CARBOHYDRATE-PROTEIN INGESTION AND CYCLING PERFORMANCE

EFFECT OF CARBOHYDRATE AND PROTEIN INGESTION DURING EXERCISE ON CYCLING TIME TRIAL PERFORMANCE AND METABOLISM IN TRAINED MEN

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ABSTRACT

Two recent studies (Ivy et al., Int J Sports Nutr Exerc Metab 13:382-395, 2003; Saunders et al., Med Sci Sports Exerc 36:1233-1238, 2004) reported dramatic (>25%) increases in endurance time to fatigue during cycling at 75-85% VO_2peak when subjects ingested a ~2% protein (PRO) plus ~7% carbohydrate (CHO) drink as compared to CHO alone. However, the research designs employed in these studies have been criticized for several reasons, including (1) the rate of CHO and fluid provided was less than what is considered optimal for endurance performance (i.e., 6% CHO, ingested at a rate of 15 g in 250 ml of fluid, every 15 min); and (2) the nature of the performance tests did not mimic the way in which athletes typically compete (i.e., a race, in which a given distance must be covered as quickly as possible). Purpose: To determine whether the addition of 2% Pro to a 6% CHO drink (CHO-Pro) improves 80 km cycling time trial performance as compared to a 6% CHO drink and a non-energetic sweetened placebo. Methods: Ten well-trained cyclists (25±5 y; VO_2peak = 63±5 ml/kg/min; means±SD) completed a simulated 80 km time trial (TT) on three separate occasions separated by 5-7 d. In a randomized, double-blind manner, subjects ingested either CHO-Pro, CHO or placebo at a rate of 250 ml every 15 min. All trials were performed on a Computrainer (RacerMate, Seattle, WA) using each subject's own bicycle with no temporal, verbal or physiological feedback. Venous blood samples were obtained periodically during exercise and subsequently analyzed for glucose, lactate, free fatty acids, ammonia and insulin concentrations. Results: Analysis of variance revealed that time to complete the 80 km

TT was lower ($P \leq 0.05$) when subjects ingested CHO (135±9 min; mean±SD) and CHO-Pro (135±9) compared to placebo (141±10), with no difference between CHO-Pro and CHO. Average power output was higher ($P<0.05$) when subjects ingested CHO and CHO-Pro versus placebo, and work intensity averaged 81±1%, 80±1% and 78±1% of heart rate reserve for the CHO, CHO-Pro and placebo rides, respectively. Improved performance of the two CHO trials was primarily attributed to maintenance of blood glucose concentration during the later stages of exercise. Conclusion: Ingestion of a 6% CHO drink at a rate of 1 L/h improves 80 km TT performance, as compared to a non-energetic placebo, in trained male cyclists. However, the addition of 2% Pro to a 6% CHO drink provides no additional performance benefit.

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- Chapter 1 -

REVIEW OF LITERATURE

1.1. Introduction

The notion of sports nutrition has changed dramatically over the years. Early Greek athletes are reported to have consumed a diet that primarily consisted of cereals, fruit, vegetables and legumes, and wine diluted with water (49). In contrast, a hundred years ago protein in the form of beef was thought to be the most important component of an athlete's diet (76). However, as the field of sports science evolved researchers began to critically evaluate the effectiveness of various nutritional strategies. Early studies from the beginning of the 20th century found that endurance exercise felt easier when subjects consumed a diet high in carbohydrate (CHO) as compared to a diet high in fat (81; 84). Moreover, Levine (1924) found that marathon running performance was significantly improved if athletes consumed CHO during the race. These findings paved the way for mechanistic studies by others documenting similar findings and today it is generally accepted that CHO consumption during exercise improves performance in endurance events lasting $\geq$ 60-90 minutes (8; 13; 23; 42; 63; 102).

Growing recognition of the ergogenic effect of CHO provided incentive for the commercial production of sports drinks. Since the 1980's, commercial sports drinks have become widely available and are now considered part of the "culture" of endurance sports (73). Consequently, with the growing number of people participating in endurance sports, the development of the "ideal" sports drink has become of serious interest to the different manufacturers competing for a share of the marketplace. Each manufacturer has its own unique combination of carbohydrates and electrolytes trying to provide the optimal performance enhancing drink. In addition, some manufacturers have opted to include other macronutrients, such as protein, under the assumption that it will provide further performance enhancement.

Protein oxidation contributes only 1-6 % to the total energy provision during exercise (101) which suggests that inclusion of this macronutrient in a sports drink would have little impact on performance. Nevertheless, two recent studies reported that adding protein to a CHO sports drink improves endurance performance (56; 93). These studies documented dramatic increases in endurance time to fatigue (>25%) during cycling at 75-85% peak oxygen uptake (VO_2peak) when subjects ingested a CHO plus protein beverage as compared to CHO alone (57; 92). However, the methodology employed in these studies makes it difficult to draw conclusions regarding the effectiveness of supplementing with protein. Moreover, the mechanism underlying the performance enhancement remains elusive. The purpose of the present review is to evaluate: i) the physiological determinants of endurance performance, ii) fuel utilization during endurance exercise, iii) nutritional strategies to improve endurance performance, iv) potential mechanisms responsible for performance improvements after nutritional manipulation, and iv) methodological considerations when evaluating the effect of nutritional supplementation on endurance performance.

1.2. Physiological determinants of endurance performance

Performance in endurance events is dependant on multiple factors including but not limited to those that are physiological, psychological, and environmental. This section of the review will solely focus on components that are physiological in nature. Specifically, it will limit the discussion to factors other than fuel utilization and availability as these will be discussed later in the review. Thus if we ignore fuel availability and utilization temporarily the physiological determinants of endurance performance may be grouped into three main factors. These factors include; the $V0_2$peak of the individual, the percent of $V0_2$peak that can be maintained throughout the endurance event, and the mechanical efficiency of translating the work produced into forward movement. (17).

An individual's $V0_2$peak is thought to set the upper limit of aerobic energy production in endurance events (20). It is well known that elite endurance athletes typically have $V0_2$peak values that are higher than the average individual suggesting this parameter is important for success in endurance sports. Indeed, Costill et al. (1973) showed a strong inverse correlation (R= -0.91) between $V0_2$peak and time to complete a 10 mile run over a wide range of $V0_2$peak values (54.8 to 81.6 ml/kg/min) (37). However, when athletes have similar $V0_2$peak values time to complete an endurance event can still vary substantially (15). The finding suggests the importance of other factors in determining endurance performance.

A second factor that influences success in endurance events is the percent of $V0_2$peak that can be maintained over the duration of the endurance event. Classically, the percent of $V0_2$peak that can be maintained is thought to be dependant upon the lactate threshold of the individual (19) where aerobic energy production is supplemented by anaerobic mechanisms, causing a sustained increase in lactate and metabolic acidosis (113). However, it should be noted that considerable debate now exists concerning whether or not acidosis is the actual cause of fatigue at exercise intensities exceeding lactate threshold (114). Regardless of the precise limiting factor, there is a certain intensity (typically expressed as a percent of $V0_2$peak) that an athlete is able to maintain and exceeding that intensity will cause fatigue to be imminent. For example, it has been shown trained individuals can work at 87% and 83% $V0_2$peak for 1 and 2 hours respectively, compared with 50% and 35% for untrained individuals (16).

The product of $V0_2$peak and percent of $V0_2$peak at lactate threshold has been termed "performance $V0_2$" (18). Essentially, performance $V0_2$ dictates the maximum rate of ATP production that can be maintained throughout an endurance event. However, the actual velocity realized by the performance $V0_2$ depends on the individual's efficiency in translating the energy produced into motion. Thus, the third major factor determining endurance performance is the mechanical efficiency the individual has in the mode of exercise being performed. The importance of this variable was illustrated by Conley and Krahenbuhl (1980) who found a relatively strong correlation (r=0.82) between running economy and 10 km run time of a group of runners with similar $V0_2$peak values but with 10 km times ranging from 30.5 - 33.5 min. Running economy was assessed via the athletes oxygen consumption at different running velocities making it reflective of their mechanical efficiency (36).

Clearly, $V0_2$peak, the percent of $V0_2$peak that can be maintained and mechanical efficiency all play a key role in determining performance in an endurance event. However, fuel availability and utilization are other key variables that affect endurance performance as discussed in detail below.

1.3. Fuel utilization during endurance exercise

The fuel used for endurance exercise is provided by the CHO, fat and protein we consume in our diet and store in our bodies. CHO and fat are the predominant fuels used during either rest or exercise (98). In contrast, protein contributes very little (1-6%) to the total energy provision during endurance exercise (87; 100). Even in extreme conditions, where an athlete may be engaged in prolonged endurance exercise in a fasted state, the contribution of protein to total substrate utilization is probably $\leq$ 10% (83).

The relative contribution of CHO and fat oxidation to energy provision is dependant on a variety of factors including the intensity and duration of exercise, diet, environmental conditions, gender, and training status. At rest and low exercise intensities fat oxidation supplies the majority of the energy needs. However, as exercise intensity increases there is a progressive shift from fat to CHO oxidation (1; 6). The absolute amount of CHO oxidized is closely related to the total energy needs of the working muscles (52). Thus, as the exercise intensity increases so does the absolute amount of CHO that is oxidized. In contrast, the absolute amount of fat that is oxidized will increase with exercise intensity up to about 65% $V0_2$peak, after which a progressive decline in fat oxidation is observed (2; 5). Currently, the mechanisms responsible for the down regulation of fat oxidation at higher exercise intensities are not fully understood (97).

The duration of exercise also plays a role in the relative contribution of CHO and fat to energy provision. As exercise duration increases there is an increase in fat oxidation parallel with a decrease in CHO oxidation (99). For example, Edwards et al. (1934) reported fat oxidation values of over 1.0-1.5 g/min after 6 hours of running compared to 0.2-0.5 g/min typically reported in shorter duration exercise (3; 4). This shift in the proportional contribution of fuel sources is likely related to a reduction of muscle glycogen stores towards the later stages of prolonged exercise (75).

1.4. Fuel availability during endurance exercise

It is now generally accepted that prolonged endurance exercise of sufficient intensity is limited by CHO availability to the working muscles (50; 58; 69). The reason for this is two-fold. First, endurance exercise is typically performed at intensities (>50% $V0_2$peak) that require the majority of energy provision to come from CHO stores. Second, CHO stores are considerably more limited than fat stores. CHO is stored as glycogen in the liver and skeletal muscle. The liver may contain 80-100 grams of glycogen in the post-absorptive state while the muscle of a well-fed endurance athlete may contain 500-900 grams of glycogen (68). After prolonged strenuous exercise liver glycogen stores can be completely depleted (55) and the total amount of glycogen in the muscles can be reduced to as little as 50 g (67). In contrast, fat is stored as triacylglycerol in the muscles and adipose tissue. While the muscles may hold ~300 grams of triacylglycerol, adipose tissue contains 5-40 kilograms, depending on the body composition of the individual. Accordingly, it is estimated that fat stores provide 92-98% of endogenously stored energy compared to 2-8% that can be stored as CHO (70). From these estimates it is clear to see why CHO is the limiting fuel source.

1.5. Nutritional Strategies to Improve Endurance Performance

Proper nutrition is essential for successful performance in sport. Getting a balanced diet consisting of the right nutrients at the right time is needed for optimal recovery from training and optimizing fuel stores before a race. Furthermore, there are certain nutritional strategies that an athlete can follow during competition. The following describes the effect of ingesting CHO and protein during exercise and discusses the potential mechanisms behind improved performance.

1.5.1 CHO Ingestion during Endurance Exercise

Since its recognition as a performance enhancer in the 1920's, CHO supplementation during endurance exercise has received considerable attention. The majority of studies that have examined the effect of manipulating CHO availability during exercise reported significant performance enhancement (7; 10; 22; 43; 64; 103), although this is not a universal finding (29; 45; 89).

Originally, evidence for the performance enhancing effect of CHO consumption during exercise came from studies demonstrating its effect on exercise lasting more than 2 hours in duration. Coyle et al. (1983) had subjects ride to exhaustion at 74% V02peak with either a placebo or a CHO intake of approximately 124 g/hour. They found endurance time to fatigue was 134 min with the placebo but was extended to 157 min with CHO ingestion (44). An ergogenic effect has also been observed in long-duration time trials (11; 104). Angus et al. (2000) had trained cyclists perform a simulated 100 km time trial and found that when subjects consumed a 6% CHO solution they were able to complete the trial in 166 min as compared to 178 min for placebo ingestion. Tsintzas et al. (1993) found that CHO intake also improved performance during long distance running. Subjects competed in a 30 km road race on two separate occasions with either a 5% CHO solution or water consumed in 150 ml doses every 5 km. Time to complete the race was significantly less with the CHO solution (128.3 min) compared to water (131.2 min) (105). Other recent studies have found a positive effect of CHO intake during relatively short (~1 hour), high intensity (>75% V02peak) endurance exercise (9; 21; 65). For example, Jeukendrup et al (1997) found that CHO ingestion during a 40 km cycling time trial improved performance by 2.3%. In contrast, Desbrow et al. (2004) found no additional performance improvement during a ~1 hour cycling time trial when subjects consumed a 6% CHO solution compared to a placebo.

The discrepancy in the literature regarding the ergogenic effect of CHO is likely related to methodological differences between studies. Some investigators have chosen performance measures that are less sensitive to changes in performance than those used in other studies. For example, showing changes with a 40 km (~1 hour) time trial is more difficult than showing changes with a 2-3 hour ride to exhaustion. The amount of CHO provided during the trial may be another reason why an ergogenic effect would not be observed. Bonen et al. (1981) found no improvement in performance during a ride to exhaustion at 80% $V0_2$peak when subjects ingested a 20% CHO solution at a rate of 234 g/hour. CHO consumption at this rate during high intensity exercise is likely to cause gastro-intestinal discomfort and may in fact be ergolytic (78). Moreover, the subjects in that study (30) exercised for less than 30 min which is likely too short to demonstrate an ergogenic effect. Palmer at al. (1998) also found that CHO ingestion did not produce an ergogenic effect during a 20 km time trial lasting approximately 30 min. Consequently, it appears exercise duration must be at least 1 hour long in order for CHO supplementation to be beneficial.

1.5.1.1. Amount of CHO

Performance improvement with CHO ingestion has been demonstrated with an intake of as little as 16 g/hour (86). However, most studies that found an ergogenic effect used an intake of 40-75 g/hour (71). Mitchell et al. (1989) examined the effect of different CHO solutions on time trial performance following 105 minutes of cycling at 70% $V0_2$peak. The investigators found that performance was improved when CHO was ingested at a rate of 74 g/hour (12% solution) but not at 37 g/hour (6% solution) or 111 g/hour (18% solution) (88). It has been suggested that ingesting CHO at a rate of 40-75 g/hour optimizes the availability of exogenous CHO at the muscle whereas consumption in excess of 75 g/hour provides no further performance enhancement (35).

Further support for an optimal range of consumption comes from studies that examined the rate of CHO oxidation during exercise. These studies indicate that the maximal rate of oxidation for a single ingested CHO is ~1g/min. For example, glucose will be maximally oxidized at a rate of ~1g/min while fructose and galactose are maximally oxidized at a rate of ~0.6g/min (82; 85). Jeukendrup (2004) compared a number of studies that examined CHO oxidation and concluded that a CHO intake rate of 1.0-1.2 g/min was enough to provide the maximal rate of CHO oxidation (Figure 1). These findings suggest that ingestion of a single CHO at a rate greater than 1-1.2 g/min would not provide further performance enhancement.

In contrast, recent research suggests that ingesting two different types of CHO simultaneously may allow oxidation rates to exceed 1.0 g/min. Jentjens et al (2003) used stable isotopic tracers to compare the exogenous CHO oxidation rates after ingesting 1.8 g/min of glucose versus an isoenergetic mixture of glucose and fructose. They found that when only glucose was ingested the oxidation rate peaked at 0.83 g/min, while the combination of glucose with fructose allowed the oxidation rate to peak at 1.26 g/min (55% higher compared to glucose only). A follow-up study by the same researchers found that a combination of glucose, fructose and sucrose, ingested at a rate of 2.4 g/ min produced peak oxidation rates of ~1.7 g/min (62). Glucose and fructose use different transporters across the intestinal wall and It has been suggested that combining different forms of CHO reduces competition for these transporters which allows a higher rate of absorption along with a higher rate of oxidation (96). Although intriguing, the practical implications of these findings are hampered by the fact that the rate of CHO ingestion in these studies is quite high which could cause gastro-intestinal distress in the majority of athletes consuming CHO at this rate. Consequently, more research examining the tolerability of mixed CHO is warranted before practical recommendations can be made.

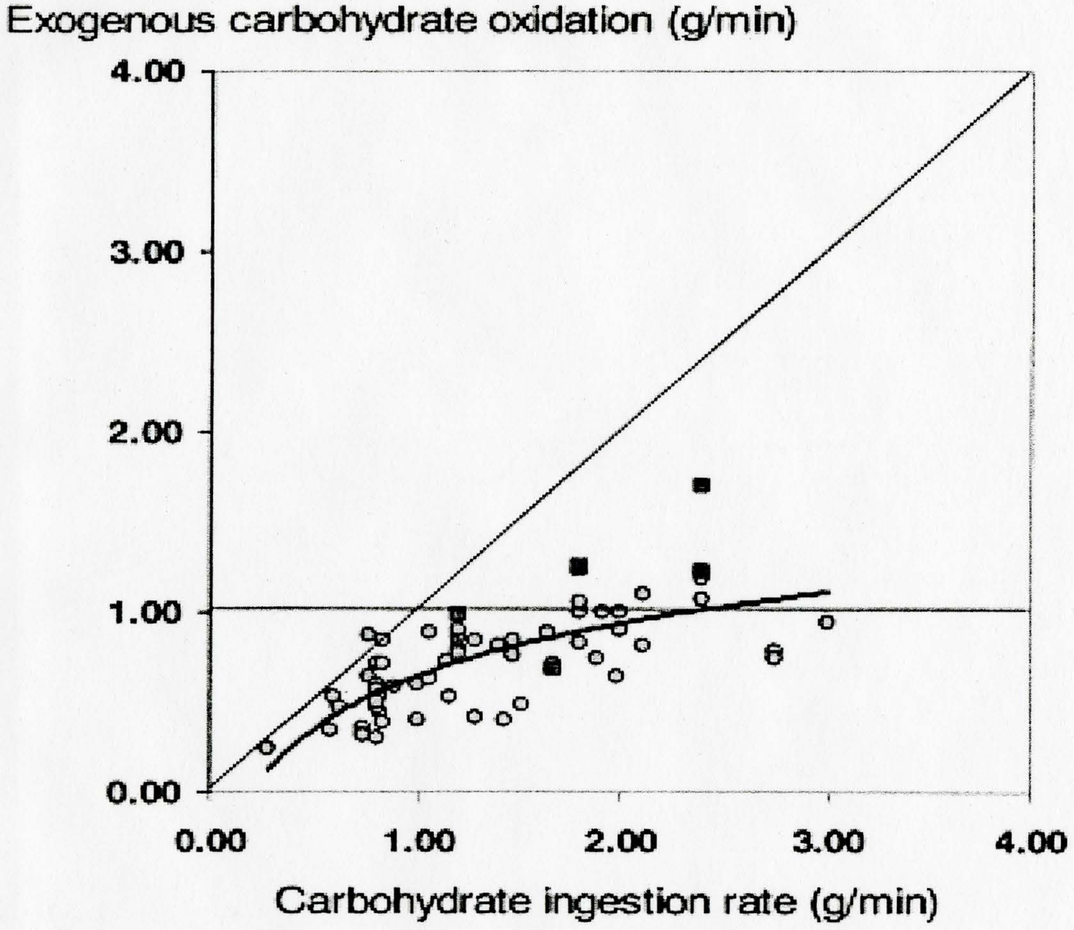

Figure 1 – Peak exogenous carbohydrate oxidation during exercise as a function of the rate of carbohydrate intake. Each dot represents the peak oxidation rate observed with one type of carbohydrate. The dotted line represents the line of identity where oxidation equals the ingestion rate. Peak oxidation rates for a single carbohydrate (circles) are typically 1.0 to 1.1 g/min. However, when multiple carbohydrates that use different intestinal transporters are ingested, oxidation rates can increase by 20% to 50% (squares). This figure is taken from a meta analysis by Jeukendrup (2004).

1.5.1.2. Mechanisms to explain the ergogenic effect of CHO ingestion during endurance exercise

There are a number of mechanisms that have been proposed to explain how CHO ingestion during exercise improves endurance performance. These include the maintenance of blood glucose concentration, sparing of endogenous glycogen, synthesizing glycogen during low intensity exercise, and the central effect of CHO. The following section of the review describes the evidence for these mechanisms.

The maintenance of blood glucose concentration is thought to allow for higher rates of CHO oxidation toward the end of prolonged exercise (12). Work done by Coyle and colleagues in the 1980's provides support for this theory (34; 40). Coyle et al. (1986) had endurance trained cyclists ride to exhaustion on two different occasions while drinking either a glucose polymer solution or a placebo. When fed CHO, which maintained plasma glucose concentration in the range of 4.2-5.2 mmol/L, the subjects were able to exercise for approximately 4 hours. In contrast, fatigue during the placebo trial occurred after 3 hours of exercise and was preceded by a decline in plasma glucose to ≤ 2.5 mmol/L. Moreover, respiratory exchange ratio was maintained throughout the CHO trial (0.86), while it dropped in the placebo trial (0.80) with no change in the rate of glycogen utilization between the trials (41). These observations suggest that the contribution of exogenous CHO improved performance by maintaining blood glucose concentration and a higher rate of CHO oxidation. The importance of maintaining blood glucose concentration for endurance capacity was further illustrated in a study by Coggan and Coyle (1987). The investigators had trained cyclists ride to exhaustion at 70%

$V0_2$peak and then rest for 20 minutes on three separate occasions while either; 1) ingesting a sweetened placebo; 2) ingesting a glucose-polymer solution; or 3) receiving a glucose infusion. After 20 min of rest the subjects performed a second ride to exhaustion which lasted significantly longer with CHO ingestion (26 min) and CHO infusion (43 min) compared to placebo (10 min). The authors attributed these findings to the fact that the placebo did not restore euglycemia after the first ride to exhaustion, while CHO ingestion restored euglycemia temporarily, and CHO infusion restored euglycemia and then maintained it. Clearly, these studies demonstrate the importance of blood glucose to energy provision during the later stages of prolonged exercise.

Most research suggests that CHO feeding does not spare muscle glycogen during moderate to high intensity exercise. Indeed, neither of the aforementioned studies examining the mechanisms of the ergogenic effect of CHO noted a difference in glycogen utilization between trials with either CHO feeding or infusion compared to placebo (33; 38). Other studies that examined glycogen depletion with CHO feeding reported similar findings with the muscle biopsy technique (47) and stable isotope infusion (79). Nevertheless, some debate still exists because a few studies have suggested that CHO feeding may spare muscle glycogen (24; 46; 106; 115). Bergsrom and Hultman (1967) infused glucose intravenously and found that muscle glycogen depletion was reduced by 25% during exhaustive one-legged cycling. However, it should be noted that the infusion produced blood glucose concentrations (21mM/L) far greater than could be reached with CHO ingestion under normal physiological conditions. Tsintzas et al. (1995) reported reduced muscle glycogen breakdown in type 1 fibres with CHO ingestion after 60 min of running at 70% V02peak. The finding suggests that the mode of exercise (i.e. cycling vs. running) may influence whether CHO feeding spares glycogen in the muscle. In addition, fibre type distribution (i.e. type 1 vs. type 2) may also have an influence whether glycogen is spared in the muscle with CHO feeding during exercise.

In contrast to the variable effect reported for muscle glycogen, most studies examining liver glycogen utilization have found that it is substantially reduced with ingestion of CHO (53; 80). For example, Jeukendrup et al. (1999) assessed endogenous glucose production from the liver via stable isotope infusion during 120 min of cycling exercise and noted a progressive decrease in gluconeogenesis and glycogenolysis with increasing CHO intake. In addition, Howlett et al. (1998) reported that endogenous glucose production returned to basal levels when glucose was infused at a rate equal to the average hepatic glucose production measured during exercise in the control trial (54). Thus, despite equivocal data with respect to muscle glycogen, exogenous CHO sources do appear to spare liver glycogen. The sparing of liver glycogen may be beneficial if exogenous CHO is not able to supply enough CHO to maintain plasma glucose concentrations toward the end of exercise (72).

It has been suggested that CHO ingestion may synthesize muscle glycogen during low intensity exercise (74). Hypothetically, this could improve endurance performance in an event involving intermittent exercise intensity where some of the exercise is performed at relatively low intensities, for example, drafting in a cycling race. Yaspelkis et al. (1993) reported higher muscle glycogen concentration following intermittent cycling (45-75% V02peak) when CHO was ingested as opposed to water. It was thought that the combination of CHO ingestion and low intensity exercise may have contributed to increased glycogen synthesis. However, the possibility exists that CHO ingestion with low intensity exercise may have simply reduced glycogenolysis. Further research is required to determine whether glycogen synthesis occurs with CHO supplementation during low intensity exercise.

Recently, the concept of a "central effect" of CHO ingestion has gained support. Studies showing improved performance during short-duration (~1 hour), high-intensity (80-85% $V0_2$peak) exercise have suggested a central effect of CHO may be at play. A number of lines of evidence support the hypothesis. First, glycogen stores are not typically limiting in fed athletes for exercise lasting approximately an hour (51). Under these conditions the body is typically able to supply all the CHO it needs from endogenous stores. Second, the contribution of muscle glycogen stores greatly exceeds contribution of blood glucose to total CHO oxidation at this relatively high exercise intensity (91; 110). Third, the amount of CHO that can be absorbed during exercise of this duration is estimated to be quite small (<15 g) (77). Finally, Carter et al. (2004) had trained cyclists ride an approximate 1 hour time trial on two occasions receiving either glucose infusion (1g/min in saline) or placebo infusion (saline). They found that despite a marked increase in plasma glucose concentration and glucose uptake, the CHO trial did not provide performance enhancement over the placebo (32). The finding suggests that the mechanism responsible for improvement in high-intensity exercise performance with exogenous CHO is not metabolic in nature. In a follow-up study Carter et al. (2004) had subjects ride the same time trial on two separate occasions with either a CHO mouth rinse or a similarly tasting, non-energetic, sweetened placebo. Interestingly, the researchers reported that time trial performance was improved when subjects rinsed their mouth with a CHO solution despite not ingesting the CHO (31). This study suggested that the performance enhancement was likely due to some form of central drive or motivational change mediated through stimulation of oropharyngeal receptors in the mouth. However, further research is required to determine the validity of this hypothesis.

1.5.2 Protein ingestion during endurance exercise

Early studies related to protein ingestion during endurance exercise looked at the effect of branched-chain amino acid (BCAA) supplementation on performance. BCAA were thought to attenuate central fatigue and therefore improve performance. This hypothesis was based on the assumption that during exercise central fatigue results from a lowering of brain activity due to an influx of tryptophan through the blood brain barrier which increases the level of serotonin. Tryptophan and BCAA compete for the same transporters across the blood brain barrier, thus the hypothesis predicts that increasing the concentration of BCAA in the blood may reduce the uptake of tryptophan and attenuate central fatigue. Indeed, limited evidence from field based studies reported both reduced mental (28) and physical (27) fatigue in some subjects. However, the majority of laboratory experiments have found no performance benefit with BCAA supplementation during exercise (25; 107; 111). In a double-blind crossover design, Varnier et al. (1994) found no differences in performance during a graded incremental exercise test to fatigue when subjects were infused with approximately 20 g of BCAA or saline 70 min before exercise. Similarly, Bloomstead et al. (1995) had endurance trained cyclists ride to exhaustion at 70% $V0_2$peak on three separate occasions with a placebo, a 6% CHO solution, or a 6% CHO + 7% BCAA solution. The investigators found that CHO only and CHO + BCAA improved performance compared to placebo, however adding BCAA provided no additional benefit over CHO only. Clearly, when examining the evidence from well-controlled lab-based studies, one would conclude that consuming BCAA during exercise does not provide endurance performance enhancement.

In contrast, two recent studies have suggested that adding whey protein to a CHO drink significantly improved performance during moderate to intense cycling (59; 95). Ivy et al. (2003) reasoned that since CHO supplementation had been shown to limit glycogen depletion in variable intensity exercise (possibly due to an altered plasma insulin response), adding protein might further increase plasma insulin levels, spare muscle glycogen and possibly improve endurance performance. To test this hypothesis, trained cyclists exercised on three separate occasions at intensities that varied between 45-75% V02peak for three hours and then performed a ride to exhaustion at 85% $V0_2$peak. Subjects performed the protocol on three separate occasions ingesting a placebo, a 7.75% CHO solution or a 7.75% CHO + 1.94% protein solution. The results indicated that time to fatigue was increased with CHO supplementation (20 min) compared to placebo (13 min) and that the addition of protein provided further performance benefit (27min). However, while blood glucose and plasma insulin levels were elevated above the placebo during the CHO and CHO + protein trials, there was no difference found between the CHO and CHO + protein trial. Thus, the researchers showed performance enhancement with the addition of protein but the reason for this improvement eluded them.

In another study, Saunders et al (2004) had moderately trained cyclists ride to exhaustion at 75% $V0_2$peak, followed 12-15 hours later by a second ride to exhaustion at 85% $V0_2$peak. The subjects performed the protocol twice (separated by 14 days), in a randomized manner with either 7.3% CHO beverage or a 7.3% CHO + 1.8% protein beverage. Interestingly, during the first ride subjects rode 29% longer with the CHO + protein beverage (106 min) compared to the CHO only beverage (82 min). In addition, during the second ride (85% $V0_2$peak), subjects were able to ride 40% longer with CHO + protein (44 min) as compared to CHO alone (31 min).

1.5.2.1 Mechanisms to explain the purported ergogenic effect of protein ingestion during endurance exercise

There are three main proposed mechanisms to explain how protein supplementation during endurance exercise may improve performance. These include altering the plasma insulin response, providing precursors to Krebs cycle intermediates, and attenuating central fatigue. However, as discussed below, the evidence supporting these hypotheses is weak.

Ivy et al. (2003) reasoned that the addition of protein to a CHO sports drink could theoretically increase plasma insulin concentration, spare muscle glycogen and thereby improve endurance time to fatigue. The researchers found that endurance time to fatigue did indeed improve with the addition of protein to a CHO drink. However, there was no difference in the plasma insulin response between the CHO only trial and the CHO + protein trial. Saunders et al. (2004) did not measure blood insulin, however the outcome would have likely been the same since the underlying rational for this purported mechanism is questionable. This is because insulin is tightly regulated during exercise to the point where even a high glycemic index sugar in a CHO beverage will only cause a small rise in insulin levels (39). Consequently, it seems unlikely that alteration of the plasma insulin response occurs with the addition of protein during endurance exercise.

It has also been proposed that supplementing with protein may provide precursors to Krebs cycle intermediates that are thought to progressively decline throughout exercise and possibly limit the ability of the mitochondria to sustain aerobic energy production (60; 112). However, Gibala et al. (2002) showed that the concentration of muscle Krebs cycle intermediates is unrelated to limb oxygen uptake during prolonged exercise. These authors showed that despite a 50% decrease in the concentration of Krebs cycle intermediates, aerobic energy provision was not compromised, as evidenced by stable $V0_2$ values during 90 minutes of leg kicking (48). Thus, even if protein supplementation contributed to the pool of Krebs cycle intermediates, it unlikely that increasing the concentration would provide an ergogenic effect.

Lastly, it has been suggested that protein supplementation may attenuate central fatigue. As already described, it is thought that central fatigue may be the result of a lowering of brain activity due to an influx of tryptophan across the blood-brain barrier producing an increased level of serotonin. The hypothesis predicts that protein supplementation raises the levels of BCAA in the blood which then compete for the same transporters on the blood-brain barrier as tryptophan, reducing the uptake of tryptophan into the brain and thereby attenuating central fatigue. However, previously discussed, current lab-based evidence for an ergogenic effect of BCAA supplementation is lacking (26; 108; 111). Moreover, van Hall et al. (1995) showed that increasing the concentration of tryptophan also did not change endurance time to fatigue. The investigators had ten endurance-trained athletes cycle to fatigue at 70-75% of maximal power output after being given either low concentrations of BCAA (6 g/L^{-1}) in 6% CHO, high concentrations of BCAA (18 g/L^{-1}) in 6% CHO, or tryptophan (36 g/L^{-1}) in a 6% CHO solution. Despite large changes in plasma concentrations of BCAA and tryptophan, exercise time to exhaustion was not different among treatments. The investigators concluded that the manipulations either had no effect on the serotonin levels of the brain or that the manipulation of serotonin activity functionally does not contribute to mechanisms of fatigue (109). Whatever the reason, these data suggest that attenuation of central fatigue is likely not a mechanism whereby protein may have an ergogenic effect.

1.6. Evaluating the effectiveness of a sports drink: Methodological Considerations

When testing the effectiveness of a sports drink on any potential ergogenic practice, the applicability of the findings is dependant upon how closely the experimental design simulates "real life" conditions. The following discusses potential strategies to help ensure meaningful results.

Controlling nutritional intake prior to the experimental trials is one key area to consider when designing an experiment. The point is particularly relevant for experiments testing differences in performance when nutritional manipulation is the variable being tested. Moreover, informed athletes will typically optimize their diet before competition. This means it is important to follow a similar optimized diet plan to make the results more transferable to a real life situation. Having subjects perform under fasted conditions does not simulate typical pre-race strategies employed by most athletes. Similarly, nutrition should be optimized during the exercise trials as well. Again, informed athletes will try to consume optimal CHO and fluid during a race to help maximize performance. As such, showing an ergogenic effect with a nutritional intervention (i.e.; the addition of protein) is only meaningful when both CHO in fluid intake has been optimized.

Other considerations are related to the methodology of performance test used. Changes in endurance performance can be assessed by a ride to exhaustion at a given workload or a time trial in which the subject completes a set distance as fast as possible. Although it may be more difficult to show changes with a time trial as compared to ride to exhaustion, a time trial is a more realistic representation of how athletes typically compete. For that reason using a time trial as a performance measure is preferred. Moreover the duration of the trial is another important factor. Nutritional manipulation during endurance exercise appears to only have an effect when the duration of exercise exceeds 60-90 min (14) As a result using a time trial that is at least that long in duration is important. Other considerations related to the performance test include environmental conditions (e.g., temperature), motivational stimuli (e.g., verbal encouragement), and performance feedback (e.g., race time) given during the trial. Ideally all of these factors should remain consistent between trials for a given subject.

1.6.1. Design limitations of previous studies that have evaluated the effectiveness of adding protein to a CHO drink.

The results from the studies conducted by Ivy et al. (2003) and Saunders et al. (2004) are intriguing; however, issues related to research design hamper the potential applicability of the experimental findings. For example, the amount of CHO provided in these studies was less than what is considered optimal for improving endurance performance. An endurance athlete that has typically trained their gastro-intestinal tract to absorb CHO rapidly will likely benefit most from a CHO intake of ~ 60 g/hour as this amount will allow maximal rates of exogenous CHO oxidation (66). However, Ivy et al (2003) had subjects ingest CHO at a rate of 46.5 g/hour, and a 70 kg subject in the Saunders (2004) study would have ingested CHO at a rate of 37.1 g/hour. While performance benefits have been shown with CHO intakes comparable to the rates employed in these studies, it is possible that further performance enhancement could be achieved in the CHO only trials had the rate of intake been optimized.

Similarly, the rate of fluid ingestion in the studies by Ivy et al. (2003) and Saunders et al. (2004) was lower than what is recommended and likely not optimal. Ivy et al. (2003) had subjects drink at a rate of 600 ml/hour (200 ml every 20 minutes) while a 70 kg subject in the study by Saunders et al. (2004) would have consumed 508 ml/hour

(127 ml every 15 minutes). However, a recent joint position statement on sports nutrition by the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada recommend that fluid intake during exercise should be 150 to 350 ml every 15 to 20 min depending on tolerance and body weight. The adult male athletes used in the aforementioned studies would have likely been able to tolerate more than they were provided and the increase in fluid could have had an effect on performance.

One problem that limits the applicability of the results from the studies conducted by Ivy et al. (2004) and Saunders et al. (2004) is that neither study used performance tests that mimic the way athletes typically compete. Instead, both used a ride to exhaustion as a measure of endurance performance. In contrast, athletes compete by trying to complete a set distance in as fast a time as possible. Consequently, using a time trial performance measure would have made the results more applicable to real life competition. In addition, Saunders et al. (2004) failed to use a control group which arguably took away from the strength of the experiment because the researchers were not able to demonstrate the performance enhancing effect of CHO *per se*. Reproducing the work of others would have added credibility to their findings.

Finally, both studies have been criticized for the fact that the CHO and CHO + protein beverages were not isoenergetic. Instead, the CHO only beverage in each study had the same amount of CHO calories as the CHO + protein beverage, while the CHO + protein beverage had additional calories from protein. Saunders et al. (2004) attempted to address the issue with a follow-up study using a similar protocol except that they used a CHO drink that was isoenergetic to the CHO + protein drink (90). Interestingly, no performance differences were observed when the total energy content of the two drinks was matched. The finding suggests that it was the total calories in the drinks that made the drink with added protein perform better in the first place. However, in Saunders et al. (2004) first study, the additional calories ingested with the protein drink (139 kcal) were considerably less than the extra calories expended (318 kcal) due to a longer performance time to fatigue. Thus, it is incorrect to simply attribute the greater time to fatigue to extra energy. Rather, it seems to put the validity of the experiments into question.

1.7. Rationale and Hypothesis

Recent findings suggest that adding protein to a CHO sports drink ingested during exercise may provide a performance benefit over CHO alone (61; 94). However, the design of these studies does not mimic the manner in which athletes typically compete. Moreover, the purported mechanisms to explain how protein may improve performance are questionable. The goal of the present work was to improve on recent studies by i) providing CHO and fluid at a rate that is near optimal for improving endurance performance; ii) including a placebo control group and thus demonstrate an effect of CHO supplementation *per se*; and iii) using a "time trial" measure of performance in order to better simulate athletic competition. We tested the following hypotheses. 1) That the drinks containing CHO plus protein and CHO alone would improve 80 km TT performance compared to the placebo. 2) That the drink containing CHO plus protein would improve 80 km TT performance compared to the drink containing CHO alone.

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- Chapter 2 -

CARBOHYDRATE INGESTION IMPROVES 80 KM TIME TRIAL PERFORMANCE IN TRAINED CYCLISTS BUT PROTEIN PROVIDES NO ADDITIONAL BENEFIT

2.1 Introduction

Carbohydrate (CHO) ingestion can significantly improve endurance performance of exercise lasting longer than 60-90 minutes (Angus *et al.*, 2000d;Coggan & Coyle, 1989;Coyle *et al.*, 1983c). Most studies documenting performance improvements with CHO ingestion have used exercise time to fatigue as a performance measure (Bjorkman *et al.*, 1984;Coggan & Coyle, 1987;Coyle *et al.*, 1983b). However, recent studies have found that CHO ingestion also improves performance in both long (~3 h) (Angus *et al.*, 2000c) and short (~1 h) duration (Jeukendrup *et al.*, 1997b) time trials. It has been suggested that time trials are a more appropriate performance measure since these tests better simulate a conventional competitive environment, in which a given distance must be completed as quickly as possible (Angus *et al.*, 2000b).

The general mechanism thought to be responsible for improvement in performance with CHO ingestion is the maintenance of blood glucose concentration and an increased rate of carbohydrate oxidation (Jeukendrup & Jentjens, 2000b). The optimal rate of CHO ingestion during exercise should therefore maximize CHO oxidation without causing gastrointestinal distress. A joint position statement by the American College of Sports Medicine (ACSM), American Dietetic Association (ADA) and Dietitians of Canada (DC) recommended a CHO intake of 30-60 grams per hour (ACSM, ADA, DC, 2000). The position statement was based on an extensive body of literature that showed ergogenic effects of CHO supplementation within this range. However, a meta analysis by Jeukendrup (2004) indicated that CHO oxidation is maximized when CHO is ingested at a rate of 1.0-1.2 grams per minute (60-70 grams per hour). Moreover, anecdotal evidence suggests that endurance athletes are able to tolerate more CHO during exercise than non-trained individuals, possibly due to adaptation of the gastrointestinal tract with CHO consumption during training. These findings suggest that endurance athletes would be better off consuming CHO at a rate on the high end of the recommended range.

The fact that CHO oxidation during exercise is limited has led some researchers to examine the effects of adding other macronutrients to CHO sports beverages. Recently, two studies reported that the addition of ~2% protein (PRO) to a CHO drink dramatically improved cycling time to exhaustion in trained cyclists (Ivy *et al.*, 2003f;Saunders *et al.*, 2004d). Ivy et al (2003) showed that cycling time to exhaustion at 85% $V0_2$peak was increased by ~55% when subjects ingested a CHO + PRO drink as compared to CHO alone during a 3 hour variable intensity cycling protocol before the ride to exhaustion. In support of these findings, Saunders et al. (2004) found that cycling time to fatigue at 75% $V0_2$peak increased by 29% when subjects ingested a CHO + PRO beverage as compared to one containing CHO alone. While these data are intriguing, the practical implications of the work are hampered by the research designs employed. First of all, the rate of CHO and fluid provided in these studies was less than what is considered optimal for improving endurance performance (ACSM *et al.*, 2000c;Jeukendrup, 2004c). In addition, the performance tests employed did not mimic the way athletes typically compete in which the challenge is to complete a set distance as quickly as possible. In the present study, we recruited highly trained cyclists and attempted to address the limitations of previous studies by (1) providing CHO and fluid at a rate that is near optimal (i.e.; a 6% solution that is ingested at a rate of 250 ml every 15 minutes) (ACSM *et al.*, 2000b;Jeukendrup, 2004b) and (2) using a time trial (TT) measure of performance in order to better simulate athletic competition.

We employed a randomized, placebo-controlled, double-blind, repeated measures design to determine whether the addition of 2% PRO to a 6% CHO beverage (CHO+PRO) ingested during exercise improves simulated 80 km TT performance as compared to a 6% CHO solution alone (CHO) or a non-energetic sweetened placebo drink (PL). We tested the following hypotheses. (1) That the CHO+PRO drink and CHO drink would improve 80 km TT performance compared to the PL. (2) That the CHO+PRO drink would improve 80 km TT performance compared to the CHO drink. We also obtained venous blood samples in order to evaluate the effect of CHO and PRO ingestion on fuel metabolism and markers of metabolic stress.

2.2. Methods

2.2.1. Subjects

Ten male endurance-trained cyclists/triathletes (24.5 ± 5.5 yr; 75.8 ± 6.7 kg; 181.8 ± 5.5 cm; $V0_2$peak = 63.1 ± 4.6 ml/kg/min; means ± SD) volunteered to participate in the study. The overall caliber of the participants was quite high given that a number of them had raced competitively at the national and international level. The experimental protocol was approved by the McMaster University and Hamilton Health Sciences Corporation

Research Ethics Board. The purpose and potential risks of the study were explained to all participants prior to their participation and all provided written informed consent.

2.2.2 Preliminary testing

At least 1 week prior to starting the experimental trials subjects performed a graded test to exhaustion on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) in order to determine peak oxygen uptake ($V0_2$peak). Exhaustion was defined as the point where subjects were no longer able to cycle at a cadence above 50 rpm. Expired gas was collected using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburg, PA). In addition, all subjects performed a simulated 80 km cycling TT in order to become familiar with the performance tests employed during the main experimental trials (see below).

2.2.3. General design

Subjects cycled a simulated 80 km TT as quickly as possible on three separate occasions while ingesting either PL, CHO or CHO+PRO. The order of the trials was randomized and individual trials were 5-7 days apart to allow adequate recovery. Venous blood samples were obtained before, during, and immediately after each trial to assess differences in metabolic stress and fuel utilization between the trials. A one time expired gas collection was used per trial to assess differences in fuel utilization between trials. Details regarding all testing procedures are outlined below and summarized in Figure 2.

2.2.4. Preexperimental procedures

Subjects were asked to standardize their workout 48 hours prior to each experimental trial and perform no strenuous activity during the 24 hour period prior to each trial. In addition, subjects were provided with a food parcel (3038 kcal, 66% CHO, 17% PRO, 21% FAT) for the 24 hours before each trial. They were instructed to only eat the meals in the food parcel, drink water ad libitum, and refrain from alcohol during this time period. Any deviation from the food included in the food parcel was recorded kept consistent for all other trials for a given subject. The only significant deviation reported was due to one of the subjects being vegetarian where adjustments were made to satisfy the individual's food preferences. All other subjects reported close adherence to the food provided.

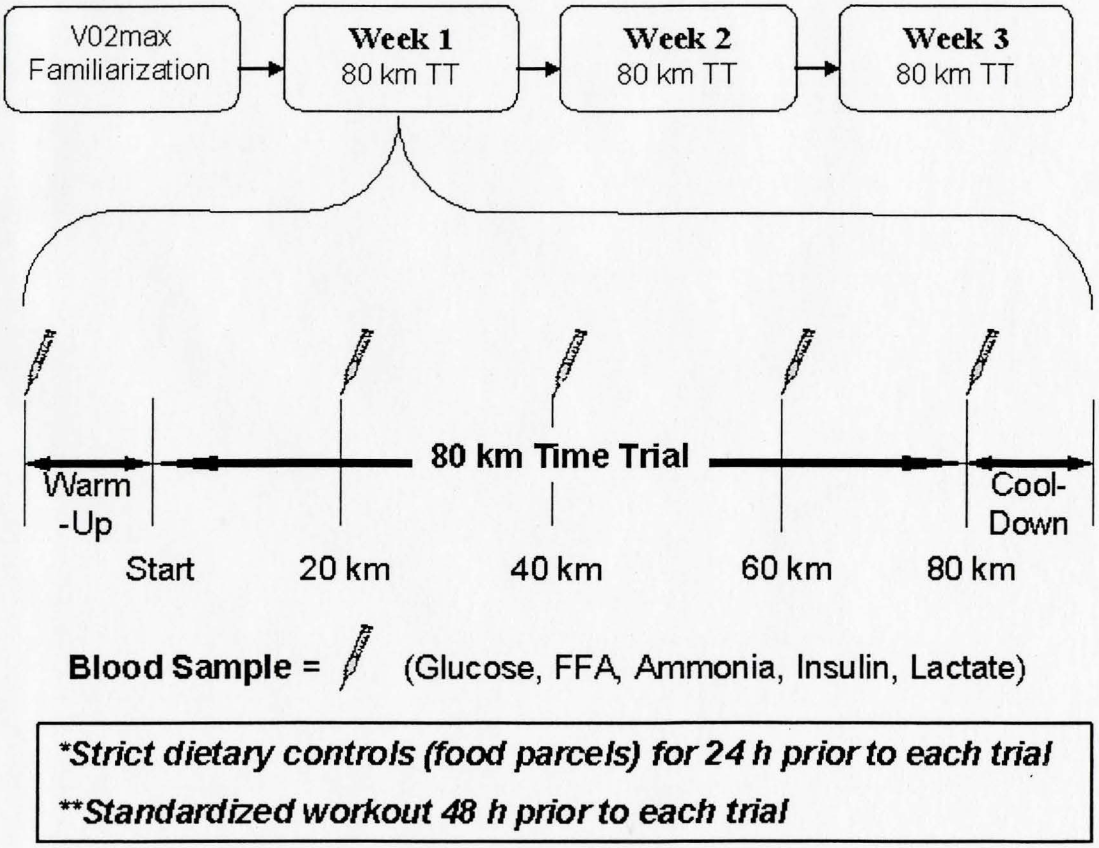

Figure 2 – Experimental design

2.2.5. Experimental trials

Upon arrival at the laboratory, subjects changed into cycling clothing, and a 22 ga. catheter (Infusion Therapy Systems Inc., Sandy, UT) was inserted into an anticubital vein for blood sampling. After a resting blood sample was obtained, subjects performed a 10 minute warm-up at a fixed workload of 150 watts. All trials were performed on a Computrainer (Lab Edition, Racermate Inc., Seattle, WA) using the subject's own bicycle. Following the warm-up, the Computrainer was calibrated according to the manufacturer's instructions and subjects were fitted with a heart rate monitor (Polar A3, Lake Success, NY). Once ready, the subjects started the time trial which consisted of 4 laps of a 20 km course (80 km total) designed specifically for the experiment using the Computrainer 3D Pro software package (Racermate Inc., Seattle, WA). The course design included several hills with a maximum grade of 4%. The software was interfaced with a projector that provided a visual display of a person riding a bicycle on a race course. The image of the cyclist was projected on a wall ~2m in front of the subject and visual changes in the grade of the terrain resulted in changes in resistance felt by the subject on the Computrainer. Accordingly, the subject had to switch gears on the bike to adjust for the changes in terrain just as they would when riding a bike outside. The only feedback provided to the subjects was the distance covered as well as the grade of the terrain. No other verbal, temporal or physiological feedback was provided. As an incentive, to complete all three time trials as quickly as possible, a monetary bonus was given to the rider that had the fastest three combined times. A bonus was also given to the rider who sustained the highest relative intensity as measured by the percent of heart rate reserve that they sustained throughout the trial. Lastly, the riders were divided up into two teams and the fastest team also received a bonus. Providing performance incentives simulated a competitive environment in the laboratory and helped keep motivation up between trials. All trials were performed at a temperature of 20-23 °C and an electric fan circulated air to minimize heat stress. Details of beverage administration, blood sampling and gas collection are described below.

2.2.6. Test beverages

All three test beverages were developed at the Gatorade Sports Science Institute (Barrington, IL), and contained the same amount of electrolytes and were similarly flavored. The only difference between the beverages was that one was artificially sweetened (PL), one contained 6% CHO (CHO) and one contained 6% CHO plus 2 % whey PRO (CHO+PRO) (Table 1). Subjects were provided with one of the beverages at the outset of each trial and were allowed to drink as desired provided 250 ml of the beverage was ingested every 15 min. A study investigator monitored the subjects drink rate to ensure that 250 ml of the beverage was consumed by the end of each 15 min period and once the bottle was empty the investigator replaced it. The beverages were administered in translucent containers in a double-blind and randomized fashion for all three experimental trials. A CHO group that was matched energetically to the CHO+PRO group (i.e.; an 8% carbohydrate group) was not included given that such a high CHO content may be ergolytic (Jeukendrup & Jentjens, 2000a).

Table 1. ***Experimental Beverage Formulations***

	grams of ingredient(1) / liter beverage		
	Placebo	**CHO**	**CHO+PRO**
Sucrose (99.5% pure)	0.0000	60.0000	60.0000
Protein (Lacprodan DI9213 from Arla Foods) (85% protein "as is")	0.0000	0.0000	20.0000
Sodium chloride (99.9% pure)	0.6730	0.6730	0.6730
Sodium citrate- hydrous ($C_6H_5Na_3O_7$; 88.5% pure)	0.5963	0.5963	0.5963
Monopotassium phosphate (KH_2PO_4; 99% pure)	0.4447	0.4447	0.4447
Citric acid (99.5% pure)	2.7621	2.7621	2.7621
Flavor	0.5115	0.5115	0.5115
Aspartame (high intensity sweetener)	0.2250	0.0000	0.0000

2.2.7. Measurements

Average heart rate was recorded over the duration of the TT using a heart rate monitor (Polar A3, Lake success, NY). Average heart rate was then used to calculate the relative intensity the subject was working at using the Karvonen formula as shown below.

$$\text{Relative Intensity (\%HRR)} = \frac{HR_{AVG} - HR_{REST}}{(HR_{MAX} - HR_{REST})}$$

Respiratory exchange ratio (RER) was measured using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburg, PA) over a 3 min period once the subject reached the 60 km mark in the TT. RER is a ratio of an individual's expired rate of carbon dioxide production ($VC0_2$) divided by the inspired rate of oxygen consumption ($V0_2$) and therefore indicates the relative contribution of fat and CHO oxidation to energy provision. Blood was sampled before the warm-up, every 20 km during the TT, and at the completion of the TT and subsequently analyzed for plasma glucose, insulin, lactate, FFA, ammonia, hematocrit and hemoglobin concentration.

2.2.8. Blood analysis

Blood samples were collected into three different 4 ml tubes that contained either no additive, heparin, or EDTA. The blood in the non-heparinized (no additive) tubes was allowed to coagulate and then centrifuged. After centrifugation the supernatant was collected and stored at -20°C for later analysis of serum insulin using a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). A drop of blood from the heparinized tubes was used to measure blood lactate with an automated lactate analyzer (Accutrend Lactate, Roche Diagnostics, Mannheim, Germany) immediately after the blood was collected. In addition, 200 μl of whole blood from the heparinized tube was combined with 1000μl of 0.6 N perchloric acid, vortexed and centrifuged, and then stored at -20 °C for subsequent analysis of blood glucose. Blood glucose was analyzed on a Hitachi F-2500 using flourometric enzyme assay described by Passoneau and Lowry (1993). The remaining blood in the heparinized tube was centrifuged and the plasma supernatant was collected and stored at -20°C, for later analysis of plasma free fatty acids (FFA) and ammonia. Plasma FFA was analyzed using a colorimetric method (Wako NEFA C test kit, Wako Chemicals, VA). Plasma ammonia was analyzed at the Hamilton Regional Laboratory Medicine Program (McMaster Hospital, Hamilton, ON). The Hamilton Regional Laboratory Medicine Program also analyzed heparinized whole blood for hematocrit and hemoglobin and these data were used to calculate plasma volume as described by Costill et al. (1974).

2.2.9. Statistical analysis

Variables that consisted of a single measure per trial were examined a using 1-factor (condition) repeated measures analysis of variance (ANOVA). Variables that included multiple measures per trial were analyzed using a two-factor (condition and time) ANOVA. When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey HSD post hoc test. Significance for all analysis was set at $P \leq 0.05$. All values are presented as means ± SEM.

2.3 Results

2.3.1. Performance measures

Time to complete the 80 km TT was lower ($P \leq 0.05$) during the CHO and CHO+PRO trials compared with the PL (PL: 141 ± 3 min; CHO: 135 ± 2 min; CHO+PRO: 135 ± 3 min), but there was no difference between the CHO and CHO+PRO trials (Figure 3). Analysis of lap times revealed that subjects performed better ($P \leq 0.05$) on lap 1 (PL: 35.2 ± 1.0 min; CHO: 33.6 ± 0.6 min; CHO+PRO: 33.7 ± 0.8 min) and lap 4 (PL: 36.1 ± 0.9 min; CHO: 33.6 ± 0.6 min; CHO+PRO: 33.6 ± 0.7 min) during the CHO+PRO and CHO trials compared to the PL trial (Figure 4). Average power output over the duration of the TT was higher during the CHO and CHO+PRO trials compared to the PL trial (PL: 265 ± 5 W; CHO: 275 ± 5 W; CHO+PRO: 275 ± 6 W) (Figure 5). No difference was found between CHO and CHO+PRO for any of the performance variables measured.

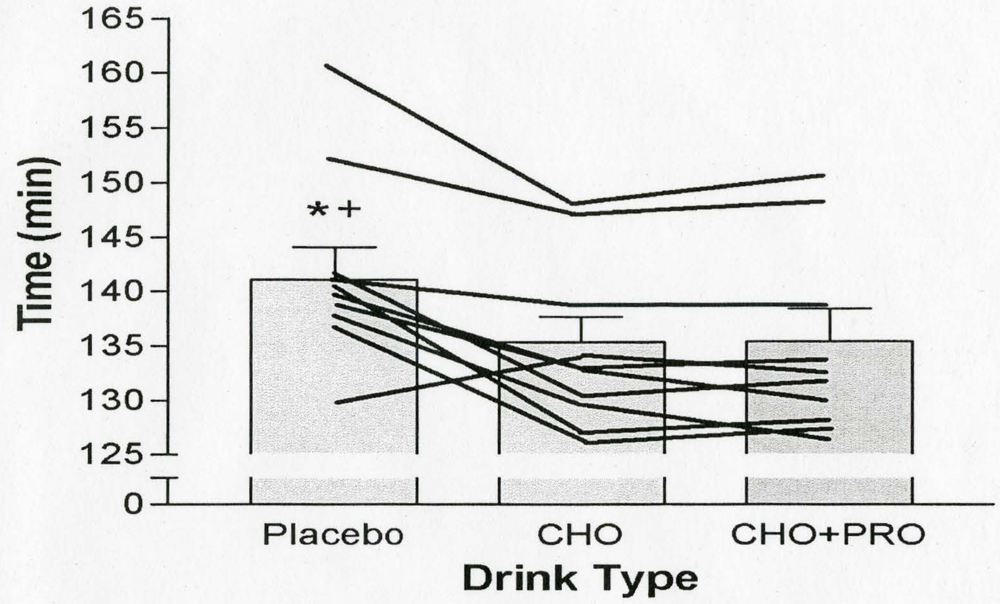

Figure 3. Time to complete the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. Solid lines represent individual subject times. * P≤0.05 vs. CHO; +P≤0.05 vs. CHO+PRO.

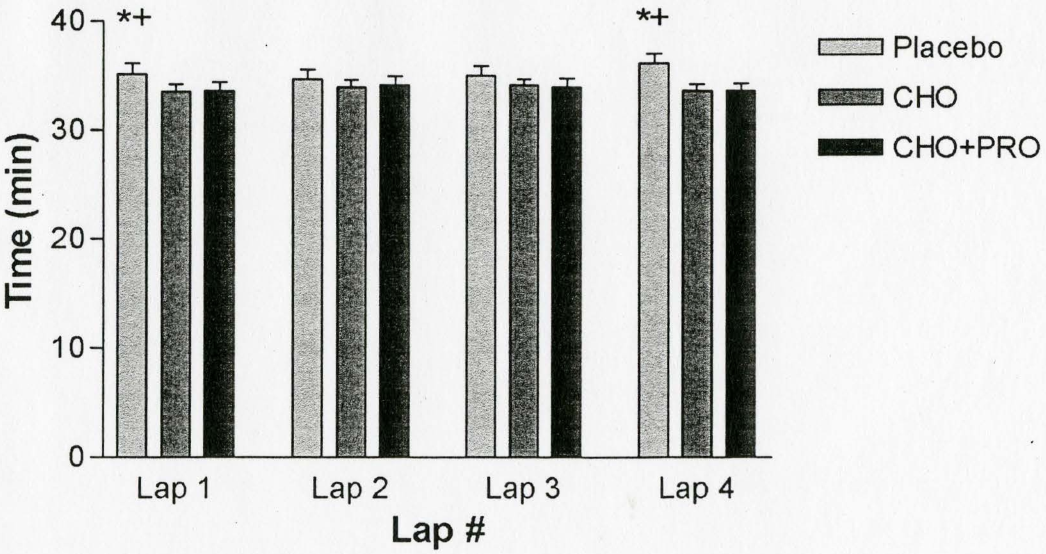

Figure 4. Time to complete the individual laps during the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. * P≤0.05 vs. CHO; +P≤0.05 vs. CHO+PRO.

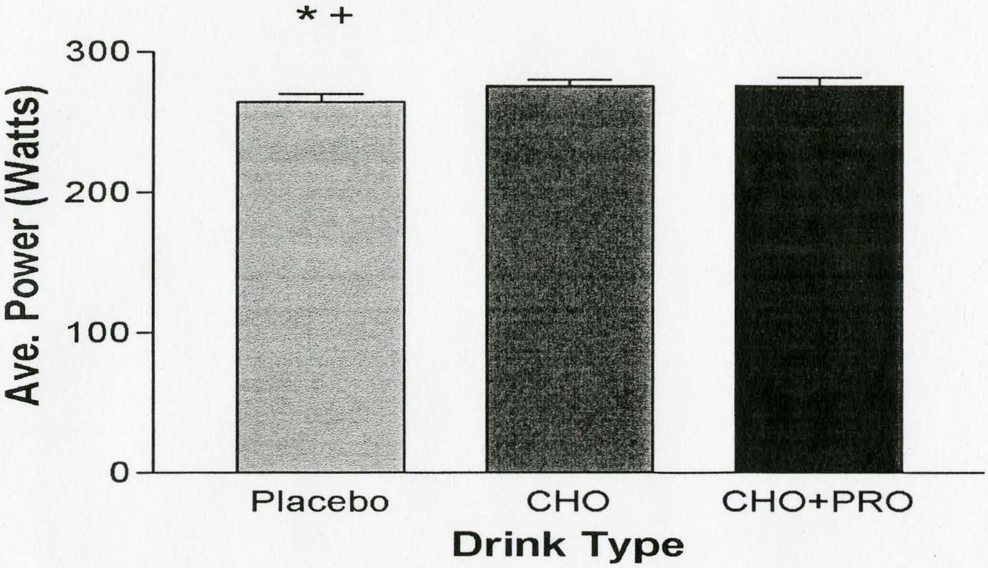

Figure 5. Average power output over the duration of the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. * $P \leq 0.05$ vs. CHO; +$P \leq 0.05$ vs. CHO+PRO.

2.3.2. Cardio-respiratory measures

Average heart rate was higher ($P \leq 0.05$) for the CHO trial (159 ± 3 bpm) compared to the PL trial (155 ± 3 bpm), with no difference between the CHO and CHO+PRO trials (159 ± 4 bpm) (Table 2). Consequently, work intensity as measured by percent of heart rate reserve (HRR) was also higher for the CHO trial (81 ± 2 %) compared to the PL trial (77 ± 2 %), with no difference between the CHO and CHO+PRO (80 ± 2 %) trials (Table 2). Breath analysis at the 60 km mark revealed no differences between the groups for oxygen uptake ($V0_2$), respiratory exchange ratio (RER), or minute ventilation (VE) (Table 3).

Table 2. ***Average Heart Rate and Work Intensity during the 80 km time trial when given Placebo, CHO, and CHO+PRO.***

	PLACEBO	CHO	CHO+PRO
Average HR (bpm)	155 ± 3 *	159 ± 3	159 ± 4
Work Intensity (%HRR)	77 ± 2 *	81 ± 2	80 ± 2

Values are mean ± SEM. * $P \leq 0.05$ vs. CHO.

Table 3. ***Respiratory exchange ratio (RER), oxygen uptake ($V0_2$), and minute ventilation (VE) at 60 km when given Placebo, CHO, and CHO+PRO.***

	PLACEBO	CHO	CHO+PRO
RER	0.89 ± 0.01	0.91 ± 0.01	0.91 ± 0.01
$V0_2$ (ml/kg/min)	44.2 ± 2.8	46.6 ± 1.9	48.7 ± 2.2
VE (L/min)	77.3 ± 5.7	79.2 ± 3.6	86.2 ± 4.5

Values are mean ± SEM.

2.3.4. Rating of perceived exertion (RPE)

Despite a gradual rise in the Borg RPE over the duration of the TT ($P \leq 0.05$), there were no differences between treatment groups at any distance point measured (Figure 6).

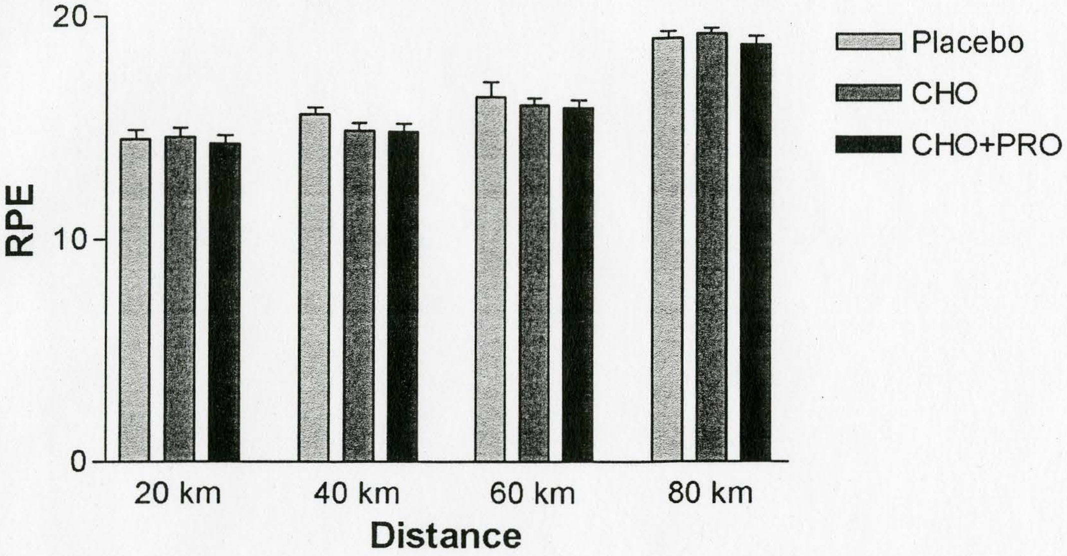

Figure 6. Borg rating of perceived exertion (RPE) throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution.

2.3.4. Metabolic measures

Blood glucose concentration remained relatively constant during exercise in the CHO and CHO+PRO trials with no difference between the two treatments at any time. In contrast, blood glucose concentration declined during the PL trial and was lower ($P \leq 0.05$) compared to CHO and CHO+PRO trials at 40, 60 and 80 km (Figure 7). Serum insulin concentration decreased during exercise in all three trials and was lower at 20, 40, 60 and 80 km compared to rest (main effect, $P \leq 0.05$).There were no differences in the serum insulin concentration at any time (Figure 8). Blood lactate concentration increased over the duration of the time trial in all three trials (main effect, $P \leq 0.05$). During all three trials there was a rapid rise in blood lactate concentration from rest to 20 km ($P \leq 0.05$)

which was then followed by a gradual decline to the 60 km mark and a rapid increase at the end of the TT where the concentration raised to an average of 4.38 mmol/L. The only difference observed for blood lactate between the trials was at the end of the time trial when blood lactate concentration for CHO (5.02 ± 0.31 mmol/L) was significantly higher ($P \leq 0.05$) than PL (3.51 ± 0.49 mmol/L) (Figure 9). The concentration of plasma FFA gradually increased throughout the duration of the trials (Figure 10). At 60 km plasma FFA was significantly higher in the PL trial (0.51 ± 0.10 mmol/L) than the CHO+PRO trial (0.29 ± 0.04 mmol/L). At 80 km plasma FFA concentration of the PL trial (0.99 ± 0.11 mmol/L) was well above ($P \leq 0.05$) both the CHO trial (0.46 ± 0.09 mmol/L) and the CHO+PRO trial (0.37 ± 0.08 mmol/L). Plasma ammonia steadily increased (main effect, $P \leq 0.05$) throughout the TT but there were no differences observed between treatment groups at any time (Figure 11). Plasma volume decreased ($P \leq 0.05$) with exercise for PL (-2.8 %), CHO (-4.5 %), and CHO+PRO (-3.9 %) but there were no significant differences between groups.

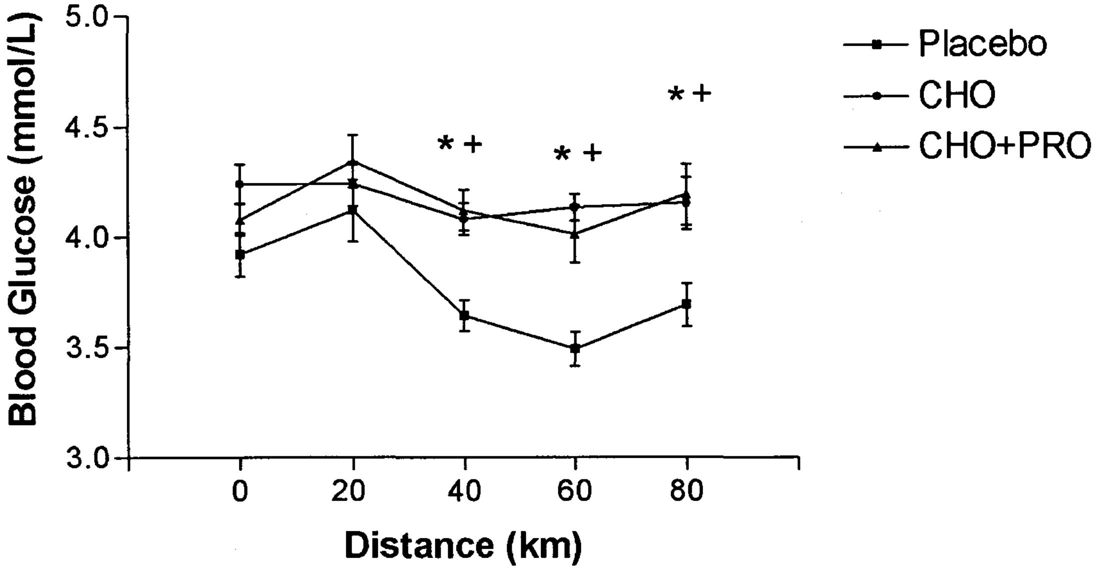

Figure 7. Blood Glucose concentration throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. * $P \leq 0.05$ vs. CHO; $+P \leq 0.05$ vs. CHO+PRO.

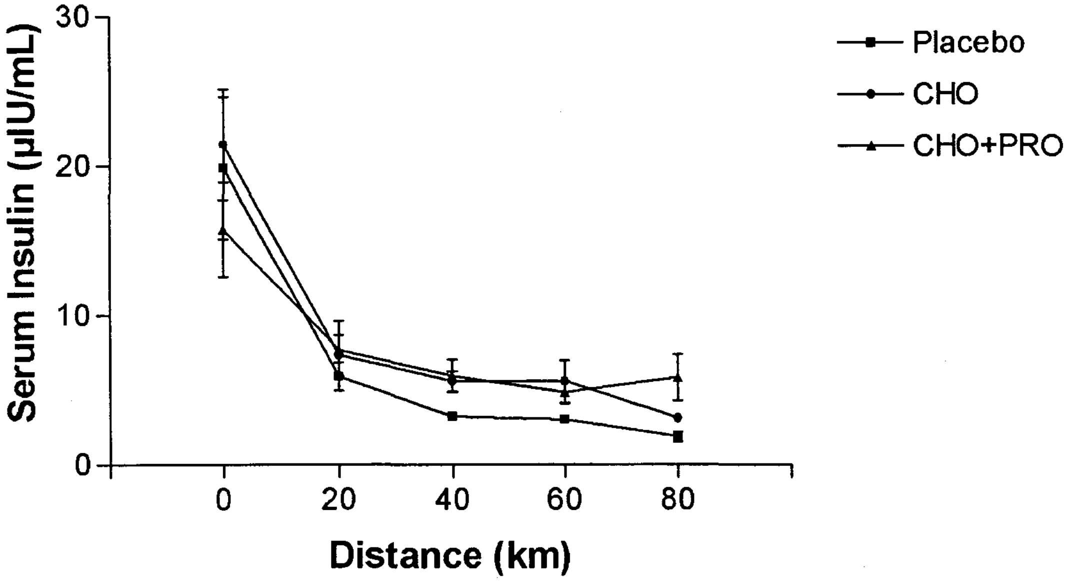

Figure 8. Serum Insulin concentration throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution.

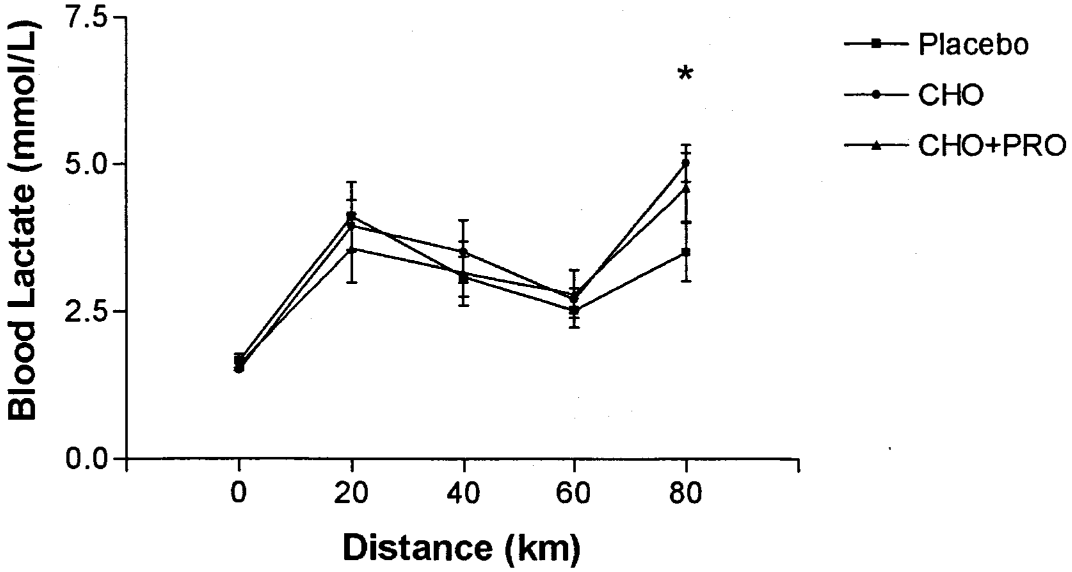

Figure 9. Blood Lactate concentration throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. * P≤0.05 vs. CHO.

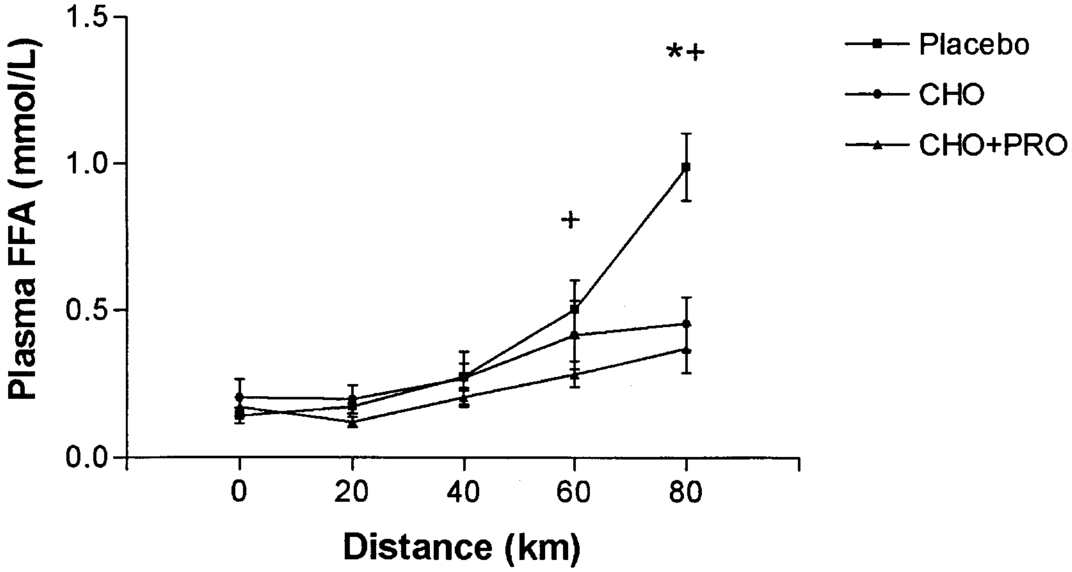

Figure 10. Plasma free fatty acids (FFA) concentration throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution. * P≤0.05 vs. CHO; +P≤0.05 vs. CHO+PRO.

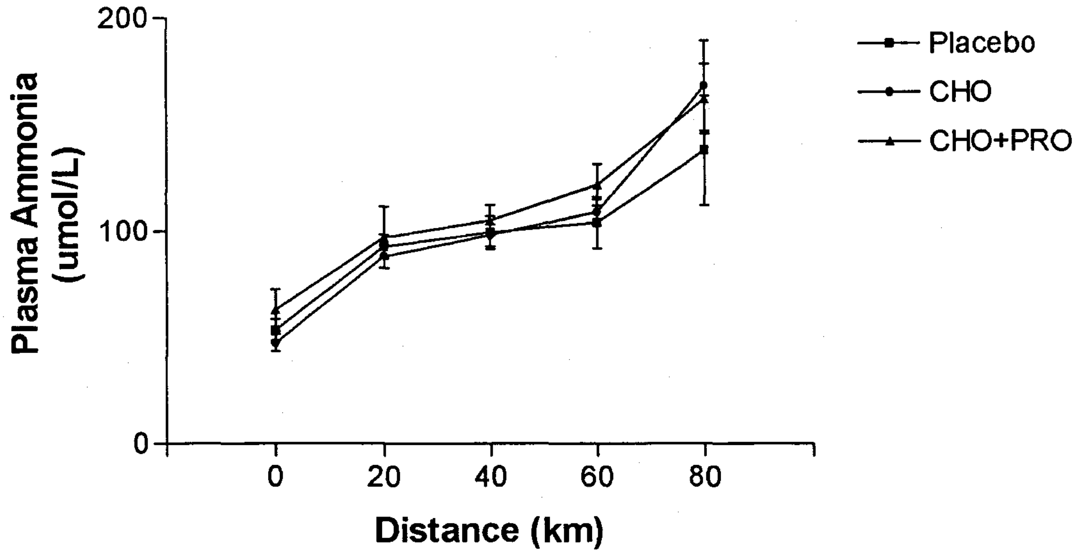

Figure 11. Plasma ammonia concentration throughout the 80 km time trial when subjects ingested a placebo, a 6% CHO solution or a 6% CHO + 2% PRO solution.

2.4. Discussion

2.4.1. 80 km time trial performance

The major finding of this study was that ingestion of a 6% CHO beverage at a rate of 1L/h improved 80 km TT performance in trained male cyclists, as compared to a non-energetic placebo. However, the addition of 2% PRO to a 6% CHO beverage provided no additional performance benefit. These findings differ in comparison with recent findings by others that reported large increases in endurance time to fatigue when PRO was added to a CHO sports drink (Ivy *et al.*, 2003e;Saunders *et al.*, 2004c). The discrepancy between the present study and others is likely related to methodological differences; however, we feel that our results are much more transferable to real athletic competition. We chose to use a TT as a measure of performance as we felt it more accurately reflects the way athletes typically compete in which the challenge is to complete a set distance as fast as possible. In contrast, both Ivy et al. (2003) and Saunders et al. (2004) used a ride to exhaustion at a given intensity as a measure of endurance performance. While both these studies showed impressive increases in time to exhaustion (>25%) with the addition of PRO to a CHO beverage, one should be careful when interpreting these results since athletes do not typically compete in this manner. Moreover, cycling at a given intensity to exhaustion can be very mentally taxing and it is possible that day to day variability in motivation could affect the duration a subject is able ride. In contrast, the day to day variability of TT performance in trained cyclists is reported to be quite low (CV = 1-3 %) (Laursen *et al.*, 2003). We found that the coefficient of variation for the present study was ~ 2.8% when comparing the familiarization trial (where subjects received water) to the placebo trial.

Another major difference between the present study and those showing an effect of adding PRO is the rate of CHO and fluid ingestion during exercise. It has been shown that exogenous CHO oxidation is maximal when CHO is ingested at a rate of 1-1.2 g/min (60-70 g/hour) (Jeukendrup, 2004a). Consequently, we chose to have our subjects ingest CHO at a rate of 60 g/hour. Since CHO is the predominant fuel source during high intensity exercise, we reasoned that a potential performance enhancing effect of adding PRO would only be meaningful if CHO ingestion was already optimized. However, Ivy et al. (2003) had subjects ingest CHO at a rate of 46.5 g/hour, while a 70 kg subject in the Saunders (2004) study would have ingested CHO at a rate of 37.1 g/hour. Performance benefits have been shown with CHO intakes comparable to the rates employed in these studies. However, the possibility exists that that further performance enhancement could have been achieved in the CHO only trials had the rate of intake been optimized which, in turn, could have obliterated the effect of protein ingestion.

2.4.2. Lap time performance

Analysis of lap times revealed that the CHO and CHO+PRO treatments performed significantly better on the first and last lap of the course compared to the PL. We expected to see this difference in the last lap of the TT since the depletion of endogenously stored CHO would likely limit performance later in the exercise bout (i.e.; after ~90 min). Numerous studies have shown that supplementing with CHO would allow maintenance of blood glucose and an increased rate of CHO oxidation (Angus *et al.*, 2000a;Coyle *et al.*, 1983a;Coyle *et al.*, 1986). Indeed, we found that blood glucose concentration was maintained and significantly higher in the CHO and CHO+PRO treatments compared to the PL from 40 km until the end of the TT (Figure 7). The RER value at 60 km for the PL (0.89) trial was slightly lower than the CHO (0.91) and CHO+PRO (0.91) trials, but the difference was not statistically significant (Table 2). This suggests that there was no difference in fuel utilization between groups at that point in the TT. It's possible that 60 km (or ~90 min) was a little too soon to detect a shift in fuel utilization of the PL trial. Analysis of the plasma FFA response appears to support this notion. While the concentration of plasma FFA in the PL trial rose slightly above the CHO+PRO trial at 60 km, it wasn't until after 60 km that the concentration of plasma FFA in the PL rose sharply above the CHO and CHO+PRO trials (Figure 10). This rapid rise of plasma FFA in the PL trial suggests increased lypolysis providing FFA to support an increased level of fat oxidation.

The more striking finding of the lap time analysis was that subjects performed better in the CHO and CHO+PRO trials compared to the PL trial during the first lap of the TT. The finding suggests that mechanisms other than the maintenance of blood glucose coupled with increased CHO oxidation are working to improve performance when CHO is ingested during exercise. Endogenous CHO stores do not typically limit performance during exercise lasting ~ 1 hour (Carter *et al.*, 2004c). Consequently, it is unlikely that well fed subjects would obtain metabolic benefit from the provision of exogenous CHO during the first lap of the TT. Interestingly, our finding that performance was improved early on in the time trial is supported by several other studies that have shown performance improvement when CHO was ingested during a 40 km TT lasting approximately 1 hour (Anantaraman *et al.*, 1995;Below *et al.*, 1995;Jeukendrup *et al.*, 1997a). Thus it appears this effect may be real and that a mechanism other than fuel provision is responsible for the observed performance improvement. Recent work by Carter et al. (2004) has found that CHO may have an effect through mechanisms that are central in nature. Carter et al. (2004) showed that infusing CHO in saline had no effect on 40 km TT performance compared to infusing saline alone (Carter *et al.*, 2004b). However, using CHO as a mouth wash significantly improved performance compared to a placebo (Carter *et al.*, 2004a) . The investigators speculated that oropharyngeal receptors sensed the presence of CHO in the mouth and sent signals to the brain which attenuated central fatigue. Further research is necessary to determine the validity of this hypothesis. However it is possible that in the present study this same mechanism improved the performance of the CHO and CHO+PRO treatment groups during the first lap of the TT.

2.4.3. Blood insulin response to protein ingestion

In the present study, PRO added to CHO provided no further performance enhancement compared to CHO alone. Ivy et al. (2003) originally hypothesized that the addition of PRO to a CHO sports drink would alter the insulin response, spare muscle glycogen and thereby improve endurance time to fatigue. Interestingly, despite showing a marked improvement in endurance time to fatigue, the researchers found that adding PRO to a CHO beverage did not alter insulin levels compared to CHO alone (Ivy *et al.*, 2003d). In our study we noted a similar response, where the serum insulin concentration of the CHO and CHO+PRO treatment group did not significantly differ from each other ($P \leq 0.05$) throughout the duration of the TT (Figure 8). The findings from both studies suggest that alteration of the insulin response is not a mechanism by which PRO could improve endurance performance.

2.4.4. Additional energy provided by protein ingestion

The two previous studies showing improved endurance performance (Ivy *et al.*, 2003c;Saunders *et al.*, 2004f) were criticized for not including a CHO group that was isoenergetic to the CHO+PRO group. Support for these criticisms came from a follow-up study by Saunders' group that showed no performance difference between a CHO group that was energy matched to a CHO+PRO group (Romano & Saunders, 2004). However, calculations from the study where Saunders et al. (2004) did show an effect of adding

PRO revealed that the extra energy expenditure of the prolonged ride to exhaustion (318 kcal) used up far more calories than was obtained via PRO ingestion (139 kcal) (Saunders *et al.*, 2004b). Thus, it is incorrect to say that the reason adding PRO improved endurance performance in these studies was simply due to the extra energy. In the present study, we measured the concentration of plasma ammonia to try and further investigate the potential for extra PRO calories to be oxidized as fuel. If PRO was being oxidized as fuel the concentration of ammonia in the blood would have likely been elevated (Rennie & Tipton, 2000). However, despite a gradual rise in the concentration of ammonia, there were no differences between any of the treatment groups (Figure 11). As a result, it is unlikely that the additional energy provided by PRO would improve performance by acting as a significant fuel source. We also chose to not include a CHO group that was matched energetically to the CHO+PRO group. We reasoned that it would be unlikely to detect a performance difference between ingestion of an 8% CHO beverage compared to a 6% CHO beverage that already had an optimized CHO content. Moreover, the extra CHO in an 8% solution may have been ergolytic by means of gastro-intestinal distress. Given that energy expended exceeded energy gained from PRO when the addition of PRO improved performance, and the fact that the plasma ammonia response was similar between treatments in the current study, we feel that not including an energy matched CHO group was justified.

2.4.5. Practical recommendations for athletes

The results from the present study indicate that ingesting a 6% CHO drink at a rate of 1 L/h is an effective way of improving performance in endurance competition.

Furthermore, we found that adding PRO to a drink that already contains an optimal amount of CHO and fluid does not further improve performance. Thus, we recommend simply ingesting a beverage that contains an optimal amount of CHO and fluid if the goal is to improve performance over a concurrent bout of exercise. That said, the addition of PRO did not appear to hinder performance and recent research has indicated that the combined ingestion of PRO and CHO during ultra-endurance exercise may improve net PRO balance of the muscles compared to ingesting CHO alone (Koopman *et al.*, 2004). The fact that net PRO balance can be increased in this way may have implications for faster recovery following exercise. However, research in this area is limited so it is difficult for us to conclusively recommend adding PRO to a beverage that will be consumed during exercise. Moreover, the addition of protein may alter the palatability of the sports drink which could have an effect on the voluntary rate of fluid consumption in exercise conditions outside of the lab. Most athletes have trouble staying hydrated through an exercise bout so optimal palatability may be more important than getting an early start on recovery. Lastly, athletes may be able to achieve similar recovery by simply eating a meal immediately following exercise that contains adequate amounts of CHO and PRO.

2.4.6. Directions for future research

Future research should look at reproducing the work of Ivy et al. (2003) and Saunders et al. (2004) while simultaneously examining the possible mechanisms these researchers have suggested may be improving performance, including the effect of PRO supplementation on (i) central fatigue, and (ii) the concentration of Kreb's cycle intermediates (Ivy *et al.*, 2003b;Saunders *et al.*, 2004a). Furthermore, future research in this area should examine the effect of combined PRO and CHO supplementation during endurance exercise on net protein balance and its effect on recovery.

2.5 Conclusion

Previous studies that reported an ergogenic effect of adding PRO to a CHO sports drink did not provide CHO and fluid at rates that are optimal for improving endurance performance (Ivy *et al.*, 2003a;Saunders *et al.*, 2004e). Furthermore these studies used a measure of endurance performance that did not mimic the way athletes typically compete. In the current study we addressed these issues and found that trained cyclists who consumed CHO and fluid at rates recommended for improving endurance performance (ACSM *et al.*, 2000a;Jeukendrup, 2004d) improved 80 km TT performance compared to PL, but adding PRO provided no further performance enhancement. Thus, we conclude that ingesting a 6% CHO drink at a rate of 1 L/h improves 80 km TT performance, as compared to a non-energetic PL. However, the addition of 2% PRO to a 6% CHO drink provides no additional performance benefit.

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APPENDIX I

CONSENT FORMS AND RESEARCH ETHICS BOARD APPROVAL FORM

McMaster
University

Exercise Metabolism Research Group
Department of Kinesiology
Ivor Wynne Centre, Room A103
1280 Main Street West
Hamilton, Ontario, Canada
L8S 4K1

Phone: 905-525-9140
EMRG Laboratory: ext. 27037
Dr. MJ Gibala: ext. 23591
Dr. MJ MacDonald: ext. 23580
Dr. SM Phillips: ext. 24465
Fax: 905-523-4025

EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

CONSENT TO PARTICIPATE IN RESEARCH

You are asked to participate in a research study being conducted by the investigators listed below at McMaster University, Hamilton, Ontario. Prior to your participation, you are asked to read and complete this form and the accompanying forms which outline the purpose, procedures, and risks associated with the study, and also provide other essential information regarding your rights and responsibilities as a subject. The accompanying forms are entitled "Invasive Procedures" and "Subject Screening Questionnaire." All experimental procedures will be conducted in Room A103 or A106, Ivor Wynne Centre.

LIST OF PRIMARY INVESTIGATORS

Name	Campus Address	Daytime Phone Number
Dr. Martin Gibala	Kinesiology, AB122	905-525-9140 ext. 23591
Martin van Essen	Kinesiology, A103	905-525-9140 ext. 27037

PROJECT TITLE

"Does the addition of protein to a carbohydrate drink improve cycling time trial performance in endurance-trained men?"

PROJECT SPONSOR

The Gatorade Sports Science Institute (GSSI).

BACKGROUND INFORMATION

An extensive body of literature has clearly established that carbohydrate (CHO) ingestion during prolonged exercise improves endurance performance. Recently, two studies reported dramatic (>25%) increases in endurance time to fatigue during cycling at 75-85% VO2peak when subjects ingested a CHO+protein supplement as compared to CHO alone. However, the research designs employed in these studies have been criticized for a number of reasons, including (1) the amount of CHO provided was less than what is usually considered optimal in order to improve endurance performance; (2) one of the studies did not include a control group; and (3) the performance test employed did not mimic the manner in which athletes typically compete (i.e., a race, in which a given distance must be covered as quickly as possible). We propose to investigate this issue using an improved research design, strict dietary controls and a testing procedure that more closely simulates athletic competition.

Hamilton Health Sciences/McMaster
JAN 12 2005
Research Ethics Board

PURPOSE OF THE STUDY

To determine whether the addition of 1.5% protein to a 6% CHO beverage ingested during exercise improves time trial performance and/or blood markers of metabolic stress, as compared to 6% CHO alone or a non-energetic placebo drink. We hypothesize that ingestion of a 6% CHO solution will improve performance during a 2000 kJ time trial (simulated 80 km race) compared to placebo, however the addition of 1.5% whey protein to CHO will not further enhance performance, in spite of the extra energy provided. The design of the proposed study represents a marked improvement over previous studies since we plan to: (1) provide CHO at a rate that is considered to be near-optimal (i.e., 6% CHO, ingested at a rate of 15 g in 250 ml of fluid every 15 min); (2) include a control group and thus demonstrate an effect of CHO supplementation *per se*; and (3) use a time trial measure of performance in order to better simulate athletic competition.

DESCRIPTION OF TESTING AND EXPERIMENTAL PROCEDURES

Following routine medical screening, you will be required to make 5 visits to the laboratory over a period of approximately 6 weeks. The first visit will be used to establish you peak aerobic capacity (VO2peak) and will last approximately 1 hour. During the second visit, which will last approximately 3 hours, you will perform a simulated 80 km Time Trial in order to become familiarized with testing procedures to be employed during the actual experimental trials. The final 3 visits will consist of the actual experimental trials, as summarized below.

VISIT 1: VO2peak Test. This test involves cycling on a stationary bike (cycle ergometer) at progressively higher workloads while the amount of oxygen taken up by your body is determined from a mouthpiece connected to a gas analyzer.

VISIT 2: "Practice" 80 km Time Trial. This test involves performing a fixed bout of exercise (2000 kJ; equivalent to a simulated 80 km cycle ride) in as fast a time as possible. This test will be performed on a Computrainer device using your own bicycle and you will be able to self-select the intensity of exercise throughout the test. You will be able to monitor "distance covered" by viewing a monitor situated in front of the bike, but will not receive an other temporal, verbal or physiological feedback (e.g., heart rate, etc.)

VISITS 3-5: Experimental Trials. Upon arrival at the laboratory, a catheter will be inserted into a forearm vein for blood sampling. The details of the blood sampling procedure and associated risks are thoroughly described on the attached forms entitled "Description of Medical Procedures." Following the resting blood sample you will be allowed to warm-up for ~5 min. Upon completion of the warm-up you will begin the simulated 80 km time trial which will be identical in all respects to the practice ride (Visit 2). An investigator will supply you will an allotted beverage at a rate of 250ml every 15 minutes in a double blind manner (neither you or the investigator will know what drink you are receiving, i.e., placebo, 6% CHO or 6% CHO+1.5% protein). Blood samples will be drawn every 30 minutes during exercise and immediately upon completion of the time trial (6 samples in total). A questionnaire will be administered following the bout in order to evaluate any potential gastrointestinal disturbances and also assess whether you were able to correctly identify the type of beverage provided during exercise. Heart will be monitored non-invasively throughout the exercise test, and periodically gas measurements will be made from a mouthpiece attached to a gas analyzer. Upon completion, you will be permitted to leave the laboratory following ~30 min of routine, post-exercise monitoring. Shower and change facilities are available should you require them.

Dietary controls. In an attempt to minimize diet-induced variability in exercise metabolism and performance, we will attempt to ensure that subjects consume the same types and quantities of food prior to each experimental trial. All subjects will be provided with pre-packaged 24 hour food parcels to be consumed on the day prior to the experiment (formulated to provide ~40 kcal/kg of energy derived from ~65% carbohydrate, 20% fat and 15% protein). Subjects will also be advised to refrain from alcohol, drugs and physical activity (aside from activities of daily living). A standardized breakfast (formulated to provide ~600 kcal of energy derived from ~65% carbohydrate, 20% fat and 15% protein) will be provided on the day of the experimental trials, ~3 h prior to exercise. Subjects will be instructed to maintain food diaries and all food diaries will be subsequently analyzed for total energy intake and proportion of energy derived from carbohydrates, fats and protein (Nutritionist Five, First Data Bank Inc., San Bruno, CA).

DESCRIPTION OF POTENTIAL RISKS AND DISCOMFORTS

Please refer to the attached form entitled "Description of Medical Procedures" for a complete description of the invasive medical procedures to be performed during the study and the potential risks and discomforts associated with these procedures.

REMUNERATION

You will receive an honorarium of $250.00 in order to compensate for your time commitment and effort. Remuneration is normally provided within one week following completion of the study.

PROVISION OF CONFIDENTIALITY

Any information that is obtained in connection with this study will remain confidential, and appropriate measures will be taken by all investigators to ensure privacy. The results from this study will be used for educational purposes and may be published in scientific journals, presented at scientific meetings or disseminated using other appropriate methods. Regardless of presentation format, subjects will not be identified by name and your personal data will be identified by a code number only. Upon completion of the study, you will have access to your own data and the group data for your own interest.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions which you do not want to and still remain in the study. The investigators also reserve the right to withdraw you from this research project if circumstances arise which warrant doing so. Should you withdraw from the study prior to its completion, a partial honorarium payment will be made based on the relative proportion of the study which was completed.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. The nature of the exercise stresses and invasive procedures to be employed in this study are routinely applied in our laboratory and been approved by the Hamilton Health Sciences / McMaster University Research Ethics Board (REB Project Number 04-388).

If you have any questions regarding your rights as a research participant you may contact the Hamilton Health Sciences Patient Relations Specialist at 905-521-2100, Ext. 75240.

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE

I have read and understand the information provided for the study as described herein and in the accompanying forms entitled "Description of Medical Procedures" and "Subject Screening Questionnaire." My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

Name of Participant

Name of Legal Representative (if applicable)

Signature of Participant or Legal Representative Date

SIGNATURE OF INVESTIGATOR

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

Signature of Investigator Date

Hamilton Health Sciences/McMaster
JAN 12 2005
Research Ethics Board

McMaster University

Hamilton Health Sciences
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www.hamiltonhealthsciences.ca

RESEARCH ETHICS BOARD

January 12, 2005

PROJECT NUMBER: 04-388

PROJECT TITLE: "Does the Addition of Protein to a Carbohydrate drink Improve Cycling Time Trial Performance in Endurance-Trained Men?"

PRINCIPAL INVESTIGATOR: **Dr. M. Gibala**

This will acknowledge receipt of your recent e-mail which enclosed a copy of the revised Consent Form and recruitment poster for the above-named study. These issues were raised by the Research Ethics Board at their meeting held on December 21, 2004. Based on this additional information, we wish to advise your study has been given ***final*** approval from the full REB. The submission, including the Consent Form and poster was found to be acceptable on both ethical and scientific grounds.

We are pleased to issue final approval for the above-named study for a period of 12 months from the date of this letter. Continuation beyond that date will require further review and renewal of REB approval. Any changes or amendments to the protocol or consent form must be approved by the Research Ethics Board.

We wish to advise the Research Ethics Board operates in compliance with ICH Good Clinical Practice Guidelines and the Tri-Council Policy Statement.

Investigators in the Project should be aware that they are responsible for ensuring that a complete consent form is inserted in the patient's health record. In the case of invasive or otherwise risky research, the investigator might consider the advisability of keeping personal copies.

A condition of approval is that the physician most responsible for the care of the patient is informed that the patient has agreed to enter the study. Any failure to meet this condition means that Research Ethics Board approval for the project has been withdrawn.

PLEASE QUOTE THE ABOVE-REFERENCED PROJECT NUMBER ON ALL
FUTURE CORRESPONDENCE.

Sincerely,

J. Holland /DM

F. Jack Holland, MD, FRCP, FRCP(C)
Chair, Research Ethics Board

/dm

All correspondence should be addressed to the REB Chair and forwarded to:
REB Secretary, Henderson Campus, 90 Wing, Room #1
711 Concession Street, Hamilton ON L8V 1C3
Telephone: 905-527-4322, ext. 42013
Fax: 905-574-5645

APPENDIX II

SUBJECT CHARACTERISTICS

Subject Characteristics

Subject #	Age (year)	Weight (kg)	Height (cm)	V02max (ml/kg/min	HR max (bpm)	HR rest (bpm)	Experience (yrs.)
S1	20	71.0	177.5	63.9	185	40	8
S2	23	81.0	190.5	62.6	191	50	8
S3	19	65.3	177.5	68.7	202	50	7
S4	28	78.0	185.5	52.3	194	50	4
S5	24	78.2	177.5	61.5	185	48	3
S6	21	80.0	183.0	62.8	175	45	5
S7	38	64.5	172.5	64.6	179	46	7
S8	25	80.0	183.0	66.4	192	46	1
S9	25	83.9	183.0	65.5	179	52	3
S10	21	76.5	188.0	54.9	184	51	4
Average	24.4	75.8	181.8	63.1	186.9	47.8	5
SD	5.5	6.7	5.5	4.6	8.6	3.6	2.4
SEM	1.7	2.1	1.7	1.5	2.7	1.1	0.8

APPENDIX III

RAW DATA – TIME TRIAL PERFORMANCE

80 km Time Trial (Overall) (hr:min:sec)

Subject #	Placebo	CHO	CHO+PRO
S1	2:11:18	2:06:32	2:08:30
S2	2:10:17	2:15:09	2:11:27
S3	2:19:04	2:13:36	2:08:55
S4	2:20:51	2:19:18	2:18:40
S5	2:20:35	2:11:56	2:13:42
S6	2:18:59	2:06:23	2:08:30
S7	2:17:49	2:14:42	2:14:14
S8	2:32:22	2:27:17	2:30:47
S9	2:17:25	2:11:10	2:06:05
S10	2:42:03	2:27:05	2:32:48
Average	**2:21:04**	**2:15:19**	**2:15:22**
SD	**0:09:30**	**0:07:21**	**0:09:24**
SEM	**0:03:00**	**0:02:19**	**0:02:58**

Average Power (Normalized) (Watts)

Subject #	Placebo	CHO	CHO+PRO
S1	283.1	293.8	289.3
S2	285.3	275.0	282.8
S3	267.3	278.2	288.3
S4	263.9	266.8	268.1
S5	264.4	281.7	278.0
S6	267.4	294.1	289.3
S7	269.7	275.9	276.9
S8	244.0	252.4	246.5
S9	270.5	283.4	294.8
S10	229.4	252.7	243.3
Average	**264.5**	**275.4**	**275.7**
SD	**16.7**	**14.6**	**18.0**
SEM	**5.3**	**4.6**	**5.7**

80 km Time Trial Lap Times (min:sec)

Subject #	Placebo				Carbohydrate				Carbohydrate + Protein			
	Lap1	Lap2	Lap3	Lap4	Lap1	Lap2	Lap3	Lap4	Lap1	Lap2	Lap3	Lap4
S1	32:23.3	32:58.5	32:54.1	33:01.8	30:57.5	31:42.4	32:19.9	31:32.1	31:28.9	33:17.4	31:56.0	31:48.1
S2	31:08.0	32:50.2	32:50.4	33:28.8	32:34.3	33:56.6	34:25.9	34:11.8	31:19.7	32:59.9	33:46.3	33:21.0
S3	32:35.6	32:44.9	36:18.6	37:25.0	33:11.8	33:26.7	34:06.7	32:50.7	31:59.8	31:51.6	31:54.8	33:08.4
S4	34:54.4	35:28.9	35:24.1	35:03.4	34:17.1	34:45.1	35:19.5	34:56.0	34:05.6	34:48.3	35:13.9	34:31.9
S5	34:46.9	35:07.6	35:28.6	35:11.8	32:58.1	33:28.2	33:10.1	32:19.5	33:45.5	33:41.6	33:12.5	33:02.8
S6	35:54.7	34:31.9	33:32.1	35:00.4	32:21.6	31:33.1	31:26.3	31:02.0	33:26.1	32:06.3	31:30.0	31:27.2
S7	32:53.6	33:05.3	33:32.1	38:18.2	32:51.5	33:35.7	34:02.5	34:12.7	32:51.2	33:41.6	33:47.8	33:53.5
S8	37:43.9	38:55.8	37:07.8	38:34.9	37:40.2	38:26.9	36:29.8	34:39.6	37:36.2	39:54.5	37:07.1	36:09.2
S9	39:33.9	31:54.2	32:22.0	33:35.2	32:36.1	32:29.7	32:42.1	33:22.3	31:56.3	31:19.0	31:34.6	31:15.0
S10	39:48.3	39:35.1	41:02.3	41:37.1	36:14.8	36:22.8	37:15.4	37:11.7	38:05.5	37:40.7	39:22.2	37:40.0
Average	**35:10.3**	**34:43.2**	**35:03.2**	**36:07.7**	**33:34.3**	**33:58.7**	**34:07.8**	**33:37.8**	**33:39.5**	**34:08.1**	**33:56.5**	**33:37.7**
SD	**0:03:03**	**0:02:39**	**0:02:39**	**0:02:46**	**0:02:00**	**0:02:07**	**0:01:50**	**0:01:49**	**0:02:24**	**0:02:42**	**0:02:37**	**0:02:03**
SEM	**0:00:58**	**0:00:50**	**0:00:50**	**0:00:52**	**0:00:38**	**0:00:40**	**0:00:35**	**0:00:34**	**0:00:46**	**0:00:51**	**0:00:50**	**0:00:39**

RPE

Subject #	Placebo				Carbohydrate				Carbohydrate + Protein			
	20 km	40 km	60 km	80 km	20 km	40 km	60 km	80 km	20 km	40 km	60 km	80 km
S1	17.000	17.000	17.000	20.000	17.000	17.000	17.000	19.000	17.000	17.000	17.000	18.000
S2	15.000	15.000	14.000	18.000	14.000	14.000	14.000	19.000	14.000	14.000	14.000	19.000
S3	16.000	17.000	20.000	20.000	14.000	14.000	17.000	20.000	15.000	16.000	17.000	20.000
S4	15.000	16.000	17.000	20.000	16.000	15.000	17.000	20.000	15.000	16.000	16.000	20.000
S5	14.000	15.000	15.000	18.000	15.000	15.000	16.000	18.000	14.000	15.000	16.000	19.000
S6	15.000	16.000	17.000	19.000	14.000	16.000	16.000	19.000	14.000	15.000	16.000	18.000
S7	13.000	15.000	17.000	19.000	15.000	14.000	15.000	18.000	14.000	14.000	15.000	16.000
S8	13.000	15.000	18.000	20.000	12.000	15.000	17.000	20.000	12.000	13.000	17.000	20.000
S9	14.000	16.000	16.000	19.000	14.000	14.000	16.000	19.000	14.000	14.000	16.000	19.000
S10	13.000	14.000	13.000	17.000	15.000	15.000	15.000	20.000	14.000	14.000	15.000	18.000
Average	**14.500**	**15.600**	**16.400**	**19.000**	**14.600**	**14.900**	**16.000**	**19.200**	**14.300**	**14.800**	**15.900**	**18.700**
SD	**1.354**	**0.966**	**2.011**	**1.054**	**1.350**	**0.994**	**1.054**	**0.789**	**1.252**	**1.229**	**0.994**	**1.252**
SEM	**0.428**	**0.306**	**0.636**	**0.333**	**0.427**	**0.314**	**0.333**	**0.249**	**0.396**	**0.389**	**0.314**	**0.396**

Average Heart Rate (beats/min)

Subject #	Placebo	CHO	CHO+PRO
S1	163	163	169
S2	165	166	171
S3	169	175	182
S4	150	153	151
S5	150	152	149
S6	150	160	158
S7	154	156	158
S8	147	147	147
S9	147	150	150
S10	152	171	155
Average	**154.7**	**159.3**	**159.0**
SD	**8.0**	**9.3**	**11.5**
SEM	**2.5**	**3.0**	**3.6**

Relative Intensity (% HRR)

Subject #	Placebo	CHO	CHO+PRO
S1	0.848	0.848	0.890
S2	0.816	0.823	0.858
S3	0.783	0.822	0.868
S4	0.694	0.715	0.701
S5	0.745	0.759	0.737
S6	0.808	0.885	0.869
S7	0.812	0.827	0.842
S8	0.692	0.692	0.692
S9	0.748	0.772	0.772
S10	0.759	0.902	0.782
Average	**0.770**	**0.805**	**0.801**
SD	**0.052**	**0.069**	**0.074**
SEM	**0.017**	**0.022**	**0.023**

Rerpiratory Exchange Ratio at 60 km

Subject #	Placebo	CHO	CHO+PRO
S1	0.94	0.94	0.91
S2	0.84	0.89	0.91
S3	0.89	0.88	0.91
S4	0.93	0.93	0.91
S5	0.85	0.88	0.91
S6	0.89	0.94	0.89
S7	0.93	0.94	0.95
S8	0.89	0.92	0.90
S9	0.88	0.84	0.84
S10	0.90	0.91	0.92
Average	**0.89**	**0.91**	**0.91**
SD	**0.03**	**0.03**	**0.03**
SEM	**0.01**	**0.01**	**0.01**

V02 at 60 km (ml/kg/min)

Subject #	Placebo	CHO	CHO+PRO
S1	52.9	51.0	52.5
S2	47.5	47.5	48.4
S3	35.6	53.4	56.9
S4	46.7	43.9	41.5
S5	47.8	43.4	48.4
S6	48.3	52.1	53.5
S7	53.9	53.9	57.4
S8	42.1	42.8	47.6
S9	42.7	42.9	46.5
S10	24.1	34.6	34.3
Average	**44.1**	**46.6**	**48.7**
SD	**8.8**	**6.1**	**7.0**
SEM	**2.8**	**1.9**	**2.2**

VE at 60 km (L/min)

Subject #	Placebo	CHO	CHO+PRO
S1	87.9	77.0	93.2
S2	70.7	77.0	85.1
S3	55.0	81.4	95.1
S4	94.0	81.7	82.0
S5	79.0	74.2	82.5
S6	85.6	96.8	93.7
S7	102.8	97.8	111.7
S8	74.9	77.2	85.6
S9	81.8	67.5	75.9
S10	41.8	61.2	57.6
Average	**77.3**	**79.2**	**86.2**
SD	**18.1**	**11.4**	**14.1**
SEM	**5.7**	**3.6**	**4.5**

APPENDIX IV

RAW DATA – BLOOD METABOLITES

Blood Lactate (mmol/L)

Subject #	Placebo					Carbohydrate					Carbohydrate + Protein				
	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km
S1	2.1	6.0	4.5	3.6	5.6	1.6	4.6	3.9	2.6	4.8	1.5	7.1	7.7	6.2	9.1
S2	1.1	4.1	1.7	1.7	2.2	1.7	4.2	3.8	1.8	5.7	1.1	2.2	1.9	2.1	3.1
S3	1.3	5.1	2.9	1.8	4.1	1.8	3.5	2.9	2.4	5.8	1.4	3.8	2.7	2.3	3.8
S4	1.6	4.3	3.3	4.1	6.7	1.2	2.4	2.7	2.7	5.8	2.0	3.0	2.6	2.4	6.2
S5	1.8	3.0	2.9	3.0	3.1	1.4	2.7	2.1	2.6	3.9	1.4	3.3	2.4	2.2	4.3
S6	1.9	1.8	2.4	1.5	2.4	1.5	3.4	3.9	3.3	4.1	1.4	2.4	2.5	2.6	3.1
S7	1.9	7.3	4.6	2.6	2.0	1.7	5.7	3.0	2.5	3.9	2.1	6.6	3.2	3.1	4.0
S8	2.1	1.8	2.8	2.4	2.7	1.6	2.1	1.7	2.4	6.1	1.8	1.6	1.8	1.7	3.3
S9	1.5	5.0	4.2	3.1	3.5	1.3	4.9	3.4	2.9	3.9	1.5	3.2	4.0	3.3	5.1
S10	1.4	2.8	1.6	1.4	2.8	1.4	6.1	7.8	3.9	6.2	1.8	2.5	2.7	2.1	4.1
Average	**1.7**	**4.1**	**3.1**	**2.5**	**3.5**	**1.5**	**4.0**	**3.5**	**2.7**	**5.0**	**1.6**	**3.6**	**3.2**	**2.8**	**4.6**
SD	**0.3**	**1.8**	**1.1**	**0.9**	**1.5**	**0.2**	**1.4**	**1.7**	**0.6**	**1.0**	**0.3**	**1.8**	**1.7**	**1.3**	**1.8**
SEM	**0.1**	**0.6**	**0.3**	**0.3**	**0.5**	**0.1**	**0.4**	**0.5**	**0.2**	**0.3**	**0.1**	**0.6**	**0.5**	**0.4**	**0.6**

Ammonia (umol/L)

Subject #	Placebo					Carbohydrate					Carbohydrate + Protein				
	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km
S1	25	90	84	85	103	47	114	119	114	136	38	78	75	88	113
S2	71	98	83	87	85	52	111	101	98	169	35	80	98	110	109
S3	64	134	139	119	108	39	107	116	130	128	71	138	143	156	178
S4	54	76	81	77	86	53	88	109	118	148	146	214	103	117	133
S5	45	77	69	70	82	44	74	76	69	84	55	77	93	95	100
S6	57	105	101	108	112	34	85	101	116	147	48	63	94	114	149
S7	43	97	95	114	223	28	73	80	101	164	51	71	95	122	177
S8	37	88	136	201	331	73	89	105	139	326	54	90	151	193	267
S9	61	89	123	102	157	56	62	73	102	145	61	71	100	114	199
S10	78	73	83	77	91	48	80	100	103	232	68	90	99	110	194
Average	**53.5**	**92.7**	**99.4**	**104**	**137.8**	**47.4**	**88.3**	**98**	**109**	**167.9**	**62.7**	**97.2**	**105.1**	**121.9**	**161.9**
SD	**16.17**	**17.83**	**24.78**	**38.03**	**80.62**	**12.56**	**17.44**	**16.30**	**19.34**	**66.77**	**31.45**	**45.98**	**23.43**	**30.77**	**51.60**
SEM	**5.11**	**5.64**	**7.83**	**12.03**	**25.49**	**3.97**	**5.51**	**5.15**	**6.12**	**21.12**	**9.95**	**14.54**	**7.41**	**9.73**	**16.32**

Blood Glucose (mmol/L)

Subject #	Placebo					Carbohydrate					Carbohydrate + Protein				
	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km
S1	3.86	4.41	3.96	3.49	3.62	3.95	4.33	4.13	4.17	3.68	3.92	4.83	4.46	4.43	4.90
S2	4.29	4.19	3.72	4.10	4.11	4.51	4.30	4.45	4.47	4.33	4.23	4.65	4.40	4.52	4.85
S3	3.80	4.30	3.34	3.28	3.20	4.15	4.49	3.81	4.15	4.19	4.19	4.16	3.97	4.08	4.23
S4	4.42	4.53	3.65	3.50	4.38	4.19	4.39	4.35	4.19	4.33	4.14	4.26	4.42	4.24	4.13
S5	4.14	3.68	3.49	3.60	3.66	4.52	3.51	3.78	3.94	4.45	3.79	4.19	4.03	3.90	4.46
S6	3.56	3.89	3.72	3.38	3.46	3.89	4.11	4.05	4.20	4.57	4.11	4.31	4.01	4.16	4.18
S7	4.22	4.92	3.92	3.35	3.46	4.75	4.73	4.30	4.13	4.44	4.47	5.02	4.41	4.17	4.02
S8	3.62	3.97	3.56	3.22	3.55	4.23	4.04	3.98	3.88	3.94	4.26	3.90	3.94	3.77	3.79
S9	3.76	3.70	3.30	3.29	3.31	4.04	4.06	4.02	3.91	4.22	3.79	4.01	3.91	3.74	3.68
S10	3.54	3.58	3.72	3.72	3.65	4.19	4.40	3.90	4.23	3.37	3.90	4.03	3.67	3.13	3.64
Average	**3.92**	**4.12**	**3.64**	**3.49**	**3.64**	**4.24**	**4.24**	**4.08**	**4.13**	**4.15**	**4.08**	**4.34**	**4.12**	**4.01**	**4.19**
SD	**0.32**	**0.43**	**0.22**	**0.26**	**0.36**	**0.27**	**0.33**	**0.23**	**0.18**	**0.38**	**0.22**	**0.37**	**0.28**	**0.40**	**0.44**
SEM	**0.10**	**0.14**	**0.07**	**0.08**	**0.11**	**0.09**	**0.10**	**0.07**	**0.06**	**0.12**	**0.07**	**0.12**	**0.09**	**0.13**	**0.14**

Serum Insulin (µIU/mL)

Subject #	Placebo					Carbohydrate					Carbohydrate + Protein				
	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km
S1	55.44	10.98	4.34	3.13	0.14	36.21	9.31	6.24	7.07	4.38	11.91	9.23	5.08	9.81	17.93
S2	7.96	3.29	2.41	2.75	2.48	18.95	3.50	5.73	7.21	2.74	16.14	6.39	6.42	4.56	4.64
S3	9.48	5.01	2.85	2.77	0.16	10.41	8.75	3.44	3.01	1.94	12.52	4.29	3.44	3.36	8.04
S4	25.99	7.11	3.34	3.27	1.85	11.82	3.49	3.35	2.97	3.13	22.02	5.23	5.04	4.42	2.70
S5	11.98	2.73	2.35	3.42	2.06	29.59	4.99	4.67	3.30	4.36	12.30	4.26	4.45	3.15	9.50
S6	18.63	4.89	3.29	3.13	2.73	39.19	13.47	10.11	5.67	3.40	33.05	13.39	6.06	7.77	4.38
S7	30.03	5.47	4.13	2.59	2.04	30.49	2.88	3.41	2.34	2.06	30.77	3.29	5.10	2.94	2.66
S8	11.03	5.02	2.44	1.95	1.71	17.09	6.22	5.27	2.74	2.31	11.05	2.85	3.05	2.42	2.03
S9	5.24	3.40	2.62	3.12	2.72	4.58	4.84	5.59	4.06	3.67	4.15	5.34	5.25	4.18	3.39
S10	22.96	11.02	4.59	4.24	2.57	15.93	15.60	7.81	17.15	3.19	3.75	22.53	15.37	5.54	3.02
Average	**19.88**	**5.89**	**3.24**	**3.04**	**1.85**	**21.43**	**7.30**	**5.56**	**5.55**	**3.12**	**15.77**	**7.68**	**5.93**	**4.81**	**5.83**
SD	**14.99**	**2.97**	**0.86**	**0.60**	**0.96**	**11.72**	**4.40**	**2.14**	**4.45**	**0.87**	**10.01**	**6.10**	**3.48**	**2.33**	**4.90**
SEM	**4.74**	**0.94**	**0.27**	**0.19**	**0.30**	**3.71**	**1.39**	**0.68**	**1.41**	**0.28**	**3.17**	**1.93**	**1.10**	**0.74**	**1.55**

Plasma Free Fatty Acids (mmol/L)

Subject #	Placebo					Carbohydrate					Carbohydrate + Protein				
	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km	Rest	20 km	40 km	60 km	80 km
S1	0.156	0.147	0.170	0.188	0.457	0.129	0.170	0.147	0.363	0.286	0.170	0.084	0.111	0.152	0.201
S2	0.295	0.228	0.443	0.479	0.896	0.717	0.448	0.973	1.358	0.677	0.129	0.129	0.183	0.300	0.412
S3	0.134	0.120	0.174	0.511	0.841	0.156	0.188	0.197	0.358	0.381	0.322	0.044	0.372	0.336	0.748
S4	0.098	0.058	0.178	0.206	0.717	0.102	0.116	0.161	0.273	0.327	0.080	0.062	0.111	0.237	0.165
S5	0.116	0.224	0.529	0.910	1.551	0.035	0.116	0.188	0.282	0.753	0.134	0.093	0.161	0.197	0.807
S6	0.028	0.264	0.166	0.178	1.053	0.034	0.015	0.157	0.144	0.287	0.033	0.117	0.113	0.260	0.282
S7	0.171	0.095	0.073	0.180	0.594	0.313	0.211	0.082	0.157	0.206	0.329	0.144	0.327	0.233	0.140
S8	0.117	0.282	0.403	0.795	1.142	0.152	0.465	0.567	0.581	0.465	0.211	0.211	0.309	0.483	0.389
S9	0.255	0.211	0.322	0.951	1.485	0.287	0.220	0.220	0.567	1.040	0.211	0.202	0.251	0.545	0.518
S10	0.046	0.108	0.296	0.657	1.156	0.104	0.046	0.033	0.082	0.144	0.100	0.126	0.131	0.108	0.042
Average	**0.142**	**0.174**	**0.275**	**0.505**	**0.989**	**0.203**	**0.199**	**0.272**	**0.417**	**0.456**	**0.172**	**0.121**	**0.207**	**0.285**	**0.370**
SD	**0.084**	**0.078**	**0.147**	**0.311**	**0.359**	**0.203**	**0.151**	**0.284**	**0.370**	**0.282**	**0.098**	**0.055**	**0.100**	**0.138**	**0.258**
SEM	**0.026**	**0.025**	**0.047**	**0.098**	**0.114**	**0.064**	**0.048**	**0.090**	**0.117**	**0.089**	**0.031**	**0.017**	**0.032**	**0.044**	**0.081**

Blood Hemoglobin and Hematocrit

Subject #	Placebo				Carbohydrate				Carbohydrate + Protein			
	Hb_{before}	Hb_{after}	Hct_{before}	Hct_{after}	Hb_{before}	Hb_{after}	Hct_{before}	Hct_{after}	Hb_{before}	Hb_{after}	Hct_{before}	Hct_{after}
S1	152	152	0.441	0.441	144	148	0.42	0.434	151	156	0.442	0.454
S2	128	134	0.378	0.392	125	125	0.363	0.363	128	131	0.367	0.378
S3	144	146	0.415	0.422	133	142	0.392	0.417	142	142	0.416	0.416
S4	130	134	0.397	0.401	136	141	0.405	0.417	140	142	0.417	0.425
S5	149	149	0.425	0.434	147	158	0.426	0.454	148	155	0.425	0.448
S6	156	161	0.445	0.462	153	162	0.432	0.461	152	162	0.436	0.464
S7	150	150	0.435	0.435	148	148	0.433	0.433	144	147	0.415	0.426
S8	156	161	0.459	0.472	152	163	0.437	0.475	149	161	0.442	0.472
S9	153	169	0.443	0.487	153	168	0.448	0.487	155	170	0.449	0.494
S10	158	164	0.449	0.466	154	162	0.451	0.471	156	163	0.453	0.465
Average	**147.6**	**152.0**	**0.429**	**0.441**	**144.5**	**151.7**	**0.421**	**0.441**	**146.5**	**152.9**	**0.426**	**0.444**
SD	**10.6**	**12.0**	**0.025**	**0.031**	**10.0**	**13.3**	**0.027**	**0.037**	**8.4**	**12.1**	**0.025**	**0.033**
SEM	**3.4**	**3.8**	**0.008**	**0.010**	**3.2**	**4.2**	**0.009**	**0.012**	**2.7**	**3.8**	**0.008**	**0.011**

Percent Change in Plasma Volume (%)

Subject #	Placebo	CHO	CHO+PRO
S1	0.00	-2.93	-2.95
S2	-4.12	0.00	-2.52
S3	-1.49	-6.20	0.00
S4	-2.19	-3.27	-1.61
S5	-0.90	-6.60	-4.79
S6	-3.37	-5.89	-6.11
S7	0.00	0.00	-2.27
S8	-2.94	-7.34	-6.94
S9	-9.26	-8.48	-8.96
S10	-3.65	-4.61	-3.50
Average	**-2.79**	**-4.53**	**-3.97**
SD	**2.71**	**2.94**	**2.72**
SEM	**0.86**	**0.93**	**0.86**

APPENDIX V

STATISTICAL TABLES

ANOVAS AND TUKEY HSD POST HOC TESTS

80 km Time Trial (Overall)

Summary of all Effects; design: (new.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	99.65321	18	8.604669	11.58129597	0.000585

Tukey HSD test; variable Var.1 (new.sta)			
Probabilities for Post Hoc Tests			
MAIN EFFECT: DRINK			
	Placebo	CHO	CHO/PRO
	141.0720	135.8840	135.3620
Placebo		0.002646	0.001168
CHO	0.002646		0.916902
CHO/PRO	0.001168	0.916902	

Average Power (Watts)

Summary of all Effects; design: (power.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	408.63797	18	40.969875	9.974108	0.001215

Tukey HSD test; variable Var.1 (power.sta)			
Probabilities for Post Hoc Tests			
MAIN EFFECT: DRINK			
	Placebo	CHO	CHO/PRO
	264.4936	275.4087	275.7162
Placebo		0.0035698	0.0028476
CHO	0.00357		0.9937414
CHO/PRO	0.002848	0.9937414	

80 km Time Trial Lap Times

Summary of all Effects; design: (laptime stats.sta)						
1-DRINK, 2-LAPTIME						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	98618.96	18	9596.348	10.27671814	0.001054
2	3	2137.933	27	8584.767	0.249038026	0.861296
12	6	7848.558	54	3026.614	2.593181133	0.027877

Tukey HSD test; variable Var.1 (laptime stats.sta)												
Probabilities for Post Hoc Tests			INTERACTION: 1 x 2									
	Placebo				Carbohydrate				Carbohydrate + Protein			
	PL - 1	PL - 2	PL - 3	PL - 4	CHO - 1	CHO - 2	CHO - 3	CHO - 4	C/P - 1	C/P - 2	C/P - 3	C/P - 4
	2110.300	2083.300	2103.200	2167.600	2014.300	2038.800	2047.800	2018.000	2019.500	2048.200	2036.600	2017.600
PL - 1		0.993601	1	0.469927	0.012918591	0.168041	0.338571	0.019959	0.023711	0.3479441	0.1383665	0.0190557
PL - 2	0.993601		0.999593	0.04863	0.207363784	0.806147	0.949107	0.277047	0.309095	0.9528733	0.7550877	0.268867
PL - 3	1	0.999593		0.296019	0.029392958	0.296019	0.521665	0.044171	0.051819	0.532693	0.2510433	0.0423109
PL - 4	0.469927	0.04863	0.296019		0.000123799	0.000269	0.000686	0.000126	0.000128	0.0007177	0.0002236	0.0001258
CHO - 1	0.012919	0.207364	0.029393	0.000124		0.997227	0.965959	1	1	0.9629713	0.9988112	1
CHO - 2	0.168041	0.806147	0.296019	0.000269	0.997226655		1	0.999384	0.999695	0.9999998	1	0.9992655
CHO - 3	0.338571	0.949107	0.521665	0.000686	0.965958595	1		0.985798	0.990587	1	0.9999987	0.984251
CHO - 4	0.019959	0.277047	0.044171	0.000126	1	0.999384	0.985798		1	0.984251	0.9997861	1
C/P - 1	0.023711	0.309095	0.051819	0.000128	1	0.999695	0.990587	1		0.9894644	0.9999055	1
C/P - 2	0.347944	0.952873	0.532693	0.000718	0.96297127	1	1	0.984251	0.989464		0.9999982	0.9825625
C/P - 3	0.138366	0.755088	0.251043	0.000224	0.998811245	1	0.999999	0.999786	0.999906	0.9999982		0.9997377
C/P - 4	0.019056	0.268867	0.042311	0.000126	1	0.999265	0.984251	1	1	0.9825625	0.9997377	

Rating of Percieved Exertion (Borg Scale)

Summary of all Effects; design: (rel int 2.sta)						
1-DRINK, 2-DISTANCE						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	2.033333	18	1.190741	1.707620502	0.209385
2	3	118.7194	27	1.929321	61.5343132	3.39E-12
12	6	0.477778	54	0.400617	1.192604065	0.324285

Tukey HSD test; variable Var.1 (rel int 2.sta)												
Probabilities for Post Hoc Tests				INTERACTION: 1 x 2								
	Placebo				Carbohydrate				Carbohydrate + Protein			
	PL-20km	PL-40km	PL-60km	PL-80km	C-20km	C-40km	C-60km	C-80km	C/P-20km	C/P-40km	C/P-60km	C/P-80km
	14.50000	15.60000	16.40000	19.00000	14.60000	14.90000	16.00000	19.20000	14.30000	14.80000	15.90000	18.70000
PL-20km		0.013531	0.000121	0.000121	0.99999994	0.95585	0.000234	0.000121	0.999889	0.995236	0.0005572	0.0001212
PL-40km	0.013531		0.198431	0.000121	0.03663522	0.378274	0.95585	0.000121	0.001553	0.1984305	0.995236	0.0001212
PL-60km	0.000121	0.198431		0.000121	0.000122607	0.000234	0.95585	0.000121	0.000121	0.00015	0.828347	0.0001212
PL-80km	0.000121	0.000121	0.000121		0.000121176	0.000121	0.000121	0.999889	0.000121	0.0001212	0.0001212	0.995236
C-20km	1	0.036635	0.000123	0.000121		0.995236	0.000557	0.000121	0.995236	0.999889	0.0015525	0.0001212
C-40km	0.95585	0.378274	0.000234	0.000121	0.995236039		0.013531	0.000121	0.611602	0.9999999	0.0366352	0.0001212
C-60km	0.000234	0.95585	0.95585	0.000121	0.000557184	0.013531		0.000121	0.000128	0.0046835	0.9999999	0.0001212
C-80km	0.000121	0.000121	0.000121	0.999889	0.000121176	0.000121	0.000121		0.000121	0.0001212	0.0001212	0.828347
C/P-20km	0.999889	0.001553	0.000121	0.000121	0.995236039	0.611602	0.000128	0.000121		0.828347	0.00015	0.0001212
C/P-40km	0.995236	0.198431	0.00015	0.000121	0.999888957	1	0.004683	0.000121	0.828347		0.0135307	0.0001212
C/P-60km	0.000557	0.995236	0.828347	0.000121	0.001552522	0.036635	1	0.000121	0.00015	0.0135307		0.0001212
C/P-80km	0.000121	0.000121	0.000121	0.995236	0.000121176	0.000121	0.000121	0.828347	0.000121	0.0001212	0.0001212	

Average Heart Rate (bpm)

Summary of all Effects; design: (Ave.HR))						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	66.23333	18	15.82593	4.185115814	0.032168

Tukey HSD test; variable Var.1 (Ave.HR)			
Probabilities for Post Hoc Tests			
MAIN EFFECT: DRINK			
	Placebo	CHO	CHO/PRO
	154.7000	159.3000	159.0000
Placebo		0.046909	0.065283
CHO	0.046909		0.984546
CHO/PRO	0.065283	0.984546	

Relative Intensity (% HRR)

Summary of all Effects; design: (new.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	0.0035201	18	0.0008452	4.164934	0.032615

Tukey HSD test; variable Var.1 (new.sta)			
Probabilities for Post Hoc Tests			
MAIN EFFECT: DRINK			
	Placebo	CHO	CHO/PRO
	.7704676	.8045114	.8011573
Placebo		0.0439313	0.0727712
CHO	0.043931		0.9641405
CHO/PRO	0.072771	0.9641405	

V02 at 60 km (ml/kg/min)

Summary of all Effects; design: (new.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	51.54894	18	17.2658	2.98561	0.0758999

Rerpiratory Exchange Ratio at 60 km (RER)

Summary of all Effects; design: (new.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	0.00049	18	0.000464	1.055866	0.3684744

VE at 60 km (L/min)

Summary of all Effects; design: (new.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	220.5203	18	72.82886	3.027925	0.0735303

Blood Lactate (mmol/L)

Summary of all Effects; design: (lactate stats.sta)						
1-DRINK, 2-DISTANCE						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	1.6616	18	3.300267	0.503474	0.612690151
2	4	35.23443	36	1.7651	19.96172	9.79127E-09
12	8	1.509683	72	0.546683	2.761532	0.010171202

Tukey HSD test; variable Var.1 (lactate stats.sta)															
Probabilities for Post Hoc Tests			INTERACTION: 1 x 2												
	PLACEBO					CARBOHYDRATE					CARBOHYDRATE+PROTEIN				
	PL-Rest	PL-20km	PL-40km	PL-60km	PL-80km	C-Rest	C-20km	C-40km	C-60km	C-80km	C/P-Rest	C/P-20km	C/P-40km	C/P-60km	C/P-80km
	1.670000	**4.120000**	**3.090000**	**2.520000**	**3.510000**	**1.520000**	**3.960000**	**3.520000**	**2.710000**	**5.020000**	**1.600000**	**3.570000**	**3.150000**	**2.800000**	**4.610000**
PL-Rest		0.000145	0.00456	0.40661	0.000177	1	0.000145	0.000173	0.128504	0.000145	1	0.0001597	0.0024925	0.064705	0.000145
PL-20km	0.000145		0.137968	0.000763	0.871298	0.000145376	1	0.884378	0.005052	0.313551	0.0001454	0.9371764	0.2065429	0.012111	0.975024
PL-40km	0.00456	0.137968		0.918556	0.993909	0.001014054	0.367866	0.992351	0.997812	0.000155	0.0022548	0.9790762	1	0.999893	0.001672
PL-60km	0.40661	0.000763	0.918556		0.181364	0.169660926	0.003733	0.169661	0.999999	0.000145	0.2800614	0.1194954	0.8426623	0.99993	0.000146
PL-80km	0.000177	0.871298	0.993909	0.181364		0.000149548	0.988268	1	0.509476	0.001847	0.0001578	1	0.9987631	0.6995	0.082026
C-Rest	1	0.000145	0.001014	0.169661	0.00015		0.000145	0.000149	0.039257	0.000145	1	0.0001472	0.0005824	0.01761	0.000145
C-20km	0.000145	1	0.367866	0.003733	0.988268	0.000145376		0.99049	0.023157	0.111055	0.0001454	0.9971082	0.4883591	0.05063	0.810863
C-40km	0.000173	0.884378	0.992351	0.169661	1	0.000149012	0.99049		0.488359	0.00204	0.0001562	1	0.998345	0.679166	0.088608
C-60km	0.128504	0.005052	0.997812	0.999999	0.509476	0.039257288	0.023157	0.488359		0.000145	0.0758609	0.3869616	0.9904896	1	0.00016
C-80km	0.000145	0.313551	0.000155	0.000145	0.001847	0.000145376	0.111055	0.00204	0.000145		0.0001454	0.0033718	0.0001668	0.000145	0.995199
C/P-Rest	1	0.000145	0.002255	0.280061	0.000158	1	0.000145	0.000156	0.075861	0.000145		0.0001509	0.0012325	0.036019	0.000145
C/P-20km	0.00016	0.937176	0.979076	0.119495	1	0.000147164	0.997108	1	0.386962	0.003372	0.0001509		0.9939094	0.573579	0.128504
C/P-40km	0.002493	0.206543	1	0.842662	0.998763	0.000582397	0.488359	0.998345	0.99049	0.000167	0.0012325	0.9939094		0.999103	0.003051
C/P-60km	0.064705	0.012111	0.999893	0.99993	0.6995	0.017609656	0.05063	0.679166	1	0.000145	0.0360193	0.5735791	0.999103		0.000192
C/P-80km	0.000145	0.975024	0.001672	0.000146	0.082026	0.000145376	0.810863	0.088608	0.00016	0.995199	0.0001454	0.1285035	0.0030506	0.000192	

Ammonia (umol/L)

Summary of all Effects; design: (new.sta)						
1-DRINK, 2-DISTANCE						
	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	1922.48	18	1238.584	1.55216	0.238841772
2	4	39963.48	36	2373.017	16.84079	7.23968E-08
12	8	600.3217	72	436.1291	1.376477	0.221476421

Tukey HSD test; variable Var.1 (new.sta)															
Probabilities for Post Hoc Tests			INTERACTION: 1 x 2												
	PLACEBO					CARBOHYDRATE					CARBOHYDRATE+PROTEIN				
	PL-Rest	PL-20km	PL-40km	PL-60km	PL-80km	C-Rest	C-20km	C-40km	C-60km	C-80km	C/P-Rest	C/P-20km	C/P-40km	C/P-60km	C/P-80km
	53.50000	92.70000	99.40000	104.0000	137.8000	47.40000	88.30000	98.00000	109.0000	167.9000	62.70000	97.20000	105.1000	121.9000	161.9000
PL-Rest		0.006278	0.000608	0.000208	0.000145	0.999997139	0.027186	0.000962	0.000151	0.000145	0.9995944	0.0012672	0.0001832	0.000145	0.000145
PL-20km	0.006278		0.999991	0.996239	0.000786	0.000736475	1	1	0.911156	0.000145	0.109314	0.9999999	0.9906868	0.134294	0.000145
PL-40km	0.000608	0.999991		1	0.008294	0.000176787	0.996867	1	0.999349	0.000145	0.0147133	1	0.9999988	0.51669	0.000145
PL-60km	0.000208	0.996239	1		0.037068	0.000148773	0.932067	0.999998	1	0.000145	0.0029838	0.9999888	1	0.836946	0.000147
PL-80km	0.000145	0.000786	0.008294	0.037068		0.000145376	0.000243	0.005097	0.14833	0.106482	0.0001454	0.0038303	0.0515913	0.925505	0.400101
C-Rest	0.999997	0.000736	0.000177	0.000149	0.000145		0.003438	0.000205	0.000146	0.000145	0.9439677	0.0002308	0.0001473	0.000145	0.000145
C-20km	0.027186	1	0.996867	0.932067	0.000243	0.003438234		0.99927	0.652473	0.000145	0.3026564	0.9997212	0.8908465	0.039393	0.000145
C-40km	0.000962	1	1	0.999998	0.005097	0.000204921	0.99927		0.997149	0.000145	0.0232024	1	0.9999809	0.414193	0.000145
C-60km	0.000151	0.911156	0.999349	1	0.14833	0.000145614	0.652473	0.997149		0.000147	0.0005379	0.9942161	1	0.986555	0.000166
C-80km	0.000145	0.000145	0.000145	0.000145	0.106482	0.000145376	0.000145	0.000145	0.000147		0.0001454	0.0001454	0.0001454	0.000589	0.999998
C/P-Rest	0.999594	0.109314	0.014713	0.002984	0.000145	0.9439677	0.302656	0.023202	0.000538	0.000145		0.0298653	0.0020166	0.000146	0.000145
C/P-20km	0.001267	1	1	0.999989	0.00383	0.000230849	0.999721	1	0.994216	0.000145	0.0298653		0.9999305	0.359436	0.000145
C/P-40km	0.000183	0.990687	0.999999	1	0.051591	0.000147343	0.890846	0.999981	1	0.000145	0.0020166	0.9999305		0.890846	0.000149
C/P-60km	0.000145	0.134294	0.51669	0.836946	0.925505	0.000145376	0.039393	0.414193	0.986555	0.000589	0.0001463	0.3594358	0.8908465		0.004754
C/P-80km	0.000145	0.000145	0.000145	0.000147	0.400101	0.000145376	0.000145	0.000145	0.000166	0.999998	0.0001454	0.0001454	0.0001485	0.004754	

Blood Glucose (mmol/L)

Summary of all Effects; design: (plasma volume difference.sta)						
1-DRINK, 2-DISTANCE						
	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	2.608008	18	0.085282	30.58088	1.62485E-06
2	4	0.55715	36	0.08311	6.703795	0.000386369
12	8	0.139646	72	0.053673	2.601793	0.01475011

Tukey HSD test; variable Var.1 (glucose stats.sta)															
Probabilities for Post Hoc Tests				**INTERACTION: 1 x 2**											
	PLACEBO					**CARBOHYDRATE**					**CARBOHYDRATE+PROTEIN**				
	PL-Rest	**PL-20km**	**PL-40km**	**PL-60km**	**PL-80km**	**C-Rest**	**C-20km**	**C-40km**	**C-60km**	**C-80km**	**C/P-Rest**	**C/P-20km**	**C/P-40km**	**C/P-60km**	**C/P-80km**
	3.920495	4.116351	3.638475	3.492591	3.640911	4.242260	4.237223	4.076597	4.125837	4.152133	4.078532	4.336338	4.120334	4.013391	4.187088
PL-Rest		0.85003	0.313451	0.007819	0.327068	0.141005754	0.157638	0.971279	0.801859	0.639054	0.9681783	0.0113172	0.8306596	0.999862	0.404878
PL-20km	0.85003		0.001589	0.000149	0.001716	0.996069729	0.99743	1	1	1	1	0.7153172	1	0.999553	0.999995
PL-40km	0.313451	0.001589		0.98401	1	0.00015533	0.000158	0.00567	0.001169	0.000537	0.0053366	0.0001454	0.0013903	0.037113	0.000245
PL-60km	0.007819	0.000149	0.98401		0.981473	0.000145376	0.000145	0.000169	0.000148	0.000146	0.0001667	0.0001454	0.0001488	0.000446	0.000145
PL-80km	0.327068	0.001716	1	0.981473		0.000156403	0.000159	0.006119	0.001262	0.000575	0.0057596	0.0001454	0.0015137	0.039676	0.000255
C-Rest	0.141006	0.99607	0.000155	0.000145	0.000156		1	0.953526	0.99827	0.999903	0.9576324	0.9998401	0.9971737	0.657586	1
C-20km	0.157638	0.99743	0.000158	0.000145	0.000159	1		0.963661	0.998928	0.999951	0.9670783	0.9997087	0.9981958	0.690699	1
C-40km	0.971279	1	0.00567	0.000169	0.006119	0.953525782	0.963661		1	0.999989	1	0.4489758	1	0.999999	0.999027
C-60km	0.801859	1	0.001169	0.000148	0.001262	0.998269975	0.998928	1		1	1	0.7728883	1	0.998804	0.999999
C-80km	0.639054	1	0.000537	0.000146	0.000575	0.999903202	0.999951	0.999989	1		0.9999918	0.8990938	1	0.989902	1
C/P-Rest	0.968178	1	0.005337	0.000167	0.00576	0.957632422	0.967078	1	1	0.999992		0.4616961	1	0.999998	0.999197
C/P-20km	0.011317	0.715317	0.000145	0.000145	0.000145	0.999840081	0.999709	0.448976	0.772888	0.899094	0.4616961		0.7400894	0.137315	0.98043
C/P-40km	0.83066	1	0.00139	0.000149	0.001514	0.997173727	0.998196	1	1	1	1	0.7400894		0.999318	0.999998
C/P-60km	0.999862	0.999553	0.037113	0.000446	0.039676	0.65758574	0.690699	0.999999	0.998804	0.989902	0.9999983	0.1373148	0.9993185		0.933409
C/P-80km	0.404878	0.999995	0.000245	0.000145	0.000255	0.999999821	1	0.999027	0.999999	1	0.999197	0.9804296	0.9999976	0.933409	

Serum Insulin (µIU/mL)

Summary of all Effects; design: (Insulin stats.sta)						
1-DRINK, 2-DISTANCE						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	42.81419	18	18.32084	2.336913	0.125236049
2	4	1228.363	36	60.43935	20.32389	7.86904E-09
12	8	32.75182	72	24.29342	1.348176	0.234249622

Tukey HSD test; variable Var.1 (Insulin stats.sta)															
Probabilities for Post Hoc Tests			INTERACTION: 1 x 2												
	PLACEBO					CARBOHYDRATE					CARBOHYDRATE+PROTEIN				
	PL-Rest	PL-20km	PL-40km	PL-60km	PL-80km	C-Rest	C-20km	C-40km	C-60km	C-80km	C/P-Rest	C/P-20km	C/P-40km	C/P-60km	C/P-80km
	19.87620	5.893160	3.239776	3.037268	1.845451	21.42671	7.304824	5.560555	5.552479	3.116510	15.76584	7.680624	5.927481	4.814910	5.829201
PL-Rest		0.000146	0.000145	0.000145	0.000145	0.999992728	0.000163	0.000146	0.000146	0.000145	0.8622121	0.0001819	0.0001463	0.000145	0.000146
PL-20km	0.000146		0.996428	0.992616	0.875041	0.000145376	0.999998	1	1	0.994388	0.0024674	0.9999581	1	1	1
PL-40km	0.000145	0.996428		1	0.999998	0.000145376	0.871564	0.999153	0.999185	1	0.0001644	0.7829949	0.9959358	0.999991	0.997224
PL-60km	0.000145	0.992616	1		1	0.000145376	0.826859	0.997899	0.997968	1	0.000158	0.7261864	0.9917228	0.999961	0.994074
PL-80km	0.000145	0.875041	0.999998	1		0.000145376	0.469605	0.930755	0.931849	0.999999	0.0001464	0.357857	0.8681039	0.989324	0.887382
C-Rest	0.999993	0.000145	0.000145	0.000145	0.000145		0.000146	0.000145	0.000145	0.000145	0.4082018	0.0001468	0.0001454	0.000145	0.000145
C-20km	0.000163	0.999998	0.871564	0.826859	0.469605	0.000145912		0.999969	0.999967	0.845237	0.0194368	1	0.9999984	0.998172	0.999996
C-40km	0.000146	1	0.999153	0.997899	0.930755	0.000145376	0.999969		1	0.998497	0.0015011	0.9996903	1	1	1
C-60km	0.000146	1	0.999185	0.997968	0.931849	0.000145376	0.999967	1		0.998549	0.0014834	0.9996766	1	1	1
C-80km	0.000145	0.994388	1	1	0.999999	0.000145376	0.845237	0.998497	0.998549		0.0001601	0.7490565	0.9936621	0.999977	0.995543
C/P-Rest	0.862212	0.002467	0.000164	0.000158	0.000146	0.408201754	0.019437	0.001501	0.001483	0.00016		0.032204	0.0025982	0.000522	0.002241
C/P-20km	0.000182	0.999958	0.782995	0.726186	0.357857	0.000146806	1	0.99969	0.999677	0.749056	0.032204		0.9999669	0.99237	0.999936
C/P-40km	0.000146	1	0.995936	0.991723	0.868104	0.000145376	0.999998	1	1	0.993662	0.0025982	0.9999669		1	1
C/P-60km	0.000145	1	0.999991	0.999961	0.989324	0.000145376	0.998172	1	1	0.999977	0.0005221	0.9923701	0.9999999		1
C/P-80km	0.000146	1	0.997224	0.994074	0.887382	0.000145376	0.999996	1	1	0.995543	0.0022411	0.9999361	1	1	

Plasma Free Fatty Acids (mmol/L)

Summary of all Effects; design: (ffa stats.sta)						
1-DRINK, 2-DISTANCE						
	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	0.435736	18	0.08752	4.978718	0.019009756
2	4	1.041187	36	0.045683	22.79134	1.89651E-09
12	8	0.21271	72	0.014958	14.22074	3.35491E-12

Tukey HSD test; variable Var.1 (ffa stats.sta)															
Probabilities for Post Hoc Tests			INTERACTION: 1 x 2												
	PLACEBO					CARBOHYDRATE					CARBOHYDRATE+PROTEIN				
	PL-Rest	PL-20km	PL-40km	PL-60km	PL-80km	C-Rest	C-20km	C-40km	C-60km	C-80km	C/P-Rest	C/P-20km	C/P-40km	C/P-60km	C/P-80km
	.1417138	.1737822	.2752962	.5052311	.9892510	.2029188	.1993508	.2723922	.4165059	.4564725	.1718253	.1214106	.2068617	.2850254	.3704135
PL-Rest		0.999999	0.493458	0.000145	0.000145	0.998344183	0.999145	0.530713	0.000449	0.000159	0.9999997	1	0.9967898	0.374694	0.006627
PL-20km	0.999999		0.866239	0.000149	0.000145	0.999999821	1	0.889187	0.002833	0.000313	1	0.9997056	0.9999989	0.771582	0.039682
PL-40km	0.493458	0.866239		0.006143	0.000145	0.990973413	0.985899	1	0.399245	0.085061	0.8493382	0.2636858	0.9947471	1	0.913347
PL-60km	0.000145	0.000149	0.006143		0.000145	0.000182867	0.000174	0.005171	0.948024	0.999871	0.0001484	0.0001454	0.0001962	0.010883	0.47778
PL-80km	0.000145	0.000145	0.000145	0.000145		0.000145376	0.000145	0.000145	0.000145	0.000145	0.0001454	0.0001454	0.0001454	0.000145	0.000145
C-Rest	0.998344	1	0.990973	0.000183	0.000145		1	0.993909	0.015866	0.001472	0.9999995	0.9738065	1	0.972143	0.155739
C-20km	0.999145	1	0.985899	0.000174	0.000145	1		0.990158	0.01296	0.00118	0.9999999	0.9822136	1	0.960504	0.133961
C-40km	0.530713	0.889187	1	0.005171	0.000145	0.993908763	0.990158		0.365522	0.07417	0.8740051	0.2919121	0.9965935	1	0.893522
C-60km	0.000449	0.002833	0.399245	0.948024	0.000145	0.015865922	0.01296	0.365522		0.999988	0.0025121	0.0002109	0.0197656	0.520379	0.999934
C-80km	0.000159	0.000313	0.085061	0.999871	0.000145	0.001472354	0.00118	0.07417	0.999988		0.0002903	0.0001479	0.0018643	0.131767	0.959068
C/P-Rest	1	1	0.849338	0.000148	0.000145	0.999999523	1	0.874005	0.002512	0.00029		0.9998097	0.9999978	0.749647	0.035841
C/P-20km	1	0.999706	0.263686	0.000145	0.000145	0.9738065	0.982214	0.291912	0.000211	0.000148	0.9998097		0.9613229	0.182398	0.001934
C/P-40km	0.99679	0.999999	0.994747	0.000196	0.000145	1	1	0.996594	0.019766	0.001864	0.9999978	0.9613229		0.981759	0.182857
C/P-60km	0.374694	0.771582	1	0.010883	0.000145	0.972143292	0.960504	1	0.520379	0.131767	0.7496468	0.182398	0.9817588		0.961551
C/P-80km	0.006627	0.039682	0.913347	0.47778	0.000145	0.155739427	0.133961	0.893522	0.999934	0.959068	0.0358412	0.0019338	0.182857	0.961551	

Percent Change in Plasma Volume (%)

Summary of all Effects; design: (plasma volume difference.sta)						
1-DRINK						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	2	7.875584	18	3.27244	2.40664	0.1185122

Tukey HSD test; variable Var.1 (plasma volume difference.sta)				
Probabilities for Post Hoc Tests				
MAIN EFFECT: DRINK				
	{1}	{2}	{3}	
	-2.79233	-4.53235	-3.96549	
1 {1}		0.107658	0.337585	
2 {2}	0.107658446		0.766238	
3 {3}	0.337584794	0.766238		