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Effects of carbohydrate supplementation on whole body and muscle substrate utilization during long duration low intensity exercise

Stephanie G. Harger

The University of Montana

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EFFECTS OF CARBOHYDRATE SUPPLEMENTATION ON
WHOLE BODY AND MUSCLE SUBSTRATE UTILIZATION,
DURING LONG DURATION, LOW INTENSITY EXERCISE

by

Stephanie G Harger

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Approved by:

Chairperson

Dean, Graduate School

Date
Effects of Carbohydrate Supplementation on Whole Body and Muscle Substrate Utilization, during Long Duration Exercise

Chairperson: Brent C. Ruby

The majority of previous research has shown that carbohydrate (CHO) supplementation during continuous exercise does not spare muscle glycogen. However, much of this research has been performed over short periods of time (<3 hours) at moderate to high intensities. **PURPOSE:** This study evaluated the effects of CHO supplementation on whole body and muscle substrate utilization during prolonged, discontinuous exercise. **METHODOLOGY:** Seven recreationally trained males performed a GXT on a treadmill (TM) and electronically braked cycle ergometer to determine ventilatory threshold (VT) and VO₂peak. In a double blind, random crossover design, subjects received either a CHO [20% maltodextrin (0.6g/kg FFM/hr)] or placebo (PLA) drink each hour. TM exercise was performed at 41±2% VO₂peak, and 69±2% VT. Cycle ergometer exercise was performed at 42±1% VO₂peak, and 72±4% VT. Hourly exercise included 9 minutes on an upper body ergometer, 19 minutes on the cycle ergometer, and 20 minutes on the treadmill, followed by a 10-minute rest and feeding period. A standardized lunch (5 g/kg BW CHO, and 1.2 g/kg BW PRO) was provided after hour five for both trials. Muscle biopsies of the vastus lateralis were performed pre- and post-exercise, and expired gases were collected every other hour during the TM segment. Blood glucose (BG) was measured continuously using an indwelling glucose sensor, and total urine void was collected. **RESULTS:** The rates of total CHO oxidation on the TM were significantly higher during the CHO trial (331.0±24.7, and 221.4±37.9g, for CHO and PLA, respectively, p=0.0001). There were no significant differences in pre-exercise glycogen between the trials (174.94±43.05, and 172.4±53.3 g/kg wet weight, for CHO and PLA respectively, p=0.7829). However, post-exercise glycogen concentration was significantly lower following the PLA (135.2±42.0, 109.35±32.2 g/kg wet weight, for CHO and PLA respectively, p=0.0274). No differences in BG or RPE between trials were observed. Total urinary nitrogen output was also similar for both trials (483.0±429.4 and 609.7±461.2 mg/dl, for CHO and PLA respectively, p=0.1696). **CONCLUSION:** These data suggest that regular CHO feedings during extended exercise increases whole body CHO oxidation and has a small glycogen sparing effect over 10 hours of exercise. This may partially explain the ability to maintain exercise intensity or self-selected work rates over long periods of activity.
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Chapter One: Introduction

Introduction

Carbohydrate (CHO) is an important fuel source for endurance activities. Sports nutritionists often advise athletes to consume diets that are largely composed of CHO (60-65% of total energy intake) in order to provide their bodies with the fuel necessary to maintain intensity for long durations. Athletes are often advised to eat CHO rich foods prior to competition, to supplement with CHO during exercise, and replenish CHO stores after exercise.

Carbohydrate stored as glycogen in muscles provides the body with a quick energy source under anaerobic conditions. When exercise intensity increases CHO is the fuel source that supplies the body with the increased energy demands (Brooks 1998; Coyle 2000; De Feo, Di Loreto et al. 2003). Fat is a more efficient fuel source, for long duration exercise because of the larger numbers of adenosine triphosphate (ATP) it can provide per fatty acid. Fats are also useful during extended activities as they are a slow burning fuel source that requires readily available O₂ for oxidation (Coyle 2000; De Feo, Di Loreto et al. 2003). The fatty acid aerobic system, called Beta-oxidation, can be increased with exercise training, but there is a limit to the adaptations that occur, the rate of fat oxidation and the availability of O₂. For this reason CHO supplementation is necessary to provide the body with energy to maintain high intensity during long duration endurance activities (Coggan 1997; De Feo, Di Loreto et al. 2003).
It has been well established that exogenous CHO supplementation during exercise increases CHO oxidation (Coyle, Hagberg et al. 1983; Coyle and Coggan 1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Jeukendrup 2004). Blood glucose levels are maintained, respiratory exchange ratio (RER) values remain elevated, muscle glycogen may be spared, rate of perceived exertion (RPE) decreases, hepatic glucose output is suppressed, a decrease in liver glycogenolysis, glycogen synthesis may occur in both muscle and liver during low intensity exercise, and there may be a central factor influence with CHO supplementation. Many researchers have shown that exogenous CHO can increase performance and time to fatigue during long duration exercise (Coyle and Coggan 1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Coyle 2000; Jeukendrup and Jentjens 2000; Hargreaves 2004; Jeukendrup 2004; Utter, Kang et al. 2004).

Exercise intensity affects the amount of exogenous CHO that is oxidized. Training related improvements in exogenous CHO oxidation occur and, it is thought that different mechanisms are responsible at different intensities (Gollnick, Piehl et al. 1974; Kuipers, Keizer et al. 1987). Maintenance of plasma glucose and sparing of liver glycogen are thought to be the main reasons for the ability to sustain performance and intensity during long duration exercise (Coyle, Hagberg et al. 1983; Bosch, Weltan et al. 1996; el-Sayed, MacLaren et al. 1997; Kjaer 1998; Burke and Hawley 1999; Horowitz, Mora-Rodriguez et al. 1999; Meyer, Gabriel et al. 2003).

Past researchers have shown conflicting evidence concerning the sparing of muscle glycogen during exercise with CHO supplementation. Most research in this area has been performed at moderate to high intensities and for no more than 5-hours of
exercise, where the varied duration and higher intensities partially explain the mixed results. Tsintzas et al. (1995, 1996) looked at specific muscle fiber types in order to determine if there was a difference in muscle glycogen use. These researchers have shown that type I fibers use much of their glycogen stores, where type II fibers are nearly unaffected, indicating that there is a fiber type difference in muscle glycogen oxidation.

Researchers have shown that during intermittent exercise CHO supplementation has a positive effect on muscle glycogen. It is not clear if these results are due to a decreased glycogenolysis or if glycogen resynthesis was able to occur during the low intensity periods built into the exercise (Kuipers, Keizer et al. 1987).

While little is known about the effects of carbohydrate feedings during long duration, low intensity exercise, or work, this knowledge can provide important information to individuals involved in long duration exercise or work such as ultra-marathon, military personnel and wildland firefighters. People working in these job settings are sometimes required to work many hours a day for multiple days. In order to help promote increased work capacity and safety for these individuals, it is important to know the mechanisms of fatigue and decreased performance in these populations.

**Problem**

The purpose of this study was to examine the effects of exogenous CHO supplementation on whole body and muscle substrate utilization during long duration, discontinuous, low intensity exercise.
Research Hypothesis

Hypothesis One

We hypothesized that CHO feedings provided during ten hours of intermittent low-intensity exercise will increase whole body CHO oxidation rates.

Rationale for Hypothesis One

Research in the past has demonstrated an increase in CHO oxidation rates when provided with exogenous sources. CHO oxidation (g·min⁻¹) and RER values have been shown to remain elevated, blood glucose levels are maintained, hepatic glucose output is suppressed, and hepatic glycogen synthesis may occur during low intensity exercise (Coyle, Hagberg et al. 1983; Coyle and Coggan 1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Jeukendrup 2004).

Hypothesis Two

We hypothesized that CHO feedings provided during ten hours of intermittent low-intensity exercise would decrease muscle glycogenolysis in exercised muscle.

Rationale for Hypothesis Two

Tsintzas et al. (1995, 1996) demonstrated that running at approximately 70% \( \text{VO}_{2\text{max}} \) while provided with a 5.5% CHO-electrolyte solution demonstrated a muscle glycogen sparing effect. The supplementation had the largest affect on the type I fibers, as they were more depleted than the type II fibers. The percent change in muscle glycogen content was lower in the CHO trial as compared to the PLA trial. It has also
been demonstrated that for the first two hours of cycling there was no muscle glycogen sparing effect. After the third hour of exercise there was a statistically significant reduction in the rate of muscle glycogen utilization with CHO supplementation. Thus, during longer duration, low intensity exercise there may be a muscle glycogen sparing effect with exogenous CHO.

**Significance of the Study**

Previous research has addressed the issue of muscle glycogen use during exercise, but most of this research has been performed at moderate to high intensities. To our knowledge no researchers have looked at long duration (>5 hours), low intensity, intermittent exercise that can be applied to an arduous work situation. Most of the previous studies have also chosen intensities that are relative to VO₂peak and not relative to the lactate threshold (Tlact) or ventilatory threshold (Tvent). Other researchers suggest that it may be more appropriate to use Tvent to prescribe exercise intensity, and that these measures are a better marker of sustainable metabolic fitness.

**Rationale for the Study**

Conducting this study in a controlled laboratory setting may allow for a better understanding of results from field research with Wildland Firefighters (WLFF) for the mechanisms explaining their improved performance with CHO supplementation. Understanding these mechanisms may also have implications for WLFF and military personnel meal plans, and feeding strategies. Recovery from fatigue in these populations
is also important in order for them to maintain work output and safety for multiple workdays. This research may provide an understanding of the mechanisms and help us to develop new strategies for recovery from arduous workdays.

Limitations

i/Non-randomized sample. Subjects will not be from a randomized sample. Subjects will be recruited from the University of Montana campus and will be volunteer subjects.

ii/Instrumentation. There is error associated with all instrumentation. By using regularly calibrated equipment and trained laboratory technicians instrumentation error will be limited.

iii/Physical Capacity of Subjects. VO\textsubscript{2peak} and T\text{vent} will be different in each subject. This limitation was minimized by adjusting the exercise workload relative to each individual T\text{vent}.

iv/Gender. The data collected will only apply to recreationally active males.

Delimitations

i/Type of subjects. Only recreationally active subjects with a VO\textsubscript{2peak} of no less than 45 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} will be used for the study.
**ii/Specific exercise intensity.** Subjects will exercise at 70% Tvent during the treadmill and cycle ergometer section of the trial.

**iv/Males only.** Only recreationally active males will be used for this study.

**v/Age of subjects.** Subjects will all be under the age of 40 in order to decrease physiological changes that occur in substrate utilization over this age.

**Definition of Terms**

**Rate of Perceived Exertion (RPE).** The subjective level of intensity a subject feels their body is exerting for any given workload during exercise. The rating is based on a 6-20 scale, and was developed by Borg (1982).

**Respiratory Exchange Ratio (RER).** Expired CO₂ divided by inspired O₂ (VCO₂/VO₂), where V is minute volume. By using this ratio, substrate utilization can be estimated in g·min⁻¹ CHO or g·min⁻¹ fat. Based on oxygen requirements to oxidize fat and CHO, it is accepted that, exclusive of CO₂ buffering an RER of 0.70 demonstrates an almost exclusive use of fats as a fuel source, where an RER of 1.00 demonstrates an almost exclusive use of CHO as a fuel source. In practice this estimate is reliable and valid with an RER range of 0.70 to 0.90

**Substrate.** The fuel source the body uses to produce ATP
ATP. Adenosine triphosphate. The molecular currency of intracellular energy.

Ventilatory Threshold (Tvent). The exercise load at which expiration, and ventilated CO₂ increases exponentially for a given increase in ventilated O₂.

Ventilatory Threshold Detection Methods. Three methods were used during this research to determine Tvent; Ventilatory Equivalencies Method, Excess CO₂ Method, and Modified V-Slope Method (Gaskill, Ruby et al. 2001).

Relative Intensity. Relative intensity is determined using the amount of oxygen consumed for a given workload, with regard to the subject’s body weight. This allows for inter-subject comparison (ml·kg⁻¹·min⁻¹). For this project, intensities are at different times reported relative to body weight (ml·kg⁻¹·min⁻¹), to VO₂peak (% VO₂peak), or Tvent (% Tvent).

Absolute Intensity. Absolute intensity is determined using the amount of oxygen consumed for a given workload, without regard to body weight (L·min⁻¹).

VO₂max. The maximum volume of oxygen a person can metabolize, despite an increase in workload.

VO₂peak. The maximum amount of oxygen a person can metabolize for any given mode of exercise.
**Low-intensity exercise.** Exercise that is performed less than 45% VO\textsubscript{2peak}. For this study low-intensity exercise is also defined as exercise less than 72% T\text{vent}.

**Intermittent Exercise.** Exercise that is interspersed with rest periods, i.e. discontinuous exercise.

**Long Duration Exercise.** Exercise that lasts longer than 5 hours in duration.

**Glycogenolysis.** The catabolism of muscle and liver glycogen.

**Exogenous Carbohydrate.** Ingested CHO. Glucose polymers, for this study maltodextrin, will be ingested in order to be used as a non-glycogenolytic fuel source for the body.

**Endogenous Carbohydrate.** Glucose stores within the liver, muscle or bloodstream that are mainly in the form of glycogen.
Chapter Two: Review of Literature

Metabolic Response to Exercise

The main goal of human body metabolism during exercise is to provide adenosine triphosphate (ATP) for all active tissue, especially the working muscles. ATP is the molecule that provides the energy for the actin/myosin cross bridge to occur during contraction of the muscle. There are many eloquent reactions that must occur to ensure the production of ATP at rates high enough to allow for muscular contraction to occur (Coyle 2000; Thong and Graham 2002; De Feo, Di Loreto et al. 2003). The intracellular stores of ATP are limited and the turnover rate for ATP in the body is high, in a normally functioning cell an ATP molecule will be consumed in about one minute. Higher rates of turnover are achieved during exercise (Brooks 1998; Febbraio and Dancey 1999; De Feo, Di Loreto et al. 2003).

There are two main classifications for muscle fiber types that occur in the human body. The two main classifications are Type I and Type II fibers. The type of fiber that a muscle is largely composed of depends on the stresses that are placed on the muscle during training. Type I muscles fibers are often called slow, oxidative fibers. These fibers have a high oxidative capacity and are often utilized during endurance exercise. Type II muscle fibers are often called fast, glycolytic fibers. Conversely, these fibers have a high glycolytic capacity and are often used during short, high intensity bursts of exercise. There are also intermediate fiber types that demonstrate both of these capacities. Each of the different fiber types have different ratios of the metabolic
pathways that are utilized to provide energy for the working muscle (Coyle 2000; De Feo, Di Loreto et al. 2003).

The first metabolic pathway that is recruited during exercise is the ATP/phosphocreatine (ATP/PCr) system. This system transfers a phosphate group, from PCr to adenosine diphosphate (ADP) to form ATP, in a reaction catalyzed by creatine kinase. This reaction occurs in the absence of oxygen, and is the main metabolic pathway that is utilized for the first 3-5 seconds of exercise. This pathway takes place mostly in Type II muscle fibers and provides the body with energy for short bursts of exercise or for the first few seconds of longer duration activity (De Feo, Di Loreto et al. 2003).

The second metabolic pathway that is engaged during muscle work is anaerobic glycolysis. This pathway is also used in the absence of oxygen and is a series of coupled reactions where glycogen, or glucose is broken down to produce molecules of ATP and pyruvate, which can be readily converted to lactate. This pathway provides that largest percentage of energy to the working muscles for approximately the first 15 seconds of intense exercise, and provides two molecules of ATP per one glucose molecule. At the onset of exercise catecholamines in the body are increased and glycolysis and glycogenolysis are upregulated to provided glucose in the form of glucose-6 phosphate molecules for this reaction (Kjaer 1998). This reaction occurs in the Type II, fast twitch muscle fibers (Brooks and Mercier 1994; Febbraio and Dancey 1999; De Feo, Di Loreto et al. 2003), and to a smaller extent the Type I fibers.

The last metabolic pathway that is activated to provide energy to working muscles is oxidative phosphorylation. In this pathway fats, CHO, and protein (PRO) are
completely oxidized into CO\textsubscript{2} and H\textsubscript{2}O. Due to the increase in catecholamines during longer duration exercise there is an increase in lipolysis that provides fat molecules for this metabolic pathway, as well as increased glycolysis resulting in pyruvate to fuel oxidative phosphorylation. This reaction takes place in the inner membrane of the mitochondria, where hydrogen ions are pumped across the membrane to form an electrochemical gradient. This gradient allows for ATP synthase to add a phosphate group to ADP to regenerate ATP for working muscles. This metabolic pathway is the most efficient at providing ATP to working muscles, because it can provide around 130 ATP molecules per fat molecule or 36 ATP molecules per glucose molecule that is oxidized completely to CO\textsubscript{2} and H\textsubscript{2}O. This reaction occurs most often in the Type I, slow oxidative muscle fibers (Brooks and Mercier 1994; Brooks 1998; De Feo, Di Loreto et al. 2003).

Fat and CHO are the primary fuel sources for working muscles during exercise, but lactate is also metabolized by oxidative phosphorylation. As exercise intensity increases there is an abundance of excess pyruvate formed which is converted to lactate, and transported out of the active muscle cells. The increase of pyruvate is due to an increased glycolytic flux causing more pyruvate to be produced than can be consumed through the Kreb’s cycle (Brooks 1998; De Feo, Di Loreto et al. 2003). This excess lactate is transported via the so called “lactate shuttle” to oxidative muscle cells where it is used directly as a fuel source. Alternatively, excess lactate can also be used to regenerate glucose molecules through gluconeogenesis in the liver (Brooks and Mercier 1994; Coggan 1997; Kjaer 1998; De Feo, Di Loreto et al. 2003).
It is widely accepted that as exercise intensity and duration increase the reliance on CHO also increases (Brooks and Mercier 1994; Coggan 1997). This concept has been described by Brooks et al. (1994) as the “crossover concept”. In this theory it is stated that at low intensity activity fats can provide up to 60% of all fuel sources for energy. As intensity of activity increases there is a greater reliance on CHO as a fuel source, until a “crossover” point is reached where the primary fuel source becomes CHO. Near maximum exercise intensity, CHO provides 100% of the energy to maintain exercise. This theory has received criticism since its introduction in 1994. The main reason for controversy is that this crossover concept is based on absolute values of VO$_{2\text{peak}}$. In this theory mild, moderate and hard exercise intensities are defined by percent VO$_{2\text{peak}}$. This theory states that endurance training reduces CHO oxidation at “mild” to “moderate” intensities, but that this adaptation does not occur at “hard” intensities. There is a large body of data that does not support this concept, including training induced decreases in RER, reduced muscle glycogen utilization, reduced glucose utilization, increase plasma FFA and increased intramuscular triglyceride (IMTG) utilization. These conflicts demonstrate the inconsistencies within the crossover concept as it is used to describe the increase in CHO oxidation during increasing intensity exercise (Coggan 1997).

**Muscle Glycogen Utilization during Exercise**

The importance of muscle glycogen during exercise in humans has been studied for many decades. In 1967 Bergstrom was able to establish the importance of muscle glycogen as a fuel source during exercise with the introduction of the muscle biopsy. This allowed for direct measure of muscle glycogen content in samples (Bergstrom and
Hultman 1967). In recent years it has been discovered that levels of muscle glycogen content may play an important role in substrate metabolism and the onset of fatigue during exercise (Febbraio and Dancey 1999; Hargreaves 2004; Johnson, Stannard et al. 2004).

There are two pathways in which glucose can enter the muscle cells, either through contraction mediated or insulin mediated pathways. Both mediated uptakes rely on the same seven-transmembrane protein, known as the glucose transport protein (GLUT 4). After glucose enters a cell it either undergoes oxidation through glycolysis or storage through glycogen synthesis (Thong and Graham 2002; Hargreaves 2004; Hargreaves, Hawley et al. 2004). It has been shown that exercise at low intensities can allow for both of these pathways to occur simultaneously. The explanation for this may be that different fiber types are recruited at different times during exercise or at varying intensities, and this would allow for resynthesis to occur in one fiber type while glycolysis is occurring in another. Different muscle fiber types may rely on different fuel sources to produce ATP for energy. During long duration, low intensity exercise multiple fiber types are recruited in order to maintain exercise. As a result of this more glycogen depletion may occur across a range of muscle tissues (Gollnick, Piehl et al. 1974; Price, Taylor et al. 1994; Tsintzas, Williams et al. 1995; Tsintzas, Williams et al. 1996; Tsintzas and Williams 1998).

The rate of glycogenolysis during exercise depends on the intensity of exercise. Total glycogen use depends on both the intensity and the duration of exercise. Glycogenolysis occurs at higher rates during intense exercise to provide the working muscles with the increased CHO that is needed to maintain the high intensity, at the onset
of exercise initial glycogenolysis is high and slows down as the muscle achieves homeostasis as the duration of exercise increases (Bergstrom and Hultman 1967; Hargreaves 1997; Watt, Heigenhauser et al. 2002; Hargreaves 2004). Price et al. (1994) demonstrated that after the initial decrease in glycogen content, during extended, low intensity exercise. A steady state was achieved in which glycogen synthesis and glycogenolysis occurred at equal rates. However, the methodology in this study did not consider the possibility that these two phenomena may occur simultaneously but in different muscle fiber types.

There are also two different molecules of glycogen that are used at different rates to provide ATP for the body. These two pools are named pro- (PG) and macro-glycogen (MG). The physiological functions and metabolic regulation of these two separate pools of glycogen have not been clearly established (Graham, Adamo et al. 2001). Graham et al. (2001) demonstrated that pro-glycogen may be the molecule that is largely responsible for metabolic regulation during exercise. In this study it was demonstrated that when glycogenolysis decreased later in exercise it was due to a decrease in MG catabolism. This may also add to the explanation for a decrease in glycogenolysis later in long duration exercise, and during repeated bouts of exercise.

**Exogenous Carbohydrate Supplementation and Performance**

A decrease in CHO availability during exercise has been shown to lead to the development of fatigue in humans. CHO supplementation before or during exercise has been shown to improve performance and increase the time to fatigue (Coyle and Coggan...
1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Coyle 2000; Jeukendrup and Jentjens 2000; Hargreaves 2004; Jeukendrup 2004; Utter, Kang et al. 2004). The mechanism for this improvement is not clear, although increased rates of CHO oxidation are observed when exogenous CHO is available (Coyle 1992; Jeukendrup, Saris et al. 1996; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Jeukendrup and Jentjens 2000; Meyer, Gabriel et al. 2003; Jeukendrup 2004). The maintenance of blood glucose, glycogen resynthesis, sparing of liver glycogen, sparing of muscle glycogen, and a central stimulating mechanism for CHO have all been used to explain this effect (Coyle, Hagberg et al. 1983; Coyle and Coggan 1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Jeukendrup 2004).

Endogenous stores of CHO are limited, and can be slightly increased with diet and training, but for many endurance activities CHO supplementation can provide additional resources to improve performance (el-Sayed, MacLaren et al. 1997; Lambert, Hawley et al. 1997; Schabort, Bosch et al. 1999; Smith, Rhodes et al. 2002; Jeukendrup 2003; Hargreaves, Hawley et al. 2004). Most studies demonstrate the ergogenic effects of supplemental exogenous CHO versus placebo (PLA), but some fail to show increased performance (Jeukendrup 2004). The reason for this may be the measure that was used to assess performance. It is difficult to compare the different ways that performance was measured in these studies, because many studies used a different measure of performance, or different mode of exercise (Jeukendrup 2004).

Maintenance of blood glucose is one of the ergogenic effects of CHO that is thought to increase measures of performance. Many studies have demonstrated that with exogenous CHO supplementation subjects maintain blood glucose levels (Coyle,
Hagberg et al. 1983; Coyle and Coggan 1984; el-Sayed, MacLaren et al. 1997; Sugiura and Kobayashi 1998; Horowitz, Mora-Rodriguez et al. 1999; Schabort, Bosch et al. 1999; Meyer, Gabriel et al. 2003). Coyle et al. (1983) demonstrated that the onset of fatigue could be delayed during exercise at 70-80% VO_{peak}. In this study subjects were asked to exercise on a cycle ergometer at approximately 74% VO_{peak} until volitional exhaustion. The exercise time to exhaustion was approximately 2-hours. Although an increase in time to exhaustion was demonstrated it was only observed in those subjects who showed a decrease in blood glucose levels during the fasted exercise trial. This early study in CHO supplementation would suggest that increased performance and time to onset of fatigue would be accountable by maintenance of blood glucose.

Another explanation for increased performance and time to fatigue during CHO feedings is glycogen synthesis during exercise. Kuipers et al. (1987) observed that supplementation of CHO during intermittent cycling exercise allowed for glycogen resynthesis to occur. This active recovery demonstrated that subjects could synthesize glycogen at maximum resynthesis rates. Upon analysis of the fiber type distribution in this study the researchers discovered that only approximately 39% of each muscle sample was Type II fibers. If the glycogen synthesis rates are adjusted for the percent of Type I fibers they may have actually been higher in these non-active fibers at this exercise intensity.

At the onset of exercise liver glycogenolysis increases in order to provide the body with CHO for ATP synthesis. During extended exercise, when the liver begins to be glycogen depleted, gluconeogenesis becomes the primary fuel source for glucose released into the blood from the liver (Coyle, Hagberg et al. 1983; Coyle, Coggan et al.
1986; Kuipers, Costill et al. 1986; Coyle 1992; el-Sayed, MacLaren et al. 1997; Kjaer 1998; Burke and Hawley 1999). Jeukendrup et al. (1999) established that with exogenous CHO supplementation liver glycogenolysis could be completely suppressed during exercise. During this study subjects were asked to ride on a cycle ergometer for 2-hours at approximately 50% VO_{2peak}. CHO rich in C^{13} was provided to the subjects in order to determine the rates of appearance (R_{a}) and rate of disappearance (R_{d}) of glucose during the trial. Using this stable isotope method it was determined that exogenous CHO in moderate amounts (35g/hr) could partially suppress glycogenolysis in the liver, and in large amounts (175g/hr) liver glycogenolysis could be completely suppressed. An interesting finding was that the rate of muscle glycogenolysis was not different between the two trials, indicating that this fuel source cannot be spared. The findings regarding muscle glycogen support previous findings by this lab (Jeukendrup, Saris et al. 1996).

There is conflicting evidence concerning the debate over muscle glycogen use during exercise when CHO supplementation is provided. Many researchers have demonstrated that there is not a muscle glycogen sparing effect when supplemental CHO is provided (Coyle, Coggan et al. 1986; Febbraio and Stewart 1996; van Zant and Lemon 1997; Febbraio and Dancey 1999; McConell, Snow et al. 1999; Schabot, Bosch et al. 1999; Karelis, Peronnet et al. 2002). Many of these studies were performed at a high intensity, for durations between two and four hours. Also, in some of these studies, subjects were in a fasted state and are not fed prior to the onset of exercise. However, van Sant et al. (1997) and Schabot et al. (1999) studied the effects of pre-exercise feeding on the rate of muscle glycogen utilization during exercise. In each of these studies researchers determined that a pre-exercise meal did not affect the rate of
glycogenolysis between the two trials. Febbraio et al. (1996) assessed the effects of different pre-exercise foods on glycogenolysis. In this study subjects were fed different glycemic index foods. Again, rates of glycogenolysis were not statistically different between trials.

Some past researchers who looked directly at the effects of CHO feedings during exercise found no difference in muscle glycogenolysis (Coyle, Coggan et al. 1986; McConnell, Snow et al. 1999; Karelis, Peronnet et al. 2002). Coyle et al. (1986) reported no difference in muscle glycogen utilization when subjects were fed CHO during 105 minutes of exercise at approximately 71% VO$_{2\text{peak}}$, and another trial that measured time to fatigue in CHO versus PLA. In the CHO trial, they found an increase in rates of CHO oxidation, blood glucose, and time to volitional exhaustion. However, there was no difference in skeletal muscle glycogen content at the end of the 105-minute trial. This study supports the finding that the rate of muscle glycogenolysis is not affected by CHO supplementation during moderate to high intensity exercise.

While this is true, Tsintzas et al. (1995, 1996) has performed numerous studies concerning specific muscle fiber types and glycogen utilization across these different fibers. In theses studies it is established that muscle fiber type plays a role in glycogenolysis (Tsintzas, Williams et al. 1995; Tsintzas, Williams et al. 1996; Tsintzas and Williams 1998). These studies have been performed in male runners, and it is thought that the reason for the sparing effect may be the mode of exercise (Jeukendrup and Jentjens 2000; Jeukendrup 2004). In the first of these two studies (Tsintzas, Williams et al. 1995) runners were asked to perform treadmill exercise for 60 minutes at approximately 70% VO$_{2\text{peak}}$. Trials were separated by at least one week. After analysis
of pro-glycogen (PG) and macro-glycogen (MG) content in fiber types it was established that more glycogen was stored, pre-exercise, in Type II fibers and that depletion occurred more in the Type I muscle fibers. There was also a higher glycogen content in the Type I fibers at the end of exercise in the CHO trial suggesting glycogen sparing in Type I fibers. In a second study (Tsintzas, Williams et al. 1996) subjects were asked to perform treadmill exercise at an intensity of approximately 60% VO$_{2peak}$ until volitional exhaustion. Time to fatigue in the CHO trial was significantly longer, consistent with findings from other labs (Coyle, Hagberg et al. 1983; Coyle and Coggan 1984; Coyle 1992; el-Sayed, MacLaren et al. 1997; Burke and Hawley 1999; Jeukendrup and Jentjens 2000; Hargreaves 2004; Jeukendrup 2004). During the CHO trial a muscle biopsy was obtained at the time that coincided with exhaustion for the PLA trial. At this time point there was a difference in muscle glycogen content, demonstrating higher muscle glycogen or reduction in glycogenolysis in the CHO trial. However, there was no difference in muscle glycogen content at the point of exhaustion between the two trials.

In a similar study Bosch et al. (1996) asked subjects to cycle at approximately 70% VO$_{2peak}$ for 180 minutes of exercise. In this study it was established that there was an increased muscle glycogen content at the end of exercise, and in the CHO trial there was no net glycogen loss between hours 2 and 3. This study supports the findings of previous research that has demonstrated sparing of muscle glycogen during exercise (Tsintzas, Williams et al. 1995; Tsintzas, Williams et al. 1996).

The last mechanism thought to be responsible for the positive results in performance and time to fatigue is a central effect of CHO. Carter et al. (2004) performed a study where subjects were asked to perform a pre-determined amount of
work as fast as possible. In this trial either 25 mL of 6.4% maltodextrin solution or PLA was rinsed in the mouth for approximately 5 seconds and then spit out. The main finding in this study was that the time to completion of the predetermined work was significantly faster in the CHO mouth rinse trial. The mechanism for this improved performance is currently not known, but the theory presented in this paper is that there may be CHO receptors in the mouth that are associated with motivation to perform work.

To our knowledge the longest study that has been performed to assess metabolism during CHO supplementation has lasted five hours in duration (Jeukendrup, Moseley et al. 2006). In this study subjects exercised at 50% of their max watts on a cycle ergometer for five hours. Labeled CHO was ingested in order to determine glucose $R_d$ and $R_a$, and to calculate exogenous and endogenous utilization. These results were consistent with previous findings, and CHO oxidation was maintained throughout this long duration exercise trial.

**Exercise Relative to Ventilatory Threshold**

Much of the current research focuses on high intensity exercise that is relative to $VO_{2peak}$. Prescribing workloads to $T_{lact}$ or $T_{vent}$ may be more appropriate to ensure similar metabolic stress across subjects and fitness levels. In this study $T_{vent}$ was determined using three concurrent methods that have been validated to accurately assess $T_{vent}$ (Gaskill, Ruby et al. 2001).
Wildland Firefighters

During long duration, arduous wildland fire suppression, Cuddy et al. (submitted for publication) showed that regular CHO feedings throughout a workday can improve work output by 28%. This research has shown that blood glucose is maintained with exogenous CHO, but there is no difference in Rate of Perceived Exertion (RPE) in these subjects. Energy expenditure in these individuals averages 3500-6000 kcal/day (Ruby, Shriver et al. 2002). Individuals working in this environment perform a variety of jobs, including but not limited to, sawing, hiking, digging line, and swamping logs. These activities use both the upper and lower body. CHO feedings may help to provide a positive energy balance during this arduous work, and further improve performance of work in these populations. Further, laboratory based studies may help to validate these findings from the field.

Summary

Metabolic response to exercise demonstrates an increased reliance on CHO as a fuel source as the intensity of exercise increases. It is well established that CHO supplementation during exercise can improve performance and increase time to exhaustion through maintenance of blood glucose, glycogen resynthesis, sparing of endogenous glycogen stores, and the possibility of a central effect of CHO on reward centers in the brain. Studies assessing the rate of muscle glycogenolysis during exercise with exogenous CHO supplementation demonstrate conflicting results. A majority of this research has been performed at high intensities, for relatively short periods of time. Also,
the exercise intensities of this prior research are largely prescribed relative to VO$_2$peak. The findings from a study assessing the effects of CHO on long duration, low intensity exercise may have considerable application to work settings, including the Wildland Firefighter or military personnel.
Chapter Three: Methodology

Setting

All exercise testing took place in the Human Performance Laboratory, in McGill Hall, Room 121 on the campus of the University of Montana, Missoula, MT.

Subjects

Seven recreationally active males served as subjects for the investigation. Prior to participation, each subject completed an Institutional Review Board (IRB) approved informed consent form. Subject descriptive characteristics are shown in Table 1.

Descriptive Data

Weight, Height, and Age

Weight in Kilograms, was measured using a calibrated, digital scale (Befour Inc, Cedarburg, WI). Height was measured using a calibrated stadiometer with shoes removed.

Anthropometric Measurements

Hydrostatic weighing, at estimated residual volume (Goldman and Becklake 1959), using a calibrated electronic under water scale (Exertech, Dresbach, MN) was used to determine body fat percent and fat free mass (Lohman 1992). Subjects performed
multiple underwater weight trials until three values within 100 grams were obtained. Body density was then used to determine body fat percent using the age specific equations of Lohman (1992).

Exercise Testing

Cycle Ergometer

All subjects were asked to report to the Human Performance Laboratory after abstaining from calories, nicotine, and caffeine for twelve hours prior to testing. A graded exercise test to volitional exhaustion, to determine maximal oxygen uptake (VO_{2peak}) and ventilatory threshold (T_{vent}) was performed using an electronically braked Cardgirus cycle ergometer (CE) (Cardgirus, Spain). The cycle ergometer was adjusted to proper seat and handle bar height for each subject. All subjects performed a warm-up prior to testing. The testing protocol consisted of a ramped protocol adding 40 Watts of resistance each minute of the test until volitional exhaustion, or until a cadence of 50 revolutions per minute (rpm) could not be maintained. Expired gas data were continuously collected and 15-second averages were analyzed using a two-way mouth piece (Hans Rudolph, Inc., Kansas City, MO) and a ParvoMedics metabolic cart (ParvoMedics, Salt Lake City, UT). Prior to each exercise test calibration was performed using a 3-Liter calibration syringe (Hans Rudolph, Inc., Kansas City, MO), and a calibration gas mixture (16.0% O_{2}, 4.0% CO_{2}, N_{2} balance) (Airgas Mid South, Inc., Tulsa, OK). Heart rate was measured using a chest strap heart rate transmitter and monitor (Polar USA, Lake Success, NY).
**Treadmill**

All subjects were asked to report to the Human Performance Laboratory after abstaining from calories, nicotine, and caffeine for twelve hours prior to testing. A graded exercise test to volitional exhaustion was performed using a Quinton Q65 treadmill (TM) (Quinton, Seattle, WA). All subjects performed a warm-up prior to testing. The testing protocol consisted of a speed set at 4.0 mph with an increase in one percent grade every 30 seconds. At a respiratory exchange ration (RER) greater than 1.0 the speed was increased 0.5 mph, every 30 seconds until volitional exhaustion. Expired gas and heart rate data were continuously collected and 15-second averages were analyzed as previously described for the cycle ergometer. Calibration was conducted as described above.

**Upper Body Ergometer**

After performing the graded treadmill test subjects were asked to use the CardiO2cycle Exercise Dynamometer, upper body ergometer (UBE) (ErgometRx, St. Paul, MN) by simulating double or single poling during cross-country skiing. Subjects were asked to find a resistance equal to a rate of perceived exertion (RPE) of 9 to 10. Watts were monitored throughout the trial using Extend Software (SMI, St. Cloud, MN).
Determination of Ventilatory Threshold

Three methods were used concurrently to determine Tvent for the purpose of determining workload for the CE and TM segments of exercise. The three methods were ventilatory equivalencies, excess CO₂ production, and a modified V-slope method (Gaskill, Ruby et al. 2001). Two researchers evaluated each volitional test for the CE and TM using these three methods and determined if a clear Tvent could be established. All three methods for determining Tvent had to occur at the same time point in order for a clear Tvent to be chosen. If a clear Tvent could not be established the data were rejected and the test was performed again. The Tvent chosen by the two researchers were then compared. Both investigators had to agree on the time point chosen for Tvent or the data were rejected and the test was performed again. Tvent for the CE was 58.4±4.2% VO₂peak and 60.3±4.3% VO₂peak for the TM.

Research Design and Control

Each subject performed two, 10-hour exercise trials in a double blind, random crossover design. Subjects received either an artificially sweetened CHO drink [20% maltodextrin (0.6g/kg FFM/hr)] or an artificially sweetened placebo (PLA) drink of the same flavor and volume each hour.

In order to ensure similar muscle glycogen levels between trials each subject was provided with a standardized meal plan, of at least 5 g/kg BW CHO, and 1.2 g/kg BW protein for the 24 hours before each trial. Subjects were allowed to add to the meal plan,
but each day before their trial was eucaloric, and subjects consumed the same diet 24-hours prior to each trial. Dietary analysis was conducted using Food Processor Nutrition Analysis Software (ESHA, Salem, OR). Subjects were also asked to refrain from any type of sustained, or hard exercise for 24 hours before each trial.

**Exercise Trial**

Subjects were asked to report to the Human Performance Lab at 0500, after abstaining from calories, nicotine, and caffeine for twelve hours prior to testing. An indwelling glucose sensor (Medtronic Minimed, Northridge, CA), with a flexible catheter was placed in the subcutaneous fat just above the right gluteus maximus, in order to continuously monitor blood glucose. One hour was set for equilibration of the glucose sensor. A OneTouch-Ultra glucometer (LifeScan, Milpitas, CA) was used to calibrate the glucose sensor immediately pre-exercise, pre-lunch, and post-exercise.

Subjects were then prompted to void their bladder and a pre-exercise weight was obtained using a calibrated, digital scale (Befour Inc, Cedarburg, WI). A pre-exercise muscle biopsy was then obtained from the midsection of the vastus lateralis. Subjects were placed in a supine position on a hospital bed, and the area was cleaned with 10% Povidine iodine swab sticks (Triad, Brookfield, WI) and draped with sterile dressings. A local anesthetic (1% lidocaine, 10mg/mL) was used prior to the 5mm incision in the vastus lateralis. A Bergstrom needle with modified suction using a three-way stopcock was used to obtain 75-100mg of muscle tissue. Visible connective tissue and fat were
dissected from the muscle tissue, and the sample was flash frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

A standardized breakfast (5 g/kg BW CHO, and 1.2 g/kg BW PRO) was provided post-biopsy. Average time from breakfast to exercise was recorded and was 23:08±8:35 min for the PLA trial and 24:36±4:57 min for the CHO trial (p=0.70). Exercise began after equilibration and calibration of the glucose sensor.

The exercise protocol during the ten hours consisted of a set schedule for the three different modes of exercise each hour. Subjects began with UBE exercise at a self-selected speed and constant resistance for 9 minutes. A one-minute transition was allowed to the CE, where exercise was performed at approximately 70% Tvent, for 19 minutes. The actual values were 72.3±4.2% Tvent, and 42.1±1.6% VO2peak, which was equivalent to 103.6±23.8 Watts. Another one-minute transition was allowed to the TM, where exercise was performed again at approximately 70% Tvent for 20 minutes. The actual values were 69.4±1.9% Tvent, and 41.8±2.4% VO2peak, which was at 4.0 mph and 5.4±1.7% grade. Following the 50 minutes of exercise a ten-minute rest and feeding period (PLA or CHO of equal volume) took place. A forty-minute lunch break was allowed between hours 5 and 6, where a standardized lunch (5 g/kg BW CHO, and 1.2 g/kg BW PRO) was provided in place of the PLA/CHO feeding.

Total urine void was collected for later analysis of urinary nitrogen, to determine protein metabolism throughout each trial. Heart rate was again monitored using a chest strap heart rate transmitter and monitor (Polar USA, Lake Success, NY) during the last five minutes of each hour of CE and TM portions of exercise. The five-minute average was recorded for PLA and CHO trials.
Immediately upon completion of the tenth hour blood glucose was measured with a glucometer, and used to calibrate the indwelling glucose sensor. Subjects were then instructed to void their bladder, and a post-exercise weight was obtained. Subjects were again placed in a supine position on the hospital bed and a second muscle biopsy was obtained from a second incision in the vastus lateralis, approximately 2cm proximal to the initial biopsy site on the same leg, using the previously described methods. Time from the end of the tenth hour to biopsy was planned at 15 minutes. The actual time for biopsy collection was 15:59±1:22 min in the PLA trial and 16:40±1:33 min in the CHO trial.

Metabolic Data

Expired gases were collected during the initial five minutes on the CE to ensure that the appropriate workload of approximately 70% $T_{vent}$ was prescribed. After this time expired gases were only collected during the last five minutes of the TM segment of the trial during hours 1, 3, 5, 6, 8, and 10. Expired gas data were continuously collected during the last five minutes of these times.

Whole Body Substrate Utilization

Whole body substrate utilization was calculated from the TM segment of exercise using steady state $VO_2$ and $VCO_2$ data. The last 2.5 minutes of expired gas data collected on the TM was averaged and RER was calculated. Fat oxidation (g·min$^{-1}$) and CHO oxidation (g·min$^{-1}$) were then calculated from the RER ($VCO_2/VO_2$) data (Frayn 1983).
Total fat and CHO oxidation for the TM segment was determined by calculating the g·min\(^{-1}\) and multiplying by 20 minutes for each hour.

**Muscle Glycogen Assay**

Muscle glycogen was analyzed using a spectrophotometric method (Infinity glucose reagent, Thermo Electron, Melbourne, Australia) after tissue preparation. Samples weighing about 20 mg wet weight (actual 19.7±3.9 mg wet weight) were weighed upon removal from a −80°C freezer. Samples were placed in 1000μL, 1 N HCl solution and homogenized using a manual mortar and pestle tissue grinder. Once homogenized, samples were incubated at 95.6°C for three hours. After the incubation, 500μL, 1 N NaOH was added to 500μL of boiled tissue sample to normalize pH. Samples were analyzed in triplicate, after pipetting 40 mL of sample into 1mL of the Infinity glucose reagent and incubated at room temperature for 15 minutes, using a spectrophotometer (Spectronic 402, Milton Roy, Rochester, NY), against glycogen and glucose controls. Muscle glycogen was expressed in mmol·kg wet weight\(^{-1}\) of muscle.

**Statistical Procedures**

Descriptive data were expressed as Mean±SD. A two-way ANOVA with repeated measures design using a series of apriori, planned comparisons were used to analyze data. Specific cell comparisons were performed using SuperANOVA (Abacus Concepts, Inc, Berkley, CA). The number of uncorrected comparisons was limited to the
degrees of freedom (df) allowed. When the number of comparisons exceeded the allowed df, a Bonferroni critical value procedure was performed (0.05/number of comparisons). The level of significance for the experiment was set at an overall alpha of 0.05. All graphed values are expressed in Mean±SE.
References


The reason for the unusual format of this thesis from traditional formatting is due to the nature of the manuscript. This thesis represents data that was collected as an individual research project. Immediately following the completion of this study the methodology was duplicated in females for another separate thesis project. It was felt that in order to complete a manuscript that would be allowed submission into a journal the data from these two separate thesis projects needed to be pooled. Chapters 1-3 and the appendices of this thesis concern only the male data, while the manuscript is the sex comparison from the pooled data. Two separate reference lists, tables, and graphs are included in this thesis because of the combined data included in the manuscript.
For Submission to Medicine and Science in Sports and Exercise

Section: Carbohydrate Metabolism

Effects of Carbohydrate Supplementation in Men and Women during a 10-hour Exercise Trial

Running Head: Carbohydrate Ingestion during Extended Exercise

Authors: Stephanie G. Harger, Anne E. McClaughry, Steven E. Gaskill, and Brent C. Ruby

Human Performance Laboratory, The University of Montana, Missoula MT 59812

Address Correspondence: Brent C. Ruby

Director, Human Performance Laboratory
Department of Health and Human Performance
The University of Montana
Missoula, MT 59812-1825

Phone: (406) 243-2117; Fax: (406) 243-6252
email: brent.ruby@mso.umt.edu
Abstract

Previous research has demonstrated conflicting results regarding sex differences in substrate utilization during exercise. In addition, although past research has shown the ergogenic benefits of exogenous carbohydrate (CHO) intake during exercise, limited work has been done to evaluate its effectiveness during extended exercise/work periods lasting longer than 4-5 hours. **PURPOSE:** The purpose of the present study was to evaluate the effects of CHO supplementation on whole body and muscle substrate utilization during 10 hours of prolonged, discontinuous exercise in men and women. **METHODOLOGY:** Thirteen recreationally trained subjects (n=7 males, n=6 females, during the follicular phase) performed a GXT on a treadmill (TM) and electronically braked cycle ergometer to determine ventilatory threshold (VT) and VO$_{2\text{peak}}$. In a double blind, randomized crossover design, subjects received either a CHO [20% maltodextrin (0.6 g·kg FFM$^{-1}$·hr$^{-1}$)] or flavored placebo (PLA) drink each hour. TM exercise was performed at 71±3% VT (44±4% VO$_{2\text{peak}}$). Cycle ergometer exercise was performed at 72±4% VT (42±1% VO$_{2\text{peak}}$). Hourly exercise included 9 minutes on an upper body ergometer, 19 minutes on a cycle ergometer, and 20 minutes on a treadmill, followed by a 10-minute rest and feeding period. A standardized breakfast and lunch (5 g·kg$^{-1}$ CHO, and 1.2 g·kg$^{-1}$ protein) were provided for both trials. Muscle biopsies of the vastus lateralis were obtained pre and post-exercise, and expired gases were collected every other hour during the TM segment of the trial to establish average rates of whole body CHO and fat oxidation for the first five (AM) and second five (PM) hours of exercise. Blood glucose (BG) was measured continuously using an indwelling glucose sensor, and
total urine void was collected. Data were analyzed using a mixed design ANOVA with repeated measures. **RESULTS:** Whole body CHO oxidation demonstrated a significant trial x time interaction (CHO: AM=138±13, PM=130±18; PLA: AM=108±17, PM=86±18 μmol/kg FFM/min, p<0.05. Muscle glycogenolysis was 78% higher for the PLA compared to the CHO trial (3.1±2.3 and 5.9±2.9 mmol kg⁻¹ wet wt·hr⁻¹ for CHO and PLA respectively, p<0.05). There were no significant sex specific differences in glycogen utilization or whole body substrate oxidation. **CONCLUSION:** Although the ingestion of CHO during long duration exercise, appears to decrease rates of muscle glycogenolysis while better maintaining whole body carbohydrate oxidation, there is limited differences between the sexes.
Key Words: Carbohydrate oxidation, fat oxidation, glycogenolysis, fat free mass, sex-specific hormones

Introduction

Paragraph 1

It has been well established that exogenous carbohydrate (CHO) supplementation during exercise increases and/or maintains rates of whole body CHO oxidation \(^4, 17, 22\). In addition, if exogenous CHO is provided, blood glucose levels are maintained \(^4, 17, 22\), RPE often decreases \(^31\), hepatic glucose output is suppressed \(^18\), and muscle glycogenolysis may \(^30\) or may not be slowed \(^5, 8\). It has also been conclusively demonstrated that exogenous CHO can increase performance and time to fatigue during exercise in both males and females \(^4, 7, 17, 18\).

Paragraph 2

The majority of past research involving the use of supplemental CHO has been conducted under a variety of exercise durations (1-5 hours) and intensities (60-80% VO\(_{2\text{peak}}\)). It appears that the longest controlled exercise trial evaluating the effects of exogenous CHO is a recent study by Jeukendrup et al. (2006) where subjects exercised for a period of 5 hours. To our knowledge, past research has not adequately addressed the effects of supplemental, exogenous CHO during exercise periods longer than five hours.
Paragraph 3

Sex specific differences in substrate metabolism have demonstrated mixed results in past research. While some studies demonstrate increased rates of fat oxidation and decreased muscle glycogenolysis in females, others demonstrate no significant differences between the sexes. Many studies demonstrate that women rely more heavily on lipid oxidation expressed as a percentage of total energy expenditure. In studies using stable isotope dilution techniques, similar rates of glucose appearance (Ra) and glucose disappearance (Rd) with carbohydrate supplementation have been observed in both men and women, when performing exercise at the same relative intensity. Moreover, our laboratory has previously demonstrated minimal differences in whole body substrate oxidation and glucose kinetics across the sexes during exercise at 70 and 90% of the lactate threshold. However, the relative contribution of blood glucose to total CHO oxidation was consistently higher in females.

Paragraph 4

Past researchers have demonstrated that females may oxidize substrates differently across the menstrual cycle and that the primary mechanism for this subtle difference is related to circulating estradiol. When amenorrheic females were provided with exogenous estradiol, plasma glucose Ra and Rd were reduced. Interestingly, this finding has been duplicated when estradiol was provided to males. These results demonstrate the importance in controlling menstrual cycle status when performing metabolism studies in female subjects. However, it is also important to note that the metabolic effects associated with the reproductive hormonal milieu are far less influential.
on substrate utilization in comparison to the effects of exercise intensity, duration, and feeding and training status.

*Paragraph 5*

While the majority of the past researchers have not demonstrated a decrease in muscle glycogenolysis when exogenous CHO is provided, the exercise durations have not been extended beyond 5 hours. In addition, the potential for a sex dependent metabolic response during extended exercise when exogenous CHO is provided has not been addressed. The purpose of this study was to determine the effects of exogenous CHO feedings on whole body substrate oxidation and muscle glycogenolysis, during long duration, low intensity exercise in males and females.

*Paragraph 6*

We hypothesized that rates of muscle glycogenolysis would be suppressed when CHO was provided during extended exercise and that there would be minimal differences in substrate metabolism across the sexes.

**Methodology**

*Subjects*

*Paragraph 7*

Thirteen recreationally trained males (n=7) and females (n=6) served as subjects for the investigation. Subject descriptive characteristics are shown in Table 1. Prior to
participation, each subject completed a University Institutional Review Board (IRB) approved informed consent form. All female subjects were eumenorrheic and had not been using any form of hormonal contraception for at least six months prior to the study. Preliminary descriptive testing was performed without regard to the menstrual cycle. However, all extended exercise trials were performed during the follicular phase of the menstrual cycle (1-4 days post-menses). All subjects were asked to maintain their normal exercise and dietary habits between the two trials. Each exercise trial was completed at least one week apart for male subjects and no more than one menstrual cycle apart for female subjects.

Anthropometric Measurements

Paragraph 8

Hydrostatic weighing, at estimated residual volume \(^{12}\) was used to determine body fat percent and fat free mass \(^{20}\). Subjects performed multiple underwater weight trials until three values within 100 grams were obtained. Net underwater weight was determined using an electric, calibrated scale (Exertech, Dresbach, MN). Body density was then used to determine body fat percent using the age and sex specific equations of Lohman (1992).

Exercise Testing

Paragraph 9

All subjects were asked to report to the Human Performance Laboratory after abstaining from calories, nicotine, and caffeine for twelve hours prior to testing. Graded exercise
tests to volitional exhaustion, to determine maximal oxygen uptake ($$\text{VO}_2\text{peak}$$) and ventilatory threshold (VT) were performed on two separate days using an electronically braked Cardgirus cycle ergometer (CE) (Cardgirus, Spain), and a Quinton Q65 treadmill (TM) (Quinton, Seattle, WA). The testing protocol for the CE consisted of a ramped protocol progressing at 40 watts per minute until volitional exhaustion, or until a cadence of 50 revolutions per minute (rpm) could not be maintained. The testing protocol for the TM consisted of a speed set at 1.8 m s\(^{-1}\), with an increase in one percent grade every 30 seconds. At a respiratory exchange ration (RER) greater than 1.0 the speed was increased 0.23 m s\(^{-1}\), every 30 seconds until volitional exhaustion.

*Paragraph 10*

Expired gas data were continuously collected using a two-way mouthpiece (Hans Rudolph, Inc., Kansas City, MO) and a ParvoMedics metabolic cart (ParvoMedics, Salt Lake City, UT). Prior to each expired gas collection calibration was performed using a 3-Liter calibration syringe (Hans Rudolph, Inc., Kansas City, MO), and a calibration gas mixture (16.0% O\(_2\), 4.0% CO\(_2\), N\(_2\) balance). Prior to each gas collection during the extended exercise trials, the metabolic system was calibrated as described above. Heart rate was continuously measured using a chest strap Polar Heart Rate monitor (Polar USA, Lake Success, NY). Three methods were used concurrently to determine the VT for the purpose of determining workload for the CE and TM segments of the extended exercise trials according to Gaskill et al. (2001).
Paragraph 11

After performing the graded treadmill test, subjects were asked to use the CardiO2cycle Exercise Dynamometer, upper body ergometer (UBE) (ErgometRx, St. Paul, MN) by simulating double or single poling during cross-country skiing. Subjects were asked to find a resistance equal to a rate of perceived exertion (RPE) of 9 to 10. Watts were monitored throughout the trial using Extend Software (SMI, St. Paul, MN).

Extended Exercise Trials

Paragraph 12

Each subject performed two, 10-hour exercise trials in a double blind, random crossover design. Subjects received either CHO [20% maltodextrin (0.6g·kg FFM⁻¹·hr⁻¹)] or an artificially sweetened placebo (PLA) drink of the same flavor, temperature and volume each hour.

Paragraph 13

In order to ensure similar muscle glycogen levels between trials, each subject was provided with a standardized meal plan, of at least 5 g·kg⁻¹ CHO, and 1.2 g·kg⁻¹ protein for the 24 hours before each trial. Although subjects were allowed to add to the meal plan prior to the first trial, the same dietary intake was duplicated prior to the second trial. Dietary analysis was conducted using Food Processor Nutrition Analysis Software (ESHA, Salem, OR). Subjects were also asked to refrain from any type of exercise for 24 hours before each trial.
Paragraph 14

Subjects were asked to report to the Human Performance Lab at 0500, after abstaining from calories, nicotine, and caffeine for twelve hours prior to testing. An indwelling glucose sensor (Medtronic Minimed, Northridge, CA), with a flexible catheter was placed in the subcutaneous fat just above the right gluteus maximus, in order to continuously monitor blood glucose. One hour was set for equilibration of the glucose sensor. The OneTouch-Ultra glucometer (LifeScan, Milpitas, CA) was used to calibrate the glucose sensor immediately pre-exercise, pre-lunch, and post-exercise.

Paragraph 15

Subjects were then prompted to void their bladder and a pre-exercise weight was then obtained using a calibrated, digital scale (Befour Inc, Cedarburg, WI). A pre-exercise muscle biopsy was then obtained from the midsection of the vastus lateralis. Subjects were placed in a supine position on a hospital bed, and the area was cleaned and draped with sterile dressings. Under local anesthetic a Bergstrom needle with modified suction was used to obtain the biopsy. Visible connective tissue and fat were dissected from the muscle tissue, and the sample was flash frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Paragraph 16

A standardized breakfast (5 g·kg\(^{-1}\) CHO, and 1.2 g·kg\(^{-1}\) protein) was provided for the subjects post-biopsy. Average time from breakfast to exercise was recorded and was 23
and 22 minutes for the PLA and CHO trials, respectively. Exercise began after equilibration and calibration of the glucose sensor.

**Paragraph 17**

The exercise protocol during the ten hours consisted of three different modes of exercise performed for varying lengths of time, over an hour. Subjects began with UBE exercise at a self-selected speed and constant resistance for 9 minutes. A one-minute transition was allowed to the CE, where exercise was performed at \( 71 \pm 4\% \, \text{Tvent} \), and \( 44 \pm 4\% \, \text{VO}_{2\text{peak}} \). Another one-minute transition was allowed to the TM, where exercise was performed at \( 71 \pm 3\% \, \text{Tvent} \), and \( 44 \pm 4\% \, \text{VO}_{2\text{peak}} \) for 20 minutes. At this time a ten-minute rest and feeding period (PLA or CHO of equal volume) occurred. A forty-minute lunch break was allowed between hours 5 and 6, where a standardized lunch (5 g \cdot kg^{-1} \, \text{CHO}, and 1.2 g \cdot kg^{-1} \, \text{protein}) was provided in place of the PLA/CHO feeding.

**Paragraph 18**

Heart rate was monitored using a chest strap Polar Heart Rate monitor (Polar USA, Lake Success, NY) during the last five minutes of each hour of CE and TM portions of exercise. The five-minute average was recorded for PLA and CHO trials.

**Paragraph 19**

Immediately upon completion of the last hour, blood glucose was measured with the glucometer, and entered into the glucose sensor for calibration. Subjects were then instructed to void their bladder, and a post-exercise weight was obtained. Subjects were
again placed in a supine position on the hospital bed and a second muscle biopsy was obtained from a second incision in the vastus lateralis, approximately 2cm proximal to the initial biopsy site on the same leg. The muscle sample was collected and stored in the same manner as the initial muscle biopsy.

Metabolic Data

Paragraph 20

Expired gases were collected during the initial five minutes on the CE to ensure that the appropriate workload \([\text{VO}_2 \ (L \cdot \text{min}^{-1})]\) was prescribed. After this time expired gases were collected during the last five minutes of the TM segment of the trial during hours 1, 3, 5, 6, 8, and 10.

Whole Body Substrate Utilization

Paragraph 21

Whole body substrate utilization was calculated from the TM segment of exercise using steady state \(\text{VO}_2\) and \(\text{VCO}_2\) data. The last 2.5 minutes of expired gas data collected on the TM was averaged to determine \(\text{VO}_2\), \(\text{VCO}_2\), fat oxidation (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\), \(\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}\)) and CHO oxidation (\(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\), \(\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}\)) \(^9\). Values were averaged for hours 1, 3, and 5 for AM and hours 6, 8, and 10 for PM estimates of whole body substrate oxidation.
**Muscle Glycogen Assay**

**Paragraph 22**

Muscle glycogen was analyzed using a spectrophotometric method (Infinity glucose reagent, Thermo Electron, Melbourne, Australia) after tissue preparation. Samples were weighed upon removal from a −80°C freezer. Samples were placed in 1000μL, 1 N HCl solution and homogenized using a manual mortar and pestle tissue grinder. Once homogenized, samples were incubated at 95.6°C for three hours. After the incubation, 500μL, 1 N NaOH was added to 500μL of boiled tissue sample to normalize pH. Samples were analyzed in triplicate, after pipetting 40 mL of sample into 1mL of the Infinity glucose reagent and incubating at room temperature for 15 minutes, using a spectrophotometer (Spectronic 402, Milton Roy, Rochester, NY), against glycogen and glucose controls. Muscle glycogenolysis was expressed in mmol·kg⁻¹ wet wt·hr⁻¹ of muscle.

**Statistical Procedures**

**Paragraph 23**

Descriptive data were expressed in Mean±SD. All descriptive data were analyzed across sex using an independent 2-tailed t-test. All other dependent variables were compared using a series of mixed design ANOVA with repeated measures (Abacus Concepts, Inc, Berkley, CA). The level of significance for the experiment was set at an overall alpha of 0.05.
Results

Descriptive Data.

Paragraph 24

Comparative baseline descriptive data are summarized in Table 1. Males were heavier, with a lower percent body fat, higher FFM, and a higher absolute (L·min⁻¹) and relative (ml·kg⁻¹·min⁻¹, ml·kg FFM⁻¹·min⁻¹) VO₂peak and absolute VT (L·min⁻¹). When values for VT were expressed relative to body mass, FFM and relative to peak oxygen consumption (%VO₂peak) there were no significant differences between sexes.

Exercise Descriptives.

Paragraph 25

The workload intensities for VT and 70% VT are summarized in Table 2. There were no differences between males or females in the exercise intensities in either mode of exercise when expressed relative to VT. Conversely, when expressed relative to VO₂peak, exercise was significantly higher for the females during the treadmill exercise. However, males performed exercise at a significantly higher absolute workload (watts) on the cycle ergometer than did females.

Paragraph 26

Heart rate (HR) and RPE data are summarized in Table 3. There were no differences between males and females in either HR or RPE for either trial. Slight increases were
seen in RPE for the PLA trial compared to the CHO trial, but values were not statistically significant.

**Blood Glucose**

**Paragraph 27**

Blood Glucose demonstrated no statistically significant difference between the sexes (Figure 1). Values for blood glucose were significantly higher for the CHO trial compared to the PLA trial for both sexes. There was a time x trial interaction that was statistically significant demonstrating decreased blood glucose across time in both CHO and PLA trials.

**Substrate Oxidation**

**Paragraph 28**

Substrate oxidation derived from expired gases and metabolic equations are summarized in Table 4. There were no differences in calculated fat oxidation when values are expressed relative to FFM. Total energy expenditure was higher in males across all times and trials. There was also a statistically significant difference CHO oxidation during the PLA trial, demonstrating a higher rate of CHO oxidation in females.

**Whole Body Substrate Oxidation**

**Paragraph 29**

Whole body carbohydrate oxidation (expressed relative to total body weight and to FFM) demonstrated a significant sex x trial interaction. Values for CHO oxidation were
significantly higher during the CHO trial compared to the PLA trial for both males and females. There was no significant difference between the males and females during the CHO trial. However, females maintained a significantly higher rate of whole body CHO oxidation during the PLA trial compared to the males (Figures 2a,b). The time x trial interaction was also statistically significant. Hours 1-5 and 6-10 demonstrated significantly higher rates of CHO oxidation values for the CHO trial compared to the PLA. In addition, CHO oxidation demonstrated a significant decrease during hours 6-10 in the PLA trial. Although not statistically significant, the CHO trial showed a trend towards lower CHO oxidation through hours 6-10 (p=0.065) (Figure 2a,b).

Paragraph 30

Whole body fat oxidation (expressed relative to total body weight and to FFM) demonstrated no sex specific significant interactions (Figure 3a,b). Values for fat oxidation were significantly higher for the PLA trial compared to the CHO trial for both sexes. The time x trial interaction was statistically significant demonstrating increased fat oxidation in hours 6-10, compared to hours 1-5 in both trials. In addition, fat oxidation was maintained at higher rates during the PLA trial compared to the CHO trial in hours 1-5 and 6-10 (Figure 3a,b).

Glycogen

Paragraph 31

For the measure of muscle glycogen, there was no significant effect of sex. However, the trial x time interaction indicated a significant decrease in muscle glycogen for both CHO
and PLA trials. In addition, pre-exercise glycogen concentration was slightly but significantly higher for the PLA trial. In contrast, the post exercise glycogen concentration was significantly lower for the PLA trial compared to CHO (Figure 4).

**Paragraph 32**

When glycogen depletion was expressed in mmol kg\(^{-1}\) wet wt hr\(^{-1}\), there were no significant differences between the males and females. However, the main effect for trial demonstrated a significantly higher (78%) rate of glycogenolysis for the PLA trial compared to the CHO (Figure 5).

**Discussion**

**Paragraph 33**

The purpose of this investigation was to determine the effects of regular CHO feedings on whole body substrate oxidation and muscle glycogenolysis in recreationally active males and females during long duration, low-intensity, intermittent exercise. The notable results from this study demonstrated no significant sex specific differences in whole body substrate oxidation when values were expressed relative to body mass or FFM. These results are in contrast to Horton et al. (1998) who demonstrated that females oxidize a higher relative amount of fat in comparison to males. However, these results are similar to the data of Riddell et al. (2003) who demonstrated a diminished effect of sex when subjects were tested in the fed vs. fasted state. Interestingly, during the PLA trial females demonstrated a significantly higher rate of CHO oxidation compared to males.
Paragraph 34

In order to standardize exercise intensities, past researchers have matched subjects based on background exercise habits, competitive history, and VO_{2peak}^{3,19,23,28,29}. Exercise intensity in the present study was expressed relative to VT in order to ensure similar workloads between sexes. Although females exercised at a significantly higher workload for the treadmill when expressed as a percent VO_{2peak}, there was no difference in the percentage of VT between the sexes. This ensures similar intensities for both modes of exercise between males and females.

Paragraph 35

In a study performed by Zderic et al. (2001) it was demonstrated that across the menstrual cycle there were differences in substrate oxidation. The results from this study demonstrated no differences in glucose kinetics, CHO and fat oxidation at low intensities. As the intensity of exercise increased, R_a and R_d, along with CHO oxidation were lower in the luteal phase. Because of these findings all of the female subjects in the present study were eumenorrheic and tested in the follicular phase (1-4 days post menses) in order to ensure low levels of circulating estrogen and progesterone.

Paragraph 36

Previous research has demonstrated that females display lower RER compared to males during exercise at the same percent VO_{2peak}. Moreover, some research has demonstrated that females use less glycogen and have lower blood glucose concentrations at the end of exercise at intensities relative to VO_{2peak}^{1,15,28,29}. Levels of estrogen and progesterone
may have an impact on the source of CHO in females during exercise\textsuperscript{6,13,33}. Ruby et al. (2002) has also suggested that females may rely more heavily on blood glucose as a CHO source for energy during submaximal exercise. This has also been suggested by Friedlander et al. (1998) in a study that trained males and females to determine changes in substrate oxidation at the same absolute and relative exercise intensities. However, in the present study, blood glucose was not significantly different across trial or between the sexes. This further suggests that static measures of blood glucose cannot adequately represent the complex patterns of glucose kinetics resulting from hepatic and gut $R_a$ as well as muscle glucose uptake and oxidation.

\textit{Paragraph 37}

In this investigation higher rates of CHO oxidation were observed for both males and females during the CHO trial. These findings are consistent with findings from past research. When exogenous CHO is provided during endurance exercise a higher RER, and therefore higher rates of CHO oxidation have been consistently demonstrated\textsuperscript{4,5,7,16-18}. However, during the CHO trial, there were no differences between the sexes. In contrast, during the PLA trial, fat oxidation increased late in the trial. Moreover, during this trial, females displayed higher rates of whole body CHO oxidation compared to males. Although females exercised at a higher \% VO\textsubscript{2peak} than males, the values for \% VT were not statistically significant (Table 2), ensuring similar workloads for both sexes. Therefore, these results are in opposition with past research\textsuperscript{1,2,14,15,28,29}. However, a possible explanation for this may be related to the design of this exercise trial. Although past research has suggested that estradiol may exert subtle metabolic effects on substrate
metabolism during submaximal exercise, these variations may be overridden by the stress associated with the present exercise. The subjects in this study were trained subjects, and did not perform exercise completely fasted. This exercise trial was also performed at an intensity below 50% VO_{peak}, and for a period of 10 hours, a much longer duration than has been studied in the past. Therefore, the influences of the exercise protocol may have a more influential effect on substrate metabolism compared to the small effects associated with the reproductive hormonal milieu \(^2,19,21-24,27,32\).

*Paragraph 38*

It has been demonstrated that CHO feedings administered at a high rate (1.0-1.7 g-min\(^{-1}\)) can completely suppress liver glycogenolysis, but there is conflicting data regarding the effects on muscle glycogenolysis \(^3,5,8,18,30,32\). In the present study the rates of muscle glycogenolysis were approximately 78% higher for the PLA trial. However, there were no differences between the sexes. This data is supported by the recent findings of Zehnder et al. (2005) where subjects cycled for three hours at 50% VO_{peak}. Muscle biopsy data indicated a main effect for time, showing a significant reduction in muscle glycogen and intramuscular triglyceride (IMTG) utilization. However, there was no sex specific difference in muscle substrate oxidation. This investigation also demonstrated a dramatic muscle glycogen "sparing" effect for both sexes, when CHO was provided at a rate of approximately 37 g-hour\(^{-1}\). Interestingly, this rate of feeding is lower than the optimal rates suggested by Jeukendrup et al. (2000) to maximize the rate of exogenous CHO.
In conclusion, the results from this study indicate that females and males exhibit similar patterns of whole body and muscle glycogen utilization when fed CHO during extended exercise. These results suggest that future research should further evaluate the potential of muscle glycogen sparing during long duration exercise and/or occupational settings when exogenous CHO is provided on a regular basis.
Acknowledgements

Paragraph 40

This investigation was supported by a grant from the Gatorade Sports Science Institute.
Table 1. Subject Descriptive Characteristics (N=13)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n=7)</th>
<th>Females (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.1± 6.1</td>
<td>22.8± 3.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.4± 9.2</td>
<td>171.6± 8.9</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>77.0± 10.1</td>
<td>62.6± 4.7*</td>
</tr>
<tr>
<td>Percent Body Fat (%)</td>
<td>9.8± 4.0</td>
<td>14.2± 2.5*</td>
</tr>
<tr>
<td>Fat Free Mass (FFM) (kg)</td>
<td>69.2± 7.5</td>
<td>53.7± 4.9*</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>7.8± 3.9</td>
<td>8.8± 1.4</td>
</tr>
<tr>
<td>$\text{VO}_2$ peak Treadmill</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/min</td>
<td>4.5± 0.6</td>
<td>3.1± 0.3*</td>
</tr>
<tr>
<td>ml/kg/min</td>
<td>58.3± 5.5</td>
<td>49.7± 4.0*</td>
</tr>
<tr>
<td>ml/kg FFM/min</td>
<td>64.7± 5.9</td>
<td>58.0± 4.9*</td>
</tr>
<tr>
<td>% $\text{VO}_2$ peak at VT Treadmill</td>
<td>60.3± 4.3</td>
<td>65.4± 7.9</td>
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<tr>
<td>$\text{VO}_2$ peak Cycle</td>
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<td></td>
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<tr>
<td>L/min</td>
<td>4.3± 0.5</td>
<td>2.8± 0.5*</td>
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<tr>
<td>ml/kg/min</td>
<td>56.2± 7.3</td>
<td>45.1± 6.9*</td>
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<tr>
<td>ml/kg FFM/min</td>
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<tr>
<td>% $\text{VO}_2$ peak at VT Cycle</td>
<td>58.4± 4.2</td>
<td>64.4± 10.5</td>
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</table>

Values are expressed Mean±SD. $\text{VO}_2$ peak, peak O$_2$ uptake, VT, ventilatory threshold, FFM, fat free mass. *p < 0.05 vs. males.
Table 2. Exercise Intensities (N=13)

<table>
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<td>Males (n=7)</td>
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<td>% VT Treadmill</td>
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<td>% VT Cycle</td>
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<td>% VO₂peak Treadmill</td>
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<td>% VO₂peak Cycle</td>
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<td>Treadmill % Grade</td>
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<td>Cycle Workload (Watts)</td>
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<td></td>
<td>Females (n=6)</td>
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<td></td>
<td>71.9± 4.1</td>
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<td>70.5± 3.8</td>
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<td>46.8± 3.9*</td>
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<td></td>
<td>45.1± 5.8</td>
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<td></td>
<td>4.7± 1.9</td>
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<tr>
<td></td>
<td>70.5± 19.0*</td>
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</table>

Values are expressed Mean±SD. *p < 0.05 vs. males.
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<th>Placebo</th>
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</thead>
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</tr>
<tr>
<td>Heart Rate Cycle</td>
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<td></td>
</tr>
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<td>Ergometer</td>
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<td>115±8.8</td>
</tr>
<tr>
<td>Heart Rate Treadmill</td>
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<td>130±8.5</td>
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<tr>
<td>RPE</td>
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<td>12±1.3</td>
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</table>

Values are expressed Mean±SD.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Carbohydrate</th>
<th>Placebo</th>
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<tbody>
<tr>
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</tr>
<tr>
<td></td>
<td>AM</td>
<td>PM</td>
</tr>
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<td>0.90±0.03</td>
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<tr>
<td>kcal/min</td>
<td>8.9±1.1</td>
<td>9.1±1.1</td>
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<tr>
<td>CHO Oxidation (umol/kg FFM/min)</td>
<td>134.3±11.1</td>
<td>128.5±10.6</td>
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<tr>
<td>Fat Oxidation (umol/kg FFM/min)</td>
<td>4.7±1.4</td>
<td>5.3±2.1</td>
</tr>
</tbody>
</table>

Values are expressed Mean±SD. *p<0.05 vs. Males
Figure 1. Blood glucose (mM) for the two exercise trials. †p<0.05 vs. pre, Main effect for time.
Figure 2a. Whole body carbohydrate oxidation (μmol·kg⁻¹·min⁻¹) for the two exercise trials. *p<0.05 vs. AM for PLA (trial x time interaction). † p<0.05 vs. Males for PLA (trial x sex interaction).
Figure 2b. Whole body carbohydrate oxidation (μmol·kg FFM⁻¹·min⁻¹) for the two exercise trials. *p<0.05 vs. AM for PLA (trial x time interaction). † p<0.05 vs. Males for PLA (trial x sex interaction).
Figure 3a. Fat Oxidation ($\mu$mol·kg$^{-1}$·min$^{-1}$) for the two exercise trials. *p<0.05 vs. AM for PLA (trial x time interaction).
Figure 3b. Fat Oxidation (μmol·kg FFM\(^{-1}\)·min\(^{-1}\)) for the two exercise trials. *p<0.05 vs. AM for PLA (trial x time interaction).
Figure 4. Muscle glycogenolysis (mmol·kg wet weight\(^{-1}\)·hour\(^{-1}\)) for the two exercise trials. *p<0.05 vs. CHO trial, main effect for trial.
References


Appendix I
Informed Consent Form

Effects of carbohydrate feedings on blood glucose and muscle glycogen during extended exercise

Principal Investigator: Brent C. Ruby, Ph.D.

Location: Human Performance Laboratory
McGill Hall #121
The University of Montana
Missoula, MT 59812
(406) 243-2117/(406) 243-4780

Purpose
The purpose of this project is to determine the effects of high carbohydrate food/drink sources on blood glucose, and changes in muscle glycogen during extended exercise. The information collected in this study will help determine if the effects of extra carbohydrate consumption will alter work performance and minimize fatigue.

As a participant in this study you will complete the following assessments. 1) a pre-screening assessment which involves a health/exercise history questionnaire (Par-Q), 2) a measure of percent body fat obtained using a underwater weighing, 3) a maximal treadmill test and maximal cycle ergometer test to measure aerobic fitness levels, 4) two ten-hour work/rest sessions involving cycling, treadmill walking and arm exercise, 5) the completion of a mood state survey (the Profile of Mood States) at six times during the study, 5) collection of expired gases to determine fuel use during the 10-hour work/rest session, 6) consumption of one of two different types of liquid carbohydrate every hour of exercise, 7) finger-stick blood samples every two hours to determine blood glucose during activity, 8) two muscle biopsies to be collected a) early in the morning before the exercise session begins, and b) late in the afternoon after the exercise session is completed.

Body Fat Measurement – Underwater Weighing:
This test session will require that subjects do not eat for a minimal of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. Subjects will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that subjects are submersed in our weighing tank (similar to a hot tub) and that they maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then subjects will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 30 minutes.
Maximal Exercise Test — Treadmill:
This test will consist of walking and running on a motorized treadmill to a maximal effort. The speed and grade of the treadmill will progress to fatigue. Subjects will be encouraged to continue to walk/run until exhaustion. During the entire testing session on the treadmill, subjects will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen the body uses during the exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 45 minutes to 1-hour. Subjects will be asked to fast for approximately 3 hours prior to this test.

Maximal Exercise Test — Cycle:
This test will consist of riding on a laboratory exercise cycle to maximal effort. The resistance of the cycle will increase each minute and will progress to fatigue. Subjects will be encouraged to continue to ride until exhaustion. During this test the subjects will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 45 minutes to 1 hour. Subjects will be asked to fast for approximately three hours prior to this test.

Maximal Exercise Test — Arm ergometer:
This test will consist of using an upper body machine with ropes and pulleys to simulate cross-country skiing to maximal effort. The resistance of the machine will increase each minute and will progress to fatigue. Subjects will be encouraged to continue until exhaustion. During this test the subjects will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 30-45 minutes. Subjects will be asked to fast for approximately three hours prior to this test.

10-hour Exercise/Rest Session:
The order of these sessions will be randomized and blinded to the study participants. Subjects will be asked to report to the laboratory after a 12 hour fast, at approximately 6:00 AM. Each hour of exercise will consist of the three activities explained above. The first 10 minutes will be self selected exercise using the upper body machine, the next 20 minutes will be performed on the cycle at a predetermined intensity, and the next 20 minutes will be walking on the treadmill at a predetermined intensity. Every other hour while walking on the treadmill expired gases will be collected for approximately 6
minutes using the nose clip, headgear, and mouthpiece mentioned above. The last 10 minutes of the hour will allow for rest and feeding.

**Muscle Biopsies:**
A total of four muscle biopsies will be obtained from your front thigh muscle (vastus lateralis - approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the sight be sterilized. After the sight is cleaned, a small amount of lidocaine will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the sight on the leg. After the area is treated with the lidocaine (approximately 5 ml, 10mg/ml), a small incision (approximately 1/4 inch long) will be made through the skin and to a depth of approximately 3/4 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips and wrapped with an ace bandage. A flexible ice pack will then be placed on the site for 10 minutes. The biopsy samples will be obtained a) prior to the 10-hour exercise/rest session, and b) immediately after the 10-hour exercise/rest session (on the same leg but approximately 2 inches above the initial sample). This will be repeated for the second trial using the opposite leg. The muscle biopsies will only be used to evaluate alterations in muscle carbohydrate stores in response to physical activity. Latex free bandages will be provided if subjects have a known allergy to latex.

**Body Weight:**
Your nude body weight will be measured using a digital scale prior to, after 5-hours, and immediately following each 10-hour exercise/rest session. All measures will be done in private.

**Surveys:**
You will be asked to complete a 60-question survey that has been developed to assess you opinions regarding your current mental state prior to, at the mid point and immediately following the each 10-hour exercise/rest session. You are asked to provide an accurate and truthful response to each question.

**Supplemental Carbohydrate Beverage:**
During each 10-hour exercise/rest session, you will be given one of two different carbohydrate containing beverages. You will be asked to drink a predetermined amount (200 ml, approximately 6.7 oz, just less than 1 cup) of the solution at the top of each hour and throughout the entire 10-hour session. You will also be provided with a small standardized lunch at the 5-hour mid point.

**Finger blood samples to determine blood glucose during activities.**
During each 10-hour exercise session, periodic finger stick blood samples will be collected prior to and following each hour of exercise. These samples are being collected to evaluate changes in blood sugar throughout each exercise session.
Risks and Discomfort

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred, as with other moderately strenuous exercise activities. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. A trained CPR technician will be on hand at all times and the laboratory has standard emergency procedures should any potential problems arise.
4. You will be informed of any new findings that may affect your decision to remain in the study.
5. The muscle biopsy and blood sampling techniques may cause some local and temporary discomfort. It is normal to have the sensation of a deep tissue bruise around the site of the muscle biopsy. This pain should be manageable and not above the pain associated from a “charlie horse” type bruise.
6. There is a minor risk of infection associated with blood sampling and the muscle biopsy. Should you notice unusual redness, swelling or drainage at the biopsy incision site, you should seek medical attention and then notify Brent Ruby, study director.
7. There are minimal risks associated with the use of lidocaine (the local anesthetic). The risk of a reaction to the lidocaine is extremely low (approximately 1/1,000,000). However to minimize this risk we will use no more than 5-7 ml of a 10% lidocaine solution per biopsy.
8. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped.

Benefits of Participating in This Study

1. There is no promise that you will receive any benefit outside of the financial payment as a result of taking part in this study.
2. The information from these tests will provide you with an accurate assessment of you aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.
3. Upon completion of the preliminary tests (body fat, treadmill, cycle and arm ergometer max tests), you will be paid $25. Upon completion of the first 10-hour exercise/rest session, you will be paid another $75. Upon completion of the second 10-hour session, you will be paid another $100. Therefore, upon completion of the entire study, you will be paid a total of $200. If you decide to withdraw at any time, you will be compensated for the test sessions you have completed or initiated.
Confidentiality
All results will be kept in strict confidence among the subject involved and the Principal Investigators and other Co-Investigators. During the entire period of data collection, subject records will be kept within the Human Performance Laboratory and will be locked under the direction of the Principal Investigator.

Compensation for Injury
Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Claim representative or University Legal Counsel.

Voluntary Participation and Withdrawal
It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive full compensation for all the test sessions you complete or initiate.

A copy of this consent form will be provided for you. In addition, the data collected during this study will be done at no cost to you.

Statement of Consent
I have read the above statements and understand the risks involved with this study. I authorize Brent C. Ruby and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Brent C. Ruby at home (406) 542-2513 or at the Human Performance Laboratory (406) 243-2117.

Participant (print) ________________________________

Signature ________________________________ Date __________

Permanent Address ________________________________

Investigator/Witness Signature ________________________________ (print)

Investigator/Witness Signature ________________________________ Date __________
Appendix II
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<th>Variable</th>
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<td>Body Weight (kg)</td>
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<td>Fat Mass (kg)</td>
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<td>Percent VO$_{2peak}$ at Tvent Treadmill (%)</td>
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<td>VO$_{2peak}$ Cycle (ml/kg/min)</td>
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<td>Percent VO$_{2peak}$ at Tvent Cycle (%)</td>
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Table 2. Ventilatory Characteristics During Exercise  
(N=7)

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*Statistically significant from Pre, main effect for time (p<0.05) (N=6) for Glucose Sensor (N=7) for Glucometer
Figure 1. Body Weight (kg) pre and post in each exercise trial. †p<0.05 vs. Pre.
Figure 2. Average Power Output (Watts) for the Upper Body Ergometer. *p<0.05 vs. CHO trial.
Figure 3. Heart Rate (bpm) for the Cycle Ergometer. *p<0.05 vs. CHO trial.
Figure 4. Heart Rate (bpm) for the Treadmill. *p<0.05 vs. CHO trial.
Figure 5. Blood Glucose (mM) from the Indwelling Glucose Sensor. *p<0.05 vs. CHO trial.
Figure 6. Blood Glucose (mM) from the Glucometer.
Figure 7. Rate of Perceived Exertion (RPE). *p<0.05 vs. CHO trial.
Figure 8. Oxygen Demand, VO₂ (L·min⁻¹) during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 9. Respiratory Exchange Ratio (RER) from Expired Gases during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 10. Carbohydrate Oxidation (g·min⁻¹) during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 11. Total Carbohydrate Oxidation (g) during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 12. Fat Oxidation (g·min⁻¹) during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 13. Total Fat Oxidation (g) during Treadmill Exercise. *p<0.05 vs. CHO trial.
Figure 14. Total water consumed (L).
Figure 15. Total Urine Void (L).
Figure 16. Urinary Nitrogen (mg·dL$^{-1}$).
Figure 17. Muscle Glycogen (mmol·kg wet weight⁻¹). †p<0.05 vs. Pre. *p<0.05 vs. CHO trial.
Appendix III
## 10 Hour Male Study - Stephanie Harger

### Baseline Testing

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% Cycle VT

| 67.07 |
| 76.79 |
| 73.40 |
| 77.85 |
| 72.89 |
| 67.40 |
| 70.95 |

72.33
4.20
1.59
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<th>VO2 (ml/kg/min) Cycle average</th>
<th>Cycle VO2max (ml/kg/min)</th>
<th>% Cycle MAX</th>
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10-hour muscle glycogen study – Fall 2003
Human Performance Lab

Trial A

Subject #: ____________________

Height: ________________ cm

Weight Pre: _____ kg
Weight Post: _____ kg

BF%: __________

VO$_{2\text{max}}$ Treadmill: _______ L/min

VT Treadmill: _______ L/min

VO$_{2\text{max}}$ Cycle: _______ L/min

VT Cycle: _______ L/min

70% VT Treadmill: _______ L/min

70% VT Cycle: _______ L/min

% Grade @ VT: _______

% Grade @ 70% VT: _______

Watts @ VT: _______

Watts @ 70% VT: _______

Amount of drink provided: ______________ ml

Average Watts on UBE (each cycle):

________________________
________________________
________________________
________________________
________________________

Average SS HR on Cycle: __________

Gas collection:

Hour 1____
Hour 3____
Hour 5____
Hour 6____
Hour 8____
Hour 10____

Average SS HR on Treadmill:

________________________
________________________
________________________
________________________
________________________

Total H$_2$O consumed: _______ L

Total Void: _______ L.

Time from breakfast to exercise: _______ min

Time from exercise to pm biopsy: _______ min
Subject #: ___________                 Height: ___________ cm

Weight Pre: _______ kg             Weight Post: _______ kg           BF%: ___________

$VO_{2\text{max}}$ Treadmill: __________ L/min

$VO_{2\text{max}}$ Cycle: __________ L/min

70% VT Treadmill: __________ L/min

70% VT Cycle: __________ L/min

% Grade@ VT: __________        % Grade @ 70% VT: __________

Watts @ VT: __________          Watts @ 70% VT: __________

Amount of drink provided: ___________ml

Average Watts on UBE (each cycle):

__________________

__________________

__________________

__________________

__________________

__________________

Average SS HR on Cycle: __________       Gas collection:   Hour 1____

                                             Hour 3____

                                             Hour 5____

                                             Hour 6____

                                             Hour 8____

                                             Hour 10____

Average SS HR on Treadmill:

__________________

__________________

__________________

__________________

__________________

Total H$_2$O consumed: _________ L

Total Void: _________ L

Time from breakfast to exercise: _________ min

Time from exercise to pm biopsy: _________ min
Appendix IV
### Type III Sums of Squares

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
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<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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Dependent: Body Weight (kg)

### Means Table

**Effect: Trial * Time**

**Dependent: Body Weight (kg)**

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**Comparison 1**

**Effect: Trial * Time**

**Dependent: Body Weight (kg)**

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<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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**Comparison 2**

**Effect: Trial * Time**

**Dependent: Body Weight (kg)**

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<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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**Comparison 3**

**Effect: Trial * Time**

**Dependent: Body Weight (kg)**

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<td>B, pre</td>
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131
### Type III Sums of Squares

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Dependent: Liters drink consumed

### Means Table

**Effect: Trial**

Dependent: Liters drink consumed

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### Type III Sums of Squares

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Dependent: Total urine void

### Means Table

**Effect: Trial**

Dependent: Total urine void

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### Type III Sums of Squares

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<th>G-G</th>
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Dependent: CHO (g/min)

### Means Table

#### Effect: trial * time

**Dependent: CHO (g/min)**

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#### Comparison 1

**Effect: trial * time**

**Dependent: CHO (g/min)**

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df 1

| Sum of Squares | .030 |
| Mean Square    | .030 |
| F-Value        | 1.624|
| P-Value        | .2123|
| G-G            | .2006|
| H-F            | .2123|

#### Comparison 2

**Effect: trial * time**

**Dependent: CHO (g/min)**

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<td>B, 3</td>
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<td>-1.000</td>
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</tbody>
</table>

df 1

| Sum of Squares | .875 |
| Mean Square    | .875 |
| F-Value        | 47.101|
| P-Value        | .0001|
| G-G            | .0001|
| H-F            | .0001|
### Comparison 3
**Effect:** trial * time  
**Dependent:** CHO (g/min)

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<tr>
<td></td>
<td>B, 5</td>
<td>-1.000</td>
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</tbody>
</table>

- **df:** 1  
- **Sum of Squares:** 1.518  
- **Mean Square:** 1.518  
- **F-Value:** 81.713  
- **P-Value:** .0001  
- **G-G:** .0001  
- **H-F:** .0001

### Comparison 4
**Effect:** trial * time  
**Dependent:** CHO (g/min)

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<tbody>
<tr>
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<td>B, 6</td>
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</tbody>
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- **df:** 1  
- **Sum of Squares:** .498  
- **Mean Square:** .498  
- **F-Value:** 26.798  
- **P-Value:** .0004  
- **G-G:** .0001  
- **H-F:** .0001

### Comparison 5
**Effect:** trial * time  
**Dependent:** CHO (g/min)

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<tbody>
<tr>
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<td>B, 8</td>
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</tbody>
</table>

- **df:** 1  
- **Sum of Squares:** 1.771  
- **Mean Square:** 1.771  
- **F-Value:** 95.356  
- **P-Value:** .0001  
- **G-G:** .0001  
- **H-F:** .0001

### Comparison 6
**Effect:** trial * time  
**Dependent:** CHO (g/min)

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<td>-1.000</td>
</tr>
</tbody>
</table>

- **df:** 1  
- **Sum of Squares:** 3.064  
- **Mean Square:** 3.064  
- **F-Value:** 164.958  
- **P-Value:** .0001  
- **G-G:** .0001  
- **H-F:** .0001
### Type III Sums of Squares

<table>
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<tr>
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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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<tbody>
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<tr>
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Dependent: total g CHO (treadmill)

### Means Table

**Effect: trial**

**Dependent: total g CHO (treadmill)**

<table>
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<tr>
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<th>Mean</th>
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<th>Std. Error</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>221.506</td>
<td>37.132</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>330.474</td>
<td>24.131</td>
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### Type III Sums of Squares

<table>
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<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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<tr>
<td>Subject</td>
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<td>.0001</td>
<td>.0001</td>
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<td>.003</td>
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<td></td>
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<td>.040</td>
<td>21.211</td>
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<td>.0001</td>
<td>.0001</td>
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Dependent: FAT (g/min)

### Means Table

**Effect: trial * time**

Dependent: FAT (g/min)

<table>
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<tr>
<th>Cell Weight</th>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
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</thead>
<tbody>
<tr>
<td>A, 1</td>
<td>7</td>
<td>.359</td>
<td>.121</td>
<td>.046</td>
</tr>
<tr>
<td>A, 3</td>
<td>7</td>
<td>.437</td>
<td>.122</td>
<td>.046</td>
</tr>
<tr>
<td>A, 5</td>
<td>7</td>
<td>.570</td>
<td>.177</td>
<td>.067</td>
</tr>
<tr>
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<td>.460</td>
<td>.150</td>
<td>.057</td>
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<tr>
<td>A, 8</td>
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<td>.057</td>
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<td>.062</td>
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<td>B, 1</td>
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<td>.310</td>
<td>.093</td>
<td>.035</td>
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<td>B, 3</td>
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<td>.069</td>
<td>.026</td>
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<td>.105</td>
<td>.040</td>
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<td>B, 8</td>
<td>7</td>
<td>.309</td>
<td>.129</td>
<td>.049</td>
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<td>B, 10</td>
<td>7</td>
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</tbody>
</table>

**Comparison 1**

**Effect: trial * time**

Dependent: FAT (g/min)

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<tr>
<th>Cell Weight</th>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 1</td>
<td>1</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, 1</td>
<td>-1.000</td>
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<td></td>
<td></td>
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</tbody>
</table>

df 1

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>.008</td>
<td>.008</td>
<td>4.363</td>
<td>.0453</td>
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**Comparison 2**

**Effect: trial * time**

Dependent: FAT (g/min)

<table>
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<tr>
<th>Cell Weight</th>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 3</td>
<td>1</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, 3</td>
<td>-1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df 1

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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</thead>
<tbody>
<tr>
<td>.123</td>
<td>.123</td>
<td>64.775</td>
<td>.0001</td>
<td>.0001</td>
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</tbody>
</table>
### Comparison 3
**Effect:** trial * time  
**Dependent:** FAT (g/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 5        | 1.000  
| B, 5        | -1.000 |

- **df:** 1  
- **Sum of Squares:** 0.280  
- **Mean Square:** 0.280  
- **F-Value:** 147.977  
- **P-Value:** 0.0001  
- **G-G:** 0.0001  
- **H-F:** 0.0001

### Comparison 4
**Effect:** trial * time  
**Dependent:** FAT (g/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
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</thead>
</table>
| A, 6        | 1.000  
| B, 6        | -1.000 |

- **df:** 1  
- **Sum of Squares:** 0.098  
- **Mean Square:** 0.098  
- **F-Value:** 51.670  
- **P-Value:** 0.0001  
- **G-G:** 0.0001  
- **H-F:** 0.0001

### Comparison 5
**Effect:** trial * time  
**Dependent:** FAT (g/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
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</thead>
</table>
| A, 8        | 1.000  
| B, 8        | -1.000 |

- **df:** 1  
- **Sum of Squares:** 0.255  
- **Mean Square:** 0.255  
- **F-Value:** 134.830  
- **P-Value:** 0.0001  
- **G-G:** 0.0001  
- **H-F:** 0.0001

### Comparison 6
**Effect:** trial * time  
**Dependent:** FAT (g/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
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</thead>
</table>
| A, 10       | 1.000  
| B, 10       | -1.000 |

- **df:** 1  
- **Sum of Squares:** 0.436  
- **Mean Square:** 0.436  
- **F-Value:** 230.281  
- **P-Value:** 0.0001  
- **G-G:** 0.0001  
- **H-F:** 0.0001
### Type III Sums of Squares

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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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<tbody>
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Dependent: total g FAT (treadmill)

### Means Table

**Effect: trial**
Dependent: total g FAT (treadmill)

<table>
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<th>Count</th>
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<th>Std. Error</th>
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<td>10.581</td>
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<td>B</td>
<td>7</td>
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<td>20.277</td>
<td>7.664</td>
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</table>
### Type III Sums of Squares

<table>
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<th>Source</th>
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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>.0109</td>
<td>.0009</td>
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<td>.9403</td>
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</table>

Dependent: Blood Glucose (mM)

### Means Table

**Effect: Trial * Time**

Dependent: Blood Glucose (mM)

<table>
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<th>Count</th>
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<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
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<tbody>
<tr>
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<tr>
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<td>.965</td>
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<td>1.067</td>
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<td>1.895</td>
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<tr>
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<td>1.959</td>
</tr>
<tr>
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<td>1.986</td>
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<tr>
<td>B, 8</td>
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<td>B, 10</td>
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</table>
### Comparison 1

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, pre      | 1.000  
| B, pre      | -1.000  

* df 1  
* Sum of Squares 2.990  
* Mean Square 2.990  
* F-Value 2.896  
* P-Value .0935  
* G-G .0917  
* H-F .0989

### Comparison 2

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 1        | 1.000  
| B, 1        | -1.000  

* df 1  
* Sum of Squares 3.827  
* Mean Square 3.827  
* F-Value 3.707  
* P-Value .0585  
* G-G .0786  
* H-F .0830

### Comparison 3

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 2        | 1.000  
| B, 2        | -1.000  

* df 1  
* Sum of Squares 8.596  
* Mean Square 8.596  
* F-Value 8.325  
* P-Value .0053  
* G-G .0408  
* H-F .0386

### Comparison 4

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 3        | 1.000  
| B, 3        | -1.000  

* df 1  
* Sum of Squares 4.093  
* Mean Square 4.093  
* F-Value 3.964  
* P-Value .0506  
* G-G .0751  
* H-F .0788

### Comparison 5

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 4        | 1.000  
| B, 4        | -1.000  

* df 1  
* Sum of Squares 2.176  
* Mean Square 2.176  
* F-Value 2.108  
* P-Value .1513  
* G-G .1091  
* H-F .1203

### Comparison 6

**Effect:** Trial * Time  
**Dependent:** Blood Glucose (mM)

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 5        | 1.000  
| B, 5        | -1.000  

* df 1  
* Sum of Squares .185  
* Mean Square .185  
* F-Value .179  
* P-Value .6733  
* G-G .2474  
* H-F .2913
**Comparison 7**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, lunch    | 1.000  
| B, lunch    | -1.000  

<table>
<thead>
<tr>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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<tbody>
<tr>
<td>1</td>
<td>1.042</td>
<td>1.042</td>
<td>1.009</td>
<td>0.3187</td>
<td>0.1509</td>
<td>0.1721</td>
</tr>
</tbody>
</table>

**Comparison 8**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 6        | 1.000  
| B, 6        | -1.000  

<table>
<thead>
<tr>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.432</td>
<td>0.432</td>
<td>0.419</td>
<td>0.5199</td>
<td>0.2008</td>
<td>0.2342</td>
</tr>
</tbody>
</table>

**Comparison 9**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
<thead>
<tr>
<th>Cell Weight</th>
</tr>
</thead>
</table>
| A, 7        | 1.000  
| B, 7        | -1.000  

<table>
<thead>
<tr>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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<tbody>
<tr>
<td>1</td>
<td>1.538</td>
<td>1.538</td>
<td>1.489</td>
<td>0.2267</td>
<td>0.1287</td>
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</table>

**Comparison 10**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
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| A, 8        | 1.000  
| B, 8        | -1.000  

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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.693</td>
<td>2.693</td>
<td>2.608</td>
<td>0.1111</td>
<td>0.0974</td>
<td>0.1058</td>
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</table>

**Comparison 11**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
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</thead>
</table>
| A, 9        | 1.000  
| B, 9        | -1.000  

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<thead>
<tr>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.083</td>
<td>3.083</td>
<td>2.986</td>
<td>0.0887</td>
<td>0.0900</td>
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</table>

**Comparison 12**  
**Effect: Trial * Time**  
**Dependent: Blood Glucose (mM)**

<table>
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| B, 10       | -1.000  

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<tr>
<td>1</td>
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<td>2.146</td>
<td>0.1477</td>
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<td>0.1191</td>
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142
## Type III Sums of Squares

<table>
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<th>Mean Square</th>
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<th>P-Value</th>
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<th>H-F</th>
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<tr>
<td>Subject</td>
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<td>3.278</td>
<td>.546</td>
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<td>1.339</td>
<td>2.090</td>
<td>.1984</td>
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<td>3.846</td>
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<td>17.398</td>
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<td>.0021</td>
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Dependent: Glucometer (mM)

## Means Table

Effect: Trial * time points  
Dependent: Glucometer (mM)

<table>
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<tr>
<th>Count</th>
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<th>Std. Error</th>
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<tbody>
<tr>
<td>A, pre</td>
<td>7</td>
<td>7.557</td>
<td>1.189</td>
</tr>
<tr>
<td>A, lunch</td>
<td>7</td>
<td>5.800</td>
<td>.416</td>
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<tr>
<td>A, post</td>
<td>7</td>
<td>5.014</td>
<td>.564</td>
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<tr>
<td>B, pre</td>
<td>7</td>
<td>7.314</td>
<td>1.286</td>
</tr>
<tr>
<td>B, lunch</td>
<td>7</td>
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<td>.888</td>
</tr>
<tr>
<td>B, post</td>
<td>7</td>
<td>5.400</td>
<td>.365</td>
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</tbody>
</table>

Comparison 1  
Effect: Trial * time points  
Dependent: Glucometer (mM)

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<th>Count</th>
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<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, pre</td>
<td>1.000</td>
<td>-1.000</td>
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<td></td>
</tr>
<tr>
<td>B, pre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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df 1  
Sum of Squares .206  
Mean Square .206  
F-Value .258  
P-Value .6204  
G-G .5168  
H-F .5453

Comparison 2  
Effect: Trial * time points  
Dependent: Glucometer (mM)

<table>
<thead>
<tr>
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<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, lunch</td>
<td>1.000</td>
<td>-1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, lunch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df 1  
Sum of Squares 3.018  
Mean Square 3.018  
F-Value 3.779  
P-Value .0757  
G-G .0969  
H-F .0917

Comparison 3  
Effect: Trial * time points  
Dependent: Glucometer (mM)

<table>
<thead>
<tr>
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<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, post</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, post</td>
<td>-1.000</td>
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<td></td>
<td></td>
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</tbody>
</table>

df 1  
Sum of Squares .521  
Mean Square .521  
F-Value .652  
P-Value .4351  
G-G .3730  
H-F .3902
Type III Sums of Squares

<table>
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<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>6</td>
<td>32724.350</td>
<td>5454.058</td>
<td>.1169</td>
<td>.1169</td>
<td>.1169</td>
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<tr>
<td>Trial</td>
<td>1</td>
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<td>2671.118</td>
<td>3.350</td>
<td>.1169</td>
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<tr>
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<td>6</td>
<td>4783.744</td>
<td>797.291</td>
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<td>.0006</td>
<td>.0006</td>
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<tr>
<td>Time</td>
<td>1</td>
<td>14463.554</td>
<td>14463.554</td>
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<td>2054.653</td>
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<td>.0282</td>
<td>.0282</td>
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</tr>
<tr>
<td>Trial * Time</td>
<td>1</td>
<td>1544.549</td>
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<td>8.271</td>
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Dependent: glycogen

Means Table
Effect: Trial * Time
Dependent: glycogen

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<tr>
<th>Count</th>
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<th>Std. Dev.</th>
<th>Std. Error</th>
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<tbody>
<tr>
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<td>169.881</td>
<td>50.950</td>
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<tr>
<td>placebo, Post</td>
<td>7</td>
<td>109.571</td>
<td>28.980</td>
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<tr>
<td>CHO, Pre</td>
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<tr>
<td>CHO, Post</td>
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Comparison 1
Effect: Trial * Time
Dependent: glycogen

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<tbody>
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</tr>
<tr>
<td>placebo, Post</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Mean Square</th>
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<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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Comparison 2
Effect: Trial * Time
Dependent: glycogen

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</tr>
<tr>
<td>CHO, Post</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>df 1</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
</tr>
</thead>
<tbody>
<tr>
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Comparison 3
Effect: Trial * Time
Dependent: glycogen

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<tr>
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<tr>
<td>CHO, Pre</td>
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</tbody>
</table>

<table>
<thead>
<tr>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>G-G</th>
<th>H-F</th>
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Comparison 4
Effect: Trial * Time
Dependent: glycogen

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<tbody>
<tr>
<td>placebo, Post</td>
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<tr>
<td>CHO, Post</td>
</tr>
</tbody>
</table>

<table>
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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
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<th>H-F</th>
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### Type III Sums of Squares

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<th>Mean Square</th>
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<th>P-Value</th>
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<th>H-F</th>
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<td>Subject</td>
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Dependent: Cycle HR (b/min)

### Means Table

**Effect: Trial * Times**

**Dependent: Cycle HR (b/min)**

<table>
<thead>
<tr>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
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<tr>
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<td>118.714</td>
<td>6.448</td>
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<td>A, 3</td>
<td>7</td>
<td>116.571</td>
<td>9.658</td>
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<td>A, 4</td>
<td>7</td>
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<td>8.538</td>
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<td>A, 5</td>
<td>7</td>
<td>119.571</td>
<td>12.804</td>
</tr>
<tr>
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<td>11.324</td>
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<td>12.212</td>
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<td>A, 8</td>
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<td>124.571</td>
<td>14.673</td>
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<td>9.013</td>
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<td>B, 8</td>
<td>7</td>
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<td>8.174</td>
</tr>
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<td>B, 9</td>
<td>7</td>
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<td>B, 10</td>
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<td>119.286</td>
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### Comparison 1
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

<table>
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<th>Cell Weight</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>A, 1</td>
<td>1.000</td>
</tr>
<tr>
<td>B, 1</td>
<td>-1.000</td>
</tr>
</tbody>
</table>

- df 1
- Sum of Squares: 3.500
- Mean Square: 3.500
- F-Value: .316
- P-Value: .5764
- G-G: .3273
- H-F: .4460

### Comparison 2
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 2</td>
<td>1.000</td>
</tr>
<tr>
<td>B, 2</td>
<td>-1.000</td>
</tr>
</tbody>
</table>

- df 1
- Sum of Squares: 1.786
- Mean Square: 1.786
- F-Value: .161
- P-Value: .6896
- G-G: .3888
- H-F: .5347

### Comparison 3
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 3</td>
<td>1.000</td>
</tr>
<tr>
<td>B, 3</td>
<td>-1.000</td>
</tr>
</tbody>
</table>

- df 1
- Sum of Squares: 60.071
- Mean Square: 60.071
- F-Value: 5.423
- P-Value: .0237
- G-G: .0586
- H-F: .0442

### Comparison 4
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 4</td>
<td>1.000</td>
</tr>
<tr>
<td>B, 4</td>
<td>-1.000</td>
</tr>
</tbody>
</table>

- df 1
- Sum of Squares: 31.500
- Mean Square: 31.500
- F-Value: 2.843
- P-Value: .0975
- G-G: .1109
- H-F: .1127

### Comparison 5
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

<table>
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<tr>
<th>Cell Weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 5</td>
<td>1.000</td>
</tr>
<tr>
<td>B, 5</td>
<td>-1.000</td>
</tr>
</tbody>
</table>

- df 1
- Sum of Squares: 97.786
- Mean Square: 97.786
- F-Value: 8.827
- P-Value: .0044
- G-G: .0299
- H-F: .0155

### Comparison 6
**Effect:** Trial * Times  
**Dependent:** Cycle HR (b/min)

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- df 1
- Sum of Squares: 540.643
- Mean Square: 540.643
- F-Value: 48.803
- P-Value: .0001
- G-G: .0003
- H-F: .0001
Comparison 7
Effect: Trial * Times
Dependent: Cycle HR (b/min)

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Sum of Squares 68.643
Mean Square 68.643
F-Value 6.196
P-Value .0159
G-G .0496
H-F .0344

Comparison 8
Effect: Trial * Times
Dependent: Cycle HR (b/min)

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Sum of Squares 126.000
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P-Value .0014
G-G .0193
H-F .0077

Comparison 9
Effect: Trial * Times
Dependent: Cycle HR (b/min)

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F-Value 16.771
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Comparison 10
Effect: Trial * Times
Dependent: Cycle HR (b/min)

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Dependent: Treadmill HR (b/min)

### Means Table

**Effect: Trial * Times**

Dependent: Treadmill HR (b/min)

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**Dependent:** Treadmill HR (b/min)  

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### Comparison 8
**Effect:** Trial * Times  
**Dependent:** Treadmill HR (b/min)  

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### Comparison 9
**Effect:** Trial * Times  
**Dependent:** Treadmill HR (b/min)  

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### Comparison 10
**Effect:** Trial * Times  
**Dependent:** Treadmill HR (b/min)  

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Dependent: RER

Means Table
Effect: trial * time
Dependent: RER

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Effect: trial * time
Dependent: RER

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Sum of Squares .001
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P-Value .0756
G-G .0954
H-F .0756

Comparison 2
Effect: trial * time
Dependent: RER

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G-G .0001
H-F .0001

Comparison 3
Effect: trial * time
Dependent: RER

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G-G .0001
H-F .0001

Comparison 4
Effect: trial * time
Dependent: RER

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G-G .0001
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Comparison 5
Effect: trial * time
Dependent: RER

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G-G .0001
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Comparison 6
Effect: trial * time
Dependent: RER

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Dependent: RPE

### Means Table

#### Effect: trial * time

Dependent: RPE

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## Comparison 1
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: 2.571
- Mean Square: 2.571
- F-Value: 9.931
- P-Value: .0037
- G-G: .0083
- H-F: .0037

## Comparison 2
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: 1.786
- Mean Square: 1.786
- F-Value: 6.897
- P-Value: .0135
- G-G: .0226
- H-F: .0135

## Comparison 3
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: .643
- Mean Square: .643
- F-Value: 2.483
- P-Value: .1256
- G-G: .1334
- H-F: .1256

## Comparison 4
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: .643
- Mean Square: .643
- F-Value: 2.483
- P-Value: .1256
- G-G: .1334
- H-F: .1256

## Comparison 5
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: .286
- Mean Square: .286
- F-Value: 1.103
- P-Value: .3019
- G-G: .2802
- H-F: .3019

## Comparison 6
**Effect:** trial * time  
**Dependent:** RPE

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- df 1
- Sum of Squares: 5.161
- Mean Square: 5.161
- F-Value: 19.931
- P-Value: .0001
- G-G: .0006
- H-F: .0001
Type III Sums of Squares

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Dependent: UBE (watts)

Means Table

Effect: Trial * Times
Dependent: UBE (watts)

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**Effect:** Trial * Times  
**Dependent:** UBE (watts)

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### Comparison 8
**Effect:** Trial * Times  
**Dependent:** UBE (watts)

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### Comparison 9
**Effect:** Trial * Times  
**Dependent:** UBE (watts)

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### Comparison 10
**Effect:** Trial * Times  
**Dependent:** UBE (watts)

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Dependent: VO2 (L/min)

### Means Table

**Effect: trial * time**

Dependent: VO2 (L/min)

<table>
<thead>
<tr>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
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<tbody>
<tr>
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Comparison 1
Effect: trial * time
Dependent: VO2 (L/min)

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<tbody>
<tr>
<td>A, 1</td>
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<tr>
<td>B, 1</td>
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</tbody>
</table>

df 1
Sum of Squares 0.004
Mean Square 0.004
F-Value 1.049
P-Value 0.3140
G-G 0.2274
H-F 0.2539

Comparison 2
Effect: trial * time
Dependent: VO2 (L/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
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<tbody>
<tr>
<td>A, 3</td>
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<tr>
<td>B, 3</td>
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</tbody>
</table>

df 1
Sum of Squares 2.571e-4
Mean Square 2.571e-4
F-Value 0.071
P-Value 0.7912
G-G 0.5012
H-F 0.5852

Comparison 3
Effect: trial * time
Dependent: VO2 (L/min)

<table>
<thead>
<tr>
<th>Cell Weight</th>
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<tbody>
<tr>
<td>A, 5</td>
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<tr>
<td>B, 5</td>
</tr>
</tbody>
</table>

df 1
Sum of Squares 0.026
Mean Square 0.026
F-Value 7.136
P-Value 0.0121
G-G 0.0430
H-F 0.0344

Comparison 4
Effect: trial * time
Dependent: VO2 (L/min)

<table>
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<tbody>
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<td>A, 6</td>
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<tr>
<td>B, 6</td>
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</table>

df 1
Sum of Squares 0.010
Mean Square 0.010
F-Value 2.714
P-Value 0.1099
G-G 0.1233
H-F 0.1249

Comparison 5
Effect: trial * time
Dependent: VO2 (L/min)

<table>
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<td>A, 8</td>
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<tr>
<td>B, 8</td>
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</tbody>
</table>

df 1
Sum of Squares 0.002
Mean Square 0.002
F-Value 0.446
P-Value 0.5093
G-G 0.3230
H-F 0.3734

Comparison 6
Effect: trial * time
Dependent: VO2 (L/min)

<table>
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<tbody>
<tr>
<td>A, 10</td>
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<tr>
<td>B, 10</td>
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</table>

df 1
Sum of Squares 0.002
Mean Square 0.002
F-Value 0.507
P-Value 0.4818
G-G 0.3089
H-F 0.3559
### Type III Sums of Squares

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<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
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<th>H-F</th>
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Dependent: Urinary Nitrogen

### Means Table

**Effect: Trial**  
**Dependent: Urinary Nitrogen**

<table>
<thead>
<tr>
<th>Count</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td>3.571</td>
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<td>B</td>
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