained

GI: glycemic index

GIP: gastric inhibitory polypeptide

GLP-1: glucagon-like peptide-1

GLUT4: glucose transporter type 4

G6Pase: glucose 6-phosphatase

G6P: glucose 6-phosphate

HIEG: hyperinsulinemic euglycemic clamp

HOMA-IR: homeostatic model assessment of insulin resistance

HR: heart rate

ISI: insulin sensitivity index

LIST: Loughborough Intermittent Shuttle Running Test

MT: moderately trained

OGTT: oral glucose tolerance test

O_2: oxygen

PLA: placebo experimental trial

PPO: peak power output

r: Pearson correlation coefficient

r^2: coefficient of determination

RCOF: caffeinated black coffee

RER: respiratory exchange ratio

RPE: rating of perceived exertion

SD: standard deviation

SE: standard error

SE: sedentary

SEM: standard error of measurement

SPSS: statistical package for the social sciences

T2DM: type 2 diabetes mellitus

VCO_2: volume of carbon dioxide

VE: ventilation

VO_2: volume of oxygen

vs.: versus

CHAPTER 1: Introduction

Carbohydrates and fats are the two major sources of energy for muscle contraction during rest and exercise. Carbohydrates are stored in the muscle and liver as glycogen, and broken down into glucose by a process called glycogenolysis. Glucose transport into the muscle during rest and during exercise is regulated by insulin and an insulin-dependent glucose transport protein called GLUT4 (Holloszy, 2008; Ivy, Zderic, & Fogt, 1999).

Type 2 diabetes mellitus (T2DM) is one of the leading causes of death in the United States and is characterized by the inability of the pancreatic beta-cells to secrete enough insulin to meet the demands of the body. Obesity and lack of regular exercise training is directly associated with increased risk for metabolic syndrome and T2DM (Church, 2011). Blood glucose disposal refers to the body's ability to take glucose from the blood and transport it into skeletal muscle and other organs, such as the liver. A chronic, high output of insulin from the pancreas in response to elevations in blood glucose is a result of insulin resistance and can lead to T2DM. Exercise training can help reduce adipose tissue, increase muscle mass, and subsequently decrease the risk for T2DM (American College of Sports Medicine & American Diabetes Association, 2010; Ivy et al., 1999). In addition, chronic and acute exercise training can improve insulin sensitivity and glucose disposal (Babraj et al., 2009; Bonen, Ball-Burnett, & Russel, 1998; Borghouts & Keizer, 1999; Hayashi et al., 2005; Holloszy, 2005; Ivy et al., 1999; Turcotte & Fisher, 2008).

In contrast, caffeine (trimethylxanthine), a naturally occurring compound that can be found in approximately 60 plants (Graham, Battram, Dela, El-Sohemy, & Thong,

2008), can counteract the effect of exercise on insulin sensitivity. Some of the most common sources of caffeine are coffee, tea, and colas. However, there are several medications, energy drinks, sports foods, and supplements that also contain caffeine (Burke, 2008). Peak concentrations of caffeine in the blood normally occur 30-90 minutes after ingestion, and half of this concentration is decreased in approximately five hours (Tarnopolsky, 2010). Caffeine supplementation can aid in the improvement of exercise performance by increasing exercise work capacity (Burke, 2008; Davis & Green, 2009; Goldstein et al., 2010; Graham, 2001), particularly through its effect on the neuromuscular system (Davis & Green, 2009; Tarnopolsky, 2008) rather than its effect on metabolism (Graham et al., 2008). Caffeine alters metabolism through several complex mechanisms (Graham, 2001; Graham et al., 2008), and it may help facilitate fat loss (Gonzalez & Stevenson, 2012; Jeukendrup & Randell, 2011; Tarnopolsky, 2010) and post-exercise glycogen resynthesis (Beelen, Burke, Gibala, & van Loon, 2010; Pederson et al., 2008).

Although pure caffeine supplementation may enhance performance by increasing exercise work capacity, facilitating fat loss, and increasing post-exercise glycogen resynthesis, it may also negate the exercise-induced insulin sensitivity response to training and the body's ability to tolerate elevated glucose levels at rest. Caffeine reduces the beneficial effects of prior exercise on insulin sensitivity and glucose transport into the muscle (Thong et al., 2002). In addition, caffeine has been shown to decrease the insulin-mediated whole body glucose disposal and insulin sensitivity in humans during an oral glucose tolerance test (OGTT) (Battram, Arthur, Weekes, & Graham, 2006; Graham et al., 2001; Petrie et al., 2004; Pizziol et al., 1998; Robinson et al., 2004). An OGTT is a

popular method used by clinicians to test for diabetes or one's ability to clear glucose from the blood.

Caffeine alters glucose homeostasis through two major mechanisms of action. Caffeine can indirectly alter glucose homeostasis by stimulating the secretion of epinephrine from the adrenal medulla (Graham, 2001). Epinephrine has been shown to play a role in the caffeine-induced impairment in glucose tolerance in humans (Battram, Bugaresti, Guspa & Graham, 2007a; Battram, Graham, & Dela, 2007b; Battram, Graham, Richter, & Dela, 2005; Thong & Graham, 2002a; Thong et al., 2002). Adenosine has been suggested to play a role in insulin- and exercise-mediated regulation of glucose transport and glycogen metabolism in the skeletal muscle of rodents (Thong & Graham, 2002b). It was shown that the activation of A_1 adenosine receptors in isolated rat soleus muscle contributes approximately 50% to insulin-stimulated muscle glucose transport (Thong et al., 2007). In addition, caffeine may directly antagonize non-selective adenosine receptors in human skeletal muscle, which may play a role in insulin-mediated glucose disposal (Graham et al., 2008; Mohr, Van Soeren, Graham, & Kjaer, 1998; Van Soeren, Mohr, Kjaer, & Graham, 1996). However, the effect of adenosine on human skeletal muscle glucose uptake is not clear (Battram et al., 2007b; Battram et al., 2005).

Although coffee contains substantial amounts of caffeine, chronic coffee consumption is associated with a decreased risk for developing T2DM (Huxley et al., 2009; Salazar-Martinez et al., 2004; van Dam, 2008). Drinking caffeinated coffee may not be as detrimental as ingesting pure caffeine because other compounds in coffee may lower intestinal absorption of glucose, help spare glycogen, or increase glucose clearance (Battram et al., 2006; de Paulis et al., 2002; Goldstein et al., 2010; Graham, Hibbert, &

Sathasivam, 1998; Johnston, Clifford, & Morgan, 2003; Tarnopolsky, 2010). As a result, these compounds in coffee may counteract caffeine's negative effect on glucose disposal and insulin sensitivity and subsequently decrease the risk of T2DM in coffee drinkers (van Dam, 2008).

Chlorogenic acid is a natural polyphenol found in green coffee beans that has been shown to increase glucose disposal during an OGTT in animals (Bassoli et al., 2008) and in humans (Thom, 2007; van Dijk et al., 2009). Research has shown that ingesting chlorogenic acid may have possible health benefits by slowing glucose absorption in the gastrointestinal tract and subsequently reducing the amount of glucose absorbed into the blood (Bassoli et al., 2008; Johnston et al., 2003; McCarty, 2005; Tarnopolsky, 2010). However, the results of a recent research study did not support the hypothesis that chlorogenic acid attenuates the absorption of glucose in the intestines (Olthof, van Dijk, Deacon, Heine, & van Dam, 2011).

Instead, a more notable hypothesis is that chlorogenic acid may inhibit hepatic glucose 6-phosphatase (G6Pase) and subsequently prevent glucose 6-phosphate (G6P) hydrolysis (Arion et al., 1997). Glucose 6-phosphatase is an enzyme that plays a major role in the homeostatic regulation of blood glucose. It catalyzes the final step of gluconeogenesis and glycogenolysis, which are the two main metabolic pathways responsible for the release of glucose in the liver (Bassoli et al., 2008). Since chlorogenic acid has been shown to inhibit G6Pase, it may also help spare glycogen. Furthermore, it was recently shown that chlorogenic acid stimulates glucose transport in rat skeletal muscle via 5' adenosine monophosphate-activated protein kinase (AMPK) activation (Ong, Hsu, & Tan, 2012). Therefore, chlorogenic acid supplementation may help spare

glycogen and enhance glucose disposal in humans. As a result, there may be less accumulation of glucose and insulin in the blood after consuming chlorogenic acid with glucose when compared to consuming caffeine with glucose.

The effect of consuming caffeine before exercise on glucose metabolism during exercise has been reviewed (Graham et al., 2008). The effect of post-exercise caffeine supplementation on glucose metabolism and homeostasis during an acute recovery has been investigated in only a few studies (Beelen et al., 2012; Pederson et al., 2008; Taylor et al., 2011; Thong et al. 2002). To our knowledge, there have not been any human studies that have investigated the effects of pre- or post-exercise chlorogenic acid supplementation on blood glucose disposal and insulin sensitivity. Based on the evidence above, post-exercise co-ingestion of chlorogenic acid with glucose may help facilitate glucose disposal and increase insulin sensitivity more than the co-ingestion of caffeine with glucose during the post-exercise recovery period when compared to ingesting glucose alone.

Purpose of Study

The purpose of this study was to investigate the effect of ingesting caffeine plus dextrose or chlorogenic acid plus dextrose immediately after an exhaustive bout of exercise on blood glucose and insulin disposal when compared to ingesting dextrose alone.

Hypotheses

This section lists the hypotheses and rationales of this research study. There are two major hypotheses (1 and 2) that are split into three sub-hypotheses (a, b, and c). The two major hypotheses refer to the dependent variables that will be analyzed: serum

glucose and serum insulin. The sub-hypotheses compare the three levels of the independent variable, which is the treatment: (a) 75 g of dextrose plus 5 mg/kg body weight of chlorogenic acid (via green coffee bean extract) vs. 75 g of dextrose plus 5 mg/kg body weight of caffeine (via powder) (b) dextrose plus chlorogenic acid vs. placebo (75 g of dextrose), and (c) dextrose plus caffeine vs. placebo. The hypotheses were tested by using differences in area under the curve (AUC) since AUC combines several data points into one value to estimate bioavailability and clearance of blood glucose and insulin.

The rationales for the hypotheses are split into three sections. The rationale for hypotheses 1a and 2a are combined into the first section; the rationale for hypotheses 1b and 2b are combined into the next; and the rationale for 1c and 2c in the last section.

Hypothesis 1a

Ingesting chlorogenic acid with dextrose immediately after an exhaustive bout of cycling will result in significantly lower serum glucose AUC during a two-hour OGTT when compared to ingesting caffeine with dextrose.

Hypothesis 1b

Ingesting caffeine with dextrose immediately after an exhaustive bout of cycling will result in significantly greater serum glucose AUC during a two-hour OGTT when compared to ingesting dextrose alone.

Hypothesis 1c

Ingesting chlorogenic acid with dextrose immediately after an exhaustive bout of cycling will not result in significantly different serum glucose AUC during a two-hour OGTT when compared to ingesting dextrose alone.

Hypothesis 2a

Ingesting chlorogenic acid with dextrose immediately after an exhaustive bout of cycling will result in significantly lower serum insulin AUC during a two-hour OGTT when compared to ingesting caffeine with dextrose.

Hypothesis 2b

Ingesting caffeine with dextrose immediately after an exhaustive bout of cycling will result in significantly greater serum insulin AUC during a two-hour OGTT when compared to ingesting dextrose alone.

Hypothesis 2c

Ingesting chlorogenic acid with dextrose immediately after an exhaustive bout of cycling will not result in significantly different serum insulin AUC during a two-hour OGTT when compared to ingesting dextrose alone.

Rationale for Hypotheses 1a and 2a

Chlorogenic acid, a compound found in coffee, may help lower intestinal absorption of glucose, help spare glycogen, or increase glucose clearance from the blood (Battram et al., 2006; de Paulis et al., 2002; Goldstein et al., 2010; Graham, Hibbert, & Sathasivam, 1998; Johnston, Clifford, & Morgan, 2003; Tarnopolsky, 2010). As a result, chlorogenic acid may counteract caffeine's negative effect on glucose disposal and insulin sensitivity (van Dam, 2008). Chlorogenic acid has been shown to inhibit hepatic G6Pase and subsequently prevent G6P hydrolysis in rats (Arion et al., 1997). Glucose-6-phosphatase is an enzyme that plays a major role in the homeostatic regulation of blood glucose. It catalyzes the final step of gluconeogenesis and glycogenolysis: the two main metabolic pathways responsible for the release of glucose in the liver (Bassoli et al.,

2008). Since chlorogenic acid has been shown to inhibit G6Pase, it may also help spare glycogen. It was recently shown that chlorogenic acid stimulates glucose transport in rat skeletal muscle via AMPK activation (Ong, Hsu, & Tan, 2012). Therefore, chlorogenic acid supplementation may help spare glycogen and enhance glucose disposal in humans. As a result, ingesting chlorogenic acid with dextrose immediately after exercise may significantly decrease glucose and insulin AUC during an OGTT when compared to ingesting caffeine with dextrose.

Rationale for Hypotheses 1b and 2b

Caffeine has been shown to decrease insulin-mediated whole body glucose disposal and insulin sensitivity in humans during an OGTT when compared to ingesting glucose alone (Battram et al., 2006; Graham et al., 2001; Petrie et al., 2004; Pizziol et al., 1998; Robinson et al., 2004). Caffeine has also been shown to reduce insulin-dependent glucose clearance in skeletal muscle (Thong & Graham, 2002a). Caffeine-induced reduction in insulin-stimulated glucose uptake in human skeletal muscle may be a major contributor to the caffeine-induced reduction in whole body glucose disposal (Thong et al., 2002). Exercising before consuming caffeine has been shown to reduce the deleterious effects of caffeine on insulin-dependent glucose clearance in skeletal muscle; however, caffeine also reduced the beneficial effects of exercise on insulin sensitivity and glucose transport into the muscle (Thong et al., 2002). Therefore, ingesting caffeine with dextrose immediately after exercise may significantly increase glucose and insulin AUC during an OGTT when compared to ingesting dextrose alone.

Rationale for Hypotheses 1c and 2c

Few studies to date have investigated the effect of pure chlorogenic acid supplementation on blood glucose and insulin in humans during an OGTT. van Dijk et al. (2009) investigated the effects of consumption of a supplement containing 12 g of decaffeinated coffee, 1 g of chlorogenic acid, 500 mg of trigonelline, and a 1 g of mannitol (placebo) on glucose and insulin concentrations during an OGTT in 15 healthy, non-smoking, overweight, and coffee-consuming men. Subjects ingested the supplements 30 minutes prior to ingesting 75 g of glucose. Glucose and insulin were measured every 15 minutes during the 120-minute OGTT. Chlorogenic acid ingestion significantly reduced glucose and insulin concentrations only at the 15-minute time-point when compared to the placebo, and there was not a significant reduction in the AUC for glucose and insulin when compared to the placebo.

Thom (2007) investigated the effect of chlorogenic acid-supplemented coffee on the glucose profile of healthy volunteers compared to normal coffee. Six healthy women and six healthy men ingested four different treatments in random order after a 12 hour overnight fast: (a) 25 g of sucrose in 400 ml of water (control), (b) 25 g of sucrose and 10 g of instant coffee (~300-400 mg of chlorogenic acid) in 400 ml of water, (c) 25 g of sucrose and 10 g of instant decaffeinated coffee (~300-400 mg of chlorogenic acid), and (d) 25 g of sucrose and 10 g of Coffee Slender (~400-450 mg of chlorogenic acid) 400 ml of water. Blood samples were drawn immediately before the treatment and throughout the two hours after the treatment (15, 30, 45, 60, 90, and 120 minutes) to measure plasma glucose concentrations. The two-hour glucose AUC was significantly lower after consuming the Coffee Slender (724 ± 8.2 mmol/L) when compared to the control (778 ±

10.2 mmol/L). The two-hour glucose AUC for the instant caffeinated coffee (788 ± 10.1 mmol/L) and instant decaffeinated coffee (818 ± 10.9 mmol/L) was not significantly different than the control. Based on the results of these two studies, ingesting chlorogenic acid with dextrose immediately after exercise is equivocal whether blood glucose and insulin are significantly altered when compared to ingesting dextrose alone. Since there is not enough clear evidence to show that chlorogenic acid significantly alters blood glucose and insulin AUC during an OGTT when compared to a placebo, we hypothesized that there would not be any significant differences between the chlorogenic acid and placebo treatments.

Scope of the Study

Ten men who were moderately to highly trained in cycling participated in this study. Subjects were recruited by word-of-mouth, email list serves and posted flyers. They were between the ages of 19 and 34 years and were free of any cardiovascular, pulmonary or metabolic disease. Subjects reported their daily consumption of caffeine using a questionnaire. Subjects who did not regularly ingest ≥ 2 caffeinated coffee or tea beverages and/or ≥ 5 caffeine-containing soft drinks per week caffeine were excluded from this study. The experimental procedures and possible risks were explained to each participant verbally and in writing. They signed an informed consent and HIPAA release prior to participation. These documents and the study were approved by the Human Research Protection Office.

This study consisted of three experimental trials conducted in the morning after a 12-hour fast from food, caffeinated products, and chlorogenic acid supplements. Participants were also asked to refrain from exhaustive exercise (greater than 80% HR

max) and avoid alcohol intake 24 hours prior to each experimental trial. Each trial was randomized and separated by at least one week. The treatments were administered in a double-blinded fashion. Participants completed a 30-minute high-intensity (60% peak power, ~90% HR max) bout of cycling during each trial. The treatment consisted of immediate post-exercise co-ingestion of 75 g of dextrose with 5 mg/kg body weight of caffeine, 75 g of dextrose with 5 mg/kg body weight of chlorogenic acid (green coffee bean extract) or a placebo (75 g of dextrose). Blood samples were drawn every 15 minutes during the first hour and every 30 minutes during the second hour of a two-hour post-exercise OGTT to measure insulin and glucose. These measurements showed how chlorogenic acid and caffeine affected insulin and the subsequent disposal of glucose during the post-exercise OGTT when compared to the placebo.

Assumptions

The following assumptions were made in this study:

1. Subjects regularly consumed ≥ 2 caffeinated coffee or tea beverages and/or ≥ 5 caffeine-containing soft drinks per week.
2. Subjects accurately reported the amount of caffeinated products they consumed.
3. Subjects accurately recorded their diet and physical activity record for 24 hours before the first experimental trial and replicated their diet and physical activity for the subsequent experimental trials.
4. Subjects refrained from exhaustive exercise and alcohol for 24 hours prior to each trial.
5. Subjects reported to the lab for each trial the morning after a 12-hour overnight fast from food, caffeine, and chlorogenic acid supplements.

6. Investigators involved with the study and the subjects were blinded to the treatment.
7. Blood samples were not contaminated with saline.

Limitations

The following are limitations in this study:

1. The study group consisted of healthy men between the ages of 19 and 34 years who were moderately to highly trained cyclists.
2. There have only been few studies that have investigated the effect of ingesting chlorogenic acid prior to an OGTT in humans with different findings (Thom, 2007; van Dijk et al., 2009). Therefore, consuming chlorogenic acid with dextrose may not be more beneficial than consuming dextrose alone in our study.
3. Due to possible limitations in funding, only glucose and insulin were measured and analyzed.
4. Green coffee bean extract (CoffeeGenic^{TM}, Life Extension®) was used as the source of chlorogenic acid. This green coffee bean extract was standardized to 50% chlorogenic acid. The other 50% of the constituents in the green coffee bean extract may have had an effect on the results from our study. Therefore, we could only speculate that chlorogenic acid was the major contributor to green coffee bean extract's effect on blood glucose and insulin.

Significance of Study

Although pure caffeine supplementation may enhance performance, facilitate fat loss, and increase post-exercise glycogen re-synthesis, it may also negate the exercise-induced insulin sensitivity response to training and the body's ability to tolerate elevated

glucose levels at rest. It has been shown that exercising before consuming caffeine reduces the deleterious effects of caffeine on insulin-dependent glucose clearance in human skeletal muscle (Thong et al., 2002). However, it was also shown in this same study that caffeine reduces the beneficial effects of exercise on insulin sensitivity and glucose transport into the muscle. In addition, it has been shown in several studies that caffeine decreases insulin-mediated whole-body glucose disposal and insulin sensitivity in humans during an OGTT (Battram, Arthur, Weekes, & Graham, 2006; Graham et al., 2001; Greer, Hudson, Ross, & Graham, 2001; Petrie et al., 2004; Pizziol et al., 1998; Robinson et al., 2004).

Chronic coffee consumption is associated with a decreased risk for developing T2DM (Huxley et al., 2009; Salazar-Martinez et al., 2004; van Dam, 2008). Drinking caffeinated coffee may not be as detrimental as ingesting pure caffeine because of other compounds in coffee that may lower intestinal absorption of glucose, help spare glycogen, or increase glucose clearance (Battram et al., 2006; de Paulis et al., 2002; Goldstein et al., 2010; Graham, Hibbert, & Sathasivam, 1998; Johnston, Clifford, & Morgan, 2003; Tarnopolsky, 2010). As a result, one or more of these compounds in coffee may counteract caffeine's negative effect on glucose disposal and insulin sensitivity and subsequently decrease the risk of T2DM in coffee drinkers (van Dam, 2008).

Chlorogenic acid is a natural polyphenol found in green coffee beans that has been shown to increase insulin sensitivity and glucose disposal during an OGTT in animals (Bassoli et al., 2008) and in humans (van Dijk et al., 2009). Our study sought to determine whether post-exercise supplementation of dextrose with chlorogenic acid

induces significantly lower glucose and insulin AUC during a two-hour OGTT when compared to consuming caffeine with dextrose. In addition, we wanted to provide evidence as to whether chlorogenic acid and/or caffeine contribute to the beneficial effects of coffee in reducing the risk for T2DM.

Definition of Terms

5' adenosine monophosphate-activated protein kinase (AMPK) is an enzyme that plays a role in cellular glucose homeostasis. When AMPK is activated, it stimulates muscle glucose uptake and modulation of insulin secretion by pancreatic beta-cells.

Adenosine is a purine nucleoside comprising a molecule of adenine attached to a ribose sugar molecule. It plays an important role in biochemical processes, such as energy transfer as well as in signal transduction.

Area under the curve (AUC) in the present study was the area under the plot of glucose and insulin concentrations during a two-hour oral glucose tolerance test. The AUC was used to estimate the bioavailability and clearance of blood glucose and insulin.

Chlorogenic acid is a family of esters of hydroxycinnamic acids with quinic acid. It can also be an ester of caffeic acid and quinic acid. It is an important intermediate of lignin biosynthesis, and it is known as an antioxidant that helps slow the absorption of glucose into the bloodstream after a meal.

Cyclic adenosine monophosphate (cAMP) is a second messenger important in many biological processes. It is derived from adenosine triphosphate and used for intracellular signal transduction.

Epinephrine (also known as adrenaline) is a hormone and a neurotransmitter that has many functions in the body, such as regulating heart rate, blood vessel and air passage diameters, and metabolic shifts.

Gluconeogenesis is the biosynthesis of new carbohydrate from non-carbohydrate precursors.

Glucose disposal or clearance is the body's ability to take glucose from the blood and transport it into organs.

Glucose 6-phophatase (G6Pase) is an enzyme that hydrolyzes glucose-6-phosphate resulting in the creation of a phosphate group and free glucose. This catalysis completes the final steps in gluconeogenesis and glycogenolysis. It plays a key role in the homeostatic regulation of blood glucose levels.

Glucose 6-phosphate (G6P) is glucose sugar phosphorylated on carbon 6. It is produced during glycolysis and glycogenolysis.

Glucose transporter type 4 (GLUT4) is a protein found in skeletal muscle and adipose tissue that is responsible for insulin-regulated glucose translocation into the cell.

Glycogen is a storage form of carbohydrates that is primarily found in the liver and skeletal muscle.

Glycogenolysis is the breakdown of glycogen first to glucose-1-phosphate and finally to glucose.

Homeostasis is the property of a system that regulates its internal environment and tends to maintain a stable, constant condition.

Homeostasis model assessment of insulin resistance (HOMA-IR) is a method used to quantify insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations.

Insulin sensitivity is a tissue's responsiveness to insulin, meaning how successfully the hormone receptor operates to permit glucose clearance or disposal.

Metabolic syndrome is the combination of medical disorders, such as obesity, high blood pressure, high triglycerides, low HDL cholesterol, and high fasting glucose that increase the risk for diabetes and cardiovascular disease.

Metabolism is the set of chemical reactions that happen in the cells of living organisms to sustain life.

Oral glucose tolerance test (OGTT) is a test to determine the ability of an individual to maintain homeostasis of blood glucose. It includes measuring blood glucose in a fasted state and at prescribed intervals before and after oral glucose intake (75 or 100 g) or intravenous infusion (0.5 g/kg of body weight).

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that results from the inability of the body to respond appropriately to insulin, and in some cases, results from an insulin deficiency.

Whole-body insulin sensitivity index is an index that was developed to predict whole-body insulin sensitivity from fasting blood glucose and insulin (HOMA-IR) or blood glucose and insulin during an OGTT.

CHAPTER 2: Review of Literature

Caffeine and Glucose Metabolism: Mechanisms of Action

Caffeine ingestion results in many different hormonal and neural responses. This can lead to difficulty in assessing which factors are most important in the understanding of caffeine's effects on the body. Caffeine alters glucose metabolism in the human body through two major mechanisms of action. Caffeine can indirectly alter glucose metabolism by stimulating the secretion of epinephrine from the adrenal medulla. Caffeine may also directly stimulate skeletal muscle and antagonize non-selective adenosine receptors. The next two sections review the research that describes how caffeine affects epinephrine and adenosine receptors, and subsequently alters glucose metabolism.

Influence of Epinephrine

Van Soeren, Mohr, Kjaer, and Graham (1996) investigated the effect of an acute dose of caffeine on metabolic and hormonal responses in resting humans with impaired epinephrine responses. Twelve men (mean ± SE: 33 ± 4 years; 74 ± 6 kg body weight) with cervical spinal cord injury (tetraplegics- aka quadriplegia) were randomly assigned into two groups of six to either ingest a capsule containing 6 mg/kg body weight of caffeine or a placebo in a single-blind fashion while at rest in the morning after consuming their normal breakfast. In addition, two paraplegic men with total leg paralysis and only minor damage to sympathetic nerves were administered the same dose of caffeine as the tetraplegics to evaluate its effect on metabolic and hormonal responses. Plasma epinephrine concentrations in both the experimental and control tetraplegics were approximately one-half the normal value found in the paraplegics. However, free fatty

acids and glycerol significantly increased, whereas plasma potassium significantly decreased in the experimental tetraplegics when compared to the control tetraplegics. This led the authors to conclude that caffeine must act directly on the tissues.

Thong and Graham (2002a) cited several studies in the introduction of their manuscript which reported that caffeine stimulates epinephrine release, and epinephrine counteracts insulin stimulation of whole-body glucose metabolism and inhibits insulin-stimulated glucose transport and uptake into skeletal muscle. Thong and Graham (2002a) hypothesized that the negative effects of caffeine ingestion on insulin sensitivity and glucose tolerance could theoretically be mediated by epinephrine rather than by adenosine receptor antagonism. Seven healthy men (24 ± 1 years; 76 ± 4 kg body weight; 23 ± 1 kg/m^2 body mass index (BMI)) received the following in a randomized, double-blind fashion on four separate occasions: (a) 5 mg/kg body weight of caffeine, (b) 80 mg of propranolol, (c) 5 mg/kg body weight of caffeine plus 80 mg propranolol, or (d) 5 mg/kg body weight of dextrose (placebo). Propranolol is a non-selective beta-adrenergic receptor blocker. After 90 minutes of rest, the men ingested 75 g of glucose and completed a 120-minute oral glucose tolerance test (OGTT). There were no significant differences in blood glucose before or during the OGTT between trials. However, caffeine administration increased insulin response by 42% and reduced the whole-body insulin sensitivity index (ISI) by 25%. These changes were significantly different when compared to all other trials. Propranolol plus caffeine administration resulted in similar levels of epinephrine and caffeine in the blood as caffeine administration alone; however, propranolol plus caffeine produced similar insulin concentrations as the placebo. The authors suggested that the greater insulin response to

caffeine was likely related to altered insulin secretion rather than altered clearance. Furthermore, the authors suggested that the negative effects associated with caffeine ingestion on insulin are coupled to increased epinephrine production and its subsequent inhibition of insulin-mediated glucose uptake in skeletal muscle. The authors concluded that the antagonistic effects of caffeine on insulin *in vivo* are mediated by elevated epinephrine levels which have profound counter-regulatory effects on insulin's diverse action in peripheral tissues.

Battram, Graham, Richter, and Dela (2005) conducted a study to determine the effect of caffeine on glucose kinetics in humans. They also wanted to ascertain whether the effects of caffeine on glucose kinetics are secondary to the accompanying increase in adrenaline concentration. Twelve healthy, recreationally active men (mean ± SEM: 23 ± 1 years; 78.8 ± 2.8 kg body weight.; 23.8 ± 0.8 kg/m^2 BMI; 16.7 ± 2.4% body fat) completed three 150-minute isoglycemic-hyperinsulinemic clamps on separate days in randomized order 30 minutes after ingesting a placebo, 5 mg/kg body weight of caffeine, and either a placebo plus a high-dose adrenaline infusion (1.2 nM) or a placebo plus a low-dose adrenaline infusion (0.75 nM). Adrenaline increased to 0.6 nM with caffeine ingestion, but there was not an effect on endogenous glucose production. Endogenous glucose production was not significantly affected by any of the treatments. However, caffeine and a high-dose adrenaline infusion significantly decreased the glucose infusion rate by 13 and 30%, respectively. The low-dose adrenaline infusion decreased the glucose infusion rate by 5% ($p > .05$). The plasma adrenaline concentration during the low-dose adrenaline infusion trial was significantly higher than during the caffeine trial. As a result, the authors concluded that acute caffeine ingestion impedes insulin-mediated

glucose disposal, but adrenaline alone does not play a dominant role in caffeine's negative effect on glucose disposal.

Battram, Graham, and Dela (2007) investigated the effect of caffeine plus adrenaline on whole-body insulin-mediated glucose disposal. On separate days and in a randomized double-blinded fashion, eight healthy men (mean ± SEM: 25 ± 2 years; 81 ± 3 kg body weight; 24 ± 1 kg/m^2 BMI; 19 ± 2% body fat) completed four 150-minute isoglycemic-hyperinsulinemic clamps 30 minutes after ingesting the following: (a) placebo plus saline, (b) 5 mg/kg body weight of caffeine plus saline, (c) placebo plus 0.006 $\mu g \cdot min^{-1} \cdot kg^{-1}$ body weight of adrenaline, and (d) 5 mg/kg body weight of caffeine plus 0.0025 $\mu g \cdot min^{-1} \cdot kg^{-1}$ body weight of adrenaline. Insulin concentrations were similar between all treatments during the clamps except the caffeine plus adrenaline trial resulted in significantly higher insulin concentration compared to the placebo plus saline (442 ± 13 pM vs. 384 ± 14 pM, respectively). Plasma adrenaline concentrations during the clamps were significantly different between all four trials, with the adrenaline plus saline trial having the highest peak concentration (1.2 nM) followed by adrenaline plus caffeine (0.93 nM) then caffeine plus saline (0.62 nM). The caffeine plus saline trial resulted in a peak plasma adrenaline concentration that was 50% lower than the adrenaline plus saline trial. The caffeine plus saline trial and adrenaline plus saline trial elicited similar reductions (26% and 24%, respectively) in whole body glucose disposal when compared to the placebo plus saline trial; however, the adrenaline plus caffeine trial elicited a 42% reduction. Since the sum of the reduced glucose disposal rate for the caffeine plus saline trial and the adrenaline plus saline trial (26% + 24% = 50%) did not equal the percent reduction for the adrenaline plus caffeine trial (42%), the authors concluded that caffeine

elicits its effects on whole-body insulin-mediated glucose disposal indirectly through adrenaline and by an additional mechanism, likely the antagonism of adenosine receptors.

Battram, Bugaresti, Guspa, and Graham (2007) conducted a study that examined the effect of caffeine on glucose tolerance when caffeine does not elicit an epinephrine response. On two separate days, 14 tetraplegics (mean ± SE: 44.9 ± 3.3 years; 82.2 ± 4.5 kg body weight) ingested in a randomized, single-blinded manner either 4 mg/kg body weight of caffeine or a placebo 60 minutes prior to an OGTT. Each subject ingested 75 g of glucose, and blood samples were collected every 30 minutes for 120 minutes. Caffeine did not impair glucose tolerance in these tetraplegics. The treatment did not have a significant effect on glucose, insulin, C-peptide, pro-insulin, glucagon-like peptide-1 (GLP-1), and epinephrine concentrations during the OGTT. However, caffeine did have a significant effect on glycerol concentrations and mean arterial pressure. The results from this study suggest that caffeine elicits epinephrine-independent effects on adipose tissue lipolysis and blood pressure, but these effects are either not responsible for or do not exert enough of an effect to alter glucose metabolism. This study provided further evidence in support of Thong and Graham (2002a) that epinephrine plays a dominant role in the caffeine-induced impairment of glucose tolerance in humans.

Based on the studies discussed above, it appears that epinephrine plays an indirect role in the caffeine-induced impairment of glucose disposal in humans. Some studies led authors to conclude that epinephrine has a dominant role, whereas others showed a lesser role. The antagonism of adenosine receptors is another mechanism that has been hypothesized to play a role in the caffeine-induced impairment of glucose tolerance in humans.

Influence of Adenosine Receptors

Caffeine may directly stimulate skeletal muscle, antagonize non-selective adenosine receptors, and subsequently alter cyclic adenosine monophosphate (cAMP) (Graham, Battram, Dela, El-Sohemy, & Thong, 2008; Mohr, Van Soeren, Graham, & Kjaer, 1998; Van Soeren, Mohr, Kjaer, & Graham, 1996). Adenosine has been suggested to play a role in insulin- and exercise-mediated regulation of glucose transport and glycogen metabolism in the skeletal muscle of rodents (Thong & Graham, 2002b). It was shown that the activation of A_1 adenosine receptors in isolated rat soleus muscle contributes approximately 50% to insulin-stimulated muscle glucose transport (Thong et al., 2007). Therefore, A_1 adenosine receptors in skeletal muscle may play an important role in insulin-mediated glucose transport in the skeletal muscle of rats. Adenosine receptors may also play a role in insulin-mediated glucose disposal in humans; however, the effects on human skeletal muscle glucose uptake is not clear (Battram, Graham, & Dela, 2007; Battram et al., 2005). More research is warranted to test this hypothesis in humans.

Caffeine and Glucose Metabolism at Rest

Ingesting caffeine on an empty stomach at rest does not significantly alter blood glucose when compared to a placebo (Table 1, Appendix 1). However, ingesting caffeine prior to an OGTT significantly increases blood glucose during the OGTT when compared to a placebo. Following is a detailed review of the literature that explains how caffeine alters glucose metabolism at rest. Most of the reviewed studies investigated the effect of caffeine or coffee ingestion on blood glucose disposal and insulin sensitivity during an OGTT. Other studies investigated the effect of caffeine on whole-body glucose uptake

using a hyperinsulinemic euglycemic (HIEG) clamp technique. This section is divided into sub-sections based on the type of treatments that were used in the studies. The first section reviews studies that investigated the effect of pure caffeine on glucose metabolism at rest compared to a placebo. Each subsequent section reviews studies that compared different treatments, such as caffeinated coffee and decaffeinated coffee.

Caffeine vs. Placebo

Graham et al. (2001) tested the hypothesis that caffeine ingestion results in an exaggerated glucose and insulin response to an OGTT in resting humans. Eighteen young men (age: 18-33 years; body weight: 77.1 ± 7.7 kg; body fat: 16.5 ± 1.6%; VO_2 max: 54.6 ± 3.1 $ml \cdot kg^{-1} \cdot min^{-1}$) ingested either 5 mg/kg body weight of caffeine in capsule form or a placebo (< 1.5 g of dextrose) with 300 ml of water in random order 60 minutes prior to ingesting 75 g of glucose. Blood samples were taken before ingesting the treatment, 60 minutes after ingesting the treatment, and 15, 30, 60, 90, and 120 minutes during the OGTT. Ingesting caffeine 60 minutes prior to an OGTT did not result in a significantly different concentration of glucose and insulin immediately prior to the OGTT when compared to the placebo; however, there was a significant increase in epinephrine, free fatty acids, and glycerol. There was no significant difference in glucose AUC during the OGTT between treatments; however, glucose AUC for the caffeine trial (209 ± 31 mM) was 24% greater than for the placebo (168 ± 27 mM). The AUC during the OGTT for insulin was 60% greater following caffeine ingestion (726 ± 107 pM) than following placebo ingestion (454 ± 75 pM). Epinephrine levels were similar during the two OGTT trials. The authors concluded that their findings support the theory that

adenosine is an important regulator of insulin's actions on glucose disposal and that caffeine inhibits this function.

Greer, Hudson, Ross, and Graham (2001) conducted the first study that investigated the influence of caffeine on whole-body glucose uptake in resting humans using an HIEG. Nine men ingested either a capsule containing 5 mg/kg body weight of caffeine or a placebo (dextrose) immediately before an HIEG clamp procedure was initiated. Blood samples were taken at baseline and every 30 minutes during the clamp. Indirect calorimetry was conducted during the last 30 minutes of the clamp to determine carbohydrate oxidation. Glucose disposal was significantly lower by 24% after caffeine ingestion (6.38 ± 0.76 mg·kg body weight^{-1}·min^{-1}) than after placebo ingestion (8.42 ± 0.63 mg·kg body weight^{-1}·min^{-1}). Epinephrine concentrations were significantly higher one hour after caffeine ingestion when compared to the placebo, and the concentration remained significantly elevated for the duration of the clamp. Insulin concentrations were not significantly different between caffeine and placebo during the 180-minute clamp. Based on indirect calorimetry, carbohydrate storage was 35% lower ($p < .05$) in the caffeine trial (4.72 ± 1.1 mg·kg body weight^{-1}·min^{-1}) than the placebo trial (7.20 ± 0.7 mg·kg body weight^{-1}·min^{-1}). Respiratory exchange ratios were similar between trials (caffeine: 0.83 ± 0.04, placebo: 0.81 ± 0.02). The authors of this study concluded that their findings support the theory that adenosine is an important regulator of insulin-mediated glucose uptake and possibly glycogen synthesis.

Petrie et al. (2004) examined the acute effects of caffeine ingestion on glucose and insulin homeostasis in obese subjects before and after a nutrition and exercise intervention. Nine sedentary, obese men (BMI ≥ 30 kg/m^2) underwent two OGTTs

before and after a 12-week supervised treadmill exercise and diet intervention. One hour prior to the OGTT, each subject consumed either 5 mg/kg body weight of caffeine or a placebo in a randomized, double-blind fashion. Venous blood samples were collected 30 minutes prior to the OGTT, immediately before the OGTT, and 60, 75, 90, 120, 150, and 180 minutes throughout the OGTT. After the 12-week intervention, VO_2 max significantly increased from 36.9 ± 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ to 45.6 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$. Body weight significantly decreased from 103.4 ± 4.7 kg to 94.8 ± 4.6 kg, and percent body fat significantly decreased from 29.3 ± 1.2% to 26.5 ± 1.2%. The 12-week intervention did not significantly alter the glucose AUC during the OGTT, and caffeine did not significantly alter blood glucose over the entire duration of the OGTT when compared to the placebo. However, the insulin AUC during the OGTT after ingesting caffeine was significantly greater than the placebo both before (caffeine: 58.41 ± 7.6 nmol/L vs. placebo: 51.14 ± 9.93 nmol/L) and after (CAF: 48.62 ± 5.90 nmol/L vs. PL: 32.50 ± 4.36 nmol/L) the intervention. Furthermore, the ISI significantly increased after the 12-week intervention for both caffeine (4 ± 1 to 6 ± 1) and placebo (5 ± 1 to 8 ± 1); however, the ISI for caffeine was significantly lower than the placebo before and after the intervention. Based on these results, the authors concluded that acute caffeine ingestion significantly dampens whole-body insulin sensitivity in obese, non-diabetic men. In addition, the 12-week diet and exercise intervention significantly improved insulin sensitivity but was unable to ameliorate the caffeine-induced deterioration in insulin sensitivity. Petrie and colleagues (2004) were also able to show that caffeine does not directly affect beta-cell secretion; rather, they suggested that caffeine directly or indirectly causes peripheral insulin resistance.

Caffeine vs. Decaffeinated Coffee

Pizziol et al. (1998) evaluated the effect of caffeine on glucose tolerance in 30 non-smoking healthy subjects (12 men and 18 women; age: 28.6 ± 4.6 years). All subjects received either 50 ml of cold black decaffeinated coffee (DECAF) without sugar or 200 mg of pure caffeine mixed in 50 ml of DECAF without sugar in random order five minutes before ingesting 75 g of glucose. Glucose and insulin were measured before and one, two, three, and four hours after ingesting the glucose. There was not a significant difference in the insulin AUC. However, the four-hour glucose AUC for the DECAF trial (22.3 ± 2.2 mmol/L) was significantly lower than the four-hour glucose AUC for the DECAF plus caffeine trial (24.5 ± 2.6 mmol/L) by 9%. The results from this study demonstrated that the mechanism of caffeine on glucose metabolism is independent of insulin.

Lane, Hwang, Feinglos, and Surwit (2007) tested the effects of caffeine administered in DECAF on plasma glucose and insulin responses to a mixed meal. Twenty habitual coffee drinkers (11 women and 9 men; age: 54 ± 13 years; height: 1.71 ± 0.11 m; weight: 94.0 ± 24.4 kg) who had a history of T2DM for at least six months consumed either 16 oz of DECAF or 250 mg of caffeine mixed with 16 oz of DECAF in a randomized double-blind fashion. Each subject then consumed Boost® Plus, a commercially available beverage containing 75 g of carbohydrate, ten minutes after consuming the treatment. Blood samples were collected prior to ingesting the treatment as well as one and two hours after ingesting Boost® Plus. At two hours, the glucose AUC for the caffeine plus DECAF trial (2.8 ± 0.3 mmol/L) was significantly higher than the DECAF trial (2.2 ± 0.3 mmol/L) by 28%. The insulin AUC for the caffeine plus

DECAF trial (50.9 ± 6.0 μU/ml) was significantly higher than the DECAF trial (42.9 ± 6.0 μU/ml) by 19%. Based on the results from this study, the authors suggested that coffee drinking in everyday life may be associated with exaggerations of the postprandial hyperglycemia and hyperinsulinemia observed in patients with T2DM.

Caffeinated Coffee vs. Decaffeinated Coffee

Moisey, Kacker, Bickerton, Robinson, and Graham (2008) investigated the effect of caffeinated coffee (RCOF) on glucose disposal and insulin sensitivity after ingestion of high and low glycemic index (GI) cereal meals. Ten healthy men (age: 23.3 ± 1.1 years; height: 176.0 ± 2.6 cm; weight: 78.5 ± 4.1 kg) ingested RCOF with 5 mg/kg body weight of caffeine or DECAF one hour before ingesting the high (Kellog's® Crispex with non-fat milk) or low (Kellog's® All Bran with non-fat milk) GI meal. The total amount of carbohydrates for each meal was 75 g, and the GIs were 81 for the high GI meal and 41 for the low GI meal. Blood samples were taken prior to ingesting RCOF and DECAF, prior to the meal, and at 15, 30, 45, 60, 90, and 120 minutes after ingesting the meal. The two-hour insulin AUC after consuming the RCOF before the low GI meal (18,611 ± 4,565 pmol/L) was significantly higher than consuming the DECAF before the low GI meal (12,904 ± 3,365 pmol/L). However, there was no significant difference in the insulin AUC between the RCOF and DECAF high GI meal trials. The glucose AUC was significantly higher when consuming the RCOF prior to the low GI meal (131 ± 23 mmol/L) than when consuming the DECAF prior to the low GI meal (41 ± 18 mmol/L). In addition, the two-hour glucose AUC was significantly higher when consuming the RCOF prior to the high GI meal (253 ± 40 mmol/L) than when consuming the DECAF prior to the low GI meal (103 ± 39 mmol/L). The ISI was significantly different between

the high GI trials (RCOF: 10.8 ± 2.2 vs. DECAF: 17.9 ± 4.2) but not the low GI trials. Based on these findings, the authors of this study concluded that the ingestion of RCOF 60 minutes prior to ingesting a high or low GI meal significantly impairs acute insulin sensitivity and glycemic control when compared to ingesting DECAF 60 minutes before these same meals.

Caffeinated Coffee vs. Placebo

Feinberg, Sandberg, De Castro, and Bellet (1968) were among the first to study the effects of coffee on glucose metabolism in humans. Twenty-three healthy adults (15 men and 8 women; age: 19-23 years) consumed, in random order, either one gram of glucose/kg of body weight that was dissolved in 400 ml of water or five grams of instant coffee containing 220 mg of caffeine administered with the glucose. Blood samples were taken before the treatment and every 30 minutes thereafter for 180 minutes. Glucose concentrations were significantly lower when instant coffee was co-ingested with glucose vs. glucose alone after 30 minutes (117.7 ± 18.6 mg % of baseline value vs. 139.1 ± 25.4 mg % of baseline value) and 60 minutes (98.6 ± 24.0 mg % of baseline value vs. 121.7 ± 24.3 mg % of baseline value). However, insulin concentrations were not significantly different at any time-point. The authors suggested that the reduced levels of glucose in the blood after the instant coffee was ingested with glucose may have occurred due to caffeine augmenting gastric secretions.

Caffeinated Coffee vs. Decaffeinated Coffee vs. Placebo

Moisey, Robinson, and Graham (2010) determined the effect of RCOF and DECAF on glucose disposal and insulin sensitivity when co-ingested with a carbohydrate meal. In addition, they determined whether prolonged effects of RCOF on glucose

management exist after ingestion of a second carbohydrate load in the form of an OGTT. Ten men (age: 18-50 years) ingested Kellogg's® Crispix cereal mixed with 150 ml skim milk along with RCOF, DECAF, or water in a randomized, crossover design. The cereal and milk contained 75 g of carbohydrates, and the RCOF contained 5 mg/kg body weight of caffeine. These meals were consumed three hours prior to undertaking a two-hour OGTT in which each subject consumed 75 g of dextrose. Blood samples were collected prior to consuming the meals, after consuming the meals, and 15, 30, 45, 60, 90, 120, 150, and 180 minutes after consuming the meals. Blood samples were also taken immediately after consuming the 75 g of dextrose and 15, 30, 45, 60, 90, and 120 minutes during the OGTT. The insulin AUC 180 minutes post-cereal ingestion was not significantly different between treatments. However, the glucose AUC for RCOF (107 ± 18 mmol/L) and DECAF (74 ± 15 mmol/L) was significantly greater than water (-0.2 ± 29 mmol/L), but RCOF and DECAF were not significantly different. The insulin AUC during the OGTT for RCOF (30,837 ± 2,879 pmol/L) was significantly greater than DECAF (21, 259 ± 2,172 pmol/L) and water (20,075 ± 1,976 pmol/L). In addition, the insulin AUC from pre-cereal ingestion to the end of the OGTT was significantly greater in RCOF (29,816 ± 2,774 pmol/L) than DECAF (19,960 ± 2,265 pmol/L) and water (19,009 ± 1,738 pmol/L). Furthermore, the glucose AUC from pre-cereal ingestion through the end of the OGTT was significantly greater in RCOF (217 ± 24 mmol/L) than DECAF (126 ± 11 mmol/L) and water (55 ± 34 mmol/L). The authors of this study concluded that individuals may experience prolonged negative postprandial caffeine-induced effects with respect to glucose and insulin responses. In addition, substituting DECAF for RCOF may improve acute glycemic control in young, healthy males.

Caffeine vs. Caffeinated Coffee vs. Decaffeinated Coffee vs. Placebo

Battram Arthur, Weekes, and Graham (2006) investigated the impact of RCOF and DECAF consumption on the blood glucose and insulin responses during an OGTT and compared these findings to those obtained from alkaloid caffeine and placebo ingestion. Eleven healthy, low to moderately active men (mean ± SEM: 23.2 ± 0.6 years; weight: 76.4 ± 1.9 kg; 15.6 ± 2.3 % body fat) consumed in a double-blind, randomized fashion one of four treatments 60 minutes prior to an OGTT: (a) 4.45 mg/kg body weight of dextrose in capsule (placebo) with 250 ml of water, (b) 4.45 mg/kg body weight caffeine in capsule form with 250 ml water, (c) RCOF containing 4.45 mg/kg body weight of caffeine, or (d) equal amount of DECAF as RCOF. The amount of glucose consumed was 75 g and the OGTT lasted for two hours. Blood samples were collected at various times during the two-hour OGTT. This study was the first to directly compare the effects of caffeine and RCOF ingestion on glucose metabolism in a single study. The glucose AUC for DECAF (90 ± 29 mmol/L) was 50% lower than the placebo (184 ± 40 mmol/L) ($p \leq 0.05$). Furthermore, the glucose AUC for RCOF (176 ± 48 mmol/L) was 40% lower than caffeine (285 ± 40 mmol/L) ($p \leq 0.05$). The insulin AUC for caffeine (32758 ± 4082 pmol/L) was significantly higher ($p \leq 0.05$) than the placebo (22087 ± 3050 pmol/L) and DECAF (20544 ± 2419 pmol/L) but not RCOF (26763 ± 2250 pmol/L). The ISI for caffeine (7.6 ± 0.6) was significantly lower ($p \leq 0.05$) than the placebo (8.7 ± 0.8), DECAF (9.0 ± 0.5) and RCOF (8.2 ± 0.7). These results led the authors to suggest that there may be some components (possibly chlorogenic acids and quinides) of DECAF and RCOF that enhance glucose tolerance and attenuate or antagonize the effects of caffeine. Therefore, the authors concluded that these

components may provide a partial explanation for the reported (Huxley et al., 2009; Salazar-Martinez et al., 2004; van Dam, 2008) decrease in incidence of T2DM in chronic coffee consumers.

Greenberg, Owen, and Geliebter (2010) assessed whether DECAF enhances glucose metabolism and whether glucose-dependent insulinotropic polypeptide (GIP), an incretin hormone that stimulates insulin secretion, plays a causal role. Eleven healthy men (23.5 ± 5.7 years; BMI = 23.6 ± 4.2 kg/m^2) ingested in a single-blinded randomized fashion one of four beverages 60 minutes prior to an OGTT (75 g of glucose in water): (a) RCOF with 6 mg/kg body weight of caffeine, (b) DECAF, (c) 6 mg/kg body weight of caffeine in warm water, and (d) placebo (warm water). Blood was drawn 30 minutes before consuming the treatment, immediately before consuming the treatment, immediately before and OGTT, and 10, 30, 60, 90, and 120 minutes during the OGTT. The AUC for glucose and insulin was calculated using the data 60 minutes prior to the OGTT through the end of the OGTT. Glucose concentrations for caffeine (7.44 ± 0.26 mmol/L), RCOF (7.63 ± 0.39 mmol/L), and DECAF (8.13 ± 0.41 mmol/L) were significantly higher during the first 30 minutes of the OGTT when compared to the placebo (6.66 ± 0.28 mmol/L); however the concentrations of glucose were not significantly different between trials during the rest of the OGTT. The three-hour glucose AUC for DECAF (4.10 ± 0.67 mmol/L) was significantly lower than caffeine (5.39 ± 0.80 mmol/L). The three-hour insulin AUCs for caffeine (882.0 ± 185.9 pmol/L), RCOF (884.9 ± 159.4 pmol/L), and DECAF (705.5 ± 109.8 pmol/L) were significantly higher than the placebo (489.4 ± 75.8 pmol/L). The ISI for DECAF (1.09 ± 0.08) was significantly higher than caffeine (0.98 ± 0.09). Based on these results, the authors

concluded that DECAF can acutely impair glucose metabolism in healthy young men, but less so than caffeine.

In summary, pure caffeine ingestion prior to an OGTT significantly decreases a person's acute tolerance to glucose. However, caffeinated coffee does not decrease glucose tolerance as much as pure caffeine. Furthermore, decaffeinated coffee has been shown to enhance glucose tolerance when compared to pure caffeine, caffeinated coffee, and a placebo (Battram et al., 2006). Several of the studies above noted that there must be some other compound in coffee that is negating caffeine's effect on glucose metabolism. The effect of this other compound found in coffee on glucose metabolism will be discussed later in this review.

Caffeine and Glucose Metabolism after Exercise

Optimal nutrition during the post-exercise recovery period helps restore muscle glycogen, stimulate muscle protein synthesis, and improve subsequent exercise performance (Beelen, Burke, Gibala, & van Loon, 2010). Co-ingesting caffeine and carbohydrates (CHO) during the post-exercise recovery period has been shown to enhance muscle glycogen resynthesis (Pedersen et al., 2008) and improve subsequent exercise performance (Taylor, Higham, Close, & Morton, 2011) when compared to ingesting CHO alone. However, ingesting CHO plus caffeine during the post-exercise recovery period did not significantly enhance glycogen repletion when compared to ingesting CHO alone (Beelen, van Kranenburg, Senden, Kuipers, & van Loon, 2012). Furthermore, it has also been shown that ingesting caffeine two hours after exercise may reduce the beneficial effects that exercise has on skeletal muscle glucose uptake (Thong et al., 2002).

Thong et al. (2002) examined the effects of caffeine ingestion on glucose uptake and glycogen synthase activity in rested and previously exercised human skeletal muscle during a euglycemic-hyperinsulinemic clamp. Seven healthy, moderately active men consumed 5 mg/kg body weight of pure caffeine or a placebo two hours after performing 60 minutes of repeated one-legged knee extensor exercise alternating every five minutes at a workload equal to 75% and 100% of knee extensor VO_2 peak. A one-step euglycemic-hyperinsulinemic clamp was initiated three hours after exercise. Blood samples were drawn 60 minutes prior to the clamp and 0, 10, 20, 30, 50, 75, and 100 minutes after the initiation of the clamp. Muscle biopsies were taken from the vastus lateralis of the rested and exercised leg 0, 30, and 100 minutes after the initiation of the clamp. Whole-body glucose disposal was significantly decreased by 30% after consuming the caffeine when compared to the placebo. Insulin-stimulated glucose uptake in the previously exercised leg was significantly higher in both trials when compared to the rested leg as a result of a higher blood glucose extraction. After caffeine ingestion, insulin-stimulated glucose uptake was significantly lower in the rested leg (55%) and exercised leg (51%) when compared to the placebo as a result of a lower glucose extraction. The caffeine-induced reduction in insulin-stimulated glucose uptake in the skeletal muscle was a significant contributor to the diminished whole-body glucose disposal ($r^2 = .93$). The results from this study also showed that exercise reduced the negative effects that caffeine had on skeletal muscle glucose uptake, but caffeine reduced the beneficial effects that exercise had on skeletal muscle glucose uptake.

Pedersen et al. (2008) determined the effect of caffeine plus CHO on rates of muscle glycogen resynthesis during recovery from exhaustive exercise. Seven endurance

trained cyclists/triathletes consumed 8 mg/kg body weight of caffeine plus 4 g/kg body weight of CHO or 4 g/kg body weight of CHO alone after cycling at 70% VO_2 peak until volitional exhaustion. The caffeine was mixed with a CHO-containing sports drink in two equal doses: 4 mg/kg body weight of caffeine plus 1 g/kg body weight of CHO immediately after exercise and 4 mg/kg body weight of caffeine plus 1 g/kg body weight of CHO two hours after exercise. The remaining 2 g/kg body weight of CHO was administered in two equal doses: 1 g/kg body weight one hour after exercise and 1 g/kg body weight three hours after exercise. A resting blood sample was drawn before exercise, and blood samples were also drawn throughout the four-hour recovery. Muscle biopsies were taken from the vastus lateralis immediately after exercise and one hour and four hours after the completion of the exercise. Blood glucose during the caffeine plus CHO trial was significantly greater after 1.5 hours, three hours, and four hours of recovery than the blood glucose during the CHO alone trial. Blood insulin during the caffeine plus CHO trial was greater than the CHO alone trial during all time points of the recovery except immediately after exercise; however, these differences were not statistically significant. Interestingly, muscle glycogen during the caffeine plus CHO trial after four hours of recovery (313 ± 69 mmol/kg dry weight) was significantly greater than muscle glycogen during the CHO alone trial (234 ± 50 mmol/kg dry weight). The authors of this study concluded that the higher blood glucose and insulin concentrations during the caffeine plus CHO trial could have contributed to the higher muscle glycogen content when compared to CHO alone.

Taylor et al. (2011) tested the hypothesis that co-ingestion of caffeine plus CHO during a four-hour recovery period after a glycogen-depleting exercise augments

subsequent high-intensity interval-running capacity compared with ingesting CHO alone. Six recreationally active men completed three trials of glycogen-depleting running on a treadmill. Following each trial, the men consumed either 1.2 g/kg body weight of CHO every hour for four hours, 1.2 g/kg body weight of CHO every hour for four hours plus 4 mg/kg body weight of caffeine immediately after exercise and two hours after exercise, or 1.2 g/kg body weight of flavored water every hour for four hours. After the four-hour recovery, the men performed a high-intensity interval-running capacity test called the Loughborough Intermittent Shuttle Running Test (LIST) to measure time to exhaustion. Blood glucose and lactate concentrations were obtained via finger-stick capillary samples immediately before and after both exercise protocols and at 30-minute intervals during the four-hour recovery. The blood glucose during the four-hour recovery for the CHO (6.2 ± 0.8 mmol/L) and caffeine plus CHO (6.7 ± 1.0 mmol/L) trials were significantly higher than the blood glucose during the water (4.6 ± 0.3 mmol/L) trial. However, there was not a significant difference between blood glucose concentrations during the four-hour recovery for the CHO and caffeine plus CHO trials. Time to exhaustion during the LIST significantly increased after ingesting caffeine plus CHO (48 ± 15 minutes) when compared to ingesting CHO alone (32 ± 15 minutes) and water (19 ± 6 minutes). The authors of this study stated that the improved time to exhaustion in the caffeine plus CHO trial may have been related to improved rates of glycogen re-synthesis, effects on the central nervous system and/or modifications in muscle metabolism through a glycogen-sparing effect.

Beelen et al. (2012) tested the hypotheses that co-ingestion of an insulinotropic amino acid/protein mixture or caffeine with 1.2 mg/kg body weight of CHO every hour

accelerates post-exercise muscle glycogen synthesis when compared to ingesting CHO alone. Fourteen well-trained male cyclists completed three glycogen-depleting cycling trials in random order followed by the ingestion of 1.2 g/kg body weight of CHO, 1.2 g/kg body weight of CHO plus 0.3 g/kg body weight of amino acid/protein mixture, or 1.2 g/kg body weight CHO plus 1.7 mg/kg body weight of caffeine every hour for six hours. Muscle biopsies were taken from the vastus lateralis immediately after exercise and the six-hour recovery period to assess changes in muscle glycogen. Blood samples were taken immediately after exercise, every 15 minutes during the first 90 minutes of recovery, and then every 30 minutes until the end of the six-hour recovery. There were no significant differences in the blood glucose and insulin concentrations during the six-hour recovery between the CHO and CHO plus caffeine trials. The blood insulin concentration during the CHO plus protein trial was significantly greater than the CHO alone and CHO plus caffeine trials. There were no significant differences in muscle glycogen synthesis rates and muscle glycogen concentrations between the three trials. Furthermore, ingesting CHO plus caffeine did not significantly alter the intestinal glucose absorption when compared to ingesting CHO alone.

There are mixed findings from the four studies presented above. When compared to the results from Pedersen et al. (2008), Beelen et al. (2012) attributed their non-significant findings in glycogen re-synthesis to differences in study design. The results from Thong et al. (2002) showed that exercise reduced the negative effects that caffeine had on skeletal muscle glucose uptake, but caffeine reduced the beneficial effects that exercise had on skeletal muscle glucose uptake. Pedersen et al. (2008) reported that blood glucose and insulin concentrations were greater during the CHO plus caffeine trial

when compared CHO alone trial. However, Taylor et al. (2011) did not find any significant differences in blood glucose concentrations, and Beelen et al. (2012) did not find any significant differences in blood glucose and insulin concentrations between the CHO plus caffeine and CHO alone trials.

Our study investigated the independent effects of post-exercise caffeine and chlorogenic acid (two major compounds found in green coffee beans) supplementation on blood glucose and insulin concentrations during a two-hour recovery. Three of the four studies reviewed above investigated post-exercise caffeine supplementation on glycogen resynthesis. Thong et al. (2002) looked at the effect of post-exercise caffeine supplementation on insulin-mediated glucose uptake into the skeletal muscle. There are no studies to date that have administered an OGTT in conjunction with oral caffeine ingestion after an intense bout of exercise to investigate how exercise and caffeine affect blood glucose and insulin concentrations during a post-exercise OGTT. In addition, no studies to date have investigated how post-exercise chlorogenic acid supplementation affects blood glucose and insulin concentrations during a post-exercise OGTT. The hypothesized mechanisms as to how chlorogenic acid may affect blood glucose and insulin concentrations during an OGTT at rest are discussed in the next section.

Chlorogenic Acid and Glucose Metabolism

Drinking caffeinated coffee may not be as detrimental as ingesting pure caffeine because of other compounds in coffee that may delay intestinal absorption of glucose, help spare glycogen, or increase glucose clearance (Battram et al., 2006; de Paulis et al., 2002; Goldstein et al., 2010; Graham et al., 1998; Johnston, Clifford, & Morgan, 2003; Tarnopolsky, 2010). Chlorogenic acid, a natural polyphenol found in green coffee beans,

has been shown to lower blood glucose during an OGTT in animals (Bassoli et al., 2008) and in humans (Thom, 2007; van Dijk et al., 2009). The majority of the research investigating the effect of chlorogenic acid on glucose metabolism has been conducted on rodents and just a few studies have been done with humans. This section reviews the research studies that have attempted to establish the chlorogenic acid-related mechanisms of action for reducing blood glucose (Figure 1, Appendix 2). In addition, this section reviews the research studies that have investigated the effect of chlorogenic acid on OGTT in both humans and rodents.

Effect on Glucose 6-Phosphatase

Chlorogenic acid may inhibit hepatic glucose 6-phosphatase (G6Pase) and subsequently prevent glucose 6-phosphate (G6P) hydrolysis (Arion et al., 1997). G6Pase is an enzyme that plays a major role in the homeostatic regulation of blood glucose. It catalyzes the final step of gluconeogenesis and glycogenolysis, the two main metabolic pathways responsible for the release of glucose by the liver (Bassoli et al., 2008). Since chlorogenic acid has been shown to inhibit G6Pase, it may also help spare glycogen.

Arion et al. (1997) sought to establish the site in the liver where chlorogenic acid and 2-hydroxy-5-nitrobenzaldehyde may inhibit hepatic glycogenolysis. In addition, they wanted to provide a detailed characterization of these possible inhibitors with the components of the G6Pase system. Liver microsomes were extracted from male Sprague-Dawley rats and exposed to 2 mM of chlorogenic acid. The results from this study showed that chlorogenic acid binds to T1, the G6P transporter, and inhibits G6Pase. The authors concluded their results demonstrated that the inhibition by chlorogenic acid

involved blocking G6Pase access to exogenous G6P without impeding its access to inorganic pyrophosphate.

Henry-Vitrac, Ibarra, Roller, Merillon, and Vitrac (2010) determined the inhibitory activity of Svetol, a decaffeinated green coffee extract containing 13 chlorogenic acid compounds, on G6P hydrolysis. Svetol significantly inhibited G6P hydrolysis in intact human liver microsomes in a competitive manner. The researchers determined that the chlorogenic acids were the principal compounds mediating the inhibition of G6P hydrolysis. They observed a 36% inhibition by Svetol, and reported that this may contribute to the anti-diabetic, glucose-lowering effects by reducing hepatic production of glucose.

Effect on Glucagon-Like Peptide-1 and Gastric Inhibitory Polypeptide

Johnston et al. (2003) investigated coffee consumption modulation of glucose uptake in healthy volunteers, and subsequent consequential effects on circulating gastrointestinal hormones and insulin concentrations. Four men and five women (age: 26.0 ± 3.2 years) consumed one of three 400 ml beverages on three separate occasions in a randomized, single-blinded crossover design: (a) 25 g of glucose dissolved in RCOF, (b) 25 g of glucose dissolved in DECAF, and (c) 25 g of glucose dissolved in water (placebo). The authors did not report the amount of caffeine in RCOF. However, they did mention that the total amount of 3-, 4-, and 5-caffeoylquinic acid, also known as chlorogenic acid, in the RCOF was 41.22 mg/g and in the DECAF was 29.44 mg/g. Blood samples were collected before ingesting the designated beverage and at frequent intervals for the following three hours. The major finding in this study was the significant attenuation of postprandial GIP secretion after drinking RCOF and DECAF

when compared to the placebo. The three-hour GIP AUC was significantly lower after ingesting RCOF (3073 ± 426 pmol/L) and DECAF (2327 ± 391 pmol/L) when compared to the placebo (3760 ± 350 pmol/L). In addition, three-hour GIP AUC for DECAF was significantly lower than RCOF. There were no significant differences in glucose and insulin concentrations between the treatments over the entire three hours. However, there was a significantly higher three-hour glucose AUC for RCOF (227.1 ± 6.9 mmol/L) when compared to DECAF (210.2 ± 6.6 mmol/L) and the placebo (212.0 ±7.3 mmol/L). Based on these findings, the authors suggested that the chlorogenic acid in coffee decreases the rate of intestinal absorption of glucose by delaying glucose absorption until it reaches the distal region of the small intestine.

Olthof, van Dijk, Deacon, Heine, and van Dam (2011) investigated the acute effects of 12 g of DECAF, 1 g of chlorogenic acid, 500 mg trigonelline, and 1 g of mannitol (placebo) on total intact GLP-1 and GIP concentrations in 15 healthy overweight men. The men ingested the supplements 30 minutes prior to ingesting 75 g of glucose. They measured plasma concentrations of total GLP-1, intact GLP-1, and total GIP immediately before ingesting the treatment, immediately before consuming the 75 g of glucose, and 15, 30, 60, 90, and 120 minutes after ingesting the glucose. There were no significant differences in the total GLP-1, intact GLP-1, and GIP AUCs. In addition, there were not any significant changes in total GLP-1, intact GLP-1, and GIP between the treatments over time. The authors concluded that their findings refuted the hypothesis that the beneficial effect of coffee on the development of T2DM could be explained by improved GLP-1 and GIP responses to meals.

Tunnicliffe, Eller, Reimer, Hittel, and Shearer (2011) implanted a catheter into the right carotid artery of 12 male Sprague-Dawley rats. The rats consumed a meal that weighed 4 g/kg of their body weight. The meal consisted of 59% carbohydrates, 25% fat, and 12% protein. They were randomly assigned to a placebo or chlorogenic acid treatment in a crossover design. During the treatment trial, 120 mg/kg body weight of chlorogenic acid was added to the meal. Baseline blood was collected five minutes prior to consuming the meal. Blood was also collected at 15, 30, 45, 60, 90, 120, and 180 minutes after consuming the meal. Blood glucose, insulin, total GIP and active GLP-1 were measured. The rate of gastric emptying was evaluated by having the rats consume acetaminophen with the meal because it is minimally absorbed in the stomach and rapidly absorbed in the small intestine. Blood glucose concentration was significantly lower 60 minutes after consuming the meal with chlorogenic acid than the meal without chlorogenic acid. Furthermore, the three-hour blood glucose AUC was significantly lower in the chlorogenic acid group (1255.6 ± 28.0 mmol/L) than the placebo group (1360.5 ± 25.9 mmol/L). Blood GIP concentration was significantly lower at 30 and 60 minutes after consuming the meal with chlorogenic acid than the meal without chlorogenic acid. Furthermore, the three-hour blood GIP AUC was significantly lower in the chlorogenic acid group (20,678.0 ± 2,694.0 pg/ml) than the placebo group (30,510.0 ± 3,683.0 pg/ml). However, there were no significant differences in the three-hour AUCs of active GLP-1 and insulin. In addition, the rate of gastric emptying was not significantly different between the treatments. Based on these findings, the authors concluded that chlorogenic acid might have a protective effect on elevated blood glucose by reducing GIP secretion and slowing the rate of glucose appearance from the intestine

into the circulation. They also concluded that chlorogenic acid might reduce the glycemic index of foods.

Effect on 5' Adenosine Monophosphate-Activated Protein Kinase

Caffeic acid and chlorogenic acid (an ester of caffeic and quinic acids) are major phenolic compounds found in coffee; both are hypothesized to reduce blood glucose concentrations in humans although no definitive research exists at this time. There are conflicting findings on the effect of chlorogenic acid on 5' Adenosine Monophosphate-Activated Protein Kinase (AMPK); one study found that chlorogenic acid increased AMPK activity in isolated skeletal muscle of mice (Ong, Hsu, & Tan, 2012) and another study found that caffeic acid rather than chlorogenic acid increased AMPK activity in isolated skeletal muscle of rats (Tsuda, Egawa, Ma, Oshima, Kurogi, & Hayashi, 2011).

It was recently shown that chlorogenic acid stimulates glucose transport in the skeletal muscle of mice via AMPK activation (Ong et al., 2012). Sixteen mice were randomly assigned to four groups: (a) lean controls, (b) diabetic controls, (c) 250 mg/kg body weight of chlorogenic acid, and (d) 250 mg/kg body weight of metformin. Each group completed an OGTT by consuming 2 g/kg body weight of glucose; blood glucose was measured over a period of two hours. After sacrificing the mice, the soleus muscle was surgically excised and isolated to investigate the effect that AMPK had on chlorogenic acid-stimulated glucose transport. The blood glucose of the diabetic mice that consumed chlorogenic acid was significantly lower than the blood glucose of the diabetic control mice ten minutes after consuming chlorogenic acid. In addition, blood glucose was significantly lower in the experimental group 15 and 30 minutes after consuming the glucose (25 and 35 minutes after consuming chlorogenic acid).

Chlorogenic acid stimulated and enhanced both basal and insulin-mediated glucose transporters and augmented glucose utilization in the muscle. The results from this study also indicated that the effect of chlorogenic acid on glucose transport might be mediated by AMPK.

Although Ong et al. (2012) showed that chlorogenic acid increased AMPK activity and subsequent glucose transport into isolated skeletal muscle of mice, Tsuda et al. (2011) showed that caffeic acid rather than chlorogenic acid stimulated AMPK activity and subsequent glucose transport into isolated rat skeletal. Isolated skeletal muscle from male Sprague-Dawley rats was incubated in a 1 mM solution of chlorogenic acid, 1mM solution of caffeic acid, or a control solution. The muscle was used to investigate glucose transport and AMPK activity. More research should be conducted before it can be concluded that chlorogenic acid (or caffeic acid) activates AMPK and subsequently increases glucose transport into skeletal muscle.

Effect on Glucose Tolerance

Thom (2007) investigated the effect of chlorogenic acid-supplemented coffee and normal coffee on the glucose profile of healthy volunteers. Six healthy women and six healthy men (Age: 24.2 ± 3.2 years; BMI: < 25 kg/m^2) ingested four different treatments in random order after a 12-hour overnight fast: (a) 25 g of sucrose in 400 ml of water (control), (b) 25 g of sucrose and 10 g of instant RCOF (~300-400 mg of chlorogenic acid) in 400 ml of water, (c) 25 g of sucrose and 10 g of instant DECAF (~300-400 mg of chlorogenic acid), and (d) 25 g of sucrose and 10 g of Coffee Slender (~400-450 mg of chlorogenic acid) 400 ml of water. Blood samples were drawn immediately before the treatment and throughout the two hours after the treatment (15, 30, 45, 60, 90, and 120

minutes) to measure plasma glucose concentrations. The two-hour blood glucose AUC was significantly lower after consuming the Coffee Slender (724 ± 8.2 mmol/L) when compared to the control (778 ± 10.2 mmol/L). The two-hour blood glucose AUC for the instant RCOF (788 ± 10.1 mmol/L) and instant DECAF (818 ± 10.9 mmol/L) were not significantly different from the control.

Bassoli et al. (2008) analyzed the effects of chlorogenic acid on hepatic glucose output, blood glucose levels, and glucose tolerance in male albino Wistar rats. The effect of chlorogenic acid on hepatic catabolism of G6Pase activity was also evaluated. Intact microsomes were obtained from the liver of the rats and perfused in chlorogenic acid or a control to assess the influence on hepatic glucose-related metabolism. In addition, some rats ingested 3.5 mg/kg body weight of chlorogenic acid and some ingested the same amount of water 10 minutes prior to ingesting 200 mg/kg body weight of glucose. Blood samples were taken immediately before ingesting the glucose and 5, 10, 15, 30, 45, 60, and 90 minutes after ingesting the glucose. The results indicated that a 0.5, 0.75, and 1.0 mM solution of chlorogenic acid significantly inhibited hepatic G6Pase when compared to the control. During the OGTT, blood glucose was significantly lower in the chlorogenic acid group after 10 and 15 minutes by 21.8% and 17.8%, respectively, when compared to the control group. However, blood glucose levels were not significantly altered 5, 10, 15, 30 and 60 minutes after an intravenous injection of chlorogenic acid into the bloodstream when compared to an intravenous injection of a phosphate buffer (control). In addition, infusing chlorogenic acid directly into the liver did not significantly affect hepatic glucose production. Therefore, the authors concluded that

consuming chlorogenic acid favors the hypothesis that the reduced blood glucose was partly attributed to the attenuation of the intestinal absorption of glucose.

van Dijk et al. (2009) investigated the effects of 12 g DECAF, 1 g of chlorogenic acid, 500 mg trigonelline, and 1 g of mannitol (placebo) on glucose and insulin concentrations during an OGTT in 15 healthy, non-smoking overweight men (BMI = 25.0-35.0 kg/m^2). The men ingested the supplements 30 minutes prior to ingesting 75 g of glucose. They measured glucose and insulin every 15 minutes during the OGTT. They found that chlorogenic acid ingestion significantly reduced glucose and insulin concentrations only at the 15-minute time-point when compared to the placebo; there was no significant reduction in the blood glucose and insulin AUC when compared to the placebo.

In summary, it has been hypothesized that chlorogenic acid may help reduce blood glucose after an OGTT or meal by one or more of the following mechanisms: (a) inhibiting G6Pase and, subsequently reducing hepatic glucose output, (b) increasing GIP and decreasing GLP-1, subsequently delaying glucose absorption in the small intestine, or (c) activating AMPK in skeletal muscle to subsequently increase glucose transport into the muscle. The results of these studies are equivocal and show that there still is no clear answer on how chlorogenic acid reduces blood glucose. In addition, most studies have been conducted on rodents and not humans. Chlorogenic acid's effect on intestinal absorption of glucose seems to be the most dominant and most studied of the three hypothesized mechanisms of action. The limited research in humans shows that chlorogenic acid may or may not help lower blood glucose levels after an OGTT or a meal. To our knowledge, there are no published studies investigating the effect of post-

exercise chlorogenic acid supplementation on glucose tolerance during an acute recovery period. More research that investigates the physiological responses and mechanisms of chlorogenic acid on blood glucose homeostasis in humans is warranted.

CHAPTER 3: Methods

Subjects

Ten men who were trained to well-trained in cycling participated in this study. Training status was determined using the criteria from Jeukendrup, Craig, and Hawley (2000). The subjects were recruited by word-of-mouth, posted flyers, and via an email list serve. They were 19-34 years of age and did not have any cardiovascular, pulmonary or metabolic disease. They reported their daily caffeine consumption on a health history questionnaire. They were regular caffeine users defined by the consumption of ≥ 2 caffeinated coffee or tea beverages and/or ≥ 5 caffeine-containing soft drinks per week (Battram et al., 2006). The experimental procedures and possible risks were explained to each participant verbally and in writing prior to their participation in this study. They signed an informed consent and HIPAA release and completed a health history questionnaire prior to participation. These documents and the study were approved by the Human Research Protection Office.

Experimental Design

This quasi-experimental, repeated measures study consisted of three experimental trials conducted in the morning. The experimental trials were randomized, and the treatments were double-blinded. Each experimental trial was separated by at least one week. During each experimental trial, an identical high-intensity 30-minute bout of cycling was completed. The treatment consisted of immediate post-exercise consumption of 5 mg/kg body weight caffeine with 75 g dextrose (CAF), 5 mg/kg body weight of chlorogenic acid via green coffee extract (CoffeeGenic^{TM}, Life Extension®) with 75 g dextrose (CGA) or 5 mg/kg body weight dextrose with 75 g dextrose (PLA). The effect

of the treatment on blood glucose and insulin concentrations was assessed during a two-hour post-exercise OGTT by taking blood samples every 15 minutes during the first hour of recovery and every 30 minutes during the second hour of recovery.

Pre-Experimental Procedures

One week prior to the first experiment, each participant reported to the exercise physiology lab to perform an incremental test to exhaustion on a cycle ergometer (Lode Excaliber, Groningen, The Netherlands) to assess VO_2 peak and peak power output (PPO). Participants were asked to void their bowel and bladder. They then put on a heart rate monitor and changed into their cycling attire. Height and body weight were measured twice while the participant was barefoot and wearing their cycling attire. Resting blood pressure was also measured twice. The duplicate measurements were averaged and reported as descriptive data. The seat and handlebar height of the cycle ergometer were adjusted to the preference of the participant. These settings were noted so that the ergometer was set up the same for each subsequent exercise trial. Each participant was utilized toe clips or clip-less pedals during the exercise, and the same pedal setup was used during each trial.

The VO_2 peak protocol was individualized based on the participant's self-reported cycling experience, and it consisted of a ramp-style increase in power (30 or 35 W/min). The workload at the start of the protocol was 70 W. Heart rate (HR) was monitored continuously along with VO_2, VCO_2 and ventilation (VE). Each participant pedaled to exhaustion until their cadence dropped below 50 rpm. At this time, the test was terminated by one of the investigators and the participant actively recovered until their HR dropped below 120 bpm. Subjects were asked what their maximal rating of

perceived exertion (Borg, 1982) was at the end of the test. The VO_2 peak was calculated as the highest 15-second average of VO_2, and the PPO was calculated as the highest single power output achieved during the exercise protocol.

Single-breath expired gas data was collected during the VO_2 peak test by open circuit spirometry using a Parvo-Medics TrueOne 2400® Metabolic Measurement System (Sandy, UT). Prior to exercise, the O_2 and CO_2 analyzers were calibrated using known gas constants, and the pneumotach was calibrated using a 3-L syringe in accordance with manufacturer guidelines. Humidity, temperature, and barometric pressure were recorded before the start of each test. The software of the metabolic cart automatically calculated 15-second averages, and these averages were used to calculate VO_2 peak. HR was monitored beat-by-beat using a Polar T31 HR monitor (Polar Electro, Inc., Lake Success, NY), and the HR data was automatically integrated into the Parvo-Medics TrueOne 2400® Metabolic Measurement System.

Each participant was instructed to do the following: (a) keep a diet and physical activity record 24 hours before the first experimental trial and replicate their diet and physical activity for the subsequent experimental trials, (b) maintain their usual caffeine intake during the study, (c) refrain from exhaustive exercise (greater than 80% HR max) and avoid alcohol intake 24 hours prior to each experimental trial, (d) abstain from consuming anything but water for the 12 hours prior to their experimental trials.

Experimental Procedures

Participants reported to the exercise physiology lab in the morning after following the pre-experimental guidelines described above. Each participant voided his bladder and bowels. A 22-gauge Teflon catheter was inserted into the antecubital vein of the

participant's arm, and a resting blood sample (6 ml) was collected using a syringe. The blood sample was immediately transferred to a tube and centrifuged once it clotted. The catheter was kept patent by flushing with 1.0-1.5 ml of saline after every blood draw. The catheter placement and all blood sampling were performed under standard sterile techniques by two investigators.

The exercise protocol consisted of high-intensity cycling. The cycle ergometer was configured to the same settings from the pre-experimental VO_2 peak test. The participant warmed-up for five minutes at 50% of his PPO. After the warm-up, each participant cycled at 60% of his PPO for 30 minutes. Beat-by-beat HR was monitored using a Polar T31 HR monitor (Polar Electro, Inc., Lake Success, NY) during each 30-minute trial, and average HR during the 30-minute trial was recorded. Immediately following the exercise, a blood sample (6 ml) was taken. The participant then immediately ingested one of the treatments (contained within a non-transparent gelatin capsule) with 75 g of dextrose mixed in 500 ml of purified water. A neutral party not involved with the data collection or analysis administered the treatment to each participant. Following this, the participant recovered for two hours in a seated position. Blood samples (6 ml) were drawn every 15-min for the first hour and every 30-min for the second hour. Prior to drawing each blood sample, 1.0-1.5 ml of blood was discarded. The catheter was kept patent by flushing with 1.0-1.5 ml of saline after every blood draw. Each participant was allowed to drink water during exercise and recovery, and they were cooled by an electric fan.

Blood Analysis

Blood samples were immediately dispensed from the syringe into two tubes: half

of the blood was transferred into a non-treated tube for later analysis of serum glucose and the other half was transferred into a non-treated tube for later analysis of serum insulin. The blood collected to analyze glucose and insulin was allowed to clot at 25º C and then centrifuged (Marathon 21K/BR centrifuge, Fisher Scientific, Pittsburgh, PA) at 3500 rpm and 4º C for 10 minutes. After centrifugation, the serum was transferred into tubes and stored at -80º C for subsequent analysis. All assays were performed by TriCore Reference Laboratories (Albuquerque, NM). Glucose assays were conducted enzymatically using a Dimension Vista® Intelligent Lab System and Dimension® EXL™ Integrated Chemistry System (Siemens AG; Munich, Germany). Insulin was assayed via two-site sandwich immunoassay of insulin using the ADVIA Centaur® XP analyzer (Siemens AG; Munich, Germany).

Calculations

The areas under the curve (AUC) for glucose and insulin were calculated in Prism (GraphPad Software, La Jolla, CA) using the trapezoidal rule (Gagnon & Peterson, 1998). The homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews, Hosker, Rudenski, Naylor, Treacher, & Turner, 1985) was calculated from pre-exercise fasting glucose and insulin values as well as immediate post-exercise glucose and insulin values using the following formula:

$$\frac{\text{glucose}\left(\frac{\text{mmol}}{\text{L}}\right) * \text{insulin}\left(\frac{\text{pmol}}{\text{L}}\right)}{22.5}$$

The Matsuda insulin sensitivity index (ISI) (Matsuda & DeFronzo, 1999) was calculated using the following formula:

$$\frac{10{,}000}{\sqrt{G0 * I0 * \frac{G0 + G30 * 2 + G60 * 2 + G90 * 2 + G120}{8} * \frac{I0 + I30 * 2 + I60 * 2 + I90 * 2 + I120}{8}}}$$

,where G0 and I0 = immediate post-exercise glucose and insulin concentrations, G30 and I30 = 30-min post-exercise glucose and insulin concentrations, G60 and I60 = 60-min post-exercise glucose and insulin concentrations, G90 and I90 = 90-min post-exercise glucose and insulin concentrations, G120 and I120 = 120-min post-exercise glucose and insulin concentrations.

Power Analysis

There is limited published research conducted on humans that has investigated the effect of chlorogenic acid supplementation on glucose and insulin during an OGTT (van Dijk et al., 2009). They found a statistical difference in glucose and insulin between chlorogenic acid and the placebo 45 minutes after ingesting the supplement (15 minutes after drinking the 75 g of glucose). The effect sizes for glucose and insulin at 15 minutes were 2.8 and 2.0, respectively. They did not find any significant differences between the glucose and insulin AUC for chlorogenic acid and the placebo.

Two studies were found that investigated the effect of ingesting a similar dose of caffeine as our study 60 minutes before an OGTT. Graham et al. (2001) did not find any significant differences in the glucose AUC between caffeine and the placebo. However, they did find significant difference in the AUC for insulin between caffeine and placebo, and the effect size was 2.99. Battram et al. (2006) found significant differences in the AUC for glucose and insulin, and the effect sizes were 2.53 and 2.99, respectively.

An *a priori* power analysis using G*Power Version 3.1.0 (Franz Faul, Universitat Kiel, Germany) was performed to determine the total sample size for this study using the average effect size of 2.66 from the studies described above, an alpha level of .05, and a power (1-beta) of 0.80. The statistical test utilized in G*Power was the difference

between two dependent means. Based on the results of the power analysis, a sample size of four men was required. However, ten men were recruited to reduce the likelihood of an insufficient sample size given the rigor of the protocol.

Statistical Analysis

Differences in blood-related parameters for glucose and insulin between the treatments and over time were analyzed using a two-way repeated-measure ANOVA. Tukey *post hoc* tests were applied if there were significant time-by-treatment effects. Differences in the glucose AUC, insulin AUC, pre-exercise HOMA-IR, post-exercise HOMA-IR, and ISI between the treatments were analyzed using a one-way repeated measures ANOVA. Tukey *post hoc* tests were applied if there were significant treatment effects. Differences were accepted as significant if $p < .05$. Values are presented as means ± SD. Data was analyzed using SPSS (version 17.0, Chicago, IL).

The relationship between BMI and insulin AUC, relative VO_2 peak and insulin AUC, absolute VO_2 peak and insulin AUC, absolute VO_2 peak and glucose AUC, and relative VO_2 peak and glucose AUC were investigated using linear regression analysis and Pearson correlations. The relationships between the amount of caffeine consumed and insulin AUC and the amount of chlorogenic acid consumed and insulin AUC were also investigated using linear regression analysis and Pearson correlations. Correlations were accepted as significant if $p < .05$.

CHAPTER 4: Results

Descriptive characteristics are summarized in Table 2, Appendix 1. Data that was collected from the VO_2 peak assessment is summarized in Table 3, Appendix 1. The average heart rate responses during each trial were not significantly different from one another (PLA = 167 ± 6 bpm, 91 ± 4% HR max; CAF = 165 ± 8 bpm, 90 ± 4% HR max; CGA = 163 ± 9 bpm, 89 ± 4% HR max). The average power output for each trial was 235 ± 30 W, 60 ± 2% PPO.

There was not a statistically significant treatment effect for fasting blood glucose and insulin concentrations before or immediately after exercise. Furthermore, there was not a statistically significant time-by-treatment effect for blood glucose or insulin concentrations during the OGTT (Table 4, Appendix 1; Figure 2, 3 and 4, Appendix 2). As a result, *post-hoc* tests were not conducted. There was also no statistically significant treatment effect for post-exercise AUC or Matsuda ISI for either glucose or insulin concentrations (Table 5, Appendix 1; Figure 5 and 6, respectively, Appendix 2). As a result, *post-hoc* tests were not conducted. HOMA-IR was significantly lower immediately after exercise during the CAF trial when compared to pre-exercise (Figure 7, Appendix 2). HOMA-IR was also lower after exercise during the PLA and CGA trials, but the difference was not statistically significant.

Glucose AUC, insulin AUC, and Matsuda ISI values were plotted in bar graphs to show the variability between subjects within each treatment and between each treatment (Figure 8, 9, and 10, respectively, Appendix 2). The glucose AUC between subjects during the PLA trial was not as variable as the glucose AUC between subjects during the CAF and CGA trials. However, the insulin AUC and Matsuda ISI between subjects

during all three treatments were highly variable.

BMI significantly ($p < .05$) and positively correlated with insulin AUC during all three trials (Figure 11, Appendix 2): however, there was no significant relationship between BMI and glucose AUC. Relative VO_2 peak was moderately and negatively correlated to insulin AUC during the PLA trial ($r = -.63$) and CGA trial ($r = -.63$) (Figure 12, Appendix 2), but absolute VO_2 peak did not significantly correlate with insulin AUC during the PLA ($r = -.25$) and CGA ($r = -.17$) trials. Relative VO_2 peak was highly and negatively correlated to insulin AUC during the CAF trial ($r = -.82$) (Figure 12, Appendix 2), but absolute VO_2 peak was moderately correlated to insulin AUC during the CAF trial ($r = -.50$). In addition, neither relative nor absolute VO_2 peak significantly correlated with glucose AUC for the PLA ($r = .33$ and $r = .49$, respectively), CAF ($r = .22$ and $r = .38$, respectively), and CGA ($r = .01$ and $r = .38$, respectively) trials. The amount of chlorogenic acid consumed during the CGA trial was moderately and significantly correlated with insulin AUC ($r = .64$), and the amount of caffeine consumed during the CAF trial was moderately correlated with insulin AUC ($r = .54$) (Figure 13, Appendix 2).

CHAPTER 5: Discussion

We investigated the effect of post-exercise caffeine and chlorogenic acid (via green coffee bean extract) supplementation on glucose disposal and insulin sensitivity in ten male cyclists. We hypothesized that consuming caffeine with dextrose immediately after exercise would significantly decrease glucose and insulin AUC when compared to consuming a placebo with dextrose or chlorogenic acid with dextrose. In addition, we hypothesized that consuming chlorogenic acid with dextrose immediately after exercise would not significantly affect glucose or insulin AUC when compared to consuming a placebo with dextrose. Based on our results, post-exercise consumption of caffeine with dextrose did not significantly affect glucose and insulin AUC when compared to consuming a placebo with dextrose or chlorogenic acid with dextrose. Furthermore, consuming chlorogenic acid with dextrose did not significantly affect glucose and insulin AUC when compared to consuming a placebo with dextrose. In other words, caffeine or chlorogenic acid did not enhance or detrimentally affect glucose or insulin clearance from the blood beyond that of control levels.

Effect of Post-Exercise Dextrose plus Caffeine Consumption on Blood Glucose Disposal and Insulin Sensitivity

The effect of post-exercise caffeine supplementation has only been investigated in four studies (Beelen et al., 2012; Pederson et al., 2008; Taylor et al., 2011; Thong et al. 2002). Three of these studies investigated the effect that caffeine had on post-exercise glycogen resynthesis rates (Beelen et al., 2012; Pederson et al., 2008; Thong et al. 2002), and the other study investigated the effect of ingesting caffeine after exercise on subsequent exercise performance (Taylor et al., 2011). No studies to date have

specifically investigated the effect of immediate post-exercise consumption of 5 mg/kg body weight of caffeine with 75 g of dextrose on a standard two-hour oral glucose tolerance test.

Pedersen et al. (2008) showed that consuming carbohydrates plus caffeine during a four-hour recovery from glycogen-depleting exercise significantly increased glycogen resynthesis rates (57.7 ± 18.5 mmol·kg dry weight^{-1}·hr^{-1}) when compared to consuming carbohydrates alone (38.0 ± 7.7 mmol·kg dry weight^{-1}·hr^{-1}). This was the first observation to show that caffeine helps facilitate glucose uptake into the muscle after a bout of glycogen-depleting exercise. Subjects consumed 1 g/kg body weight of carbohydrates in the form of sports bars, gels, and carbohydrate-containing sports drinks immediately post-exercise and 60, 120, and 180 minutes post-exercise. During the trial with caffeine, subjects consumed 4 mg/kg body weight of caffeine immediately post-exercise and 120 minutes post-exercise. In the present study, our subjects consumed 75 g of dextrose with 5 mg/kg body weight of caffeine immediately post-exercise, and we measured blood glucose and insulin for 120 minutes. Previous investigators measured blood glucose and insulin for 240 minutes (Pedersen et al., 2008). Similar to Pedersen's findings, ours revealed no significant differences in blood glucose and insulin during the first hour of recovery. However, Pedersen fed subjects greater amounts of carbohydrates following exercise making comparisons of data after one-hour post-exercise unreliable. In addition, Pedersen measured muscle glycogen content during the post-exercise recovery period, allowing inferences to be made about effects of caffeine and carbohydrates on glycogen re-synthesis.

Beelen et al. (2012) reported that consuming 1.2 g/kg body weight of carbohydrates with 1.7 mg/kg body weight of caffeine per hour after glycogen-depleting exercise did not significantly change the glucose or insulin AUC during six hours of recovery compared with consuming carbohydrates only. Our results indicated that there was not a significant difference in the glucose and insulin AUC between the CAF and PLA trials. In contrast to Pedersen et al. (2008), Beelen et al. (2012) failed to show a significant improvement in muscle glycogen resynthesis rates between the CAF plus CHO and CHO only trials.

Caffeine decreases insulin-mediated whole-body glucose disposal and subsequent skeletal muscle uptake at rest in humans (Battram, Graham, & Dela, 2007; Battram et al. 2005; Greer et al., 2001; Keijzers, Galan, Tack, & Smits, 2002; Thong & Graham, 2002), and it has been shown that this reduction is largely attributed to a reduction in insulin-stimulated glucose uptake in human skeletal muscle (Thong et al., 2002). However, Thong et al. (2002) showed that exercise can reduce the caffeine-induced impairment of insulin-mediated glucose uptake into the skeletal muscle (Thong et al., 2002). Additionally, they showed that caffeine reduced the beneficial effects of exercise on insulin-mediated glucose uptake into the skeletal muscle. As a result of higher glucose extraction during 60 minutes of repeated one-legged knee extensions alternating every five minutes at a workload eliciting 75% and 100% of localized thigh muscle VO_2 peak, glucose uptake into the thigh muscle was significantly increased during a euglycemic-hyperinsulinemic clamp that was initiated three hours after exercise. Ingesting caffeine two hours after exercise and one hour prior to the clamp significantly reduced glucose

extraction. This inhibitory effect of caffeine on glucose uptake occurred only in the presence of high insulin (510 ± 45 pmol/L).

Consuming caffeine during or after exercise does not impede post-exercise glycogen resynthesis (Battram, Shearer, Robinson & Graham, 2004; Thong et al., 2002) and may enhance glycogen resynthesis (Pedersen et al., 2008). An exhaustive bout of exercise may override caffeine's negative effects on glucose metabolism by enhancing insulin-independent mechanisms of glucose transport into the muscle as a result of lower levels of glycogen (Battram et al., 2004; Richter, Derave, & Wojaszewski, 2001). When an OGTT is administered with caffeine without prior exercise, the glucose AUC and insulin AUC are significantly elevated when compared to a placebo (Battram et al., 2006; Graham et al., 2001; Greenberg et al., 2010; Petrie et al., 2004; Pizziol et al., 1998; Robinson et al., 2004). Our data showed that ingesting 5 mg/kg body weight of caffeine combined with 75 g of dextrose immediately after 30 minutes of high-intensity cycling did not significantly elevate the glucose or insulin AUC during a two-hour OGTT when compared to ingesting only 75 g of dextrose. The two-hour glucose AUC for the CAF trial (658 ± 74 mmol/L) and PLA trial (661 ± 77 mmol/L) were very similar. However, more insulin was required for glucose disposal in the CAF trial (AUC = 30,005 ± 13,304 pmol/L) than in the PLA trial (AUC = 27,020 ± 12,339 pmol/L). As a result, the ISI for the CAF trial (9.7 ± 5.2) was slightly, but not significantly lower than the ISI for the PLA trial (10.0 ± 7.3). Our results support the findings of Thong et al. (2002), who showed that exercise reduced the deleterious effects of caffeine on glucose tolerance.

Based on our results and the results from Pedersen et al. (2008) and Beelen et al. (2012), consuming caffeine after exercise does not significantly affect blood glucose and

insulin. However, consuming caffeine with carbohydrates without prior exercise has consistently been shown to decrease glucose tolerance and insulin sensitivity in humans during an OGTT (Battram et al., 2006; Graham et al., 2001; Greenberg et al., 2010; Petrie et al., 2004; Pizziol et al., 1998; Robinson et al., 2004).

Effect of Post-Exercise Dextrose plus Chlorogenic Acid Consumption on Blood Glucose Disposal and Insulin Sensitivity

The effect of chlorogenic acid supplementation on blood glucose and insulin in humans during an OGTT has been investigated in a few studies (Thom, 2007; van Dijk et al., 2009). However, we are among the first to investigate the effect of post-exercise dextrose with chlorogenic acid from green coffee bean extract (50% chlorogenic acid) on blood glucose and insulin during an OGTT.

Thom (2007) investigated the effect of chlorogenic acid-supplemented coffee and normal coffee on the glucose profile of six healthy women and six healthy men. The two-hour glucose AUC was significantly lower after consuming the Coffee Slender (724 ± 8.2 mmol/L) when compared to the control (778 ± 10.2 mmol/L). The two-hour glucose AUC for the instant caffeinated coffee (788 ± 10.1 mmol/L) and instant decaffeinated coffee (818 ± 10.9 mmol/L) was not significantly different than the control. Bassoli et al. (2008) analyzed the effects of chlorogenic acid on hepatic glucose output, blood glucose levels, and glucose tolerance in male albino Wistar rats. During the OGTT, blood glucose was significantly lower in the chlorogenic acid group after 10 and 15 minutes by 21.8% and 17.8%, respectively, when compared to the placebo. van Dijk et al. (2009) investigated the effects of 12 g of decaffeinated coffee, 1 g of chlorogenic acid, 500 mg trigonelline, and 1 g of mannitol (placebo) on glucose and insulin concentrations

during an OGTT in 15 healthy, non-smoking, overweight men (BMI = 25.0-35.0 kg/m^2). The men ingested the supplements 30 minutes prior to ingesting 75 g of glucose. They measured glucose and insulin every 15 minutes during the OGTT. They found that chlorogenic acid ingestion significantly reduced glucose and insulin concentrations only at the 15-minute time-point when compared to the placebo, and there was no significant reduction in the glucose or insulin AUC when compared to the placebo.

We demonstrated that 5 mg/kg body weight of chlorogenic acid (via green coffee bean extract) consumed simultaneously with 75 g dextrose immediately after 30 minutes of high-intensity cycling (60% PPO) did not significantly lower blood glucose after 15 minutes compared to the placebo as seen in previous observations (Bassoli et al., 2008; van Dijk et al., 2009). After 60 minutes of the post-exercise OGTT, blood glucose was lower in the CGA trial than in the PLA and CAF trials after 60 minutes, but was not statistically significant (Table 4, Appendix 1; Figure 3 and 4, respectively, Appendix 2). The insulin AUC in our study was highest in the CGA trial, whereas the insulin AUC was lowest in the study conducted by van Dijk et al. (2009). This possibly indicates that chlorogenic acid may have a different effect on insulin's role in glucose homeostasis when ingested after exercise. The inter-subject variability of insulin AUC was elevated during the CGA trial when compared to the CAF and PLA trials, which perhaps could be attributed to differences in doses of chlorogenic acid given to the participants (5 mg/kg body weight).

To control for variability between the trials, treatments were administered in a double-blinded counterbalanced fashion. However, there was still large variability in blood glucose and insulin between the three trials during the post-exercise OGTT. This

was particularly notable in the blood insulin values. Therefore, we attributed the reduced effect of treatment during our observations in part to high variability in the insulin AUC between trials. The blood insulin AUC during the CGA trial was ~91% greater than the PLA trial and ~77% greater than the CAF trial (Table 6, Appendix 1). The increased variability in the insulin AUC during the CGA trial could be the result of a moderate dose-response relationship (r = .64) between the amount of chlorogenic acid that was consumed during the trial and the insulin AUC (Figure 13; Appendix 2). Subjects who consumed the most chlorogenic acid tended to have the greatest insulin AUC although the dosage was uniform in terms of g/kg of body weight.

BMI and VO_2 peak's Contribution to Variability in Insulin AUC

The variability and non-significant findings in the present study may have also been attributed to the high variability in BMI and/or VO_2 peak among subjects. Instead of recruiting a small homogenous sample of cyclists with similar body composition and VO_2 peak, we recruited a small heterogeneous sample of cyclists with large ranges in BMI (19.6 - 34.5 kg/m^2), VO_2 peak (38.1 – 65.3 $ml \cdot kg^{-1} \cdot min^{-1}$), and PPO (326-452 W). Our data revealed that BMI and VO_2 peak were significantly correlated with insulin AUC. This is discussed in further detail below.

BMI significantly ($p < .05$) and positively correlated with insulin AUC during all three trials (Figure 11, Appendix 2). However, there was no significant relationship between BMI and glucose AUC. Pratley, Hagberg, Dengel, Rogus, Muller, and Goldberg (2000) evaluated the effects of physical activity on body fat distribution and insulin secretion and action in 17 men 45-75 years of age. The men followed a progressive aerobic training program for nine months. Body composition analysis, VO_2 max tests,

hyperglycemic glucose clamps, and OGTTs were conducted before and after the training. BMI decreased from 26.6 ± 0.6 kg/m^2 to 25.9 ± 0.6 kg/m^2, and VO_2 max increased from 42.2 ± 1.9 ml·kg^{-1}·min^{-1} to 47.8 ± 1.7 ml·kg^{-1}·min^{-1}. The physical training intervention did not significantly change the two-hour glucose AUC; however, the two-hour insulin AUC decreased from 43,956 ± 4,470 pmol/L to 37,008 ± 4,709 pmol/L. The average plasma insulin concentrations during the late-phase insulin response during the hyperglycemic glucose clamp (20-120 minutes) significantly decreased from 268 ± 23 pmol/L to 233 ± 25 pmol/L. Additionally, the late-stage insulin response significantly correlated with changes in waist circumference (r = 0.84), percent body fat (r = 0.65), waist-to-hip ratio (r = 0.56), and hip circumference (r = 0.52). However, it did not significantly correlate with the change in VO_2 max (r = 0.26). The significant change in insulin AUC did not significantly correlate with changes in VO_2 max or body composition. Pratley and associates concluded that the effects of aerobic exercise training in lowering insulin responses to glucose are partially mediated by loss of fat in the abdominal region and not to changes in VO_2 max. We demonstrated that while BMI and VO_2 peak did not significantly correlate with glucose AUC, BMI and post-exercise insulin AUC were significantly related. We hypothesize that waist circumference, body fat, waist-to-hip ratio, or hip circumference may have also influenced insulin responses during post-exercise OGTT in our study as previously demonstrated (Pratley et al. 2000).

Obesity increases intramyocellular lipid concentration and decreases mitochondrial oxidative capacity. As a result, obesity is associated with insulin resistance and lower insulin sensitivity (Goodpaster & Brown, 2005). In the present study, subject 1 had the highest BMI (34.5 kg/m^2) and subject 3 had the lowest BMI

(19.6 kg/m^2). In addition, from visual inspection, subject 1 had the greatest abdominal fat and subject 3 had the least abdominal fat of all the subjects. It is interesting to note that the two-hour glucose AUC during the PLA trial for subject 1 (674.3 mmol/L) and subject 3 (686.4 mmol/L) were very similar (Figure 8, Appendix 2). However, subject 1 had the highest two-hour insulin AUC (47,926 pmol/L) and lowest insulin sensitivity (3.91), while subject 3 had the lowest insulin AUC (7,782 pmol/L) and second highest insulin sensitivity (17.74) during the PLA trial (Figure 9 and 10, Appendix 2). Subject 1 had the lowest relative VO_2 peak (38.1 ml·kg^{-1}·min^{-1}) but his absolute VO_2 peak was the fifth lowest (4.22 L/min). Subject 3 had the third highest relative VO_2 peak (63.9 ml·kg^{-1}·min^{-1}), but his absolute VO_2 peak was the sixth lowest (4.40 L/min). The correlation between BMI and insulin AUC (r = .73) was high and significant. Furthermore, the correlation between relative VO_2 peak and insulin AUC (r = -.63) was moderate and significant. However, the correlation between absolute VO_2 peak and insulin AUC (r = -.25) was low and non-significant. As a result, we concluded that the large range of variability in BMI (19.6-34.5 kg/m^2) rather than relative VO_2 peak (38.1-65.3 ml·kg^{-1}·min^{-1}) among our subjects contributed the most to the variability in insulin AUC (Figure 9, Appendix 2) during the PLA trial.

Chlorogenic Acid's Effect on Blood Glucose Disposal and Insulin Sensitivity when Compared to Caffeine

Although there were no significant main effects between the three trials for blood glucose and insulin, there are some interesting and notable differences between the three trials. First, blood glucose during the CAF trial was reduced between 0 and 30-min of the post-exercise OGTT when compared to the PLA and CGA trials (Figure 2 and 4,

respectively, Appendix 2). Additionally, blood glucose was reduced during the CGA trial between 30 and 120 minutes of the post-exercise OGTT when compared to the PLA and CAF trials. Second, during the first 30 minutes of the OGTT, blood insulin during the CAF trial was attenuated when compared to the PLA and CGA trials (Figure 2 and 4, respectively, Appendix 2). Interestingly, blood insulin was greater between 30 and 90 minutes during the CGA and CAF trials when compared to the PLA.

Muscle glycogen synthesis following exercise-induced glycogen depletion occurs in two phases which is reviewed in detail elsewhere (Jentjens and Jeukendrup, 2003). The initial phase (insulin-independent phase) is rapid and lasts for 30-60 minutes and is dependent on glycogen depletion during exercise, and subsequent glycogen synthase activity and GLUT4 translocation (Richter et al., 2001). After 60 minutes, glycogen synthesis activity is reduced and is dependent on insulin sensitivity and muscle glucose uptake. The increase in insulin sensitivity following exercise is regulated by muscle glycogen concentration, serum factors, AMPK, and insulin-signaling molecules.

The above evidence suggests that when compared to consuming caffeine after exercise, chlorogenic acid may affect blood glucose disposal through a different mechanism. During the first 30 minutes of the OGTT, blood glucose and insulin were lowest in the CAF trial suggesting caffeine may have stimulated insulin-independent glucose transport. Conversely, blood glucose during the first 30 minutes was similar between the CGA and PLA trials but higher than the CAF trial. However, insulin was relatively higher in the CGA trial despite reduced blood glucose in the CAF and PLA trials. After 30 minutes, insulin remained elevated in the CGA trial when compared to the PLA and CAF trial, but glucose was lower. This is further supported by the higher

ISI during the CGA trial (12.1 ± 7.9) when compared to the CAF (9.7 ± 5.2) and PLA (10.0 ± 7.3) trials (Table 8; Appendix 1; Figure 6, Appendix 2) although trials were statistically similar.

Previous observations suggest that chlorogenic acid may delay glucose absorption in the small intestine possibly through reduction in gastric inhibitory polypeptide (GIP) and increased glucagon-like peptide-1 (GLP-1) (Bassoli et al., 2008; Johnston et al., 2003) (Figure 1, Appendix 2). However, Olthof et al. (2011) showed that chlorogenic acid did not affect GIP and GLP-1 during an OGTT. Chlorogenic acid may also inhibit glucose-6-phosphatase, decrease hepatic glucose, and lower blood glucose (Arion et al., 1997). Furthermore, chlorogenic acid stimulated AMPK-mediated glucose transport into rat muscle (Ong et al., 2012). However, chlorogenic acid's effect on glucose-6-phosphatase and AMPK has not been verified in humans and was beyond the scope of our study.

Based on the glucose and insulin responses during the post-exercise OGTT, consuming 5 mg/kg body weight of chlorogenic acid via green coffee bean extract (50% chlorogenic acid) may stimulate AMPK-mediated transport of glucose into skeletal muscle. Our results do not support the hypotheses that chlorogenic acid may inhibit hepatic glucose output and delay intestinal glucose absorption because blood glucose between the first 30 minutes of the OGTT during the CGA trial was similar to the PLA trial. However, blood insulin during the CGA trial was higher between the first 30 minutes when compared to the PLA trial. Pedersen et al. (2008) showed that consuming caffeine with carbohydrates during glycogen-depleting exercise significantly enhanced glycogen synthesis compared with ingesting carbohydrates alone. Additional research is

warranted to evaluate the effect of coffee, caffeine, and chlorogenic acid consumption with carbohydrates following exercise and their effect on glycogen synthesis. Coffee or green coffee bean extract (50% chlorogenic acid) with concurrent carbohydrates during the post-exercise recovery period may significantly enhance glycogen synthesis when compared to consuming caffeine with carbohydrates or carbohydrates alone.

In conclusion, we found that consuming caffeine with dextrose or chlorogenic acid with dextrose immediately after exercise did not significantly affect blood glucose and insulin when compared to consuming dextrose alone. BMI and relative VO_2 peak were highly related to insulin AUC during all three treatments. As a result, body composition and training status should be similar between subjects for investigations of glucose tolerance and insulin sensitivity. Caffeine and chlorogenic acid may affect the body's ability to regulate post-exercise insulin-mediated glucose transport into the exercised skeletal muscle through different mechanisms; however more research is warranted to verify this hypothesis.

CHAPTER 6: Summary, Conclusions, Findings, and Recommendations

Summary

The purpose of this study was to investigate the effect of ingesting dextrose and caffeine or dextrose and chlorogenic acid immediately after an exhaustive bout of exercise on the blood glucose and insulin responses during a two-hour OGTT when compared to ingesting dextrose alone (placebo). We hypothesized that chlorogenic acid would significantly increase glucose clearance from the blood during the OGTT when compared to caffeine. In addition, we hypothesized that caffeine would significantly decrease glucose clearance from the blood during the OGTT when compared to a placebo. Finally, we hypothesized that consuming chlorogenic acid would not significantly affect glucose clearance from the blood during the OGTT when compared to a placebo.

Ten moderately to highly trained male cyclists participated in this study. Subjects were recruited by word-of-mouth, email list serves and posted flyers. They were between the ages of 19 and 34 years and were free of any cardiovascular, pulmonary or metabolic disease. The participants reported their daily consumption of caffeine using a questionnaire; they consumed on average 88 ± 55 oz of coffee, tea, or soda per week. The experimental procedures and possible risks were explained to each participant verbally and in writing prior to their participation in this study. They signed an informed consent and HIPAA release prior to participation, and these documents and the study were approved by the Human Research Review Committee.

This quasi-experimental, repeated measures study consisted of three experimental trials conducted in the morning after a 12-hour fast from food, beverages, or supplements

containing caffeine and chlorogenic acid. Each trial was randomized and separated by at least one week. The treatments were administered in a double-blinded fashion. During the experimental trials, a high intensity (60% PPO) 30-minute bout of cycling was completed. The three treatments consisted of immediate post-exercise co-ingestion of 75 g of dextrose with 5 mg/kg body weight of caffeine, 75 g of dextrose with 5 mg/kg body weight of chlorogenic acid (green coffee bean extract), or a placebo (75 g of dextrose). Blood samples were drawn every 15 minutes during the first hour and every 30 minutes during the second hour of a two-hour post-exercise OGTT to measure insulin and glucose. These measurements showed how chlorogenic acid and caffeine altered the blood glucose and insulin during the post-exercise OGTT when compared to the placebo.

A two-way (treatment and time) repeated measures ANOVA was conducted to evaluate the effects of the treatments on blood glucose and insulin responses during the post-exercise OGTT. In addition, a one-way (treatment) repeated measures ANOVA was conducted to evaluate the effect of the treatment on AUC for blood glucose and insulin.

All of our hypotheses were rejected except the hypotheses that chlorogenic acid would not significantly affect blood glucose and insulin AUC when compared to the placebo. Our results indicated that blood glucose and insulin during the CGA trial were not significantly different than the CAF and PLA trials; the blood glucose and insulin during the CAF trial were not significantly different than the PLA trial.

We sought to evaluate why there were not any statistically significant treatment effects as had been predicted. We noted the high variability between and within the treatments having large standard deviations. We speculate that this is one reason why we failed to show differences between treatments. We investigated which variables may

have contributed to the high variability by running several Pearson correlation analyses. We found that BMI and VO_2 peak significantly correlated with insulin AUC and that standard deviations for insulin AUC during the CGA trial were higher than in the CAF and PLA trials. Additionally, we observed that the relative amount of chlorogenic acid consumed during the CGA trial (5 mg/kg body weight) significantly correlated with insulin AUC. We speculate that there may have been a dose-response relationship between the amount of chlorogenic acid consumed and the insulin response during the CGA trial that contributed to the high variability in insulin AUC during the CGA trial. We calculated the Matsuda Insulin Sensitivity Index (ISI) and pre-exercise and post-exercise homeostasis model assessment of insulin resistance (HOMA-IR) to see if these were affected by the treatments. A one-way (treatment) repeated measures ANOVA showed that ISI and pre-exercise and post-exercise HOMA-IR were not significantly different between treatments.

Conclusions

Based on the data analysis, the following conclusions were made:

1. There was not a statistically significant time-by-treatment or treatment effect for blood glucose and insulin.
2. There was not a statistically significant treatment effect for blood glucose and insulin AUC.
3. The hypotheses substantiated by our data were numbers 1c and 2c, which stated that there would be no significant difference between blood glucose and insulin during the CGA trial when compared to the PLA trial.

Additional Findings

After investigation of our data, we concluded that the major reason we failed to show statistically significant findings was due to the high between-subject and between-trial variability. We discussed why inter-subject differences in BMI, VO_2 peak, and amount of chlorogenic acid consumed during the CGA trial may have contributed to the high variability. The high variability also could have been ascribed to the following factors: (a) training status, (b) diet the day before the trials, (c) amount and intensity of exercise performed the day or days prior to OGTT, and (d) glycogen levels after exercise. Each of these factors will be further discussed below.

Training Status

The insulin AUC during a resting OGTT is significantly greater in young men who have a higher VO_2 max than who have a lower VO_2 max, though glucose AUC is not significantly different. Fifteen men with a higher VO_2 max (65.8 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$) who were running 50 ± 6 miles per week had a lower 180-minute insulin AUC (~2,500 µU/ml) during an OGTT than 15 men (AUC = ~7,000 µU/ml) with a lower VO_2 max (44.4 ± 1.3 $ml \cdot kg^{-1} \cdot min^{-1}$) who had not performed any regular physical activity for several years (Seals et al., 1984). In another study, seven men with a higher VO_2 max (57.9 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$) who trained six days per week for 60 minutes per day had a lower 180-minute insulin AUC (~3,500 µU/ml) during an OGTT than seven men (AUC = ~7,000 µU/ml) with a lower VO_2 max (48.6 ± 2.2 $ml \cdot kg^{-1} \cdot min^{-1}$) who trained three days per week for 30 minutes per day (Young, Enslin, & Kuca, 1989).

Ten sedentary (SE; VO_2 max= 42.6 ± 6.7 $ml \cdot kg^{-1} \cdot min^{-1}$) men, twelve moderately trained (MT; VO_2 max = 47.1 ± 8.0 $ml \cdot kg^{-1} \cdot min^{-1}$) men who were training 163 ± 55

minutes per week, and twelve endurance trained men (ET; VO_2 max = 55.7 ± 6.6 ml·kg^{-1}·min^{-1}) who were training 659 ± 192 minutes per week were administered a 50 g OGTT the morning after an overnight fast (Mettler, Lamprecht-Rusca, Stoffel-Kurt, Wenk, & Colombani, 2007). The venous glucose and insulin responses and incremental AUC were lowest in the ET and highest in the SE.

Our data showed that subjects who had a higher VO_2 peak tended to have a lower insulin AUC than those who had a lower VO_2 peak (Figure 12, Appendix 2), but there was no relationship between glucose AUC and VO_2 peak. Based on our results and the results of these prior studies, the high variability in training status and VO_2 peak may have contributed to the large variation in insulin response during the post-exercise OGTT.

Diet the Day before an OGTT

In our study, subjects were asked to record their diet for 24 hours before their first trial and repeat it for the following two trials. The amount of carbohydrates, protein, and fat consumed the day prior to the trials was not controlled and this may have accounted for some of the variability in our data. Ivy, Frishberg, Farrell, Miller and Sherman (1985) showed that consuming a high-carbohydrate diet (75% carbohydrates, 14% fat, and 11% protein) for three days after an exhaustive bout of exercise can significantly increase insulin responses and significantly decrease glucose responses during an OGTT when compared to consuming a high-fat-protein diet (12% carbohydrates, 49% fat, and 39% protein) and mixed diet (41% carbohydrates, 30% fat, and 29% protein). Some of the subjects in our study who had the highest ISI (> 10.0) during the PLA trial (subjects 2, 3, and 9) could have been consuming a high carbohydrate diet up to three days prior to the

trial. However, this does not explain why six out of the ten subjects (subjects 2, 3, 4, 5, 8, and 10) during the CGA trial and four out of the ten subjects (2, 3, 5, and 8) during the CAF trial had an ISI > 10.0. Therefore, diet may not have contributed substantially to the variability between trials and subjects.

Amount and Intensity of Exercise Performed before an OGTT

Subjects were asked to refrain from exhaustive exercise (> 80% HR max) for 24 hours prior to their trials and to perform the same type of exercise (if any) the day before each trial. Insulin sensitivity can be affected by the amount and type of exercise performed prior to an OGTT. Young et al. (1989) investigated the effect of 40 minutes of cycling at 40% VO_2 max and 80% VO_2 max in the afternoon (4-5 pm) prior an OGTT the next morning (8-9 am). They found that a single bout of exercise at 40% VO_2 max and 80% VO_2 max significantly reduced the insulin AUC in the men with a lower VO_2 max (48.6 ± 2.2 ml·kg^{-1}·min^{-1}) who trained three days per week for 30 minutes per day to a similar level as the men with a higher VO_2 max (57.9 ± 2.5 ml·kg^{-1}·min^{-1}) who trained six days per week for 60 minutes per day. It is interesting to note that the insulin AUC for the more highly trained men was slightly higher as the intensity of exercise increased, whereas the insulin AUC for the men who trained less decreased with increasing intensity. There was not a training effect on glucose AUC.

Detraining for ten days significantly increased plasma insulin and glucose responses of six men and two women (VO_2 max = 58.6 ± 2.2 ml·kg^{-1}·min^{-1}) during an OGTT (Heath, Gavin III, Hinderliter, Hagberg, Bloomfield & Holloszy, 1983). However, one bout of exercise performed 11 days after detraining significantly reduced the glucose and insulin responses, returning them to their values before they de-trained.

These results provide evidence that chronic adaptations to improved glucose tolerance and insulin sensitivity can be lost with ten days of detraining; however, the adaptation can be regained after a single bout of exercise.

The results from the above referenced studies indicate that the amount of exercise the subjects in our study performed ten days prior, as well as the day prior, may have contributed to the variability in glucose and insulin responses during the post-exercise OGTT. Though subjects were asked to perform the same exercise before each trial, some of the participants may not have abided by these guidelines. This may have somewhat contributed to the variability in our data.

Glycogen Levels before an OGTT

The exercise that was performed in our study (30 minutes of cycling at 60% PPO) was not likely to deplete glycogen. Exercise can acutely reduce insulin resistance and improve glucose tolerance, and these improvements may be related to muscle glycogen depletion (Jensen, Rustad, Kolnes, & Lai, 2011; Richter et al., 2001; Wojtaszewski, Jorgensen, Frosig, MacDonald, Birk, & Richter, 2003). Muscle glucose uptake is higher when exercising with low glycogen and after a glycogen-depleting bout of exercise. The enhanced glucose uptake is attributed partially to an enhancement of glycogen synthase activity and GLUT4 translocation to the surface membrane of the muscle cell (Richter et al., 2001, Wojtaszewski et al, 2003).

Some of our subjects may have started with lower glycogen and/or ended exercise with lower glycogen than other subjects. This may have contributed to the variability in insulin responses during the post-exercise period. In particular, this phenomenon may have occurred with subject six during all three trials. He had one of

the highest insulin responses during all three trials, and also had a difficult time finishing the exercise trial due to muscle fatigue (Figure 9, Appendix 2). The exercise workload had to be lowered from 60% PPO to 50% PPO halfway through the trials in order for the subject to be able to complete the exercise bout. However, his BMI (24.3 kg/m^2) and VO_2 peak (57.4 $ml \cdot kg^{-1} \cdot min^{-1}$) were around average for our sample. His data points were the furthest from the regression lines in the figures depicting the relationship between BMI and insulin AUC (Figure 11, Appendix 2) as well as VO_2 peak and insulin AUC (Figure 12, Appendix 2) for all three trials. This could indicate that he was exercising with low glycogen and ended exercise with low glycogen, and as a result, his insulin response led to transport of the ingested dextrose into the glycogen-depleted muscles of his legs.

Recommendations

Future research on the effect of post-exercise ingestion of caffeine and chlorogenic acid on metabolism should consider the following:

1. Implement a controlled diet and exercise regimen 48 hours prior to the day of the trial. The type of diet can affect subsequent results during an OGTT.
2. If possible, verify that muscle glycogen concentrations are similar before and after exercise for each treatment by taking muscle biopsies from the vastus lateralis.
3. Control and record RPE and RER during the exercise trials because this may provide insight regarding the amount and type of substrate utilized.
4. Conduct a baseline resting OGTT while fasted to verify that glucose tolerance and insulin sensitivity are similar between subjects.

5. Recruit a homogeneous sample with similar training status, body composition, and VO_2 max because research has shown that these variables can affect insulin sensitivity.
6. Measure hip and waist circumferences as well as conduct body fat analysis to investigate how these measurements affect and contribute to glucose clearance and insulin sensitivity.
7. Investigate the effects of the simultaneous consumption of caffeine and chlorogenic acid on post-exercise blood glucose disposal, insulin sensitivity, blood glucose uptake into the muscle, and the rate of glycogen synthesis.
8. Investigate the effect of caffeinated and decaffeinated coffee on post-exercise blood glucose disposal, insulin sensitivity, blood glucose uptake into the muscle, and the rate of glycogen synthesis
9. Investigate the effect of an absolute amount (e.g. 1 g) and a relative amount (e.g. 5 mg/kg body weight) of chlorogenic acid on post-exercise blood glucose disposal, insulin sensitivity, blood glucose uptake into the muscle, and the rate of glycogen synthesis. This will help identify if there is a dose-response relationship.

Other questions to be answered in the future:

1. Does chlorogenic acid stimulate AMPK mediated glucose uptake into the muscle during post-exercise recovery? Is there a higher rate of glycogen synthesis per hour when compared to consuming caffeine?
2. Does gut motility affect blood glucose disposal, insulin sensitivity, blood glucose uptake into the muscle, and the rate of glycogen synthesis?

3. What are the chronic effects of consuming caffeine, caffeinated coffee, decaffeinated coffee, green coffee bean extract, and chlorogenic acid on blood glucose disposal, insulin sensitivity, blood glucose uptake into the muscle, and the rate of glycogen synthesis?

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APPENDIX 1: Tables

Table 1. Resting blood glucose response before and after ingesting caffeine

Study	Subjects[a]	Treatment[b]	Caffeine Dose[c]	Time Treatment Ingested	Glucose Before Ingestion (mmol/L)[d]	Glucose After Ingestion (mmol/L)[d]
Casal and Leon (1985)	9 M well-trained marathon runners	CAF DC PL (water)	400 mg	60 min before running on a treadmill for 45-min at 75% VO_2max	5.1 ± 0.2 5.0 ± 0.2 5.1 ± 0.1	5.1 ± 0.2 4.9 ± 0.1 4.9 ± 0.1
Graham and Spriet (1991)	1 F and 6 M well-trained distance runners	CAF PL	9 mg/kg 1 h before exercise	60 min before running on a treadmill to exhaustion at ~85% VO_2max	3.60 ± 0.25 3.47 ± 0.37	3.79 ± 0.19 3.55 ± 0.14
Graham and Spriet (1991)		CAF PL	9 mg/kg 1 h before exercise	60 min before cycling to exhaustion at ~85% VO_2max	4.00 ± 0.37 3.42 ± 0.31	3.85 ± 0.23 3.48 ± 0.16
Graham and Spriet (1995)	8 M trained in distance running	CAF CAF CAF PL (Dextrose)	3 mg/kg 6 mg/kg 9 mg/kg N/A	60 min before running on a treadmill to exhaustion at ~85% VO_2max	3.59 ± 0.34 3.83 ± 0.30 3.26 ± 0.29 3.39 ± 0.47	3.27 ± 0.26 3.62 ± 0.27 3.95 ± 0.17 3.35 ± 0.29
Graham, Hibbert, and Sathasivam (1998)	8 M and 1 F actively training endurance runners	CAF DC+C RC DC PL (Dextrose)	4.45 mg/kg 4.45 mg/kg 4.45 mg/kg NA NA	60 min before running on a treadmill to exhaustion at ~85% VO_2max	3.6 ± 0.4 3.6 ± 0.2 3.6 ± 0.2 3.4 ± 0.2 3.7 ± 0.2	4.0 ± 0.2 4.2 ± 0.3 4.2 ± 0.2 3.7 ± 0.3 3.7 ± 0.3
Greer, Friars, and Graham (2000)	8 healthy, active M	CAF PL (Dextrose)	6 mg/kg NA	90 min before cycling to exhaustion at ~80-85% VO_2max	3.90 ± 0.22 3.88 ± 0.19	4.36 ± 0.41 4.24 ± 0.19
Greer, Friars, and Graham (2000)	7 healthy, active M	CAF PL (Dextrose)	6 mg/kg NA	90 min before cycling for 45 min at ~65-70% VO_2max	4.72 ± 0.33 4.33 ± 0.42	4.52 ± 0.29 4.35 ± 0.23

Spriet et al. (1992)	7 M and 1 F recreational cyclists	CAF PL	9 mg/kg 1 h before exercise	60 min before cycling to exhaustion at ~80% VO_2max	3.54 ± 0.32 4.05 ± 0.28	3.04 ± 0.34 3.29 ± 0.27
Van Soeren, Sathasivam, Spriet, and Graham (1993)	7 M habitual users of caffeine (400-750 mg/day)	CAF/CAF PL/CAF PL	5 mg/kg CAF per day for 6 days then 5 mg/kg CAF before trial 5 mg/kg per day of PL for 6 days then 5 mg/kg CAF before trial NA	60 min before cycling for 60 min at 50% VO_2max	3.33 ± 0.39 3.58 ± 0.22 3.74 ± 0.08	3.13 ± 0.35 3.75 ± 0.35 3.16 ± 0.28
Van Soeren et al. (1993)	7 M nonusers of caffeine (0-20 mg/week)	CAF PL	5 mg/kg NA	60 min before cycling for 60 min at 50% VO_2max	3.53 ± 0.36 3.68 ± 0.24	3.56 ± 0.26 3.39 ± 0.22
Greenberg, Owen, and Geliebter (2010)	11 healthy M	CAF RC DC PL	6 mg/kg 6 mg/kg NA NA	60 min before an OGTT	4.18 ± 0.14 4.29 ± 0.09 4.34 ± 0.16 4.25 ± 0.14	4.31 ± 0.12 4.62 ± 0.11 4.44 ± 0.18 4.35 ± 0.19

[a] M = male, F = female; [b] CAF = caffeine, PL = placebo, DC + C = decaffeinated coffee + caffeine, RC = caffeinated coffee, DC = decaffeinated coffee, CAF/CAF = caffeine ingestion each day for 6 days before trial/caffeine 60 min before trial, PL/CAF = placebo ingestion each day for 6 days before trial/caffeine 60 min before trial; [c] caffeine dose in mg/kg body wt.; [d] There were not any significant differences in glucose concentration between the treatments.

Table 2. Descriptive data (N = 10)

Variable	Mean ± SD	Range
Age (years)	26 ± 5	19-34
Height (cm)	179.9 ± 5.4	168.8-184.4
Weight (kg)	77.6 ± 13.3	66.4-110.8
BMI (kg/m^2)	24.0 ± 4.3	19.6-34.5
Resting systolic blood pressure (mmHg)	119 ± 15	96-144
Resting diastolic blood pressure (mmHg)	75 ± 8	62-84
Caffeinated coffee, tea, or soda per week (oz)	88 ± 55	30-224

BMI = body mass index

Table 3. Data from VO_2 peak test (N = 10)

Variable	Mean ± SD	Range
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	55.9 ± 8.4	38.1-65.3
VO_2 peak ($L \cdot min^{-1}$)	4.28 ± 0.45	3.62-4.92
Maximum Heart Rate (bpm)	183 ± 7	171-194
Maximum RER	1.26 ± 0.02	1.23-1.29
Peak Power (W)	392 ± 47	326-452
Max RPE	19 ± 1	17-20
Heart rate at 60% of peak power (bpm)	151 ± 11	133-167
Percentage of maximum heart rate at 60% peak power	82 ± 4	76-88
RER at 60% of peak power	0.99 ± 0.04	0.91-1.04

RPE = rating of perceived exertion; RER = respiratory exchange ratio

Table 4. Glucose and insulin concentrations before exercise and during a post-exercise OGTT (N = 10)

	Time (min)						
Variable and Treatment	**-30**	**0**	**15**	**30**	**60**	**90**	**120**
Glucose (mmol/L)							
Placebo	4.7 ± 0.2	5.6 ± 0.6	7.1 ± 1.0	7.1 ± 1.1	5.4 ± 1.4	4.4 ± 0.7	3.7 ± 0.7
Caffeine	4.7 ± 0.4	5.3 ± 0.5	6.7 ± 0.9	7.2 ± 0.7	5.4 ± 1.3	4.5 ± 0.9	3.8 ± 0.8
Chlorogenic Acid	4.8 ± 0.3	5.2 ± 0.8	7.0 ± 1.1	7.1 ± 1.5	5.2 ± 1.3	4.2 ± 0.9	3.5 ± 0.9
Insulin (pmol/L)							
Placebo	67.8 ± 45.6	46.0 ± 23.5	217.0 ± 103.1	517.7 ± 361.9	246.1 ± 95.2	134.7 ± 69.4	49.9 ± 34.2
Caffeine	69.9 ± 37.8	39.0 ± 28.6	184.7 ± 81.4	489.3 ± 296.8	363.4 ± 191.8	144.5 ± 49.6	46.4 ± 24.7
Chlorogenic Acid	76.1 ± 59.9	47.0 ± 43.1	262.0 ± 252.9	562.1 ± 537.5	336.6 ± 257.0	137.8 ± 68.2	53.6 ± 29.7

There was no statistically significant treatment effect for glucose and insulin. Therefore, *post-hoc* analyses were not conducted. Time-point –30 represents fasting values immediately before exercise. Time-point 0 represents values immediately after exercise and before ingesting the treatment + 75 g dextrose. Time-points 15, 30, 60, 90, and 120 represent the time after ingesting the treatment + 75 g dextrose. Values are Mean ± SD.

Table 5. Glucose and insulin area under the curve (AUC) during a 120-min post-exercise OGTT (N = 10) using American and International units

Variable and Treatment	AUC (120-min)	Variable and Treatment	AUC (120-min)
Glucose (mmol/L)		Glucose (mg/dL)	
Placebo	661 ± 77	Placebo	11,912 ± 1,386
Caffeine	658 ± 74	Caffeine	11,853 ± 1,335
Chlorogenic Acid	637 ± 100	Chlorogenic Acid	11,483 ± 1,804
Insulin (pmol/L)		Insulin (µIU/mL)	
Placebo	27,020 ± 12,339	Placebo	3,891 ± 1,777
Caffeine	30,005 ± 13,304	Caffeine	4,321 ± 1,916
Chlorogenic Acid	31,965 ± 23,586	Chlorogenic Acid	4,603 ± 3,396

There was no statistically significant treatment effect for glucose and insulin. Therefore, *post-hoc* analyses were not conducted. AUC = area under the curve. The international units are in the second column, and the American units are in the fourth column. Values are Mean ± SD

APPENDIX 2: Figures

Figure 1. Hypothesized mechanisms of chlorogenic acid for lowering blood glucose

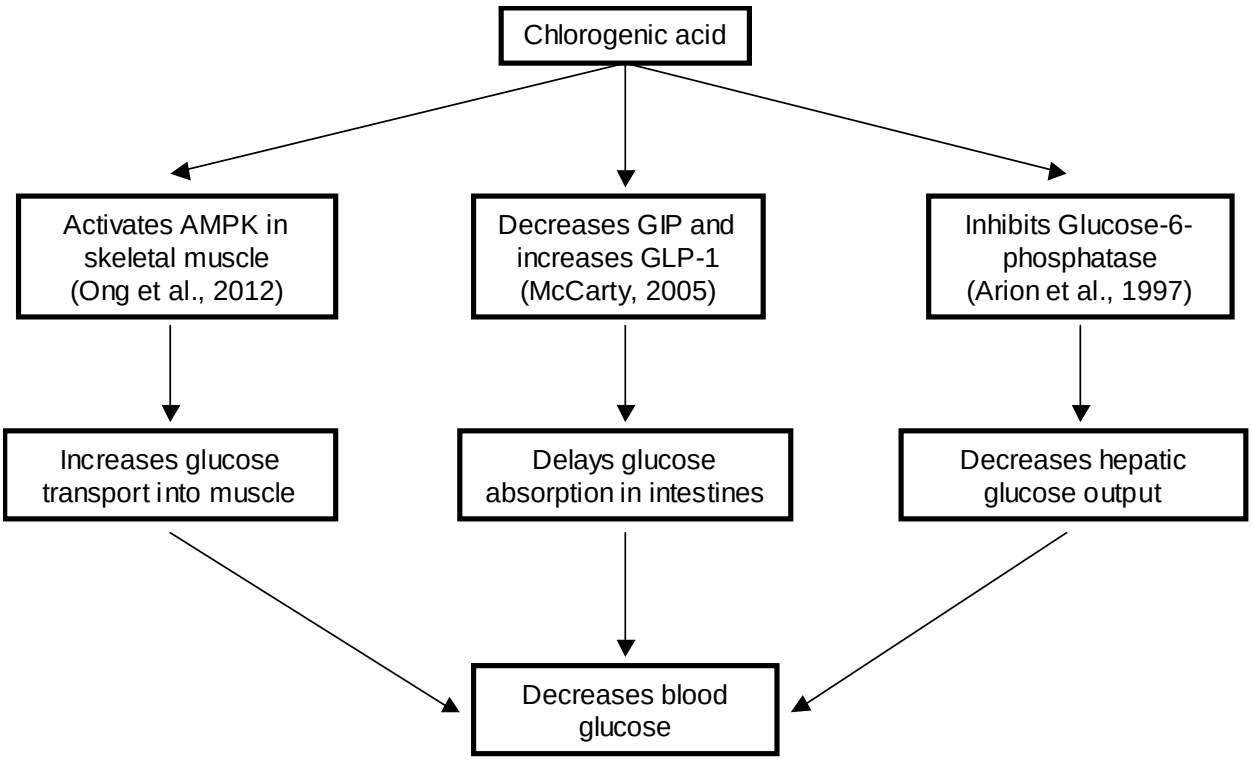
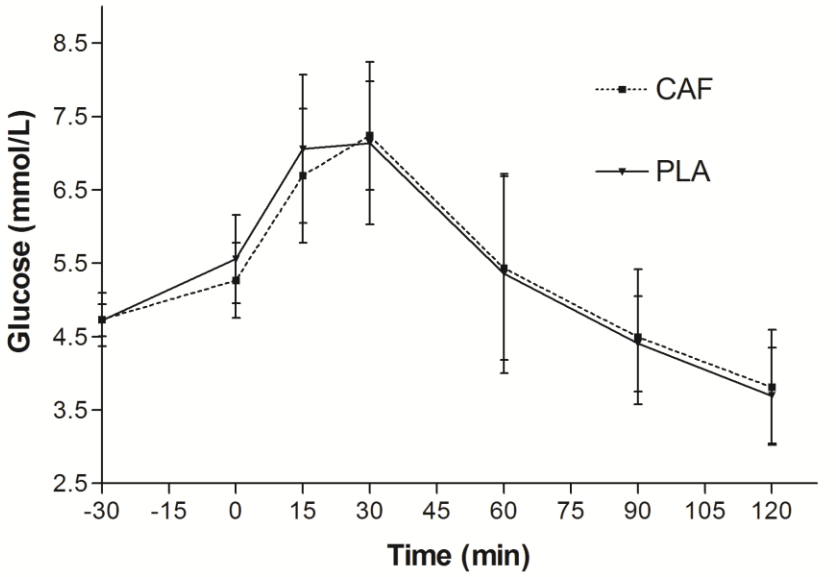


Figure 2. Time course for glucose and insulin concentrations in men before and during the post-exercise OGTT for the caffeine (CAF) and placebo (PLA) trials. Values are means ± SD, n = 10. There were not any significant differences over time between the trials.



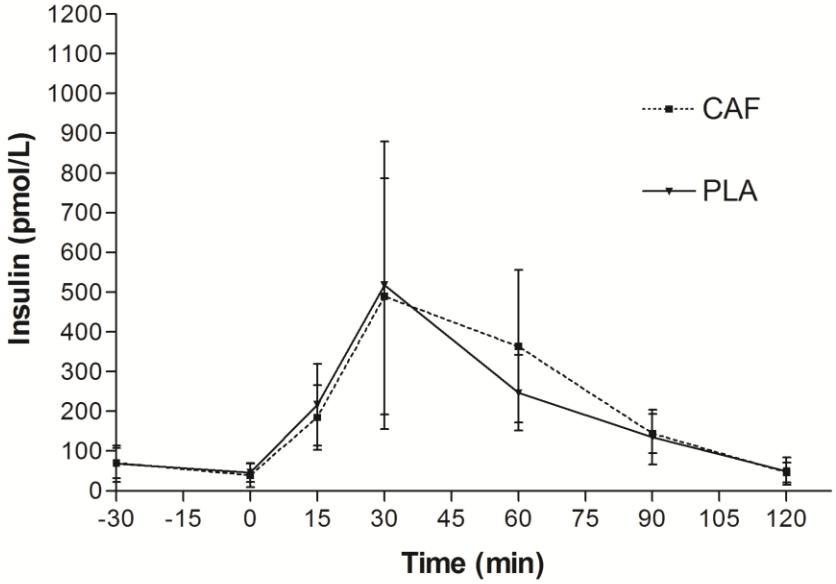
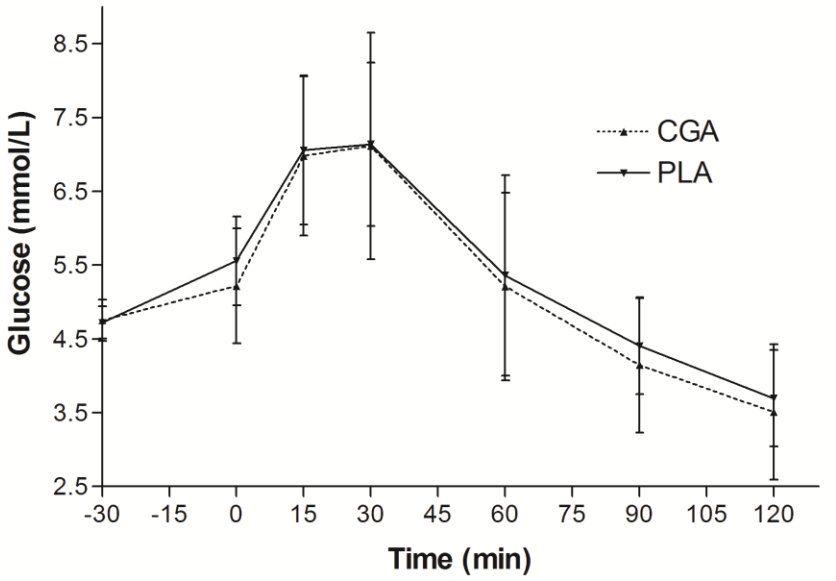


Figure 3. Time course for glucose and insulin concentrations in men before and during the post-exercise OGTT for the chlorogenic acid (CGA) and placebo (PLA) trials. Values are means ± SD, N = 10. There were not any significant differences over time between the trials.



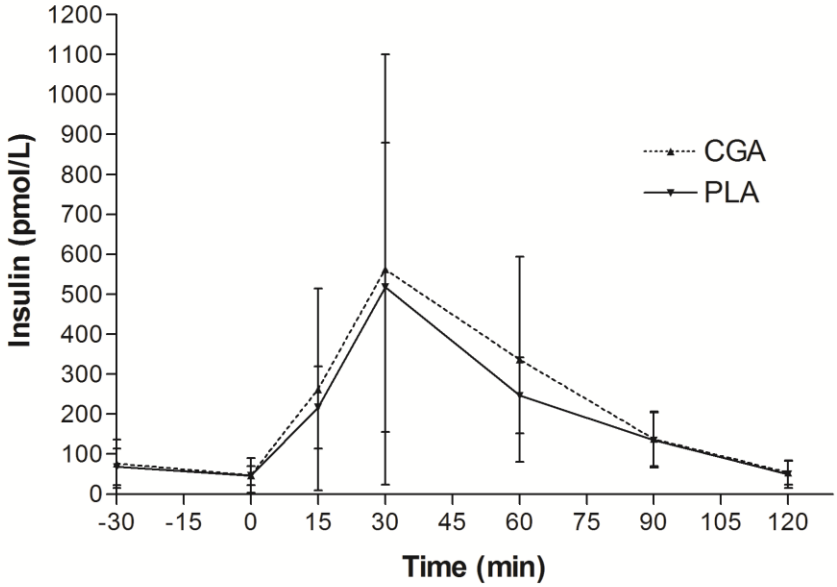
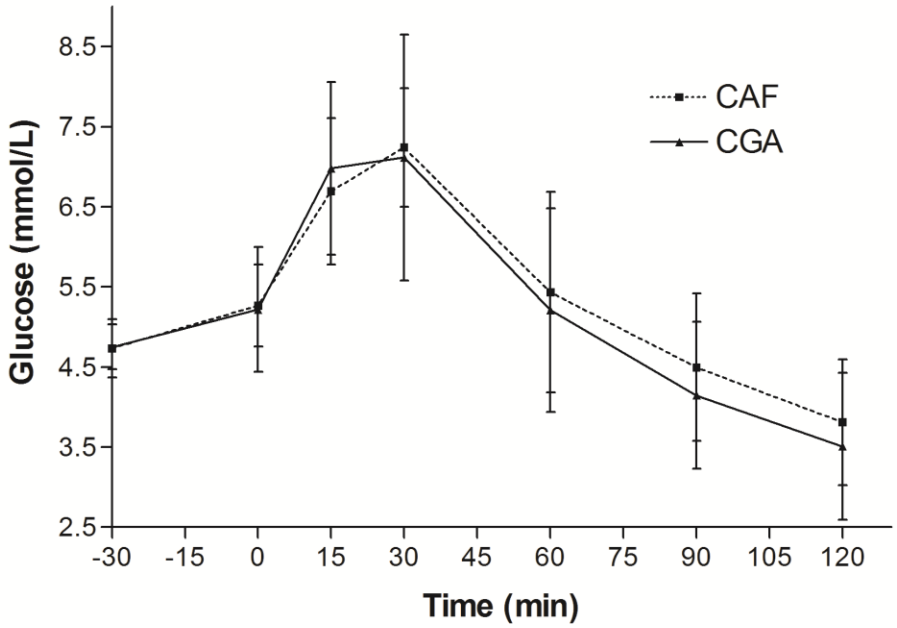


Figure 4. Time course for glucose and insulin concentrations in men before and during the post-exercise OGTT for the caffeine (CAF) and chlorogenic acid (CGA) trials. Values are means ± SD, N = 10. There were not any significant differences over time between the trials.



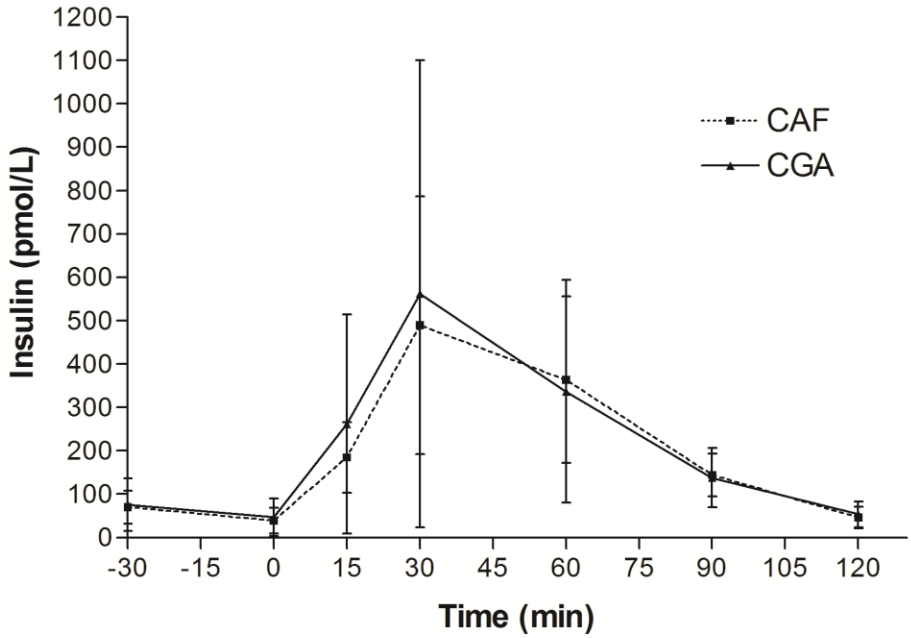
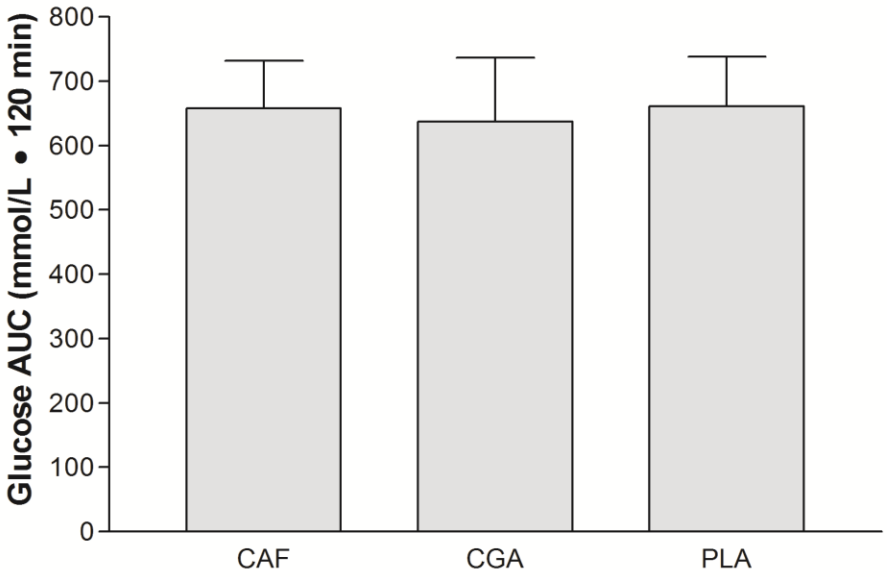


Figure 5. Area under the curve (AUC) for glucose and insulin during the post-exercise 120-minute OGTT for the caffeine (CAF), chlorogenic acid (CGA), and placebo (PLA) trials. Values are means ± SD, N = 10. There were no significant differences between trials.



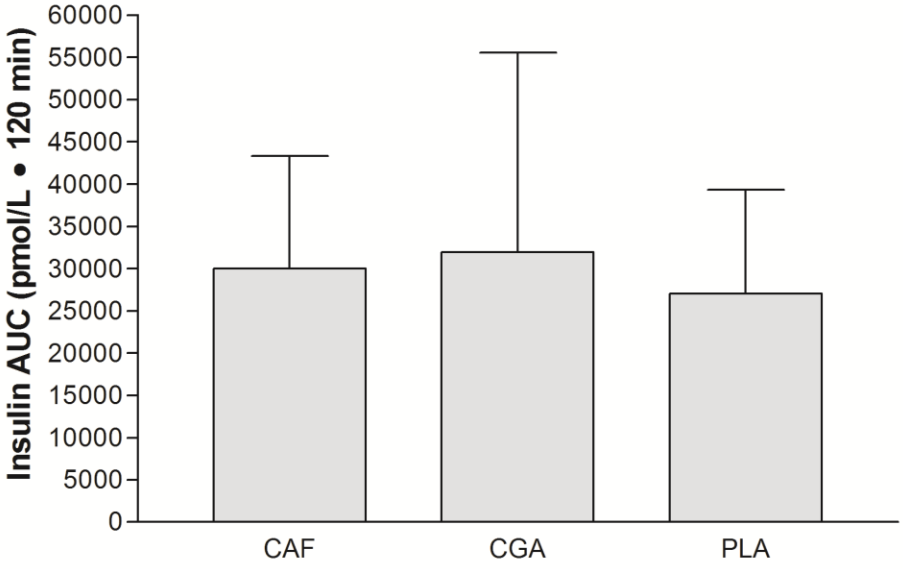


Figure 6. Matsuda Insulin Sensitivity Index (ISI) during the post-exercise OGTT for the caffeine (CAF), chlorogenic acid (CGA), and placebo (PLA) trials. Values are means ± SD, N =10. There were not any significant differences between trials.

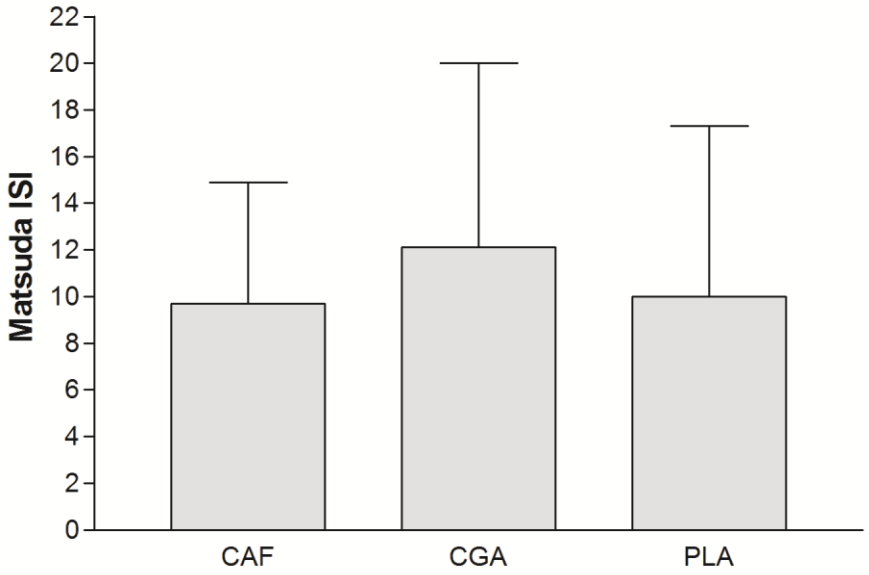


Figure 7. Homeostasis model assessment of insulin resistance (HOMA-IR) from pre- (left bar) and post (right bar)-exercise blood glucose and insulin during the caffeine (CAF), chlorogenic acid (CGA), and placebo (PLA) trials. * indicates a significant difference between pre and post-exercise HOMA-IR. Values are means ± SD, N = 10.

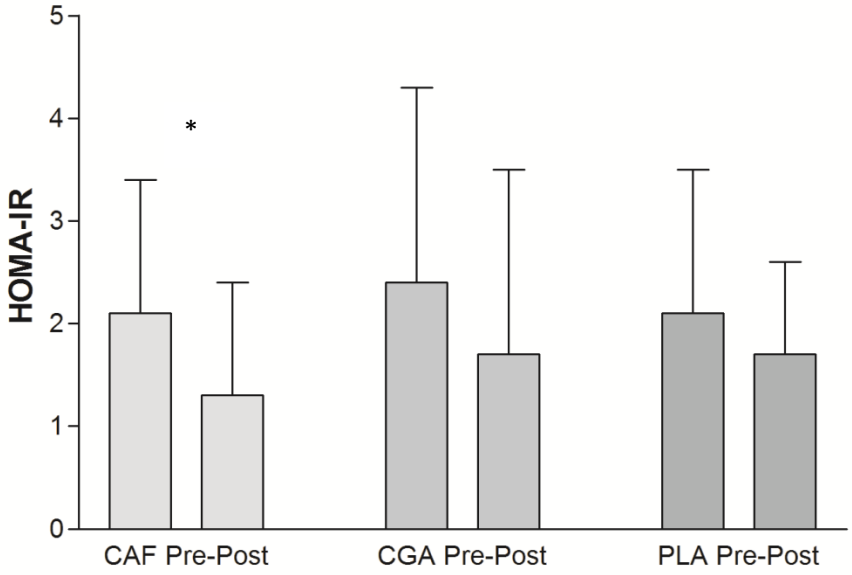


Figure 8. Glucose area under the curve (AUC) for each subject during the placebo (PLA), caffeine (CAF), and chlorogenic acid (CGA) trials.

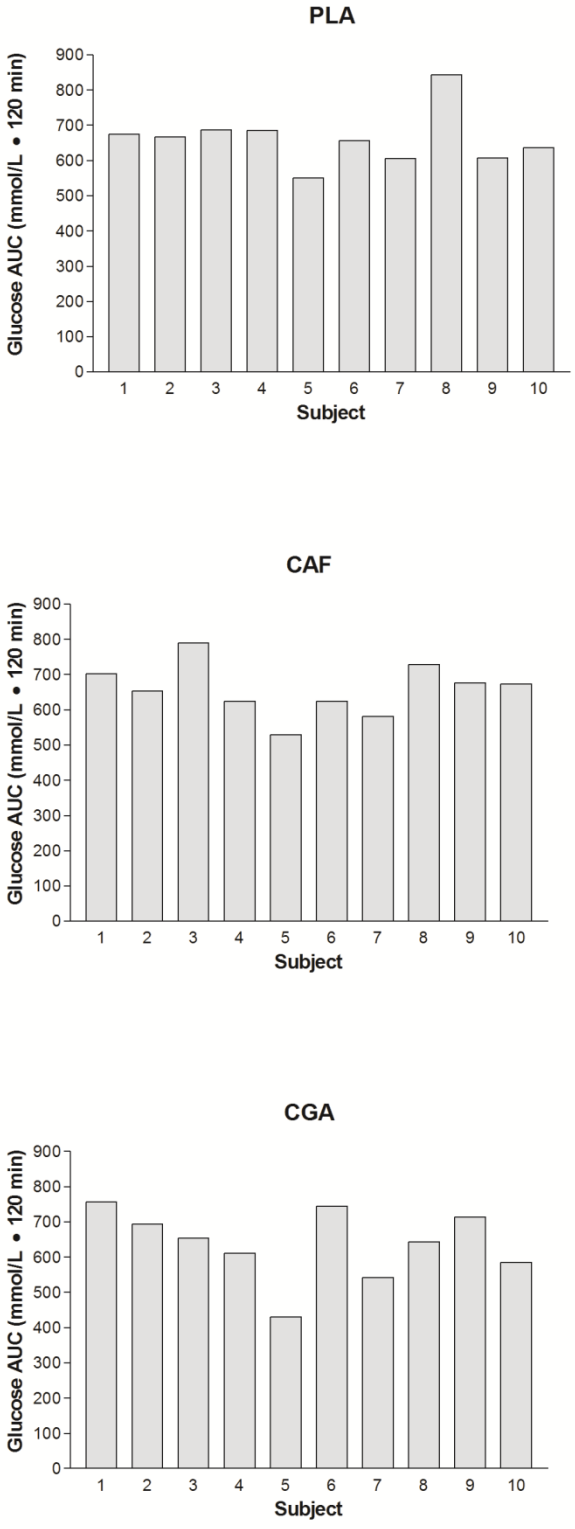


Figure 9. Insulin area under the curve (AUC) for each subject during the placebo (PLA), caffeine (CAF), and chlorogenic acid (CGA) trials.

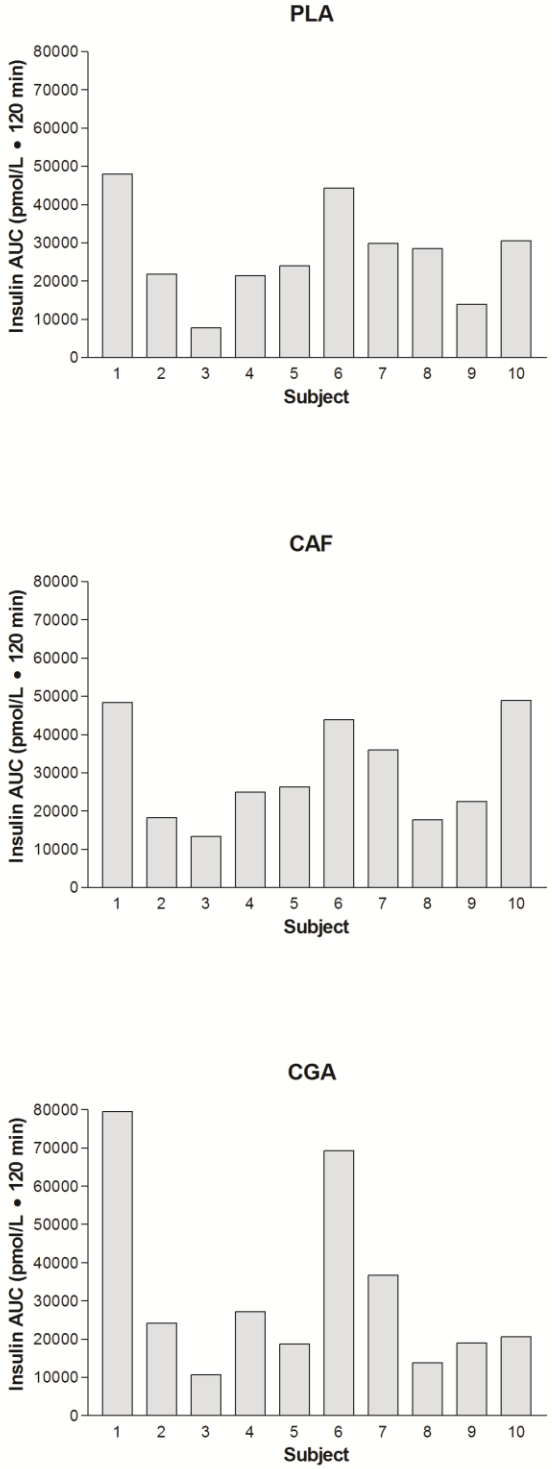


Figure 10. Matsuda Insulin Sensitivity Index (ISI) for each subject during the placebo (PLA), caffeine (CAF), and chlorogenic acid (CGA) trials.

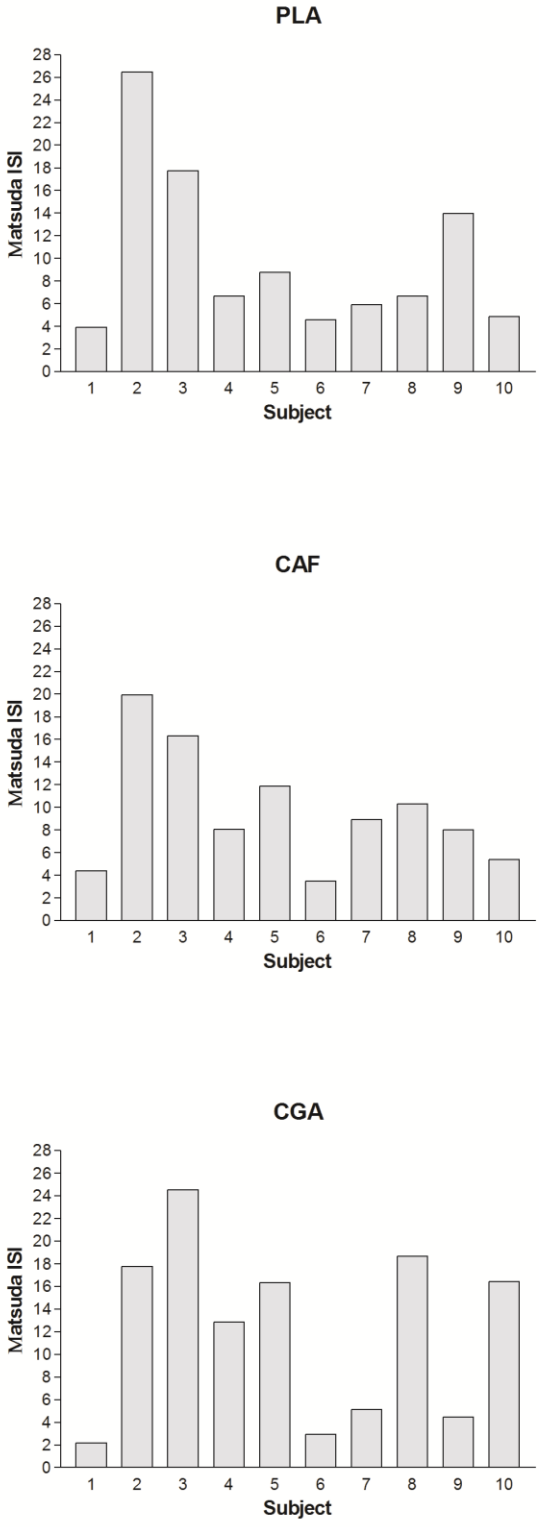


Figure 11. Relationship between body mass index (BMI) and insulin area under the curve (AUC) during the placebo (PLA), caffeine (CAF), and chlorogenic acid (CGA) trials.

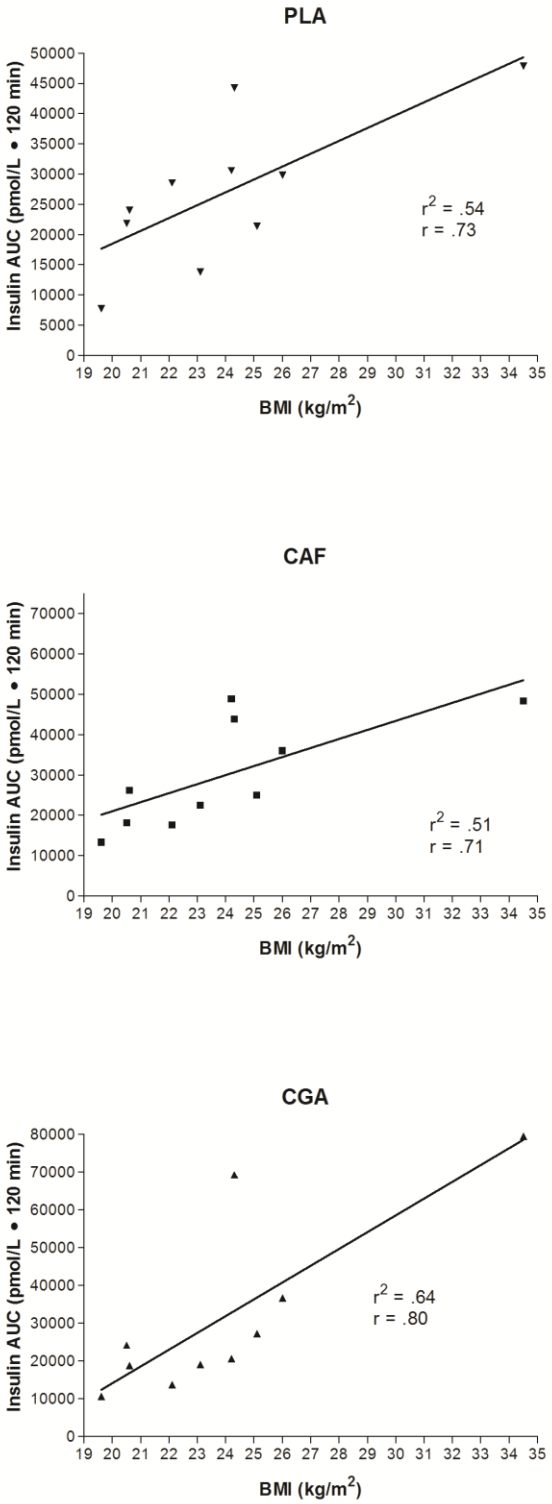
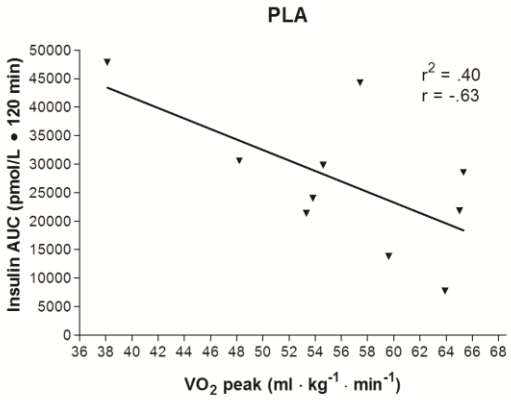
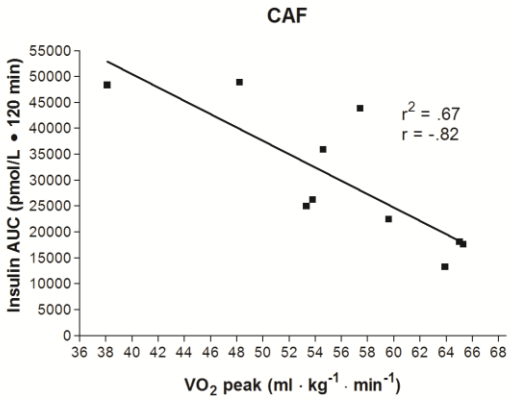


Figure 12. Relationship between peak oxygen consumption (VO_2 peak) and insulin area under the curve (AUC) during the placebo (PLA), caffeine (CAF), and chlorogenic acid (CGA) trials.





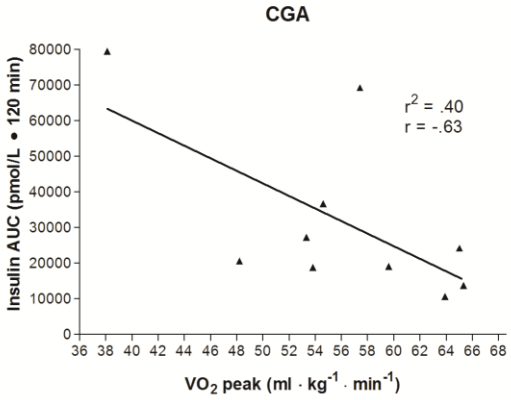
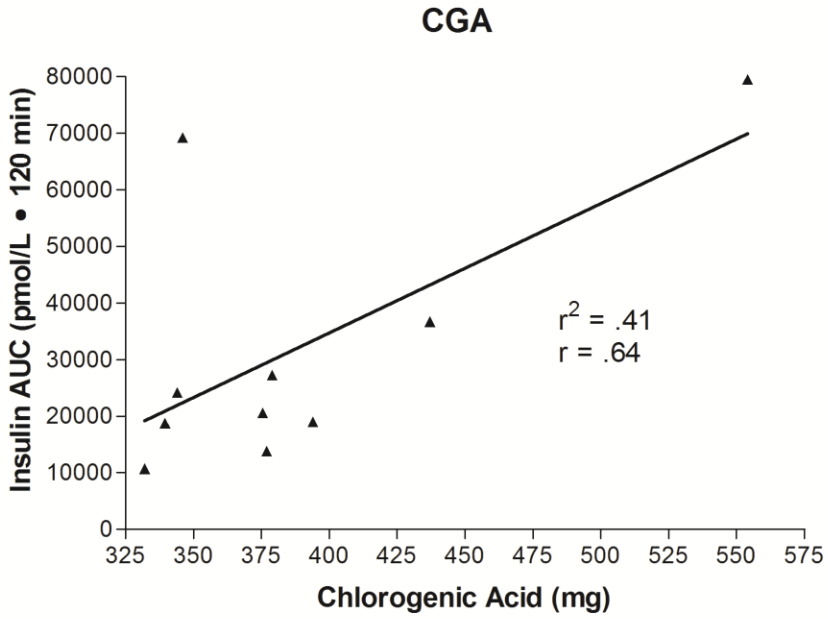
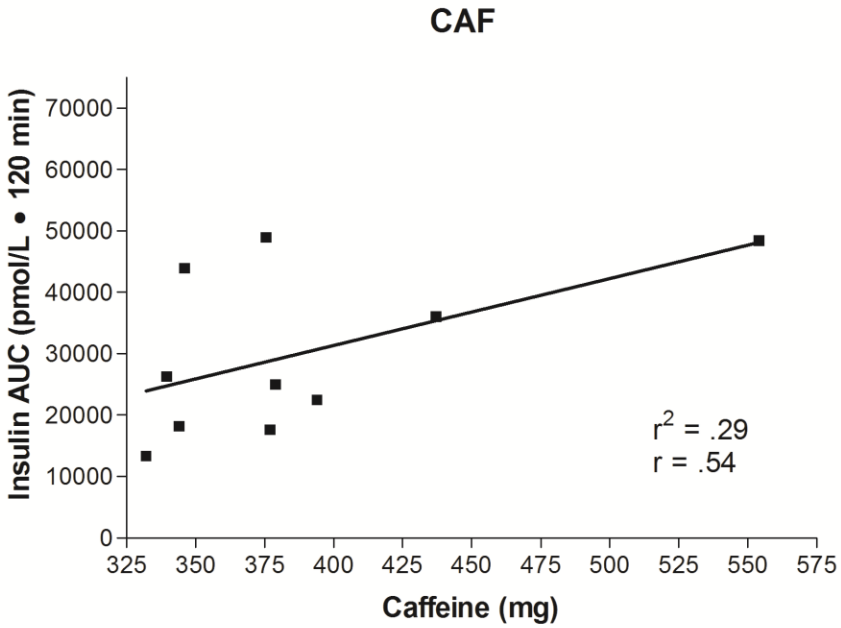


Figure 13. Top Figure: Relationship between the relative amount of chlorogenic acid consumed (5 mg/kg body weight) during the chlorogenic acid trial (CGA) and insulin area under the curve (AUC). Bottom Figure: Relationship between the relative amount of caffeine consumed (5 mg/kg body weight) during the caffeine trial (CAF) and insulin AUC.





APPENDIX 3: Health History Questionnaire

HEALTH & PHYSICAL ACTIVITY QUESTIONNAIRE

Name______________________________ Date___/___/___

Phone (H or cell)____________________

Date of Birth ___/___/___ Age____ Gender____ Ethnicity_______

Address (home)____zip__________

Email_______________________________________

Emergency contact (name, phone #)____

♦♦♦

MEDICAL HISTORY

Physical injuries:____

Limitations____

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction____	Heart surgery ____	Valve problems _____
Chest pain or pressure ____	Swollen ankles ____	Dizziness _____
Arrhythmias/Palpitations ____	Heart murmur ____	Shortness of breath _____
Congestive heart failure ____		

Have you ever had any of the following? Please check all that apply.

Hepatitis/HIV _____	Depression _____	Cancer (specify type) __________
Rheumatic fever _____	High blood pressure_____	Thyroid problems _____
Kidney/liver disease _____	Obesity _____	Total cholesterol >200 mg/dl _____
Diabetes (specify type) _____	Asthma _____	HDL cholesterol <35 mg/dl _____
Emphysema _____	Stroke _____	LDL cholesterol >135 mg/dl _____
		Triglycerides>150 mg/dl _____

Do immediate blood relatives (biological parents & siblings **only**) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

____

Do you currently have any other medical condition not listed?
Details____

____

Indicate level of your overall health. Excellent ____ Good ____ Fair ____ Poor_____

Are you taking any medications, vitamins or dietary supplements now? Y N

If yes, what are they?____

Do you have allergies to any medications or supplements? If yes, what are they?

____

Are you allergic to latex? Y N

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)? Y N If yes, elaborate.____

____

♦♦♦

LIFESTYLE FACTORS

Do you now or have you ever used tobacco? Y N If yes: type ________________

How long?______ Quantity____/day Years since quitting______________

How often do you drink the following?

Caffeinated coffee or tea _______oz/wk

Decaffeinated coffee or tea ________oz/wk

Caffeinated soft drink _________oz/wk

Other substances containing caffeine- please describe______________________________

♦♦♦

EXERCISE

Cycling

Times per week (circle one): 2-3 3-7 5-8

Minutes/Day (circle one): 30-60 min 60-240 min 60-360 min

Training background (circle one): 1 y 3-5 yr 5-15 yr 5-30 yr

Race days/yr (circle one): 0-10 0-20 20-100 >100

Do you participate in other sports? (describe) ____

APPENDIX 4: Daily Food and Activity Record

DAILY FOOD AND ACTIVITY RECORD

Name: _____ Date: _____ Day of the week:_____

Please list all food/beverages/water and exercise. Measure all food/drink amounts accurately.

TIME	AMOUNT	DESCRIPTION	NOTES

Was this a typical day? If not, why?_____

APPENDIX 5: Criteria for Classifying Subjects

Category	Trained cyclists	Well-trained	Élite	World Class
Training and race status				
Training frequency	2-3 times a week	3-7 times a week	5-8 times a week	5-8 times a week
Training duration	30-60 min	60-240 min	60-360 min	60-360 min
Training background	1 year	3-5 years	5-15 years	5-30 years
Race days per year	0-10	0-20	50-100	90-110
UCI ranking	-	-	first 2000	first 200
Physiological variables				
W_{max} (W)	250-400	300-450	350-500	400-600
W_{max} (W/kg)	4.0-5.0	5.0-6.0	6.0-7.0	6.5-8.0
$VO2_{max}$ (L/min)	4.5-5.0	5.0-5.3	5.2-6.0	5.4-7.0
$VO2_{max}$ (ml/kg/min)	64-70	70-75	72-80	75-90
Economy (W/L/min)	72-74	74-75	76-77	>78

Table 1: Criteria for the classification of trained, well-trained, élite and World Class road cyclists.

Origin of table: Jeukendrup, A. E., Craig, N. P., Hawley, J. A. (2000). The bioenergetics of world class cycling. *Journal of Science and Medicine in Sport, 3*(4), 414-433.

APPENDIX 6: Consent Form

The University of New Mexico Health Sciences Center
Consent to Participate in Research

The effect of post-exercise caffeine and chlorogenic acid supplementation on blood glucose homeostasis during two hours of recovery

07/23/2012

Introduction

You are being asked to participate in a research study that is being done by Dr. Christine Mermier, who is the Principal Investigator and Jason Beam, M.S., and their associates from the Department of Health, Exercise, and Sports Sciences. This research is studying the effect of ingesting caffeine and chlorogenic acid after exercise on the ability to alter blood sugar.

Ingesting caffeine + carbohydrates (sugars) after exercise has recently been shown in a research study conducted by Pedersen and colleagues in 2008 to help the body absorb sugar and store it in the muscle. Having too much sugar in the blood over a long period of time may eventually lead to type 2 diabetes. According to the Center for Disease Control, type 2 diabetes is the seventh leading cause of death in the United States as of 2007. Obesity and lack of regular exercise training may increase the risk for type 2 diabetes. Exercise training can help reduce body fat, increase muscle mass, and decrease the risk for type 2 diabetes. Drinking coffee has been shown in several research studies to help decrease the risk for developing type 2 diabetes. Some research has shown that drinking caffeinated or decaffeinated coffee may help the body take sugar out of the blood and help lower the chance of getting type 2 diabetes. Several studies have been conducted that analyzed caffeine consumption before exercise and its effect on blood sugar during exercise. Our study will look at the effect of consuming two compounds that are found in coffee (caffeine and chlorogenic acid) on blood sugar <u>after</u> exercise.

You are being asked to participate in this study because you are a trained cyclist who regularly consumes coffee and does not have any cardiovascular, pulmonary, or metabolic diseases. Approximately 15 people will take part in this study at the University of New Mexico.

This form will explain the research study, and will also explain the possible risks as well as the possible benefits to you. We encourage you to talk with your family and friends before you decide to take part in this research study. If you have any questions, please ask one of the study investigators.

What will happen if I decide to participate?

If you agree to participate, the following things will happen:

Overview

1. You will be asked to visit the Exercise Physiology Lab in the Johnson Center on the University of New Mexico main campus four times over the course of 4-6 weeks.
2. Each visit will be separated by at least one week.

3. During your first visit, you will perform a maximal exercise test on a stationary cycle to measure your maximal rate of oxygen consumption and the highest workload you can reach.
4. During your second, third, and fourth visits, you will perform a high intensity cycling bout for 30 minutes followed by 2 hours of recovery. Immediately after exercise, you will consume one of three supplements combined with 75 grams of sugar. During the two hour recovery, we will periodically draw your blood so that we can measure how well your body can tolerate the sugar that you consumed.

First Visit

1. During your first visit, you will sign this consent form and HIPAA form and fill out a health history questionnaire.
2. After filling out paperwork, you will void your bladder and bowels.
3. Following this, you will put on a heart rate monitor. You will then change into your cycling attire and we will measure your height, weight, and resting blood pressure.
4. We will adjust the handlebars, seat, and pedals of the cycle ergometer to your comfort.
5. You will perform a maximal exercise test that will last 8-12 minutes.
6. During the maximal exercise test, we will measure the maximal rate that your body can consume oxygen and the maximal amount of power that you can produce during the test.
7. Your maximal oxygen consumption will be measured using a special system. You will have a mouthpiece in your mouth and a nose clip over your nose during the entire test.
8. After you complete this test, we will give you a food and physical activity log so that you can keep track of your food and physical activity one day before your second visit to the Exercise Physiology Lab.
9. We will explain to you how to complete this log, and we will also schedule the date and time for your second visit before you leave.
10. We will also give you a sheet with pre-test guidelines that you should follow before each exercise trial.

Second, Third, and Fourth Visits

EXERCISE

1. Upon your second, third, and fourth arrival to the Exercise Physiology Lab, you will void your bladder and bowel, put on a heart rate monitor, and change into your cycling attire.
2. We will measure your height, weight, and blood pressure.
3. We will prep either your right or left arm for a needle stick.
4. We will insert an indwelling venous catheter into your arm. This will allow us to take your blood without having to stick you multiple times. This catheter will stay in your arm for the entire duration of your exercise and recovery.
5. We will draw 5 ml (1 tsp) of blood. We will then insert 1 ml (1/5 tsp) of saline into the catheter to keep it from getting clotted by blood.
6. We will set you up on the cycle ergometer with the same handle bar, seat, and pedal settings from your first visit.
7. You will warm up on the cycle ergometer for 5 minutes at 50% of your peak power output.
8. You will then cycle on the cycle ergometer at 70% of your peak power output for 30 minutes.
9. During exercise, twice we will put 1 ml (1/5 tsp) of saline into your catheter to keep it from clotting.

RECOVERY

10. Immediately after exercise, we will draw 1 ml (1/5 tsp) of blood and discard it. We will then draw 5 ml (1 tsp) of blood for later analysis of blood sugar and insulin concentrations.
11. You will ingest one of the three treatments (capsule) with 75 grams of a sugar solution. The capsule will either contain caffeine, chlorogenic acid (CGA) made from green coffee, or a placebo (sugar pill). The dose of caffeine and CGA will be around the same amount you would get in 1-2 cups of coffee.
12. You will sit and rest for 2 hours while we draw 5 ml (1 tsp) of blood every 15 minutes for the first hour and then 5 ml (1 tsp) of blood every 30 minutes during the second hour.
13. For each blood draw, we will draw 1 ml (1/5 tsp) of blood and discard it. We will then draw 5 ml (1 tsp) of blood for later analysis of blood sugar and insulin concentrations. We will clear out the catheter with 1 ml (1/5 tsp) saline to keep the catheter from clotting.
14. After the 2 hours of recovery, we will take the catheter out of your arm.
15. We will schedule the date and time of your third visit.
16. We will ask you to accurately consume the same food and substances that you recorded on your food diary and perform the same amount and type of exercise 2 days prior to your next trial.
17. You will repeat the above procedures when you come to the lab for your third and fourth visit.

How long will I be in this study?

Participation in this study will take a total of 10 hours over a period of 4-6 weeks.

What are the risks or side effects of being in this study?

Exercise Risks

- Possible side effects of maximal exertion include feelings of nausea, lightheadedness, muscle cramps, or dizziness after completion of the exercise.
- In patients with cardiovascular disease, exercise testing to the point of fatigue has a very low risk of sudden death (1 in 10,000) and complications of the heart (4 in 10,000) associated with it.
- Because you are a trained to well-trained cyclist who is accustomed to high intensity exercise, the risk is expected to be much less.

Blood Drawing Risks

- Risks of blood drawing include fainting (<0.01%), lightheadedness (<0.01%), bruising at the site of needle puncture (~2%), and localized infection (<0.01%).

Glucose Tolerance Test Risks

- Some people feel nauseated, sweaty, light-headed, or faint after drinking the sugar for the test. However, this is uncommon.

Supplement Risks

- According to the National Institute of Health, the doses of caffeine and chlorogenic acid that you will be ingesting after exercise are no more than what would be found in regular consumption of food and drink

There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

For more information about risks and side effects, ask the investigator.

What are the benefits to being in this study?

You will learn more about how your body responds to high intensity exercise. You may also benefit from learning your maximal oxygen consumption and peak power output. Furthermore, you may benefit from learning how your body tolerates elevated blood sugar after exercise.

What other choices do I have if I do not want to be in this study?

Your participation is voluntary, and if you decide to not be in the study then you will not be contacted again.

How will my information be kept confidential?

We will take measures to protect the security of all your personal information, but we cannot guarantee confidentiality of all study data.

Information contained in your study records is used by study staff. The University of New Mexico Health Sciences Center Human Research Review Committee (HRRC) that oversees human subject research, and the Food and Drug Administration and/or other entities may be permitted to access your records. There may be times when we are required by law to share your information. However, your name will not be used in any published reports about this study.

Your information will be stored in a locked cabinet in the Exercise Physiology Lab. In addition, you will be given a study number that will be used for data collection and analysis. Your information will be destroyed after the completion of data analysis.

What are the costs of taking part in this study?

The only cost for participating in this study is your time.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNMHSC will provide you with emergency treatment, at your cost.

No commitment is made by the University of New Mexico Health Sciences Center (UNMHSC) to provide free medical care or money for injuries to participants in this study.

In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, (505) 272-1129 for more information.

Will I be paid for taking part in this study?

You will be paid $100 if you complete all four exercise trials. If you complete the first two exercise trials before you withdraw from the study, you will be paid $50.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study, such as changes in the risks or benefits resulting from participating in the research or new alternatives to participation that might change your mind about participating.

Can I stop being in the study once I begin?

Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without affecting your future health care or other services to which you are entitled.

Whom can I call with questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Dr. Christine Mermier , or his/her associates will be glad to answer them at (505) 277-2658.

If you need to contact someone after business hours or on weekends, please call (318)-453-1943 and ask for Jason Beam.

If you would like to speak with someone other than the research team, you may call the UNMHSC HRRC at (505) 272-1129.

Whom can I call with questions about my rights as a research participant?

If you have questions regarding your rights as a research participant, you may call the UNMHSC HRRC at (505) 272-1129. The HRRC is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving human participants. For more information, you may also access the HRRC website at http://hsc.unm.edu/som/research/hrrc/.

CONSENT

You are making a decision whether to participate (or to have your child participate) in this study. Your signature below indicates that you/your child read the information provided (or the information was read to you/your child). By signing this consent form, you are not waiving any of your (your child's) legal rights as a research participant.

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this consent form, I agree to participate (or let my child participate) in this study. A copy of this consent form will be provided to you.

_____ _____ _____

Name of Adult Subject (print) Signature of Adult Subject Date

INVESTIGATOR SIGNATURE

I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

_____
Name of Investigator/ Research Team Member (type or print)

_____ _____

(Signature of Investigator/ Research Team Member) Date