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Effect of a Low Carbohydrate – Moderate Protein Supplement on Endurance Performance in Female Athletes

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Effect of a Low Carbohydrate – Moderate Protein Supplement on Endurance Performance in Female Athletes

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Abstract

Effect of a Low Carbohydrate – Moderate Protein Supplement on Endurance Performance in Female Athletes

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The purpose of this study was to investigate if a low mixed carbohydrate plus moderate protein supplement, provided during endurance exercise, would improve time to exhaustion in comparison to a traditional 6% carbohydrate supplement in female athletes exercising at or below their ventilatory threshold. Fourteen (n = 14) trained female cyclists and triathletes cycled on two separate occasions for three hours at intensities varying between 45% - 70% VO_2max, followed by a ride to exhaustion at an intensity approximating the individual's VT (average 75.06% VO_2max). Supplements (275ml) were provided every 20 min during exercise and were composed of a 3% carbohydrate mixture + 1.2% protein (CHO+PRO) or a 6% carbohydrate-only (CHO).

The CHO+PRO treatment contained a mixture of dextrose, maltodextrin, fructose, and whey protein isolate. The CHO treatment was composed of dextrose only. Time to exhaustion (TTE) was significantly greater with CHO+PRO in comparison to CHO (49.94 ± 7.01 vs 42.36 ± 6.21 min, respectively, $p < 0.05$). Blood glucose was signifcantly lower during the CHO+PRO (4.07 ± 0.12 $mmol \cdot L^{-1}$) trial compared to CHO (4.47 ± 0.12 $mmol \cdot L^{-1}$), with treatment x time interactions occurring from 118 minutes of exercise until exhaustion ($p < 0.05$). Heart rate was significantly lowered in the CHO+PRO treatment during exercise as compared to CHO ($p < 0.05$). There were no significant differences for other blood measures, ratings of perceived exertion or carbohydrate and fat oxidation between trials. Results from the present study suggest that the addition of a moderate amount of protein to a low mixed carbohydrate supplement improves endurance performance in females above that of a traditional 6% carbohydrate supplement. Improvement in performance occurred despite CHO+PRO containing a lower carbohydrate and caloric content. It is likely the greater performance seen with CHO+PRO was a result of the carbohydrate protein combination and the use of a mixture of carbohydrate sources.

KEY WORDS: nutritional supplementation, time to exhaustion, aerobic capacity.

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INTRODUCTION

Previous research has demonstrated that consuming a carbohydrate supplement during prolonged endurance exercise improves performance compared to water or placebo (2, 9-11, 16, 23, 26, 49-51). The addition of protein to a carbohydrate (CHO+PRO) supplement, however, has demonstrated enhanced performance beyond that of carbohydrate (CHO) alone (24, 37-39), but these findings are not universal (29, 34, 44).

Investigations from our laboratory recently found protein added to either a low carbohydrate (3% CHO + 0.75% PRO) or moderate carbohydrate supplement (4.5% CHO + 1.2% PRO) maintained endurance performance efficacy relative to a traditional 6% carbohydrate supplement (32). In an effort to improve effectiveness of our carbohydrate plus protein supplement, we altered the carbohydrate source from a single source of dextrose to a mixture of glucose, fructose and maltodextrin. Multiple sources of CHO appear to increase CHO oxidation rates above that achieved by a single CHO source. The maximal rate of exogenous carbohydrate utilization during prolonged exercise while providing a single CHO source is about 1.0 - 1.1 $g \cdot min^{-1}$ (27, 28, 46). The oxidation rate, however, can increase to 1.7 $g \cdot min^{-1}$ when a combination of CHO is ingested during exercise (25). Furthermore, the combination of multiple carbohydrate sources has been shown to further enhance endurance performance above that of glucose alone (12, 43).

We previously reported that a 3% mixed carbohydrate plus 1.2% protein supplement did not improve time to exhaustion in comparison to a traditional 6% carbohydrate treatment. However, on closer inspection, it was noted that improvement in performance did occur in subjects exercising at or below their ventilatory threshold (18). Based on these findings, we sought to determine if this improved performance could be further demonstrated when utilizing a larger subject sample exercising at an intensity approximating their ventilatory threshold.

Therefore, the purpose of this study was to investigate if the addition of a moderate protein (1.2%) concentration to a low carbohydrate (3%) mixture (glucose, maltodextrin and fructose) (CHO+PRO) would improve time to exhaustion in comparison to a traditional 6% carbohydrate (CHO) supplement, in female athletes exercising at or slightly below their ventilatory threshold. It was hypothesized that performance would be enhanced when consuming CHO+PRO in comparison to a traditional 6% carbohydrate (CHO) supplement, despite a 50% lower carbohydrate concentration and approximately 30% lower caloric intake with the CHO+PRO treatment.

METHODS

Experimental Design

This study followed a randomized, double-blinded, repeated measures design. After initially completing a VO_2max test and familiarization trial, subjects performed two experimental trials in order to test the effect of a mixed 3% carbohydrate supplement with 1.2% added protein (CHO+PRO), against a traditional 6% carbohydrate (CHO) supplement. The experimental protocol was composed of varying intervals between 45% and 70% VO_2max, followed by a ride to exhaustion at an intensity approximating the individual's VT. Supplements (275ml) were consumed immediately prior to commencing the trial, and every 20 minutes thereafter.

CHO+PRO contained a mixture of dextrose (glucose), maltodextrin and fructose, and a whey protein isolate. The CHO treatment was composed of dextrose only. CHO+PRO contained 50% the carbohydrate content in comparison to CHO, and 33% lower caloric content. Both treatments contained equal amounts of electrolytes (Table 1). Supplements were supplied by the Human Performance Laboratory (Austin, TX), and were prepared by a laboratory technician not directly involved in the study. All supplements were similar in taste, color and texture.

Subjects

Fourteen female cyclists and triathletes were recruited via email announcement from local triathlon and cycling teams in Austin, TX. A detailed explanation of the experimental procedures and the potential risks of the study were given both verbally and

in writing to all subjects prior to initial testing. Subjects were given the opportunity to ask questions before signing the informed consent, according to the protocol described in the University of Texas at Austin's 'Institutional Review Board Procedures Manual for Faculty, Staff and Student Researchers with Human Participants'. The University of Texas at Austin Institutional Review Board approved the study before it commenced. Subject characteristics are found in Table 2.

Preliminary Testing

Subjects initially reported to the laboratory for determination of maximum oxygen consumption (VO_2max) and ventilatory threshold (VT). All trials were conducted on the same ergometer (Veletron Dynafit Pro, Racermate, Seattle, WA)**.** Prior to testing, body weight was recorded and subjects were outfitted with a Polar Heart Rate monitor (Polar Beat, Polar Electro Oy, Finland).

Maximal oxygen consumption was determined via a ramped protocol, consisting of a four minute warm up stage (range 75 - 130 W), followed by four stages of 2 minute duration. Each 2 minute stage increased in intensity by 35 W increments. Thereafter, workloads increased by 10 W each minute until the subject could no longer continue. Subjects breathed through a Hans Rudolf valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Inspired air volumes were measured using a dry gas meter (ParvoMedics TrueOne2400, ParvoMedics, Sandy, UT). A laboratory computer collected gas meter outputs, and used values for calculation of oxygen uptake (VO_2), carbon dioxide production (VCO_2), and Respiratory Exchange

Ratio (RER) every 15 seconds. The criteria for establishing VO_2max was a plateau in oxygen consumption (VO_2) with increasing exercise intensity, in addition to a respiratory exchange ratio (RER) greater than 1.10. The two highest 30 second values were averaged to determine VO_2max (ml $O_2 \cdot kg^{-1}$ min^{-1}). VT values were determined from the VO_2max test and a computer-generated plot (ParvoMedics TrueOne2400 software). VT was defined as the point at which the minute ventilation (V_E) increased in a nonlinear fashion compared to increases in VO_2 and was confirmed by an increase in the V_E/VCO_2 to V_E/VO_2 ratio. Subjects were given constant verbal encouragement throughout the VO_2max test.

Testing Protocol

All trials were conducted in the Exercise Physiology Metabolism Laboratory at The University of Texas at Austin. Within the seven days following preliminary testing, subjects reported to the laboratory for a familizaration trial in order to accustom subjects with the testing protocol and equipment. This trial simulated the experimental protocol, exclusive of blood draws and treatment beverages. Water (275ml) was substituted for the experimental beverages.

The cycling protocol consisted of varying intervals between 45% and 70% VO_2max, followed by a ride to exhaustion at an exercise intensity approximating the individual's VT. The first 30-min of cycling was conducted at 45% VO_2max, followed by six intervals of 8-min duration. Interval duration was then reduced to 3-min. At 3 hours into the cycling protocol, subjects began the performance ride at an intensity

relative to their VT, and this intensity was held until exhaustion. Refer to Figure 1 for cycling protocol. Exhaustion was determined as the point at which subjects could no longer maintain a pedaling cadence of 60 revolutions per minute (rpm), despite constant verbal encouragement.

On the morning of the experimental trials, subjects arrived at the laboratory between 7am and 8am, following a 12-hour fast during which they were permitted to consume water only. Diet and activity logs were collected and verified, and body weight was obtained. Subjects were fitted with a Polar Heart Rate Monitor and a Teflon catheter, fitted with a three-way stopcock and a catheter extension, was inserted into an antecubital vein. Subjects were instructed to sit quietly for 2 minutes, after which a baseline HR was recorded and a 5 ml baseline blood draw (PRE) was taken. After baseline blood sampling, participants consumed the first supplement (275ml), before mounting the ergometer. Supplements were provided every 20-min during the exercise protocol. Upon nearing exhaustion, however, subjects were asked to consume as much as they felt comfortable.

All timing devices were removed from subject's sight, blinding participants to the length of ride completed. Personal music devices were permitted, however devices were required to be on a random song shuffle setting to eliminate any indication of time.

Cardio-Respiratory Measures, Ratings of Perceived Exertion

Respiratory gas samples and ventilation were collected five different times throughout the protocol. Collections occurred at 10-min (low intensity), 46-min (high),

130-minute (low/high) and 184-min (start of exhaustion ride). Collection periods were 5 minutes in length, excluding the collection at time point 130-136 minutes, which consisted of two, three minute recordings at low and high intensities respectively. To ensure a steady state VO_2 and RER, only the last 2 minutes of each collection were recorded. Carbohydrate and fat oxidation rates ($g \cdot min^{-1}$) were calculated from VCO_2, VO_2, and RER according to Frayn (19). It was assumed that protein oxidation during exercise was negligible. Heart Rate (HR) and Ratings of Perceived Exertion were recorded 12 times throughout the exercise protocol. RPE was recorded using the Borg Scale (6).

Blood Sampling

Blood samples (5 ml) were collected pre-exercise (PRE), 118-min (T-118) and 177-min (T-177) into the exercise protocol, and at the point of exhaustion (END). 0.3 ml of the sample was transferred into a seperate tube containing 1 ml 10% perchloric acid (PCA). The remaining sample was divided into two tubes and mixed with 0.3ml of EDTA (24 $mg \cdot ml^{-1}$, pH 7.4) to prevent coagulation. Tubes were centrifuged for 10-mins at 3,000 rpm in a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). Plasma extracts were transferred and all tubes were stored at -80^0C until analysis.

Prior Diet and Exercise

Subjects were required to record activity levels for 3 days and diet for the 2 days prior to each trial. Diet and exercise were recorded in supplied logs, and subjects were required to replicate diet and exercise before each trial. Subjects were asked to keep diet and activity levels as close to their regular routine as possible, and asked to refrain from strenuous exercise in the 24 hours prior to each trial. Logs were reviewed prior to each trial to ensure compliance. All subjects abided with requirements.

Tissue Analysis

At each blood collection, one drop of blood was used to measure blood glucose concentration (One Touch Basic glucose analyzer, LifeScan Inc., Milipitas, CA). This sample was used to ensure subjects were fasted upon arrival and additionally, as an indicator of blood glucose levels as the trial progressed. Prior to each trial, the analyzer was calibrated using standards provided by LifeScan Inc.

For data anaylsis, plasma samples were measured for glucose in duplicate using a modified Trinder procedure at 37^{o}C (42). Samples were read at 500nm using a Beckman DU640 Spectrophometer (Coulter, Fullerton, CA) and had a coefficient of variation (CV) of 3.7%.

Blood lactate concentrations were measured from the PCA extracts using enzymatic analysis according to Hohorst (22). The assay was run in duplicate and had a CV of 1.2%. Samples were read at 340 nm using a Beckman DU640 Spectrophometer (Coulter, Fullerton, CA).

Plasma insulin was analyzed via radioimmunoassay based on the principles of Goetz (Goetz et al., 1961) (MP Biomedicals ^{125}I RIA, Solon, OH, USA) and had a CV of 6.0% Duplicate tubes were prepared and counted in a Wallac 1470 Wizard Gamma Counter (Wallac 1470 Wizard Gamma Counter, PerkinElmer Life and Analytical Sciences, Boston, MA), which had been calibrated for insulin ^{125}I.

Myoglobin was measured in duplicate by solid phase ELISA (Myoglobin Enzyme Immunoassay Test Kit, BioCheck, Inc, Foster City, CA, USA), with a CV of 5.4%. Wells were read at 450 nm with a microtiter well reader (Bio-Tek ELx800, Biotek Instruments Inc, Winooski, VT, USA).

Statistical Analysis

Data were analyzed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL). Time to exhaustion was analyzed using a paired sample *t*-test. Average HR and substrate (carbohydrate and fat) utilization across the variable intensity protocol were also compared between trials using paired-samples *t*-test. All other variables were measured using a two-way (treatment x time) repeated measures analysis of variance (ANOVA). Where significance was found, post hoc comparisons were conducted using a Least Significant Difference (LSD) adjustment. Significance was determined at $p < 0.05$. Data were expressed as mean ± SE.

RESULTS

Endurance Performance

Time to exhaustion (TTE) was significantly greater with CHO+PRO, with a 15.2% increase in performance in comparison to CHO (CHO+PRO: 49.94 ± 7.01 vs CHO: 42.36 ± 6.21 minutes, $p < 0.05$) (Figure 2). Subjects performed the exhaustion ride at an average of 75.06% VO_2max, ~1.5% lower than the calculated average group VT (76.57 ± 1.24 %VO_2max). Intensities for individual subjects ranged from 7.25% below VT to 5.1% above VT.

Tissue Analysis

There were no significant differences in the pre-exercise plasma glucose levels. Glucose dropped slightly at 118-min with CHO+PRO, in comparison to PRE. Plasma glucose levels increased significantly from PRE to END only in the CHO treatment. Additionally, mean blood glucose for CHO (4.47 ± 0.12 mmol·L^{-1}) was significantly greater than for CHO+PRO (4.07 ± 0.12 mmol·L^{-1}) with treatment by time differences occurring at minutes 118 and 177, and at the point of exhaustion ($p < .05$) (Figure 3).

Plasma insulin levels decreased as exercise progessed during both exercise trials, however there was no significant differences between treatments (Figure 4). Average plasma insulin was 73.56 ± 7.37 pmol·L^{-1} during the CHO trial and 70.00 ± 7.78 pmol·L^{-1} during the CHO+PRO trial.

Average blood lactate concentration was 1.21 ± 0.139 mmol·L^{-1} for the CHO treatment and 1.22 ± 0.147 mmol·L^{-1} for the CHO+PRO treatment. No significant differences were found among the treatments or treatment by time (Figure 5). In both treatments, blood lactate concentrations significantly increased from 177-min to exhaustion ($p < 0.05$)

The average plasma myoglobin concentration was 26.28 ± 7.28 ng·ml^{-1} during the CHO trial and 19.64 ± 1.79 ng·ml^{-1} during the CHO + PRO trial. However, statistical analysis revealed no significant overall difference in treament or treatment by time interaction for myoglobin (Figure 6)

Respiratory Exchange Ratio and Substrate Utilization

The average respiratory exchange ratio (RER) across the first three hours of the CHO treatment was 0.924 ± 0.011 and 0.939 ± 0.012 for CHO+PRO (Table 3). Oxygen consumption (VO_2) over the same period was slightly, but significantly higher during the CHO+PRO trial (1.779 ± L·min^{-1}) as compared to CHO (1.755 ± 0.09 L·min^{-1}, $p < 0.05$). Carbohydrate and fat utilization were calculated from VO_2, VCO_2, and RER data. Collections occurred only during the 3 hour variable intensity ride, thus results are not indicative of utilization rates during the performance ride to exhaustion. Average carbohydrate oxidation was 1.76 ± 0.12 g·min^{-1} for the CHO trial and 1.75 ± 0.12 g·min^{-1} for the CHO+PRO trial. Average fat oxidation for the CHO and CHO+PRO trials were 0.24 ± 0.04 g·min^{-1} and 0.22 ± 0.04 g·min^{-1} respectively. There were no significant

treatment differences in either carbohydrate or fat oxidation rates ($g \cdot min^{-1}$) between CHO+PRO and CHO treatments (Table 3).

Heart Rate and Ratings of Perceived Exertion

During exercise, average HR was significantly lower during the CHO+PRO (130.17 ± 3.13 bpm) trial in comparison to CHO (132.80 ± 2.92 bpm, $p < 0.05$). There were no significant differences between treatments for RPE (Table 4).

DISCUSSION

The primary purpose of this study was to determine if a moderate protein low mixed carbohydrate (CHO+PRO) sports drink could increase endurance performance in comparison with a traditional 6% carbohydrate sports drink (CHO) in trained female athletes. The primary finding was that CHO+PRO enhanced time to exhaustion above that of CHO when exercising at an intensity at or slightly below VT (CHO+PRO, 49.94 ± 7.01 minutes vs CHO, 42.36 ± 6.21 minutes, $p < 0.05$). This represents a 15.2% improvement in performance with CHO+PRO. Improvement in time to exhaustion occurred despite CHO+PRO containing a 50% lower carbohydrate content and approximately 30% fewer calories. This maybe an important consideration for individuals concerned about body weight and caloric intake.

This study is in agreement with previous findings that the addition of PRO to a CHO supplement enhances endurance performance in comparison with traditional 6% CHO supplement (24, 37-39). Our laboratory recently found that a low CHO plus PRO sports drink maintained efficacy in comparison to a traditional 6% CHO sports drink (32). In an effort to improve our CHO/PRO sport drink, we altered the CHO source to contain a mixture of glucose, fructose and maltodextrin, rather than a single CHO source (glucose). We recently compared the effects of our 3% mixed CHO plus 1.2% PRO sports drink with a traditional 6% CHO sports drink during variable intensity cycling to exhaustion (18). Improvements in performance, however, were observed only in those individuals performing at or below their VT during the exhaustion portion of the cycling

protocol. Recently, it was suggested that the performance effects of CHO+PRO supplementation may be related to the intensity of exercise (8). In the present study, we sought to further determine whether the improved performance with CHO+PRO was related to exercise intensity.

Individuals completed the performance ride at an average of 75.06% VO_2max, approximately 1.5% lower than the average VT (76.57 ± 1.24 % VO_2max). Individualizing the performance ride to the subject's VT is novel in comparison to prior studies investigating the performance effect of CHO+PRO supplementation. Time to exhaustion rides have used intensities ranging from 70% - 85% VO_2max (24, 35, 37). While workloads were adjusted relative to an individual's VO_2max, these studies did not account for individual differences in VT or corresponding lactate threshold (LT). Performance during endurance sporting events, such as marathons, are performed at self-selected intensities that approximate LT (17). Therefore, adjusting performance trials relative to LT or VT may potentially decrease some of the variability in results across subjects.

In the present study, performance was defined as the point at which individuals could no longer maintain their cycling cadence above 60 rpm during an exhaustive exercise bout. Previous criticisms of exhaustive exercise bouts are that they are not as representative of sporting events in comparison to a set distance time trial (13, 44). However, supplementation benefits are not limited to endurance races such as marathons, distance road cycling, and long-distance triathlons. High levels of endurance are required in numerous situations inside and outside the sporting world. Sports such as tennis,

volleyball, and baseball have the capacity to last several hours, with success reliant on lasting endurance during the later stages. Professionals such as firefighters and military personnal are routinely required to maintain high levels of physical and mental performance for prolonged periods, and prolonging time to exhaustion can be critical for the success of their mission or even survival.

The improvement in performance with CHO+PRO over CHO is potentially explained by a number of mechanisms. Exogenous CHO alone has previously demonstrated increased glucose uptake above that of placebo (1, 30) and suggested to be associated with sparing of endogenous CHO (51). Insulin and muscle contraction are considered the major stimulators of glucose transport (7, 21) and both CHO and PRO have been shown to have a stimulatory effect on insulin levels. Greater insulin response has been found with the combined ingestion of CHO with either PRO (40, 52) amino acids (45). However, plasma glucose uptake has been shown to be stimulated independently of insulin in the presence of the amino acids, leucine and isoleucine in animal models (14, 15, 33). Additionally, CHO/PRO supplementation in humans has demonstrated further stimulation of leg glucose uptake above a CHO only treatment (31). Levenhagen et al. (31), found subjects had 3.5 fold increase in leg glucose uptake when consuming a CHO plus PRO treatment immediatey post-exercise, in comparison to CHO only. Treatments were isocarbohydrate, thus the 3.5 fold increase in glucose uptake with CHO/PRO was likely attibuted to the PRO content of the treatment.

In the present study, plasma glucose levels were significantly lower during exercise as compared to CHO. However, plasma insulin levels were similar between

trials; therefore, the lower plasma glucose levels cannot be attributed to an increase in insulin availability. The combination of CHO and PRO could have increased glucose clearance from the blood at a greater rate than CHO alone, resulting in lower blood glucose levels and increased exogenous CHO availability to the working muscle. However, it is also possible that the lower plasma glucose values of the CHO+PRO treatment were associated with its lower CHO concentration. The CHO+PRO treatment delivered carbohydrate at a rate of 24.75 g CHO·h $^{-1}$ (0.413 g CHO·min^{-1}), in comparison to 49.5 g CHO·h^{-1} (0.83 g CHO·min^{-1}) in the CHO treatment.

The CHO+PRO supplement in the present study utilized a mixture of CHO sources. CHO+PRO was composed of equal amounts of dextrose, maltodextrin and fructose, as opposed to the single source of dextrose in the 6% CHO treatment. Oxidation rates up to 1.7 g·min^{-1} can occur when ingesting a combination of CHO at a high rate of 2.4 g·min^{-1} (25), in comparison a maximum rate of 1.0 – 1.1 g·min^{-1} when ingesting a single CHO source (27, 28, 46). The increased exogenous oxidation with the multiple CHO sources appears to be related to the use of different intestinal transporters. Glucose and its derivatives are absorbed into the small intestine via the sodium-dependent glucose cotransporter (SGLT1), while fructose absorption utilizes GLUT 5.

In comparison to a single CHO, mixed CHO supplements have previously demonstrated improved time trial performance (12, 43). After cycling for 120 minutes at 55% Wmax, Currell and Jeukendrup (12) found cyclists had an 8% time trial improvement while ingesting a glucose plus fructose mixture, in comparison to glucose only. Similar results were seen in a recent study comparing isocarbohydrate glucose and

glucose plus fructose during a 100-km cycling time trial (43). As in the study by Currell and Jeukendrup (12), the glucose + fructose mixture improved performance by 8% in comparison to glucose only. Previous investigations in our laboratory have additionally found improved efficacy when utilizing a mixture of carbohydrates, in combination with a moderate protein concentration (18). Therefore, it appears this is a likely mechanism contributing to the improved time to exhaustion we observed.

Heart rate during the CHO+PRO trial (130.17 ± 3.13 bpm) was slightly, but significantly lower, during the 3-hour variable intensity ride, as compared to CHO (132.80 ± 2.92 bpm, $p < 0.05$). However, this cannot be contributed to subjects working at a lower intensity during the CHO+PRO trial, as evidenced by a significantly higher treatment effect for VO_2 during the same exercise period (CHO: 1.755 ± 0.09 $L \cdot min^{-1}$ vs CHO+PRO: 1.779 ± $L \cdot min^{-1}$, $p < 0.05$). Potentially, this difference could be explained by a greater efficiency of the heart during the CHO+PRO trial, however, we are unable to conclude the exact mechanism behind this result. The higher VO_2 during exercise does give evidence that the improved time to exhaustion with CHO+PRO was not related to subjects riding at a lower intensity during the stages preceeding the time to exhaustion exercise bout.

During the present study, we measured plasma myoglobin levels to indirectly assess muscle damage. Previous studies have found CHO/PRO supplementation can decrease muscle damage response to exercise (35, 37-39) . This mechanism to enhance performance was first proposed by Saunders et al. (37), when a CHO/PRO supplementation enhanced time to exhaustion by 29% compared with a traditional 6%

supplement. In addition, levels of the muscle damage marker creatine phosphokinase (CPK) were found to be 83% lower 20-24 hours post-exercise following CHO/PRO supplementation. In a subsequent exhaustive exercise bout 12-15 hours later, performance was 40% greater in comparison to CHO only supplementation. In a later study, Saunders et al. (38) found subjects cycled 13% longer when consuming a CHO/PRO gel compared to CHO alone during a ride to exhaustion at 75% VO_2peak. Plasma CPK levels were significantly lower 12 - 15 hour post exercise in the CHO/PRO treatment in comparison to CHO. Despite these findings, reduced muscle damage with CHO/PRO supplementation has not always corresponded with improved performance. Romano-Ely et al. (35) found that in comparison to CHO alone, a CHO/PRO supplement attenuated the increase in two markers of muscle damage - CPK and LDH (lactate dehydrogenase) post-exercise. However, there were no performance differences during two exhaustive exercise bouts (35). In contrast to previous findings by Saunders et al. (37, 38) and Romano-Ely et al. (35), we found no difference in markers of muscle damage between treatments. However, measures were only taken during and immediately post exercise, with the last collection taken at the point of exhaustion. It is possible the times we selected to assess muscle damage via measurement of plasma myoglobin were inadequate.

Another mechanism by which CHO+PRO may have enhanced performance is related to aerobic energy production. It has been proposed that consuming a CHO/PRO supplement during exercise maintains Krebs cycle intermediates and aerobic energy production, and may enhance endurance performance (24, 47). Krebs cycle intermediates

increase at the onset of exercise and progressively decline as exercise continues (20, 36). A decrease in CHO availability has been proposed to further decrease Krebs cycle intermediates, possibly limiting the mitochondria's ability to maintain aerobic energy capacity (36). Maintaining Krebs cycle intermediates is critical in the maintenance of aerobic energy production (36, 47). The addition of PRO to CHO may further enhance CHO ability to maintain Krebs cycle intermediates during exercise (47). A study recently conducted by Cermak et al. (8) found no difference in the Krebs cycle intermediates citrate and malate while ingesting a 6% CHO plus 2% PRO or an isocarbohydrate CHO treatment. However, there was no measure of α-ketoglutarate, which is likely to be the rate limiting Krebs cycle intermediate during prolonged exercise bouts. Although not directly assessed in the present study, it is possible improved endurance performance with CHO+PRO could be attributed to its ability to maintain aerobic energy capacity.

Finally, it has been proposed that the ability of a CHO+PRO supplement to increase the efficacy above a traditional CHO supplement may be related to the central fatigue hypothesis (5, 24). Briefly, this hypothesis states that brain 5-hydroxytryptamine (5-HT, serotonin) regulates arousal, mood, motivation and fatigue in humans. Free tryptophan (Trp) is a precursor for serotonin production. Trp shares the same blood transporter as plasma free fatty acids, and numerous amino acids. An increase in blood free fatty acid production during exercise, in addition to an increased amino acid uptake into the muscle results in increased free Trp in the blood. This increases the free Trp that can move across the blood brain barrier, potentially increasing 5-HT production and feelings of fatigue (41, 48). Carbohydrate supplementation during exercise decreases

levels of plasma free fatty acids and free Trp (3). Supplementing with branched chain amino acids during exercise has been found to decrease mental fatigue (5), and improve endurance performance (4). Possibly, CHO+PRO may prevent a greater rise in serotonin production through decreased free Trp levels during exercise in comparison to CHO supplementation alone, therefore, allowing individuals to exercise longer without experiencing the sensation of exhaustion. However, the similar RPE between treatments in the present study likely indicates differences in performance cannot be attributed to central fatigue.

In summary, the addition of a moderate PRO concentration to a low concentration CHO mixture improved endurance performance in comparison to a traditional 6% CHO sports drink in trained female athletes. This improvement occurred despite CHO+PRO containing 50% less carbohydrate and approximately 30% fewer calories than the traditional 6% CHO supplement. It is likely the greater performance seen with CHO+PRO was a result of the combination of protein and the use of a mixture of carbohydrate sources.

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TABLE 1: Supplement Composition

	CHO	**CHO+PRO**
Kcals	24	16.9
% total CHO	6.0	3.0
% dextrose	6	1
% fructose	0	1
% maltodextrin	0	1
% PRO	0	1.2
Ratio of CHO:PRO	--	2.5:1
CHO g	6	3
PRO g	0	1.2

Per 100 ml. Both treatments contained the same amounts of electrolytes (Na^{+}, K^{+}, and Mg^{2+}).
275 ml was provided immediately before exercise and every 20 min thereafter.

TABLE 2: Subject Characteristics (n=14)

Measure	Mean
Age (years)	30.4 ± 1.6
Height (m)	1.67 ± 2.7
Body Mass (kg)	61.5 ± 2.2
VO_2max (L $O_2 \cdot min^{-1}$)	2.90 ± 0.15
VT (L $O_2 \cdot min^{-1}$)	2.23 ± 0.13

Data presented as mean ± SE

TABLE 3: Respiratory Exchange Ratio and Substrate Utilization

	10 min	50 min	130 min	135 min	184 min
	RER				
CHO+PRO	0.90 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.96 ± 0.01
CHO	0.89 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.97 ± 0.02
	CHO Utilization ($g \cdot min^{-1}$)				
CHO+PRO	1.16 ± 0.07	1.98 ± 0.14	1.23 ± 0.06	1.85 ± 0.13	2.51 ± 0.18
CHO	1.12 ± 0.07	2.01 ± 0.15	1.26 ± 0.07	1.86 ± 0.13	2.55 ± 0.20
	Fat Utilization ($g \cdot min^{-1}$)				
CHO+PRO	0.23 ± 0.03	0.25 ± 0.05	0.22 ± 0.03	0.29 ± 0.05	0.19 ± 0.05
CHO	0.24 ± 0.03	0.23 ± 0.04	0.20 ± 0.02	0.28 ± 0.04	0.16 ± 0.04

Data presented as mean ± SE.

TABLE 4: Heart Rate and Ratings of Perceived Exertion

	Low Intensity				High Intensity			
	25 min	90 min	130 min	161 min	50 min	115 min	159 min	184 min
	Heart Rate (bpm)							
CHO+PRO	111.79 ± 2.60	117.79 ± 2.89	123.29 ± 2.65	127.21 ± 3.62	138.79 ± 2.24	139.57 ± 3.57	138.79 ± 3.14	151.00 ± 3.48
CHO	113.29 ± 2.65	121.64 ± 2.55	124.00 ± 3.26	128.93 ± 3.20	141.43 ± 2.46	144.86 ± 3.28	143.50 ± 3.14	151.00 ± 3.48
	Ratings of Perceived Exertion							
CHO+PRO	9.36 ± 0.44	11.57 ± 0.27	11.93 ± 0.32	12.00 ± 0.41	12.57 ± 0.25	13.50 ± 0.23	13.36 ± 0.44	15.04 ± 0.32
CHO	8.82 ± 0.42	11.93 ± 0.50	11.89 ± 0.29	12.07 ± 0.34	11.93 ± 0.50	13.46 ± 0.31	14.04 ± 0.40	15.21 ± 0.38

Data presented as Mean ± SE.

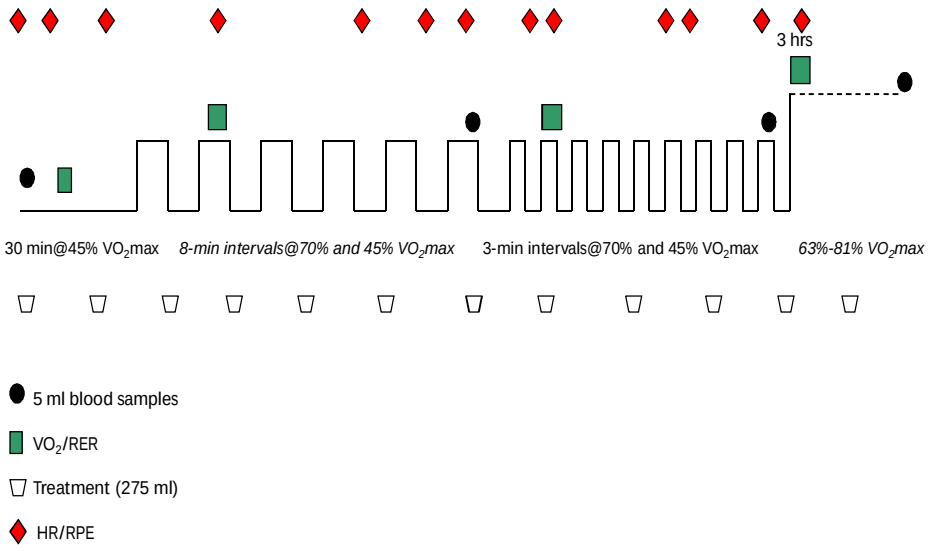

FIGURE 1: Experimental protocol. Three-hour variable intensity ride between 45-70%, followed by ride to exhaustion. Blood samples, VO_2, RER, Heart Rate and RPE measured where noted.

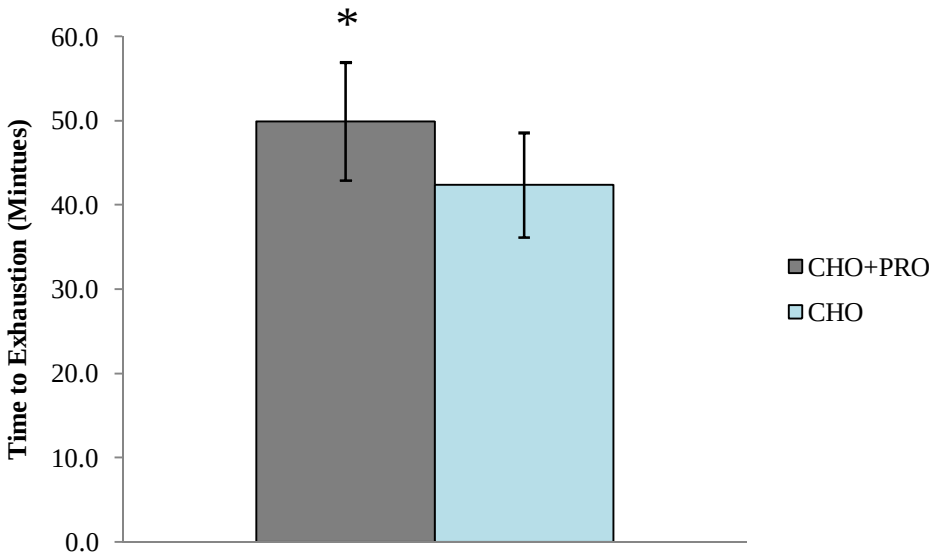

FIGURE 2: Time to Exhaustion (TTE). Exhaustion measured as the point at which subject could no longer hold cycling cadence above 60 RPM. TTE was 49.94 ± 7.01 min for CHO+PRO and 42.36 ± 6.21 min for CHO. *Significantly different from CHO ($p < .05$). Values are mean ± SE

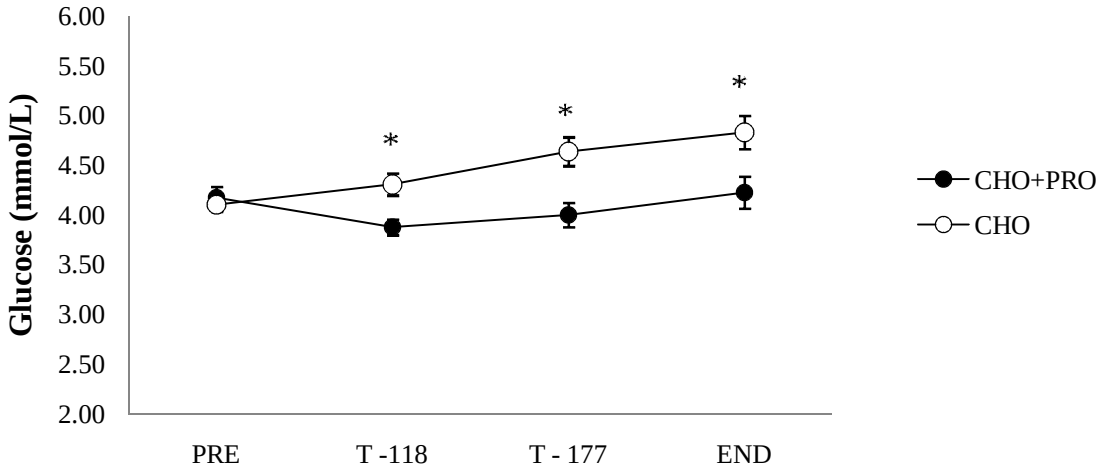

FIGURE 3: Plasma glucose was measured from samples taken pre exercise (PRE), exercise time points 118-min and 177-min, and at exhaustion (END). Significant treatment differences with CHO in comparison to CHO+PRO (CHO: 4.47 ± 0.12 mmol·L^{-1}; CHO+PRO: 4.07 ± 0.12 mmol·L^{-1} $p < 0.05$). Additionally, treatment by time difference occurred at 118-min and 177-min, and END ($p < .05$). *Significant difference between treatments ($p < .05$). Values are mean ± SE

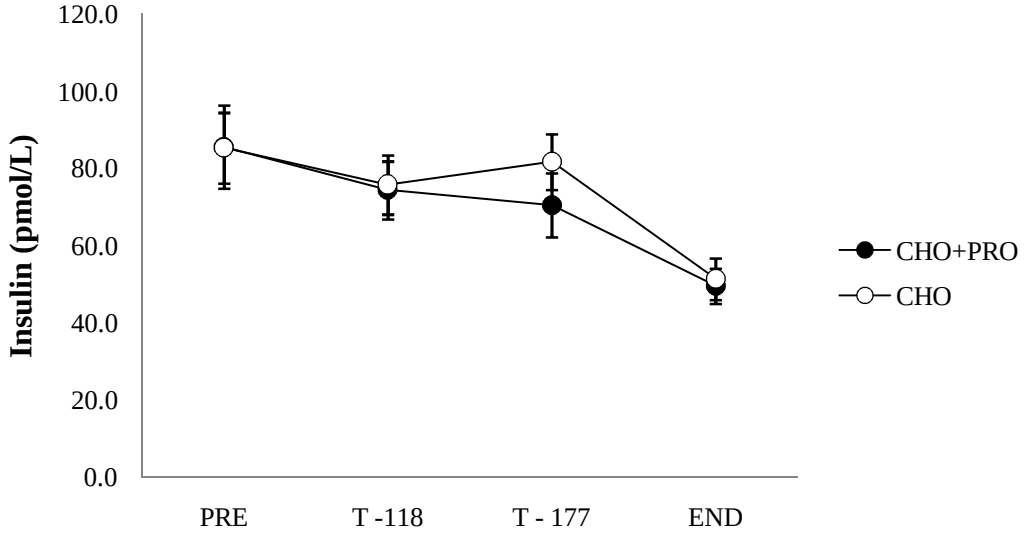

FIGURE 4: Plasma insulin levels at pre-exercise (PRE), 118-min and 177-min of exercise, and at exhaustion (END). Average plasma insulin was 73.56 ± 7.37 pmol·L^{-1} during the CHO trial and 70.00 ± 7.78 pmol·L^{-1} during the CHO+PRO trial. No overall significant treatment differences or treatment by time interactions. Values are mean ± SE

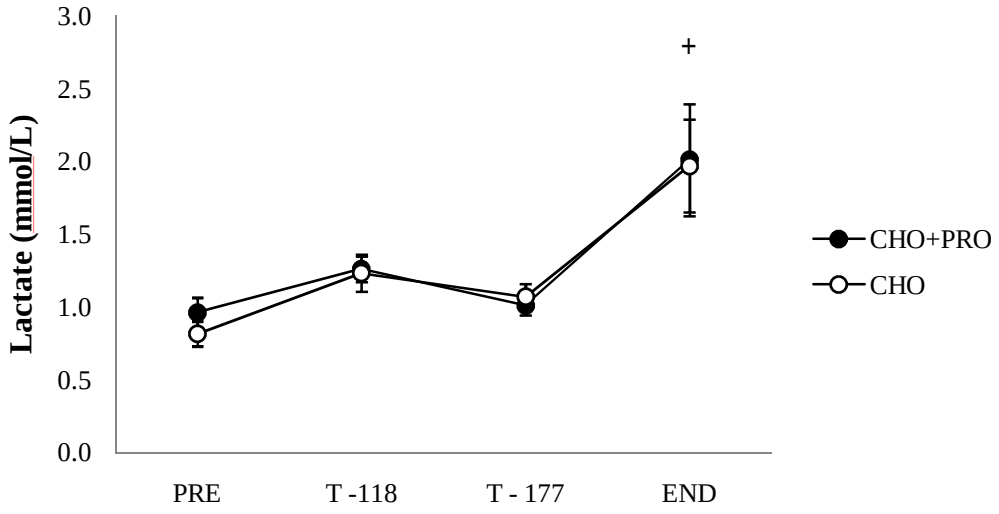

FIGURE 5: Blood lactate concentrations at pre exercise (PRE), 118-min and 177-min of exercise, and at exhaustion (END). No significant differences among treatments or treatment by time interactions. In both treatments, blood lactate concentrations significantly increased from 177-min to exhaustion ($p < .05$) $^{+}$ Significantly different from PRE to END. Values are mean ± SE

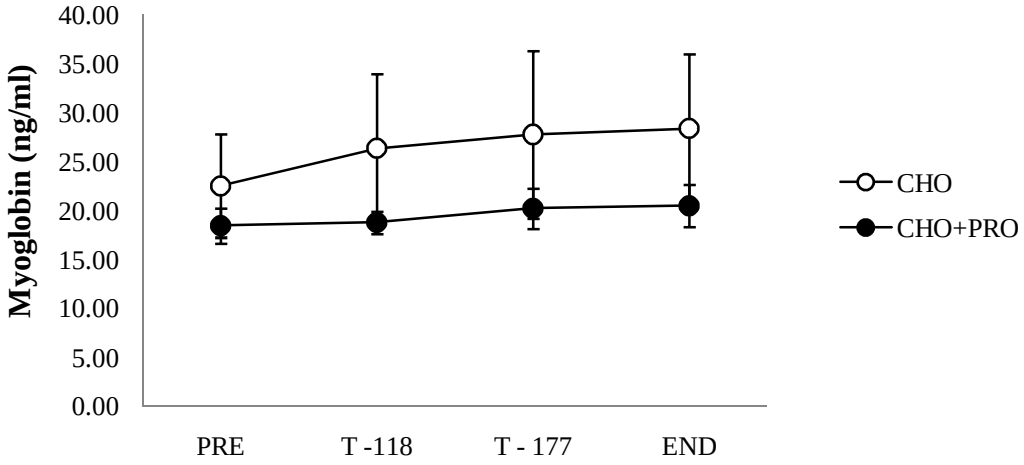

FIGURE 6: Plasma myoglobin at pre exercise (PRE), 118-min and 177-min of exercise, and at exhaustion (END). The average plasma myoglobin concentration was 26.28 ± 7.28 $ng \cdot ml^{-1}$ during the CHO trial and 19.64 ± 1.79 $ng \cdot ml^{-1}$ during the CHO + PRO trial. No significant overall differences in treament or the treatment by time interaction. Values are mean ± SE

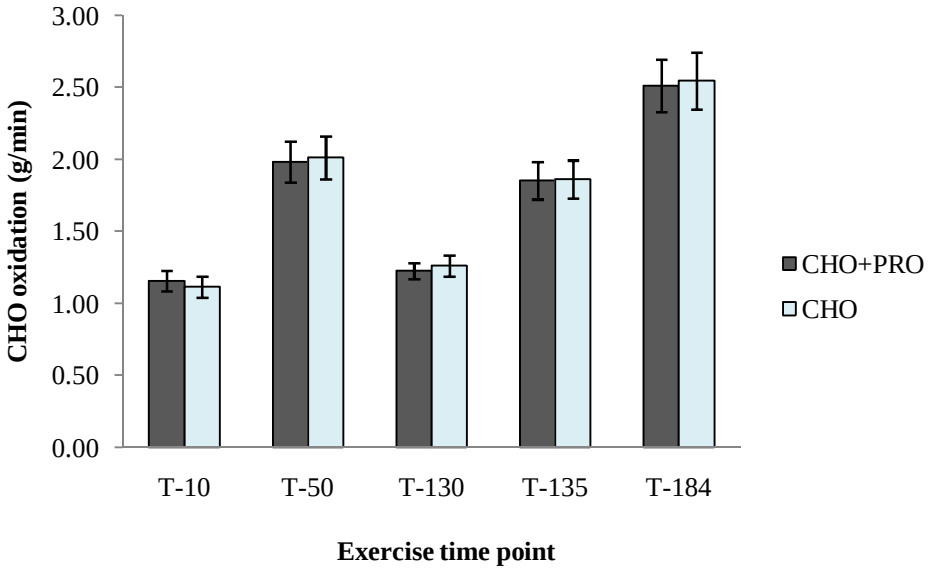

FIGURE 7: Carbohydrate utilization during exercise. Time points correlate to low intervals (10 min and 130 min), high intervals (50 min and 135 min), and at the beginning of the performance ride (184 min). No significant differences between treatments or treatment by time interactions. Values are mean ± SE.

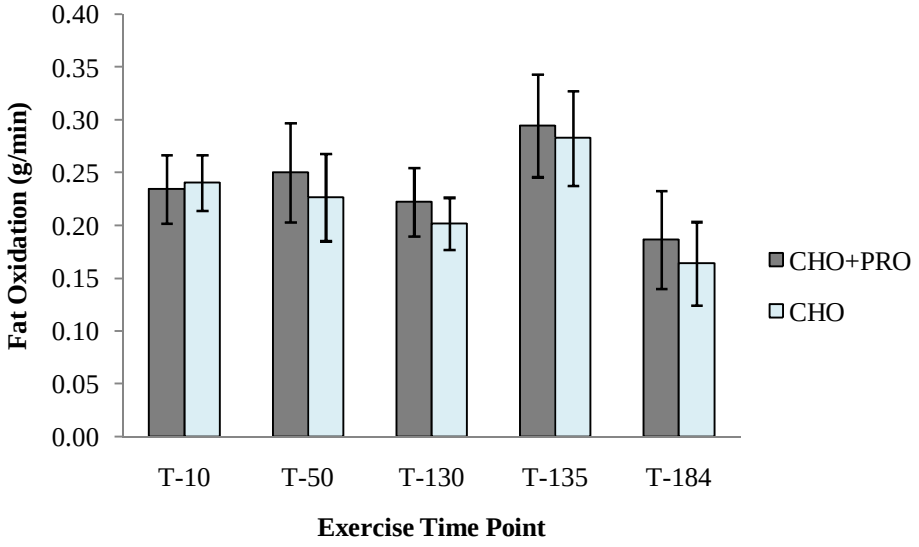

FIGURE 8: Fat utilization during exercise. Time points correlate to low intervals (10 min and 130 min), high intervals (50 min and 135 min), and at the beginning of the performance ride (184 min). No significant differences between treatments or treatment by time interactions. Values are mean ± SE.

LITERATURE REVIEW

Introduction

Participation in endurance sports such as marathons and triathlons has experienced an enormous increase in popularity over the past decade. This participation rise has resulted in increased awareness of the importance of nutritional supplementation and the demand for nutritional products among both professional and recreational athletes. A large body of research during the past decade has focused on the optimization of aerobic endurance performance through nutritional supplementation to fuel this growing market.

Exhaustion from exercise is ultimately a result of the body's inability to provide sufficient fuel to the working muscle. Depletion of liver and muscle glycogen, in combination with low blood glucose levels, are major factors related to the onset of exhaustion. The importance of carbohydrate as a fuel source during exercise became apparent in the 1960s, when investigators observed the importance of muscle glycogen in delaying fatigue. Investigators proposed that an increase in exercise intensity paralleled decreases in muscle glycogen stores, and that the capacity to perform prolonged exercise bouts was dependent on initial muscle glycogen stores (7). Since then, numeorus studies have led to the consensus that nutritional supplementation during exercise, in particular carbohydrate supplementation, will delay the onset of exhaustion by maintaining glucose levels.

Recently, the addition of amino acids (AA) or protein (PRO) to carbohydrate (CHO/PRO) has been proposed to increase the ergogenic effect of a traditional

carbohydrate treatment (CHO). Several investigators have found supplemention with CHO/PRO during exercise, improves endurance performance in comparison to CHO-only supplementation (14, 15, 50, 76-78).

This literature review aims to investigate the performance effect of nutritional supplementation and related physiological mechanisms during exercise. First, CHO supplementation and its impact on endurance performance will be reviewed, followed by a discussion of the possible mechanisms involved. The focus of the review will then shift to the effect of CHO/PRO supplementation on endurance performance, and possible physiological mechanisms by which CHO/PRO exerts its ergogenic effect. Finally, I will directly compare supplementing with CHO or CHO/PRO during endurance exercise. For the purpose of this review, prolonged endurance exercise will be defined as continuous exercise lasting more than 2 hours. Low-intensity exercise will be defined as exercise at or below 45% VO_2max; moderate intensity exercise will be defined between 45% and 75% VO_2max. Exercise intensity above 75% VO_2max will be classified as high intensity.

Carbohydrate Supplementation During Endurance Exercise

CHO Supplementation and Endurance Performance.

Supplementing with CHO during endurance exercise has demonstrated improved endurance performance, as compared to placebo or water (20, 22, 25, 26, 49, 92-94). Although the general agreement in the literature is that CHO supplementation has an ergogenic effect during prolonged aerobic exercise, benefits do not appear to be limited to a unique endurance exercise protocol. Improved performance has been demonstrated

during prolonged exercise bouts of similar intensity (22, 25, 49, 92), variable intensity (50, 61, 94), intermittent exercise (41, 45, 82), and during team sports such as soccer (3, 27).

In an early study investigation, Coyle et al. (25) found cyclists rode for one hour longer (4.02 h v 3.02 h) when fed a 6% glucose polymer during prolonged steady state moderately-high intensity exercise (71% VO_2max), in comparison to a placebo. During the additional hour of exercise with the CHO treatment, CHO oxidation rates were maintained, yet there was little reliance on muscle glycogen stores. This led the authors to suggest the postponement of fatigue resulted from the ability of CHO to maintain blood glucose levels in a euglycemic state, combined with the ability of the body to utilize exogenous CHO sources late in exercise.

Yaspelkis et al. (94) later investigated the effect of CHO supplementation during prolonged variable intensity exercise. Trained cyclists rode for 3 hours at intensities varying between 45% and 75% VO_2max, followed by a ride at 80% VO_2max until exhaustion. Subjects received either placebo, a 10% liquid CHO supplement (L: 3 x 18 g CHO·h^{-1}) or a 10% solid CHO supplement (S: 2 x 25 g CHO·h^{-1}). There were no differences between S and L for any variables measured. Time to exhaustion was significantly longer in both CHO treatments (233.4 min) as compared to placebo (204.4 min), with a positive correlation between muscle glycogen levels after 190-mins of exercise and time to exhaustion. These findings led investigators to conclude that CHO supplementation during variable-intensity exercise spares muscle glycogen stores, potentially delaying the onset of exhaustion.

In addition to prolonged steady state and variable intensity exercise, CHO supplementation appears benefical during intermittent exercise bouts (41, 45, 82). Hargreaves et al. (45) had subjects perform 4-hours of intermittent exercise, during which they consumed either a placebo or CHO (sucrose; 43 $g \cdot h^{-1}$) at one hour intervals. The intermittent exercise protocol involved repeated 30-min cycling bouts. Each 30-min bout consisted of 20-min at 50% VO_2max, followed by 10-min of 30-second bouts at 100% VO_2max with 2-min rest after each sprint. Muscle glycogen utilization was significantly lower during the CHO trial, and authors concluded the sparing of glycogen stores as the reason for a 45% improvement in a sprint to exhaustion (100% VO_2max) at the trial conclusion, as compared to the placebo trial. Furthermore, glucose supplementation during 90-min of intermittent, high-intensity cycling bouts was found to increase power output and sprint performances (82). The above results give evidence that CHO supplementation is benefical in a wide variety of endurance exercise activities, including prolonged steady-state, variable intensity and intermittent exercise bouts

Although a large proportion of the literature indicates a strong ergogenic effect of CHO supplementation, several studies have found no additional performance benefits of CHO supplementation above that of placebo and/or water (42, 54). Flynn et al. (42) investigated the effect of varying CHO mixtures during a 2-hour exercise bout. Subjects consumed one of four treatments (150 ml) every 20-min; placebo, maltodextrin plus fructose (45 $g \cdot h^{-1}$), maltodextrin plus high fructose corn syrup (45 $g \cdot h^{-1}$) or maltodextrin plus glucose (22.5 $g \cdot h^{-1}$). Despite riding for 120-min at a self-selected pace, there were no significant differences in total work output or muscle glycogen utilization between the

four trials. The lack of additional performance benefit of the CHO supplements compared to placebo may be related to a pre-trial high CHO diet elevating initial glycogen stores to an average of 185.35 $mmol \cdot kg^{-1}$ wet weight. Even after 120-min of riding, the average glycogen values across the four treatments was 91.93 $mmol \cdot kg^{-1}$ wet weight. The exercise intensity selected by the individuals and/or the duration of exercise did not appear sufficient to significantly deplete liver and muscle glycogen. Furthermore, the amount of CHO supplied was at the low end of the 30 – 60 g $CHO \cdot h^{-1}$ recommended for optimal performance during prolonged exercise (17, 24). As a result, the availability of exogenous CHO may have been insufficient to elicit a significant improvement in performance with the CHO trials in comparison to placebo. Recently, Jeukendrup et al. (54) found no improvement in performance time or power output compared to placebo when ingesting a 6% CHO supplement during a 16 km cycling time trial. The short duration and high intensity of the 16 km time trial is likely related to the lack of improvement in performance with the CHO supplement. The effect of exercise duration and intensity will be discussed in a later section of this review.

It must be noted that CHO is often supplemented in combination with water, thus fluid consumption alone may be responsible for an ergogenic effect independent of CHO. Indeed, fluid replacement during exercise has previously demonstrated improved endurance performance (6, 62). Maughan et al. (62) demonstrated slight improvement in endurance performance when subjects ingested water (100 ml at 10-min intervals) during an exhaustive ride at 70% VO_2max in comparison to no fluid replacement. However, when given the same volume of a glucose-electrolyte beverage, subjects rode

significantly longer in comparison to both water and no fluid treatments. In a study conducted by Below et al. (6), cyclists completed four, 50-minute high-intensity (80% VO_2max) cycling bouts, followed immediately by a performance ride. On each occasion, subjects ingested either a small or a large volume of water-only or water with added maltodextrin during exercise. Performance was improved 6.5% with large fluid consumption (~1330 ml) in comparison to small fluid consumption (~200ml), and a further 6.3% when maltodextrin was added to the large fluid volume. Authors concluded that fluid and CHO independently improved cycling performance, and together this improvement was additive. Although fluid replacement alone may elicit improved performance, particularly during prolonged exercise in a hot environment, the macronutrients provided by CHO increases the availability of an additional energy source for working muscles. Therefore, CHO can be considered an ergogenic aid independent of fluid replacement.

Mechanisms For Improved Performance With CHO Supplementation

Numerous mechanisms have been proposed by which CHO supplementation delays exhaustion. Muscle and liver glycogen depletion during prolonged exercise, in conjunction with hypoglycemia, are the major factors in the individual's inability to continue exercise at the same intensity (26). Previous research has indicated that improved performance may be related to a maintenance of blood glucose levels during exercise (20, 25, 49, 51, 63, 94), a sparing of glycogen stores (45, 80, 93, 94) and/or increased CHO oxidation rates (23, 25, 49, 51). Majority of these mechanisms interact

with one another, thus overlapping between mechanisms is likely. For example, increased availability of blood glucose contributes to increased CHO oxidation rates and a decreased reliance on muscle glycogen stores. The mechanisms will be discussed in the following paragraphs.

CHO Supplementation and Plasma Glucose Levels

One frequent mechanism in the literature by which CHO supplementation improves performance is its potential to maintain blood glucose levels in a euglycemic state (20, 25, 49, 51, 63, 94). There is an increased reliance on blood glucose for ATP production as exercise duration continues, and it can account for up to 100% of carbohydrate metabolism during the later stages of exercise (2, 25). Without exogenous supplementation, blood glucose levels will remain above 3 mmol for only 30 - 40 mins after the depletion of glycogen stores (25). This is important, as at low to moderate intensities, plasma glucose uptake provides sufficient fuel to continue exercising, even in a glycogen depleted state (20-22, 25). Therefore, delivery of an exogenous CHO source increases plasma glucose concentrations, thus increasing the availabilty of glucose to be taken up by the working muscle and allows for the continuation of exercise (20, 25, 49, 51, 63, 94). Coyle et al. (25) found a 20 – 40% increase in blood glucose concentrations while ingesting a 6% CHO, resulting in subjects riding for an additional hour at moderately high intensity, as compared to placebo.

While blood glucose is a critical component related to the onset of exhaustion, particularly during low to moderate intensities, it does not appear to be the sole factor.

Ivy et al. (49) found individuals walked 11.5% longer when consuming a glucose polymer at a rate of 24 - 29 $g \cdot h^{-1}$ during prolonged low intensity walking as compared to a placebo (GP: 299.0 ± 9.8 min; Placebo: 268.3 ± 11.8 min). Glucose ingestion increased plasma glucose concentrations during exercise, and individuals remained in a euglycemic state for the remainder of the protocol, even at the point of exhaustion (4.59 mM), demonstrating that hypoglycemia alone does not cause exhaustion at low intensities. Investigators concluded that the maintenance of higher CHO oxidation rates with GP (0.53 $g \cdot min^{-1}$ higher in comparison to control) was a contributing factor to the postponement of exhaustion.

CHO Supplementation and CHO Oxidation

As noted above, a second mechansim by which CHO supplementation is suggested to delay exhaustion is via the maintenance of high CHO oxidation rates (23, 49, 51)**.** CHO oxidation provides 60 – 70% of total energy requirements during moderate intensity exercise (25) and CHO oxidation rates increase with exercise intensity (23, 73). Thus, supplying CHO exogenously increases the availability of CHO to meet the oxidation requirements of exercise. Coggan and Coyle (20) found that when subjects were fed CHO every 30-mins (1.2 $g \cdot kg^{-1}$ h^{-1}) during moderate-high exercise (60 – 85% VO_2max), plasma glucose concentration increased to approximately 6mM, and CHO oxidation rates were as high as 2 $g \cdot min^{-1}$ during the later stages of exercise. As a result, subjects completed 19% more work in a time-to-exhaustion bout, as compared to placebo. The investigators concluded that delayed exhaustion with the CHO treatment

could be contributed to both euglycemic blood glucose levels, and a higher rate of CHO oxidation.

CHO Supplementation and the Sparing of Glycogen Stores

The sparing of muscle glycogen stores has been proposed as a potential mechanism by which CHO supplementation enhances endurance performance (45, 80, 93, 94). As mentioned earlier, Hargreaves et al. (45) found reduced muscle glycogen utilization during prolonged intermittent exercise when supplementing with CHO. Likewise, Stellingwerff et al. (80) found reduced rates of muscle glycogen utilization with the ingestion a of glucose supplement (0.7 $g \cdot kg^{-1}$ h^{-1}) during a 3-hour ride at 63% VO_2max. In comparison to a control treament, CHO lowered muscle glycogen use by 38% in type I fibers and 57% in type II fibers during the first hour of cycling. Using a slightly higher intensity protocol, De Bock et al. (33) found a sparing of glycogen while consuming CHO before (150 g) and during (1 $g \cdot kg\ bw^{-1} \cdot h^{-1}$), a 2-hour steady state ride at 75% VO_2max.

During a low-moderate intensity exercise bout (48.8% VO_2max), Yaspelkis et al. (93) compared the effectiveness of two different glucose polymers (2.0% and 8.5%) to water. The 8.5% CHO treatment increased plasma glucose and insulin levels, maintained CHO oxidation rates and resulted in a reduction in the rate of muscle glycogen utilization, as compared to water (206.5 ± 23.6 mu $mol \cdot g^{-1}$ protein and 342.3 ± 41.9 mu $mol \cdot g^{-1}$ protein, respectively). This suggests supplementing with CHO during low

intensity exercise is a potential mechanism to improve endurance performance, via the more efficient utilization of glycogen stores.

During rest or low intensity exercise, plasma glucose and insulin concentrations are elevated (1, 49, 93, 94). Both of these factors are major stimulators of muscle glucose uptake, contributing to reduced muscle glycogenolysis. Also, low intensity exercise decreases muscle fiber recruitment, and it has been previously suggested that muscle glycogen resynthesis can occur during low intensity exercise, as long as the fibers are nonactive, glycogen depleted and exogenous CHO is supplemented (56, 94). This has lead to the proposal that intermittent and variable intensity exercise facilitates glycogen resynthesis during an exercise bout (45, 93, 94). Yaspelkis and his team (94) investigated the impact of a 10% liquid CHO supplementation (3 x 18 $g \cdot h^{-1}$) on glycogen sparing and endurance performance during variable intensity exercise. Plasma insulin concentrations were significantly increased with CHO supplementation (70.6 ± 17.2 $\mu U \cdot ml^{-1}$) as compared to placebo (17.7 ± 1.6 $\mu U \cdot ml^{-1}$). Investigators found a positive correlation ($r = 0.76$, $p < .05$) between the CHO supplement and placebo in regards to muscle glycogen utilization and time to exhaustion after 190-mins of exercise. Given these results, it is possible that glycogen resynthesis during low intensity or rest periods will increase glycogen availability as a fuel source during the energy demanding, higher intensity intervals.

In addition to muscle glycogen sparing, some have proposed that CHO supplementation may spare liver glycogen stores (20, 53). Hepatic glucose output is highly regulated, and the increased availability of exogenous CHO is believe to decrease

the reliance on liver glycogen, as long as the exogenous glucose is sufficient to meet the demands of the muscle.

In summary, it is apparent that CHO supplementation has the capacity to increase the effectiveness of glycogen utilization. Whether CHO supplementation spares muscle glycogen during exercise via maintaining blood glucose concentrations and CHO oxidation rates, by decreasing liver glycogen utilization, or potentially resynthesizing muscle glycogen stores, still remains somewhat unclear in the literature. Further investigations are required to further confirm these theories.

Additional Factors Influencing CHO Supplementation and Performance

In addition to the factors mentioned above, the capacity to achieve optimal performance through nutritional supplementation is associated with a number of variables, such as exercise intensity and the amount of CHO supplied. Also, the use of either a single or a multiple source of CHO and the form of supplementation (solid v liquid); contribute to the effectiveness of a CHO treatment. Each of these factors will be discussed below.

CHO Supplementation and Exercise Intensity

The effectiveness of CHO supplementation to improve performance appears associated with the intensity of the exercise protocol. During low to moderate exercise intensities, CHO supplementation can maintain or increase blood glucose and insulin

concentrations, potentially decreasing glycogen utilization and thereby, prolonging time-to-exhaustion.

In an early study, Ahlborg et al. (1) investigated the physiological impact of prolonged low intensity exercise and CHO supplementation. Subjects rode for 4-hours at 30% VO_2max, and at 90-min into the exercise protocol, subjects consumed either 200 g glucose or a placebo. Post supplementation, there was a 35% increase in plasma glucose, 60 - 70% decrease arterial free fatty acids and glycerol, and a two-three fold increase in arterial insulin. Muscle glucose uptake increased by 60% and additionally, liver uptake of gluconeogenic precursors (lactate, pyruvate, glycerol) decreased by 70 - 100%. Given these results, CHO supplementation at low exercise appears to increase exogenous CHO utilization and decrease the reliance on endogenous energy mechanisms, such as glycogen breakdown, gluconeogensis and lipid oxidation. This could potentially increase glycogen availability for utilization in the later stages of prolonged exercise bouts, or during moderate to high intensity intervals where glycogen is the major energy source utilized (50, 94). In addition, Ivy et al. (49) found glucose ingestion (~30 $g \cdot h^{-1}$) maintained plasma glucose in a euglycemic state during prolonged low intensity walking.

During moderate intensity exercise, CHO supplementation appears to decrease the reliance on muscle glycogen stores (26, 33, 50, 80, 94). Coyle et al. (26) concluded that ability to perform the additional hour of exercise at a moderate to high intensity was a result of the utilization of fuel sources other than muscle glycogen.

CHO supplementation during high intensity exercise appears to be less effective when compared to low and moderate intensities. The ability to exercise at high exercise

intensities is highly dependent on glycogen stores, as the rate of blood glucose uptake is too slow to meet exercise demands (20). As a result, glycogen stores are rapidly decreased, and exogenous CHO supplementation may provide no further performance benefits above that of endogenous CHO sources. As a result, once glycogen stores have been depleted, intensity must be decreased in order to continue exercising.

CHO Supplementation Rates

When ingesting a single source of CHO (such as glucose, maltodextrin or sucrose), the maximum rate of exogenous CHO oxidation is approximately 1.0 – 1.1 $g \cdot min^{-1}$, or 60 - 70 $g \cdot h^{-1}$ (52, 53, 88). The ingestion of more than 75 $g \cdot h^{-1}$ does not appear to further increase performance (53). Consuming CHO at a rate of 30 – 60 $g \cdot h^{-1}$ is generally recommended to improve performance (45, 49), however, improvement in performance has been demonstrated while ingesting lower concentrations of CHO (41, 62). Fielding et al. (41) found ingesting 10.75 g sucrose every 30-minutes during a 4-hour intermittent, moderate intensity ride (21.5 $g \cdot h^{-1}$) improved sprint time to exhaustion after 4-hours, in comparison to placebo.

Supplementing With a Mixture of CHO Sources

Varying forms of CHO given independently, including glucose, sucrose or maltodextrin, do not appear to elicit different effects on performance (25). One exception is possibly fructose, which is reported to have lower oxidation rates, possibly related to slower intestinal absorption and its conversion to glucose in the liver before it can be

fully metabolised. However, increased CHO oxidation is evident with a mixture of CHO sources as compared to a single source. As stated previously, the maximal rate of CHO utilization during prolonged exercise while providing a single CHO source is approximately 1.0 - 1.1 $g \cdot min^{-1}$ (53, 88). Consumption of multiple types of CHO appears to increase CHO oxidation rates above that achieved by a single CHO source. Oxidation rates up to 1.7 $g \cdot min^{-1}$ have been observed when ingesting a combination of CHO at a high rate of 2.4 $g \cdot min^{-1}$ (51). The increased exogenous oxidation appears related to different intestinal transporters. Glucose and maltodextrin are both absorbed into the small intestine via the sodium-dependent glucose cotransporter (SGLT1) and saturation of transporters is believed to occur at ingestion rates greater than 1.2 $g \cdot min^{-1}$ (52). However, fructose transport into the intestine occurs via GLUT5 transporters (40). Sucrose is hydrolysed into fructose and glucose at the intestinal brush border (30). Due to the capacity to utilize multiple transporters at one time, ingestion of mutiple carbohydrates is proposed to increase the rate of intestinal absorption, resulting in greater exogenous CHO oxidation (52). Wallis et al. (89) found ingesting maltodextrin plus fructose at a high rate (1.8 $g \cdot min^{-1}$), resulted in exogenous oxidation rates of ~1.5 $g \cdot min^{-1}$, which was 40% higher than maltodextrin alone (89).

Jentjens et al. (51) further illustrated the ability of a combination of CHO sources to increase exogenous CHO oxidation rates. Cyclists rode for 150-min at 62% VO_2max while ingesting glucose, or an isocaloric treatment containing a mixture of glucose, fructose and sucrose, at a high rate of 2.4 $g \cdot min^{-1}$. Increased exogenous CHO oxidation rates were found with the CHO mixture in comparison to isocaloric glucose (1.7 $g \cdot min^{-1}$ v

1.18 g·min^{-1} respectively). In addition, the mixture of CHO lowered endogenous CHO oxidation rates (0.76 ± 0.12 g·min^{-1}) as compared to glucose (1.05 ± 0.06 g·min^{-1}). Higher exogenous CHO oxidation rates likely reduce the reliance on liver and muscle glycogen during exercise, potentially delaying glycogen depletion and hypoglycemia. This can delay the onset of exhaustion, as demonstrated by the team of Currell and Jeukendrup (28). Following 120-mins of cycling at 55% Wmax, Currell and Jeukendrup found cyclists had an 8% improvement during a performance time trial while ingesting an isocaloric glucose plus fructose mixture (2:1 ratio), in comparison to glucose only. Both treatments were delivered at a high rate of 1.8 g·min^{-1}. Total CHO oxidation rates were similar between trials. However, investigators hypothesized a possible increase in exogenous CHO oxidation and a resulting decrease in endogenous oxidation rates, given that previous findings have found increased exogenous CHO oxidation when consuming a CHO mixture (51). In conclusion, an increase in performance with a mixed CHO supplement is associated with a decreased utilization of endogenous carbohydrate stores during exercise, due to an increased availability and oxidation of exogenous carbohydrate.

CHO Supplementation Form (solid vs liquid)

Although nutritional supplementation during exercise is traditionally given in a liquid form, the ergogenic capabilities of exogenous CHO does not appear to be affected by the supplementation delivery method (6, 45, 59, 66, 94). Yaspelkis et al. (94) had trained cyclists supplement during a 3 hour continuous variable intensity ride with either

a placebo, 10% liquid CHO (3 x 18 g CHO·hr^{-1}) or a 10% solid CHO supplement (2 x 25 g CHO·hr^{-1}). Both CHO supplements enhanced performance above that of placebo, however, there were no significant differences between the liquid and solid CHO supplement. Similarily, Lugo et al. (59) found no time difference during a performance time trial, nor any differences in blood parameters when consuming a liquid 7% CHO drink or a solid sports bar.

Despite the above articles illustrating no difference between fluid and solid supplementation, as mentioned earlier, fluid replacement and CHO appear to have an additive effect (6). In addition, fluid replacement is crucial during exercise in hot and stressful conditions to replace fluids and electrolytes lost in sweat. Thus, exercise conditions become an important consideration when choosing an appropriate supplementation method.

Gender and Endurance Performance

Potentially, the capacity of individuals to perform prolonged endurance bouts maybe related to gender differences, specifically in regards to substrate utilization, and the effect of menstrual cycle on endurance performance. Previous studies have cited gender differences regarding to the contribution of fat, carbohydrate and protein to total body energy requirements during exercise (35, 43, 48, 57, 64, 69, 83, 84). When matched for training status, VO_2max and fat free mass, women appear to oxidize proportionally more fat in comparison to men during aerobic endurance exercise (43, 69, 83). Tarnopolosky et al. (83) found that during a 15.5 km run, female athletes had

increased lipid and lower CHO and PRO utilization in comparison to equally trained males. However, these findings are not universal (71, 73). Once corrected for lean body mass, Romijn et al. (73), found no differences in carbohydrate or fat oxidation in men and women during moderate to high intensity exercise.

Similarly, no gender differences in respiratory exchange ratio (RER) or leg respiratory quotient (RQ) results were seen by Roepstorff et al. (71). Male and female subjects performaned 90-min of cycling at 58% VO_2peak, and were matched for lean body mass and training status. In addition, all subjects followed a standarized diet for the 8 days preceding the trial. This raises the possibility that many of the gender differences seen in previous studies may be related to factors outside of the exercise protocol, such as dietary intake.

In addition to substrate utilization, the lack of studies in the literature utilizing female subjects is likely related to concerns of the influence of the menstrual cycle on performance. Despite studies showing performance differences (16), the general consensus in the literature is that there is no effect of menstrual cycle phase or oral contraceptive use on either endurance exercise performance or VO_2max (4, 13, 34, 47, 60, 70). Additionally, it has been proposed that nutritional supplementation during exercise may possibly reduce any substrate utilization differences across the menstrual cycle. Bailey et al. (4) found no difference in time-to-exhaustion between the follicular or luteal phases when supplementing with CHO. Campbell et al. (16) found a 13% improvement in a time-trial performance during the follicular phase in comparison to the luteal phase in fasted conditions. However, CHO supplementation eliminated differences

between mentrual cycle phases on time trial performance. These results indicate that any potential performance differences across the menstrual cycle maybe diminshed with CHO supplementation.

In addition to supplementing with a CHO-only treatment, there are few studies in the literature investigating the effect of CHO/PRO with females. Saunders et al. (77) were the first to investigate gender differences in performance during prolonged endurance exercise while consuming either CHO or CHO/PRO. No significant differences were found between genders in regards to time to exhaustion, blood glucose, creatine kinase, perceived exertion, heart rate, VO_2 or RER. A current lack of research utilizing female subjects warrants further investigations to fully understand the ergogenic effect of both CHO and CHO/PRO supplementation in females during exercise.

Summary of CHO Supplementation

There is no question of the capacity of CHO supplementation to enhance performance during prolonged, moderate intensity endurance exercise. This capacity can be attributed to a variety of mechanisms, namely its ability to increase exogenous CHO oxidation, the maintenance of blood glucose levels in an euglycemic state and decreasing the reliance on liver and muscle glycogen stores. Supplementing with a combination of carbohydrates appears to be more beneficial in comparison to a single source of CHO, and this should be taken into consideration when choosing a sports drink to optimize performance and subsequent recovery.

Carbohydrate Plus Protein Supplementation During Endurance Exercise

While the ergogenic effects of CHO supplementation during prolonged endurance exercise have been studied extensively, only recently has it been proposed that the addition of PRO to CHO may further enhance the ergogenic benefit of a CHO supplement. Thus, the following sections will discuss the potential benefits of adding PRO or AA to a CHO supplement. This section will begin with a review of the studies investigating the impact on endurance performance when PRO or AA is added to CHO. Then, it will examine possible mechanisms by which CHO/PRO or AA may improve performance above that of a CHO-only treatment.

CHO plus PRO or AA and Endurance Performance

Several studies have demonstrated that the addition of PRO or AA to CHO, will improve endurance performance as compared to CHO alone (14, 15, 38, 50, 76-78). However, several studies have found contradicting results (61, 72, 85).

Our laboratory was the first to demonstrate that CHO/PRO can improve time to exhaustion above that of CHO (50). Subjects cycled for three hours at intensities varying between 45% and 75% VO_2max, followed by a performance ride to exhaustion at 85% VO_2max. Every 20 minutes throughout the exercise bout, subjects received 200 ml of either a placebo, a 7.75% CHO, or a 7.75% CHO + 1.94% PRO beverage. CHO increased time to exhaustion above placebo (19.7 min v 12.7 min, respectively), while CHO/PRO further improved performance (26.9 min). Investigators were not able to conclude the exact mechanism behind the increased performance with CHO/PRO as

compared to CHO, however hypothesized it may be related to maintenance of Krebs cycle intermediates, the central nervous fatigue hypothesis and/or sparing of glycogen stores. Despite the addition of approximately 2% PRO resulting in a 36% improvement in time to exhaustion, the treatments were not isocaloric, nor were the treatments standized by individual body weight. Potentially, the improved performance was a result of the higher caloric content of CHO/PRO, rather than the added PRO specifically.

Similarly, Saunders et al. (76) found individuals rode 29% longer during an exhaustive exercise bout at 75% VO_2max, when supplementing with a 7.5% CHO + 1.8% PRO beverage in comparison to an isocarbohydrate CHO treatment (106.3 min v 82.3 min, respectively). As in the previous study by Ivy and his team, the CHO/PRO treatment had a higher caloric content than the CHO-only. However, Saunders calculated the volume of each treatment calculated relative to body weight (1.8 $ml \cdot kg^{-1}$ BW^{-1}). During a second bout to exhaustion performed 12 - 15 hours later (85% VO_2max), the CHO/PRO treatment once again improved time to exhaustion as compared to CHO (43.6 min v 31.2 min, respectively). Authors concluded that the increased endurance performance was likely releated to reduced muscle fiber damage, indicated by an 83% reduction in plasma creatine phosphokinase (CPK) levels post-exercise. In a later study, Saunders and his team again found CHO/PRO enhanced performance during an exhaustive exercise bout at 75% VO_2max (77). Instead of consuming the supplements in a traditional beverage form, individuals ingested either CHO/PRO or isocarbohydrate CHO gels. Once again, CPK levels were significantly higher in the CHO treatment post-

exercise, while there was no signficant increase with the CHO/PRO treatment. The potential impact of reduced levels of muscle damage markers will be discussed later.

Recently, our laboratory found improved time-to-exhaustion when consuming a low concentrated CHO (3%) with moderate whey protein concentration (1.2%); in comparison to a traditional 6% CHO supplement (38). The CHO treatment was composed of dextrose only, while the carbohydrate source utilized in the CHO/PRO treatment was an equal mixture of dextrose, maltodextrin and fructose. Supplements (275 ml) were given every 20-mins during a 3-hour variable intensity ride, with intensities varying between 45% and 70% VO_2max. At the completion of 3-hours, subjects rode to exhaustion at approximately 80% VO_2max. While there was no overall difference in performance, CHO/PRO improved time-to-exhaustion in those individuals that were riding at or slightly below their lactate threshold (CHO/PRO: 45.64 ± 7.38; CHO: 35.47 ± 5.94 min, $p < .05$). This study not only gives evidence of a potential interaction between CHO/PRO supplementation and exercise intensity, but also that the addition of PRO to a low mixed CHO content has the capacity to improve endurance performance above that of a higher caloric CHO supplement.

While the previous studies have investigated the impact on performance when adding a PRO source (typically whey protein) to CHO, investigations have also been carried out utilizing AA. In regards to performance, AA administration has demonstrated improvement in performance in both animal (14, 15) and human models (10). The three BCAAs (leucine, isoleucine and valine) are essential amino acids that are major contributors to skeletal muscle make-up in the body. Calders et al. (15) injected 30 mg

branched chain amino acids (BCAA) into Wister rats prior to completing a treadmill run to exhaustion. Time to exhaustion was significantly enhanced after BCAA administration in comparison to the placebo group (99 ± 9 min v 76 ± 4 min, respectively). In a follow-up study, Calders et al. (14) compared exhaustive exercise performance in Wister rats when injecting 100 mg glucose, 30 mg BCAA or the combination of 100mg glucose and 30 mg BCAA (14). Treatments were injected 45 mins prior to running to exhaustion. Performance was improved with BCAA in comparison to placebo (158 min v 118 min, respectively) however; there were no additional performance improvements in comparison to the glucose treatment (179 min). It was concluded by the authors that consuming glucose prior to exercise, may negate the additional benefits of supplementary BCAA admininstration.

Despite AA administration indicating performance benefits in animal models, there is only a small body of evidence supporting these results during exercise in humans with improved performance demonstrated only during field studies (10). Blomstrand et al. (10) investigated the effect of ingesting BCAA during a marathon (16 g BCAA) or a 30 km cross country race (7.5 g BCAA). Participants completed a Stroop Color and Word test pre- and post-race in order to test for mental performance. While no change in mental performance occurred with placebo, those subjects ingesting the BCAA supplement had increased post-race mental performance. As a whole group, physical performance was not significantly different, however those individuals that were 'slower' marathon runners (3.05 h – 3.30 h) experienced significantly enhanced performance. It must be noted that while runners did not consume CHO directly in combination with

BCAA, they were supplied CHO and water ad libitum during the runs. This study suggests that AA supplementation has the potential to improve mental performance in addition to physical performance. Yet the improved endurance performance with AA alone has yet to be replicated in a laboratory setting (9, 32). Likely, PRO or AA supplementation in humans may depend on the co-supplementation with CHO.

Despite the above studies indicating improved performance with the addition of PRO or AA to CHO, several studies have demonstrated no further increases in performance above CHO-only (68, 75, 84, 85). Osterberg et al. (68) found no additional performance benefits of CHO/PRO when cyclists rode for 2 hours at an intensity slightly below lactate threshold (~5 % below that at which subjects elicted a 4 mM lactate response). After this time, they completed a time trial where subjects were required to complete a set amount of work as quickly as possible (7 $kJ \cdot kg^{-1}$). Treatments were identical to those that produced enhanced performance in a previously mentioned study by Saunders et al. (6% CHO mixture, a 7.5% CHO + 1.6% whey protein, or placebo) (76). Performance was significantly improved with CHO (37.1 min) treatments in comparison to placebo (39.7 min); however, there was no significant difference between either treatments when compared with CHO/PRO (38.8 min). The lack of difference between treatments can likely be attributed to the short duration of the time trial, which was likely not long enough to significantly rely on exogenous fuel sources.

Van Essen and Gibala (85) failed to find any additional performance benefits during an 80 km cycling time trial when comparing a traditional 6% sucrose drink (60 g $CHO \cdot h^{-1}$) to an isocarbohydrate 6% sucrose + 2% whey protein mixture. Treatments

(250 ml) were provided every 15 min. In comparison to placebo (141 min), both treatments had a 4.4% improvement in performance, with both treatments having an average time trial time of 135 min. This led investigators to hypothesize that the addition of PRO to CHO may only enhance performance when sub-optimal doses (less that 60 – 70 g CHO·hour^{-1}) of CHO are provided.

Mechanisms for Improved Performance With CHO/PRO Supplementation

Mechanisms by which the addition of PRO or AA to CHO improves endurance performance are still not clearly understood. Several possible mechanisms have been proposed, including the sparing of endogenous CHO stores (50, 65), the maintenance of Krebs Cycle intermediates (44, 50), a reduction in exercise induced muscle damage (72, 76-78) and finally the maintance of plasma AA in regards to the Central Fatigue Hypothesis (10, 15, 31, 50). Each of these mechanisms will be discussed below in further detail.

CHO/PRO and the Sparing of Glycogen Stores

Supplying exogenous CHO with PRO during exercise may enhance performance via its ability to decrease the reliance on endogenous CHO stores, in particular muscle and liver glycogen. Ivy et al. (50) proposed that a sparing, or more efficient use of muscle glycogen, may have resulted in the 36% improvement in time-to-exhaustion with CHO/PRO in comparison to an isocarbohydrate CHO treatment. No muscle biopsies

were taken, thus authors could not make direct conclusions of the role of muscle glycogen.

As discussed earlier, CHO supplementation has demonstrated increased insulin concentration and sparing of muscle glycogen stores in comparison to placebo (93, 94). The combination of CHO with PRO (79, 95) or AA (86, 87) has been shown to further enhance the insulin response.

In addition, previous work in animal models have demonstrated stimulated glucose uptake in the presence of BCAAs, independent of CHO (36, 37, 67). Isoleucine and leucine appear to be the primary amino acids that lower blood glucose, and have been shown in animal models to stimulate muscle glucose uptake in insulin-free conditions (36, 67). Human studies have additionally demonstrated that CHO/PRO increases blood glucose uptake into the muscle as compared to a CHO only or placebo. In a study conducted by Levenhagen et al. (58), subjects consumed one of three treatments immediately post 60-min of cycling (60% VO_2max). Supplements were composed of either 8 g CHO, 10 g PRO + 8 g CHO or a placebo. CHO alone did not significantly increase leg glucose uptake above placebo, however the CHO/PRO supplement significantly increase glucose uptake 3.5 fold above both CHO and placebo. As discussed previously in regards to CHO supplementation, increasing exogenous glucose uptake not only increases fuel availability for the working muscle, but also decreases the reliance on endogenous carbohydrate stores such as liver and muscle glycogen for energy production.

CHO/PRO and the Maintenance of Krebs Cycle Intermediates.

The Krebs cycle is a crucial component in the oxidative phosphorylation of carbohydrates, fats and protein in the body. Krebs cycle intermediates (i.e. oxaloacetate and malate) increase at the onset of exercise and progressively decline as exercise continues (44, 74). A decrease in CHO availabilty during exercise has been proposed to further decrease Krebs cycle intermediates, and may be a limiting factor in the mitochondria's ability to maintain aerobic energy production (74). Exogenous PRO increases the availability of amino acids that are precursors for anaplerotic reactions needed to maintain Krebs cycle intermediates. Ivy et al. (50) proposed the maintenance of Krebs cycle intermediates as a possible mechanism by which CHO/PRO improved performance above that of CHO alone in their study. To test this hypothesis, Cermak et al. (18) measured the Krebs cycle intermediates citrate and malate both before and after a 90-min ride at 69% VO_2peak. Every 15-mins, subjects consumed a 6% CHO + 2% PRO or an isocarbohydrate CHO treatment at a rate of 60 g CHO·h^{-1}. Both treatments increased the citrate and malate concentrations as compared to pre-exercise levels; however, there was no significant difference between CHO and CHO/PRO. However, the only performance measure was a subsequent 20-km time trial 24 hours later, during which there was no significant performance differences between treatments. Potentially, if performance was measured in a similar manner to Ivy et al. (50), and CHO/PRO was found to improve performance, then different results in regards to Krebs cycle intermediates may have been achieved. Additionally, the measured intermediates, citrate and malate, may have not been the most revelant to the exercise protocol. The Krebs

cycle intermediate most likely to be rate limiting under the conditions imposed is α ketogluterate, which was not measured. Evidently, more research is required to confirm the role this proposed mechanism in relation to performance.

CHO/PRO Supplementation and Muscle Damage

Muscle damage is a well-documented result of strenous exercise. In comparison to concentric muscle contractions, eccentric contractions result in higher levels of muscle damage due to the increased stress from lengthening the muscle fibers (19). However, muscle damage occurs during prolonged cycling bouts, despite the majority of the contractions being concentric. This is partially due to the rise in cortisol and other catobolic hormones during exercise (90). Due to the catabolic state of the body immediately post-exercise, muscle damage can occur not only during exercise, but can continue for several hours post-exercise.

Two common indicators of muscle damage mentioned in the literature are creatine phosphokinase (CPK) and myoglobin. Levels of CPK in the blood are low during normal conditions; however, damage to the muscle fibers will increase CPK leakage from the muscle into the blood. CPK levels will remain high in the blood for up to 24 hours, before slowly returning to basal levels with recovery (12).

Myoglobin is an oxygen-binding protein found primarily in the heart and skeletal muscle. Myoglobin plays an important role during exercise, primarily related to its capacity to store oxygen in the form of oxymyoglobin in the muscle, and to release this reserve during exercise. In addition, myoglobin is proposed to increase oxygen

utilization in the muscle by increasing oxygen distribution through the muscle fiber (46). Along with CPK, myoglobin is released into the blood from damaged muscle tissue.

In comparison to CHO, CHO/PRO during cycling protocols has been found to decrease muscle damage (72, 76-78). The capacity of CHO/PRO supplementation to decrease muscle damage was first proposed by Saunders et al. (76) when a CHO/PRO supplementation lowered post-exercise plasma CPK levels by 83% in comparison to CHO. Similar reduced levels of CPK post exercise have been seen in subsequent studies from the same laboratory, when consuming CHO/PRO as a traditional beverage or in a CHO/PRO gel (77, 78). During an exhaustive exercise bout at 75% VO_2max, Saunders and his team had individuals consume CHO/PRO or CHO gels, as opposed to traditional sports drinks used in previous studies. CHO/PRO again appeared to decrease muscle damage during exercise, with CPK levels significantly higher in the CHO treatment post-exercise, while there was no signficant increase with the CHO/PRO treatment (77). Reduced levels of muscle damage markers with CHO/PRO supplementation have additionally been found during eccentric resistance exercise protocols. Baty et al. (5) had subjects complete a selection of resistance exercises until volitional fatigue. In comparison to placebo, CHO/PRO consumption reduced levels of plasma myoglobin during and 6-hours post-exercise and CPK levels 24-hours post-exercise.

Despite these findings, reduced muscle damage with CHO/PRO supplementation has not always corresponded with improved performance. Romano-Ely et al. (72) found that in comparison to an isocaloric CHO, a CHO/PRO supplement attenuated the increase in CPK post-exercise. However, there were no performance differences between

treatments during an exhaustive cycling bout, or a subsequent exercise bout 24 hours later (72). It appears that reduced levels of muscle damage may not always impact performance during an intial exercise bout, but its ability to decrease the levels of muscle damage markers, may correspond with delayed exhaustion either during subsequent exercise bouts (76) and/or during the later stages of prolonged exercise (78).

CHO/PRO and Central Nervous System Fatigue Hypothesis

It has been proposed that the ability of CHO/PRO to delay exhaustion might relate to central nervous system (CNS) fatigue (8, 11). Increased levels of serotonin (5-hydroxytryptamine, 5-HT) in the brain stimulate increased perceptions of fatigue, resulting in decreased mood and motivation levels. The amino acid tryptophan (Trp) is a precursor for 5-HT production, and the rate-limiting step in serotonin synthesis is the transport of Trp across the blood–brain barrier. Trp and plasma free fatty acids compete for the same albumin-binding site in plasma (8, 39). During prolonged exercise, increased level of plasma free fatty acids will decrease the availibility of binding sites for Trp to bind to albumin. This results in an increase in free Trp movement across the blood-brain barrier, potentially increasing serotonin production and feelings of fatigue (81, 91). Carbohydrate supplementation has the capacity to decrease serotonin production during exercise, by reducing the reliance on fat oxidation and free fatty acids as energy sources during exercise (8). In addition to increasing plasma free fatty acid concentration, exercise increases the uptake of plasma AA into the muscle. As both free Trp and several large AA (including BCAA) use the same carrier system, exercise

increases the ratio of free Trp/BCAA, potentially increasing sertonin production (8, 39). BCAA supplementation during exercise will increase the amino acid pool, and has been associated with decreased levels of mental fatigue post-exercise and improved running performance (10). Given these results, it appears the combination of CHO with PRO or AA may not only be associated with reducing peripheral fatigue, but also reduces CNS factors involved with the perception of fatigue.

Summary of Carbohydrate Plus Protein Supplementation

In summary, CHO/PRO has shown enhanced performance during a prolonged cycling bout (50, 76, 77). The addition of PRO to CHO may enhance performance above that of CHO alone by several possible mechanisms. Maintenance of Krebs cycle intermediates during exercise may prevent a limitation of aerobic energy production during the later stages of exercise. Additionally, increased glucose uptake, glycogen sparing and a reduction of central nervous system fatigue are other possibilities. Decreased muscle damage following an exercise bout indicates that CHO/PRO may not only improve performance in the immediate exercise bout, but may also enhance the speed of recovery, possibly improving performance in subsequent bouts (76, 77). Likely, it is not one single mechanism, but their combination resulting in the ergogenic effect of CHO/PRO. From reviewing the literature, CHO/PRO will at a minimum, maintain the efficiacy of CHO supplementation, and possibly enhance performance above that of CHO. Further investigations are required in order to define the physiological mechanisms by which the addition of PRO can improve the efficiacy of a CHO treatment.

Comparisons of Carbohydrate and Carbohydrate Protein Supplementation

This literature review has examined both CHO and CHO/PRO supplementation in regards to endurance performance. It is apparent that both CHO and CHO/PRO have the ability to delay exhaustion and improve performance when compared to placebo or water treatments. The ability of CHO/PRO to futher enhance performance above that of CHO supplementation is debatable in the current literature. The variability in results regarding CHO/PRO supplementation has been suggested to be related to the type of exercise protocol used to measure performance. The two major methods utilized are set distance time trials and exercise bouts until exhaustion. However, the benefits of CHO/PRO supplementation do not appear limited to a specific exercise protocol. Improved performance has been demonstrated during time-to-exhaustion bouts (38, 50, 76), as well as in set distance time trials (78). Failure of CHO/PRO to futher improve performance above CHO has been observed in exhaustive exercise bouts (61, 72), in addition to set distance time trials (55, 68, 75, 85). Given these results, it appears the performance measure utilized is likely not the major influence of the success of CHO/PRO enhancing performance. However, exercise protocol should be taken into consideration when comparing across studies. For example, Osterberg et al. (68) failed to find improved performance with CHO/PRO, despite replicating the previous treatments utilized by Saunders et al. (76) which was found to enhance time-to-exhaustion. Differences between the studies are likely related to different performance measures utilized. Osterberg et al. (68) measured performance as the time to complete a set amount of work during a short duration, high intensity bout. Saunders et al. (76) utilized exhaustive exercise bouts as

their performance measure, which were of longer duration and lower intensity than Osterberg et al. (68). Thus, when making comparisons across studies, it is important to take into consideration the type, duration, and intensity of the exercise protocol.

A criticism of exhaustive exercise bouts in the literature is that they are not as representative of sporting events in comparison to a set distance time trial (29, 68, 85). However, supplementation benefits are not limited to timed endurance races such as marathons, distance road cycling, and long-distance triathlons. High levels of endurance are required in numerous situations inside and outside the sporting world. Sports such as tennis, volleyball, and baseball have the capacity to last several hours, with success reliant on lasting endurance during the later stages. Professions such as firefighting and military personnal are routinely required to maintain high levels of physical and mental performance for extended periods, and prolonging time to exhaustion can be critical for the success of their mission or even survival. It must be noted, that due to the variabilty of these situations, they are often difficult to create in a laboratory setting.

Likely, the success of CHO/PRO enhancing performance above CHO related more to the concentration of the CHO supplemented. Several studies demonstrating a lack of improvement with CHO/PRO have used high concentrations of CHO (85). As stated earlier, 60 - 70 g $CHO \cdot h^{-1}$ is suggested to optimize exogenous CHO oxidation (53, 88). In summary, it is important to note that while there appears to be variable results in with the addition of PRO to CHO, no studies have shown a decrease in performance when supplementing with CHO/PRO in comparison to CHO; all studies have shown that

CHO/PRO at least maintains the efficiacy of CHO supplementation, even when supplements are isocaloric.

Conclusion

The capacity to maintain exercise performance for extended periods involves the integration of multiple mechanisms in the human body. Nutritional supplementation is crucial in optimizing performance during endurance exercise bouts. While the ability of CHO supplementation to enhance performance has been extensively ingestivated for several years, it is apparent that CHO supplemented in combination with PRO or AA has the potential to further enhance performance above that of CHO alone. The specific mechanisms for this improvement still requires extensive research, however it is suggested to involve several mechanisms, namely a sparing of muscle glycogen stores, maintanence of Krebs cycle intermediates, a reduction in muscle damage, and/or a reduction in CNS fatigue. Some have suggested that the addition of PRO may only enhance a CHO supplement when lower than optimal CHO amounts are provided. This is an important consideration, as a high concentration of CHO given at regular intervals during exercise appears as effective as CHO/PRO in delaying the onset of fatigue. However, for individuals participating in exercise, competitively or recreationally, lowering the carbohydrate and caloric content, in combination with additional PRO is perhaps more pratical, particularly for those individuals concerned about weight management. In addition, utilizing a mixture of CHO sources in combination with PRO appears to maintain or even improve the efficiacy of a CHO only treatment In summary,

supplementing with a mixture of CHO sources, in combination with added PRO, appears to optimize the benefits of nutritional supplementation during endurance exercise.

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DETAILED METHODS

Experimental Design

This study followed a randomized, double-blinded, repeated measures design. After initially completing a VO_2max test and familiarization trial, subjects performed to two experimental trials in order to test the effect of a mixed 3% carbohydrate supplement with 1.2% added protein (CHO+PRO), against a traditional 6% carbohydrate (CHO) supplement. The experimental protocol was composed of varying intervals between 45% and 70% VO_2max, followed by a ride to exhaustion at an intensity approximating the individual's VT. Supplements (275ml) were consumed immediately prior to commencing the trial, and every 20 minutes thereafter.

CHO+PRO contained a mixture of dextrose (glucose), maltodextrin and fructose, and a whey protein isolate. The CHO treatment was composed of dextrose only. CHO+PRO contained 50% the carbohydrate content in comparison to CHO, and 33% lower caloric content. Both treatments contained equal amounts of electrolytes (Table 1). Supplements were supplied by the Human Performance Laboratory (Austin, TX), and were prepared by a laboratory technician not directly involved in the study. All supplements were similar in taste, color and texture.

Subjects

Fourteen female cyclists and triathletes were recruited via email announcement from local triathlon and cycling teams in Austin, TX. A detailed explanation of the experimental procedures and the potential risks of the study were given both verbally and

in writing to all subjects prior to initial testing. Subjects were given the opportunity to ask questions before signing the informed consent, according to the protocol described in the University of Texas at Austin's 'Institutional Review Board Procedures Manual for Faculty, Staff and Student Researchers with Human Participants'. The University of Texas at Austin Institutional Review Board approved the study before it commenced.

Preliminary Testing

Subjects initially reported to the laboratory for determination of maximum oxygen consumption (VO_2max) and ventilatory threshold (VT). All trials were conducted on the same ergometer (Veletron Dynafit Pro, Racermate, Seattle, WA). Prior to testing, body weight was recorded and subjects were outfitted with a Polar Heart Rate monitor (Polar Beat, Polar Electro Oy, Finland).

Maximal oxygen consumption was determined via a ramped protocol, consisting of a four minute warm up stage (range 75 - 130 W), followed by four stages of 2 minute duration. Each 2 minute stage increased in intensity by 35 W increments. Thereafter, workloads increased by 10 W each minute until the subject could no longer continue. Subjects breathed through a Hans Rudolf valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Inspired air volumes were measured using a dry gas meter (ParvoMedics TrueOne2400, ParvoMedics, Sandy, UT). A laboratory computer collected gas meter outputs, and used values for calculation of oxygen uptake (VO_2), carbon dioxide production (VCO_2), and Respiratory Exchange Ratio (RER) every 15 seconds. The criteria for establishing VO_2max was a plateau in

oxygen consumption (VO_2) with increasing exercise intensity, in addition to a respiratory exchange ratio (RER) greater than 1.10. The two highest 30 second values were averaged to determine VO_2max (ml $O_2 \cdot kg^{-1}$ min^{-1}). VT was defined as the point at which the minute ventilation (V_E) increased in a nonlinear fashion compared to increases in VO_2 and was confirmed by an increase in the V_E/VCO_2 to V_E/VO_2 ratio. Values were determined from the VO_2max test and a computer-generated plot (ParvoMedics TrueOne2400 software). Subjects were given constant verbal encouragement throughout the VO_2max test.

Testing Protocol

All trials were conducted in the Exercise Physiology Metabolism Laboratory at The University of Texas at Austin. Within the seven days following preliminary testing, subjects reported to the laboratory for a familizaration trial in order to accustom subjects with the testing protocol and equipment. This trial simulated the experimental protocol, exclusive of blood draws and treatment beverages. Water (275ml) was substituted for the experimental beverages.

The cycling protocol consisted of varying intervals between 45% and 70% VO_2max, followed by a ride to exhaustion at an exercise intensity approximating the individual's VT. The first 30 minutes of cycling was conducted at 45% VO_2max, followed by six intervals of 8-min duration. Interval duration was then reduced to 3-min. At 3-hours into the cycling protocol, subjects began the performance ride at an intensity relative to their VT, and this intensity was held until exhaustion. Refer to

Figure 1 for cycling protocol. Exhaustion was determined as the point at which subjects could no longer maintain a pedaling cadence of 60 revolutions per minute (rpm), despite constant verbal encouragement.

On the morning of the experimental trials, subjects arrived at the laboratory between 7am and 8am, following a 12-hour fast during which they were permitted to consume water only. Diet and activity logs were collected and verified, and body weight was obtained. Subjects were instructed to sit quietly for 2-min, after which a baseline HR was recorded. A Teflon catheter, fitted with a three-way stopcock and extended with a catheter extension, was inserted into the antecubital vein for blood sampling, and 5 ml baseline blood draw (PRE) taken. After baseline blood sampling, participants consumed the first supplement (275ml), before mounting the ergometer. Supplements were provided every 20-min during the exercise protocol. Upon nearing exhaustion, however, subjects were asked to consume as much as they felt comfortable.

All timing devices were removed from subject's sight, blinding participants to the length of ride completed. Personal music devices were permitted, however devices were required to be on a random song shuffle setting to eliminate any indication of time.

Cardio-Respiratory Measures

Respiratory gas and ventilation measures were collected five different times throughout the protocol. Collections occurred at 10 minutes (low intensity), 46 minutes (high), 130 minutes (low/high) and 184 minutes (start of exhaustion ride). Collection periods were 5 minutes in length, excluding the collection at time point 130-136 minutes,

which consisted of two, three minute recordings at low and high intensities respectively. To ensure a steady state VO_2 and RER, only the last 2 minutes of each collection were recorded. Carbohydrate and fat oxidation rates ($g \cdot min^{-1}$) were calculated from VCO_2, VO_2, and RER according to Frayn (3). It was assumed that protein oxidation during exercise was negligible. Heart rate (HR) was taken prior to the commencement of exercise. Heart Rate (HR) and RPE were measured 12 times throughout the exercise protocol. RPE was recorded using the Borg Scale ranging from 6 – 20 (2).

Blood Sampling

Blood samples (5ml) were collected pre-exercise (PRE), 118 minutes (T-118) and 177 minutes (T-177) into the exercise protocol, and at the point of exhaustion (END). 0.3 ml of the sample was transferred into a seperate tube containing 1 ml 10% perchloric acid (PCA). The remaining sample was divided into two tubes and mixed with 0.3ml of EDTA (24 $mg \cdot ml^{-1}$, pH 7.4) to prevent coagulation. Tubes were centrifuged for 10 mins at 3,000 RPM in a HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT). Plasma extracts were transferred and all tubes were stored at -80^0C until analysis.

Prior Diet and Exercise

Subjects were required to record activity levels for 3 days and diet for the 2 days prior each to trial. Diet and exercise were recorded in supplied logs, and subjects were required to replicate diet and exercise before each trial. Subjects were asked to keep diet

and activity levels as close to their regular routine as possible, and asked to refrain from strenuous exercise in the 24 hours prior to each trial. Logs were reviewed prior to each trial to ensure compliance. All subjects abided with requirements.

Tissue Analysis

At each blood collection, one drop of blood was used to measure blood glucose concentration (One Touch Basic glucose analyzer, LifeScan Inc., Milipitas, CA). This sample was used to ensure subjects were fasted upon arrival and additionally as an indicator of blood glucose levels as the trial progressed. Prior to each trial, the analyzer was calibrated using standards provided by LifeScan Inc. Prior to each trial, the analyzer was calibrated using standards provided by LifeScan Inc.

For data anaylsis, plasma samples were measured for glucose in duplicate using a modified Trinder procedure at 37^{o}C (6). Dry powder glucose reagent was dissolved in 100 ml deionized water. Tubes were run in duplicates, with 1.5 ml of reagent added to each tube (blank, standards and blood samples). Twenty microliters of standard (50, 100, 200, 300 mg·dL^{-1}) and blood samples were added to their respective tubes, vortexed, and incubated in a shaking water bath for 10 minutes at 37^{o}C. Samples were read at 500 nm using a Beckman DU640 Spectrophometer (Coulter, Fullerton, CA). Values reported and a CV of 3.7%.

Blood lactate concentration was measured from the PCA extracts using enzymatic analysis according to Hohorst (5). Glycine-hydrazine buffer (Fisher G-46 glycine, Fisher Scientific, Pittsburg, PA; Sigma H-9507 Hydrazine, Sigma- Aldrich, Inc.,

St Louis, MO) totaling 1000 mililiters was mixed, and used to prepare the cocktail reagent prior to beginning the assay. Added to each tube was 1 ml glycine- hydrazine buffer, 0.83 mg β-Nicotinanide adenine dinucleotide (NAD) (Sigma N-7004, Sigma-Aldrich, Inc., St Louis, MO) and 5 µl L-Lactate Dehydrogenase (LDH) (Sigma 826-6 or L-3916, Sigma-Aldrich, Inc, St. Louis, MO). 50 µl 10% PCA was added to the blank, two tubes were designated for low and high lactic acid standards (1.1 mM and 2.2mM respectively). 50µl of blood PCA samples added to each remaining tube. Following vortexing, tubes were incubated at 37^{o}C for 45mins in a shaking water bath. Samples were read at 340 nm using a Beckman DU640 Spectrophometer (Coulter, Fullerton, CA). All tubes were run in duplicates and had a CV of 1.2%.

Plasma insulin was analyzed via radioimmunoassay and based on the principles of Goetz (4). The radioimmunoassay kit used was MP Biomedicals ^{125}I RIA, Solon, OH, USA and had a CV of 6.0%. Duplicate tubes were prepared and incubated at room temperature for 18 hours. Tubes were decanted, blotted with absorbant paper, and rinsed with 4 ml of deionized water before a second decantation. Empty tubes were counted in a Wallac 1470 Wizard Gamma Counter (Wallac 1470 Wizard Gamma Counter, PerkinElmer Life and Analytical Sciences, Boston, MA), which had been calibrated for insulin ^{125}I.

Myoglobin was measured in duplicate by solid phase ELISA (Myoglobin Enzyme Immunoassay Test Kit, BioCheck, Inc, Foster City, CA, USA), with a CV of 5.4%. Myoglobin standards (0, 25, 100, 250, 500 and 1000ng/ml), control and specimen samples (20µl) were added to appropriate wells, with 200µl of Enzyme Conjugate

Reagent (horseradish peroxidase in Tris-Buffer-BSA solution). Wells were mixed and left to incubate at room temperature for 45 minutes. At this time, tubes were rinsed with deionized water for five times, and blotted to remove additional residual water drops. Tetramethyl-benzidine (TMB) reagent (100µl) was added to wells, and after 5 mins of mixing, wells were incubated for 20 further minutes. To stop the reaction, 100µl of 1N hydrochloric acid was added and mixed, following which, wells were read at 450 nm with a microtiter well reader (Bio-Tek ELx800, Biotek Instruments Inc, Winooski, VT, USA).

Calculations

CHO and fat utilization rates ($g \cdot min^{-1}$) were calculated according to Frayn (4); with the assumption that PRO oxidation during exercise was negligible.

$$\text{CHO utilization } (g \cdot min^{-1}) = (4.55 * VCO_2) - (3.21 * VO_2)$$

$$\text{Fat utilization } (g \cdot min^{-1}) = (1.67 * VO_2) - (1.67 * VCO_2)$$

Maximum power output in Watts (W_{max}) and relative work rates (W) during the experimental rides. The formala for calculating maximal Watts was adapted from Astrand and Rodahl (1).

$$Wmax = (VO_2max\ mL - 300\ mL\ O_2) / 12.5\ W \cdot mL^{-1}\ O_2$$

The experimental workloads were set as percentages of the Wmax:

$$W = [(VO_2max\ mL \times \%VO_2max\ desired) - 300\ mL\ O_2] / 12.5\ W \cdot mL^{-1}\ O_2$$

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5. Hohorst H. *Determination of L-lactate with LDH and DPN*. New York: Academic, 1965, p. 266-270.

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APPENDIX A

Individual Subject Data

Subject	Age (years)	Height (cm)	Body Weight (kg)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	VT ($L \cdot min^{-1}$)	Watts Max (W)
1	37	152	56.1	2.17	38.65	1.58	149
2	31	173	59.4	3.57	60.15	2.86	262
3	42	157	59.4	2.78	46.75	1.99	198
4	22	178	55.7	2.57	46.10	1.98	182
5	27	164	62.6	2.89	46.15	2.14	207
6	27	159	58.7	3.13	53.25	2.31	226
7	31	157	61.2	2.77	45.15	2.05	197
8	26	163	51.2	2.28	44.45	1.73	158
9	35	160	46.0	1.86	40.40	1.40	125
10	32	179	70.4	3.31	47.00	2.58	241
11	30	183	73.7	3.45	46.80	2.59	252
12	24	178	70.6	3.85	54.45	2.61	176
13	23	166	72.4	3.41	47.05	2.52	248
14	23	178	68.5	2.60	38.00	1.77	184
AVERAGE	**30.43**	**167.59**	**61.85**	**2.90**	**46.74**	**2.15**	**200.29**
SE	**1.63**	**2.72**	**2.23**	**0.15**	**1.61**	**0.12**	**11.10**

Heart Rate (bpm) **Time of Collection (minutes)**

	Subject	Pre	10	25	50	90	105	115	130	135	159	161	175	184
CHO + PRO	1	62	109	111	138	112	113	132	120	139	139	119	138	142
	2	67	105	100	134	104	111	137	123	138	139	125	140	145
	3	82	110	108	131	119	113	131	119	125	131	120	130	142
	4	52	112	111	142	119	121	134	114	131	135	120	129	148
	5	66	106	101	134	105	106	132	109	133	122	110	134	142
	6	70	128	135	159	137	138	162	140	168	164	147	165	176
	7	87	120	119	146	131	138	143	131	144	145	137	148	156
	8	85	128	120	138	132	139	154	136	148	150	148	154	153
	9	62	113	110	140	115	120	145	127	151	151	138	135	173
	10	66	112	114	138	115	123	143	129	142	142	134	143	153
	11	45	92	96	124	100	100	107	108	109	104	103	122	139
	12	71	111	109	143	118	123	147	123	144	142	123	143	148
	13	75	116	118	145	125	132	153	133	153	153	139	150	166
	14	80	115	113	131	117	116	134	114	136	126	118	132	131
	AVG	**69.29**	**112.64**	**111.79**	**138.79**	**117.79**	**120.93**	**139.57**	**123.29**	**140.07**	**138.79**	**127.21**	**140.2**	**151.00**
	SE	**3.23**	**2.45**	**2.60**	**2.24**	**2.89**	**3.27**	**3.57**	**2.65**	**3.71**	**3.99**	**3.62**	**3.48**	**3.48**

	Subject	Pre	10	25	50	90	105	115	130	135	159	161	175	184
CHO	1	66	120	112	132	115	118	138	117	140	123	120	135	142
	2	64	105	101	131	110	113	132	113	131	132	116	128	143
	3	72	111	111	138	113	120	140	116	137	140	121	137	138
	4	45	111	114	138	126	122	138	126	142	147	135	141	156
	5	57	114	109	143	112	112	142	111	139	137	121	133	145
	6	64	133	134	165	135	133	174	154	174	172	151	173	180
	7	81	118	120	133	115	132	134	125	138	140	138	136	152
	8	83	123	122	145	143	128	154	129	145	143	140	145	150
	9	58	108	105	139	117	120	151	120	151	151	121	145	168
	10	57	113	117	144	122	131	143	128	143	144	134	148	158
	11	45	101	100	138	120	107	137	110	137	137	110	137	143
	12	72	106	102	145	123	123	153	131	152	147	131	148	151
	13	73	128	126	154	132	135	161	140	160	158	145	161	168
	14	80	114	112	135	120	115	131	116	130	138	122	133	137
	AVG	**65.50**	**114.64**	**113.21**	**141.43**	**121.64**	**122.07**	**144.86**	**124.00**	**144.21**	**143.50**	**128.93**	**142.86**	**152.21**
	SE	**3.27**	**2.41**	**2.65**	**2.46**	**2.55**	**2.33**	**3.28**	**3.26**	**3.16**	**3.14**	**3.20**	**3.23**	**3.38**

Rating of Perceived Exertion (RPE) **Time of Collection (minutes)**

	Subject	**10**	**25**	**50**	**90**	**105**	**115**	**130**	**135**	**159**	**161**	**175**	**184**
CHO+PRO	1	9	11	13	12	11	12	12	13	11	12	14	14
	2	9	9	10	9	10	12	10	12	12	10	11	13
	3	11	11	12	12	13	14	12	14	15	13	15	16
	4	6.5	9	13	12	12	14	11.5	13	12	11	13.5	16
	5	8	7	11	11	10	12	10	12	13	10	13	13
	6	10	11	15	12	13	16	13	15	16	12	16	17
	7	7	9	11	12	13	13	14	15	15	14	16	16
	8	9	11	13	12	11	13	12	13	15	12	14	15
	9	6	6	7	11	12	13	11	13	15	11	15	17
	10	8	9	12	11.5	12.5	13.5	13	14	13.5	13	13	14
	11	9	10	12	11	12	14	12	15	15	13	15	16
	12	10	10	13	11	11	14	12	14	15	12	11	14
	13	11	11	13	12	13	15	12	14	14	12	15	15
	14	10	10	12	12	11	13	12	13	15	14	14	17
	AVG	**9.36**	**9.89**	**12.57**	**11.57**	**11.61**	**13.50**	**11.93**	**13.36**	**13.36**	**12.00**	**13.79**	**15.04**
	SE	**0.48**	**0.44**	**0.25**	**0.27**	**0.28**	**0.23**	**0.32**	**0.22**	**0.44**	**0.41**	**0.41**	**0.32**

	Subject	**10**	**25**	**50**	**90**	**105**	**115**	**130**	**135**	**159**	**161**	**175**	**184**
CHO	1	9	9	12	11	11	13	12	13	12	11	12	13
	2	8	7.5	12	9	9	12	10	12	12	9	12	15
	3	11	11	13	12	12.5	13	11	13	12	12	14	15
	4	9	10	13	13	12	14	12	13.5	14	12	15	17
	5	10	10	12	11	11	13	10	13	10	10	13	13
	6	11	12	15	12	13	14.5	12	14	16	13	16	15
	7	7	9	11	12	12	13	13	13.5	14	13	14	15.5
	8	9	11	13	11	11	13	12	14	13	12	13	14
	9	6	6	12	11	11	13	11	12	12	10	12	15
	10	7	9	12	11	12	13.5	12	13	14	13	14	15
	11	11	11	13	13	13	14	14	14	14	14	13	16
	12	11	11	13	12	11	13	12	13	14	12	13	15
	13	11	11	13	12	12	15	12	15	16	13	15	15
	14	11	11	12	12	12	15	14	14	14	14	17	17
	AVG	**8.82**	**9.57**	**11.93**	**11.46**	**11.75**	**13.46**	**11.89**	**13.57**	**14.04**	**12.07**	**13.96**	**15.21**
	SE	**0.42**	**0.42**	**0.50**	**0.23**	**0.29**	**0.31**	**0.29**	**0.27**	**0.40**	**0.34**	**0.42**	**0.38**

Respiratory Exchange Ratio (RER)

		Time of Collection (minutes)				
	Subject	**10**	**50**	**130**	**135**	**184**
CHO+PRO	1	0.87	0.87	0.89	0.85	0.94
	2	0.82	0.85	0.84	0.82	0.90
	3	0.85	0.88	0.90	0.90	0.93
	4	0.94	0.92	0.99	0.94	0.98
	5	0.94	1.01	0.90	0.95	1.03
	6	0.91	0.98	0.92	0.95	1.01
	7	0.95	1.01	0.97	0.97	1.01
	8	0.91	0.91	0.90	0.88	0.89
	9	0.92	0.97	0.95	0.98	1.10
	10	0.92	0.92	0.89	0.91	0.98
	11	0.90	0.94	0.91	0.91	0.94
	12	0.87	0.90	0.87	0.89	0.90
	13	0.91	0.93	0.89	0.95	0.97
	14	0.89	0.92	0.90	0.89	0.89
	AVG	**0.90**	**0.93**	**0.91**	**0.91**	**0.96**
	SE	**0.01**	**0.01**	**0.01**	**0.01**	**0.02**

	Subject	**10**	**50**	**130**	**135**	**184**
CHO	1	0.88	0.87	0.91	0.89	0.91
	2	0.83	0.88	0.88	0.85	0.90
	3	0.85	0.89	0.87	0.88	0.93
	4	0.90	0.92	0.96	0.92	0.95
	5	0.95	1.02	0.91	0.99	1.03
	6	0.91	0.97	0.93	0.97	1.00
	7	0.91	0.97	0.98	0.94	1.01
	8	0.88	0.90	0.88	0.88	0.91
	9	0.91	0.99	0.93	0.96	1.12
	10	0.91	0.92	0.88	0.89	0.97
	11	0.92	0.97	0.92	0.92	0.95
	12	0.88	0.92	0.91	0.88	0.93
	13	0.90	0.94	0.93	0.96	1.01
	14	0.89	0.89	0.90	0.89	0.90
	AVG	**0.89**	**0.93**	**0.91**	**0.92**	**0.97**
	SE	**0.01**	**0.01**	**0.01**	**0.01**	**0.02**

Oxygen Consumption ($L \cdot min^{-1}$)

Time of Collection (minutes)

	Subject	10	50	130	135	184
CHO+PRO	1	1.02	1.53	1.04	1.52	1.60
	2	1.63	2.43	1.69	2.42	2.73
	3	1.33	1.90	1.39	1.90	2.04
	4	1.33	2.04	1.30	1.91	2.18
	5	1.12	1.72	1.13	1.61	1.79
	6	1.46	2.13	1.50	2.21	2.46
	7	1.08	1.48	1.11	1.46	1.74
	8	1.27	1.84	1.30	1.88	1.99
	9	0.78	1.31	0.83	1.33	1.68
	10	1.44	2.19	1.50	2.23	2.62
	11	1.54	2.47	1.63	2.48	2.57
	12	1.98	2.87	1.96	2.74	2.80
	13	1.55	2.24	1.53	2.38	2.80
	14	1.27	1.73	1.25	1.70	1.65
	AVG	**1.34**	**1.99**	**1.37**	**1.98**	**2.19**
	SE	**0.08**	**0.12**	**0.08**	**0.12**	**0.12**

	Subject	10	50	130	135	184
CHO	1	1.01	1.48	0.98	1.47	1.52
	2	1.63	2.37	1.64	2.40	2.63
	3	1.30	1.87	1.43	1.89	1.99
	4	1.34	1.96	1.37	1.99	2.22
	5	1.08	1.76	1.09	1.69	1.80
	6	1.46	2.14	1.51	2.21	2.46
	7	1.11	1.52	1.11	1.44	1.75
	8	1.28	1.81	1.26	1.81	1.85
	9	0.77	1.27	0.81	1.30	1.64
	10	1.37	2.19	1.53	2.22	2.70
	11	1.56	2.35	1.54	2.38	2.49
	12	1.79	2.74	1.97	2.71	2.77
	13	1.57	2.29	1.48	2.29	2.72
	14	1.24	1.75	1.23	1.70	1.71
	AVG	**1.32**	**1.96**	**1.35**	**1.97**	**2.16**
	SE	**0.07**	**0.11**	**0.08**	**0.11**	**0.12**

CHO Utilization ($g \cdot min^{-1}$)

		Time of Collection (minutes)				
	Subject	**10**	**50**	**130**	**135**	**184**
CHO+PRO	1	0.74	1.14	0.87	0.99	1.68
	2	0.83	1.59	1.01	1.24	2.39
	3	0.87	1.51	1.21	1.69	2.04
	4	1.18	1.66	1.45	1.70	2.21
	5	1.41	2.83	1.15	2.12	3.21
	6	1.36	2.66	1.48	2.46	3.40
	7	1.20	2.04	1.31	1.76	2.41
	8	1.15	1.70	1.13	1.47	1.68
	9	0.76	1.58	0.92	1.70	3.02
	10	1.40	2.14	1.26	2.07	3.26
	11	1.36	2.62	1.51	2.31	2.74
	12	1.44	2.54	1.48	2.35	2.42
	13	1.42	2.26	1.28	2.64	3.37
	14	1.07	1.52	1.09	1.45	1.34
	AVG	**1.16**	**1.98**	**1.23**	**1.85**	**2.51**
	SE	**0.07**	**0.14**	**0.06**	**0.13**	**0.18**

	Subject	**10**	**50**	**130**	**135**	**184**
CHO	1	0.80	1.11	0.90	1.26	1.42
	2	0.89	1.83	1.30	1.58	2.33
	3	0.84	1.53	1.09	1.50	1.99
	4	0.95	1.75	1.26	1.62	1.99
	5	1.49	2.80	1.30	2.58	3.28
	6	1.34	2.58	1.54	2.66	3.30
	7	1.01	1.82	1.38	1.54	2.42
	8	0.99	1.58	1.00	1.44	1.75
	9	0.72	1.65	0.83	1.51	3.05
	10	1.25	2.14	1.21	1.86	3.25
	11	1.55	2.80	1.51	2.34	2.73
	12	1.38	2.65	1.79	2.14	2.83
	13	1.37	2.44	1.48	2.60	3.77
	14	1.04	1.50	1.07	1.44	1.53
	AVG	**1.12**	**2.01**	**1.26**	**1.86**	**2.55**
	SE	**0.07**	**0.15**	**0.07**	**0.13**	**0.20**

Fat Utilization ($g \cdot min^{-1}$)

		Time of Collection (minutes)				
	Subject	**10**	**50**	**130**	**135**	**184**
CHO+PRO	1	0.23	0.33	0.19	0.38	0.17
	2	0.50	0.61	0.46	0.74	0.47
	3	0.34	0.38	0.24	0.31	0.25
	4	0.12	0.24	0.02	0.17	0.07
	5	0.13	0.00	0.22	0.16	0.00
	6	0.22	0.07	0.19	0.18	0.00
	7	0.09	0.00	0.06	0.07	0.00
	8	0.20	0.28	0.23	0.38	0.36
	9	0.10	0.07	0.07	0.03	0.00
	10	0.19	0.29	0.28	0.33	0.09
	11	0.26	0.26	0.24	0.37	0.26
	12	0.45	0.48	0.42	0.49	0.49
	13	0.23	0.27	0.28	0.20	0.14
	14	0.23	0.23	0.21	0.30	0.32
	AVG	**0.23**	**0.25**	**0.22**	**0.29**	**0.19**
	SE	**0.03**	**0.05**	**0.03**	**0.05**	**0.05**

	Subject	**10**	**50**	**130**	**135**	**184**
CHO	1	0.20	0.32	0.15	0.26	0.23
	2	0.47	0.49	0.33	0.60	0.44
	3	0.33	0.36	0.30	0.38	0.25
	4	0.18	0.22	0.07	0.24	0.15
	5	0.11	0.00	0.20	0.03	0.00
	6	0.23	0.11	0.18	0.11	0.00
	7	0.18	0.08	0.04	0.14	0.00
	8	0.27	0.31	0.25	0.36	0.27
	9	0.11	0.02	0.09	0.09	0.00
	10	0.22	0.29	0.31	0.41	0.14
	11	0.20	0.13	0.20	0.31	0.22
	12	0.37	0.37	0.31	0.55	0.32
	13	0.27	0.23	0.19	0.17	0.00
	14	0.23	0.31	0.21	0.31	0.28
	AVG	**0.24**	**0.23**	**0.20**	**0.28**	**0.16**
	SE	**0.03**	**0.04**	**0.02**	**0.04**	**0.04**

APPENDIX B

Tissue Analysis

Glucose ($mmol \cdot L^{-1}$)

Time of Collection (minutes)

	CHO+PRO				CHO			
Subject	**PRE**	**118**	**177**	**END**	**PRE**	**118**	**177**	**END**
1	3.54	3.57	3.90	4.06	3.51	4.03	3.99	4.66
2	4.16	4.25	3.92	4.61	4.29	5.42	5.71	6.21
3	3.83	4.31	4.44	3.77	3.71	3.96	4.34	4.44
4	4.23	3.73	3.74	5.50	4.14	4.50	4.86	5.67
5	3.95	3.53	3.05	3.76	3.83	3.73	3.82	4.43
6	4.28	3.97	3.84	3.88	4.29	4.41	4.60	4.62
7	4.64	4.01	3.91	4.29	4.19	4.03	4.31	3.87
8	3.82	3.37	4.30	4.70	4.31	4.31	5.06	5.01
9	3.64	3.68	3.84	3.79	4.12	4.44	4.28	4.05
10	4.59	4.05	4.26	4.48	4.01	4.09	4.28	5.16
11	3.91	3.91	4.30	4.26	3.97	4.72	4.97	4.80
12	5.04	3.69	3.51	3.01	4.27	4.32	4.35	5.38
13	4.52	4.34	5.03	4.91	4.60	4.44	5.42	4.53
14	4.28	3.89	3.99	4.11	4.26	3.92	4.95	4.80
AVG	**4.17**	**3.88**	**4.00**	**4.22**	**4.11**	**4.31**	**4.64**	**4.83**
SE	**0.11**	**0.08**	**0.12**	**0.16**	**0.08**	**0.11**	**0.14**	**0.17**

Insulin ($pmol \cdot L^{-1}$)

Time of Collection (minutes)

	CHO+PRO				CHO			
Subject	**PRE**	**118**	**177**	**END**	**PRE**	**118**	**177**	**END**
1	86.46	52.28	92.54	60.91	71.98	111.64	86.41	71.01
2	43.52	90.47	82.87	56.62	90.32	70.96	93.29	41.92
3	97.42	100.34	122.73	39.55	148.93	128.64	139.97	94.06
4	56.12	54.87	41.88	47.43	68.82	55.84	76.67	57.78
5	115.29	92.95	67.00	49.80	100.08	91.83	69.38	69.49
6	55.56	36.67	27.50	12.78	73.27	23.40	47.78	10.83
7	141.40	116.33	75.63	58.41	90.56	100.91	80.35	42.71
8	50.43	60.94	72.77	51.02	60.18	64.10	85.37	46.29
9	56.46	59.24	40.98	36.48	48.48	73.62	74.59	46.25
10	68.06	60.28	58.27	39.86	57.50	51.60	40.07	27.15
11	74.24	47.43	50.21	39.59	58.82	61.95	87.51	42.78
12	90.92	64.89	53.49	48.62	61.05	67.21	83.52	64.26
13	189.46	132.79	138.90	88.55	162.93	108.62	125.57	57.37
14	72.95	71.97	62.57	63.54	101.84	50.98	53.52	47.26
AVG	**85.59**	**74.39**	**70.52**	**49.51**	**85.34**	**75.81**	**81.71**	**51.37**
SE	**10.79**	**7.48**	**8.30**	**4.57**	**9.16**	**7.71**	**7.20**	**5.40**

Lactate ($mmol \cdot L^{-1}$)

Time of Collection (minutes)

	CHO+PRO				CHO			
Subject	**PRE**	**118**	**177**	**END**	**PRE**	**118**	**177**	**END**
1	0.889	1.028	0.696	0.722	0.758	0.887	0.814	0.907
2	0.673	1.128	0.924	1.106	0.763	0.891	0.803	0.882
3	1.533	1.167	0.897	0.752	1.363	1.287	1.023	1.018
4	0.852	1.957	1.579	4.349	0.884	2.267	1.819	4.357
5	0.559	0.757	0.701	0.636	0.666	0.903	0.644	0.942
6	0.708	1.121	0.952	1.685	0.637	0.961	0.767	1.627
7	1.469	1.279	0.975	2.054	1.223	1.043	0.996	2.107
8	0.786	0.911	0.895	0.866	0.634	0.941	0.865	1.122
9	1.034	1.531	1.063	3.558	0.682	0.994	0.903	3.355
10	0.857	1.244	0.969	2.298	0.610	1.094	1.245	3.074
11	0.371	1.003	0.928	1.311	0.337	1.374	1.134	1.406
12	0.869	1.633	1.101	1.050	0.820	1.951	1.415	1.484
13	1.469	1.228	1.254	3.763	1.171	1.171	0.997	2.254
14	0.712	1.041	0.804	0.764	0.678	0.931	0.877	0.926
AVG	**0.913**	**1.216**	**0.981**	**1.779**	**0.802**	**1.192**	**1.021**	**1.819**
SE	**0.094**	**0.083**	**0.060**	**0.349**	**0.074**	**0.112**	**0.082**	**0.291**

Myoglobin ($ng \cdot ml^{-1}$)

Time of Collection (minutes)

	CHO+PRO				CHO			
Subject	**PRE**	**118**	**177**	**END**	**PRE**	**118**	**177**	**END**
1	10.25	13.54	15.84	18.37	16.89	17.31	15.00	14.37
2	19.60	17.43	16.77	18.53	20.52	22.74	27.09	24.38
3	37.94	24.41	40.51	39.10	90.17	124.44	137.70	125.05
4	14.83	15.36	15.04	17.88	18.18	18.91	18.18	20.15
5	13.33	20.94	17.10	15.00	13.75	14.37	14.79	17.73
6	22.27	22.35	22.30	22.28	22.27	22.32	22.47	22.74
7	15.72	17.06	17.17	16.08	15.23	15.15	15.58	15.44
8	25.29	26.61	30.18	29.06	23.76	32.89	30.40	34.71
9	14.44	11.82	10.73	11.36	11.92	11.73	12.30	12.79
10	18.66	22.25	23.47	29.43	18.39	18.39	25.21	31.04
11	17.80	17.15	17.88	17.80	16.81	17.09	17.15	16.34
12	16.37	21.18	25.02	28.05	13.04	18.36	19.60	25.44
13	16.75	17.27	18.12	19.57	16.65	16.65	18.45	20.49
14	15.52	15.75	13.17	12.21	18.27	18.88	15.22	16.93
AVG	**18.48**	**18.79**	**20.23**	**21.05**	**22.56**	**26.37**	**27.80**	**28.40**
SE	**1.80**	**1.14**	**2.06**	**2.15**	**5.28**	**7.66**	**8.57**	**7.62**

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