THE INFLUENCE OF HIGH FRUCTOSE CORN SYRUP ON ENDURANCE EXERCISE METABOLISM

by

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ABSTRACT

Athletes are advised to ingest adequate amounts of carbohydrate (CHO) prior to competitions, during exercise, and after to replenish glycogen storages. Performance outcomes can be determined by the amount, timing, and sources of CHO ingested. High fructose corn syrup (HFCS) is thought to be one of the main contributors in the development of obesity and chronic disease, therefore making this CHO source controversial. The study purpose was to determine if a HFCS-sweetened beverage is an acceptable CHO source for prolonged moderate intensity exercise by determining its effects on substrate metabolism during exercise when compared to a dextrose-sweetened beverage (DEXT). Eleven (6 male, 8 female: Mean±SD; 24 ± 3.9 yrs, 71.8 ± 12.5 kg, 173.8 ± 9.5 cm) active healthy adults volunteered to participate in for this study. A baseline blood sample was drawn prior to ingesting half of a 400 mL beverage sweetened with 50 grams of HFCS or DEXT 15 min prior to a 120 min cycle and the other half was consumed 10 min of exercise. This was a randomized, double-blinded study. Blood was collected at -20, 58, and 140 min, tested for glucose, lactate, hemoglobin and hematocrit, then coagulated for 20 min, centrifuged for 10 min for serum separation and analyzed for lipids. Respiratory exchange ratio, HR, and RPE were collected at 20, 50, and 115 min during exercise. Treatment interaction effects were determined using repeated measures ANOVA and a one-way ANOVA, with a Tukey (HSD) post hoc test (alpha level < 0.05). All statistics were mean ± SD. There were no significant time by treatment interactions or main treatment effects for any dependent variable RER ($p=0.996$), triglycerides ($p=0.451$), CHOL ($p=0.230$), LDL ($p=0.875$), HDL ($p=0.753$), VLDL (p=0.439), glucose (p=0.802), HR (p=0.749), RPE (p=0.719), or lactate ($p=0.065$). However, there was a significant time effect identified for decreasing RER, increasing HR and RPE that were not related to the treatments, but the physical needs of exercise. In conclusion, the current study demonstrates an acute 50 g dose of HFCS at the beginning of prolonged moderate intensity exercise does not induce hypertriglyceridemia and is an adequate CHO source for supplementation.

CHAPTER 1

INTRODUCTION

Athletes are usually advised to consume adequate amounts of carbohydrates (CHO) prior to participating in competitions, during those events if they last longer than one hour, and afterwards to replenish glycogen storages in preparation for future workouts (Jeukendrup & Gleeson, 2010). Determining what sport specific products (SSP) to consume, in what amounts, and at what times can influence performance outcomes. Sport specific products containing CHO are of interest to endurance athletes, coaches, sports related clinical staff (i.e. doctors, dietitians, etc.), and SSP manufacturers. According to the USDA Nutrient Data Laboratory (2005a, b), SSP contain a variety of monosaccharides (i.e. glucose, galactose, and fructose) and disaccharides (i.e. sucrose).

Over the last few decades both fructose consumption and obesity have gradually increased, especially with the introduction of high fructose corn syrup (HFCS) as an inexpensive substitution for table sugar (sucrose) (Havel, 2005; National Center for Health Statistics, 2013). The most common form of high fructose corn syrup contains a mixture of 45% glucose and 55% fructose monosaccharides (Hanover & White, 1993). Sucrose is a bonded disaccharide of 50% glucose and 50% fructose.

Fructose metabolism tends to promote lipogenesis when consumed in excess of energy requirements (Bray, Nielsen, & Popkin, 2004; Havel, 2005; Lustig, 2010; Melanson, Zukely, Lowndes, Von Nguyen, Angelopoulos, & Rippe, 2007; Stanhope, Griffen, Bair, Swarbrick, Keim, & Havel, 2008; Teff, Elliott, Tschop, Keiffer, Rader, Heiman, Townsend, Keim, D'Alessio, & Havel, 2004). An increase of lipogenesis

without equivalent energy expenditure can lead to dyslipidemia, metabolic syndrome, type 2 diabetes, and cardiovascular disease (Bray et al., 2004; Havel, 2005; Lustig, 2010). Athletes and active individuals are not considered high risk for developing chronic diseases, because they tend to expend more energy than they store (Jeukendrup & Gleeson, 2010). Therefore, the consumption of fructose is unlikely to induce lipogenesis and more likely to be converted into metabolic energy substrates (i.e. glucose and lactate) or directly utilized for energy during exercise (Leoultre, Benoit, Carrel, Schutz, Millet, Tappy, & Schneiter, 2010). The metabolic substrates produced during prolonged endurance exercise after consumption of HFCS may significantly increase blood lipid concentrations, specifically triglycerides. The implications of blood lipid increases may lead to elevated risk for chronic disease development, but at this time it is not known if these elevations take place and it is the intent of this study to assess the risk to active individuals.

Purpose Statement

The purpose of this study is to investigate whether the ingestion of high fructose corn syrup alters substrate metabolism (i.e. lipids, glucose, and lactate) during prolonged moderate intensity exercise when compared to dextrose.

Rationale

High fructose corn syrup (HFCS) is an inexpensive mixture of glucose and fructose monosaccharides (45% glucose to 55% fructose) that is regularly added to sport specific products (SSPs) (Hanover & White, 1993). Manufacturers claim their products

containing HFCS enhance athletic performance, but not all SSPs contain HFCS as the carbohydrate (CHO) source. Yet, manufacturers, such as Gatorade and Powerade, still claim their products enhance athletic performance. According to the Nutrient Data Laboratory (2005a, b), Powerade contains HFCS and Gatorade a mixture of glucose, sucrose, and fructose, yet both manufacturers support their products even though they are quite dissimilar from one another. Powerade has a 1:1.3 ratio of glucose to fructose and Gatorade has a 1.3:1 ratio. Also, because HFCS is the subject of scrutiny with its potential link to the rise in obesity, cardiovascular disease, and metabolic disorders, some manufacturers may avoid utilizing this carbohydrate source (Bray, Nielsen, & Popkin, 2004; Hanover & White, 1993; Havel, 2005; Saad, Khan, Sharma, Michael, Riad-Gabriel, Boyadjian, Jinagouda, Steil, Kamdar, 1998; Soenen & Westerterp-Plantenga, 2007; Stanhope, Griffen, Bair, Swarbrick, Keim, & Havel, 2008; Teff, Elliott, Tschöp, Keiffer, Rader, Heiman, Townsend, Keim, D'Alessio, & Havel, 2004). By avoiding HFCS athletes may lose the performance enhancing benefits that HFCS could have to offer. Therefore, the intent of this study is to determine if a HFCS-sweetened beverage is an acceptable CHO source for prolonged moderate intensity exercise by determining its effects on substrate (i.e. lipids, triglycerides, glucose, and lactate) metabolism during exercise when compared to a dextrose-sweetened beverage in healthy adults.

Delimitations

The study will be delimited to low risk moderately active adults with a recent history of cycling experience, ≥18 years of age, and without signs or symptoms of

cardiovascular and/or metabolic disease as determined via health history questionnaire (Thompson, Gordon, & Pescatello, 2009).

Limitations

This study was dependent on the participants' ability to follow instructions while recording oral intake the day prior to their first cycling test and then replicating that same diet each day before all other required test days. Participants were asked to refrain from exercise and consuming alcohol for 24 hours and caffeine for 12 hours prior to cycling. Also, depending on the relative energy requirements of each participant, after ingestion of a 50 gram carbohydrate beverage, there may have been differences in substrate utilization from person to person.

Assumptions

It will be assumed that the participants will follow all instructions prior to testing and the day of testing. All instrumentation utilized for measuring metabolic substrates will be assumed to be reliable and valid. It will also be assumed that the individual mixing the carbohydrate beverages will prepare similar tasting and concentrated solutions each day of testing.

Hypotheses

NULL HYPOTHESIS: There will be no difference between HFCS and dextrose treatments for blood glucose, lactate, lipids, RER, HR, and RPE during prolonged moderate intensity exercise.

H_O: μDEXT (lipids, glucose, lactate, RER, HR, RPE) = μHFCS (lipids, glucose, lactate, RER, HR, RPE)

ALTERNATIVE HYPOTHESIS: There will be an increase in blood lipid and lactate concentrations with a decrease in blood glucose, RER, HR, and RPE after the consumption of HFCS when compared to dextrose during prolonged moderate intensity exercise.

H_A: μHFCS (glucose, RER, HR, RPE) > μDEXT (glucose, RER, HR, RPE)

H_A: μHFCS (lipids, lactate) > μDEXT (lipids, lactate)

CHAPTER 2

REVIEW OF RELATED LITERATURE

Introduction

According to Hanover and White (1993), HFCS was created in the 1960s in a stable form and quickly became an acceptable sweetener used by food and beverage manufacturers. When the authors compared HFCS to sucrose (table sugar), HFCS was similar in sweetness, less expensive, easier to handle, more condensed (utilizes less storage space), and more resistant to spoilage, indicating that HFCS was the more economical selection of the two CHOs for decreasing manufacturing costs. Sports specific products, especially beverages, according to Hanover and White (1993), included HFCS because of its sweetness, ability to dissolve in liquids, and flavor enhancing properties. Sucrose is a disaccharide composed of glucose and fructose bonded together in a 50:50 mixture (Hanover and White, 1993). The most common form of high fructose corn syrup is composed of glucose and fructose monosaccharides were 45% is made up of dextrose and 55% comes from fructose (Hanover and White, 1993). Fructose is sweeter than glucose by 50 points on the relative sweetness scale and since HFCS contains 5% more fructose SSPs required less syrup to provide the same sweetness as sucrose in foods and beverages (Hanover and White, 1993). The usage of HFCS as a sweetener has been scrutinized with the increasing trends of obesity, cardiovascular disease, and metabolic disorders (Havel, 2005). This scrutiny may be influencing the use of HFCS in SSPs and has potentially influenced the replacement of this sweetener with

alternative CHO sources. The intent of this study is to determine if a HFCS-sweetened beverage is an acceptable CHO source for prolonged moderate intensity exercise by determining its effects on substrate (i.e. lipids, triglycerides, glucose, and lactate) metabolism during exercise when compared to a dextrose-sweetened beverage in healthy adults. It is hypothesized that there will be a difference in substrate metabolism during moderate to high intensity endurance exercise between HFCS and dextrose.

Substrate Utilization

To understand how athletic performance is enhanced, the sources of fuel utilized during exercise must be reviewed and the metabolic limitations determined. Glucose, condensed into glycogen, is stored in muscle and organs (mainly liver and heart) (Jeukendrup & Gleeson, 2010). Muscle glycogen stores are the primary fuel oxidized to produce energy during moderate to high intensity exercise (Jeukendrup & Gleeson, 2010). Fat is also oxidized as a fuel source in the form of fatty acids transported to muscle from adipose tissue and intra muscular fatty acids (Jeukendrup & Gleeson, 2010). Fatty acid oxidation in combination with glucose slows the depletion of glycogen storages at low to moderate exercise intensities (Jeukendrup & Gleeson, 2010). Exercise intensity is defined as a percentage of an individuals' maximal oxygen consumption (VO_{2max}), otherwise known as the largest volume of oxygen an individual is able to consume per minute at high intensity exercise just prior to exhaustion (Brooks, Fahey, & Baldwin, 2005). According to Achten and Jeukendrup (2003), the maximum amount of fat oxidized occurs around 62-63% of VO_{2max} and declines as exercise intensity increases. Blood flow is routed predominantly to the muscles and organs needed to support high

intensity exercise and delivery of fat via the blood stream to muscle decreases above 78% of VO_{2max} (Shi & Passe, 2010). Therefore, as exercise intensity increases glycogen utilization also increases to maintain the energy demand and because of this, finding alternative fuel sources to spare glycogen or alter the central nervous systems (CNS) rate of perceived exertion (RPE) during exercise are of interest to all endurance athletes, coaches, sports related clinical staff (i.e. doctors, dietitians, etc.), and SSP manufactures (Currell & Jeukendrup, 2008; Jentjens, Moseley, Waring, Harding, & Jeukendrup, 2004b; Jentjens et al., 2006).

Once glycogen stores are depleted the body will not be able to perform at high intensities and will be forced to slow down (Jeukendrup & Gleeson, 2010). Exercise performance is dependent on the body's ability to oxidize fuel efficiently, spare glycogen, and absorb exogenous (ingested nutrients) CHO through the small intestine while participating in prolonged moderate to high intensity exercise (Currell & Jeukendrup, 2008; Jentjens et al., 2004b; Jentjens et al., 2006). The ingestion of CHOs during prolonged exercise has several biological limitations slowing its absorption through the small intestine. Athletes that overcome these limitations are potentially able to exercise for longer, at greater speeds, and lower intensities than their fellow competitors giving them an advantage.

Exogenous Carbohydrate Absorption

Researchers have concluded that continuous CHO supplementation enhances performance and postpones fatigue during prolonged (>45 minutes) moderate to high intensity (58-78% VO_{2max}) exercise (Jentjens, Achten, & Jeukendrup, 2004a; Jentjens &

Jeukendrup, 2005a; Jentjens et al., 2004b; Jentjens et al., 2005b; Jentjens, Venables, & Jeukendrup, 2004c; Jentjens et al., 2006; Jeukendrup, 2008; Jeukendrup & Jentjens, 2000; Shi & Passe, 2010; &Wallis, Rowlands, Shaw, Jentjens, & Jeukendrup, 2005). The importance of CHO supplementation during exercise was discovered after researchers investigated the biological limitations to absorption of CHOs during moderate intensity exercise: fluid osmolality and stomach emptying, CHO absorption and metabolism, CHO monosaccharide, disaccharide, and polysaccharide ratios, and CHO ingestion rates.

The rate at which food empties from the stomach into the small intestine is the first limitation conflicting exogenous CHO absorption (Shi & Passe, 2010). The rate of stomach emptying depends on the concentration (osmolality) of the ingested food bolus or liquid entering the stomach and how long it takes to properly digest (Shi & Passe, 2010). For this review, the absorption and metabolism of beverages will be described. According to Jeukendrup and Gleeson (2010), beverages with an osmolality between 186-417 mOsm/kg of water (i.e. Powerade, Gatorade, 50:50 mixture of fruit juice and water) will not significantly affect the rate of stomach emptying and are more readily absorbed by the small intestine, but beverages containing greater than 500 mOsm/kg of water (i.e. soda and fruit juices) will slow stomach emptying and decrease the rate of exogenous CHO available to spare glycogen.

The optimal sports beverage would have an osmolality near 290 mOsm/kg (concentration of blood) for proper rehydration during exercise, but according to Jeukendrup and Gleeson (2010), and Shi and Passe (2010), in order for beverages to enhance endurance performance the solution should contain larger concentrations of CHOs (~60 g or less vs. 100-150 g/L CHO). Because of these conflicting

recommendations the authors discussed the importance of athletes training with beverages containing different osmolalities to determine what works best for their body and does not cause unwanted distress to their gastrointestinal (GI) tract.

The reasons it is beneficial to consume beverages with similar osmolalities as blood is to first minimize the need for digestion in the stomach and reduce the potential for stomach aches, nausea, belching, and vomiting during exercise (Shi & Passe, 2010). At moderate to high intensity exercise the GI tract does not receive the usual amount of blood flow and an adequate flow is required to digest and absorb food properly, thus the stated signs and symptoms (Jeukendrup & Gleeson, 2010). The second reason is to prevent water influx from the blood into the lumen of the small intestine during exercise (Shi & Passe, 2010). Water influx is a process the body uses to dilute the contents of the small intestine and increase the osmotic pull of water and nutrients into the body (Shi & Passe, 2010). If individuals consume beverages with >500 mOsm/kg they may first saturate the ability of the small intestine to absorb nutrients, therefore leaving a high concentration of molecules in the gut, and secondly having a large influx of water increases the risk of bloating and diarrhea (Shi & Passe, 2010). Maintaining hydration during exercise may be difficult, so by ingesting beverages that decrease the influx of water into the GI tract therefore allowing for continuous rehydration during prolonged exercise, the athlete will reduce the risk for event ending complications (Jentjens & Jeukendrup, 2005a; Jentjens et al., 2004b; Jentjens et al., 2005b; Jentjens et al., 2004c; Jeukendrup & Gleeson, 2010; Jeukendrup, 2008; Shi & Passe, 2010; Wallis et al., 2005).

In order for the ingested beverage to reach portal circulation, leading to the liver, glucose and galactose are transported across the intestinal epithelial cell border by active

transport via sodium-glucose co-transporter (SGLT1) and fructose by the facilitated sodium-independent sugar transporter (GLUT5) (Shi & Passe, 2010; Wood & Trayhurn, 2003). Once glucose, galactose, or fructose enters the epithelial cells it is then transported into portal circulation via GLUT2, travels to the liver, and the liver releases the appropriate amount of CHO into circulation destined to fuel muscle cells (Jeukendrup & Jentjens, 2000).

Carbohydrate Dose Response

Glucose is the main fuel source utilized for energy production during moderate to high intensity exercise. Jentjens et al. (2004b), hypothesized continuously feeding athletes a glucose-sweetened beverage during exercise would increase performance and they were correct. According to Jentjens et al. (2004b), eight trained athletes completed four cycling trials all lasting 120 minutes at 63% of VO_{2max}. During each trial they ingested either 1.2 grams per minute (g/min) of glucose, 1.8 g/min of glucose, or a 2:1 ratio of 1.2 g/min glucose plus 0.6 g/min fructose. The amount of exogenous and endogenous (glycogen) oxidation was measured by labeling the carbons in exogenous glucose and fructose prior to ingestion with a carbon thirteen isotope (C^{13}). Each subject performed an exhausting bout of exercise several days prior to testing in order to deplete their glycogen stores of existing C^{13} and then asked not to consume foods known to contain C^{13}. This allowed the researchers to collect the subjects expired air during testing and calculate the specific carbon oxidation rates of each isotope.

Jentjens et al. (2004b) discovered four new findings regarding the absorption of glucose. First, the ingestion of 1.2 g/min of glucose saturated SGLT1, essentially maxing

out the work capacity of this transporter, and produced an exogenous oxidation rate of 0.8 ± 0.04 g/min. Prior to determining the SGLT1 saturation level, the researchers attempted to feed subjects 1.8 g/min of glucose only to find an exogenous oxidation rate of 0.83 ± 0.05 g/min (not significantly different from 0.8 g/min). Several other researchers discovered similar results after feeding 1.2, 1.5, or 1.8 g/min of glucose to study subjects, the oxidation rates ranged from 0.77-1.06 g/min of exogenous glucose oxidation (Jentjens & Jeukendrup, 2005a; Jentjens et al. 2005b; Jentjens et al., 2004c; & Jentjens et al., 2006). The maximal exogenous oxidation rate has been determined to be 1.0 g/min of glucose when ingested at a rate of 1.2 g/min (Jentjens & Jeukendrup, 2005a; Jentjens et al. 2004b; Jentjens et al. 2005b; Jentjens et al., 2004c; Jentjens et al., 2006). Therefore, associating glucose consumption over 1.2 g/min with the potential of GI tract discomfort as glucose concentrates within the gut causing more harm to performance than good. The second finding was an increase in exogenous oxidation to 1.26 ±0.07 g/min after the co-ingestion of glucose and fructose with an endogenous oxidation rate of 1.33 ± 0.07 g/min, trending towards more glycogen sparing effects than the other CHO trials, but with no significant differences. The third finding indicated that the co-ingestion of glucose and fructose trended towards sparing a portion of the glycogen storages with the oxidation of labeled C^{13} from fructose. The fourth finding was reported in a survey taken by each subject while they cycled. The subjects reported that an ingestion of glucose and fructose in a 2:1 ratio and 1.2 g/min glucose alone decreased the development of GI symptoms when compared to the ingestion of 1.8 g/min of glucose. The authors hypothesized that the subjects developed less symptoms because the ingestion of fructose with glucose somehow enhanced the osmotic pull of CHO from the small intestine into portal

circulation. Indicating that even with an increase in osmolality, because the GLUT5 receptor was not saturated and the SGLT1 was saturated, the epithelial cells lining the small intestine could absorb this concentration without complications.

Jentjens et al. (2004c) investigated the 5:1 ratio of glucose to fructose in a solution of glucose plus sucrose ingested at 1.8 g/min (1.2 g/min glucose + 0.6 g/min sucrose). This 5:1 ratio produced an exogenous oxidation rate of 1.25 ± 0.07 g/min and an endogenous CHO oxidation rate that was significantly lower when compared to water, indicating potential for performance enhancing glycogen sparing effects. The authors hypothesized that ingesting sucrose, which has glucose and fructose bonded together, enhanced the absorption of the two monosaccharides because the enzyme (sucrase) that cleaves the bond between the molecules originates at the cell wall and this close cellular proximity allowed for faster absorption.

In a similar study by Wallis et al. (2005), the ratio of glucose to fructose was 2:1 and the solution ingested contained 1.2 g/min of maltodextrin (a chain of glucose) plus 0.6 g/min of fructose for a total of 1.8 g/min. This combination produced an exogenous oxidation rate of 1.5 ± 0.07 g/min and an endogenous oxygenation rate similar to the results for Jentjens et al. (2004c). Again, the authors' stated this increased oxidation rate was most likely due to the location of enzymatic digestion relative to the intestinal wall.

Jentjens et al. (2004a) investigated the 1.7:1 ratio of glucose to fructose in a solution containing 1.2 g/min glucose, 0.6 g/min fructose, and 0.6 g/min sucrose for a total of 2.4 g/min. This combination produced an exogenous oxidation rate of 1.70 ± 0.07 g/min and a decreased endogenous oxidation rate of 0.76 ± 0.12 g/min compared to glucose only trial. Then, Jentjens and Jeukendrup (2005a) investigated the 1:1 ratio of

glucose to fructose in a solution containing 1.2 g/min of glucose plus 1.2 g/min of fructose for a total of 2.4 g/min. The tolerance of the GI tract to the ingestion of a 1:1 ratio of glucose to fructose indicated that saturation of GLUT5 was not achieved, but this ratio did produce the largest exogenous oxidation rate to date of 1.75 ± 0.11 g/min with an endogenous oxidation rate of 1.07 ± 0.15 g/min (no significant difference between the glucose only trials). See Table 2.1 for description of the CHO dose responses described above.

Table 2.1 Carbohydrate Dose Response (Jentjens, 2005a, Jentjens, 2004a, 2004b, 2005b, Jeukendrup, 2008, Wallis, 2005).

Carbohydrate Source	Ratio of Glucose to Fructose	Total Ingested Carbohydrate (g/min)	Exogenous Oxidation Rate (g/min)
Glucose (1.2 g/min)	1 : 0	1.2	0.8 ± 0.04
Glucose (1.8 g/min)	1 : 0	1.8	0.83 ± 0.05
Glucose (1.2 g/min) + Fructose (0.6 g/min)	2 : 1	1.8	1.26 ± 0.07
Glucose (1.2 g/min) + Sucrose (0.6 g/min)	5 : 1	1.8	1.25 ± 0.07
Maltodextrin (1.2 g/min) + Fructose (0.6 g/min)	2 : 1	1.8	1.5 ± 0.07
Glucose (1.2 g/min) + Sucrose (0.6 g/min) + Fructose (0.6 g/min)	1.7 : 1	2.4	1.7 ± 0.07
Glucose (1.2 g/min) + Fructose (1.2 g/min)	1 : 1	2.4	1.75 ± 0.11

As discussed above, the oxidation rate of CHO during prolonged endurance exercise was amplified as the concentration of ingested fructose increased, but are there metabolic consequences to the human body after ingesting high concentrations of fructose in HFCS? Is disease risk increased after moderate or chronic consumption of fructose? Does exercise reduce disease risk after ingestion of fructose?

Chronic Fructose Induced Lipid Formation

Fructose travels from the epithelial cells of the small intestine through portal circulation to the liver, where it is taken up by GLUT5 and metabolized according to energy requirements at that time (Jeukendrup & Jentjens, 2000; Shi & Passe, 2010; Wood & Trayhurn, 2003). Once fructose is inside the liver fructokinase converts fructose to fructose-1-phosphate (F-1-P) by removing a phosphate group from adenosine triphosphate (ATP) in an energy utilizing reaction (Gropper et al., 2009). Unfortunately, fructokinase does not have a negative feedback loop that inhibits the metabolism of fructose in the liver (Havel, 2005). At least within glycolysis there is a negative feedback loop that inhibits phosphofructokinase from converting Fructose 6-P to Fructose-1,6-bisPhosphate when the cytoplasm concentrations of citrate and ATP increase to levels that signal a reduction in glycolysis (Havel, 2005) (Figure 2.1). Fructose-1-P is transferred to the Embden-Meyerhoff glycolytic cascade and mostly formed into pyruvate. The pyruvate is then formed into acetyl-CoA and entered into the tricarboxylic acid (TCA) cycle for energy production. If there is an excess of pyruvate/acetyl-CoA than required at that time, it is converted to citrate in the mitochondria and shuttled into the cytoplasm. Havel (2010); and Sun and Empie (2012), discussed an alternative pathway for pyruvate which converted the excess to lactate and released into the blood stream to be utilized by muscle for energy production. Once the muscle had acquired enough energy producing substrates the excess lactate is removed from the blood stream by the liver. The hepatic Cori Cycle converts lactate to glucose for utilization in the synthesis of

glycogen, adenosine triphosphate (ATP), glycerol, or lipids to name a few metabolic end products (Gropper et al., 2009; Havel, 2010; Sun & Empie, 2012).

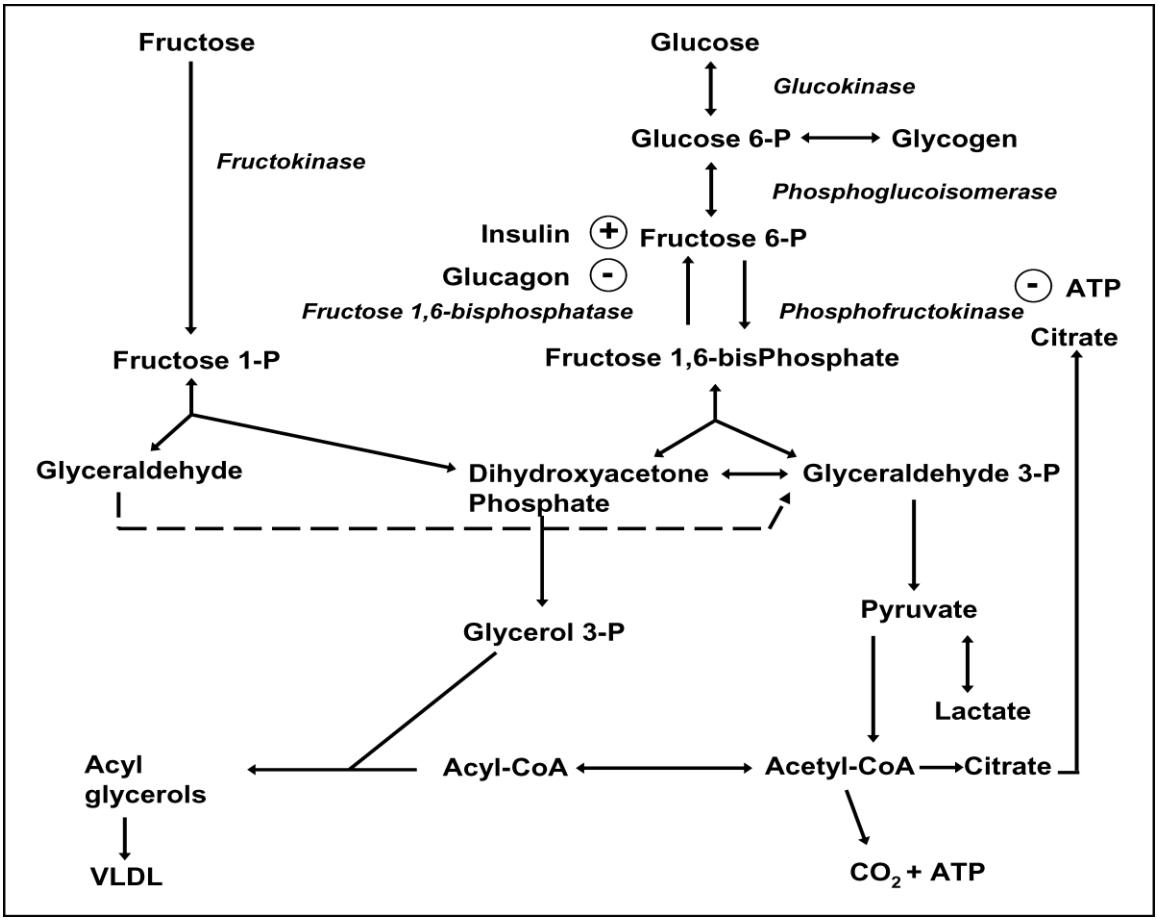

Figure 2.1 Hepatic carbohydrate metabolism (Havel, 2005). Fructose metabolism does not have a negative feedback loop as glucose does in glycolysis, therefore fructose metabolized for energy utilization in negative energy balance and for energy storage in positive energy balance.

If the Embden-Myerhoff glycolytic cascade is bombarded by an excess of F-1-P, it will be converted into fructose-1,6-bisphosphate, and combined with glyceraldehyde to form xylulose-5-phosphate (Lustig, 2010) (Figure 2.2). The xylulose-5-phosphate (xylulose-5-P) stimulates protein phosphatase 2A (PP2A), which signals carbohydrate response element binding protein (ChREBP) to activate *de novo* lipogenesis (DNL)

enzymes (adenosine triphosphate citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS)) (Iizuka, Bruick, Liang, Horton, & Uyeda, 2004; Lustig, 2010) (Figure 2.2). The DNL enzymes convert excess citrate in the cytoplasm into fatty acyl-CoA and free fatty acids (FFA) (citrate → acetyl-CoA → malonyl-CoA → acyl-CoA) (Gropper et al., 2009). High concentrations of fructose alone will also stimulate DNL enzymatic activity via peroxisomal proliferator-activated receptor-γ coactivator-1β (PGC–1β), which induces transcription of SREBP-1c and more DNL activity (Iizuka et al., 2004; Lustig, 2010; Stanhope et al., 2008) (Figure 2.2). Interestingly, Iizuka et al. (2004) stated that only 50% of the DNL enzymatic activity is stimulated by SREBP-1c. The authors found that ChREBP deficient mice died after being fed a diet of fructose for seven days because of their inability to stimulate the production of enough ALC, ACC, and FAS via ChREBP to metabolize all the consumed fructose. Other potential sources of lipogenesis come from the production of lactate and glucose synthesized from fructose. These energy substrates can be taken up by multiple cell types for conversion to pyruvate, then acetyl-CoA, and in excess stimulate DNL for lipogenesis after conversion to citrate in the mitochondria (Gropper et al., 2009; Havel, 2010; & Sun & Empie, 2012).

According to Faeh, Minehira, Schwarz, Periasami, Seongsu, and Tappy (2005), healthy men fed a diet high in fructose for six days significantly increased their fasting glucose and lactate concentrations when compared to the control group consuming a low fructose diet. This may indicate the liver utilized fructose to re-synthesize glycogen storages and then converted the excess into lactate and glucose. The liver released the extra glucose and lactate into the blood for extrahepatic (any energy requiring cells

outside the liver) energy production and/or storage. Once the extrahepatic tissues are satisfied with stored energy the circulating lactate and glucose would be taken up by the liver and converted to lipids in DNL for storage.

The hexosemonophosphate shunt (pentose phosphate shunt) utilizes glucose to form pentose phosphates for the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and nucleotides in general (Gropper et al., 2009). This shunt also produces NADPH for use in the biosynthesis of FA (Gropper et al., 2009; Sun & Empie, 2012). A portion of exogenous fructose will more than likely be converted to glucose and routed into the hexosmonophosphate shunt to maintain FA synthesis (Gropper et al., 2009; Sun & Empie, 2012).

Faeh et al. (2005), found a 79% increase in plasma TG concentrations after overfeeding healthy men a diet high in fructose. According to Gropper et al. (2009), the liver synthesizes triacylglycerols (triglycerides, TG) from excess glycerol formed from glucose or fructose which is phosphorylated by ATP, combined with excess fatty acyl-CoA, to produce phosphatidic acid which is dephosphorylated to DAG and then combined with another fatty acyl-CoA to produce TG. In order to remove excess fatty acyl-CoA, FFA, and TG from the liver hepatic peroxisome proliferation-activated receptor-α activity is reduced, signaling an increase in apolipoprotein B100 (apoB100) production, provoking the formation of very-low-density lipoprotein (VLDL) (Lustig, 2010). If the amount of ingested fructose continues to exceed total body energy requirements its contribution to lipogenesis and storage will remain constant. As a consequence of excessive energy intake hepatocyte lipid droplets (hepatic steatosis) will

form from fatty acyl-CoA escaping VLDL packaging prior to its release into the blood (Lustig, 2010).

Free fatty acids, lactate, and glucose released from the liver are taken up by skeletal muscle as energy producing substrates (Gropper et al., 2009). Lipoprotein lipase (LPL) on the cell membrane transport FFA and TG, removed from VLDL, into skeletal muscle (Gropper et al., 2009). Cellular lipid concentrations are thought by Cororan, Lamon-Fava, and Fielding (2007) to be increased by reduced mitochondrial lipid oxidation. The authors conclude that lipid accumulation is a symptom of inactivity.

Chronic fructose consumption without adequate energy expenditure can lead to an accumulation of stored fat and increased risk for disease progression (Lustig, 2010; Sun & Empie, 2012) (Figure 2.2). Physical activity provides one of the most important cellular benefits in disease reversal and prevention. Activity requires the utilization of stored energy (i.e. glycogen and FA), which induces mitochondrial oxidation of stored energy substrates. After exercise the liver and skeletal muscle re-synthesize cellular glycogen storages by increasing the cell membrane permeability to glucose, therefore producing a more insulin sensitive cell and lowering the risk for diabetes (Sun & Empie, 2012).

Fructose Metabolism during Endurance Exercise

As discussed, in order to enhance exercise performance during endurance activities (>45 hour) a combination of glucose and fructose ingestion is beneficial. In healthy active individuals, the ingestion of fructose for utilization during exercise will, according to Lecoultre et al. (2010), be converted into lactate (28%), glucose (29%), and

a portion will be retained for utilization after exercise (~40% non-oxidative). The authors found that as fructose reached the liver via portal circulation from the small intestine the liver converted 30% of it to lactate right away and released it into the blood for tissues

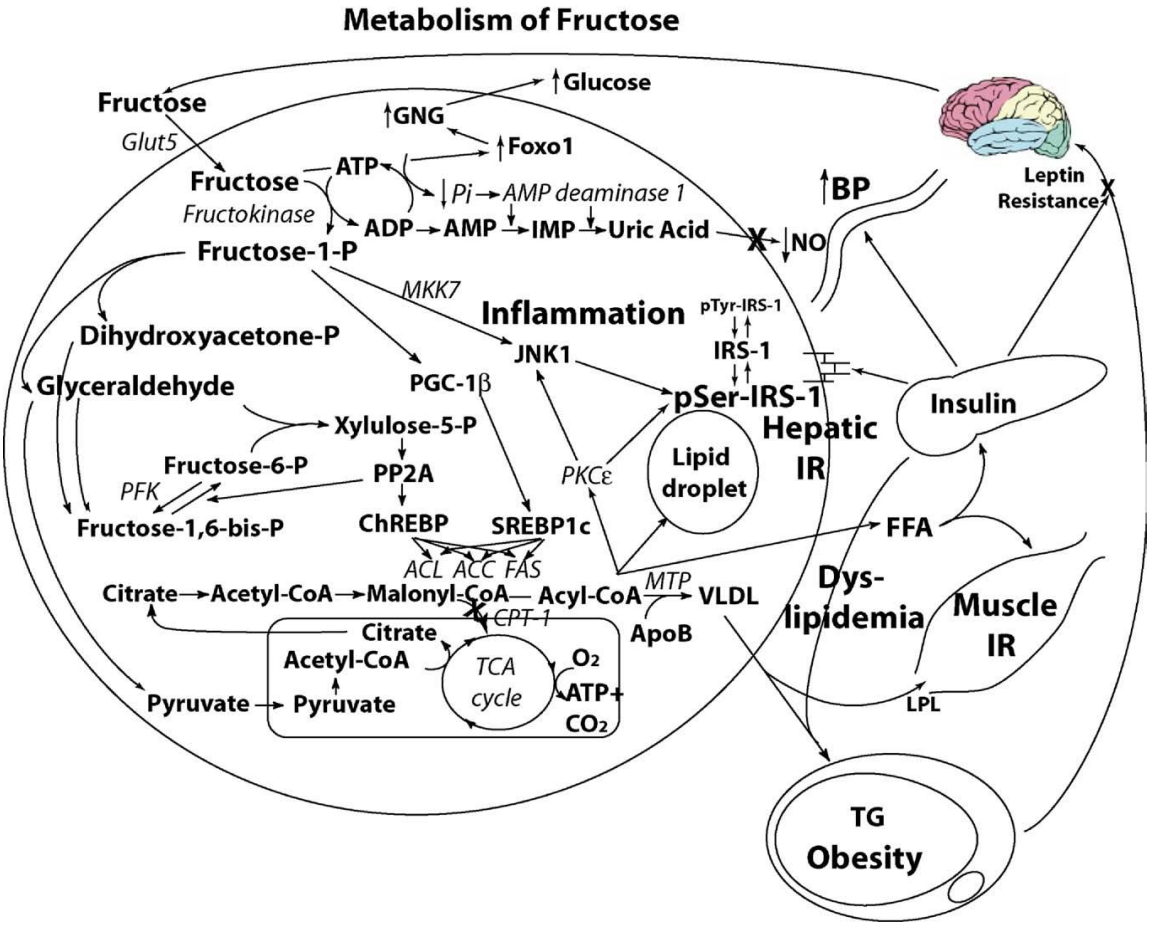

Figure 2.2 Potential fructose chronic disease development pathways (Lustig, 2010)

requiring substrate for energy production. As exercise continued the liver converted the excess lactate into glucose via gluconeogenesis. Forty percent of the 96 grams (g) of ingested fructose was said to be retained and not oxidized during exercise, potentially for glycogen re-synthesis after exercise. It is possible that the excess non-oxidative fructose

entered DNL to produce fat for storage, but it is more likely that it was utilized for glycogen re-synthesis.

Lecoultre et al. (2010), found that 96 g of fructose and 144 g of glucose produced total (exogenous plus endogenous) CHO oxidation rates that were larger than those of glucose (3.3 ± 0.3 g/min vs. 3.1 ± 0.4 g/min) and fat oxidation rates for the fructose/glucose that were lower than glucose (0.27 ± 0.18 g/min fructose vs. 0.35 ±0.16 g/min glucose). The respiratory exchange ratio (RER) for fructose and glucose ingestion was larger than for glucose ingestion alone, 0.95 ± 0.03 vs. 0.93 ± 0.03. Therefore, indicating that more CHO (i.e. glucose and lactate) was oxidized than FA when fructose was co-ingested with glucose. The authors' findings indicate that FA and TG synthesis from fructose during exercise is highly unlikely because energy substrate (i.e. glucose and lactate) production is required to continue performing physical activity, especially prolonged endurance exercise, and DNL requires energy utilization to produce fat from CHO. If TG are formed it will probably take place after exercise, but not until glycogen storages have been replenished.

Conclusions

The findings of this review indicate that chronic consumption of fructose has the potential to increase gluconeogenesis and *de novo* lipogenesis, both of which in excess are shown to enhance the development of certain diseases. Even though the consumption of fructose appears to be harmful when consumed in excess for sedentary individuals, it is believed to be beneficial for endurance athletes and active individuals who want to fuel their body while participating in prolonged bouts of exercise.

CHAPTER 3

THE INFLUENCE OF HIGH FRUCTOSE CORN SYRUP INGESTION ON ENDURANCE EXERCISE METABOLISM

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Aided in development of study methodology, implemented data collection, processing, and analysis, and authored manuscript.

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Contributions: Dr. Seifert aided in development of study methodology, assisted with data collection, processing, and analysis, and edited the manuscript.

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Contributions: Dr. Harmon reviewed and recommended alterations to study design; reviewed study results and statistical analysis; and reviewed the study proposal and final manuscript.

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Abstract

Athletes are advised to ingest adequate amounts of carbohydrate (CHO) prior to competitions, during exercise, and after to replenish glycogen storages. Performance outcomes can be determined by the amount, timing, and sources of CHO ingested. High fructose corn syrup (HFCS) is thought to be one of the main contributors in the development of obesity and chronic disease, therefore making this CHO source controversial. The study purpose was to determine if a HFCS-sweetened beverage is an acceptable CHO source for prolonged moderate intensity exercise by determining its effects on substrate metabolism during exercise when compared to a dextrose-sweetened beverage (DEXT). Eleven (6 male, 8 female: Mean±SD; 24 ± 3.9 yrs, 71.8 ± 12.5 kg, 173.8 ± 9.5 cm) active healthy adults volunteered to participate in for this study. A baseline blood sample was drawn prior to ingesting half of a 400 mL beverage sweetened with 50 grams of HFCS or DEXT 15 min prior to a 120 min cycle and the other half was consumed 10 min of exercise. This was a randomized, double-blinded study. Blood was collected at -20, 58, and 140 min, tested for glucose, lactate, hemoglobin and hematocrit, then coagulated for 20 min, centrifuged for 10 min for serum separation and analyzed for lipids. Respiratory exchange ratio, HR, and RPE were collected at 20, 50, and 115 min during exercise. Treatment interaction effects were determined using repeated measures ANOVA and a one-way ANOVA, with a Tukey (HSD) post hoc test (alpha level < 0.05). All statistics were mean ± SD. There were no significant time by treatment interactions or main treatment effects for any dependent variable RER ($p=0.996$), triglycerides ($p=0.451$), CHOL ($p=0.230$), LDL ($p=0.875$), HDL ($p=0.753$), VLDL (p=0.439), glucose (p=0.802), HR (p=0.749), RPE (p=0.719), or lactate ($p=0.065$). However, there was a significant time effect identified for decreasing RER, increasing HR and RPE that were not related to the treatments, but the physical needs of exercise. In conclusion, the current study demonstrates an acute 50 g dose of HFCS at the beginning of prolonged moderate intensity exercise does not induce hypertriglyceridemia and is an adequate CHO source for supplementation.

Introduction

Athletes are usually advised to consume adequate amounts of carbohydrates (CHO) prior to participating in competitions, during those events if they last longer than one hour, and afterwards to replenish glycogen storages in preparation for future workouts (Jeukendrup & Gleeson, 2010). Determining what sport specific products (SSP) to consume, in what amounts, and at what times can influence performance outcomes. Sport specific products containing CHO are of interest to endurance athletes, coaches, sports related clinical staff (i.e. doctors, dietitians, etc.), and SSP manufacturers. According to the USDA Nutrient Data Laboratory (2005a, b), SSP contain a variety of monosaccharides (i.e. glucose, galactose, and fructose) and disaccharides (i.e. sucrose).

Over the last few decades both fructose consumption and obesity have gradually increased, especially with the introduction of high fructose corn syrup (HFCS) as an inexpensive substitution for table sugar (sucrose) (Havel, 2005; National Center for Health Statistics, 2013). The common form of high fructose corn syrup contains a mixture of 45% glucose and 55% fructose monosaccharides (Hanover & White, 1993). Sucrose is a bonded disaccharide of 50% glucose and 50% fructose.

Fructose metabolism tends to promote lipogenesis when consumed in excess of energy requirements (Bray, Nielsen, & Popkin, 2004; Havel, 2005; Lustig, 2010; Melanson, Zukely, Lowndes, Von Nguyen, Angelopoulos, & Rippe, 2007; Stanhope, Griffen, Bair, Swarbrick, Keim, & Havel, 2008; Teff, Elliott, Tschop, Keiffer, Rader, Heiman, Townsend, Keim, D'Alessio, & Havel, 2004). An increase of lipogenesis without equivalent energy expenditure can lead to dyslipidemia, metabolic syndrome,

type 2 diabetes, and cardiovascular disease (Bray et al., 2004; Havel, 2005; Lustig, 2010). Athletes and active individuals are not considered high risk for developing chronic diseases, because they tend to expend more energy than they store (Jeukendrup & Gleeson, 2010). Therefore, the consumption of fructose is unlikely to induce lipogenesis and more likely to be converted into metabolic energy substrates (i.e. glucose and lactate) or directly utilized for energy during exercise (Leoultre, Benoit, Carrel, Schutz, Millet, Tappy, & Schneiter, 2010). The metabolic substrates produced during prolonged endurance exercise after consumption of HFCS may significantly increase blood lipid concentrations, specifically triglycerides. The implications of blood lipid increases may lead to elevated risk for chronic disease development, but at this time it is not known if these elevations take place. The intent of this study is to determine if a HFCS-sweetened beverage is an acceptable CHO source for prolonged moderate intensity exercise by determining its effects on substrate (i.e. lipids, triglycerides, glucose, and lactate) metabolism during exercise when compared to a dextrose-sweetened beverage in healthy adults. It is hypothesized that there will be a difference in substrate metabolism during moderate to high intensity endurance exercise between HFCS and dextrose.

Methodology

Informed Consent

The study protocol was be submitted to the Institutional Review Board (IRB) for approval prior to testing. All participants received verbal and written information regarding the purpose, protocol, risks, and benefits of this study prior to signing the

informed consent. In order to participate in this study all participants signed and abided by the terms stated within the informed consent.

Subjects

Eleven subjects were selected on a volunteer basis after completion of a health history form indicating their physical activity level, cycling experience, age (≥18), and low risk for cardiovascular and/or metabolic disease (Thompson, Gordon, & Pescatello, 2009).

Experimental Design

Study subjects served as their own controls by utilizing a repeated measures crossover design. Each subject completed an initial maximal oxygen consumption (VO_{2max}) test to determine fitness level and exercising workloads, and then two, 120-min cycle ergometer trials. Subjects were required to initially record their dietary habits for one day and then consume that same diet, while refraining from exercise and alcohol, the day before each trial. A 12 hour fast with caffeine avoidance was required prior to each trial. Experimental treatments consisted of a 12.5% HFCS solution and a 12.5% glucose solution. The order of CHO beverage consumption was randomized and blinded to the study subject and investigators. The dependent variables measured during the trials were the respiratory exchange ratio (RER), serum concentrations of triglycerides (TG), total cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoprotein (VLDL), blood glucose, heart rate (HR), rating of perceived exertion (RPE), and blood lactate.

Procedures

On the first visit subjects arrived at the Movement Science Laboratory (MSL) in the morning and anthropometrics (height and body mass) were measured. A cycle ergometer cardiorespiratory VO_{2max} test was conducted to determine experimental (60% and 50% VO_{2max}) workload and cadence. Each subject warmed-up for five minutes at their preferred cadence with a workload of 0.5 kg. As the test proceeded the subjects preferred cadence, selected during warm-up, was maintained while the workload increased by 0.5 kg every three minutes until volitional exhaustion.

At least one week after VO_{2max} testing participants arrived at MSL in the morning for CHO experimental testing. Each subject was asked to consume the same diet 24-hrs prior to each test, excluding caffeine, and arrived fasted for 12-hrs. Body mass was measured and the subject was fitted with a HR monitor, to the seat height on the cycle ergometer, and with a respiratory mask. A baseline venous blood sample was taken prior to the 120 min cycle ergometer trial (at -20 min). Subjects then consumed a randomly selected experimental CHO beverage in equal amounts at -15 and 10 min of exercise. In an attempt to maximize lipid substrate utilization subjects initially cycled at 50% VO_{2max} for 25 min increasing to 60% VO_{2max} for the majority of the 120 minute ride, with one break in intensity at 93 min of exercise cycling at 50% VO_{2max}. A second blood sample was collected at 58 min of exercise, allowing ten min for sampling, fluid consumption, and rest. The final blood sample was collected at the conclusion of each trial. Subjects were required to sit for 20 minutes prior to the baseline and concluding blood samples to allow for fluid compartment equilibration (Costill, Branam, Eddy, & Fink, 1985). Rate of perceived exertion (RPE), HR, and expired air (RER, VO_2, CO_2, and ventilation) were

collected at 20, 50, 115 min of exercise to identify metabolic substrate utilization (respiratory exchange ratio; RER). Additionally, subjects ingested 150 mL water at 25, 45, 62, 93, and 110 min of exercise for a total of 1,150 mL of fluid including the experimental beverage. See table 3.1 for the procedure cycling timeline.

Table 3.1 Procedure cycling timeline

Time	**Speed**	**Resistance**	**HR**	**RPE**	**RER**	**Task**
min	**rpm**	**kg**	**bpm**			
-50:00						Bathroom & Pre-weight
-40:00						20 min sit
-20:00						Baseline blood draw
-15:00						Feed 200 mL CHO beverage
00:00						Start Test at 50% VO_{2max}
00:01-9:59						
10:00-14:59						Feed 200 mL CHO beverage
15:00-19:59						
20:00-24:59						RPE, HR, and RER
25:00-29:59						Increase to 60% VO_{2max}; 150 mL water
30:00-44:59						
45:00-49:59						150 mL water
50:00-55:00						RPE, HR, and RER
55:01-57:59						
58:00-1:08:00						2^{nd} blood draw; 150 mL water
1:08:01-1:32:59						Start at 60% VO_{2max}
1:33:00-1:37:59						Decrease to 50% VO_{2max}; 150 mL water
1:38:00-1:49:59						Increase to 60% VO_{2max}
1:50:00-1:54:59						150 mL water
1:55:00-2:00:00						RPE, HR, and RER
2:00:01-2:20:01						End test; 20-min sit
2:20:02						Final blood draw

Approximately 5 mL of blood was required for each collection. Samples were collected by a venous puncture of an antecubital vein using a 21 g x 1.5" needle (Becton Dickinson, Franklin Lakes, NJ) and a SST Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ). Blood glucose and lactate were analyzed prior to separating the blood. An aliquot of blood was collected for the analysis of hemoglobin and a second aliquot used for the measurement for hematocrit prior to separation of serum from red blood cells. The rest of the blood sample processing consisted of coagulation for 20 minute blood, 10 minute centrifugation, and then freezing at -80°Celcius for future analysis. Each participant returned at least one week after their initial CHO cycling trial.

Exogenous Carbohydrate Beverage

The CHO beverages were prepared with either 50 g of dextrose (DEXT) or 50 g of HFCS and water, in a 400 mL solution (12.5% CHO), utilizing an ordinary flavoring (Kool-Aid) to mask the flavor. Solutions were prepared the day prior to the trial and were served chilled. For convenience during training and competition, it is typical for individuals to purchase beverages that are bottled containing a specific CHO concentration (6-8% sports drinks and 11% soft drinks) and fluid volume. Consuming a 10% CHO solution with a volume of 300 mL was accomplished by Bloemendal (2012), in a resting state, with no gastrointestinal complications while a 12.5% CHO beverage was consumed without complications in this study.

Biochemical Analyses

Triglycerides (TG), total cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoprotein (VLDL) were analyzed

utilizing a Piccolo® Xpress™ Chemistry Analyzer (Abaxis, Inc., Union City, CA). Blood glucose was measured with a Contour Blood Glucose System (Bayer Healthcare, Mishawaka, IN). Blood lactate concentrations were measured using a LactatePro portable blood lactate analyzer (Arkray Factory, Inc., Shiga, Japan). Alterations in hematocrit concentrations were analyzed by subtracting the length of the separated red blood cells from the total length of the whole blood sample after centrifugation. Hemogolobin was analyzed utilizing a HemoCue® blood hemoglobin analyzer (HemoCue Inc., Cypress, CA).

Statistical Analysis

A 2 x 3 time by treatment analysis of variance (ANOVA) with repeated measures (α level of 0.05) was utilized in data processing for RER, glucose, lactate, HR, and RPE. A 2 x 2 time by treatment ANOVA with repeated measures (α level of 0.05) was utilized in data processing for TG, CHOL, LDL, HDL, and VLDL. A one-way ANOVA with a Tukey-Kramer post hoc test was used for determination of significance of time main effects. A paired two sample for means t-test was utilized for determination of significance in the percentage of plasma volume change (%ΔPV). All statistics are reported as mean ± standard deviation (SD).

Table 3.2 Hypotheses, dependent measured variables, and predictions.

Hypotheses	Dependent Measured Variables	Predictions
There will be no difference between HFCS and dextrose treatments for blood glucose, lactate, lipids, RER, HR, and RPE during prolonged moderate intensity exercise.	RER, lipids (TG, CHOL, LDL, HDL, VLDL), glucose, HR, RPE, and lactate.	μHFCS (RER, lipids, glucose, HR, RPE, lactate) = μDEXT (RER, lipids, glucose, HR, RPE, lactate)
There will be an increase in blood lipid and lactate concentrations with a decrease in blood glucose, RER, HR, and RPE after the consumption of HFCS when compared to dextrose during prolonged moderate intensity exercise.	Glucose, RER, HR, and RPE.	μHFCS (glucose, RER, HR, RPE) < μDEXT(glucose, RER, HR, RPE)
	Lipids (TG, CHOL, LDL, HDL, VLDL) and lactate.	μHFCS (lipids, lactate) > μDEXT (lipids, lactate)

Results

Subject Characteristics

Subject study characteristics are reported in Table 3.3.

Table 3.3 Descriptive data for study subject (Mean ± SD)

	n	Age (yrs)	Body Mass (kg)	Body Height (cm)
Total	11	24.0 ± 3.9	71.8 ± 12.5	173.8 ± 9.5
Men	6	24.0 ± 3.7	77.7 ± 11.7	180.5 ± 5.9
Women	5	24.0 ± 4.5	64.7 ± 10.3	165.8 ± 5.9

Percent Plasma Volume

A total of 1150 mL of fluid was ingested by each subject while cycling in an attempt to maintain fluid balance. Percent change in plasma volume (%ΔPV) was maintained throughout this study (DEXT=0.09±1.8%, HFCS= -0.6±4.46%).

Measured Maximal Oxygen Consumption

Table 3.4 contains the percent of VO_{2max} that the subject cycled at for the two experimental rides.

Table 3.4 Maximal HR, relative and absolute VO_{2max}, and $\%VO_{2max}$ for the cycling trials (Mean±SD)

	n	HR_{max} (bpm)	VO_{2max} (ml/kg/min)	VO_{2max} (L/min)	Upper $\%VO_{2max}$	Lower $\%VO_{2max}$
Total	11	188 ± 11.9	47.2 ± 7.3	3.4 ± 0.6	62 ± 5.5	52 ± 5.2
Men	6	195 ± 7.2	50.0 ± 8.6	3.8 ± 0.4	63 ± 4.3	52 ± 4.3
Women	5	179 ± 10.7	43.8 ± 3.8	2.8 ± 0.3	61 ± 7.2	51 ± 6.8

Respiratory Exchange Ratio

There was no significant interaction after comparing the time by treatment effect of DEXT and HFCS ingestion for RER *(p=0.996)* (See Figure 3.1). There was no treatment main treatment effect on RER *(p=0.651)*. However, there was a significant time effect identified for RER *(p<0.001)*. The RER at 20 minutes was significantly greater than 115 minutes (*20 min=0.92±0.02, 115 min=0.86±0.03, p<0.001*) and RER was significantly greater at 50 minutes than 115 minutes *(50 min=0.90±0.02, p<0.05)*. See Appendix B for Mean±SD of dependent variables.

Serum Lipids

There were no significant time by treatment interactions for blood serum TG concentrations (p=0.451) (Figure 3.2), CHOL *(p=0.230)* (Figure 3.3), LDL *(p=0.875)* (See Figure 3.4), HDL *(p=0.753)* (Figure 3.5), or VLDL *(p=0.439)* (Figure 3.6). The carbohydrate treatments had no significant main effect on the concentrations of TG *(p=0.215)*, CHOL *(p=0.705)*, LDL *(p=0.123)*, HDL *(p=0.111)*, and VLDL *(p=0.222)*.

Additionally, there were no main time effects found within the concentrations of TG *(p = 0.721)*, LDL *(p = 0.271)*, or VLDL *(p = 0.698)*. However, there were significant time effects identified in the concentrations of CHOL *(p = 0.008)* and HDL *(p = 0.002)*. The significance was determined to be an increase from the pre cycling baseline blood draw to the final blood draw 20 minutes post exercise from 181.5 mg/dL±27.7 to 186.2 mg/dL±27.7 for CHOL and from 55.4 mg/dL±12.5 to 57.9 mg/dL±13.4 for HDL.

Blood Glucose

There was no significant time by treatment interaction for blood glucose concentrations *(p=0.802)* (Figure 3.7). Also, there were no significant main effects of treatment *(p=0.757)* or time *(-20 min=4.7 mmol/L±0.44, 60 min=4.5 mmol/L±0.59, 140 min=4.0 mmol/L±0.58)* on the concentrations of blood glucose.

Heart Rate

There was no significant time by treatment interaction for heart rate (HR) *(p=0.749)* (Figure 3.8). There was no significant treatment main effect on HR *(p=0.192)*. However, there was a significant time effect where HR increased from 20 minutes of cycling to 115 minutes (*129.7 bpm±16.98 to 156.7 bpm±14.92; p<0.05*).

Rate of Perceived Exertion

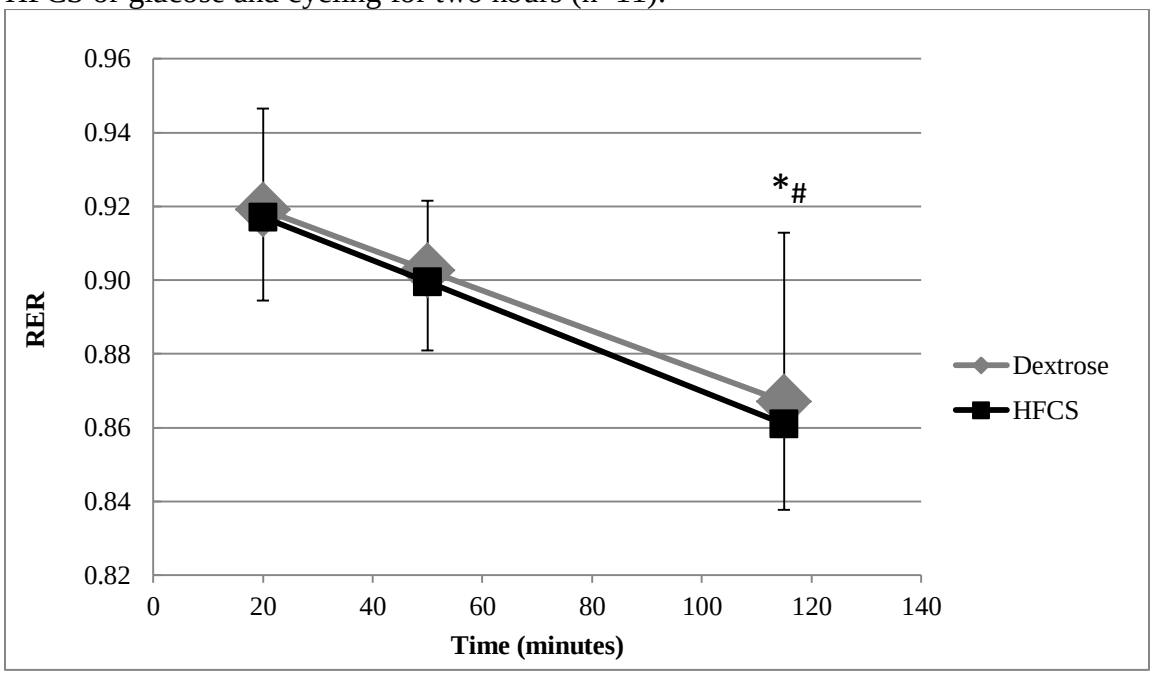
There was no significant time by treatment interaction within participants for their ratings of perceived exertion (RPE) *(p=0.719)* (Figure 3.9). Also, the carbohydrate treatments had no significant main effect on RPE *(p=0.607)*. However, there was a

significant time effect identified in RPE *(p<0.001)*. The RPE was significantly lower at 20 minutes of cycling than at 115 minutes *(10.9±1.6 to 14.0±1.4, p<0.001)*.

Blood Lactate

There was no significant time by treatment interactions for blood lactate concentrations *(p=0.065)* (Figure 3.10). Also, there was neither a significant treatment had main effect on the concentrations of lactate *(p=0.217)* nor a significant time effect identified for lactate *(p=0.061)*.

Figure 3.1 Mean (±SD) respiratory exchange ratio prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).



*=Significantly different from 20 minutes.
#=Significantly different from 50 minutes.

Figure 3.2 Mean (±SD) serum triglyceride concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

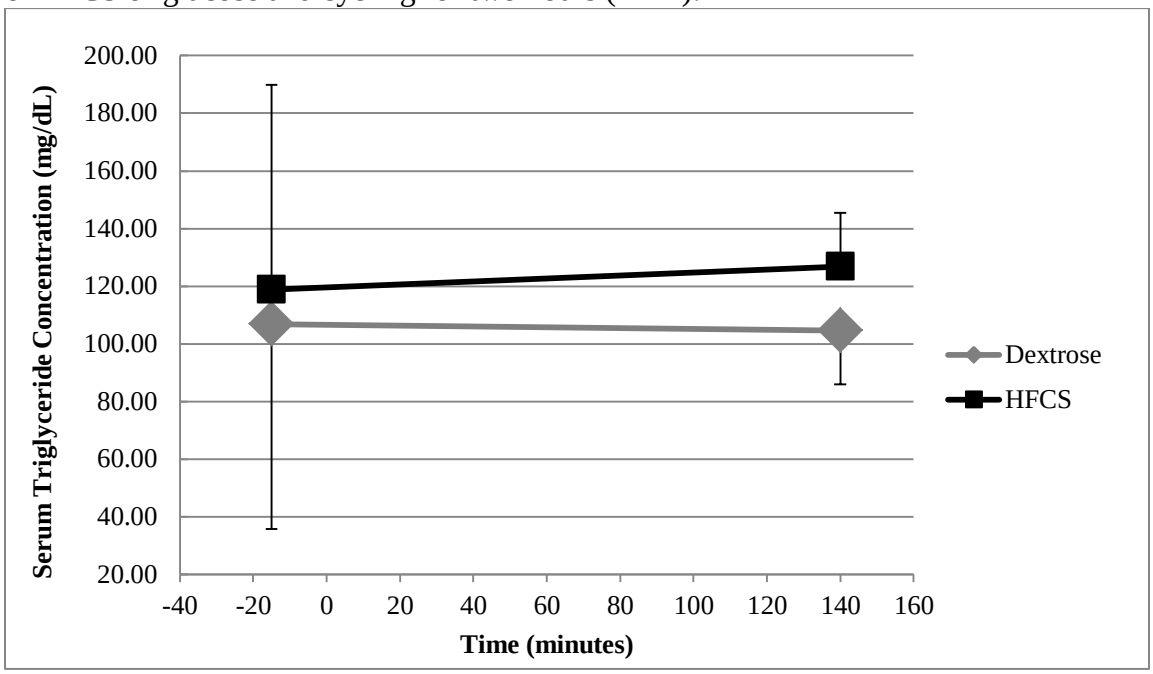


Figure 3.3 Mean (±SD) serum total cholesterol concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

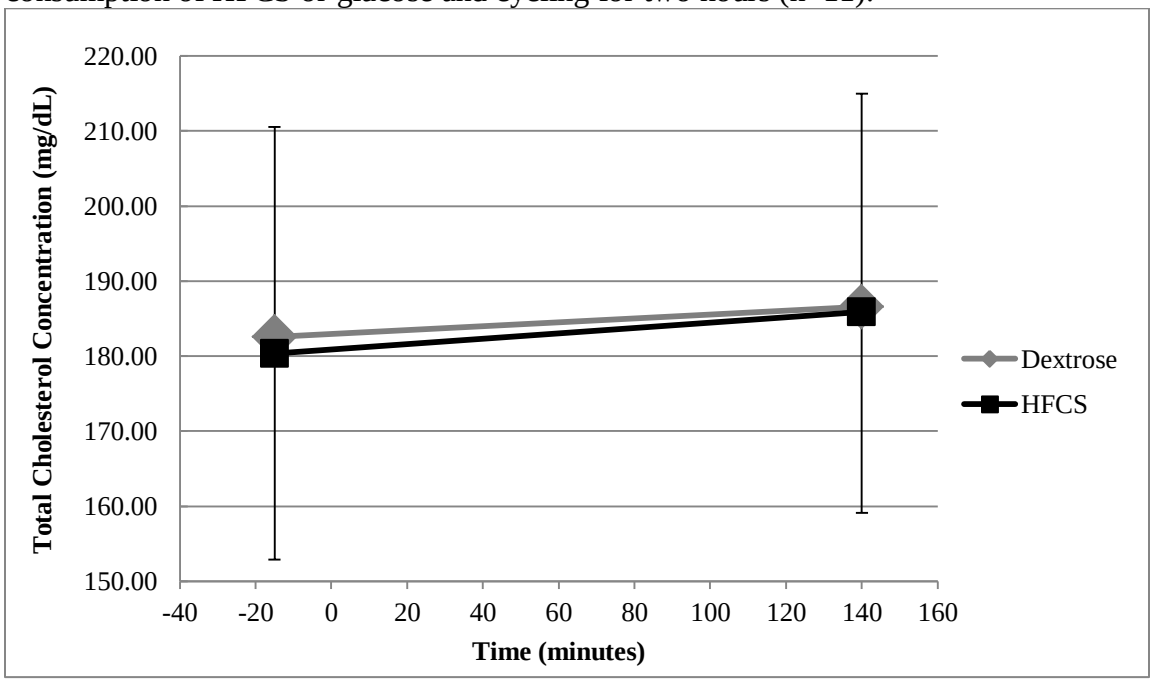


Figure 3.4 Mean (±SD) serum low density lipoprotein concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

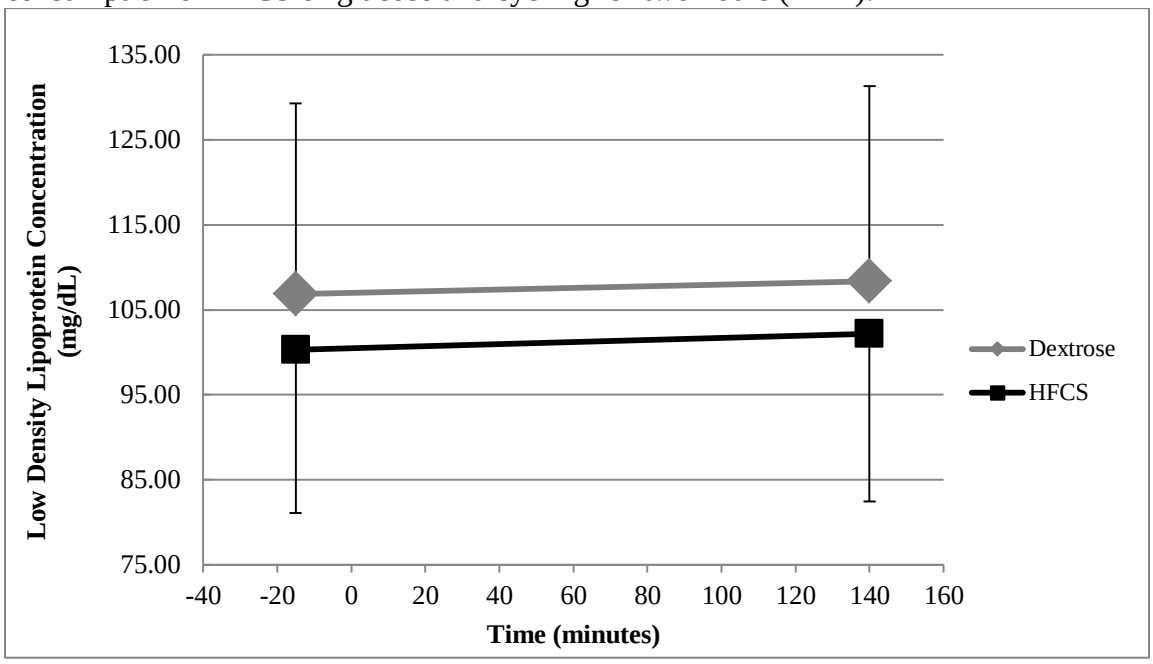


Figure 3.5 Mean (±SD) serum high density lipoprotein concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

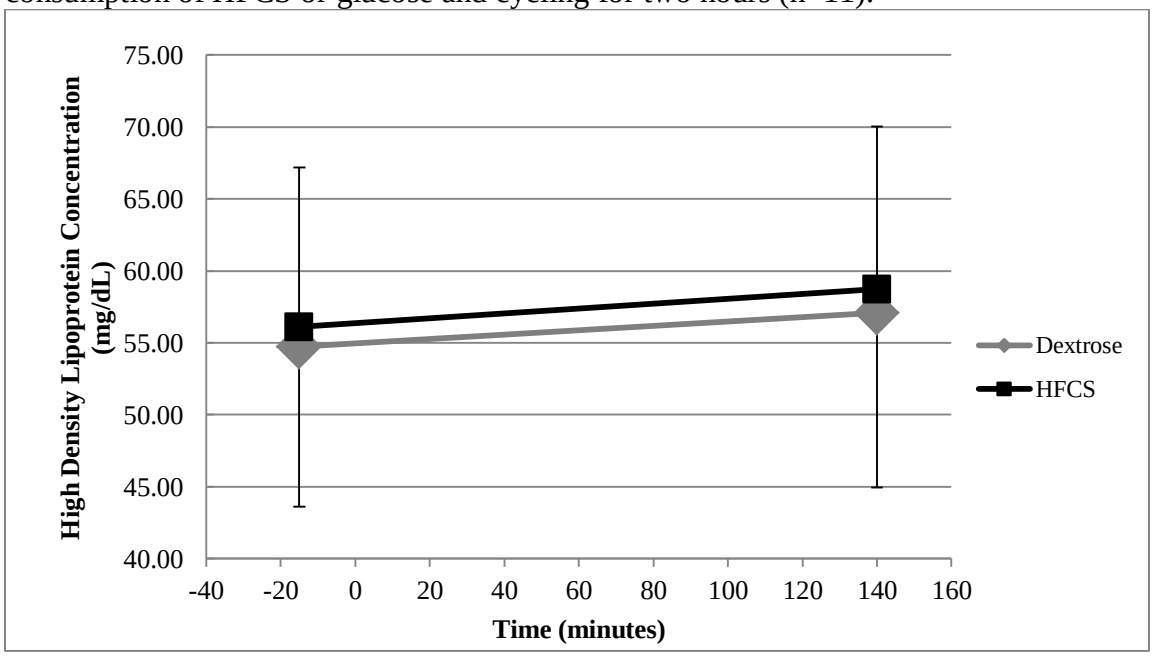


Figure 3.6 Mean (±SD) serum very low density lipoprotein concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

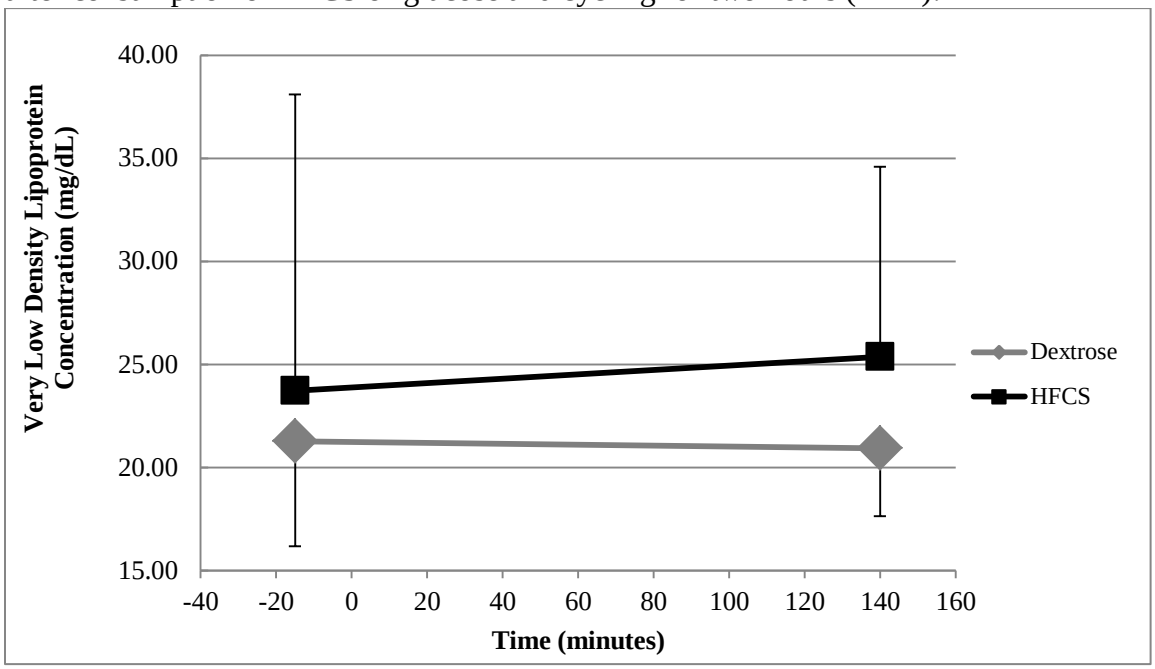


Figure 3.7 Mean (±SD) serum glucose concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).

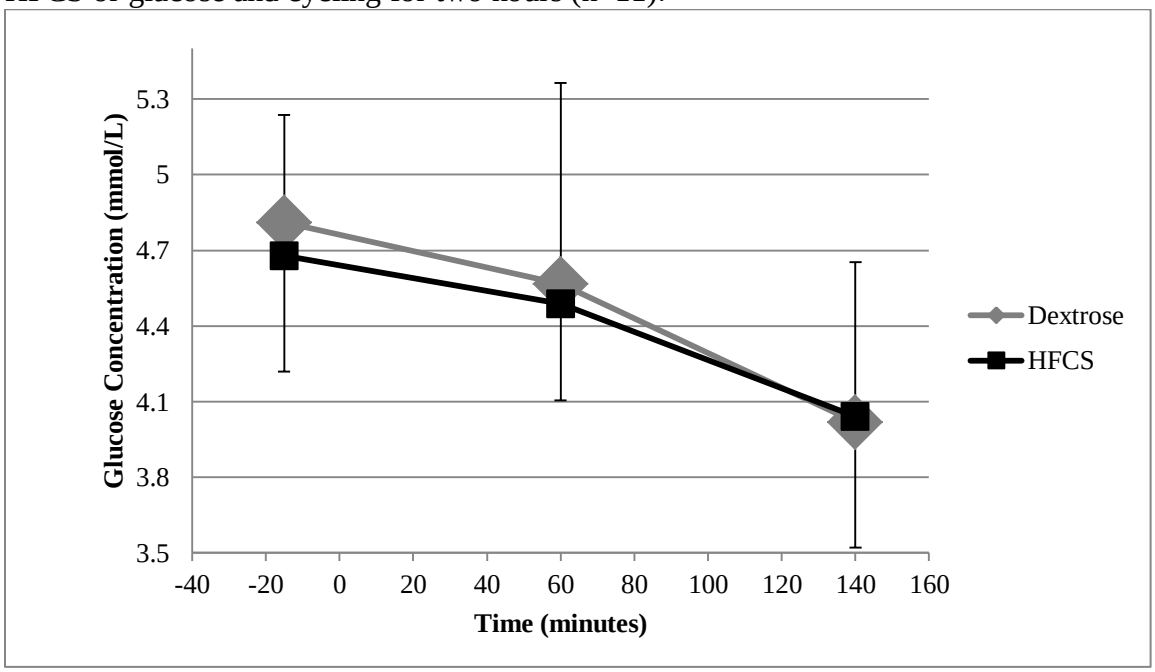
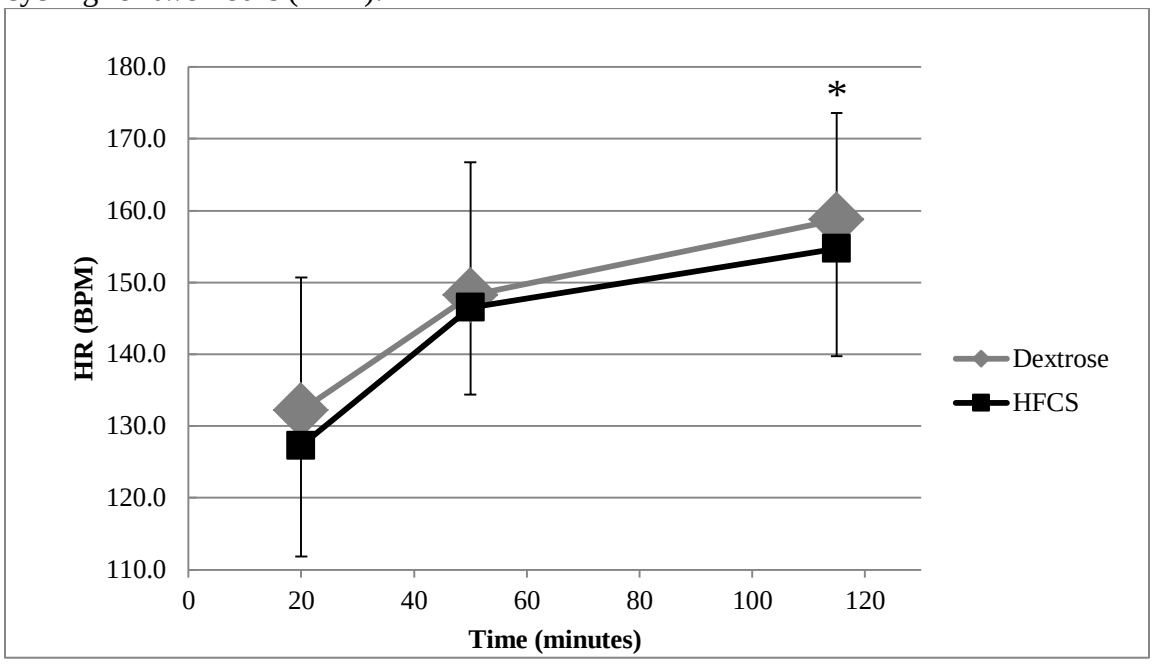
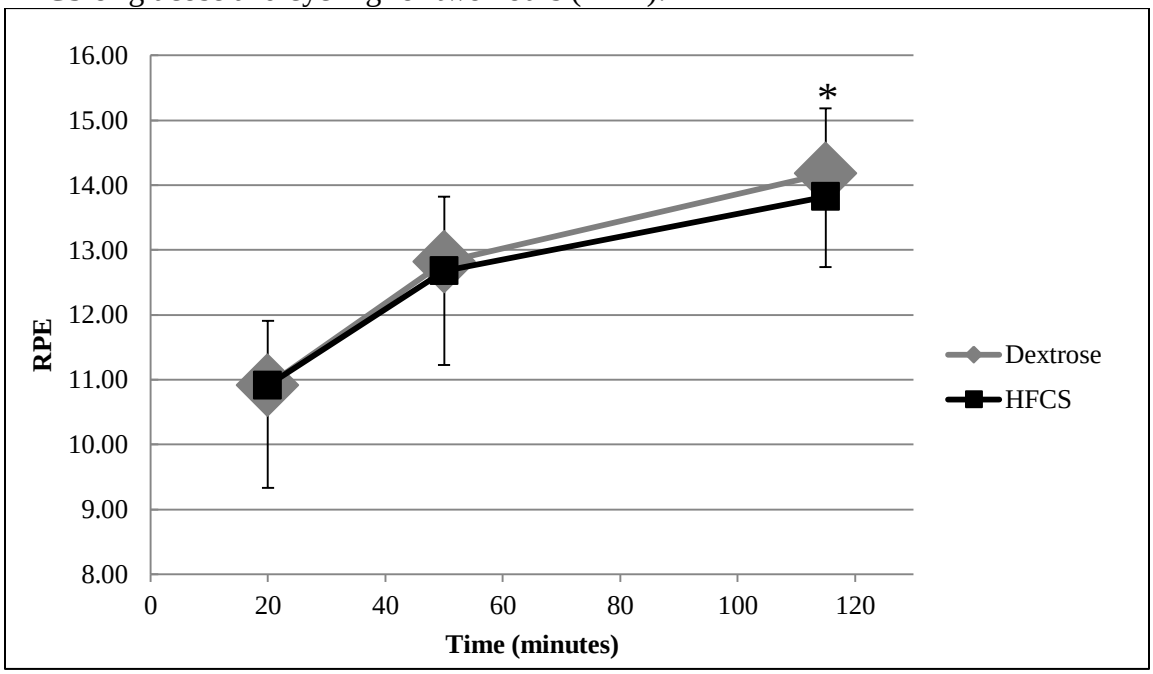


Figure 3.8 Mean (±SD) heart rate prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).



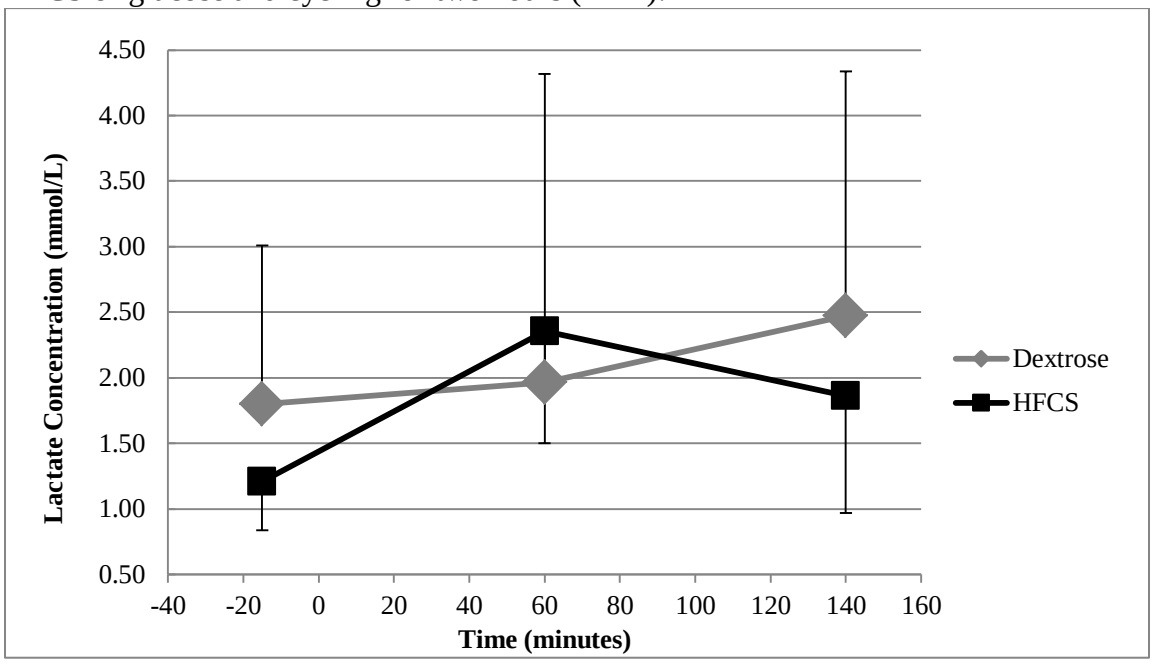
*=Significantly different from 20 minutes.

Figure 3.9 Mean (±SD) rate of perceived exertion prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).



*=Significantly different from 20 minutes.

Figure 3.10 Mean (±SD) serum lactate concentrations prior to and after consumption of HFCS or glucose and cycling for two hours (n=11).



Discussion

The current study focused on the metabolism of acute 50 g feedings of DEXT or HFCS during moderate intensity endurance cycling. High fructose corn syrup has been purported to increase lipid production and mobilization after ingestion, therefore an attempt was made to create an environment which maximized lipolysis by having subjects cycle at 50-60% of VO_{2max} when deciding to feed subjects 50 g of HFCS during exercise. Several variables were taken into account. Another similar research study by Lecoultre et al. (2010) fed subjects a high CHO dose of 240 g combining dextrose (144 g) plus fructose (96 g) and compared their findings to 240 g of dextrose. In a controlled lab environment, feeding subjects 240 g of any CHO source is acceptable because the biological limitations of CHOs absorption can be monitored. Outside of the

lab, it is important to consider fluid osmolality and gastric emptying, how to maximize CHO absorption by the combination of monosaccharides, disaccharides, and polysaccharides in the food or beverage, and at what rate CHOs can be ingested without causing intestinal discomfort. In reality, active individuals have access to sports drinks containing 5-8% CHO, powdered sports drink mixes, and gummies or gels containing 20-30 g CHO plus the recommended fluid intake per package. According to Noakes (1993), *ad libitum* fluid ingestion for runners was about 375 mL/hr and cyclists about 960 mL/hr during competition. Cyclists were "visibly uncomfortable" after being encouraged to ingest 1600 mL/hr and 25% of the subjects developed diarrhea after exceeding their fluid absorption rate (Noakes, 1993). To emphasize how unrealistic ingesting 240 g of CHO in 120 minutes is for endurance exercise, roughly 4000 mL of sports drinks, a 1200 mL 20% CHO solution of powdered sports drink, or 8-12 gel packages plus fluid would need to be consumed without inducing intestinal discomfort. Therefore, the dose of CHO fed during the current study was low (50 g vs. 240 g), but was a more reasonable dose that an active individual may consume prior to or during cycling.

The other purpose of providing a 50 g CHO beverage (half of the beverage 15 min prior to cycling and the rest 10 min into cycling) at the beginning of each experimental ride, while fasted, was to ensure the metabolism observed during 120 minutes of cycling was induced by an ordinary dose of CHO and the energy requirements of the activity. The goal was to investigate how the body reacted to a single CHO dose over time, not continuous feedings over time as other investigators have demonstrated. There was also interest in determining if HFCS posed any potential health risks to active individuals utilizing this energy source for supplementation during exercise. Previous

investigators have reported significant differences in the metabolism of dextrose and HFCS after high dose (96 grams of fructose) feedings paired with exercise (Lecoultre, 2010) and how diets high in fructose paired with exercise can counteract the hypertriglyceridemic effects of fructose (Egi, L, Lecoultre, V, Theytaz, F, Campos, V, Hodson, L, Schneiter, P, Mittendorfer, B, Patterson, BW, Fielding, BA, Gerber, PA, Giusti, V, Berneis, K, & Tappy, L, 2013). In contrast to Lecoultre et. al (2010), there were no significant differences observed between the ingestion of acute 50 g doses of HFCS and DEXT during prolonged moderate intensity exercise for RER, serum lipids, blood glucose, HR, RPE, and blood lactate concentrations.

During each experimental ride 750 mL of water in addition to the 400 mL provided in the CHO beverage were ingested by the subjects. Fluid was provided in hopes of buffering hydration and thermoregulation. There were no significant differences in %ΔPV between the two experimental rides. The %ΔPV were very small, 0.09±1.8% for DEXT and -0.6±4.46% for HFCS. An increase in HR was observed overtime for each subject, these increases were more likely a symptom of fatigue than dehydration or hyperthermia, because the %ΔPV was so small.

Dextrose and HFCS ingestion did not have any main effect on the significant decrease in RER over time during the experimental rides. Even though there was no significant time by treatment or main treatment effect, there was a significant decrease from 20 to 115 minutes for RER, regardless of the treatment. This would indicate the subjects were utilizing far more fatty acids for energy at the end of the ride than at the beginning.

The concentration of TGs in the blood did not significantly change during each trial. The controversy surrounding HFCS and its contribution to increasing blood lipid concentrations after ingestion was not found to be true in this current study. Total cholesterol increased significantly (2.6%) and slightly along with HDL (4.5%) over the duration of each experimental trial, but as expected, neither increase was linked to the treatments. Low density lipoproteins and VLDL concentrations also did not change. Very low density lipoproteins are packaged with TGs made in hepatocytes and transported into the blood. If fructose ingestion was going to induce a change in blood lipids an increase in VLDL after HFCS feeding would have been expected (Egli, 2013), along with an increase in TGs. The significant decrease of RER over time may have indicated an increase in lipid utilization after HFCS ingestion if the DEXT test results were not also the same. This suggests that the dose of CHO fed to each subject may have been utilized quickly after being converted to glucose or lactate in the liver and the blood draw at 60 minutes was too late in the exercise to observe fructose metabolism. Egli et al. reported that exercise minimized the effects of high fructose diet induced hypertriglyceridemia. Comparing Egli's et al. findings to this current study, the subjects probably did not ingest a large enough amount of fructose to increase lipid production in such a short period of time and the energy requirements of a 2 hour cycle ergometer ride probably oxidized the metabolic products of fructose prior to TG formation.

Figure 3.2 reports a large standard deviation (SD) for baseline TG levels (±70.99 mg/dL). The large SD can be attributed to one subject's baseline TG of 318 mg/dL and final TG of 208 mg/dL. This individual is the perfect example of what endurance exercise can do to help lower the concentration of blood substrates that are larger than

recommended even after the consumption of HFCS. Also, the lipid analyzer has a recommended monthly calibration and was calibrated by the investigator the day serum testing started. All serum samples were analyzed within four days of the calibration. Each serum sample was mixed by rotating the tube with a windshield wiper motion at least twenty times back and forth prior to pipetting into the testing cassette. There could have been lipid drops that may have formed in the serum, but every serum tube was mixed this same way after thawing.

There were no significant time by treatment, main treatment and time differences in the concentrations of blood glucose over the course of the experimental ride, but there was a decreasing trend (Figure 3.7). The trending reduction in glucose aligned appropriately with the significant decrease in RER (Figure 3.1), the significant increase in HR (Figure 3.8), and the significant increase in RPE (Figure 3.9). The significant decrease in RER represented a shift from oxidizing larger concentrations of glucose to utilizing more fatty acids as fuel instead. As HR increases so does cardiac output (CO), by amplifying the amount blood passing through the heart more energy substrates and oxygen will be available for oxidation, and more fatigue inducing oxidative byproducts can be buffered and removed from circulation by the lungs in an attempt to maintain pH balance as long as possible. The cyclists were definitely feeling the negative effects of pedaling for an extended period of time in a fasted state at 50-60% of their VO_{2max}, according to their significantly increasing RPE values, but these effects described above were not linked to either treatment beverage just to the demands of the body to maintain work output during moderate intensity exercise.

It was hypothesized that fructose feedings would increase the concentration of blood lactate providing a secondary and beneficial source of pooled carbon for energy during exercise as blood glucose concentrations naturally decreased. There were no significant differences between treatments for blood lactate during the experimental rides, but there was a trend, as seen in Figure 3.10, in the concentration of lactate to increase an hour after ingestion of HFCS. An increase in lactate was anticipated after the ingestion of HFCS (Lecoultre, 2010), but 27.5 grams of fructose seems to not have been a large enough dose to induce a significant increase in lactate production during exercise. Lecoultre et al. however, found a significant increase in lactate production when 96 g of fructose and 144 g of dextrose were fed throughout 120 minutes of cycling at a moderate 60% of VO_{2max} intensity when compared to a 240 g dextrose feeding. Fructose is thought to be easily converted to lactate and released by the liver into the blood during exercise. In the current study, any lactate produced after fructose ingestion may have been oxidized prior to the 60 minute blood draw. As previously discussed a lower dose of CHO was selected to study the substrate metabolism of sport specific products available to the general public containing fructose. Increasing the dose of fructose would also not be beneficial because of the potential for inducing intestinal discomfort.

An interesting, but not surprising side effect after the first hour of cycling was asymptomatic hypoglycemia. Subjects did not display or report any lack of coordination, confusion, lightheadedness or dizziness, nausea, impaired vision, trembling or chills, anxiety, irritability, rapid heartbeat, tingling or numbness in the mouth or tongue, headaches, or seizures when hypoglycemic (American Diabetes Association, 2014). Nine out of the eleven subjects developed hypoglycemia (blood glucose <3.9 mmol/L or

<70 mg/dL). The cyclists were more likely to become hypoglycemic around 90 minutes of exercise after ingesting DEXT (7 vs. 4 subjects, two of which developed hypoglycemia for both rides) than HFCS. Two of these same DEXT subjects were also hypoglycemic at 60 minutes of exercise and another cyclist only developed hypoglycemia at 60 minutes then regained normal glucose concentrations. At the final blood draw, four subjects experienced hypoglycemia after ingestion of HFCS and four after DEXT ingestion. Only one subject was hypoglycemic at the final blood draw for both treatment rides.

A 90 minute blood glucose check was implemented as a precaution to inform the subjects about their blood glucose concentrations, allowing informed decisions to be made as exercise continued. Active individuals usually consume a meal 2-3 hours prior to exercise, especially after a night of fasting. Subjects, in the present study, were required to fast for 12 hours prior to cycling. They were also encouraged to consume a meal with CHO before the fasting cut off the night before their experiments ride.

In a fasted state depleted liver glycogen creates the perfect environment for exercise induced hypoglycemia. Carbohydrate absorption would not be inhibited at moderate intensity exercise and it can be assumed that the entire dose of DEXT and HFCS was transported through the small intestine into circulation. Although, insulin was not measured it could be hypothesized that the first dose of DEXT would have initially spiked insulin levels as the concentration of blood glucose increased at rest, causing myocytes and hepatocytes to take up glucose for re-synthesis of fasting induced depletion of glycogen. The second half of the beverage was ingested during exercise and was more than likely stored as glycogen in the liver, with some delivery to working muscle.

Glycogen storages would continue to decrease within muscle and the liver as exercise progressed eventually causing hypoglycemia without further supplementation of CHO. Blood glucose concentrations were maintained for longer after the consumption of HFCS according the 90 minute finger sticks. Fructose has been shown to be converted to lactate fairly quickly upon uptake in the liver and released into circulation (Lecoultre, 2010). Extra lactate would allow muscle an additional energy source slowing the glycolysis and maintaining high blood glucose concentrations. Therefore, the ingestion of HFCS may be even more beneficial than previously thought, providing a longer lasting source of energy.

Conclusion

In conclusion, since there were no significant differences in time by treatment or main treatment effects for RER, serum TGs, CHOL, LDL, HDL, and VLDL, blood glucose, HR, RPE, and blood lactate after ingestion of DEXT and HFCS, and completion of a 2 hour moderate intensity cycle ergometer ride, the null hypothesis, that there would be no difference between treatments for the dependent variables listed above during prolonged moderate intensity exercise, cannot be rejected at this time. Additionally, the acute consumption of HFCS as a CHO supplement for endurance activities lasting 2 hours does not appear to have been harmful to the subjects' health as there were no significant increases in serum lipid concentrations. The subjects' significant main time effects for RER, HR, and RPE appear to be consistent with typical patterns observed over the course of endurance exercise for alterations in substrate metabolism and exercise physiology. According to research by Jeukendrup et al. (2008), it is beneficial for

athletes and active individuals to ingest CHO sources containing larger concentrations of fructose, paired with glucose as previously recommended. The current research supports Jeukendrup's recommendations and ingestion of mixed sources of monosaccharides (dextrose + fructose) as fuel for endurance activities. Active individuals need to remember to supplement CHO according to the activity, if the number of calories consumed are in excess of the energy expended, the same results observed in this study may not apply to wellbeing of the individual.

CHAPTER 4

CONCLUSIONS

In the current study, RER, serum TGs, CHOL, LDL, HDL, and VLDL, blood glucose, HR, RPE, and blood lactate were measured during a 120 minute moderate (50-60% VO2max) intensity endurance cycle ergometer exercise after ingestion of a beverage containing 50 g of HFCS or dextrose. The results indicated there were no significant differences identified after ingestion of HFCS when compared to DEXT. However, RER decreased while HR and RPE significantly increased as exercise progressed from 20 to 115 minutes. These changes were expected as a result of the physical demands required to complete the exercise. RER should decrease over time as the body utilizes more fatty acids as a substrate for energy production during moderate intensity exercise. Heart rate and RPE will increase because the physical and metabolic requirements of exercise become more demanding over time. The metabolism and physical responses observed during this study are within the parameters expected for substrate utilization and exercise demands.

The test subjects all reported increasing exertion rates and hunger by the end of the experimental rides. This is consistent with exercising after a 12 hour fast and ingestion of 50 g CHO supplement at the start of exercise. The subjects were able to maintain their designated cadence throughout the ride, but were feeling the effects of exercise-induced fatigue.

None of the subjects reported any intestinal discomfort after ingestion of either CHO beverage. The 50g CHO beverages were prepared in a 400 mL water solution

(12.5 % CHO), utilizing an ordinary flavoring (Kool-Aid) to mask the flavor. Surprisingly, most of the subjects stated that DEXT was sweeter than HFCS. This supported the double-blind structure of the study masking the sweeter fructose flavors with Kool-Aid.

Chronic consumption of fructose has the potential to increase gluconeogenesis and *de novo* lipogenesis, both of which in excess are shown to enhance the development of disease. Even though the consumption of fructose appears to be harmful when consumed in excess for sedentary individuals, according to this current study ingestion of HFCS paired with exercise did not significantly induce an increase in serum lipid concentrations. According to these results 50 g of HFCS can be utilized as a CHO supplement for exercise lasting 2 hours to benefit endurance athletes and active individuals.

As discussed, Lecoultre et al previously conducted a similar study feeding highly trained cyclists either 240 g of dextrose or 144 g dextrose plus 96 g of fructose (DEXFRU) (1.2 g dextrose/min + 0.8 g dextrose or fructose/min) over 120 minute of cycling at ~60% VO_{2max}. Lecoultre et al. found significant increases in lactate production after the consumption of dextrose plus fructose vs. dextrose alone. One hundred percent of the lactate taken up by muscle during Lecoultre et al.'s study was oxidized and believed to be larger than previous reports of ~80% lactate oxidation, because the oxidative capacity of highly trained athletes is higher. Also, free fatty acids did not significantly increase over baseline for DEXFRU feeding and were not significantly different from the dextrose only feedings. Even after ingesting a large dose (96 g vs. 27.5 g) of fructose during exercise the energy substrates that were formed (lactate and

glucose) were efficiently utilized and the harmful effects of increased lipid production and mobilization were not observed in Lecoultre's study.

For future research, modifications to the protocol would include a meal with carbohydrates consumed directly before the 12 hour cut off for fasting, emphasizing subjects refrain from high intensity exercise 48 hours prior to testing, and collection of blood samples every 20 to 30 minutes of the 120 minute cycle ergometer ride to establish a more in depth description of substrate metabolism. It would also be interesting to investigate the effects of HFCS on substrate metabolism in a fed state, performing this same study, after providing a standardized meal to each subject 2-3 hours prior to exercise.

In conclusion, the current study demonstrates that an acute 50 gram dose of HFCS at the beginning of exercise does not induce hypertriglyceridemia or dyslipidemia in active individuals cycling at a moderate intensity for two hours. Additionally, prolonged moderate intensity exercise may reduce disease risk by utilizing stored energy and exogenous energy to fuel the activity, especially in the case of this current study when the individuals were in a negative energy balance. In a fasted state, the ingestion of HFCS was more prone to prevent hypoglycemia when compared to DEXT. Furthermore, active individuals' should not avoid fructose containing sport specific products when participating in endurance exercise lasting greater than sixty minutes. High fructose corn syrup has proven to be an adequate CHO source for moderate intensity endurance cycling.

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APPENDICES

APPENDIX A

ADDITIONAL TABLES

Table A.1. Mean (±SD) of dependent variables.

Carbohydrate Source	Collection Time (min)	Dependent Variables	Mean	SD	N
Dextrose	20	RER	0.92	0.03	11
Dextrose	50	RER	0.90	0.02	11
Dextrose	115	RER	0.86	0.04	11
HFCS	20	RER	0.92	0.02	11
HFCS	50	RER	0.90	0.02	11
HFCS	115	RER	0.86	0.02	11
Dextrose	-15	TG	106.7	26.18	11
Dextrose	140	TG	104.6	17.08	11
HFCS	-15	TG	118.9	70.99	11
HFCS	140	TG	126.8	45.65	11
Dextrose	-15	CHOL	182.6	28.04	11
Dextrose	140	CHOL	186.6	28.48	11
HFCS	-15	CHOL	180.4	27.44	11
HFCS	140	CHOL	185.9	26.82	11
Dextrose	-15	LDL	106.8	22.47	11
Dextrose	140	LDL	108.4	22.92	11
HFCS	-15	LDL	100.3	19.18	11
HFCS	140	LDL	102.2	19.75	11
Dextrose	-15	HDL	54.7	12.47	11
Dextrose	140	HDL	57.1	12.92	11
HFCS	-15	HDL	56.1	12.48	11
HFCS	140	HDL	58.7	13.78	11
Dextrose	-15	VLDL	21.3	5.1	11
Dextrose	140	VLDL	20.9	3.27	11
HFCS	-15	VLDL	23.7	14.37	11
HFCS	140	VLDL	25.4	9.21	11
Dextrose	-15	Glucose	4.8	0.43	11
Dextrose	60	Glucose	4.6	0.80	11
Dextrose	140	Glucose	4.0	0.64	11
HFCS	-15	Glucose	4.7	0.46	11
HFCS	60	Glucose	4.5	0.38	11
HFCS	140	Glucose	4.0	0.52	11
Dextrose	-15	Lactate	1.8	1.25	11
Dextrose	60	Lactate	2.0	0.7	11
Dextrose	140	Lactate	2.5	1.39	11
HFCS	-15	Lactate	1.2	0.37	11
HFCS	60	Lactate	2.4	0.85	11
HFCS	140	Lactate	1.9	0.89	11
Dextrose	20	HR	132.2	18.5	11
Dextrose	50	HR	148.2	18.6	11
Dextrose	115	HR	158.7	14.8	11
HFCS	20	HR	127.3	15.5	11
HFCS	50	HR	146.5	12.2	11
HFCS	115	HR	154.7	15	11
Dextrose	20	RPE	10.9	1.51	11
Dextrose	50	RPE	12.8	1.47	11
Dextrose	115	RPE	14.2	1.78	11
HFCS	20	RPE	10.9	1.58	11
HFCS	50	RPE	12.7	1.45	11
HFCS	115	RPE	13.8	1.08	11

Table A.2. Mean ± SD of the dependent variables after combining treatments. For RER, HR, and RPE the initial sample was taken at 20 min of exercise, midway sample at 50 min, and the final sample at 115 minutes. For TG, CHOL, LDL, HDL, VLDL, glucose, and lactate the initial sample was taken 20 min prior to the start of exercise, midway sample at 60 minutes of exercise, and the final sample at 140 min after the start of exercise.

Dependent Variable	Initial Sample	Initial SD	Midway Sample	Midway SD	Final Sample Mean	Final SD
RER	0.92	0.02	0.90	0.02	0.86	0.02
TG	112.8	48.6	-	-	115.7	17.8
CHOL	181.5	27.7	-	-	186.2	27.7
LDL	103.6	20.8	-	-	105.3	21.3
HDL	55.4	12.5	-	-	57.9	13.4
VLDL	22.5	9.7	-	-	23.1	6.2
Glucose	4.7	0.4	4.6	0.6	4.0	0.6
HR	129.7	17.0	147.4	15.4	156.7	14.9
RPE	10.9	1.6	12.8	1.5	14.0	1.4
Lactate	1.5	0.8	2.2	0.8	2.2	1.1

APPENDIX B

SUBJECT CONSENT FORM

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

Title: The Influence of High Fructose Corn Syrup on Endurance Exercise Metabolism

You are being asked to participate in a research study on nutritional supplementation and metabolic substrate utilization. You will be asked to ride a cycle ergometer for 120 minutes, consume a carbohydrate beverage, and participate in venous blood samples and respiratory gas collections throughout the ride to determine fuel utilization. High fructose corn syrup (HFCS) is under scrutiny for its potential link to the rise in obesity, cardiovascular disease, and metabolic disorders, so much that some manufacturers may avoid utilizing this carbohydrate source in their sports specific products. By avoiding HFCS athletes may lose the performance enhancing benefits that this particular carbohydrate combination may provide. Although the effects of carbohydrate supplementation during exercise have been researched, there has been minimal research documenting substrate production after ingestion of HFCS during an endurance exercise bout. The purpose of this study is to investigate whether the ingestion of high fructose corn syrup alters substrate (i.e. lipids, glucose, and lactate) metabolism during prolonged moderate intensity exercise when compared to glucose.

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John Seifert, 994-751, Movement Science Laboratory

Funding Agency: Sports, Cardiovascular, and Wellness Nutrition Graduate Student Research Grant

Procedures. If you agree to participate you will be asked to visit the Movement Science Laboratory in Romney Gym on three separate occasions (about eight hours total). You will arrive in the morning and anthropometrics (height and body mass) will be measured. You will be asked to complete a maximal oxygen uptake test (VO_2max test) on your first visit. You will start cycling at a low workload. The workload will increase every three minutes until you can no longer maintain your self-selected pedaling cadence. Expired air, rate of perceived exertion, and heart rate will be collected throughout the exercise. This visit will take about an hour to an hour and a half. The second and third visits will be the experimental rides. The day prior to testing you will be required to record your dietary habits for one day and then consume that same diet, while refraining from exercise and alcohol for 24 hours, the day before each trial. A 12 hr fast with caffeine avoidance will be required prior to each trial. A venous blood sample will be collected prior to the trial. The experimental treatments consist of two carbohydrate-based beverages, one drink containing glucose (50 grams) and the other containing high fructose corn syrup (50 grams). You will ingest 200 mL (about 7 fluid oz.) of the given treatments 15 minutes prior to the start of exercise and at 10 minutes of exercise. The experimental rides will last for 120 minutes. You will cycle at 50% VO_{2max} for the first 25 minutes, then increase to 60% VO_{2max} at 25 minutes, with breaks in intensity at 58 (10 minute seated blood draw) and 93 minutes of exercise cycling at 50% VO_{2max}. A second blood sample will be collected off the

bike sitting in a chair at 58 min of exercise, allowing ten minutes for sampling, fluid consumption, and rest. The final blood sample will be collected at the conclusion of each trial. Approximately 5 mL of blood will be required for each collection. You will be asked to sit for 20 minutes prior to the baseline and concluding blood samples. Rating of perceived exertion, heart rate, and expired air will be collected for three minutes at 20, 50, and 115 minutes to identify what metabolic substrates (i.e. fat, carbohydrates, or a mixture of the two) you are utilizing to fuel the exercise bout. Additionally, you will drink 150 mL (about 5 fluid oz.) water at 25, 45, 62, 93, and 110 minutes of exercise for a total of 1150 mL of fluid including the experimental beverage. You will be asked to return to complete the second experimental ride approximately one week later. You will have time on the first visit to become familiar with the timing apparatus and movement patterns.

Time Commitment. Total time for your participation is about eight hours. This is broken into about 1.5 hours for the maximal oxygen uptake test and about 3.0 hours per experimental test. You are free to discontinue this study at any time.

Confidentiality. Personal information and data will be kept in a cabinet in a locked office. Data used in analysis will be coded so that data sheets will not be identifiable.

Benefits. Results of this study may help us understand how the body utilizes high fructose corn syrup during prolonged moderate to high intensity exercise and may in turn alter the nutritional recommendations for endurance activities in order to enhance performance.

Compensation. You will receive a free maximal oxygen uptake test and a $50 gift card from Town and Country grocery store for completion of the project.

Risks. There are risks to participating in this study. These include: possible infection from blood sampling, soreness from the blood sampling, and fatigued or sore muscles from the cycling exercise. Heart rate and perceived exertion will be monitored throughout the testing. The maximal oxygen uptake test will require that you cycle to volitional exhaustion. There is the risk of sudden death, but this risk is extremely low considering your training status. You will be required to complete a health history form prior to participating. This form will help screen potential health problems before the exercise. Reasonable precautions will be taken for your safety. There is no compensation available from MSU for injury.

Questions. Your decision whether or not to participate will not jeopardize your relationship with the Movement Sciences Laboratory. You are free to discontinue participation at any time without negative effects on your relationship with Montana State University or the researchers. Privacy and confidentiality of data will not be breached. If you have any questions, please ask us.

If you have any additional questions later, Erika Rauk (406-529-1097) or John Seifert (406-994-7154) will be happy to answer them. Additional questions about the rights of human subjects can be answered by the Chairman of the Institutional Review Board, Mark Quinn, (406) 994-4707.

The Influence of High Fructose Corn Syrup on Endurance Exercise Metabolism

Freedom of Consent

I have been given ample opportunity to read this document in its entirety and to ask questions which have been answered to my satisfaction. I hereby consent to become a participant in this study knowing the health risks involved and that I may withdraw my consent at any time, for any reason. I also understand that project personnel may screen me from this study for any reason deemed appropriate.

I consent to photographs being taken during testing, please initial here if you consent: _____

AUTHORIZATION: I have read the above and understand the discomforts, inconvenience and risk of this study. I, _____ *(name of subject),* agree to participate in this research. I understand that I may later refuse to participate, and that I may withdraw from the study at any time. I have received a copy of this consent form for my own records.

Signed: _____

Witness: _____

Investigator: _____

Date: _____ .

APPENDIX C

THESIS DATA COLLECTION PACKET

Visit 1

Participant Information **Tech Initials: _____**

Participant Name: _____

Phone Number: _____

Email: _____

Address: _____

Participant ID: _____

Visit 1

Qualification Questionnaire **Tech Initials: _____**

Participant ID: _____

Time and date of visit: _____, _____

Completed consent form? Date of signature _____

Completed Health History form? Date completed _____

Exclusion Criteria

Is cycling a regular part of your exercise program? ☐ YES ☐ NO

If yes, please describe: _____

Do you currently have any muscle or skeletal limitations/injuries? ☐ YES ☐ NO

If yes, please describe: _____

Do you take lipid, cholesterol, and/or blood pressure-lowering medications? ☐ YES ☐ NO

If yes, please describe: _____

Are you currently utilizing some form of contraceptive? ☐ YES ☐ NO

If yes, please describe: _____

Are you pregnant? ☐ YES ☐ NO

Do you regularly use any other types of medications? ☐ YES ☐ NO

If yes, please describe: _____

Date of Birth: _____

Would you like reminders prior to testing? ☐ YES ☐ NO

If yes, please check all that apply: ☐ EMAIL ☐ PHONE

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health status by marking all *true* statements

History
You have had:
___ a heart attack
___ heart surgery
___ cardiac catheterization
___ coronary angioplasty (PTCA)
___ pacemaker/implantable cardiac
___ defibrillator/rhythm disturbance
___ heart valve disease
___ heart failure
___ heart transplantation
___ congenital heart disease

Symptoms
___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness.
___ You experience dizziness, fainting, or blackouts.
___ You take heart medications.

Other health issues
___ You have diabetes.
___ You have asthma or other lung disease.
___ You have burning or cramping sensation in your lower legs when walking short distances.
___ You have musculoskeletal problems that limit your physical activity.
___ You have concerns about the safety of exercise.
___ You take prescription medication(s).
___ You are pregnant.

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

Cardiovascular risk factors
___ You are a man older than 45 years.
___ You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal.
___ You smoke, or quit smoking within the previous 6 months.
___ Your blood pressure is >140/90 mm Hg.
___ You do not know your blood pressure.
___ You take blood pressure medication.
___ Your blood cholesterol level is > 200 mg/dL.
___ You do not know your cholesterol level.
___ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).
___ You are > 20 pounds overweight.

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

___ None of the above

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

24 Food Record and Pre-Experimental Ride Directions

The second and third visits will be the experimental rides. The day prior to testing please record your dietary habits for 24 hours and then consume that same diet, while refraining from exercise and alcohol for 24 hours, and caffeine for 12 hours, the day before each experimental ride. Please also fast for 12 hours fast prior to each experimental ride.

***No Alcohol/Exercise for 24 hours & Caffeine/Food 12 hours prior to testing.**

Example - 24 HOUR DIET RECORD – DATE: 11/22/13

Time	Amount Consumed	Description of Food and Drink Consumed
8:00 AM	1 cup	Coffee
	1 cup	Oatmeal
	0.5 cup	Milk
	1/4 cup	Raisins
	1/2 tsp	Cinnamon
	2	Scrambled eggs
	2 Slices	Whole wheat toast
	1 tbsp	Strawberry jelly
	16 oz	Water
12:30 PM	1 cup	Lettuce
	1/4 cup	Tomatoes
	1/4 cup	Carrots
	1/2 cup	Cucumber
	1/2 cup	Red Cabbage
	1/2 cup	Croutons
	1/8 cup	Sunflower Seeds
	1 - 8"	Flour Tortilla
	1 oz	Swiss Cheese
	3 oz	Turkey
	1 tsp	Stone ground mustard
	16 oz	Water
4:00 PM	1 - Medium Size	Banana
	1 tbsp	Creamy Peanut Butter
	16 oz	Water
7:00 PM	1 - 5"x5" Slice	Lasagna (Use the same recipe next week)
	8 oz	Skim Milk
	1/2 cup	Quinoa
	1/4 cup	Red Pepper
	1/4 cup	Peas
	1 tsp	Olive Oil
	1 tsp	Vinegar
	2 - Medium Sized	Chocolate Chip Cookies
9:00 PM	8 oz	Water

***No Alcohol/Exercise for 24 hours & Caffeine/Food 12 hours prior to testing.**

24 HOUR DIET RECORD – DATE: ___________

Time	Amount Consumed	Description of Food and Drink Consumed

***No Alcohol/Exercise for 24 hours & Caffeine/Food 12 hours prior to testing.**

24 HOUR DIET RECORD – DATE: ___________

Time	Amount Consumed	Description of Food and Drink Consumed

Visit 1 – Maximal Testing
ParvoMedics TrueOne Metabolic System Daily Calibration

Tech. Initials _____

Date: _____

Time: _____

Barometric Pressure: _____

Room Temperature: _____

Humidity: _____

Flowmeter Calibration Difference (< ± 1.0%): _____

Gas Calibration (Change < ± 1%): _____

Notes: _____

_____

_____

_____

Visit One

Tech Initials: _____

Date of visit: _____

Time of visit: _____

<u>Anthropometrics Measures</u>

Body mass: _____ (lbs) Height: _____ (in)

_____ (kg) _____ (cm)

<u>Body Mass Index</u>

BMI (kg/m^2) = _____ ___ Normal (18.5-24.9 kg/m^2)

___ Overweight (25.0-29.9 kg/m^2)

___ Obese I (30.0-34.9 kg/m^2)

___ Obese II (35.0-39.9 kg/m^2)

___ Obese III (>40.0 kg/m^2)

Visit 1 – Maximal Testing

Tech. Initials _____

Bike #: _____ Bike Seat Height: _____

Age: _____

Age-Predicted HRmax = 220 - _____ = _____

RAMP Protocol							
Stage	**Time**	**Resistance**	**Cadence**	**HR**	**RPE**	**RER**	**Notes**
	min	**kg**	**rpm**	**bpm**			
Warm-up	0:00-4:59	0.5					
1	5:00-7:59	1.0					
2	8:00-10:59	1.5					
3	11:00-13:59	2.0					
4	14:00-16:59	2.5					
5	17:00-19:59	3.0					
6	20:00-22:59	3.5					
7	23:00-25:59	4.0					
8	26:00-28:59	4.5					
9	29:00-31:59	5.0					

$VO2_{max}$:_____

Print out Metabolic Cart Data and add to packet. **$Workload_{max}$:_____**

HR_{max}:_____

Visit 1 – Maximal Testing - Calculating 60% & 50% VO2max

Tech. Initials _____

<u>Calculating %VO_{2max}</u>

_____L/min VO_{2max} x 0.60 = _____ (60% VO_{2max}) Workload = _____ kg

_____L/min $VO2_{max}$ x 0.50 = _____ (50% VO_{2max}) Workload = _____ kg

60% VO_{2max} Workload for Experimental Rides = _____ kg

50% VO_{2max} Workload for Experimental Rides = _____ kg

Notes: _____
_____
_____
_____
_____

Visit 2 – Experimental Testing
ParvoMedics TrueOne Metabolic System Daily Calibration
Tech Initials: _____

Date: _____

Time: _____

Barometric Pressure: _____

Room Temperature: _____

Humidity: _____

Flowmeter Calibration Difference (< ± 1.0%): _____

Gas Calibration (Change < ± 1%): _____

Notes: _____

_____

_____

_____

Visit 2 – Experimental Testing

Carbohydrate Beverage Code _____ **Tech Initials: _____**

Test Date: _____ Weight: _____kg Bike #: _____ Seat Height: _____
Age: _____ Height: _____
60% VO_{2max} HR: _____ 50% VO_{2max} HR: _____
60% VO_{2max} Cadence: _____ 50% VO_{2max} Cadence: _____
60% VO_{2max} Resistance: _____ 50% VO_{2max} Resistance: _____

Time	Speed	Resistance	HR	RPE	RER	Task	Notes
min	rpm	kg	bpm				
-50:00						Bathroom & Pre-weight	
-40:00						20 min sit	
-20:00						Baseline blood draw	
-15:00						Feed 200 mL CHO beverage	
00:00						**Start Test at 50% VO_{2max}**	
00:01-9:59							
10:00-14:59						Feed 200 mL CHO beverage	
15:00-19:59							
20:00-24:59						**RPE, HR, and RER**	**RER Time =**
25:00-29:59						**60% VO_{2max}**; 150 mL water	
30:00-44:59							
45:00-49:59						150 mL water	
50:00-55:00						**RPE, HR, and RER**	**RER Time =**
55:01-57:59							
58:00-1:08:00						**2nd blood draw;** 150 mL water	
1:08:01-1:32:59							
1:33:00-1:37:59						**50% VO_{2max};** 150 mL water	
1:38:00-1:49:59						**60% VO_{2max}**	
1:50:00-1:54:59						150 mL water	
1:55:00-2:00:00						**RPE, HR, and RER**	**RER Time =**
2:00:01-2:20:01						20-min sit	
2:20:02						Final blood draw	

NOTES: _____

***Print out Metabolic Cart Data and add to packet.**

Visit 2 - Venus Blood Draw Carbohydrate Testing

Carbohydrate Beverage Code _____ Tech. Initials _____

Date of visit: _____

Time of visit: _____

Baseline – Blood Draw (-10 minutes)

Antecubital venous:

Time sitting starts: _____

Time of draw: _____Arm: L / R

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Glucose (1): _____

Glucose (2): _____

Lactate: _____

Notes:

Serum Vial #1: _____
Serum Vial #2: _____

Second – Blood Draw (58 minutes)

Antecubital venous:

Time sitting starts: _____

Time of draw: _____Arm: L / R

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Glucose (1): _____

Glucose (2): _____

Lactate: _____

Notes:

Serum Vial #1: _____
Serum Vial #2: _____

Visit Two - Venus Blood Draw Carbohydrate Testing
Carbohydrate Beverage Code _____ Tech. Initials _____

Final – Blood Draw (140 minutes)

Antecubital venous:

Time sitting starts: _____

Notes:

Time of draw: _____Arm: L / R

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Serum Vial #1: _____
Serum Vial #2: _____

Glucose (1): _____

Glucose (2): _____

Lactate: _____

Visit 2 - Baseline Venous Blood Sample Lipid Testing Tech. Initials

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes:

Visit 2 - Second Venous Blood Sample Lipid Testing Tech. Initials

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes:

Visit 2 - Final Venous Blood Sample Lipid Testing **Tech. Initials**

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes:

Visit 3 – Experimental Testing
ParvoMedics TrueOne Metabolic System Daily Calibration

Tech Initials: _____

Date: _____

Time: _____

Barometric Pressure: _____

Room Temperature: _____

Humidity: _____

Flowmeter Calibration Difference (< ± 1.0%): _____

Gas Calibration (Change < ± 1%): _____

Notes: _____

_____

_____

_____

Visit 3 – Experimental Testing

Carbohydrate Beverage Code _____ **Tech Initials: _____**

Test Date: _____ Weight: _____kg Bike #: _____ Seat Height: _____
Age: _____ Height: _____
60% VO_{2max} HR: _____ 50% VO_{2max} HR: _____
60% VO_{2max} Cadence: _____ 50% VO_{2max} Cadence: _____
60% VO_{2max} Resistance: _____ 50% VO_{2max} Resistance: _____

Time	**Speed**	**Resistance**	**HR**	**RPE**	**RER**	**Task**	**Notes**
min	**rpm**	**kg**	**bpm**				
-50:00						Bathroom & Pre-weight	
-40:00						20 min sit	
-20:00						Baseline blood draw	
-15:00						Feed 200 mL CHO beverage	
00:00						**Start Test at 50% VO_{2max}**	
00:01-9:59							
10:00-14:59						Feed 200 mL CHO beverage	
15:00-19:59							
20:00-24:59						**RPE, HR, and RER**	**RER Time =**
25:00-29:59						**60% VO_{2max}**; 150 mL water	
30:00-44:59							
45:00-49:59						150 mL water	
50:00-55:00						**RPE, HR, and RER**	**RER Time =**
55:01-57:59							
58:00-1:08:00						**2nd blood draw;** 150 mL water	
1:08:01-1:32:59						**60% VO_{2max}**	
1:33:00-1:37:59						**50% VO_{2max};** 150 mL water	
1:38:00-1:49:59						**60% VO_{2max}**	
1:50:00-1:54:59						150 mL water	
1:55:00-2:00:00						**RPE, HR, and RER**	**RER Time =**
2:00:01-2:20:01						20-min sit	
2:20:02						Final blood draw	

***Print out Metabolic Cart Data and add to packet.**

Visit 3 - Venus Blood Draw Carbohydrate Testing

Carbohydrate Beverage Code _____ **Tech. Initials _____**

Date of visit: _____

Time of visit: _____

<u>Baseline – Blood Draw (-10 minutes)</u>

<u>Antecubital venous:</u>

Time sitting starts: _____

Time of draw: _____Arm: L / R

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Glucose (1): _____

Glucose (2): _____

Lactate: _____

Notes:

Serum Vial #1: _____

Serum Vial #2: _____

<u>Second – Blood Draw (58 minutes)</u>

<u>Antecubital venous:</u>

Time sitting starts: _____

Time of draw: _____Arm: L / R

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Glucose (1): _____

Glucose (2): _____

Lactate: _____

Notes:

Serum Vial #1: _____

Serum Vial #2: _____

Visit 3 - Venus Blood Draw Carbohydrate Testing
Carbohydrate Beverage Code _____ Tech. Initials _____

Final – Blood Draw (140 minutes)

Antecubital venous:

Time sitting starts: _____

Time of draw: _____Arm: L / R Notes:

Hematocrit: _____ (1) _____ (2)

Hemoglobin: _____

Glucose (1): _____ Serum Vial #1: _____
Serum Vial #2: _____

Glucose (2): _____

Lactate: _____

Visit 3 - Baseline Venous Blood Sample Lipid Testing Tech. Initials

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes:

Visit 3 - Second Venous Blood Sample Lipid Testing Tech. Initials

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes:

Visit 3 - Second Venous Blood Sample Lipid Testing Tech. Initials

TRIG	
CHOL	
HDL	
LDL	
VLDL	

Place Piccolo Chemistry Analyzer Printout Here

Notes: