THE IMPACT OF INCREASING CARBOHYDRATE INTAKE DOSES ON

EXOGENOUS CARBOHYDRATE OXIDATION, SUBSTRATE

UTILIZATION, AND EXERCISE PERFORMANCE

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THE IMPACT OF INCREASING CARBOHYDRATE INTAKE DOSES ON EXOGENOUS CARBOHYDRATE OXIDATION, SUBSTRATE UTILIZATION, AND EXERCISE PERFORMANCE

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JohnEric W. Smith, son of Kenneth Rodger and Gail Underwood Smith, was born December 15, 1976, in LaGrange, Georgia. He graduated from Central High School, Carrollton, Georgia, in 1995. JohnEric married Kimberly Odum, daughter of James and Jeanie Odum, on June 10, 2000. He graduated with a Bachelor of Science degree in Exercise Science in August 2000. In May of 2003, JohnEric received his Masters of Science degree in Exercise Science and began his doctoral studies at Auburn University. In November 2004, JohnEric accepted a scientist position at the Gatorade Sports Science Institute.

DISSERTATION ABSTRACT

THE IMPACT OF INCREASING CARBOHYDRATE INTAKE DOSES ON EXOGENOUS CARBOHYDRATE OXIDATION, SUBSTRATE UTILIZATION, AND EXERCISE PERFORMANCE

JohnEric William Smith

Doctor of Philosophy, May 10, 2008
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This study assessed: (1) the impact of increasing carbohydrate dosages on carbohydrate oxidation and (2) the impact of increasing carbohydrate dosages on exercise performance. Twelve male cyclists/triathletes ingested a placebo or glucose drinks delivering 15, 30, and 60 $g \cdot hr^{-1}$ during 120 minutes of constant load cycling at ~75% $VO_{2\,PEAK}$. Glucose drinks were extrinsically labeled with 1.8 $mg \cdot g^{-1}$ U-^{13}C-glucose and a 20-km time-trial followed each constant load ride. Blood glucose and insulin were highest when ingesting 60 $g \cdot hr^{-1}$ while free fatty acids were the lowest. Insulin and free fatty acid responses for placebo and the 15 $g \cdot hr^{-1}$ trial were virtually identical. Exogenous glucose oxidation rates during the last 30 minutes of the constant load cycling (mean ±

SE) were 0.26 ± 0.05, 0.44 ± 0.04 and 0.66 ± 0.07 g•min^{-1} for 15, 30 and 60 g•hr^{-1} ingestion rates, each being significantly different from each other ($p \leq 0.05$). Liver glucose oxidation rate was highest when consuming 15 g•hr^{-1} (0.63 ± 0.13 g•min^{-1}) followed by 30 g•hr^{-1} (0.51 ± 0.12 g•min^{-1}) and 60 g•hr^{-1} (0.42 ± 0.08 g•min^{-1}), all significantly different from one another at a $p \leq 0.05$ level. There was also significant reduction in muscle glycogen oxidation during the last hour of the 2-hour constant load ride with no significant differences between the 15, 30, and 60 trials. Relative to placebo, glucose ingestion improved time-trial performance ($p \leq 0.05$) with no statistical difference between glucose doses. These findings suggest the ingestion of glucose at increasing rates, between 0 g•hr^{-1} and 60 g•hr^{-1}, reduce the demand on liver glucose and carbohydrate ingestion rates as low as 15 g•hr^{-1} can improve cycling time-trial performance.

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Style manual or journal used	Journal of Applied Physiology
Computer Software used	Microsoft Word for Windows XP

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I. INTRODUCTION

Fatigue during prolonged exercise has often be associated with hypoglycemia and depletion of muscle glycogen. There is a great deal of evidence demonstrating the ergogenic effect of consuming carbohydrate before and during endurance exercise (2; 12; 20; 21; 29; 31; 42; 43; 56; 121; 130; 142; 144). The American College of Sports Medicine (ACSM) and the National Athletic Trainers Association (NATA) both have fluid replacement position stands that promote the benefit of carbohydrate intake during exercise. The position stands both suggest carbohydrate ingestion rates of 30-60 $g \bullet hr^{-1}$ (18; 123). These positions and experts' findings have led to the use of carbohydrate drinks during prolonged exercise as common practice.

A great deal of research has been conducted to show carbohydrate ingestion rate that optimizes exercise performance and substrate utilization (20; 29; 31; 34; 49; 61; 70; 125). Results indicate that when carbohydrate is consumed during exercise, time to exhaustion is prolonged (12; 20; 21; 29; 31; 42; 43; 56; 121; 130; 142; 143). Carbohydrate intake during exercise has also been shown to reduce time required to complete a set distance or work volume (2; 4; 6; 8; 92; 93; 95; 97). Improvements in exercise performance have been reported with carbohydrate intakes during exercise ranging from 18 to 180 $g \bullet h^{-1}$ (31; 34; 42; 49; 89; 90; 92; 95; 143).

The improvements in performance experienced with carbohydrate ingestion have been attributed to the maintenance of carbohydrate oxidation late during exercise (14; 21; 29; 136; 142). Carbohydrate oxidation is maintained through the oxidation of exogenous carbohydrate sources (85; 98; 115), the maintenance of blood glucose,(4; 5; 14; 21; 29; 49; 57; 136) and the sparing of endogenous carbohydrate (80; 98; 115). Most research suggests that liver glycogen is spared through the ingestion of carbohydrate prior to and during exercise (5; 49; 55; 57; 70; 85). The sparing of muscle glycogen is not typically reported during cycling exercise when carbohydrate is ingested (14; 20; 29; 31; 42; 43; 89; 125).

Many studies have tested carbohydrate ingestion rates above those recommended by the ACSM and NATA position stands in hopes of optimizing exercise performance and exploring the possibility of a carbohydrate/performance dose response. These studies have failed to demonstrate greater enhancements in performance with carbohydrate ingestion rates above ACSM and NATA recommended ingestion rates compared to the recommended rate range (33; 89; 93). Much less research has been conducted to determine if there is a minimum level of carbohydrate intake needed for improved exercise performance and if there is a dose response to varying carbohydrate levels below what is typically found in sports drinks (17; 42; 138; 143).

While it might be logical that providing increased levels of carbohydrate will provide additional energy for exercise, research has shown that high carbohydrate ingestion rates have been associated with an increased incidence of stomach discomfort and stomach upset which may result in diminished exercise performance (114; 119; 135). Research has also demonstrated that when only glucose is ingested the body is able to

utilize it at a rate of about 1 g•min^{-1} (14; 64; 70; 80; 136). When multiple carbohydrates (i.e. glucose and fructose) are consumed, glucose oxidation rates have been reported to be up to and above 1.5 g•min^{-1} (14; 61; 64; 69; 70; 80; 136).

This study examined the physiological and exercise performance responses of cyclists during a 2-hour constant load ride followed by a simulated 20-km time trial to determine the impact of carbohydrate delivery rates below what is typically recommended (60 g•hr^{-1}) for prolonged exercise. Participants completed four trials including a trial with no carbohydrate and three trials where carbohydrate was ingested in a drink at a rate of 15 g•h^{-1}, 30 g•h^{-1}, and 60 g•h^{-1}.

OPERATIONAL DEFINITIONS

^{13}C – an isotope of carbon containing an additional neutron with a molecular weight of 13

CompuTrainer™ – a bicycle trainer and computer system that allows cyclists to perform cycling exercise while simulating the variations in work that one would experience riding on the road

Endogenous Carbohydrate – carbohydrate that is consumed and stored within the body outside of a testing period

Endogenous Carbohydrate Oxidation – a process in which endogenous carbohydrate stored within the body, prior to the testing period, is being metabolized for energy

Exogenous Carbohydrate – carbohydrate that is consumed during a testing period

Exogenous Carbohydrate Oxidation – a process in which exogenous carbohydrate being ingested is being metabolized for energy

Glucose Oxidation – the metabolism of glucose for energy

Pee Dee Bellemnitella (PDB) – a description of the ratio of $^{13}C/^{12}C$ compared to the Chicago Standard

Plasma – Fluid portion of blood remaining after the removal of red blood cells

Respiratory Exchange Ratio – the ratio of $\dot{V}CO_2$ and $\dot{V}O_2$, often used to describe carbohydrate and fat contribution to energy

Serum – Fluid portion of blood remaining after the removal of red blood cells and clotting factors

Stable Isotope – an isotope of an atomic molecule that is not radioactive

Urine Specific Gravity – a measure of particle concentration in urine as compared to distilled water

$\dot{V}O_{2\ PEAK}$ – the highest oxygen uptake measured during a graded exercise test

The working hypotheses for this investigation are as follows:

Exogenous Carbohydrate Oxidation:

H_O: The null hypothesis states there is no difference in exogenous carbohydrate oxidation as carbohydrate ingestion rate increases.

H_A: The alternative hypothesis states there is an increase in exogenous carbohydrate oxidation as carbohydrate ingestion rate increases.

Exogenous carbohydrate oxidation is calculated from the change in $^{13}C/^{12}C$ ratio in expired CO_2 with the ingestion of ^{13}C labeled glucose.

Endogenous Carbohydrate Oxidation:

H_O: The null hypothesis states there is no difference in endogenous carbohydrate oxidation as carbohydrate ingestion rate increases.

H_A: The alternative hypothesis states there is a decrease in endogenous carbohydrate oxidation as carbohydrate ingestion rate increases.

Endogenous carbohydrate oxidation is calculated by subtracting exogenous carbohydrate oxidation rate from total carbohydrate oxidation.

Time Trial Performance:

H_O: The null hypothesis states there is no difference in time required to complete a 20-km time-trial following 2-hours of constant load cycling when carbohydrate ingestion rate is increased.

H_A: The alternative hypothesis states there is a decrease in the time required to complete a 20-km time-trial following 2-hours of constant load cycling when carbohydrate ingestion rate is increased.

20-km time-trial completion time is measured by the CompuTrainer computer program.

Delimitations

1. Only experienced male cyclists and triathletes were included as subjects
2. Only 12 subjects were tested
3. Subjects performed the 20-km time-trial after 2-hours of constant load cycling exercise
4. Subjects performed the 20-km time-trial on a computer simulated cycling course
5. Subjects began the 2-hour constant load cycling exercise after a 10-hour fast

Limitations

1. Endogenous carbohydrate oxidation was not directly measured
2. Subjects were not allowed to drink ad libitum

II. REVIEW OF LITERATURE

Fatigue during prolonged exercise has been associated with hypoglycemia and depletions of muscle glycogen (20; 29; 53; 129). There is a great deal of evidence demonstrating the ergogenic effect of consuming carbohydrate before and during endurance exercise (2; 12; 14; 20; 21; 29; 31; 42; 43; 56; 121; 130; 142; 143). Sport drinks provide a good avenue to replenish fluids lost during exercise as well as an opportunity to take in an exogenous source of energy (20; 89). The American College of Sports Medicine (ACSM) and the National Athletic Trainers Association (NATA) both have fluid replacement position stands that promote the benefit of carbohydrate intake during exercise (18; 123). Both position stands recommend carbohydrate ingestion rates of 30-60 $g \bullet hr^{-1}$. These research studies and position statements have led to the common use of carbohydrate supplementation during prolonged exercise. A practical hypothesis would suggest greater carbohydrate ingestion rates would enhance exercise performance.

The ergogenic effect of carbohydrate feeding is thought to be partially related to the maintenance of plasma glucose levels and to an increased contribution of glucose as substrate for working muscle (4; 14; 20; 21; 29; 89; 115; 130; 136). During prolonged exercise blood glucose and intramuscular glycogen are the two major sources of carbohydrate utilization by the active muscles (40). A continuous decline in plasma glucose can be observed during exercise when not consuming exogenous energy. This decline demonstrates that hepatic glucose output cannot match glucose uptake by

exercising muscles (93). Carbohydrate oxidation is maintained at higher rates when circulating blood glucose is better maintained (136). Massicotte et al (1986) found that blood glucose is maintained with glucose and fructose ingestion (80). Many cycling studies have found that when carbohydrate is ingested there is a reduction in the decline in blood glucose levels and total carbohydrate oxidation observed during prolonged exercise when consuming a placebo (12; 22; 29; 31; 89). Murray et al (1991) found that the ingestion of carbohydrate during 2-hours of cycling exercise helped maintain blood glucose levels and resulted in improved subsequent exercise performance (93). Tsintzas et al (1998) suggested that the supplementation of carbohydrate will delay fatigue by preventing the reduction in blood glucose and providing an immediately usable fuel source to the exercising muscles (129).

Massicotte and colleagues (1986) also reported elevated insulin levels following glucose ingestion as compared to water and fructose (80). In 1985, Koivisto reported that glucose ingestion can lead to a seven fold rise in plasma insulin levels (73). The increase of serum insulin concentrations will promote an increase in the amount of glucose uptake by the muscle (85; 143). Nicholas (1999) suggested the increased glucose uptake by the exercising muscle would reduce the depletion of muscle glycogen stores by providing an alternative energy source (98).

Increased plasma glucose, as a result of carbohydrate ingestion, reduces the rise in plasma free fatty acids (29; 31). Increases in plasma insulin levels also result in a decline circulating free fatty acid levels (140). This suggests that with increased carbohydrate availability the need to utilize fat for energy to sustain performance is reduced. Massicotte et al (1986) reported that the ingestion of both exogenous glucose and

fructose reduced endogenous carbohydrate and fat utilization as compared to placebo. However, fat utilization was significantly higher when fructose was ingested as compared to ingesting glucose (80).

Muscle glycogen is a crucial energy source to prevent exhaustion during prolonged exercise (10; 11; 54). Both exercise duration and intensity affect the rate of glycogen breakdown during exercise (10; 11; 72). Research shows significant muscle glycogen depletion occurs only after 90-120 minutes of exercise at ~70% $\dot{V}O_{2max}$ (29). It has been suggested that ingested carbohydrate can be used for energy protecting intramuscular glycogen stores which can then be used later during exercise (5; 9; 11; 12; 25; 38; 46; 48-50; 75; 78-81; 98; 104; 108; 110; 113; 124; 126; 130; 131; 143). Researchers suggest that carbohydrate ingestion during exercise does not result in sparing of muscle glycogen stores (14; 20; 29; 31; 42; 43; 73; 89; 100; 125). Most of the experiments that have demonstrated muscle glycogen sparing have used muscle biopsy techniques. A reason for the different findings may be the single muscle group analysis with the biopsy technique compared to the total body analysis with the tracer technique. Another possible explanation for the differences in metabolism between the studies may be accounted for by the exercise form chosen for the studies (130). These differences may help account for the differences found in the literature.

While there is debate concerning muscle glycogen sparing, research demonstrates hepatic glucose output is reduced with carbohydrate ingestion (14; 70; 85). Exogenous carbohydrate appears to supplement hepatic glucose production and maintain blood glucose levels (5; 29; 49; 57; 136). With exogenous glucose aiding in the maintenance of plasma glucose less demand is placed on the liver for glucose production (14; 70; 85).

With available carbohydrate stores diminishing there is a reduction in carbohydrate oxidation during prolonged exercise. Carbohydrate oxidation can be maintained during the latter stages of prolonged exercise with carbohydrate feeding (14; 21; 29; 142). McConnell et al (1994) suggested that when glycogen stores are depleted in adults the primary source of energy in prolonged exercise comes from exogenous carbohydrate sources (85). In 1998, Sugiura et al reported that carbohydrate oxidation was lower in placebo trials as compared to trials when subjects received either glucose or fructose (127).

Depending on the type of carbohydrate ingested benefits can vary. Massicotte et al (1986) reported that 75% of ingested glucose was oxidized over a 3-hour exercise period whereas only 56% of ingested fructose was oxidized (80). However, there is no difference in carbohydrate delivery or oxidation when glucose polymer is ingested as compared to the ingestion of a simple glucose solution (52; 81). Hawley et al (1992) found that there was no difference in carbohydrate oxidation rates when either glucose or maltose was ingested (52). Wagenmakers et al (1993) found no difference in the oxidation rates of sucrose and maltodextrin (136). In a series of papers by Jentjens and Jeukendrup, it was found that ingesting multiple forms of carbohydrate during exercise resulted in a significantly higher oxidation rate as compared to ingesting a single carbohydrate form (61; 64; 69).

The amount and type of carbohydrate ingested will also play a part in its availability to muscle as fuel. For ingested carbohydrate to be utilized by the exercising muscle, it must first be emptied from the stomach and absorbed by the intestines. Early research suggested that beverages designed to rehydrate should not contain more 2.5%

carbohydrate concentrations levels because carbohydrate beverages emptied from the stomach more slowly than water (7; 27; 30; 44). Later research determined that carbohydrate (glucose, fructose, sucrose, and maltodextrin) concentrations between 5 and 7.5% emptied from the stomach at a rate no different than water (90; 96). Other researchers have reported that carbohydrate concentrations in excess of 6% can reduce the rate of gastric emptying (114; 116; 135). Caloric content has been determined to be the primary determinant of gastric emptying (86; 91).

Once the ingested carbohydrate reaches the intestine, the type of carbohydrate can impact the rate absorption. Carbohydrate concentrations in excess of 6% can slow the rate of absorption (119). Glucose is absorbed in the intestine by a sodium dependent glucose transporter (SGLT 1) in the brush border membrane and paracellular absorption (41; 116). Fructose is reportedly absorbed via facilitated diffusion and GLUT 5 transporters (15; 41; 47; 117). The rate at which fructose is absorbed in humans is slower than that seen with glucose (112). Due to the delayed absorption rate with fructose, ingestion can lead to gastric discomfort and distress (94; 127).

Not all ingested carbohydrate is oxidized for energy. When a single form of carbohydrate is ingested the percentage being oxidized ranges from 32-80% (61; 64; 69; 70; 80; 136). When multiple carbohydrate forms are ingested the percentage that is being oxidized ranges from 55-93% (61; 64; 69; 136). Jeukendrup (1999) and Jentjens (2004) demonstrated that increased ingestion rates resulted in lower percentages of the ingested carbohydrate being oxidized (61; 69). Questions arise as to the reason all of the ingested carbohydrate is not oxidized even when carbohydrate ingestion rate is low. It has been reported that most carbohydrate ingested is emptied from the stomach, meaning gastric

emptying is not the rate limiting step for exogenous carbohydrate oxidation (114). It has been suggested that some of the carbohydrate that is ingested may remain in the intestines or may go to the liver or inactive muscles to be stored (70).

Research has shown that there is a threshold to carbohydrate intake that when exceeded does not improve endogenous carbohydrate sparing or performance. Wagenmakers et al (1993) found that carbohydrate intakes above 74 $g \bullet hr^{-1}$ did not improve carbohydrate oxidation or endogenous carbohydrate sparing (136). Jentjens et al reported that exogenous glucose oxidation rates do not increase when glucose intake is increased from 1.2 $g \bullet min^{-1}$ to 1.8 $g \bullet min^{-1}$ (61; 136).

Methodology has been developed and tested demonstrating the ability to use isotopes of carbon to non-invasively analyze the oxidation rates of exogenous carbohydrate and estimate endogenous substrate utilization during exercise (35; 48; 50; 52; 58; 61; 64; 69; 74; 78-82; 102; 104-106; 108-110; 113; 115; 122; 136). This type of research has demonstrated that various forms of carbohydrate consumed immediately prior to and during exercise is readily used for energy (61; 64; 80; 81; 104; 108). The rate at which glucose enters the systemic system seems to be the limiting factor for exogenous carbohydrate oxidation (70).

When a single type of carbohydrate is ingested peak oxidation rates are approximately 1 $g \bullet min^{-1}$ (51; 65; 70; 109; 120; 136). This is likely the result of intestinal SGLT 1 glucose transporters being saturated. It has been reported that SGLT 1 may be saturated at 1.0 $g \bullet min^{-1}$ (111). Exogenous oxidation rates have been reported to exceed 1.2 $g \bullet min^{-1}$ when multiple forms of carbohydrate are ingested at high rates (61; 64; 69).

The increase in exogenous oxidation rate may be the result of the ability to use both glucose and fructose intestinal transporters (61).

When using carbon isotope techniques several assumptions are made and need to be considered before making conclusions. The computation of the oxidation rate of exogenous glucose is made assuming that, in response to exercise, ^{13}C is not irreversibly lost in pools of the tricarboxylic acid cycle intermediates and/or bicarbonates and that $^{13}CO_2$ recovery in expired gases is complete or almost complete (23; 76). Since some of the ingested ^{13}C is going into the bicarbonate pools, there is a delay in researchers ability to utilize the $^{13}CO_2$ being collected at the mouth (144) and the baseline shift in $^{13}CO_2$ production from endogenous sources (79). It has also been suggested that the use of ^{13}C may lead to overestimations of CHO oxidation while the use of ^{14}C may result in underestimations of CHO oxidation (106).

In some instances the estimates of exogenous carbohydrate oxidation may exceed the amount of carbohydrate ingested. Peronnet et al (1990) described this error as an over simplification of the assumption that all of the excess $^{13}CO_2$ is the result of the ingestion of the exogenously labeled substrate (106). The North American diet consists of many food ingredients, such as maze and cane sugar, which naturally contain high levels of ^{13}C. This can result in increased amounts of ^{13}C being stored in endogenous carbohydrate stores. As the endogenous stores of carbohydrate are oxidized for energy, the ^{13}C in it is expired as additional $^{13}CO_2$. This has been reported by Wolfe (1984) (141) and repeatedly by Massicotte (78; 80; 81). Overestimations of exogenous carbohydrate are less likely when the exogenous carbohydrate is highly enriched (106). Researchers have restricted participants' food selections and artificially enriched beverages with

labeled ^{13}C-glucose and ^{13}C-fructose to overcome the naturally occurring elevations in ^{13}C (50; 79-81; 107; 108). Since products containing naturally high levels of ^{13}C are not as widely used in Europe, many ^{13}C carbohydrate oxidation studies conducted in Europe have utilized corn-derived glucose, crystalline fructose, and/or sugar cane-derived sucrose to analyze exogenous carbohydrate oxidation successfully (59; 60; 63; 64; 67-69).

Not all positive physiological changes resulting from carbohydrate ingestion translate to improved performance. Mitchell (1989) found that ingestion of a beverage delivering 111 g•hr^{-1} of carbohydrate elevated blood glucose more than other treatments but this treatment's performance measure was no better than placebo (89).

Reviewing 73 studies with 110 carbohydrate treatments, carbohydrate improved performance compared to a non-carbohydrate treatment in 61 of the 110 treatments (1; 3; 4; 24; 37; 39; 45; 66; 77; 88; 93; 98; 99; 101; 116; 127; 133; 134; 139; 143). Unfortunately, variations in carbohydrate type, concentration, delivery form, delivery schedule, and testing protocol used in many of the studies make comparisons of dosage benefits difficult. A number of studies have directly or indirectly addressed the question of whether a dose-response relationship exists between carbohydrate intake and performance (84; 89; 90; 92; 95). A dose-response relationship has yet to be found (92; 93).

Carbohydrate's impact on exercise performance has been assessed using various methods. The most common method to analyze the impact of carbohydrate on exercise performance is cycling exercise. Reasons cycling is the most common endurance exercise mode to study carbohydrate feeding may result from difficulty of ingesting fluid

and increased incidence of gastric discomfort when running (19). The most common measures used to assess exercise performance are rides to exhaustion (12; 17; 20; 21; 29; 31; 34; 42; 49; 56; 57; 84; 99; 121; 132; 142; 143) amount of work completed in a set time (36; 37; 43; 89; 90; 97),and time to complete a set amount of work (3; 13; 33; 39; 87; 88; 92; 93; 95; 98; 116).

Studies using time to exhaustion to analyze the impact of nutrient ingestion on exercise performance have found many performance enhancements when ingesting carbohydrate as compared to a placebo (12; 17; 20; 21; 29; 31; 34; 42; 49; 56; 57; 84; 99; 121; 132; 142; 143). Coyle et al (1986) and Nicholas et al (1995) reported carbohydrate ingestion increased time to fatigue by 33% (29; 99). In 1984, Hargreaves et al reported a 45% increase in time to exhaustion when carbohydrate was ingested during exercise (49). Similarly, using the amount of work has repeatedly demonstrated a performance improvement when carbohydrate is ingested (36; 37; 43; 89; 90; 97). While time to exhaustion and amount of work completed in a set amount of time seem to be good methods to demonstrate relationships in nutrient ingestion and performance, they both lack related exercise situations.

Based on the findings of this previous research, this study examined the physiological and exercise performance responses of cyclists during a 2-hour constant load ride followed by a simulated 20-km time trial to determine the impact of carbohydrate delivery rates below what is typically recommended (60 $g \bullet hr^{-1}$) for prolonged exercise. Participants completed four trials including a trial with no carbohydrate and three trials where carbohydrate was ingested in a drink at a rate of 15 $g \bullet h^{-1}$, 30 $g \bullet h^{-1}$, and 60 $g \bullet h^{-1}$.

III: METHODS

Participants:

Twelve trained male cyclists and triathletes participated in this study. Mean and standard error of age, height, mass, and peak oxygen uptake ($\dot{V}O_{2\ PEAK}$) were 31.7 ± 1.1 yrs, 1.82 ± 0.02 m, 77.6 ± 2.0 kg, and 4.12 ± 0.09 $l \bullet min^{-1}$, respectively. Participants served as their own controls for the study. All participants read and signed an informed consent approved by the Human Subject Review Committee prior to beginning the study.

Preliminary Testing:

Peak oxygen uptake ($\dot{V}O_{2\ PEAK}$) was determined using an increasing resistance, multistage cycling test with 30 second Douglas Bags collected during the last 30 seconds of each stage. Expiratory gases were analyzed using an Ametek S-3A/I Oxygen and Ametek CD-3A Carbon Dioxide Analyzers (Naperville, IL). Expired volume was measured with a spirometer (Vacumed Inc., Ventura CA). A regression analysis of the $\dot{V}O_2$-workload relationship determined the exercise workloads for lactate threshold testing.

The participants' two hour ride workload was set at 95% of the workload that would elicit a 4 $mmol \bullet L^{-1}$ blood lactate. Preliminary research in our lab found that this was the highest intensity most participants could maintain for two hours of cycling. Subjects exercised for 3-minute stages at 55, 60, 65, 70, 75, 80, 85, and 90% of their

$\dot{V}O_{2PEAK}$ with blood samples taken for lactate analysis at the end of each stage. Blood was analyzed using a whole blood analyzer (Gem Premier 3000, Instrumentation Laboratory, Lexington, MA). All testing was performed with the participants exercising on their own bicycle affixed to a CompuTrainer™ Pro (RacerMate Inc, Seattle WA).

Participant Orientation:

Participants were required to perform three familiarization course rides prior to beginning the actual trials, with at least seven days between course rides. The first familiarization was performed to allow the participant to become familiarized with the 20-km time trial course. The second familiarization was performed to allow the participant to familiarize himself with the feel of a 2-hour ride followed by the 20-km time trial. This model is commonly used in metabolic fuel investigations but also has application to competitive cycling events. The third and final familiarization was performed to allow the participant to experience the testing procedures.

Experimental Protocol:

Participants completed four exercise trials with randomized interventions separated by at least seven days. Trials were conducted in a laboratory maintained at 20-25˚C, 35-40% relative humidity. Participants were instructed to record and maintain the same diet and abstain from exercise 24 hours before each trial. Participants reported to the lab after a 10-hour overnight fast.

After arriving at the lab, participants voided and body weights were recorded. A registered nurse then inserted an intravenous catheter (BD Insyte™ Autoguard™,

Becton, Dickinson Infusion Therapy Systems Inc., Sandy UT) into an antecubital vein with a three-way stopcock (Solution-Plus™, Mansfield, MA) to collect baseline blood samples and allow for blood sampling throughout the trial.

Participants performed a standardized ten-minute ride at 100 watts to warm up and prepare the trainers for calibration. Following the 10-minute ride the CompuTrainers™ were calibrated according to the manufacturer's recommendations.

On average, participants workload was set at 227.5 ± 2.2 watts, after which they began a two hour constant load ride on the CompuTrainer™ with $\dot{V}O_2$, ratings of perceived exertion, and heart rate collected every 15 minutes and blood samples collected every 15 minutes during the final hour (Figure 1).

After completing the 2-hour ride participants stopped pedaling and were allowed off the bike for one and a half minutes. Two-minutes after the cessation of the two hour constant load ride, participants began the simulated 20-km time trial. For the time trial participants were instructed to complete the 20-km course (Figure 2) as quickly as possible. The undulating course was designed using the CompuTrainer™ 3D computer program.

Participants were aware of the approximate distance traveled and remaining from a course profile showing their position on the course but no verbal stimuli or other information was given. Resistance varied throughout the ride based on the inclination of the course and the participants' speed and gearing, similar to that experienced when cycling outdoors. Participants were allowed to change gears at will throughout the course of the ride.

Intervention Beverages:

Participants completed four trials during this study. The intervention beverages tested during the trials were 1) 250 mL of Placebo with electrolytes (PLA); 2) 250 ml of 1.5% glucose with electrolytes (15 $g \bullet hr^{-1}$); 3) 250 ml of 3.0% glucose with electrolytes (30 $g \bullet hr^{-1}$); and 4) 250 ml of 6.0% glucose with electrolytes (60 $g \bullet hr^{-1}$). Beverages were consumed every 15 minutes during the 2-hour ride.

All beverages were formulated to be identical in flavor and sweetness, and kept at a temperature of 1˚C to minimize differences in taste. The test beverages were given to the participant in opaque plastic containers.

To allow for the calculation of exogenous glucose oxidation, uniformly labeled ^{13}C-glucose (Isotec™, Miamisburg, OH) was added at 1.8 $mg \bullet g^{-1}$ of glucose contained within the experimental beverage. This resulted in beverages with high abundances of ^{13}C (15: 121 Pee Dee Bellemnitella (‰ [δ-^{13}C]PDB-1), 30: 136 ‰ [δ-^{13}C]PDB-1, and 60: 151 ‰ [δ-^{13}C]PDB-1).

Data Collection:

Heart Rate and Perception

Heart rate was measured with a chest strap and watch telemetry system (Polar Electro Inc., Lake Success, NY). Heart rate and a Borg Scale was used to determine athletes' perceived exertion was recorded every 15 minutes during the two hour ride.

Physiological Measures and Metabolites

A 60-second expired air sample was collected every 15 minutes during the 2-hour ride. The expired air sample was analyzed for oxygen and carbon dioxide partial pressures and the expired volume was measured using a flow meter. With the expired air measures, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were calculated, neglecting the small contribution of protein oxidation to the energy yield (71; 107). Total carbohydrate (TCO) and fat oxidation rates were calculated using equation 1 and 2, respectively: (28)

$$(g \bullet min^{-1}) = (4.59 \bullet \dot{V}CO_2) - (3.23 \bullet \dot{V}O_2) \quad \text{(Equation 1)}$$

$$(g \bullet min^{-1}) = 1.70 \bullet (\dot{V}O_2 - \dot{V}CO_2) \quad \text{(Equation 2)}$$

Whole blood samples were used to measure plasma lactate, hematocrit, and hemoglobin using a Gem Premier 3000 whole blood analyzer from Instrumentation Laboratory. Plasma glucose was measured using a liquid glucose (Hexokinase) Reagent Set kit (Pointe Scientific, Canton, MI). Insulin was measured using a Human Insulin ELISA kit (Millipore Corp, St. Charles, MO). Free-fatty acids were measured using a non-essential fatty acid HR2 series reagents kit (Wako Diagnostics, Richmond, VA). Cortisol was measured using a Cortisol kit (Pointe Scientific, Canton, MI). All samples were analyzed on a Multisample Spectrophotometer (Synergy HT, Bio-tek Instruments, Winooski, MA).

Tracer

To determine exogenous carbohydrate oxidation rate expired gas was captured in a 10 mL vacutainer (BD Vacutainer™, Franklin Lakes, NJ) for analysis of expired $^{13}CO_2/^{12}CO_2$. Expired $^{13}CO_2/^{12}CO_2$ ratio was measured using a BreathMat Plus (Finnigan MAT). Exogenous glucose oxidation rate (EGO) was calculated using equation 3: (70; 108)

$$(g \bullet min^{-1}) = \dot{V}CO_2 \bullet \frac{R_{OBS} - R_{REF}}{R_{ING} - R_{REF}} \bullet \frac{1}{k} \quad \text{(Equation 3)}$$

Where R – isotopic ratio, R_{OBS} – The observed $^{13}C/C$ ratio in the breath, R_{ING} – The $^{13}C/C$ ratio of the beverage ingested, R_{REF} – The baseline breath $^{13}C/C$ ratio, and k (0.747 $L \bullet g^{-1}$) is the amount of $\dot{V}CO2$ provide by the oxidation of 1 g of glucose.

During the second hour of exercise, plasma samples were collected to allow for the calculation of exogenous glucose present in the blood, plasma glucose oxidation, liver glucose oxidation, and muscle glucose oxidation. These calculations were made only during the final 60 min of the 2-hour constant load ride allowing 60 min of equilibration. This 60 min equilibration is based on observations that $^{13}C/^{12}C$ in expired CO_2 equilibrates slowly with the $^{13}C/^{12}C$ in the CO_2 produced in tissues (103), that ^{13}C provided from ^{13}C-glucose is not irreversibly lost in tricarboxylic acid cycle intermediates (118) and/or bicarbonate (128) pools, and that the $^{13}CO_2$ recovery in expired gases is complete or almost complete.

Plasma was deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). Glucose was then separated from the plasma by double-bed ion-exchange chromatography (AG 50W-X8 H^+ and AG 1-X8 chloride, 200-400 mesh; Bio-Rad, Mississauga, ON, Canada). The elute was then evaporated to dryness (Virtis Research Equipment, New York, NY) and combusted with copper oxide for 60 minutes at 400°C. The CO_2 was recovered from the glucose combustion for isotopic analysis. The measurement of $^{13}C/^{12}C$ in the CO_2 coming from combusted glucose was then performed using mass spectrometry (Prism, Manchester, UK). The isotopic ratio of the combusted plasma glucose was expressed in percentage difference (Equation 4) by comparison with the PDB Chicago Standard:

$$‰\ [\delta\text{-}^{13}C]PDB\text{-}1 = [Rspl/Rstd) - 1] \text{ X } 1{,}000 \quad \text{(Equation 4)}$$

Where Rspl – $^{13}C/C$ ratio in the sample and Rstd – $^{13}C/C$ ratio in the standard (1.1237 ‰), respectively (16).

The percentage of plasma glucose derived from exogenous glucose was calculated using equation 5:

$$(\%) = \frac{R_{GLU} - R_{REF}}{R_{ING} - R_{REF}} \bullet 100 \quad \text{(Equation 5)}$$

Where R – isotopic ratio, R_{GLU} – The observed $^{13}C/C$ ratio in the blood, R_{ING} – The $^{13}C/C$ ratio of the beverage ingested, and R_{REF} – The baseline blood $^{13}C/C$ ratio.

Plasma glucose oxidation (PGO) was calculated using equation 6: (108)

$$(g \bullet min^{-1}) = \frac{\text{Plasma Glucose being Oxidized}}{\text{Exogenous Plasma Glucose Concentration}} \quad \text{(Equation 6)}$$

Liver glucose oxidation (LGO) was calculated using equation 7: (28; 50)

$$(g \bullet min^{-1}) = PGO - EGO \quad \text{(Equation 7)}$$

Muscle glycogen oxidation (expressed in grams of glucose/min) was calculated using equation 8: (28; 50)

$$(g \bullet min^{-1}) = TCO - PGO \quad \text{(Equation 8)}$$

Statistical Analysis:

All data are expressed as mean ± standard error. Statistical analysis was performed using SPSS version 13 (SPSS Inc. Chicago, IL). Data were analyzed with a univariate ANOVA. When ANOVA reported significant interactions, a Duncan post-hoc analysis was performed. Significance was set at $p \leq 0.05$.

IV: RESULTS

Physiological Measures:

Heart rate increased significantly over the duration of the 2-hour constant load ride (Figure 3). RPE significantly increased over the duration of the 2-hour constant load ride (Figure 4). RPE was significantly higher during 15 g•hr^{-1} as compared to all other trials and 30 g•hr^{-1} was significantly lower than PLA. $\dot{V}O_2$ during the 2-hour constant load ride averaged 3.17 ± 0.08, 3.21 ± 0.09, 3.19 ± 0.10, and 3.16 ± 0.09 for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1}, respectively (Figure 5). This average $\dot{V}O_2$ of the four trials represented 77.2 ± 0.9% of the participants' $\dot{V}O_{2\ PEAK}$. There was a significant reduction in RER (Figure 6) and carbohydrate oxidation (Figure 7) while fat oxidation significantly increased (Figure 8) during the 2-hour constant load ride ($p \leq 0.05$). RER and carbohydrate oxidation was significantly higher while fat oxidation were significantly lower in the 60 g•hr^{-1} trial as compared to all other trials ($p \leq 0.05$).

Blood, Hormone, and Metabolite Measures:

There was no difference in baseline blood glucose, insulin, or free-fatty acid measures. There were significant reductions in plasma glucose (Figure 9) and insulin (Figure 10) levels during the last hour of the 2-hour constant load ride. With reductions in carbohydrate ingestion below 30 g•hr^{-1}, plasma glucose levels declined at a significantly greater rate as carbohydrate ingestion declined. Plasma glucose levels were

significantly lower in the PLA trial as compared to the 15 g•hr^{-1} trial. Plasma insulin levels were significantly higher in the 60 g•hr^{-1} trial as compared to all other trials. There was a significant rise in plasma free-fatty acid levels during the last hour of the 2-hour constant load ride (Figure 11). There was no difference in plasma free-fatty acid levels when comparing the PLA and 15 g•hr^{-1} trials. As carbohydrate ingestion increased above 15 g•hr^{-1}, plasma free-fatty acid levels significantly decreased as glucose ingestion increased. There was a significant increase in plasma lactate levels during the 2-hour constant load ride (Figure 12), but no difference during exercise and no difference between treatments. Average lactate levels during exercise were 2.96 ± 0.47 mmol•L^{-1}, 2.99 ± 0.55 mmol•L^{-1}, 2.75 ± 0.53 mmol•L^{-1}, and 2.83 ± 0.52 mmol•L^{-1} for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1}, respectively. Plasma cortisol levels rose significantly during the second hour of the 2-hour constant load ride (Figure 13). There was no difference in plasma cortisol levels. Average cortisol levels during the 2-hour constant load ride were 243.05 ± 41.24, 249.33 ± 39.15, 236.40 ± 37.95, and 242.28 ± 35.09 for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1}, respectively.

Exogenous and Endogenous Glucose Utilization:

The expired $^{13}C/C$ ratio significantly increased during the 2-hour constant load ride with all treatments being significantly different at the beginning of the second hour of exercise (Figure 14). The percentage of exogenous glucose in the plasma increased significantly as exogenous carbohydrate intake increased (Figure 15). During the 2-hour constant load ride, exogenous glucose oxidation significantly increased as glucose ingestion rate increased. Exogenous glucose oxidation rates during the last 30 minutes of

the constant load cycling were 0.26 ± 0.05, 0.44 ± 0.04 and 0.66 ± 0.07 g•min^{-1} for 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} ingestion rates, each being significantly different from all others ($p \leq 0.001$). There was no difference in total plasma glucose oxidation in the 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} trials. Since ^{13}C was not given in the PLA trial we are unable to compare plasma glucose oxidation in the PLA trial. There was, however, a significant increase in liver glycogen oxidation during the last hour of the 2-hour constant load ride. Liver glycogen oxidation rate was highest when consuming 15 g•hr^{-1} (0.63 ± 0.13 g•min^{-1}) followed by 30 g•hr^{-1} (0.51 ± 0.12 g•min^{-1}) and 60 g•hr^{-1} (0.42 ± 0.08 g•min^{-1}), all significantly different from one another at a $p \leq 0.05$ level. There was also significant reduction in muscle glycogen oxidation during the last hour of the 2-hour constant load ride with no significant differences found between the 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} trials. No tracer was given in the PLA to examine muscle glycogen oxidation during the PLA trial. (Figures 16)

Performance Measures:

The time required to complete the 20-km time trial was 36.39 ± 0.84 min, 35.23 ± 0.80 min, 34.99 ± 0.75 min, and 34.69 ± 0.62 min for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1}, respectively. The change in time required to complete the 20-km time trial compared to PLA are shown in Figure 17. The average wattage during the time trial for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} was 212.0 ± 9.5 W, 227.9 ± 10.7 W, 229.7 ± 10.6 W, and 234.2 ± 9.4 W, respectively. The change in average wattage during the 20-km time trial compared to PLA are shown in Figure 18. PLA required significantly more time to complete the time trial and resulted in a significantly lower average wattage

during the time trial compared to 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} ($p \leq 0.05$). However, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} were not significantly different from one another.

Figure 1: Order of test measures

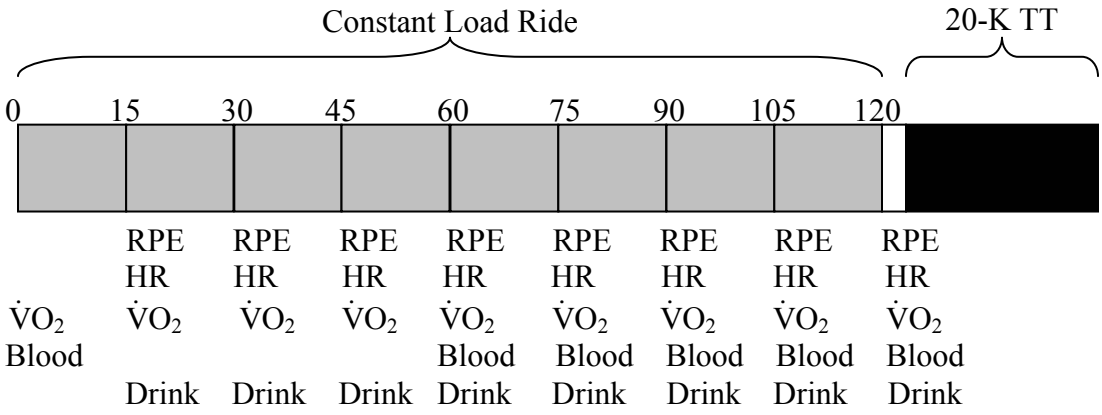
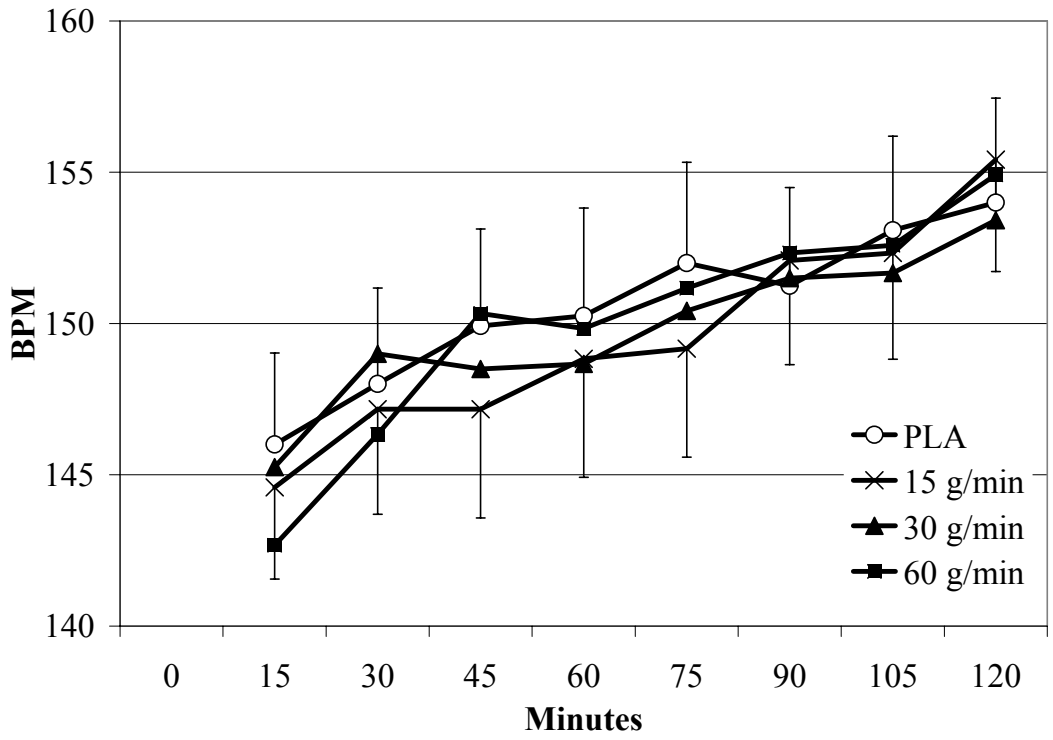


Diagram of the testing protocol.

Figure 2: 20-kilometer course profile

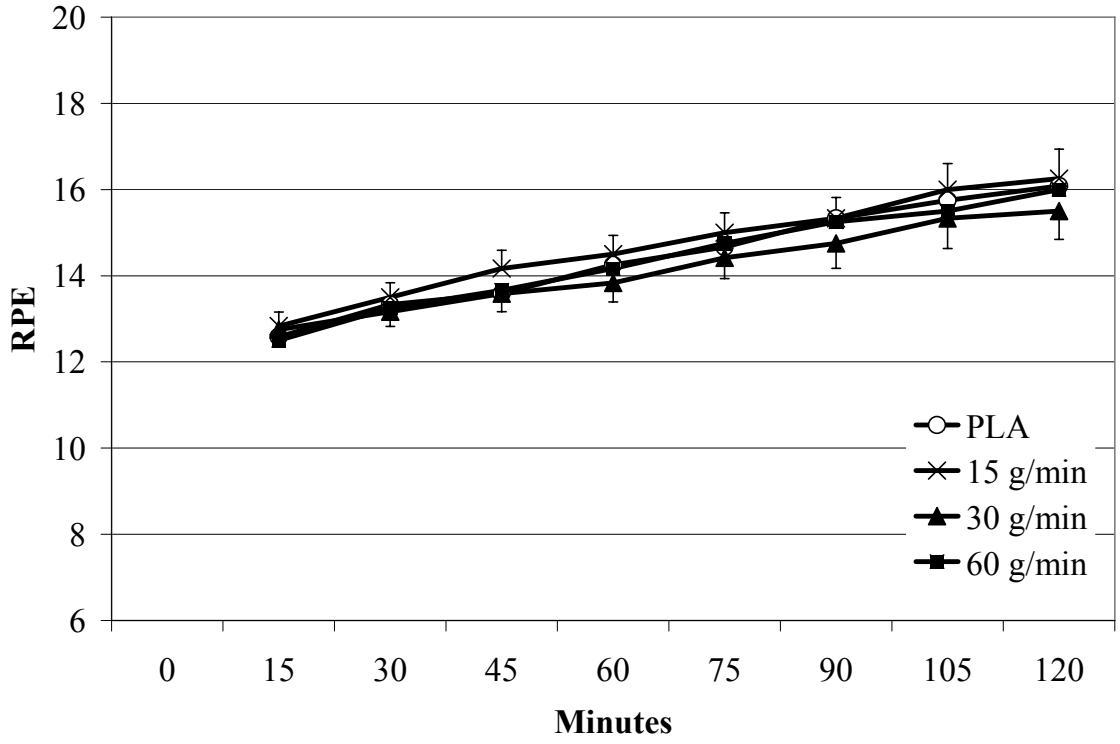
Course profile of the 20-km time trial.

Figure 3: Heart rate during 2 hour constant load ride



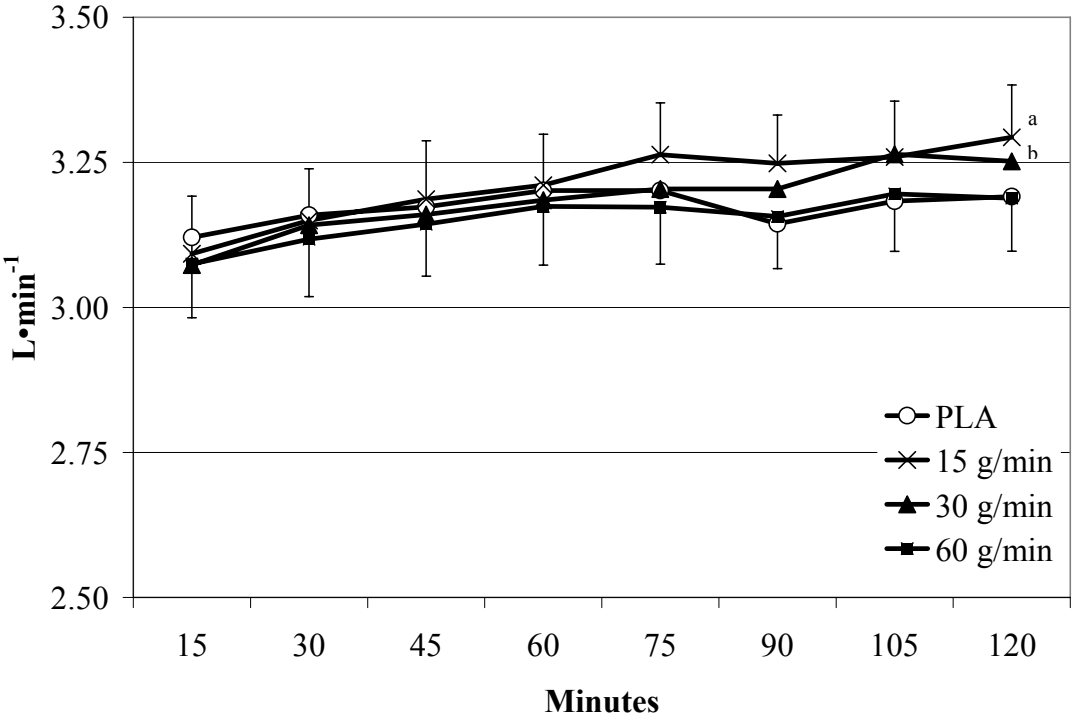
Heart rate response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). There were no significant differences seen between treatments. There is a significant main effect for time ($p < 0.05$).

Figure 4: Ratings of perceived exertion during the 2-hour constant load ride



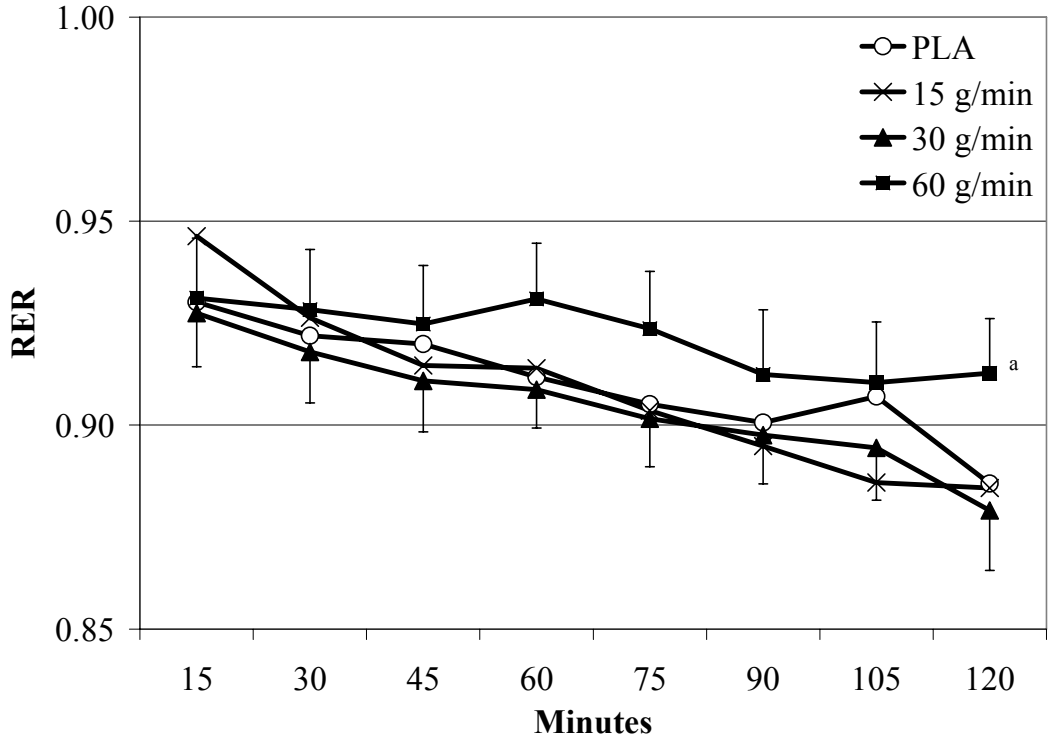
Ratings of perceived exertion during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). PLA is significantly different than 15 g•hr^{-1} and 30 g•hr^{-1}, 15 g•hr^{-1} is significantly different than PLA, 30 g•hr^{-1}, and 60 g•hr^{-1}, There is a significant main effect for time ($p < 0.05$).

Figure 5 – $\dot{V}O_2$ during exercise



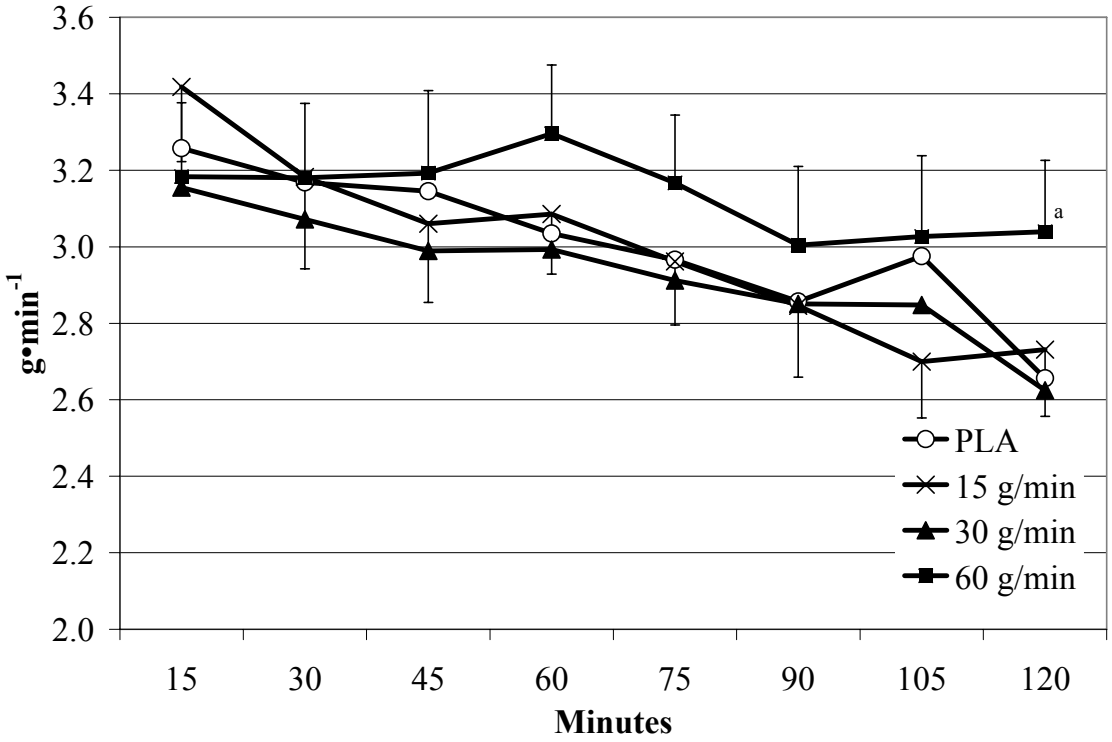
Oxygen uptake response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA and 60 g•hr^{-1}. [b] significantly higher than 60 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 6 – Respiratory exchange ratio during exercise



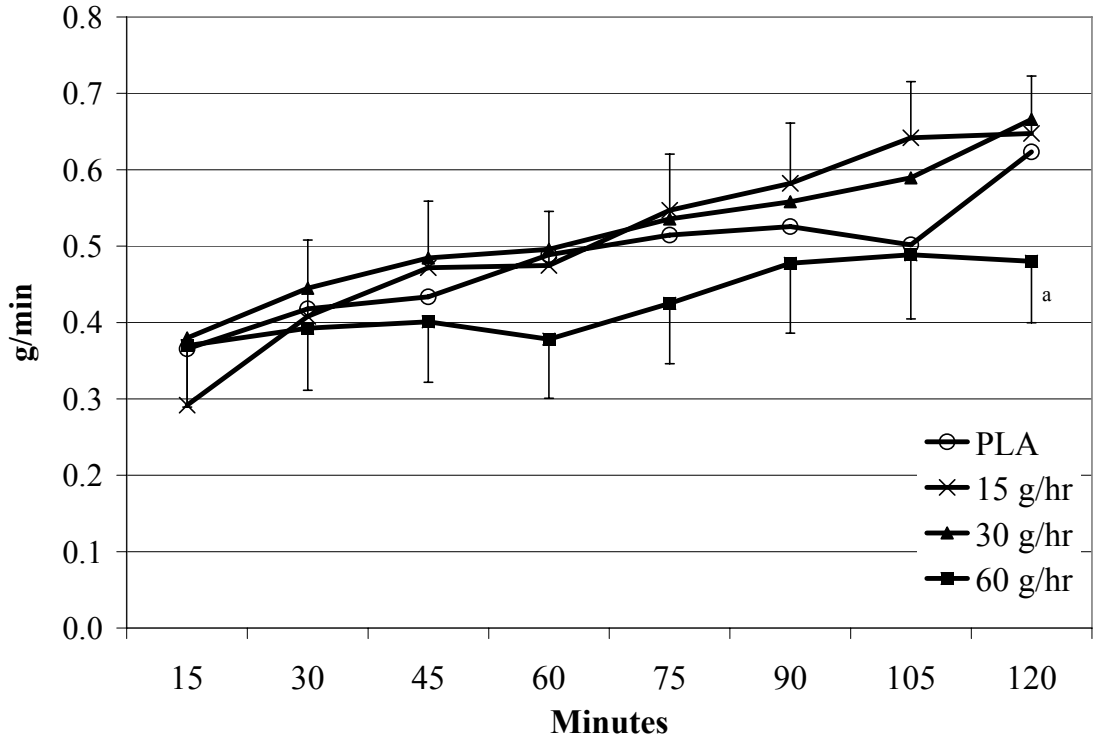
Respiratory exchange ratio response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA, 15 g•hr^{-1} and 30 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 7 – Carbohydrate oxidation during exercise



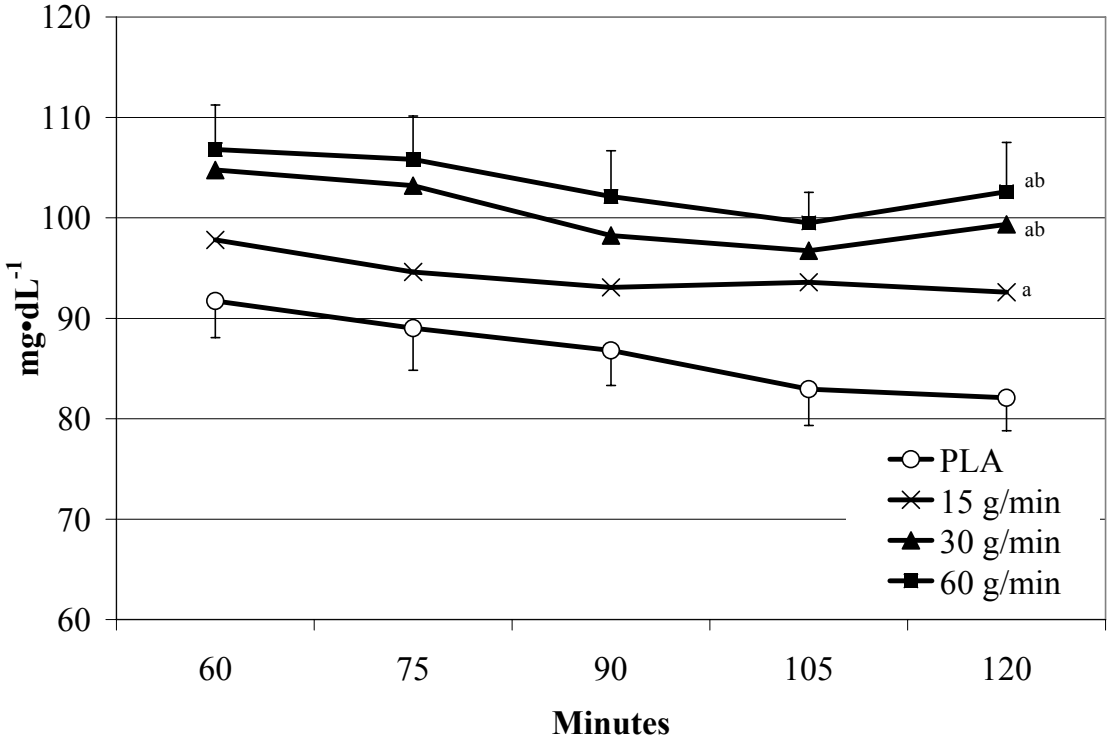
Carbohydrate oxidation response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA, 15 g•hr^{-1},and 30 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 8 – Fat oxidation during exercise



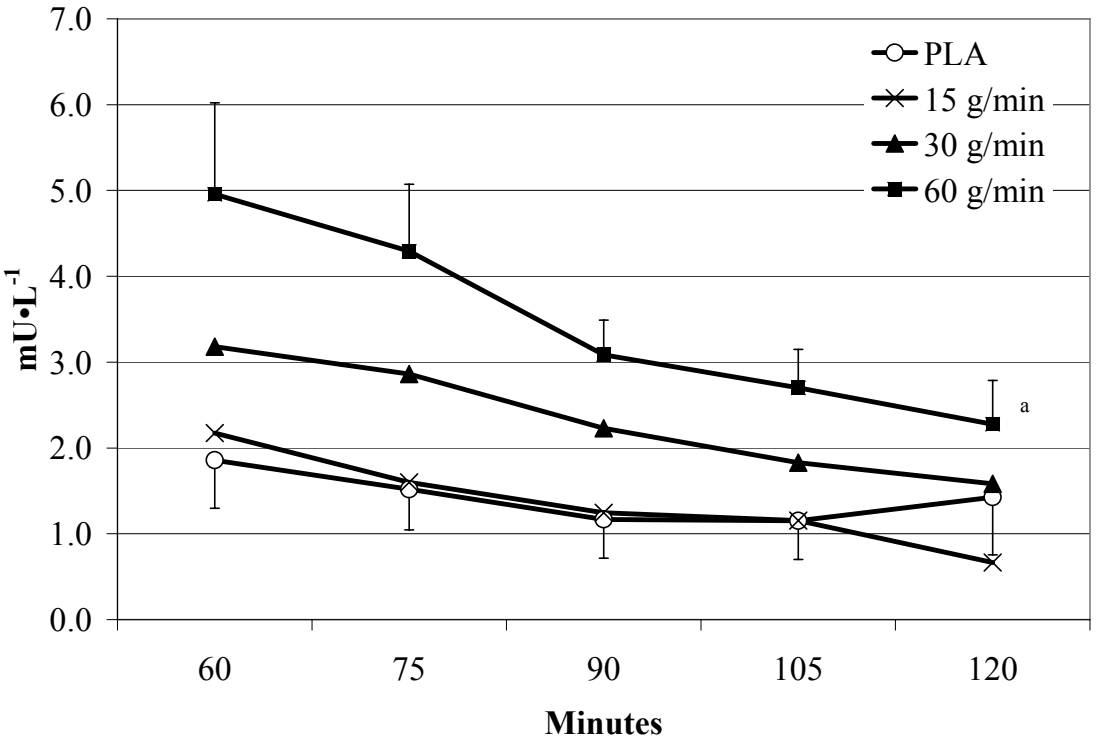
Fat oxidatoion during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly lower than PLA, 15 g•hr^{-1},and 30 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 9 – Blood glucose response during exercise



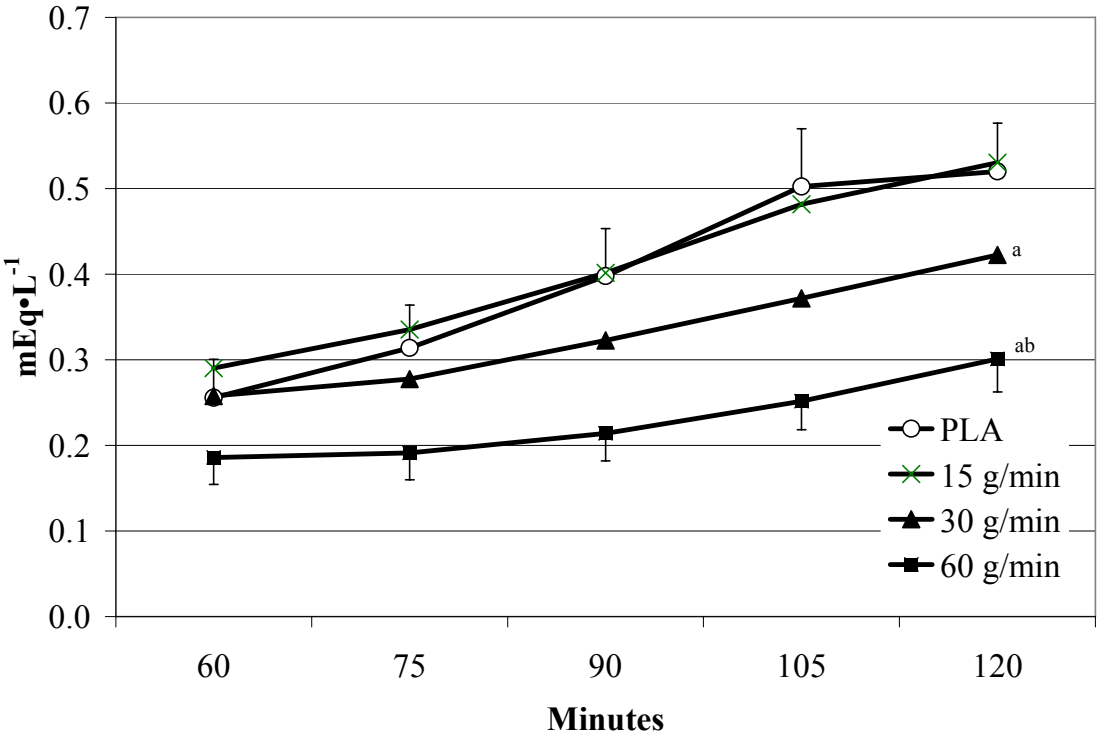
Plasma glucose response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than 0; [b] significantly higher 15. There is a significant main effect for time ($p < 0.05$).

Figure 10 – Insulin response during exercise



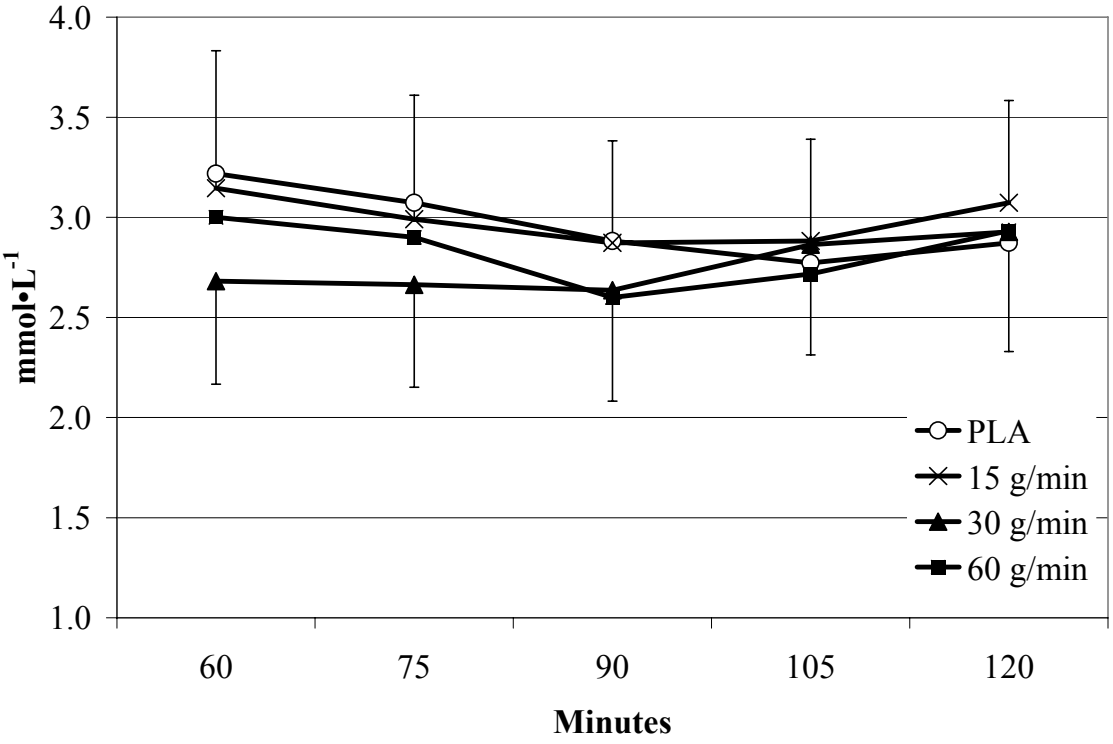
Plasma insulin response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than all other treatments ($p < 0.05$). There is a significant main effect for time ($p < 0.05$).

Figure 11 – Free fatty acid response during exercise



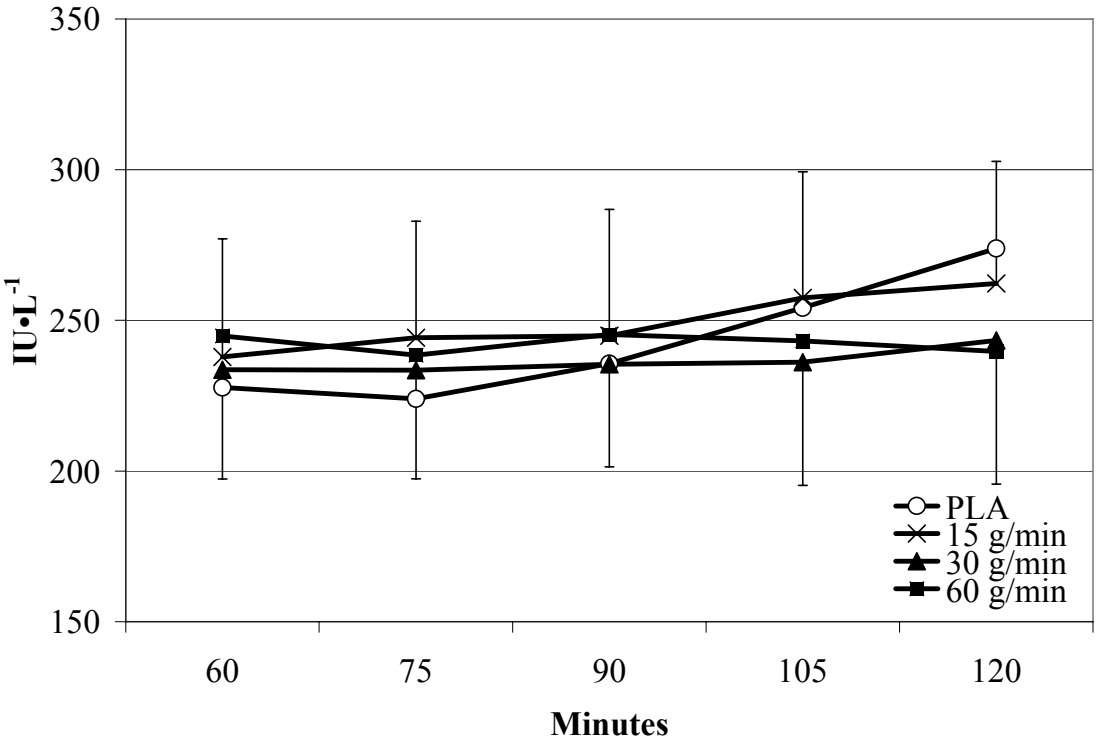
Serum free-fatty acid response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly lower than 0 and 15; [b] significantly lower 30. ($p < 0.05$). There is a significant main effect for time ($p < 0.05$).

Figure 12 – Lactate during 2-hour constant load exercise



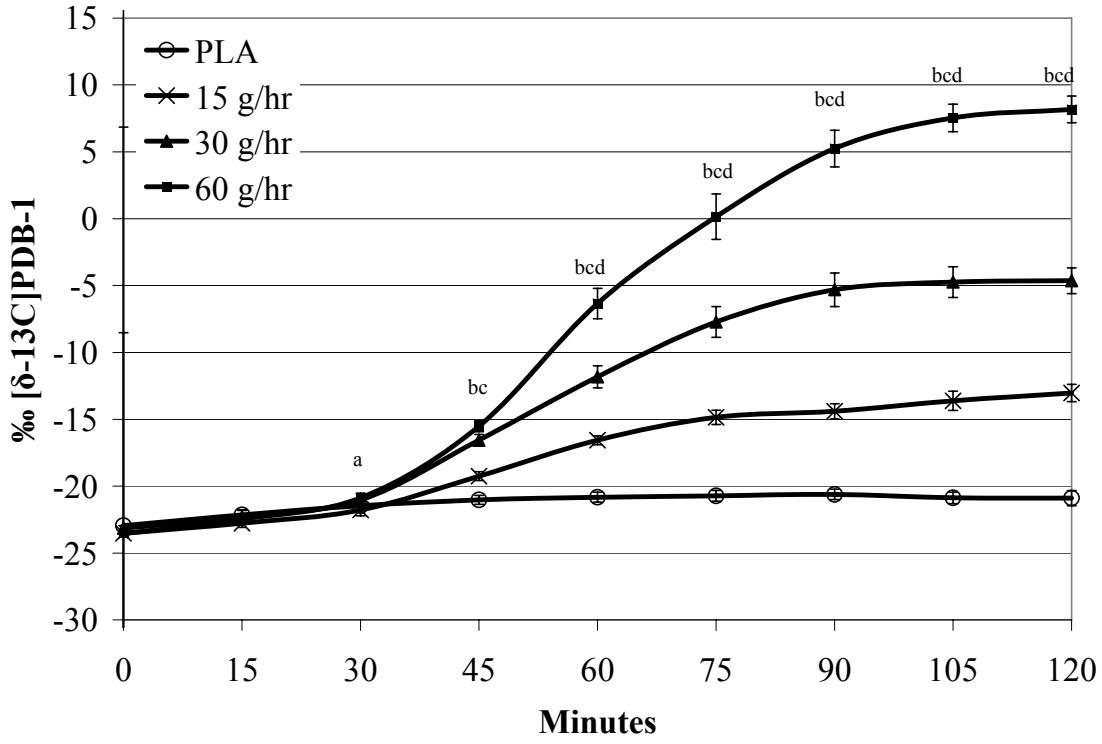
Plasma lactate response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). There were no significant differences seen between treatments. There is a significant main effect for time ($p < 0.05$).

Figure 13 – Cortisol response during exercise



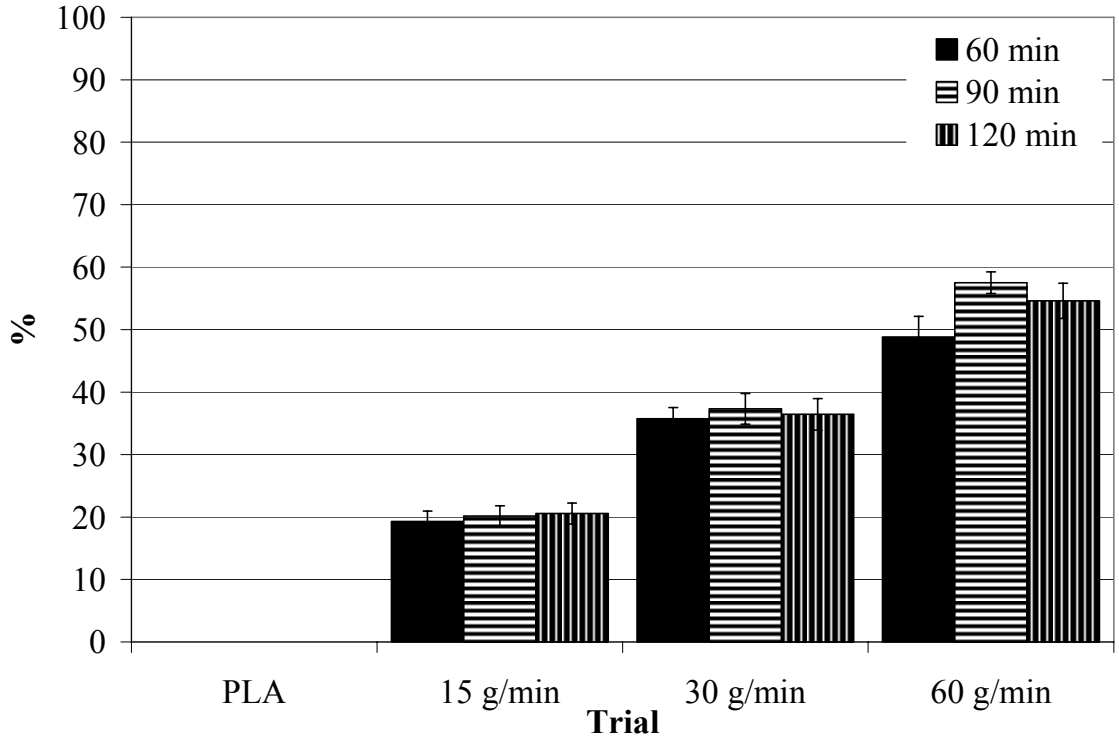
Plasma cortisol response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). There were no significant differences seen between treatments. There is a significant main effect for time ($p < 0.05$).

Figure 14 – $^{13}C/C$ ratio in expired air during exercise



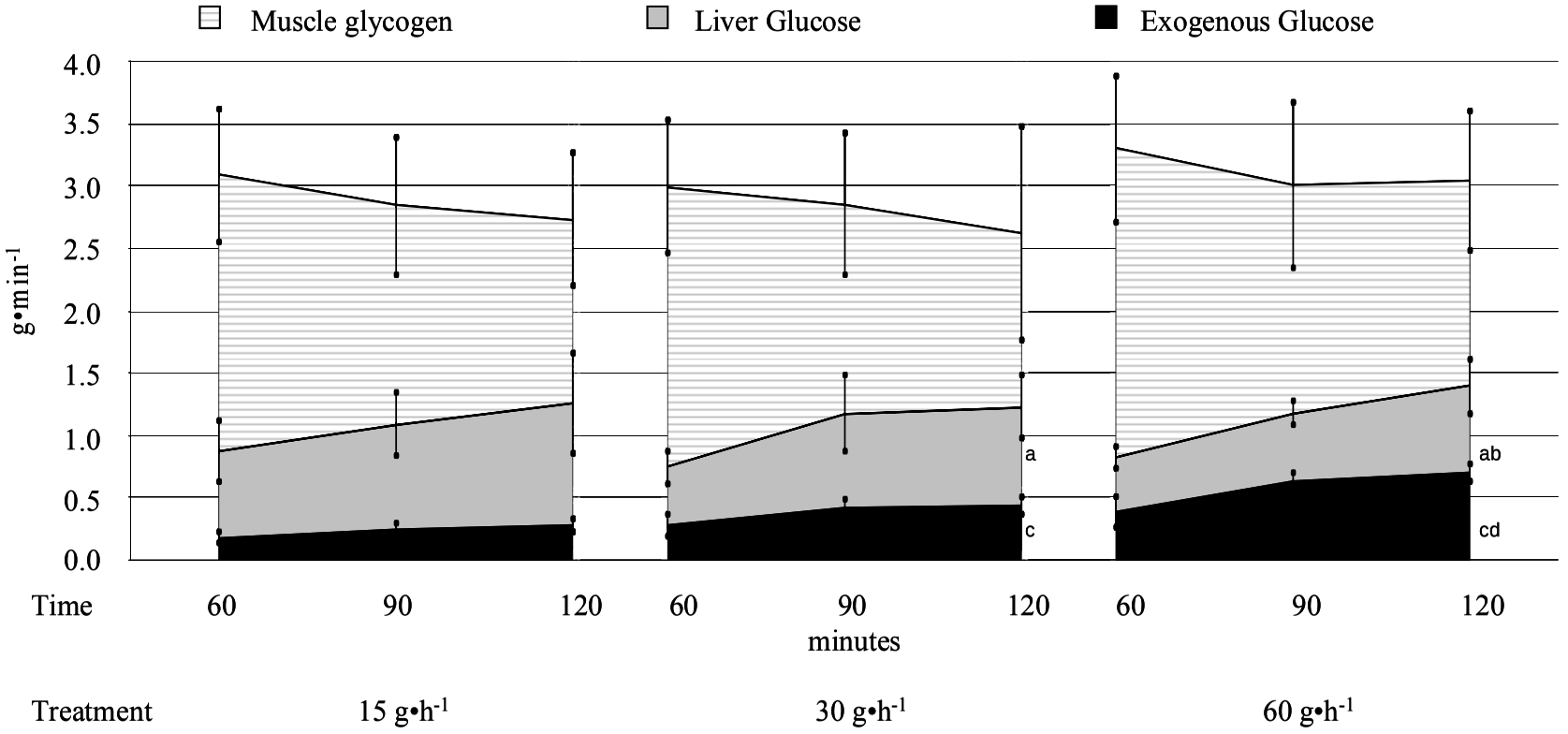
Expired PDB in the breath during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] 15 g•min^{-1} significantly lower than PLA, 30 g•min^{-1} and 60 g•min^{-1}, [b] PLA significantly lower than 15 g•min^{-1}, 30 g•min^{-1}, and 60 g•min^{-1}, [c] 15 g•min^{-1} significantly lower than 30 g•min^{-1} and 60 g•min^{-1}, and [d] 30 g•min^{-1} significantly lower than 60 g•min^{-1}.

Figure 15 – Percent plasma glucose coming from an exogenous source



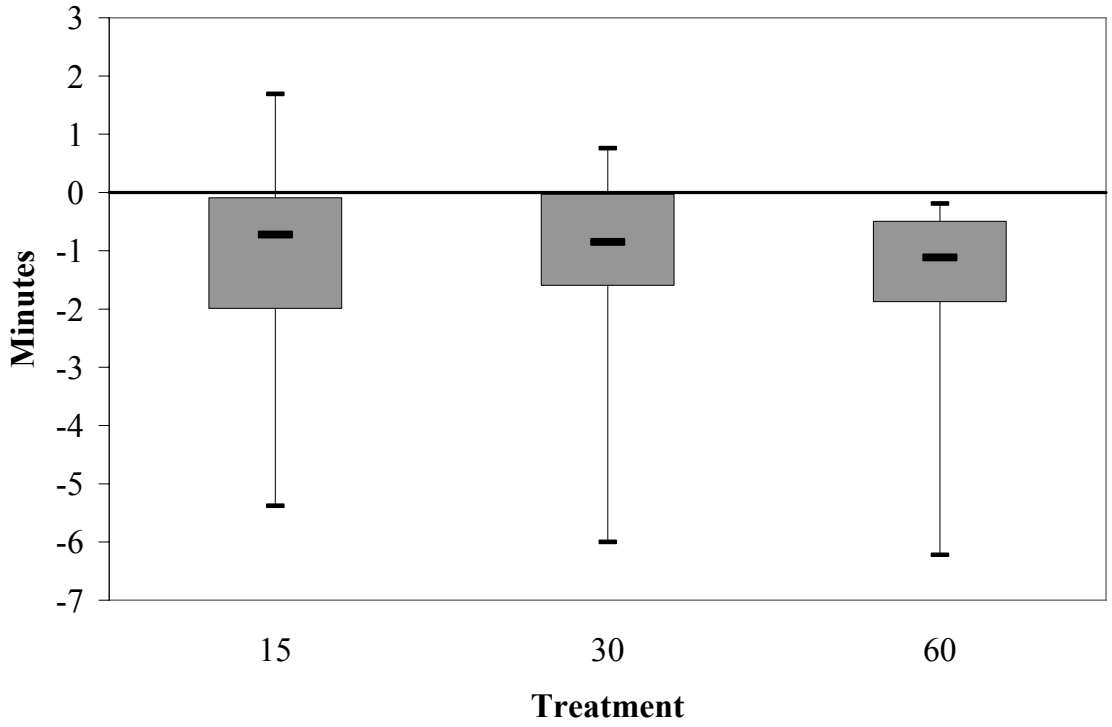
Percent plasma glucose coming from an exogenous source during the last hour of a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). All trials are significantly different from each other. There were no significant time differences within trials.

Figure 16 – Source of oxidized carbohydrate during the last hour of exercise when consuming glucose at 15, 30, and 60 g•h^{-1}.



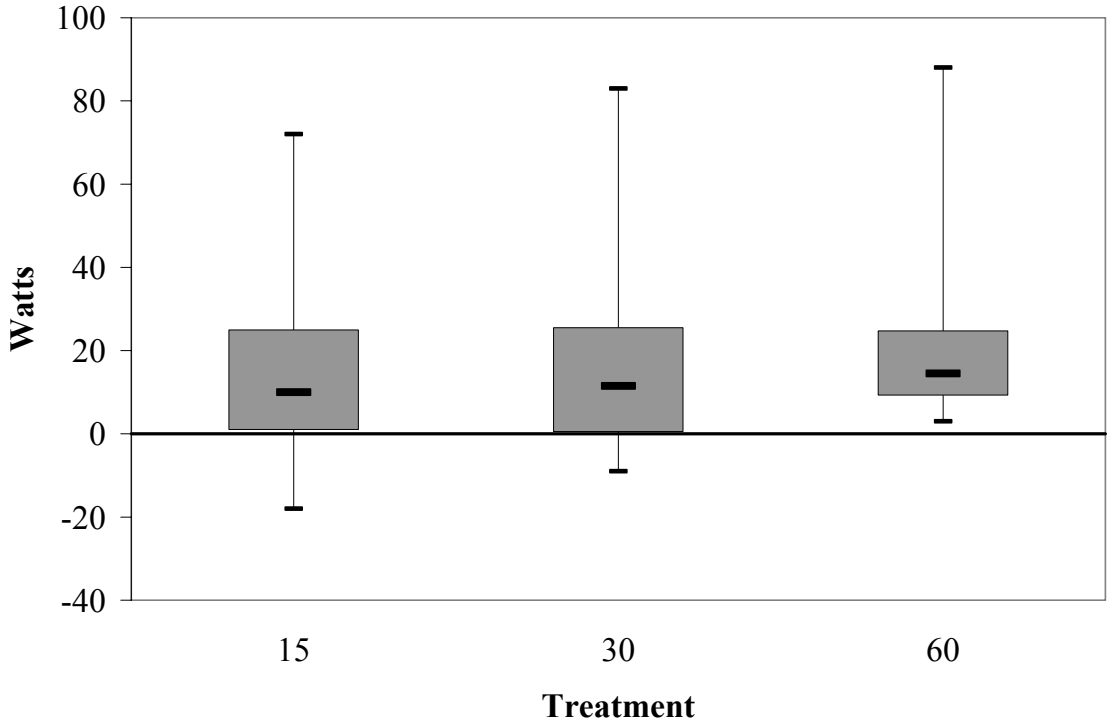
Carbohydrate utilization during the final hour of a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response when consuming 15, 30, and 60 g of glucose an hour. Values are means (SE). [a] significantly less than 15 g•h^{-1}; [b] significantly less than 30 g•h^{-1}; [c] significantly greater than 15 g•h^{-1}; [d] significantly greater than 30 g•h^{-1}. ($p < 0.05$)

Figure 17: Change in 20-km time trial completion time in relation to PLA



Box plots of change in duration in the 20-km time trial performance following a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response when consuming glucose at various rates. The top and bottom of the box represents the 75th and 25th percentile. The whiskers capture the range of performance times for the entire group of subjects. The black line in the box corresponds to the median performance value. No differences were observed between glucose treatments. No differences were observed between glucose treatments. All glucose treatments were significantly faster than placebo.

Figure 18: Change in 20-km time trial average wattage in relation to PLA



Box plots of change in average wattage in the 20-km time trial workrate following a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response when consuming glucose at various rates. The top and bottom of the box represents the 75th and 25th percentile. The whiskers capture the range of performance times for the entire group of subjects. The black line in the box corresponds to the median performance value. No differences were observed between glucose treatments. No differences were observed between glucose treatments. All were significantly faster than placebo.

V: IMPACT OF LOW DOSE GLUCOSE INGESTION ON EXOGENOUS GLUCOSE OXIDATION, SUBSTRATE UTILIZATION, AND EXERCISE

ABSTRACT

This study investigated the impact of glucose ingestion at rates of 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} on carbohydrate metabolism and exercise performance. Twelve cyclist/triathletes cycled at ~75% $\dot{V}O_{2\,PEAK}$ for two hours while ingesting glucose drinks delivering 15, 30, and 60 g•hr^{-1} or a placebo. Glucose drinks were extrinsically labeled with 1.8 mg•g^{-1} U-^{13}C-glucose. Expired breath samples and blood samples were collected every 15 minutes for future analyses. Immediately following the two hour constant load exercise session, participants completed a 20-km time-trial as quickly as possible. Exogenous glucose oxidation rose significantly as ingestion rate increased. Blood glucose and insulin were highest when ingesting 60 g•hr^{-1} while free fatty acids were the lowest. Insulin and free fatty acid responses for placebo and the 15 g•hr^{-1} trial were virtually identical. Liver glucose oxidation was significantly reduced by increasing glucose ingestion. Relative to placebo, glucose ingestion improved ($p < 0.01$) time-trial performance with no statistical difference between glucose doses. The findings of this study indicate that increasing glucose ingestion rate increases oxidation of exogenous glucose and spares liver-derived glucose. This study also demonstrates that 60 g•hr^{-1} provided the most consistent improvements in performance, even though performance

can be improved when ingesting only 15 g•hr^{-1} during exercise lasting approximately 150 minutes.

Key Words: cycling, exogenous carbohydrate oxidation, substrate utilization, performance

INTRODUCTION

Fatigue during prolonged exercise has been associated with hypoglycemia and depletion of muscle glycogen (6; 11; 19; 49). There is a great deal of evidence demonstrating the ergogenic effect of consuming carbohydrate before and during endurance exercise (6; 8; 11; 12; 16; 24; 51; 57; 58). The American College of Sports Medicine (ACSM) and the National Athletic Trainers Association (NATA) both have fluid replacement position stands that promote the benefit of carbohydrate intake during exercise. Both position stands suggest carbohydrate ingestion rates of 30-60 g•hr^{-1} (5; 46). These position statements and experts' findings have suggested the need for carbohydrates during prolonged exercise, but the specific carbohydrate ingestion rate for optimal performance benefits have yet to be defined.

Many researchers using various techniques have investigated the question to identify the carbohydrate ingestion rate that optimizes exercise performance and substrate utilization (6; 11; 12; 21; 26). Results indicate that time to exhaustion is prolonged when carbohydrate is consumed during exercise (6; 11; 12; 16; 51; 57; 58). Carbohydrate intake during exercise has also been shown to reduce time required to complete a set distance or work volume (1; 2; 35; 36).

Many of the improvements in performance experienced with carbohydrate ingestion have been associated to the maintenance of carbohydrate oxidation late during exercise (11; 53; 57). Carbohydrate oxidation is maintained through the oxidation of exogenous carbohydrate sources (30; 37; 43), the maintenance of blood glucose (1; 11; 53), and the sparing of endogenous carbohydrate (27; 37; 43). Most research suggests that liver glucose and not muscle glycogen is the endogenous carbohydrate source spared through exogenous carbohydrate ingestion. Liver glucose has been repeatedly shown to be spared through the ingestion of carbohydrate prior to and during exercise (3; 26; 30). While some running studies and a few cycling studies have demonstrated a sparing of muscle glycogen (37; 50; 51), most cycling studies do not report the sparing of muscle glycogen when carbohydrate is ingested during exercise (6; 11; 12; 16; 32).

Improvements in exercise performance have been reported with carbohydrate intakes ranging from 18 to 180 $g \bullet h^{-1}$ during exercise (12; 16; 32; 33; 36; 58). Many studies have tested carbohydrate ingestion rates above those recommended by the ACSM and NATA position stands in hopes of optimizing exercise performance and exploring the possibility of a carbohydrate/performance dose response. These studies have failed to demonstrate greater enhancements in performance with carbohydrate ingestion rates above ACSM and NATA recommended ingestion rates compared to the recommended rate range (14; 32; 35). While the impact of increasing carbohydrate ingestion rate has not been demonstrated, less is known about metabolic changes occurring at lower carbohydrate ingestion rates, the minimum level of carbohydrate intake needed for improved exercise performance, or if there is a dose response to varying carbohydrate

levels within and below the ingestion rates recommended by the ACSM and NATA position stands (16; 35; 55; 58).

While it might be logical that providing increased levels of carbohydrate will provide additional energy for exercise, carbohydrate ingestion rates above those defined in the ACSM and NATA position stands have also been associated with an increased incidence of stomach discomfort and stomach upset which may result in diminished exercise performance (42; 45; 52). Scientists have demonstrated increasing carbohydrate ingestion rate above 1.2 $g \bullet min^{-1}$, with a single carbohydrate form, does not further increase exogenous carbohydrate oxidation (21; 53). Research has also demonstrated that when only glucose is ingested the body is able to utilize that glucose at a rate of about 1 $g \bullet min^{-1}$ (23; 26; 27; 53). When multiple forms of carbohydrates (i.e. glucose or glucose polymers, and fructose) are consumed, glucose oxidation rates have been reported to be up to and above 1.5 $g \bullet min^{-1}$ (21; 23; 26; 27; 53).

This study examined the physiological and exercise performance responses of cyclists during a 2-hour constant load ride followed by a simulated 20-km time trial to determine the impact of carbohydrate delivery rates below what is typically recommended (60 $g \bullet h^{-1}$) for prolonged exercise. Participants completed four trials including a trial with no carbohydrate and three trials where carbohydrate was ingested in a drink at a rate of 15 $g \bullet h^{-1}$, 30 $g \bullet h^{-1}$, and 60 $g \bullet h^{-1}$.

METHODS

Participants:

Twelve trained male cyclists and triathletes participated in this study. Mean and standard deviation age, height, mass, and peak oxygen uptake ($\dot{V}O_{2\ PEAK}$), were 31.7 ± 1.1 yrs, 1.82 ± 0.02 m, 77.6 ± 2.0 kg, and 4.12 ± 0.09 l•min^{-1}, respectively. Participants served as their own controls for the study. All participants read and signed an informed consent approved by the Human Subject Review Committee prior to beginning the study.

Preliminary Testing:

$\dot{V}O_{2\ PEAK}$ was determined using an increasing resistance, multistage cycling test with 30 second Douglass Bags collected during the last 30 seconds of each stage. Expiratory gases were analyzed using Ametek S-3A/I Oxygen and Ametek CD-3A Carbon Dioxide Analyzers (Naperville, IL) and expired volume was measured with a spirometer (Vacumed Inc., Ventura CA). A regression analysis of the $\dot{V}O_2$-workload relationship determined the exercise workloads for lactate threshold testing.

The participants' two hour ride workload was set at 95% of the workload that would elicit a 4 mmol•L^{-1} blood lactate. Preliminary research in our lab found that this was the highest intensity most participants could maintain for two hours of cycling. Subjects exercised for 3-minute stages at 55, 60, 65, 70, 75, 80, 85, and 90% of their $\dot{V}O_{2\ PEAK}$ with blood samples taken for lactate analysis at the end of each stage. Blood was analyzed using a whole blood analyzer (Gem Premier 3000, Instrumentation Laboratory, Lexington, MA). All testing was performed with the participants exercising on their own bicycle affixed to a CompuTrainer™ Pro (RacerMate Inc, Seattle WA).

Participant Orientation:

Participants were required to perform three familiarization course rides prior to beginning the actual trials, with at least seven days between course rides. The first familiarization was performed to allow the participant to become familiarized with the 20-km time trial course. The second familiarization was performed to allow the participant to familiarize himself with the feel of a 2-hour ride followed by the 20-km time trial. This model is commonly used in metabolic fuel investigations but also has application to competitive cycling events. The third and final familiarization was performed to allow the participant to experience the testing procedures.

Experimental Protocol:

Participants completed four exercise trials with randomized interventions separated by at least seven days. Participants were instructed to record and maintain the same diet and abstain from exercise 24 hours before each trial. Participants reported to the lab after a 10-hour overnight fast. After arriving at the lab, participants voided and body weight were recorded. A registered nurse then inserted an intravenous catheter (BD Insyte™ Autoguard™, Becton, Dickinson Infusion Therapy Systems Inc., Sandy UT) into an antecubital vein with a three-way stopcock (Solution-Plus™, Mansfield, MA) to collect baseline blood samples and allow for blood sampling throughout the trial.

Environmental conditions were maintained at 20-25˚C, 35-40% relative humidity throughout the experiment. Participants performed a standardized ten-minute ride at 100 watts to warm-up and prepare the CompuTrainers™ for calibration. Following the 10-minute ride the CompuTrainers™ were calibrated according to the manufacturer's

recommendations. On average, participants 2-hour workload was set at 227.5 ± 2.2 watts after which they began a two hour constant load ride on the CompuTrainer™ with $\dot{V}O_2$ and heart rate collected every 15 minutes and blood samples collected every 15 minutes during the final hour (Figure 1).

After completing the 2-hour ride participants stopped pedaling and were allowed off the bike for one and a half minutes. Two-minutes after the cessation of the two hour constant load ride, participants began the simulated 20-km time trial. For the time trial participants were instructed to complete the 20-km course (Figure 2) as quickly as possible. This undulating course was designed using the CompuTrainer™ 3D computer program. Participants were aware of their position on the course but no verbal stimuli or other information was given by the investigators. Resistance varied throughout the ride based on the inclination of the course and the participants' speed and gearing, similar to that experienced when cycling outdoors. Participants were allowed to change gears at will throughout the course of the ride.

Intervention Beverages:

Participants completed the 2-hour exercise sessions consuming one of the four treatments over four trials during this study. The beverage treatments tested during the trials were 1) 250 mL of placebo with electrolytes (PLA); 2) 250 ml of 1.5% glucose with electrolytes (15 $g \bullet hr^{-1}$); 3) 250 ml of 3.0% glucose with electrolytes (30 $g \bullet hr^{-1}$); and 4) 250 ml of 6.0% glucose with electrolytes (60 $g \bullet hr^{-1}$) consumed every 15 minutes of the 2-hour ride. All beverages were formulated to be identical in flavor, sweetness and kept at a temperature of 1˚C to minimize differences in taste. The test beverages were given to

the participant in opaque plastic containers. To allow for the calculation of exogenous glucose oxidation, uniformly labeled ^{13}C-glucose (Isotec™, Miamisburg, OH) was added at 1.8 mg•g^{-1} of glucose contained within the experimental beverage. This resulted in beverages with high abundances of ^{13}C (15: 121 Pee Dee Bellemnitella (‰ [δ-^{13}C]PDB-1), 30: 136 ‰ [δ-^{13}C]PDB-1, and 60: 151 ‰ [δ-^{13}C]PDB-1).

Data Collection:

Physiological Measures and Metabolites

Heart rate was measured every 15 minutes during the two hour ride with a chest strap and watch telemetry system (Polar Electro Inc., Lake Success, NY). A 60-second expired air sample was collected every 15 minutes during the 2-hour ride. The expired air sample was analyzed for oxygen and carbon dioxide fraction and the expired volume was measured using a flow meter. With the expired air measures, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were calculated. Total carbohydrate (TCO) and fat oxidation rates were calculated, neglecting the small contribution of protein oxidation to the energy yield (75; 116), using equation 1 and 2, respectively: (10)

$$(g \bullet min^{-1}) = (4.59 \bullet \dot{V}CO_2) - (3.23 \bullet \dot{V}O_2) \quad \text{(Equation 1)}$$

$$(g \bullet min^{-1}) = 1.70 \bullet (\dot{V}O_2 - \dot{V}CO_2) \quad \text{(Equation 2)}$$

Plasma glucose was measured using a liquid glucose (Hexokinase) Reagent Set kit (Pointe Scientific, Canton, MI). Insulin was measured using a Human Insulin ELISA kit (Millipore Corp, St. Charles, MO). Free-fatty acids were measured using a non-

essential fatty acid HR2 series reagents kit (Wako Diagnostics, Richmond, VA). All samples were analyzed on a Multisample Spectrophotometer (Synergy HT, Bio-tek Instruments, Winooski, MA).

Tracer

To determine exogenous carbohydrate oxidation rate expired gas was captured in a 10 mL vacutainer (BD Vacutainer™, Franklin Lakes, NJ) for analysis of expired $^{13}CO_2/^{12}CO_2$. Isotopic tracer in the expired $^{13}CO_2/^{12}CO_2$ ratio was analyzed using a BreathMat Plus (Finnigan MAT). Exogenous glucose oxidation rate (EGO) was calculated using equation 3: (26; 39)

$$(g \bullet min^{-1}) = \dot{V}CO_2 \bullet \frac{R_{OBS} - R_{REF}}{R_{ING} - R_{REF}} \bullet \frac{1}{k} \qquad \text{(Equation 3)}$$

Where: R – isotopic ratio, R_{OBS} – The observed $^{13}C/C$ ratio in the breath, R_{ING} – The $^{13}C/C$ ratio of the beverage ingested, R_{REF} – The baseline breath $^{13}C/C$ ratio, and k (0.747 $L \bullet g^{-1}$) is the amount of $\dot{V}CO2$ provide by the oxidation of 1 g of glucose.

During the second hour of exercise, plasma samples were collected to allow for the calculation of exogenous glucose present in the blood, plasma glucose oxidation, liver glucose oxidation, and muscle glucose oxidation. These calculations were made only during the final 60 min of the 2-hour constant load ride allowing 60 min of equilibration. This 60 min equilibration is based on observations that $^{13}C/^{12}C$ in expired CO_2 equilibrates slowly with the $^{13}C/^{12}C$ in the CO_2 produced in tissues (38), that ^{13}C provided from ^{13}C-glucose is not irreversibly lost in tricarboxylic acid cycle intermediates (44)

and/or bicarbonate (48) pools, and that the $^{13}CO_2$ recovery in expired gases is complete or almost complete. Plasma was deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). Glucose was then separated from the plasma by double-bed ion-exchange chromatography (AG 50W-X8 H^+ and AG 1-X8 chloride, 200-400 mesh; Bio-Rad, Mississauga, ON, Canada). The elute was then evaporated to dryness (Virtis Research Equipment, New York, NY) and combusted with copper oxide for 60 minutes at 400°C. The CO_2 was recovered from the glucose combustion for isotopic analysis. The measurement of $^{13}C/^{12}C$ in the CO_2 coming from combusted glucose was then performed using mass spectrometry (Prism, Manchester, UK). The isotopic ratio of the combusted plasma glucose was expressed in per mil difference by comparison with the PDB Chicago Standard (equation 4):

$$‰\ [\delta\text{-}^{13}C]PDB\text{-}1 = [R_{spl}/R_{std}) - 1] \text{ X } 1{,}000 \quad \text{(Equation 4)}$$

Where: R_{spl} – ^{13}C-to-^{12}C ratios in the sample and R_{std} – ^{13}C-to-^{12}C ratios in the standard (1.1237 ‰), respectively (4).

The percentage of plasma glucose derived from exogenous glucose was calculated using equation 5:

$$(\%) = \frac{R_{GLU} - R_{REF}}{R_{ING} - R_{REF}} \bullet 100 \quad \text{(Equation 5)}$$

Where: R – isotopic ratio, R_{GLU} – The observed $^{13}C/C$ ratio in the blood, R_{ING} – The $^{13}C/C$ ratio of the beverage ingested, and R_{REF} – The baseline blood $^{13}C/C$ ratio.

Plasma glucose oxidation (PGO) was calculated using equation 6: (39)

$$(g \bullet min^{-1}) = \frac{\text{Plasma Glucose being Oxidized}}{\text{Exogenous Plasma Glucose Concentration}} \quad \text{(Equation 6)}$$

Liver-derived glucose oxidation (LGO) was calculated using equation 7: (10; 18)

$$(g \bullet min^{-1}) = PGO - EGO \quad \text{(Equation 7)}$$

Muscle glycogen oxidation (expressed in grams of glucose/min) was calculated using equation 8: (10; 18)

$$(g \bullet min^{-1}) = TCO - PGO \quad \text{(Equation 8)}$$

Statistical Analysis:

All data are expressed as means ± standard errors. Statistical analysis was performed using SPSS version 13 (SPSS Inc. Chicago, IL). Data were analyzed with a univariate ANOVA. When ANOVA reported significant interactions, a Duncan post-hoc analysis was performed. Significance was set at $p \leq 0.05$.

RESULTS

Physiological Measures:

$\dot{V}O_2$ increase significantly during the 2-hour constant load ride and was significantly higher in the 30 g•hr^{-1} and 60 g•hr^{-1} trials (Figure 3). This average $\dot{V}O_2$ for the four trials represented 77.2 ± 0.9% of the participants' $\dot{V}O_{2\,PEAK}$. There was a significant reduction in RER (Figure 4) and carbohydrate oxidation (Figure 5) while fat oxidation (Figure 6) significantly increased during the 2-hour constant load ride ($p \leq 0.05$). RER and carbohydrate oxidation were significantly higher while fat oxidation was significantly lower in the 60 g•hr^{-1} trial as compared to all other trials ($p \leq 0.05$).

Blood, Hormone, and Metabolite Measures:

There were no differences in baseline blood glucose, insulin, or free-fatty acid measures. There were significant reductions in plasma glucose (Figure 7) and insulin (Figure 8) levels during the last hour of the 2-hour constant load ride. With reductions in carbohydrate ingestion below 30, plasma glucose levels declined at a significantly greater rate as carbohydrate ingestion declined. Plasma glucose levels were significantly lower in the PLA trial as compared to the 15 g•hr^{-1} trial. Plasma insulin levels were significantly higher in the 60 g•hr^{-1} trial as compared to all other trials. There was a significant rise in plasma free-fatty acid levels during the last hour of the 2-hour constant load ride. Plasma free-fatty acids increased during the second hour of the 2-hour constant load ride (Figure 9) There was no difference in plasma free-fatty acid levels when comparing the PLA and 15 g•hr^{-1} trials. As carbohydrate ingestion increased above 15,

serum free-fatty acid levels significantly increased as glucose ingestion increased (Figure 5).

Exogenous and Endogenous Glucose Utilization:

The expired $^{13}C/C$ ratio significantly increased during the 2-hour constant load ride with all treatments being significantly different at the beginning of the second hour of exercise (Figure 10). The percentage of exogenous glucose in the plasma increased significantly as exogenous carbohydrate intake increased (Figure 11). During the 2-hour constant load ride, exogenous glucose oxidation significantly increased as glucose ingestion rate increased. The areas under the curves for exogenous glucose, liver-derived glucose, and muscle glycogen were calculated for the second hour of the constant load ride (Figure 12). There was no difference in plasma glucose oxidation in the 15 $g \bullet hr^{-1}$, 30 $g \bullet hr^{-1}$, and 60 $g \bullet hr^{-1}$ trials. Since ^{13}C was not given in the PLA trial we are unable to compare plasma glucose oxidation in the PLA trial. Exogenous glucose oxidation rates during the second hour of the constant load cycling were 0.24 ± 0.01, 0.39 ± 0.01 and 0.58 ± 0.02 $g \bullet min^{-1}$ for 15 $g \bullet hr^{-1}$, 30 $g \bullet hr^{-1}$ and 60 $g \bullet hr^{-1}$ ingestion rates, each being significantly different from each other ($p \leq 0.001$). Exogenous glucose oxidation peaked in the final 30 minutes of the two hour constant load ride at 0.26 ± 0.05, 0.44 ± 0.04 and 0.66 ± 0.07 $g \bullet min^{-1}$ for 15 $g \bullet hr^{-1}$, 30 $g \bullet hr^{-1}$ and 60 $g \bullet hr^{-1}$ trials, respectively ($p \leq 0.001$). There was also a significant increase in liver glycogen oxidation during the last hour of the 2-hour constant load ride. Liver glucose oxidation rate was highest when consuming 15 $g \bullet hr^{-1}$ (0.84 ± 0.07 $g \bullet min^{-1}$) followed by 30 $g \bullet hr^{-1}$ (0.70 ± 0.07 $g \bullet min^{-1}$) and 60 $g \bullet hr^{-1}$ (0.56 ± 0.03 $g \bullet min^{-1}$), all significantly different from one another at a $p \leq 0.05$ level.

There was also significant reduction in muscle glycogen oxidation during the last hour of the 2-hour constant load ride with no significant differences found between the 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} trials. No tracer was given in the PLA to examine muscle glycogen oxidation during the PLA trial.

Performance Measures:

The time required to complete the 20-km time trial was 36.39 ± 0.84 min, 35.23 ± 0.80 min, 34.99 ± 0.75 min, and 34.69 ± 0.62 min for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1}, respectively. The change in time required to complete the 20-km time trial compared to PLA are shown in Figure 13. The average wattage during the time trial for PLA, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} was 212.0 ± 9.5 W, 227.9 ± 10.7 W, 229.7 ± 10.6 W, and 234.2 ± 9.4 W, respectively. PLA required significantly more time to complete the time trial and resulted in a significantly lower average wattage during the time trial compared to 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} ($p \leq 0.05$). However, 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} were not significantly different from one another.

DISCUSSION

This study investigated the impact of glucose ingestion at rates of 15 g•hr^{-1}, 30 g•hr^{-1}, and 60 g•hr^{-1} on carbohydrate metabolism and exercise performance. The main findings of this investigation were: 1) exogenous glucose oxidation increased as glucose ingestion rate increased, 2) the ingestion of glucose at increasing rates provided increased protection of endogenous carbohydrate stores, and 3) ingesting glucose at rates equal to and greater than 15 g•hr^{-1} in improved cycling time-trial performance.

Many studies have examined the impact of carbohydrate ingestion on carbohydrate metabolism, substrate utilization, and exercise performance. This study has combined all three to determine the impact of carbohydrate on whole body carbohydrate metabolism, substrate utilization, and exercise performance.

It has been demonstrated that exogenous carbohydrate quickly enters into the bloodstream providing an additional source of glucose (1; 11; 12; 50; 51; 58). The ingestion of carbohydrate has been reported to result in an elevation in plasma insulin as compared to the ingestion of water (27; 37). Elevations in circulating insulin and glucose promote increased glucose uptake by the exercising muscle (30; 58). In this investigation, plasma glucose was increased when ingesting carbohydrate at a rate of 30 $g \bullet hr^{-1}$. Increasing rate to 60 $g \bullet hr^{-1}$ did not provide further elevations in plasma glucose. Interestingly, this study also demonstrated a significant rise in plasma insulin with a glucose ingestion rate of 60 $g \bullet hr^{-1}$ but no difference when comparing PLA, 15 $g \bullet hr^{-1}$, and 30 $g \bullet hr^{-1}$. Elevations in circulating insulin levels associated with glucose ingestion reduce the lipolytic rate and limit the availability of circulating free-fatty acids (56). Ingestion of carbohydrate and the impact on free-fatty acids response has previously been studied by Coyle's laboratory (11; 12). We found circulating free-fatty acid levels during the 30 $g \bullet hr^{-1}$ trial were reduced as compared to the PLA and 15 $g \bullet hr^{-1}$ trial with the 60 $g \bullet hr^{-1}$ trial providing a further reduction. Costill et al (1977) demonstrated a sparing of muscle glycogen when plasma free-fatty acid levels were higher (9).

It has been demonstrated that exogenous glucose oxidation rates rise as glucose intake rises until a threshold is reached (typically 1 $g \bullet min^{-1}$). The exogenous glucose oxidation rates found in this investigation are similar to those found in other

investigations (26; 54). This study suggests the percentage of exogenous glucose being oxidized is influenced by ingestion rate. Exogenous oxidation rates peaked during the last 30 minutes of the two-hour constant load ride. The peak percentage of exogenous glucose oxidation was 104%, 88%, and 66% of the ingestion rate during the 15 $g \bullet hr^{-1}$, 30 $g \bullet hr^{-1}$, and 60 $g \bullet hr^{-1}$, respectively. The reason peak glucose oxidation rate in the 15 $g \bullet hr^{-1}$ exceeds the ingestion rate may be explained by the oxidation of glucose ingested earlier in the trial since peak rates were observed during the last 30 minutes of each trial. Wallis et al (2007) reported the percentage of exogenous glucose being oxidized with ingestion rates of 30 $g \bullet hr^{-1}$ and 60 $g \bullet hr^{-1}$ was 66% and 50%, respectively in women (54). They suggested that some of the carbohydrate that is ingested may remain in the intestines or may go to the liver or inactive muscles to be stored (26; 54). With exogenous glucose oxidation rates in the 30 $g \bullet hr^{-1}$ exceeding ingestion rates in the 15 $g \bullet hr^{-1}$, as well as exogenous glucose oxidation rates in the 60 $g \bullet hr^{-1}$ exceeding ingestion rates in the 30 $g \bullet hr^{-1}$, it seems intestinal absorption is not a major limiting factor for the oxidation of exogenous glucose at rates below 60 $g \bullet hr^{-1}$. Several studies have also demonstrated that exogenous carbohydrate oxidation rates peak at approximately 1.0 $g \bullet min^{-1}$ when a single form of carbohydrate is ingested in large amounts (25; 26; 53). Therefore, ingestion rates of 60 $g \bullet hr^{-1}$ (1.0 $g \bullet min^{-1}$) are likely able to be absorbed by the intestine at a rate allowing most of the ingested carbohydrate to be quickly oxidized. This suggests that when glucose is ingested at greater than 15 $g \bullet hr^{-1}$ rates the difference in ingestion rate and exogenous oxidation rate is likely being taken-up or stored by the liver and muscle.

It seems that when exogenous glucose is quickly made available to the exercising muscle, the demand to utilize endogenous stores of carbohydrate for energy is not as

great (20; 22; 23; 27; 37; 43). Although, there continues to be some debate as to what endogenous carbohydrate sources are spared with carbohydrate ingestion. The sparing of liver-derived glucose when consuming carbohydrate has been repeatedly demonstrated (26; 27; 30; 39; 54). In agreement with this we demonstrated a reduction in the liver-derived glucose oxidation during the last 30 minutes of exercise as carbohydrate ingestion rate increased. Several studies using running and variable intensity cycling as the exercise modes have demonstrated muscle glycogen sparing (37; 50; 51; 58). Constant load cycling studies have failed to demonstrate a sparing of muscle glycogen stores with exogenous carbohydrate (7; 13; 54). Since ^{13}C was not added to the placebo treatment in this study, no comparison can be made concerning the changes in muscle glycogen oxidation between carbohydrate ingestion trials and the placebo trial. However, increasing the rate of glucose ingestion did not alter the rate of muscle glycogen oxidation. The alterations of available glucose, insulin, and free-fatty acids, seen with the three glucose treatments, may have led to the same amount of fuel supplied to the working muscle, resulting in observation of similar rates of muscle glycogen oxidation.

The impact of carbohydrate as an ergogenic aid in exercise lasting longer than 1-hour is well accepted. Many researchers have demonstrated that carbohydrate ingestion improves exercise performance as compared to not receiving carbohydrate (1; 11; 16; 17; 29; 32; 33; 57; 58). Several studies have investigated the impact of increasing carbohydrate intake rate on exercise performance. It would seem that large carbohydrate ingestion rates would provide the exercising muscle more fuel to continue work. Mitchell et al found carbohydrate ingestion rates, ranging from 34 to 111 $g \bullet hr^{-1}$, did not vary in their impact on exercise performance (32; 33). Gastric emptying rates (31; 34)

and intestinal absorption rates (41) have been identified as potential reasons large carbohydrate intakes during exercise do not provide additional performance benefits.

While many have tried to maximize exercise performance by increasing carbohydrate ingestion rate, fewer studies tried to determine the minimal amount of carbohydrate needed to elicit performance benefits. There have been several studies suggesting performance can be enhanced with carbohydrate ingestion rates near 30 $g \bullet hr^{-1}$ (33; 35; 36; 47; 55) and other studies have demonstrated carbohydrate ingestion below 30 $g \bullet hr^{-1}$ can elicit performance benefits (15; 16; 28; 29). This study found carbohydrate ingestion rates ranging from 15 $g \bullet hr^{-1}$ to 60 $g \bullet hr^{-1}$ provide performance benefits. In the 60 $g \bullet hr^{-1}$ trial all twelve subjects completed the simulated time-trial faster as compared to the PLA trial. When ingesting glucose at rates of 15 $g \bullet hr^{-1}$ and 30 $g \bullet hr^{-1}$, nine subjects finished the time trial faster than PLA trial.

Exogenous carbohydrate oxidation plateaus when exercise intensity exceeds 50% $\dot{V}O_2max$ (40). Since there are only limited stores of carbohydrate, the body must utilize carbohydrate from external sources or reduce intensity to continue work. As the carbohydrate available to the exercising muscle declines during prolonged exercise, the utilization of fat for energy increases. As fat's role in energy production increases, work intensity declines due to the slower rate of ATP production from fat metabolism. Exogenous carbohydrate's incorporation into the bloodstream provides another source of carbohydrate which allows for maintained work output and spares some of the hepatic stores of carbohydrate.

These findings as well as those of many other researchers demonstrate the ability of carbohydrate ingestion to sustain work and improve performance. In 1987, Coggan

and Coyle suggested that fatigue during cycling was due to an insufficient supply of carbohydrate reaching the exercising muscle (6). This data adds to the literature demonstrating athletes can maintain higher exercise intensities and improve exercise performance if exogenous carbohydrate sources maintain blood glucose and reduce the body's reliance on endogenous carbohydrate sources for energy.

In summary, this study found carbohydrate ingestion increased plasma glucose with ingestion rates as low as 15 $g \bullet hr^{-1}$. Ingesting glucose at 30 and 60 $g \bullet hr^{-1}$ resulted in an increase in plasma glucose greater than what was observed when ingesting 15 $g \bullet hr^{-1}$. Plasma insulin did not demonstrate a significant elevation until carbohydrate ingestion reached 60 $g \bullet hr^{-1}$. A reduction in serum free-fatty acid was observed when glucose ingestion reached 30 $g \bullet hr^{-1}$ with a further reduction during the 60 $g \bullet hr^{-1}$ trial. Exogenous carbohydrate oxidation rates increased as carbohydrate ingestion rate increased from 15 $g \bullet hr^{-1}$ to 30 $g \bullet hr^{-1}$, with a further increase observed during the 60 $g \bullet hr^{-1}$ trial. As glucose ingestion rate increased a smaller percentage of exogenous glucose was oxidized. Liver-derived glucose oxidation was reduced as glucose ingestion rate increased. Glucose ingestion did not alter muscle glycogen oxidation. Glucose ingestion resulted in improved time-trial performance but alterations in carbohydrate dose did not impact the performance benefit. In conclusion, the findings of this study indicated that increasing glucose ingestion rate increases oxidation of exogenous glucose and spares liver-derived glucose. This study also demonstrates that 60 $g \bullet hr^{-1}$ provided the greatest protection on liver-derived glucose and resulted in all subjects completing the time trial faster than placebo, even though performance was improved in nine of the twelve subjects when ingesting glucose at a rate as low as 15 $g \bullet hr^{-1}$ during exercise lasting approximately 150

minutes. Future research is needed to explore the potential benefits and drawbacks of ingesting carbohydrate at rates lower than 30-60 g•hr^{-1}.

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Figure 1: Order of test measures

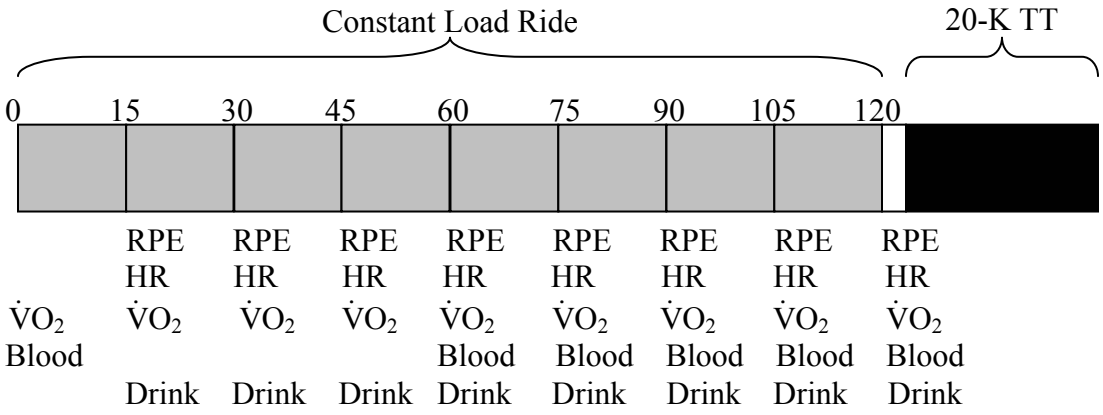
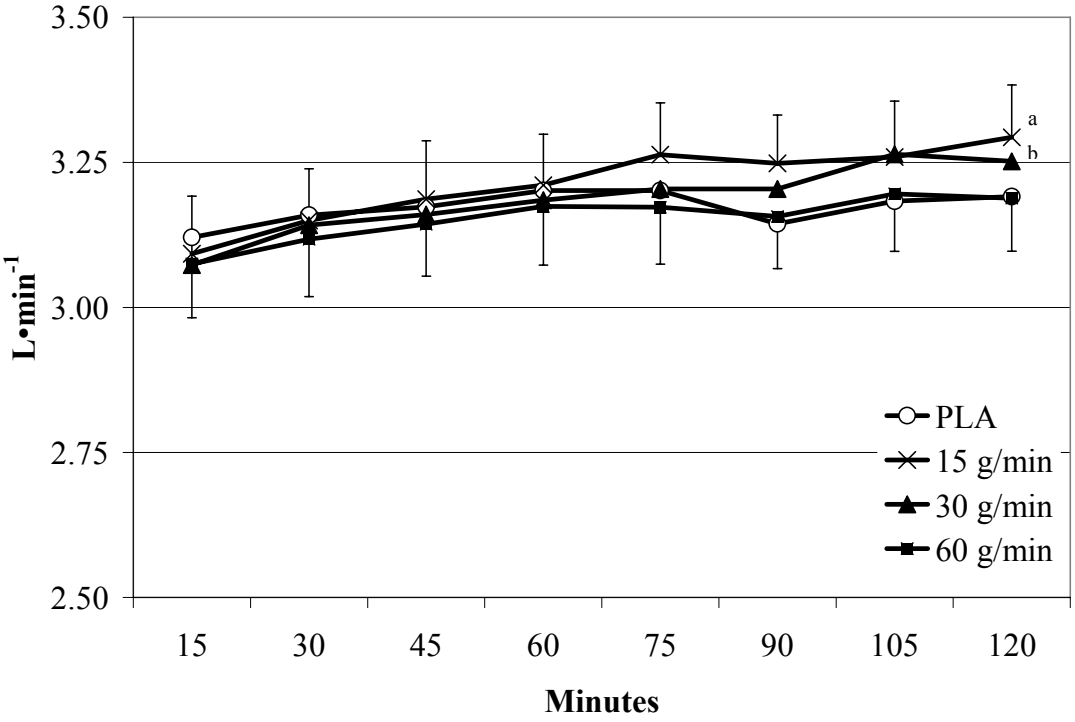


Diagram of the testing protocol.

Figure 2: 20-kilometer course profile

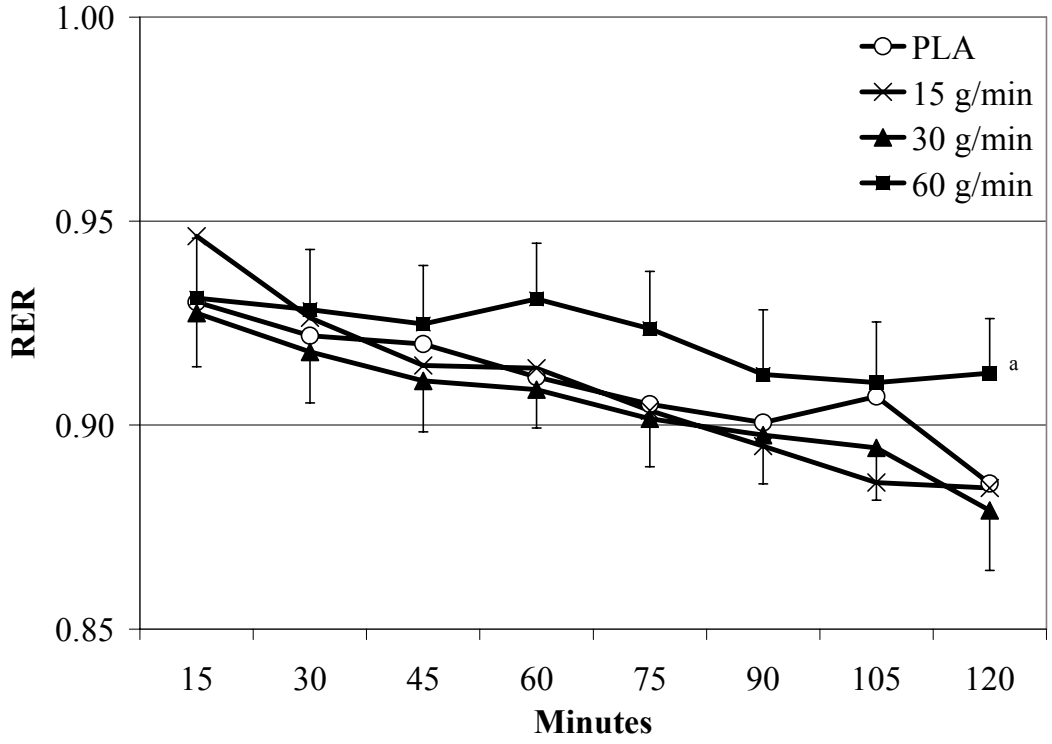
Course profile of the 20-km time trial.

Figure 3 – $\dot{V}O_2$ during exercise



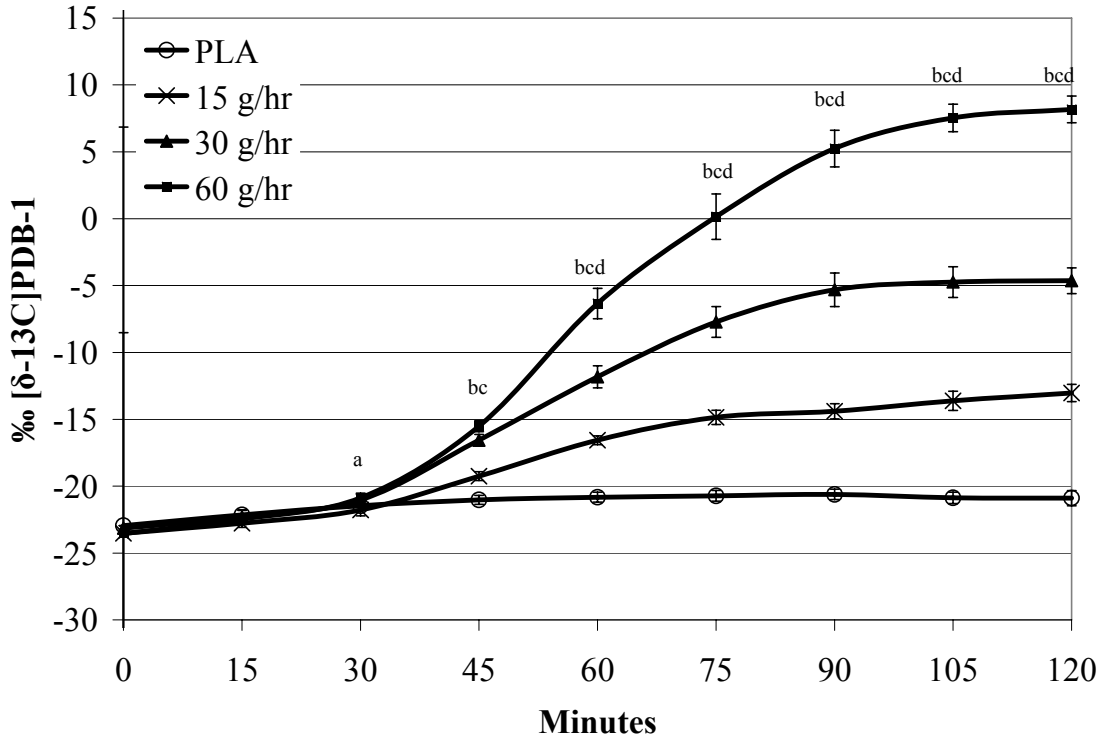
Oxygen uptake response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA and 60 g•hr^{-1}. [b] significantly higher than 60 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 4 – Respiratory exchange ratio during exercise



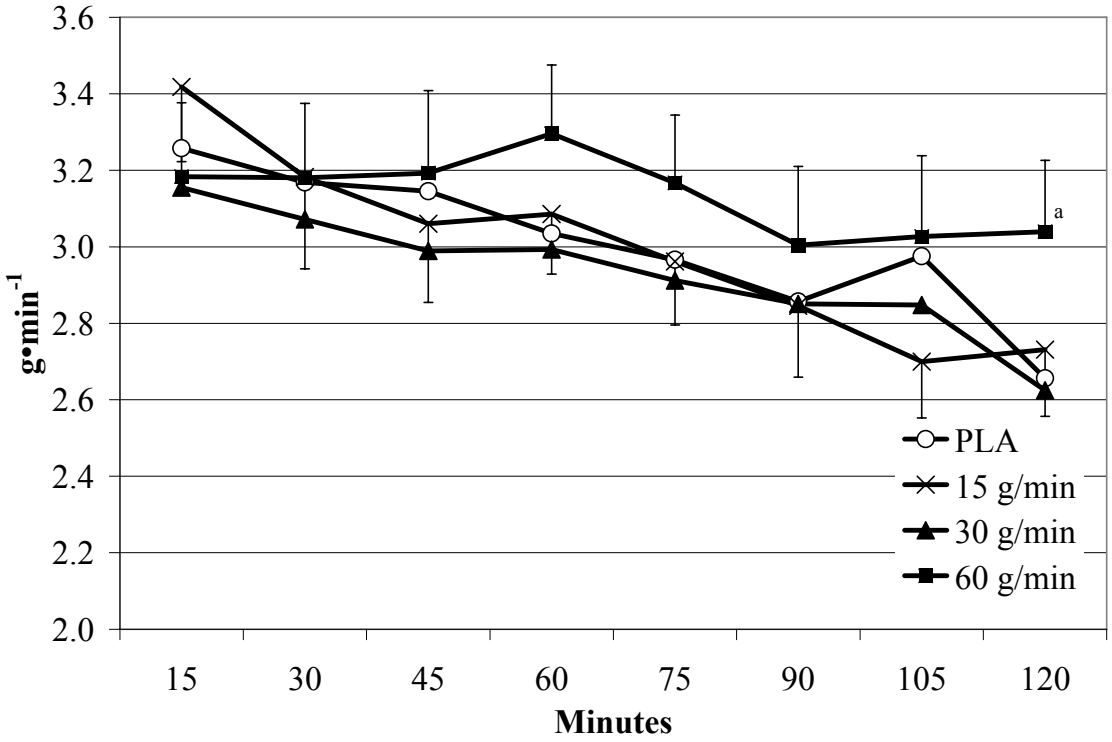
Respiratory exchange ratio response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA, 15 g•hr^{-1}, and 30 g•hr^{-1} There is a significant main effect for time ($p < 0.05$).

Figure 5 – $^{13}C/C$ ratio in expired air during exercise



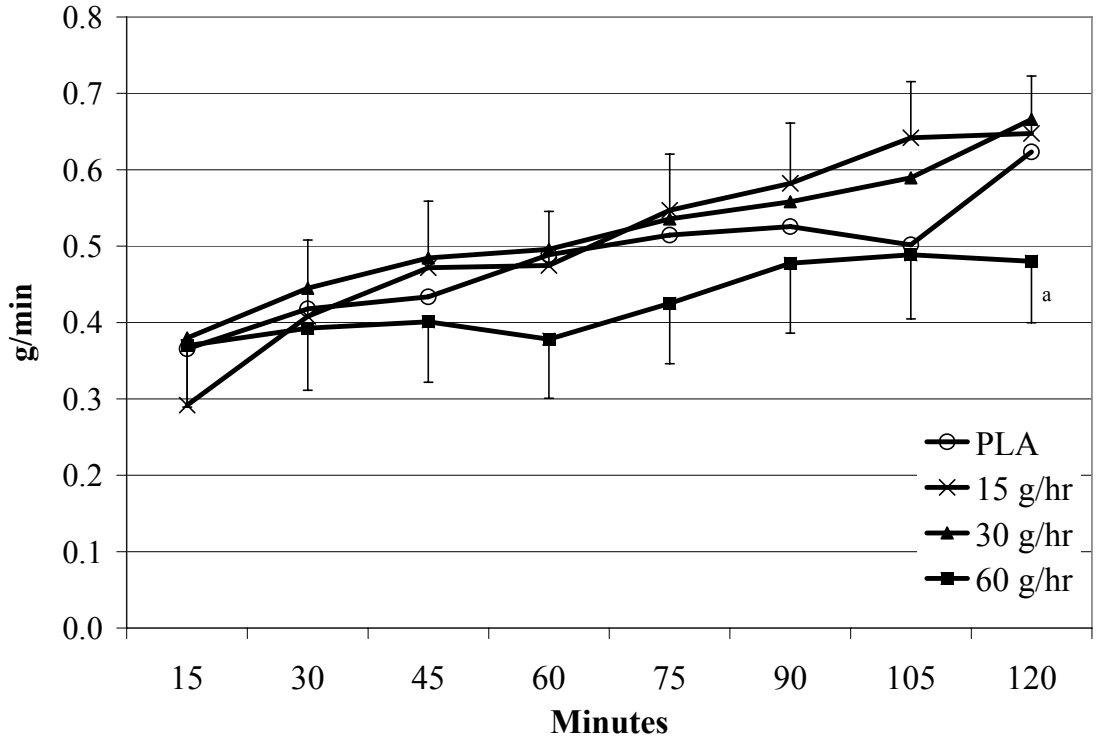
Expired PDB in the breath during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] 15 g•min^{-1} significantly lower than PLA, 30 g•min^{-1} and 60 g•min^{-1}, [b] PLA significantly lower than 15 g•min^{-1}, 30 g•min^{-1}, and 60 g•min^{-1}, [c] 15 g•min^{-1} significantly lower than 30 g•min^{-1} and 60 g•min^{-1}, and [d] 30 g•min^{-1} significantly lower than 60 g•min^{-1}.

Figure 6 – Carbohydrate oxidation during exercise



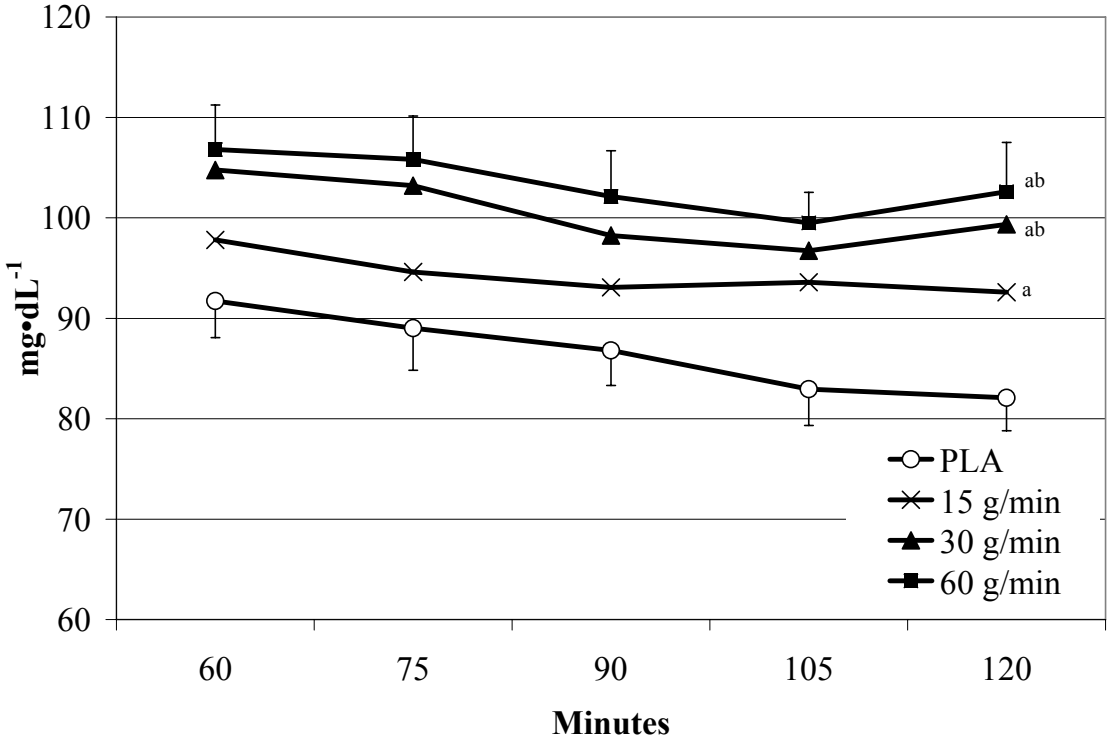
Carbohydrate oxidation response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than PLA, 15 g•hr^{-1},and 30 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 7 – Fat oxidation during exercise



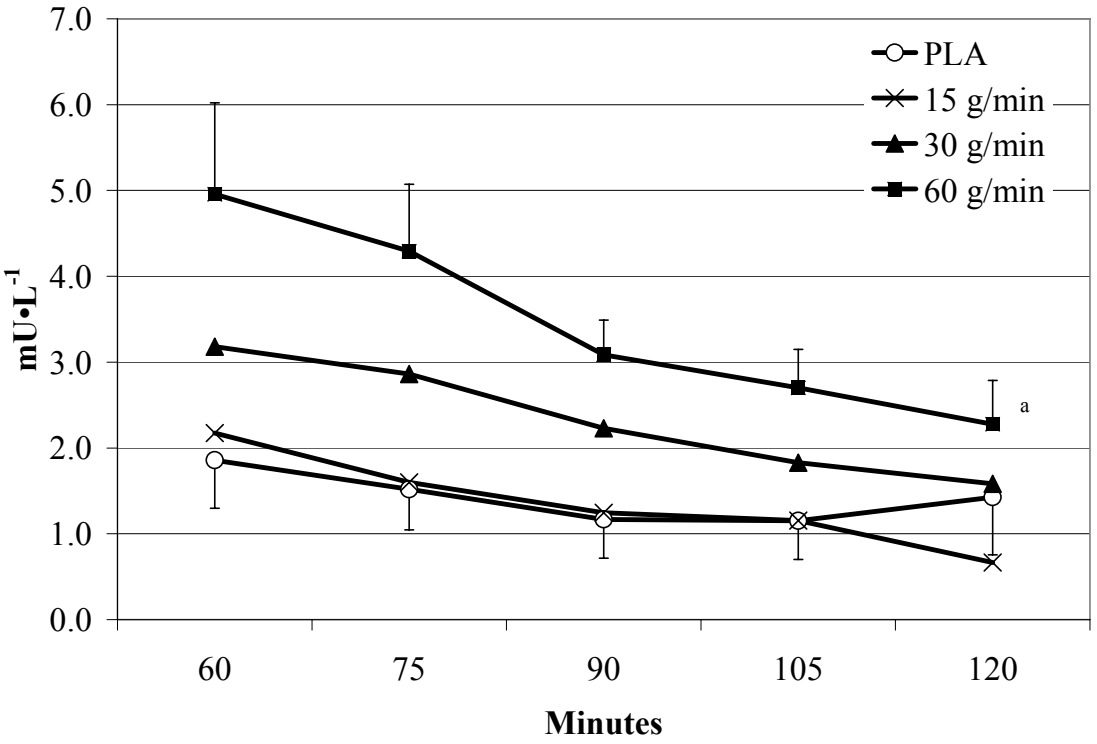
Fat oxidatoion during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly lower than PLA, 15 $g \bullet hr^{-1}$,and 30 $g \bullet hr^{-1}$. There is a significant main effect for time ($p < 0.05$).

Figure 8 – Blood glucose response during exercise



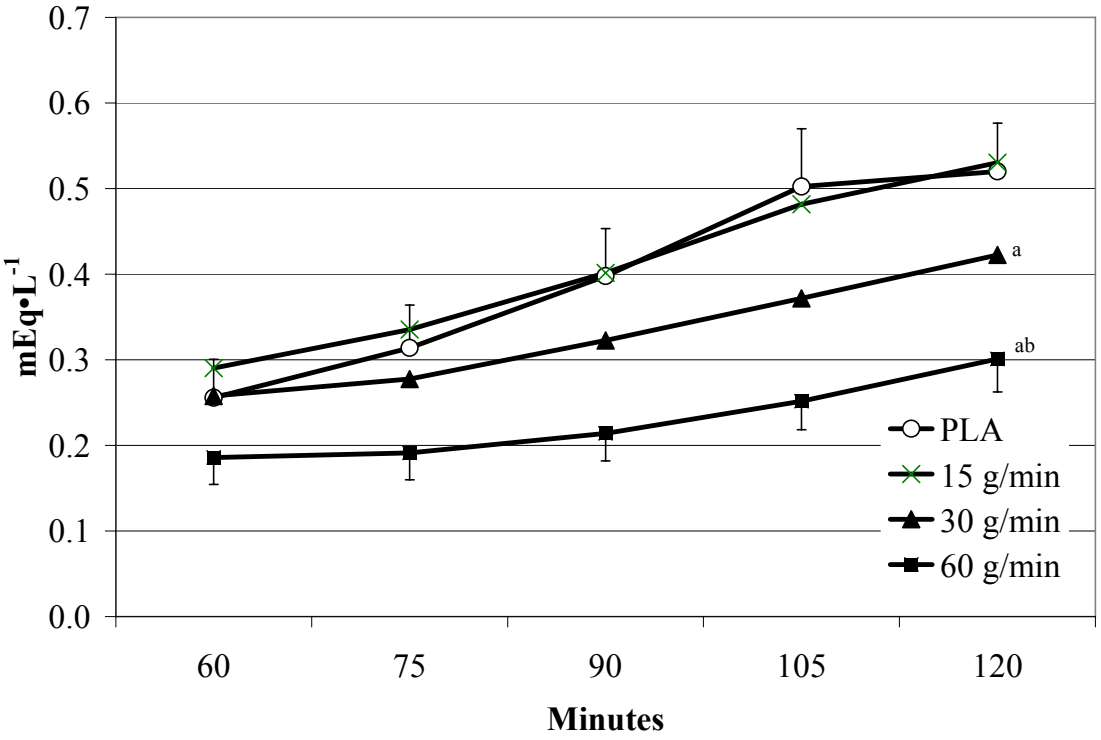
Plasma glucose response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than 0; [b] significantly higher than 15 g•hr^{-1}. There is a significant main effect for time ($p < 0.05$).

Figure 9 – Insulin response during exercise



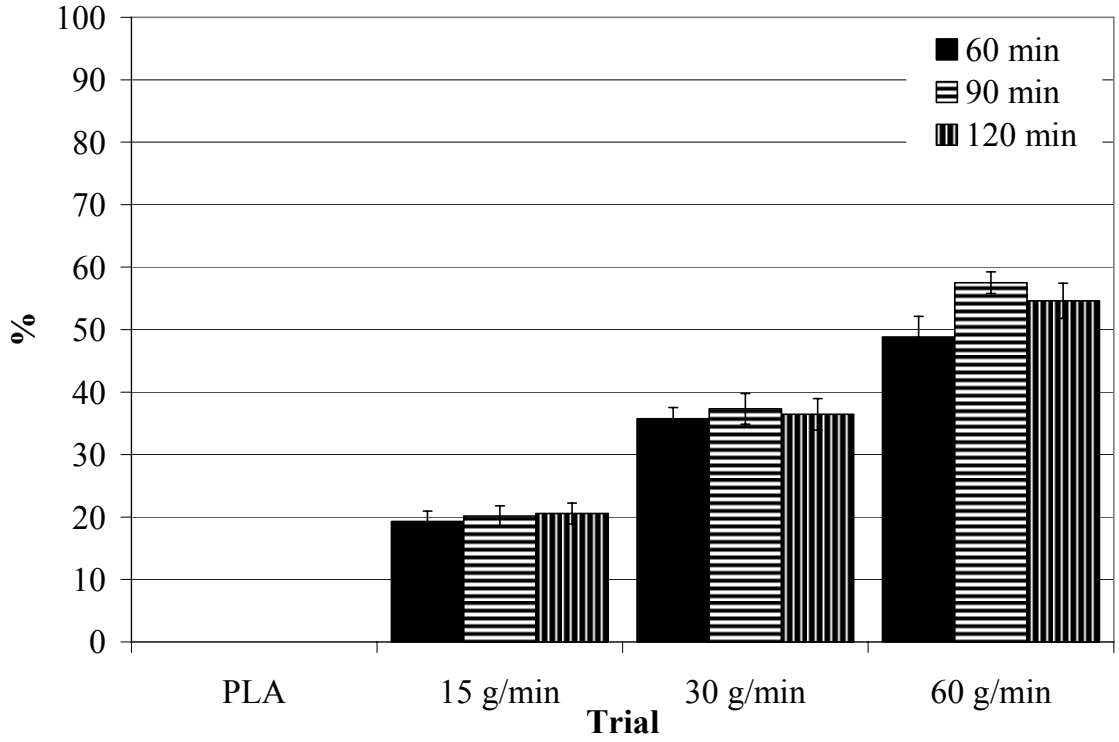
Plasma insulin response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly higher than all other treatments ($p < 0.05$). There is a significant main effect for time ($p < 0.05$).

Figure 10 – Free fatty acid response during exercise



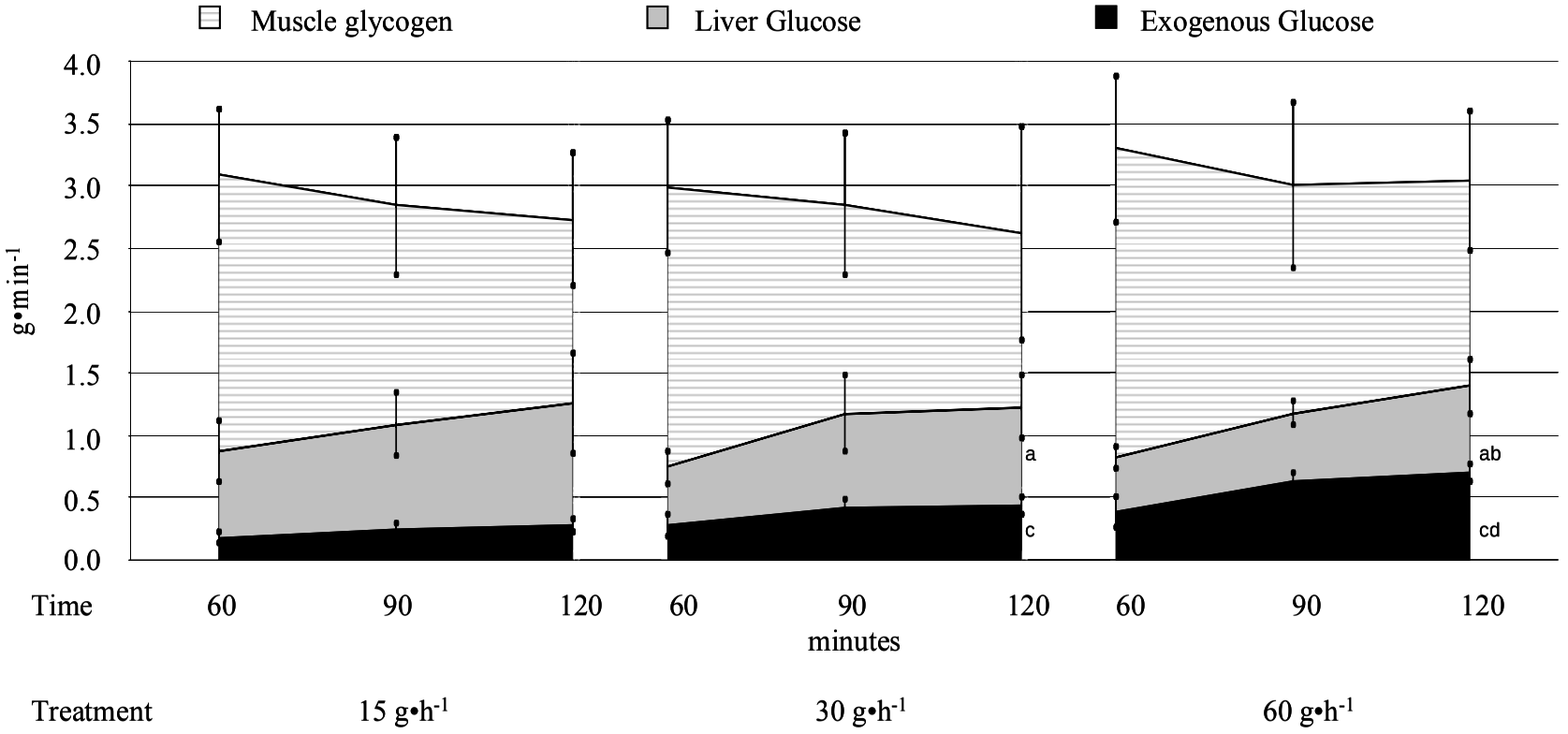
Serum free-fatty acid response during a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). [a] significantly lower than 0 and 15; [b] significantly lower 30. ($p < 0.05$). There is a significant main effect for time ($p < 0.05$).

Figure 11 – Percent plasma glucose coming from an exogenous source



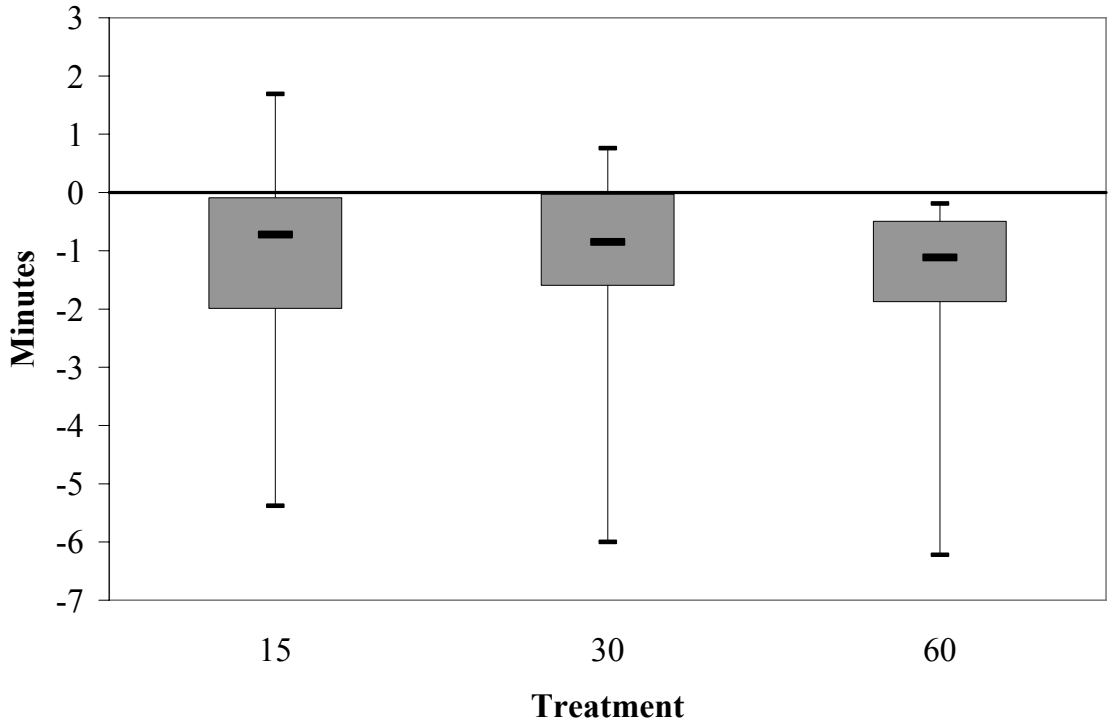
Percent plasma glucose coming from an exogenous source during the last hour of a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response. Values are means (SE). All trials are significantly different from each other. There were no significant differences in regards to time within trials.

Figure 12 – Source of oxidized carbohydrate during the last hour of exercise when consuming glucose at 15, 30, and 60 g•h^{-1}.



Carbohydrate utilization during the final hour of a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response when consuming 15, 30, and 60 g of glucose an hour. Values are means (SE). [a] significantly less than 15 g•h^{-1}; [b] significantly less than 30 g•h^{-1}; [c] significantly greater than 15 g•h^{-1}; [d] significantly greater than 30 g•h^{-1}. ($p < 0.05$)

Figure 13: Change in 20-km time trial completion time in relation to PLA



Box plots of change in duration in the 20-km time trial performance following a 2-hour ride at 95% of the workload that elicits a 4 mmol blood lactate response when consuming glucose at various rates. The top and bottom of the box represents the 75th and 25th percentile. The whiskers capture the range of performance times for the entire group of subjects. The black line in the box corresponds to the median performance value. No differences were observed between glucose treatments. All treatments were significantly faster than placebo.

VI: SUMMARY

This study examined the physiological and exercise performance responses of cyclists during a 2-hour constant load ride followed by a simulated 20-km time trial to determine the impact of beverages with glucose concentrations at and below what is recommended by the American College of Sports Medicine and the National Athletic Trainer Association. Participants completed four trials including an electrolyte containing placebo beverage trial with no carbohydrate and three trials during which a glucose/electrolyte beverage was ingested at a rate of 15 $g \bullet h^{-1}$, 30 $g \bullet h^{-1}$, and 60 $g \bullet h^{-1}$.

This study investigated the impact of glucose ingestion at rates of 15 $g \bullet hr^{-1}$, 30 $g \bullet hr^{-1}$, and 60 $g \bullet hr^{-1}$ on carbohydrate metabolism and exercise performance. The main findings of this investigation were: 1) exogenous glucose oxidation increased as glucose ingestion rate increased, 2) the ingestion of glucose at increasing rates provides increased protection of liver glucose stores, and 3) ingesting glucose at rates equal to and greater than 15 $g \bullet h^{-1}$ result in improved cycling time-trial performance.

Exogenous glucose oxidation rates increased as glucose ingestion rates increased. This demonstrates that the glucose ingested during exercise can be readily used for energy. With exogenous glucose oxidation rates in the 30 $g \bullet hr^{-1}$ exceeding ingestion rates in the 15 $g \bullet hr^{-1}$, as well as exogenous glucose oxidation rates in the 60 $g \bullet hr^{-1}$ exceeding ingestion rates in the 30 $g \bullet hr^{-1}$, it seems that intestinal absorption is not a major limiting factor to the oxidation of exogenous glucose at low ingestion rates.

As glucose ingestion increased the reliance on muscle glycogen did not change but the utilization of liver-derived glucose for energy was reduced. Delaying the utilization of endogenous sources of carbohydrate would provide the body greater energy later in exercise allowing the maintenance of workloads further into the duration of exercise.

Glucose ingestion did not alter heart rate, ratings of perceived exertion, respiratory exchange ratio, blood lactate, or cortisol. Blood glucose levels were significantly elevated compared to placebo with glucose ingestion rates as low as 15 $g \bullet hr^{-1}$ and were further elevated with ingestion rates of 30 and 60 $g \bullet hr^{-1}$. Blood insulin levels were significantly elevated compared to placebo only when glucose was ingested at 60 $g \bullet hr^{-1}$. Free-fatty acid levels were reduced with a glucose ingestion rate of 30 $g \bullet hr^{-1}$ and further reduced with a glucose ingestion rate of 60 $g \bullet hr^{-1}$.

Time to complete the 20-km time-trial and average wattages of the 20-km time-trial were significantly improved with a glucose ingestion rate of 15 $g \bullet hr^{-1}$ as compared to placebo. Increasing glucose ingestion rate provided no further improvements.

Potential areas for future research in this area include: 1) determining the impact of other carbohydrate and fat fuel source on physiological and performance measures; 2) exploring the impact multiple carbohydrate sources delivered in reduced carbohydrate beverages on physiological and performance measures; 3) exploring the mechanism that allows for similar improvements in exercise performance with variations in the utilization of exogenous and endogenous energy sources; and 4) exploring methods to maximize endogenous and exogenous substrate utilization to enhance exercise performance.

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APPENDICES

APPENDIX A:

INFORMED CONSENT

GATORADE SPORTS SCIENCE INSTITUTE

SUBJECT INFORMED CONSENT –

PHYSIOLOGY RESEARCH

PROJECT LIGHT WEIGHT: Two hour fixed-load cycling followed by a simulated 20-kilometer time trial.

RESEARCHERS: JohnEric Smith, Jeff Zachwieja

Please read the following information carefully and feel free to ask questions. Sign the final page only when you are satisfied that all procedures and risks have been sufficiently explained to you.

A. REQUIREMENTS

This study requires that you meet the following criteria:

- You must have undergone stress-testing and be cleared for participation by the medical director of GSSI.
- You must be a non-smoking male between the ages of 18-40 with no history of diabetes or PKU (phenylketonuria). You must be used to cycling for long periods of time at a moderate to high intensity.

Subjects may be excluded following one or more of the pre-tests based on fitness level or inability to complete the protocol.

B. PURPOSE OF THE STUDY

This study is designed to determine the effect of drinking sports drinks with different nutrient compositions on cycling performance.

C. TEST PROCEDURES

On nine (9) separate occasions, you will be asked to ride your own bike on a CompuTrainer in our laboratory.

NINE VISITS TO THE LAB WILL BE REQUIRED, AS SUMMARIZED BELOW:

1. VO_2 Max Test: This test will involve cycling for 2-3 minute periods at an increasingly higher resistance until you can no longer pedal. We will measure your peak oxygen consumption rate and peak cycling wattage. To assess oxygen consumption rate, you will be required to perform the test with a mask placed over your mouth and nose. You will be able to easily inhale room air while your expired air will be analyzed for oxygen and carbon dioxide content (Total time: 45 minutes).
2. Lactate Threshold Test: This test will consist of riding for 3 minute stages at 50, 55, 60, 65, 70, 75, 80, 85, and 90 percent of your VO_2 max (determined in the test above). Blood samples will be taken to measure the amount of lactate in your blood. A registered Nurse will insert a small sterile flexible tube into one of the arm veins that is close to the skin to get blood samples. The sterile tube will remain in your arm vein

for the length of the experiment. Approximately 4 ml of blood will be drawn at each stage of the test totaling about 30 ml (1 oz) of blood for the entire test. All equipment used in the procedure is sterile. You will wear a mask for this test in order to assess your oxygen consumption as in the VO_2 Max Test. (Total time: 60 minutes)

3. Familiarization I: The purpose of this visit is to allow you to become familiar with the CompuTrainer 20 kilometer time trial course. (Total time 1 hour)
4. Familiarization II: The purpose of this visit is to allow you to become familiar with riding the CompuTrainer 20 kilometer time trial course after a two hour constant resistance ride that will be set just below your lactate threshold. (Total time: 3 hours)
5. Familiarization III: The purpose of this visit is for you to become familiar with the drinking schedule and physiological measures that will be made during the experiment. During the 2 hour constant resistance rise (resistance set just below your lactate threshold) you will receive about 8.5 oz of water every 15 minutes. A registered Nurse will insert a small sterile flexible tube into one of the arm veins that is close to the skin to get blood samples. The sterile tube will remain in your arm vein for the length of the experiment. Approximately 8 ml of blood will be drawn before the initiation of the constant resistance ride and then every 15 minutes during the last hour of the constant resistance ride totaling 48 ml (~1.6 oz) of blood for the entire test. All equipment used in the procedure is sterile. Heart rate and oxygen consumption will be monitored and ratings of perceived exertion will be recorded throughout. When the 2-hour constant resistance ride is complete, you will receive a final 8.5 oz of water and a two minute rest. You will then complete a simulated 20-kilometer time

trial. The goal of the time trial will be to complete the distance as quickly as you can. Heart rate will be monitored throughout the time trial. (Total time: 4 hours)

6. Experimental treatment I - IV: Performance trials with test beverages. The 2 hour constant resistance ride will be set just below your lactate threshold. You will receive about 8.5 oz of a beverage every 15 minutes during the constant resistance ride. A registered Nurse will insert a small sterile flexible tube into one of the arm veins that is close to the skin to get blood samples. The sterile tube will remain in your arm vein for the length of the experiment. Approximately 8 ml of blood will be drawn before the initiation of the constant resistance ride and then every 15 minutes during the last hour of the constant resistance ride totaling 48 ml (~1.6 oz) of blood for the entire test. All equipment used in the procedure is sterile. Heart rate and oxygen consumption will be monitored and ratings of perceived exertion will be recorded throughout. When the 2-hour constant resistance ride is complete, you will receive a final 8.5 oz of beverage and a two minute rest. You will then complete a simulated 20-kilometer time trial. The goal of the time trial will be to complete the distance as quickly as you can. Heart rate will be monitored throughout the time trial. (Total time: 4 hours)

EXPERIMENTAL SESSIONS: Each session will *require* the following:

- You MUST arrive 15 minutes prior to the start time (5:45 am).
- You will be asked to fast overnight leading up to the day of an experimental trial. On the morning of an experimental trial you are permitted water, but no other food or beverage.

- No exercise for 24 hours prior to the testing.
- You will be asked to keep a written food record of your diet for 24- hours prior to the first experimental trial. You will repeat that diet for each of the 3 subsequent experiments.
- You will be required to empty your bladder just prior to each experiment. If you need to urinate during a test you will collect it in a plastic container, which will be weighed and then disposed.
- Nude bodyweights will be measured before and after the experiment. This occurs in privacy behind a screened off area. Your weight is recorded from a digital readout outside of the privacy area.
- You will be asked to consume each of the beverages given to you in their entirety during exercise.

D. HEALTH RISKS

All experimental procedures used in this study have been routinely used in this and other exercise physiology laboratories, and present minimal risk to your health. However, you should be aware that there are risks involved in any laboratory procedure.

- Exercise: Abnormal responses to exercise include unusual blood pressure responses, disturbances in heart function, nausea, and fainting. Risks also include muscle cramps, strains, tears, joint and/or muscle pain, sweating, breathlessness, breathing difficulty, changes in heart rate, stroke and death. If any of these symptoms occur during the exercise session, stop exercising and notify a staff

member immediately. In addition a fall from the bike may result in bruises, broken bones, dislocations, or head injury.

- Dehydration: Risks associated with dehydration include abnormal feelings of fatigue, irritability, headache, lightheadedness, abnormal heart rate and blood pressure response and heat illness. Despite drinking fluids during exercise modest dehydration may occur. This level of dehydration ($\leq 1\%$) is commonly experienced by athletes with no associated symptoms. However, every precaution, including provision of beverage will be taken to reduce all risks of dehydration.
- Blood Draw: Pain or infection may occur.
- Beverage Ingredients: The beverages used in this study will contain electrolytes and various levels of glucose. Glucose ($C_6H_{12}O_6$) is made up of carbon (C), hydrogen (H), and oxygen (O). Most of the carbon in nature has a mass of 12, a small portion of the glucose you will ingest will be enriched with carbon 13 (^{13}C), a naturally occurring isotope of carbon that is non-radioactive. The ^{13}C will allow us to monitor how much of the ingested glucose is being used by the muscle during the exercise. Some beverages contain FDA approved artificial sweeteners, which are harmful to individuals who have the metabolic condition PKU.

E. NOTIFICATION TO STAFF

For your health and safety, it is imperative that you notify the lab staff of any acute and/or chronic medical conditions, and your current use of all medications (even over-the-counter drugs). You are also required to notify us of any allergies you may have.

It is also important that you immediately notify the lab staff during exercise of any unusual symptoms or abnormal responses arising from the testing. Emergency procedures are coordinated through the Barrington Paramedics and Good Shepherd Hospital. All precautions needed to minimize risks to your health will be taken.

F. ACCESS TO TEST RESULTS

Upon specific request, you may have access to certain test results, provided those results do not reveal any information that is confidential to GSSI research.

G. CONFIDENTIALITY OF TEST RESULTS

Your *test results* will be kept in the Gatorade Sports Science Institute laboratories. Grouped or blind-coded data may be used for publication in scientific journals or for marketing claims. Personal medical confidentiality will not be breached. Only the staff will have access to your individual test results.

H. PUBLICITY RELEASE

As part of my participation in testing by the Gatorade Sport Science Institute, I understand that photographs, videotapes and/or drawings or other likenesses of me may be taken and used from time to time by the press or by Gatorade for public relations or other publicity or advertising purposes. I hereby grant full permission to Stokely-Van Camp, Inc. and The Quaker Oats Company, and their parents, affiliates, successors and assigns or anyone authorized by any of them, to use my name, photograph, video image, voice, likeness and biographical data, in whole or in part, in any and all media for the

purposes of publicity, advertising, trade or news purposes and in connection therewith I hereby release them and each of them from all liability.

I have read each and every word of the foregoing prior to my execution of this release and am fully familiar with the contents thereof.

I. SUBJECT COMPENSATION

After all testing has been completed, you will receive an honorarium that includes:

$ 25 for VO_2 max test

$ 25 for lactate threshold test

$ 25 for familiarization I trial

$ 75 for familiarization II trial

$100 for familiarization III trial

$100 for each of 4 experimental sessions (4 tests X $100 = $400)

$ 50 finishing bonus

Total compensation upon completion of all tests = $700

You may withdraw at any time from any or all sessions without prejudice to your status at PepsiCo, or as a subject in the GSSI research program. If you withdraw, your honorarium will be prorated based on the number of sessions completed.

The honorarium will also be prorated if a test is partially completed and must be rescheduled due to staff constraints or experimental failure of our equipment.

J. INFORMED CONSENT FORM AND WAIVER FORM

By reading and signing this document, I acknowledge my consent to participate based on sections "A" through "I" above. I also acknowledge that my participation in this exercise activity is not without risk of injury. I understand that muscular or orthopedic injury may result from my improper use of the equipment, from poor exercise technique, and from overuse or overtraining. Although I have been screened by GSSI staff or physician, including a symptom-limited graded exercise test, there exists the possibility of certain changes occurring during exercise. These include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances, heart attack, stroke or death. I am aware that every effort will be made to minimize these risks by provision of appropriate supervision during exercise. Emergency equipment and trained personnel are available to deal with unusual situations that may arise. I declare that I am in good physical and mental health, and have no heart, lung, kidney, musculoskeletal disease or problems, or diabetes mellitus or any disorder that would make my participation medically inadvisable. I hereby release from liability and promise not to sue Pepsico, Inc, Stokely Van-Camp, and the Gatorade Company, and any of their subsidiaries, affiliates, officers and employees, for all loss or damage suffered by me or to my bicycle, and I promise not to make any claim on account of injury to my person or property or resulting in my death whether caused by negligence or otherwise to myself, my personal representatives, heirs and next of kin.

I have been given ample opportunity to read this document and to ask questions which have been answered to my satisfaction. I understand the intent of this document

and acknowledge the possibility of exercise-related injury. I hereby consent to participate in this exercise activity, under the terms and conditions stated above. I know that I may withdraw my consent and stop participation in the exercise session at anytime, for any reason.

I agree to the terms set forth in the subject informed consent document and further agree to keep confidential any information learned or obtained in connection with my participation in the exercise activity and to not disclose to others my impression of or use in any way any information that is confidential or proprietary to PepsiCo, Inc concerning any concepts, proposals, test results, improvements to existing products or any other confidential or proprietary information. I sign this document voluntarily.

Signature _____ Date:_____

Name (Printed) _____

Signature of Investigator: _____ Date:_____

APPENDIX B:

DATA COLLECTION SHEET

Participant _____ Trial Number _____ Date _____

- Get dietary record from participant.
- Have the participant empty their bladder record a nude body weight

  Body Weight _____ kg Urine Volume _____ g

  Urine Specific Gravity _____
- Have nurse insert catheter
- Collect 1 red top vacutainer of blood

  2 green top vacutainers of blood

  1 hepranized syringe of blood

After Catheter Insertion

- Collect and analyze one 2 minute Douglas Bag sample.

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____
- Set the bike up on the trainer.
- Remove participant's cyclocomputer.
- Remove participant's watch.
- Have participant put on Polar Heart Rate monitor.
- Change the rider to the participant's name.
- Provide the participant with a towel.

- Have the participant ride 10 minutes at 100 Watts for a warm-up.
- Following the warm-up have the participant calibrate the trainer.

  Set the number between 2.0 and 2.1 _____
- Have the participant mount the bike and begin the 2 hour ride at the predetermined workload

  Workload _____ Watts.

  Gearing: Front _____

  Rear _____
- Turn front fan on 1.

At 13 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample.

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____
- Give 1st Beverage

At 28 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____

- Give 2nd Beverage

At 43 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____

- Give 3rd Beverage

At 58 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____

- Collect 1 red top vacutainer of blood

  2 green top vacutainers of blood

  1 hepranized syringe of blood

- Give 4th Beverage

At 73 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

Barometric Pressure _____

- Collect 1 red top vacutainer of blood
  2 green top vacutainers of blood
  1 hepranized syringe of blood
- Give 5th Beverage

At 88 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____
- Collect 1 red top vacutainer of blood
  2 green top vacutainers of blood
  1 hepranized syringe of blood
- Give 6th Beverage

At 103 minutes

- Record RPE rating _____
- Record Heart Rate _____

- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____

- Collect 1 red top vacutainer of blood

  2 green top vacutainers of blood

  1 hepranized syringe of blood

- Give 7th Beverage

At 118 minutes

- Record RPE rating _____
- Record Heart Rate _____
- Collect and analyze two 30 second Douglas Bag sample. .

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  O_2% _____ CO_2% _____ Sampling Time _____

  Initial Volumetric Reading _____ Final Volumetric Reading _____

  Room Temperature _____ Room Humidity _____

  Barometric Pressure _____

- Collect 1 red top vacutainer of blood

2 green top vacutainers of blood

1 hepranized syringe of blood

- Give 8th Beverage

At 120 minutes

- Allow the participant to dismount the bike
- Turn off any music
- Lock doors
- Select 20 k time trial 3 and desert view

At 121:30

- Have participant mount bike
- Have participant finish beverage

At 121:57

- Press start to begin time trial
- Record completion time

: : .

_____

hrs mins sec hundredths

- Record participant's nude body weight

Body Weight _____ kg

- Have the participant empty their bladder into a cup and record urine volume

Urine Volume _____ g Urine Specific Gravity _____

- Allow the participant to cool down and provide with their choice of beverage.

Research Initials: 1. _____ 2. _____ 3. _____