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Effects of carbohydrate + protein co-ingestion during and following exercise on cardiovascular adaptations and exercise tolerance during intensified cycle training

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Effects of carbohydrate + protein co-ingestion during and following exercise on cardiovascular adaptations and exercise tolerance during intensified cycle training

Justin Faller

A Thesis Submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

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Abstract

Introduction: This study investigated the effects of carbohydrate and protein (CHO+Pro) supplementation during intensified training on cardiovascular adaptations and cycling performance. Methods: Five cyclists (VO\textsubscript{2peak} = 62.6 ± 10.7 ml/kg/min) performed two 27-day training protocols while receiving either CHO or CHO+Pro supplements. The exercise protocols consisted of 7 days of normal training (NT), 10 days of intensified cycle training (ICT; 100% increase in average training duration versus NT), and 10 days of reduced volume training (RVT; reduction in training duration to 60% of NT). Performance was assessed by a 30-km time trial following 120 min of constant-load cycling and VO\textsubscript{2peak} testing. Other physiological measures were assessed at rest (heart rate, hematocrit, hemoglobin, and albumin) and during exercise (heart rate, ventilation, RER, RPE, VO\textsubscript{2}, glucose, and lactate). Supplements were consumed during exercise (750 ml·hr\textsuperscript{-1}; CHO = 6% concentration; CHO+PRO = 6% CHO, 2% Pro concentrations) and immediately following exercise (9.93 ml of fluid·kg BW\textsuperscript{-1}; CHO = 1.2 g CHO·kg BW\textsuperscript{-1}; CHO+PRO = 1.2 g CHO·kg BW\textsuperscript{-1} + 0.3 g Pro·kg BW\textsuperscript{-1}). Data was analyzed using magnitude-based inferences. Results: Time to complete the 30-km TT and average TT power were ‘likely’ impaired following ICT, with no differences between treatments. Following RVT, time to complete the 30-km TT and average TT power were ‘likely’ improved versus ICT, with no differences between treatments. Between NT and ICT constant-load HR significantly decreased (independent of treatment), with a ‘very likely’ larger reduction with CHO+Pro (135±13 bpm to 128±10 bpm) versus CHO (133±15 bpm to 132±13 bpm). Between NT and RVT, constant-load HR also ‘very likely’ declined to a greater extent with CHO+Pro (135±13 bpm to 132±14) than with
CHO (133±15 to 138±16 bpm). Serum albumin was ‘likely’ increased following ICT and RVT (independent of treatment), but differences between treatments were ‘unclear’.

**Conclusion:** These findings suggest exercising heart rates in well trained-cyclists are attenuated with carbohydrate and protein supplementation, although the cause of the reduced in HR is unclear. In addition, the implications of these findings require further study, as overall exercise tolerance and adaptations following intensified training were similar to those observed with carbohydrate supplementation.
Chapter I

Introduction

Periods of intensified training are often used by endurance athletes in an attempt to enhance performance (20). When intensified training is followed by adequate recovery, it is common for athletes to experience improvements in performance compared to baseline, known as a “supercompensation” effect (20, 26). During intensified training it is common to experience reductions in global mood scores, maximum heart rate, VO2max, muscle glycogen levels, and an increase in perceived exertion during exercise (19, 26). This can result in short-term decrements in performance, known as overreaching (18, 20). However, continued heavy training while experiencing overreaching may ultimately lead to overtraining (19–21, 26). Overtraining is classified as an accumulation of training stress that leads to long-term decrements in performance (18–21, 26).

Nutritional interventions have commonly been implemented to promote recovery during and following periods of intensified training. High carbohydrate (CHO) diets have been shown to improve glycogen re-synthesis (40, 41), and improve performance in subsequent exercise following heavy training (1, 18, 41). Timing of CHO consumption is also important during heavy training. Dietary CHO consumption immediately after training results in higher rates of muscle glycogen re-synthesis, versus when CHO consumption is delayed (18, 23, 40).

A particular concern during periods of intensified training is muscle glycogen stores. Muscle glycogen depletion can occur after repeated bouts of high-intensity exercise, and it is believed that this, along with the other stressors of overtraining, can
lead to fatigue and decrements in performance (9, 20). Evidence suggests that increased dietary CHO intake during heavy training may attenuate overtraining symptoms (1, 18). Achten et al. (1) observed that a high carbohydrate diet (8.5 g/kg/day) resulted in better maintenance of physical performance and mood scores compared to lower carbohydrate intake (5.4 g/kg/day) following a period of intensified training. Similarly, Halson et al. (18) observed that 8-days of intensified cycle training with low-carbohydrate supplementation (2% solution before, during, and immediately following each exercise session) resulted in significantly greater declines in time to fatigue, and mood scores when compared to high-carbohydrate supplementation (6% solution before and during exercise and a 20% solution immediately following each exercise session). These findings suggest that higher CHO intakes, particularly when fed during/post-exercise may help to improve tolerance to periods of intensified training.

The effects of supplemental protein on recovery from heavy aerobic training have also been investigated in recent years. Numerous studies have reported that co-ingestion of carbohydrate and protein (CHO+Pro) during short-term recovery following heavy exercise improves performance in subsequent exercise versus CHO alone (2, 3, 5, 7, 25, 27, 32, 38, 43). Enhanced glycogen re-synthesis (4, 24, 28, 46), and/or reduced muscle damage (15, 17, 29–31, 37, 42, 44) are possible explanations for improvements in performance, though not all studies have reported these benefits (6, 36, 37, 39).

The short-term recovery benefits of CHO+Pro beverages are potentially important for athletes. However, the effects of CHO+Pro ingestion on exercise tolerance, training adaptations and performance during sustained periods of heavy training/competition are poorly understood. A few studies have investigated CHO+Pro supplementation during 3-
6 days of training, and reported attenuated creatine kinase (CK) levels (15, 29, 42) and decreased muscle soreness (29). However, it is unclear whether regular intake of CHO+Pro beverages improves performance following heavy training to a greater degree than CHO beverages. Witard et al. (45) examined the effects of increased dietary protein consumption, during a 1 week period of intensified training and the subsequent effects on performance and mood. When compared to a normal protein intake of 1.5 g·kg BW\(^{-1}\), increasing dietary protein intake to 3.0 g·kg BW\(^{-1}\) “possibly attenuated” psychological symptoms of stress experienced during training, and “possibly reduced” decrements in performance following intensified training. Although the aforementioned findings were not statistically significant, this study highlights the potential importance of protein supplementation during periods of intensified training.

A few recent studies have suggested that CHO+Pro supplementation may also augment cardiovascular adaptations following training (16, 33–35). CHO+Pro supplementation has been associated with increased serum albumin content and plasma volume, resulting in increased stroke volumes, lower heart rates, and enhancements in thermoregulatory responses to heat. These results have been observed following both short (~5 days) and long (~8 wks) training periods (7, 12, 16, 34, 35). An increase in plasma albumin content will draw fluid from the extravascular space into the intravascular space due to oncotic pressure leading to an increase in plasma volume (8, 14). The increase in plasma volume appears to enhance thermoregulatory responses to heat, such as an increased sweat rate and a lower esophageal temperature (16). The rate of albumin synthesis has been linked to dietary protein intake (13). Therefore the increase in serum albumin may be partly due to the added protein of the CHO+Pro supplement.
However these results have only been directly observed in studies comparing CHO+Pro supplements to placebo treatments (as opposed to CHO), and only in studies examining untrained subjects.

There is also indirect evidence suggesting that CHO+Pro may augment cardiovascular adaptations. Ferguson-Stegall et al. (11) reported that CHO+Pro (chocolate milk) supplementation throughout 4.5 weeks of aerobic training resulted in greater increases in VO$_{2\text{max}}$ values compared to CHO alone. The authors speculated that these effects were the result of enhanced cardiovascular adaptations with CHO+Pro ingestion, as discussed previously. Furthermore, Cathcart et al. (7) recently investigated the effects of CHO+Pro supplementation on thermoregulatory capacity, exercise performance, and recovery during eight days of competitive mountain biking in a hot environment. Subjects competing in the TransAlp mountain bike race were divided into a CHO group (76 g·L$^{-1}$ CHO) and a CHO+Pro group (18 g·L$^{-1}$ Pro and 72 g·L$^{-1}$ CHO) and given enough beverages to consume *ad libitum* during each stage of the race. Subjects consuming the CHO+Pro supplement completed stages 2-8 of the race significantly quicker and maintained both body mass and body temperature more effectively throughout the multi-day race. These findings provide preliminary evidence that trained subjects may derive cardiovascular benefits from CHO+Pro supplementation of sufficient magnitude to improve their performance in subsequent exercise.

Few studies have investigated the effects of CHO+Pro supplementation on exercise tolerance during intensified training periods longer than a few days duration. Furthermore, we are aware of no studies that have systematically investigated the impact of CHO+Pro supplementation on cardiovascular responses and performance in well-
trained subjects. Therefore the primary aims of this study are to determine if a) CHO+Pro supplementation during and following exercise results in better maintenance of exercise performance during 10 days of intensified cycle training in trained cyclists compared to CHO b) CHO+Pro supplementation during and following exercise enhances exercise performance following 10 days of reduced volume training in trained cyclists compared to CHO and c) changes in cardiovascular physiology may explain potential performance differences between treatments. We hypothesize that the use of CHO+Pro supplementation during and following exercise will attenuate impairments in endurance performance following intensified training. We also hypothesize that CHO+Pro supplementation will amplify improvements in endurance performance following reduced-volume training. We expect that changes in cardiovascular responses with training will be positively affected by the CHO+Pro supplementation and will (at least partially) explain the augmented performance response observed with CHO+Pro supplementation.

**Assumptions:**

- Subjects will accurately complete all self-reported forms.
- Intensified cycle training will result in a state of overreaching.

**Limitations:**

- Small sample size
- May not be generalizable to athletes who perform less....

**Delimitation:**

- Cyclists with training of \( \geq 7 \) hours a week, for at least two months prior to the study
- No dairy allergies.
**Operational Definitions:**

1. **Intensified cycle training (ICT):** Training volume that is 100% greater than normal training.

2. **Overtraining:** A state in which performance decrements, global mood disruptions, and increased feelings of fatigue occur after repeated bouts (~7 days) of high intensity exercise with little recovery.

3. **Performance Decrement:** Time trial performance that has become worse than baseline levels. An increase in time trial times.
Chapter II

Methodology

Subjects

Five endurance-trained cyclists (4 males and 1 female) were recruited to participate in this study. All cyclists were between the ages of 18-55 and had a training history of $\geq 7$ hours per week of cycle training for $\geq 2$ months prior to the study. A general health questionnaire was used to assess each participant’s health status, and informed consent was obtained before participation in this study. Subjects were required to demonstrate a peak aerobic capacity ($\text{VO}_2\text{peak}$) of $\geq 50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ prior to entry into the study. Participants were given a detailed explanation of the experimental procedures and required commitment. Individuals with preexisting injuries, or with milk allergies were excluded from study participation. The James Madison University’s Institutional Review Board approved all procedures prior to the start of the study.

Experimental Design

The study consisted of two 27-day training periods. The training periods consisted of 7 days of normal training (NT), 10 days of intensified cycle training (ICT) characterized by ~100% increase in average daily training volume relative to NT, and 10 days of reduced volume training (RVT) (Figure 3.1). RVT commenced immediately after ICT and consisted of ~60% of the average daily training volume relative to NT. A 2-week or greater washout period was provided between the training periods. Familiarization and preliminary testing was conducted during the seven days preceding the initial training block (i.e. after 1 week of NT). During the ICT and RVT training
periods subjects were provided with either a carbohydrate (CHO) or carbohydrate plus protein (CHO+Pro) recovery beverage during and immediately following each cycling session. Subjects completed this design in a random treatment order and both the subjects and researchers were blinded to the CHO and CHO+Pro treatments.

Figure 1. Experimental Design

Preliminary Testing

Prior to the beginning of this study subjects performed an incremental test to exhaustion on an electrically braked cycle ergometer (Velotron, RacerMate Inc, Seattle WA) to determine VO_{2peak} and maximal power output (W_{max}). Briefly, subjects warmed-up for 5 minutes at a self-selected, moderate workload. The workload was increased by 25 W every 2 minutes until subjects voluntarily requested to stop due to fatigue or were unable to continue at a cadence >50 rpm. Metabolic measurements (including VO_{2}, CO_{2}, RER, and ventilation) were obtained at 30-second intervals during each stage of the test using indirect calorimetry via an automated Moxus Modular Metabolic System (AEI Technologies, Bostrop TX). Heart rate was obtained using a Polar heart rate monitor.
(Brooklyn, NY). Heart rate and RPE, using Borg’s 6 to 20 scale, were recorded every 60 s. This initial test was used to obtain the VO$_{2peak}$ used for inclusion/exclusion, and as a familiarization trial for subsequent VO$_{2peak}$ testing. Subjects also completed a familiarization time-trial (TT). This took place the week before ICT and was completed on a computerized electrically braked cycle ergometer (Velotron, Racermate Inc, Seattle WA). The TT consisted of 120 minutes of cycling at 50% $W_{max}$ (obtained from VO$_{2peak}$ testing) followed by a simulated 30-km time trial (as described below). No measurements were taken during this TT, other than power output and time to completion.

**Experimental Training**

Following baseline data collection, subjects began NT. Subjects were asked to complete and record a typical week of training during this time. Each subject was provided with a training diary to record all training sessions, and a rear bicycle wheel equipped with an integrated PowerTap system (Saris Cycling Group Inc, Madison WI), allowing subjects to monitor daily workouts with the recording of power output ($W_{Avg}$). These tools were used to quantify power output, HR, and baseline training loads (distance covered, and time). From this data, training duration (minutes) was used to quantify training volume. Immediately following NT, subjects performed 10 days of ICT. During the ICT phase, subjects were required to complete a 100% work increase of their daily average training volume relative to NT. This magnitude of overload is comparable to previously used training protocols that resulted in impaired performance following ICT (10, 19). On days 1, 4, 7, & 10 of ICT, and day 10 of RVT an endurance performance test (time-trial) was conducted, lasting approximately three hours (described below). These
endurance performance tests contributed to the total training load during ICT. The remaining days of ICT were performed outside of the laboratory. During this time subjects were provided with detailed training guidelines that were based on individualized training volume to ensure a 100% increase in training volume was being met. Outside of the lab, to ensure proper training volume, the subject monitored and recorded HR, power output, and training duration, using the same methods discussed previously. Immediately following ICT, subjects performed 10 days of RVT consisting of a 60% reduction in average training volumes (relative to NT). Ten days of reduced volume training has been shown to restore/improve cycling performance (18, 45). During all exercise sessions and immediately post exercise, CHO and CHO+Pro beverages were provided, as described below. A two-week washout period (WO) followed the completion of the RVT phase. WO consisted of an individualized initial period of recovery, with the intent of restoring normal training loads by the end of the washout period. Following the washout period, subjects completed one week of NT (matching the training volumes/intensities from the first NT period), before initiating a second phase of ICT and RVT training (while consuming the second beverage treatment).

**Nutritional Supplements**

During ICT and RVT subjects were provided with CHO or CHO+Pro beverages to consume during and immediately following each training session. During all laboratory TT sessions (Figure 2), subjects ingested 250 ml of fluid every 20 minutes until TT completion (750 ml·hr⁻¹; details provided below). For all rides performed in the field (ICT days 2, 3, 5, 6, 8, 9 and RVT days 2-10), participants were provided with 500
ml bottles filled with the appropriate beverage, and instructed to ingest 1 bottle during each 40-minute block of training (750 ml·hr⁻¹). Following each ride, participants were given bottles containing an individualized volume of fluid (see details below) and instructed to finish the beverage within 30 minutes of terminating exercise. Participants avoided any other beverage or food intake for 2 hrs following the completion of each exercise session, with the exception of *ad libitum* water consumption.

The during-exercise CHO treatment was a commercial sports drink containing 6% CHO by volume (Gatorade®). During all training sessions CHO was ingested at a rate of 45 g CHO·hr⁻¹ (750 ml·hr⁻¹), providing equal carbohydrate content to CHO+Pro. The post exercise CHO recovery beverage provided 1.2 g CHO·kg BW⁻¹. The beverage was created by mixing the appropriate amount of commercially available chocolate flavored carbohydrate gels (Clif Shots) with water, providing a similar taste and color to the post exercise CHO+Pro beverage (low-fat chocolate milk).

The during-exercise CHO+Pro treatment was Gatorade® (6% CHO by volume) with additional hydrolyzed whey protein isolate (2% Pro by volume). The CHO+Pro beverage was ingested at a rate of 750 ml·hr⁻¹, providing equal fluid and carbohydrate content to the CHO treatment. The post-exercise CHO+Pro beverage was 9.93 ml·kg BW⁻¹ of a low-fat chocolate milk beverage, providing 1.2 g CHO·kg BW⁻¹. This provided equal calories and carbohydrates to the post-exercise CHO beverage.

**Dietary Controls**

Subjects met with a dietician prior to the beginning of the study. During this meeting a food scale was supplied and a general orientation on how to weight and record
daily intakes was provided. Also, 72-hour dietary records were gathered during NT, whereupon individualized feedback was provided about total caloric- and macronutrient intake; participants with inadequate daily carbohydrate intake (<6.5 g·kgBW$^{-1}$·day$^{-1}$) were encouraged to increase their dietary carbohydrate levels prior to the training intervention. Dietary intake was also recorded throughout ICT (10 days) and RVT (10 days). The dietary intake was analyzed using Nutrition Data System for Research software (NDSR, University of Minnesota) producing a detailed outline of macro and micro nutrient intake. During the time period between the onset of each training session and 2 hours following each training session, participants did not receive any nutrients other than the CHO or CHO+Pro beverages. All laboratory testing (i.e. VO$_{2\text{peak}}$ tests, and TT) was performed after an 8-10 hr overnight fast (ad libitum water consumption). Following each laboratory test a standardized lunch was provided. The lunch was chosen prior to starting the study according to participate preferences. Each lunch contained 1-2 standard sandwiches, chips or a cookie, and water. Using copies of dietary records obtained from the first intervention phase, subjects were instructed to replicate their dietary habits during the second phase of the cross-over design. Subjects were also provided with nutritional choices to match previous intake, helping to maintain a similar diet between phases. 

**Performance Measurements**

**Endurance Performance:** To determine endurance performance subjects completed TTs on a computerized cycle ergometer (VeloTron, Racermate Inc., Seattle WA), on days 1, 4, 7, & 10 of ICT and on day 10 of RVT. Upon arrival at the laboratory, subjects were fitted with a HR monitor and instructed on the procedures of the TT. Subjects initially
performed a preload consisting of 120 minutes of cycling at 50% $W_{\text{max}}$ followed by a simulated 30-km time trial. The only information provided to subjects during the time trial was distance completed. 30-km TT finishing times and average power output were used as performance measures.

**VO$_{2\text{peak}}$ (aerobic capacity):** Subjects performed an incremental exercise test to exhaustion on a bicycle ergometer (Velotron), as described above, on day 7 of NT, day 9 of ICT, and day 9 of RVT (Figure 2). The highest sustainable (30 seconds) power output ($W_{\text{max}}$) was used to calculate exercise intensities for the 120-minute preload segment of the TT (50% $W_{\text{max}}$). Test duration (time to fatigue) was also used as a measure of performance.

**Metabolic/Cardiovascular Measurements**

**Respiratory Exchange Ratio, VO$_2$, Blood Lactate and Glucose, and Heart Rate:** Breath samples were obtained at minutes 25-30, 55-60, 85-90, and 115-120 of the 120-min preload TT during all 10 TT. The final three minutes of gas collection at each time point were aggregated to represent a mean for VO$_2$, expired ventilation (VE), and respiratory exchange ratio (RER). Finger stick blood samples were also obtained at these same time points. Blood glucose (Glu) and lactate levels (Lac) were immediately analyzed using an automated tabletop analyzer (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, OH). Submaximal and peak heart rate (HR) were assessed during each VO$_{2\text{peak}}$ test and throughout the 120-minute submaximal preload segment of each TT on days 1 and 10 of ICT and day 10 of RVT. Heart rate was obtained using Polar heart-rate monitors (Brooklyn, NY).
Plasma Albumin, Hemoglobin, and Hematocrit: Fasting venous blood samples were obtained from an antecubital vein prior to TT on days 1 and 10 of ICT and day 10 of RVT. Plasma albumin was assessed using standard enzyme-linked immunosorbent assay (ELISA) procedures. Finger stick blood samples were also obtained at these same time points. Hemoglobin (Hgb) and hematocrit (Hct) were immediately analyzed using a Hemo Control hemoglobin and hematocrit analyzer (EKF Diagnostics, London, United Kingdom).

Ratings of Perceived Exertion (RPE): RPE was recorded every 30 minutes during the 120-minute preload during each of the 10 TT.

Statistical Analysis
Statistical testing was conducted using SPSS version 17.0 (Thomson Learning, Pacific Grove, CA), using an alpha level of p < 0.05 for all analyses. Two-way ANOVAs (treatment*time) were used to determine differences between treatments for all variables.

Due to limitation in examining performance-related measurements with traditional null hypothesis testing, magnitude-based inferences about the data were made using methods described by Hopkins and colleagues (22). 90% confidence intervals (CI) are presented to illustrate uncertainty in treatment effects. Threshold values for substantial change were calculated as 0.2 x SD (from CHO trial). A published spreadsheet (5) was use to classify treatment effects as beneficial/positive, harmful/negative, or trivial/negligible. Likelihoods of reaching the substantial change threshold were classified as <1% almost certainly no chance, 1-5% = very unlikely, 5-
25% = unlikely, 25-75% = possible, 75-95% = likely, 95-99% = very likely, and >99% = almost certain (45). All data is presented as percent difference, 90% CI, and p-value.
Figure 2. Study Schematic with Corresponding Data Collection

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VO₂pk = VO₂peak, TT = preloaded time trial
Note: This design was repeated twice (Figure 1). Therefore, days -1, -2, and -3 correspond to the final 3 days of normal training, whereas day +1 corresponds to day 1 of washout or study completion.
Chapter III

Manuscript

Effects of carbohydrate + protein co-ingestion during and following exercise on cardiovascular adaptations and exercise tolerance during intensified cycle training.

Abstract

Introduction: This study investigated the effects of carbohydrate and protein (CHO+Pro) supplementation during intensified training on cardiovascular adaptations and cycling performance. Methods: Five cyclists (VO_{2peak} = 62.6 ± 10.7 ml/kg/min) performed two 27-day training protocols while receiving either CHO or CHO+Pro supplements. The exercise protocols consisted of 7 days of normal training (NT), 10 days of intensified cycle training (ICT; 100% increase in average training duration versus NT), and 10 days of reduced volume training (RVT; reduction in training duration to 60% of NT). Performance was assessed by a 30-km time trial following 120 min of constant-load cycling and VO_{2peak} testing. Other physiological measures were assessed at rest (heart rate, hematocrit, hemoglobin, and albumin) and during exercise (heart rate, ventilation, RER, RPE, VO_{2}, glucose, and lactate). Supplements were consumed during exercise (750 ml·hr^{-1}; CHO = 6% concentration; CHO+PRO = 6% CHO, 2% Pro concentrations) and immediately following exercise (9.93 ml of fluid·kg BW^{-1}; CHO = 1.2 g CHO·kg BW^{-1}; CHO+PRO = 1.2 g CHO·kg BW^{-1} + 0.3 g Pro·kg BW^{-1}). Data was analyzed using magnitude-based inferences. Results: Time to complete the 30-km TT and average TT power were ‘likely’ impaired following ICT, with no differences between treatments. Following RVT, time to complete the 30-km TT and average TT power were ‘likely’ improved versus ICT, with no differences between treatments. Between NT and ICT
constant-load HR significantly decreased (independent of treatment), with a ‘very likely’ larger reduction with CHO+Pro (135±13 bpm to 128±10 bpm) versus CHO (133±15 bpm to 132±13 bpm). Between NT and RVT, constant-load HR also ‘very likely’ declined to a greater extent with CHO+Pro (135±13 bpm to 132±14) than with CHO (133±15 to 138±16 bpm). Serum albumin was ‘likely’ increased following ICT and RVT (independent of treatment), but differences between treatments were ‘unclear’.

Conclusion: These findings suggest exercising heart rates in well trained-cyclists are attenuated with carbohydrate and protein supplementation, although the cause of the reduced in HR is unclear. In addition, the implications of these findings require further study, as overall exercise tolerance and adaptations following intensified training were similar to those observed with carbohydrate supplementation.
Introduction

Endurance athletes often utilize periods of intensified training in an attempt to enhance performance. However, sustained periods of heavy training with inadequate recovery can lead to symptoms of non-functional overreaching/overtraining, such as impaired performance, and increases in negative mood states and perceived exertion during exercise (16, 17, 21). Nutritional interventions have been successfully utilized to promote recovery during periods of intensified training. For example, increased carbohydrate (CHO) intake immediately following exercise has been shown to improve glycogen re-synthesis (39, 40), and result in better maintenance of performance and mood following heavy training (1, 15, 39, 40).

In recent years numerous studies have reported that co-ingestion of carbohydrate and protein (CHO+Pro) during short-term recovery following heavy exercise improves performance in subsequent exercise versus CHO alone (2, 4, 6, 8, 20, 22, 30, 37, 42). Enhanced glycogen re-synthesis (5, 19, 24, 45), and/or reduced muscle damage (12, 14, 25–27, 36, 41, 43) are possible explanations for improvements in performance, though not all studies have reported these benefits (7, 34, 36, 38). Despite evidence from short-term studies, the effects of CHO+Pro ingestion on exercise tolerance, training adaptations and performance during sustained periods of heavy training/competition are poorly understood. A few studies have investigated CHO+Pro supplementation during 3-6 days of training, and reported reductions in markers of muscle damage, such as creatine kinase (CK) (12, 25, 41) and muscle soreness (25). However, it is unclear whether regular intake of CHO+Pro beverages has a positive impact on performance following heavy training to a greater degree than CHO beverages. Witard et al. (44) reported increased dietary
protein led to “possibly attenuated” psychological stress symptoms, and “possibly reduced” decrements in performance following a week of intensified training. However, this study compared large differences in dietary protein intake (CON: 1.5 g Pro·kg BW⁻¹; Pro: 3.0 g Pro·kg BW⁻¹), and did not specifically examine supplemental protein intake provided during and/or following exercise.

Recent studies have suggested that CHO+Pro supplementation may also augment cardiovascular adaptations following training (13, 31–33). Specifically, CHO+Pro supplementation has been associated with increased serum albumin content and plasma volume, resulting in increased stroke volume, lower heart rate, and enhancements in thermoregulatory responses to heat (8, 11, 13, 32, 33). However these results have only been directly observed in studies comparing CHO+Pro supplements to placebo treatments (as opposed to CHO), and only in studies examining untrained subjects. Ferguson-Stegall et al. (10) reported that subjects receiving CHO+Pro supplementation throughout 4.5 weeks of aerobic training had greater increases in VO₂max compared to those receiving CHO. The authors speculated that these effects were probably the result of enhanced cardiovascular adaptations with CHO+Pro ingestion, but cardiovascular variables were not directly assessed. Furthermore, Cathcart and associates (8) reported that cyclists receiving CHO+Pro supplementation during eight days of competitive mountain biking had better performance times and enhanced thermoregulatory responses compared to those receiving CHO (8). However, this was a field-based study, which prevented the standardization of a variety of factors, including exercise intensity, which presumably varied substantially throughout each race stage. This makes it impossible to determine the effects of the supplements on physiological responses during constant-load exercise.
Therefore, these findings provide preliminary evidence that trained subjects may derive cardiovascular benefits from CHO+Pro supplementation, but this hypothesis has yet to be examined in controlled studies. Therefore, the purpose of this study was to examine the effects of CHO+Pro supplementation (versus CHO) on cardiovascular responses and exercise performance during intensified cycle training, and following a subsequent period of reduced volume training in trained cyclists.

**Methods**

**Subjects**

Five endurance-trained cyclists (4 males and 1 female) were recruited to participate in this study. Inclusion criteria for this study were that all cyclists were between the ages of 18-55 and had a training history of ≥ 7 hours per week of cycle training for ≥ 2 months prior to the study. A general health questionnaire was used to assess each participant’s health status, and informed consent was obtained before participation in this study. Subjects were required to demonstrate a peak aerobic capacity (VO$_{2peak}$) of ≥ 50 ml·kg$^{-1}$·min$^{-1}$ prior to entry into the study. Participants were given a detailed explanation of the experimental procedures and required commitment. Individuals with preexisting injuries or milk allergies were excluded from participation. The James Madison University’s Institutional Review Board approved all procedures prior to the start of the study.

**Experimental Design**

The study consisted of two 27-day training periods. The training periods consisted of 7 days of normal training (NT), 10 days of intensified cycle training (ICT) characterized by a training prescription of ~ 100% increase in average daily training
volume relative to NT, and 10 days of reduced volume training (RVT) (Figure 3.1). RVT commenced immediately after ICT and consisted of a training prescription of ~60% of the average daily training volume relative to NT. A 2-week or greater washout period was provided between the training periods. Familiarization and preliminary testing was conducted during the seven days preceding the initial training block (i.e. after 1 week of NT). During ICT and RVT training subjects were provided with either carbohydrate (CHO) or carbohydrate plus protein (CHO+Pro) beverages during and immediately following each cycling session. Subjects completed this design in a random treatment order and both the subjects and researchers were blinded to the CHO and CHO+Pro treatments. Female subjects started each 27-day training block on the same day of their menstrual cycle to minimize the impact of menstrual phase on responses between treatments.

Figure 1. Experimental Design

![Experimental Design Diagram]
Preliminary Testing

Prior to the beginning of this study subjects performed an incremental test to exhaustion on an electrically braked cycle ergometer (Velotron, RacerMate Inc, Seattle WA) to determine VO_{2peak}, and maximal power output (W_{max}). Briefly, subjects warmed-up for 5 minutes at a self-selected, moderate workload. The workload was increased by 25 W every 2 minutes until subjects voluntarily requested to stop due to fatigue or were unable to continue at a cadence >50 rpm. Metabolic measurements (including VO_{2}, CO_{2}, RER, and ventilation) were obtained at 30-second intervals during each stage of the test using indirect calorimetry via an automated Moxus Modular Metabolic System (AEI Technologies, Bostrop TX). Heart rate was obtained using a Polar heart rate monitor (Brooklyn, NY). Heart rate and RPE, using Borg’s 6 to 20 scale, were recorded every 60s. This initial test was used to obtain the VO_{2peak} used for inclusion/exclusion, and as a familiarization trial for subsequent VO_{2peak} testing. Subjects also completed a familiarization time-trial (TT). This took place the week before ICT and was completed on a computerized electrically braked cycle ergometer (Velotron, RacerMate Inc, Seattle WA). The TT consisted of 120 minutes of cycling at 50% W_{max} (obtained from VO_{2peak} during preliminary testing) followed by a simulated 30-km time trial (as described below). No measurements were taken during this TT, other than power output and time to completion.

Experimental Training

Following preliminary testing, subjects began NT. Subjects were asked to complete and record a typical week of training during this time. Each subject was provided with a training diary to record all training sessions, and a rear bicycle wheel
equipped with an integrated PowerTap system (Saris Cycling Group Inc, Madison WI), allowing subjects to monitor daily workouts with the recording of power output ($W_{Avg}$). These tools were used to quantify power output, HR, and baseline training loads (distance covered and time). From this data, training duration (minutes) was used to quantify training volume. Immediately following NT, subjects performed 10 days of ICT. During the ICT phase, subjects were prescribed a training load which doubled their daily average training volume during NT. This magnitude of overload is comparable to previously used training protocols that resulted in impaired performance following ICT (9, 16). On days 1, 4, 7, & 10 of ICT, and day 10 of RVT, an endurance performance test (time-trial) was conducted, lasting approximately three hours (described below). These endurance performance tests contributed to the total training load during ICT and RVT. The remaining days of ICT were performed outside of the laboratory. During this time subjects were provided with detailed training guidelines that were based on individualized training volume to ensure a 100% increase in training volume was being met. Outside of the lab, to ensure proper training volume, the subject monitored and recorded HR, power output, and training duration, using the same methods discussed previously. Immediately following ICT, subjects performed 10 days of RVT, which consisted of a reduction in average training volume to 60% of NT. Ten days of reduced volume training has been shown to restore/improve cycling performance (15, 44). During all exercise sessions and immediately post exercise, CHO and CHO+Pro beverages were provided, as described below. A two-week washout period (WO) followed the completion of the RVT phase. WO consisted of an individualized initial period of recovery, with the intent of restoring normal training loads by the end of the washout
period. Following the washout period, subjects completed one week of NT (matching the training volumes/intensities from the first NT period), before initiating a second phase of ICT and RVT training (while consuming the second beverage treatment).

**Nutritional Supplements**

During ICT and RVT subjects were provided with CHO or CHO+Pro beverages to consume during and immediately following each training session. During all laboratory TT sessions (Figure 2), subjects ingested 250 ml of fluid every 20 minutes until TT completion (750 ml of fluid·hr⁻¹; details provided below). For all rides performed in the field (ICT days 2, 3, 5, 6, 8, 9 and RVT days 2-10), participants were provided with 500 ml bottles filled with the appropriate beverage, and instructed to ingest 1 bottle during each 40-minute block of training (750 ml of fluid·hr⁻¹). Following each ride, participants were given bottles containing an individualized volume of fluid (see details below) and instructed to finish the beverage within 30 minutes of terminating exercise. Participants avoided any other beverage or food intake for 2 hrs following the completion of each exercise session, with the exception of *ad libitum* water consumption.

The during-exercise CHO treatment was a commercial sports drink containing 6% CHO by volume (Gatorade®). During all training sessions CHO was ingested at a rate of 45 g CHO·hr⁻¹ (750 ml of fluid·hr⁻¹), providing equal carbohydrate content to CHO+Pro. The post exercise CHO recovery beverage provided 1.2 g CHO·kg BW⁻¹. The beverage was created by mixing the appropriate amount of commercially available chocolate flavored carbohydrate gels (Clif Shots) with water, providing a similar taste and color to the post exercise CHO+Pro beverage (low-fat chocolate milk).
The during-exercise CHO+Pro treatment was Gatorade® (6% CHO by volume) with additional hydrolyzed whey protein isolate (2% Pro by volume) (American Casein Company, Burlington NJ, USA). The CHO+Pro beverage was ingested at a rate of 750 ml of fluid·hr⁻¹, providing equal fluid and carbohydrate content to the CHO treatment. The post-exercise CHO+Pro beverage was 9.93 ml of fluid·kg BW⁻¹ of a low-fat chocolate milk beverage, providing 1.2 g CHO·kg BW⁻¹. This provided equal carbohydrates to the post-exercise CHO beverage.

**Dietary Controls**

Subjects met with a registered dietician prior to the study. During this meeting, a food scale was supplied and specific information regarding how to weigh and record daily intakes was provided. 72-hour dietary records were gathered during NT, whereupon individualized feedback was provided about total caloric- and macronutrient intake; participants with inadequate daily carbohydrate intake (<6.5 g·kgBW⁻¹·day⁻¹) were encouraged to increase their dietary carbohydrate levels prior to the training intervention. Dietary intake was also recorded throughout ICT (10 days) and RVT (10 days). During the time period between the onset of each training session and 2 hours following each training session, participants did not receive any nutrients other than the CHO or CHO+Pro beverages. All laboratory testing (i.e. VO₂peak tests, and TT) was performed after an 8-10 hr overnight fast (*ad libitum* water consumption). Following each laboratory test a standardized lunch was provided. The lunch was chosen prior to starting the study according to participant preferences. Each lunch contained 1-2 standard sandwiches, chips or a cookie, and water. Using copies of dietary records obtained from the first intervention phase, subjects were instructed to replicate their dietary habits during the
second phase of the cross-over design. Subjects were also provided with nutritional choices to match previous intake, helping to maintain a similar diet between phases.

**Performance Measurements**

*Endurance Performance:* To determine endurance performance subjects completed TTs on a computerized cycle ergometer (VeloTron, Racermate Inc., Seattle WA), on days 1, 4, 7, and 10 of ICT and on day 10 of RVT. Subjects initially performed a preload consisting of 120 minutes of cycling at 50% $W_{\text{max}}$ followed by a simulated 30-km time trial. The only information provided to subjects during the time trial was distance completed. 30-km TT finishing times and average power output were used as performance measures.

$VO_{2\text{peak}}$ (aerobic capacity): Subjects performed an incremental exercise test to exhaustion on a bicycle ergometer (Velotron), as described above, on day 7 of NT, day 9 of ICT, and day 9 of RVT (Figure 2), under both treatment conditions. The highest sustainable (30 seconds) power output ($W_{\text{max}}$) was used to calculate exercise intensities for the 120-minute preload segment of the TT ($50\% W_{\text{max}}$ obtained during preliminary $VO_{2\text{peak}}$ test). Test duration (time to fatigue) was also used as a measure of performance.

**Metabolic/Cardiovascular Measurements**

*Respiratory Exchange Ratio, $VO_2$, Blood Lactate and Glucose, and Heart Rate:* Breath samples were obtained at minutes 25-30 and 115-120 of the 120-min preload TT during all 10 TT. The final three minutes of gas collection at each time point were aggregated to represent a mean for $VO_2$, expired ventilation (VE), and respiratory exchange ratio (RER). Finger stick blood samples were also obtained at these same time points. Blood glucose (Glu) and lactate levels (Lac) were immediately analyzed using an automated
tabletop analyzer (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, OH). Submaximal and peak heart rate (HR) were assessed during each VO$_{2}^{\text{peak}}$ test and throughout the 120-minute submaximal preload segment of each TT on days 1 and 10 of ICT and day 10 of RVT. Heart rate was obtained using Polar heart-rate monitors (Brooklyn, NY).

*Plasma Albumin, Hemoglobin, and Hematocrit:* Fasting venous blood samples were obtained from an antecubital vein prior to TT on days 1 and 10 of ICT and day 10 of RVT. Plasma albumin was assessed using standard enzyme-linked immunosorbent assay (ELISA) procedures. Finger stick blood samples were also obtained at these same time points. Hemoglobin (Hgb) and hematocrit (Hct) were immediately analyzed using a Hemo Control hemoglobin and hematocrit analyzer (EKF Diagnostics, London, United Kingdom).

*Ratings of Perceived Exertion (RPE):* RPE was recorded at minutes 30 and 120 during the 120-minute preload during each of the 10 TT.

**Statistical Analysis**

Statistical testing was conducted using SPSS version 17.0 (Thomson Learning, Pacific Grove, CA). Two-way repeated measures ANOVAs (treatment*time) were used to determine effects between time-points (i.e. NT vs. ICT), and treatment*time effects for all variables.

Due to limitations in examining performance-related measurements with traditional null hypothesis testing, magnitude-based inferences about the data were made using methods described by Hopkins and colleagues (18). Threshold values for substantial change were calculated as 0.2 x SD (from CHO trial). A published
spreadsheet (18) was used to classify treatment effects as beneficial/positive, harmful/negative, or trivial/negligible. Likelihoods of reaching the substantial change threshold were classified as <1% almost certainly no chance, 1-5% = very unlikely, 5-25% = unlikely, 25-75% = possible, 75-95% = likely, 95-99% = very likely, and >99% = almost certain (44). Effects were described as unclear if confidence intervals overlapped onto both positive and negative values. P-values are presented for all data.
Figure 2. Study Schematic with Corresponding Data Collection

<table>
<thead>
<tr>
<th>Days</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>+1</th>
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</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>VO\textsubscript{2pk}</td>
<td>X</td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
</tbody>
</table>

VO\textsubscript{2pk} = VO\textsubscript{2peak}, TT = preloaded time trial
Note: This design was repeated twice (Figure 1). Therefore, days -1, -2, and -3 correspond to the final 3 days of normal training, whereas day +1 corresponds to day 1 of washout or study completion.
Results

Subjects

Statistical analyses were conducted on data from the 5 subjects (4 male and 1 female) who completed all testing. Average (±SD) age, height, weight, maximal oxygen consumption (VO$_{2\text{peak}}$), and work max (W$_{\text{max}}$) of the participants were 27.6 ± 8.2 y, 176.4 ± 8.3 cm, 74.4 ± 10.2 kg, 62.6 ± 10.7 ml/kg/min, 330 ± 60 watts, respectively.

Training Duration and Intensity

As illustrated in Table 1, training duration increased (100%) between NT and ICT (‘mostly likely’, p < 0.001) in both treatments. With respect to training intensity, average training heart rate ‘very likely’ decreased (p = 0.025) during ICT (independent of treatment). Similarly, average power output during ICT was ‘likely’ (p = 0.072) lower than NT. Treatment*time interactions between NT-ICT were ‘very likely’ for power (p = .063) and ‘possible’ for duration (p = .396). Changes were ‘unclear’ for HR.

Training duration during RVT was reduced to approximately 60% of NT duration (‘mostly likely’, p < 0.001) with no differences between treatments. With respect to training intensity, heart rate responses during RVT were similar to NT (‘unclear’), while average power output was ‘possibly’ higher (p = .149) during RVT (independent of treatment). A treatment*time interaction between NT-RVT was ‘very likely’ for power (p = .068) and ‘possible’ for durations (p = .696. All other variables were ‘unclear’.
### Table 1: Training Data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal Training</th>
<th></th>
<th>Intensified Training</th>
<th></th>
<th>Reduced Volume Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration (min/day)</td>
<td>Heart rate (bpm)</td>
<td>Power (watts)</td>
<td>Duration (min/day)</td>
<td>Heart rate (bpm)</td>
</tr>
<tr>
<td>CHO</td>
<td>72±6</td>
<td>142±17.4</td>
<td>184±34</td>
<td>143±13</td>
<td>134±12</td>
</tr>
<tr>
<td>CHO+Pro</td>
<td>74±6</td>
<td>140±12</td>
<td>173±32</td>
<td>147±13</td>
<td>132±10</td>
</tr>
</tbody>
</table>

CHO=carbohydrate; CHO+Pro=carbohydrate + protein

**Time-effects:**
Changes between NT-ICT were ‘Mostly Likely’ for: Duration; Changes were ‘Very Likely’ for: HR; Changes were ‘Likely’ for: Power
Changes between NT-RVT were ‘Mostly Likely’ for: Duration; Changes were ‘Possible’ for: Power; Changes were ‘Unclear’ for: HR

**Treatment*time effects:**
Changes between NT-ICT were ‘Very Likely’ for: Power; Changes were ‘Possible’ for: Duration; Changes were ‘Unclear’ for: HR
Changes between NT-RVT were ‘Very Likely’ for: Power; Changes were ‘Possible’ for: Duration; Changes were ‘Unclear’ for: HR
Effects of Training and Treatment Beverages

Endurance Performance and Aerobic Capacity

Table 2 displays cycling performance variables following NT, ICT, and RVT under both treatment conditions. Independent of treatment, VO_{2peak} Time was ‘very likely’ decreased (p = 0.050) following ICT. Similarly, Average TT Power was ‘likely’ decreased (p = 0.144 and TT Time ‘likely’ increased (p = 0.215) following ICT. Changes in VO_{2peak} were ‘possible’ between NT-ICT (p=0.473), and changes in W_{max} were ‘trivial’ (p = 0.426). All other variables demonstrated ‘unclear’ differences between these time-points. A treatment*time interaction between NT-ICT was ‘likely’ for VO_{2peak} (p =0.178), with all other treatment*time effects ‘unclear’.

The RVT period also altered cycling performance measurements. Average TT Power (p = 0.061), VO_{2peak} time (p = 0.089), and TT time (p=.071) were ‘likely’ increased from ICT to RVT (independent of treatment). Changes from ICT to RVT were ‘trivial’ for VO_{2peak} (p = 0.640) and W_{max} (p = 0.305), with changes in all other variables ‘unclear’. Treatment*time interactions between ICT-RVT were ‘possible’ for Average TT Power (p = 0.570) and W_{max} (p = 0.621), with changes in all other variables ‘unclear’.

When comparing performance variables from NT to RVT there was a ‘likely’ increase in VO_{2peak} (p =0.187). Changes in TT Time (p = 0.667) and Average TT Power (p = 0.624) were ‘trivial’, and all other variable were ‘unclear’. Treatment*time interactions between NT-RVT were ‘unclear’ for all performance variables.
Table 2: Performance Measurements Following Each Training Period

<table>
<thead>
<tr>
<th></th>
<th>Normal Training</th>
<th></th>
<th>Intensified Training</th>
<th></th>
<th>Reduced Volume Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
<td>CHO+Pro</td>
<td>CHO</td>
<td>CHO+Pro</td>
<td>CHO</td>
</tr>
<tr>
<td>TT Time (min)</td>
<td>54.9±5.9</td>
<td>54.8±7.2</td>
<td>57.3±10.1</td>
<td>57.6±9.5</td>
<td>55.1±6.4</td>
</tr>
<tr>
<td>Average TT Power (watts)</td>
<td>204±52</td>
<td>213±60</td>
<td>194±63</td>
<td>190±57</td>
<td>203±48</td>
</tr>
<tr>
<td>VO₂peak (ml/kg/min)</td>
<td>63.4±10.8</td>
<td>63.7±12.0</td>
<td>66.9±8.7</td>
<td>62.6±13.3</td>
<td>67.0±12.0</td>
</tr>
<tr>
<td>W₉₉₉₉max (watts)</td>
<td>330±65</td>
<td>330±76</td>
<td>320±57</td>
<td>325±61</td>
<td>330±57</td>
</tr>
<tr>
<td>VO₂peak Time (min)</td>
<td>14.3±3.2</td>
<td>14.7±4.6</td>
<td>12.4±2.1</td>
<td>12.6±2.4</td>
<td>13.4±2.6</td>
</tr>
</tbody>
</table>

CHO=carbohydrate; CHO+Pro=carbohydrate + protein.

Time-effects:
Changes between NT-ICT were ‘Very Likely’ for VO₂peak Time; Changes were ‘Likely’ for: TT Time and Average TT Power; Changes were ‘Possible’ for: VO₂peak; Changes were ‘Trivial’ for: W₉₉₉₉max; All other variables were ‘Unclear’.

Changes between ICT-RVT were ‘Likely’ for: Average TT Power, VO₂peak Time, and TT Time; Changes were ‘Trivial’ for: VO₂peak, and W₉₉₉₉max.

Changes between NT-RVT were ‘Trivial’ for: TT Time, Average TT Power, and VO₂peak; All other variable were ‘Unclear’

Treatment*time effects:
Changes between NT-ICT were ‘Likely’ for: VO₂peak; All other other variables were ‘Unclear’.

Changes between ICT-RVT were ‘Possible’ for: Average TT Power and W₉₉₉₉max; All other variables were ‘Unclear’

Changes between NT-RVT were ‘Unclear’ for all variables

Cardiovascular Measurements at Rest

Table 3 displays resting cardiovascular measurements after NT, ICT, and RVT for both treatment conditions. Albumin was ‘likely’ increased (p = 0.122) from NT to ICT, independent of treatment. All other variables displayed ‘unclear’ effects over time, and all treatment*time effects between NT-ICT were also ‘unclear’.

Changes between ICT-RVT were ‘likely’ for hemoglobin (p = 0.294), and ‘possible’ for albumin (p = 0.395) and hematocrit (p = 0.587) (independent of treatment).
All other variables were ‘unclear’. A treatment*time interaction between ICT-RVT was ‘possible’ for hematocrit (p = 0.587), with effects for all other variables ‘unclear’.

Albumin (p = 0.120), hemoglobin (p = 0.096), and hematocrit (p = 0.140) were all ‘likely’ increased from NT to RVT (independent of treatment). All other effects between these time-points were ‘unclear’. Treatment*time effects between NT-RVT were ‘likely’ for hemoglobin (p = 0.343), and ‘possible’ for hematocrit (p = 0.586). All other treatment*time effects between NT-RVT were ‘unclear’. 
Table 3: Cardiovascular Measurements at Rest

<table>
<thead>
<tr>
<th></th>
<th>Normal Training</th>
<th>Intensified Training</th>
<th>Reduced Volume Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
<td>CHO+Pro</td>
<td>CHO</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>64±5</td>
<td>59±3</td>
<td>61±7</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>14.8±1.0</td>
<td>15.0±1.5</td>
<td>15.1±1.5</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>43.6±3.0</td>
<td>43.3±3.9</td>
<td>44.1±4.6</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.5±1.0</td>
<td>5.4±0.8</td>
<td>6.1±0.6</td>
</tr>
</tbody>
</table>

CHO=carbohydrate; CHO+Pro=carbohydrate + protein; HR=heart rate; Hgb=hemoglobin; Hct=Hematocrit.

Time-effects:
Changes between NT-ICT were ‘Likely’ for: Albumin; All other variables were ‘Unclear’
Changes between ICT-RVT were ‘Likely’ for: Hgb; Changes were ‘Possible’ for: Albumin, and Hct; All other variables were ‘Unclear’
Changes between NT-RVT were ‘Likely’ for: Albumin, Hgb, and Hct; All other variables were ‘Unclear’.

Treatment*time effects:
Changes between NT-ICT were ‘Unclear’ for all variables
Changes between ICT-RVT were ‘Possible’ for: Hct; All other variables were ‘Unclear’
Changes between NT-RVT were ‘Likely’ for: Hgb; Changes were ‘Possible’ for: Hct; All other variables were ‘Unclear’
Constant Load Metabolic/Cardiovascular Measurements

Table 4 and Figure 3 display the metabolic and cardiovascular responses during constant-load exercise during NT, ICT, and RVT. Early (30 min) and late (120 min) exercise responses are shown in Table 4. However, because we observed no likely treatment*time*timepoint interactions, we removed timepoint (30 min versus 120 min) from subsequent analyses to allow simpler interpretation. Therefore, the treatment* time interactions reported below represent an aggregate value for the early and late time-points for all variables. Following ICT, there were ‘likely’ increases in RPE (p = 0.166), and ‘likely’ decreases in VE (p = 0.112) and HR (p = 0.139) versus NT, independent of
treatment. All other time effects between NT-ICT were ‘unclear’. Treatment*time interactions from NT to ICT were ‘very likely’ for HR (p = 0.045), and ‘likely’ for VE (p = 0.366) and RER (p = 0.293). All other treatment*time effects during this time-point were ‘unclear’.

Following the RVT period, there were ‘likely’ changes in VE (p = 0.087), RER (p = 0.424), and HR (p = 0.107) in comparison to ICT (independent of treatment). Changes were also ‘possible’ for VO₂ (p = 0.968). All other variables were ‘unclear’.

Treatment*time interactions between ICT-RVT were ‘very likely’ for lactate (p = 0.065), ‘likely’ for glucose (p = 0.279), possible’ for VE (p = 0.892), and ‘trivial’ for VO₂ (p = 0.864); All other variables were ‘unclear’.

Changes in responses between NT-RVT were ‘trivial’ for VE (p = 0.486), VO₂ (p = 0.060) and HR (p = 0.758) (independent of treatment). Effects for all other variables between NT-ICT were ‘unclear’. Treatment*time interactions were ‘very likely’ for HR (p = 0.054), and ‘likely’ for RER (p = 0.146). In addition, changes were ‘possible’ for VE (p = 0.358), and glucose (p = 0.369). All other treatment*time effects were ‘unclear’.
Table 4: Physiological Responses During Constant-Load Cycling

<table>
<thead>
<tr>
<th></th>
<th>Normal Training</th>
<th></th>
<th>Intensified Training</th>
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<th>Reduced Volume Training</th>
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<tr>
<td></td>
<td>CHO</td>
<td>CHO+Pro</td>
<td>CHO</td>
<td>CHO+Pro</td>
<td>CHO</td>
<td>CHO+Pro</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>131±15</td>
<td>134±13</td>
<td>132±13</td>
<td>127±10</td>
<td>139±18</td>
<td>131±15</td>
</tr>
<tr>
<td>Late</td>
<td>135±15</td>
<td>135±12</td>
<td>131±13</td>
<td>128±10</td>
<td>136±13</td>
<td>132±13</td>
</tr>
<tr>
<td><strong>VE (L/min)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Early</td>
<td>64.8±11.0</td>
<td>67.0±9.1</td>
<td>64.3±8.8</td>
<td>64.3±10.0</td>
<td>70.4±4.7</td>
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</tr>
<tr>
<td>Late</td>
<td>68.8±9.7</td>
<td>74.1±12.4</td>
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<td><strong>RPE</strong></td>
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<tr>
<td>Early</td>
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<tr>
<td>Late</td>
<td>12.8±0.8</td>
<td>12.2±0.1</td>
<td>13.6±1.7</td>
<td>13.2±1.6</td>
<td>12.6±2.5</td>
<td>12.4±2.7</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>0.81±.04</td>
<td>0.82±.02</td>
<td>0.83±.14</td>
<td>0.79±.04</td>
<td>0.87±.02</td>
<td>0.83±.03</td>
</tr>
<tr>
<td>Late</td>
<td>0.81±.04</td>
<td>0.84±.03</td>
<td>0.82±.13</td>
<td>0.79±.05</td>
<td>0.85±.03</td>
<td>0.83±.04</td>
</tr>
<tr>
<td><strong>VO₂ (ml/kg/min)</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Early</td>
<td>38.0±6.6</td>
<td>37.9±8.1</td>
<td>36.5±3.9</td>
<td>36.4±6.9</td>
<td>37.2±3.9</td>
<td>36.2±9.0</td>
</tr>
<tr>
<td>Late</td>
<td>38.8±7.3</td>
<td>39.4±9.0</td>
<td>37.2±4.7</td>
<td>37.4±6.5</td>
<td>36.7±6.4</td>
<td>37.3±10.7</td>
</tr>
<tr>
<td><strong>Glu (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>90.3±8.1</td>
<td>79.2±10.6</td>
<td>93.7±8.7</td>
<td>78.9±11.5</td>
<td>89.9±4.7</td>
<td>83.8±8.1</td>
</tr>
<tr>
<td>Late</td>
<td>81.8±7.3</td>
<td>78.6±2.8</td>
<td>81.7±4.6</td>
<td>76.2±6.7</td>
<td>76.7±0.5</td>
<td>73.9±6.5</td>
</tr>
<tr>
<td><strong>Lac (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>1.3±0.4</td>
<td>1.1±0.5</td>
<td>0.9±0.1</td>
<td>1.1±0.4</td>
<td>1.2±0.6</td>
<td>0.7±0.5</td>
</tr>
<tr>
<td>Late</td>
<td>1.7±1.1</td>
<td>0.9±0.2</td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
<td>1.1±0.4</td>
<td>1.1±0.7</td>
</tr>
</tbody>
</table>

CHO=carbohydrate; CHO+Pro=carbohydrate + protein; HR=heart rate; VE=ventilation; RPE=rating of perceived exertion; RER=respiratory exchange ratio; Glu=glucose; Lac=lactate; Early=30 minutes; Late=120 minutes.

Time-effects:
Changes between NT-ICT were ‘Likely’ for: RPE, VE, and HR; All other variables were ‘Unclear’.
Changes between ICT-RVT were ‘Likely’ for: VE, RER, and HR; Changes were ‘Possible’ for: VO₂; All other variables were ‘Unclear’
Changes between NT-RVT were ‘Trivial’ for: VE, VO₂, and HR; All other variable were ‘Unclear’

Treatment*time effects:
Changes between NT-ICT were ‘Very Likely’ for: HR; Changes were ‘Likely’ for: VE and RER; All other variables were ‘Unclear’
Changes between ICT-RVT were ‘Very Likely’ for: Lac; Changes were ‘Likely’ for: Glu; Changes were ‘Possible’ for: VE; Changes were ‘Trivial’ for: VO₂; All other variables were ‘Unclear’
Changes between NT-RVT were ‘Very Likely’ for: HR; Changes were ‘Likely’ for: RER; Changes were ‘Possible’ for: VE, Glu; All other variables were ‘Unclear’
Discussion

Effects of the Intensified Training Model

The intensified training period was intended to elicit a state of overreaching in subjects, characterized by short-term decrements in exercise performance. Average daily training duration was increased by ~100% compared to NT for 10 days. Subjects were instructed to maintain their normal training intensity during ICT. However, we observed small but ‘very likely’/’likely’ decrements in average training HR and power,
respectively. These changes probably reflect the subjects’ inability to maintain training intensity due to fatigue incurred by the more than two-fold increase in training duration that occurred during ICT. This is similar to findings by Costill et al. (9), Jeukendrup et al. (21), and Halson et al. (16) who implemented similar training protocols to the one utilized in this study and observed significant decrements in training intensity after completion of a period of heavy training. Following the ICT period, we observed ‘likely’ impairments in TT time, average TT power, and VO2peak time; and higher constant load RPE. These findings are similar to other studies that have examined the effects of intensified training loads on performance (1, 9, 16, 44), and are consistent with a state of ‘overreaching’ in athletes.

The RVT period was utilized to allow subjects to recover following ICT, and was intended to restore or improve performance compared to baseline measurements. During RVT subjects reduced average training duration to ~60% of NT, for 10 days. This reduction in training load was similar to prior studies which have effectively restored performance levels following intensified training (15, 44). Following RVT, there were ‘likely’ improvements in TT time, average TT power and VO2peak time, compared to ICT. As a result of these changes, markers of endurance performance generally returned to baseline levels following RVT. However there did not appear to be any significant improvements in performance compared to baseline levels, which has been observed by some (21, 28). The reasons for this lack of performance improvement are unclear. However it is possible that the proportional increases in training volume, or length of the ICT period were excessive, leading subjects into a state of ‘non-functional’, rather than ‘functional’ overreaching (29). ‘Functional’ overreaching is described as a decrement in
performance that is reversed when an appropriate period of recovery is implemented, as opposed to ‘non-functional’ overreaching, which is when performance decrements persist following a planned recovery period (29). Similarly, the RVT period may not have been long enough to allow full recovery from the increased training stimulus, allowing a return to baseline performance but no improvements through training adaptations. Recovery periods are planned with the intent that subjects will derive a supercompensation response; however, there is substantial inter-individual variation in required recovery times. Therefore, the absence of performance improvements following intensified training periods is not uncommon, and has been reported in studies using similar training protocols to those used in this study (15, 16). Nevertheless, the training protocols elicited the desired effects for the purposes of this study, with reduced performance following ICT and restored performance following RVT - allowing a meaningful comparison in responses between treatments.

Effects of Treatment Beverages on Training Responses

The primary purpose of this study was to examine the effects of CHO+Pro supplementation on exercise tolerance and training adaptations, particularly cardiovascular adaptations, to this intensified training/recovery model in well-trained cyclists. The most important finding was that CHO+Pro supplementation attenuated constant load HR following ICT and RVT in comparison to CHO (Figure 4 and Table 4). Ventilation and RER were also ‘likely’ decreased after ICT with CHO+Pro supplementation, but the magnitude of these changes were quite small, and both returned to near-baseline levels following RVT. A lowered exercising HR response with CHO+Pro supplementation has also been reported in prior studies. For example Goto el
al. (13) examined the effects of CHO+Pro supplementation on cardiovascular adaptations in untrained subjects, who completed five consecutive days of aerobic exercise (30 minutes at 70% VO_{2peak}). Subjects who received CHO+Pro supplementation (CHO: 8.3 g/100 mL, Pro: 5.6 g/100 mL) following the exercise sessions had significantly greater decreases in HR during submaximal exercise than those receiving a placebo. Similarly Okazaki et al. (33) reported lower exercise HR’s in older males who consumed CHO+Pro following exercise during an 8-week training program (60 min at 60-75% VO_{2peak}, 3 days a week). These studies illustrate the potential influence of CHO+Pro supplementation on HR responses during exercise. However, both studies were conducted using previously untrained subjects, and CHO+Pro supplementation was not compared to a supplement containing equal CHO content. Therefore it is unclear whether these effects would persist in trained athletes, or if the adaptations were elicited by protein per se. Cathcart et al. (8) examined the effects of CHO+Pro supplementation in cyclists during a competitive mountain biking race. CHO or CHO+Pro supplements (in liquid and solid form) were consumed during each day of an 8-day competition. Mean HR’s during each stage of the race tended to be lower with CHO+Pro ingestion, but were not significantly different between groups. However, the highly applied (and field-based) nature of this study prevented the standardization of a variety of factors (such as the amount of nutrients consumed, exercise intensities, etc.) that could have influenced study outcomes, making direct interpretations of these findings difficult. To our knowledge, our study is the first to report that CHO+Pro supplementation lowers constant load HR in trained subjects in a controlled setting.
A possible explanation for reduced constant-load HR may be protein-mediated increases in plasma volume. Goto et al. (13) and Okazaki et al. (33) reported increases in plasma albumin content with CHO+Pro supplementation. This has been proposed to cause an oncotic pressure gradient, resulting in fluid shifts from the extra-vascular space to the intra-vascular space, expanding plasma volume. Plasma volume expansion leads to an enhanced venous return to the heart, which stretches cardiopulmonary mechanoreceptors, resulting in enhancements in stroke volume, and reductions in HR (31). Plasma albumin levels were assessed in the present study (Figure 3 and Table 3); however we did not observe any significant differences between treatments. Plasma volume and stroke volume were not directly assessed in this study so we can only speculate as to whether this had an effect on exercising HR. In addition to decrements in HR, protein-mediated changes in stroke volume have also been reported to augment VO2peak in prior studies (10, 33); but VO2peak was not increased with CHO+Pro in our study, despite the suppressed constant-load HR. Therefore, out data does not directly support an increased plasma volume and stoke volume as a mechanism for the lower HR response during constant load exercise.

The observed reduction in exercising HR is presumed to be a positive adaptation to training-supplementation because of its association with reduced cardiac strain and improved thermoregulation (8, 13, 33). Goto et al. (13) and Okazaki et al. (33) observed improvements in vasodilation and sweat rate following CHO+Pro supplementation, which were associated with an increase in plasma volume. As a result, CHO+Pro supplementation has been reported to enhance the maintenance of body temperature during exercise (8, 33). However in the context of the present study design, it is also
possible that overtraining led to hypothalamic dysfunction, which could have caused the lower exercising heart rates. Lehmann et al. (23) reported elevated catecholamine levels and decreased constant-load HRs following a period of increased training volume that was intended to elicit overtraining. The authors speculated that the lower exercising HR might have been due to decreased sensitivity to catecholamines. This decrement in sensitivity is indicative of decreased intrinsic sympathetic activity, a dysfunction which has been described as ‘central fatigue’ in exhausted athletes (3). Therefore, it is possible that the decrement in constant load HR, which occurred following ICT in the CHO+Pro condition, was due to ‘central fatigue’. However, if this were the case, subjects would likely have also reported an increased RPE during exercise (16). In addition, we would expect that the decrease in HR would have been restored, along with the observed improvements in exercise performance, following RVT, if the cause of the lowered HR response had been central fatigue. Because neither of these changes occurred, we propose that the decrement in constant load HR was not due to hypothalamic dysfunction. In support of this theory, Halson et al. (15) reported a decrease in exercising heart rate following ICT and RVT with supplemental carbohydrate consumption. However, the decreased heart rate was only statistically significant in the higher-carbohydrate condition, which is notable because various markers of overtraining were also attenuated in this condition. This suggests that the reduced heart rate was a positive training adaptation, rather than a response to central fatigue. However, further study is required to fully support this position.

CHO+Pro supplementation during and/or following heavy exercise has been reported to improve performance in subsequent exercise versus CHO in short-term
studies (2, 4, 6, 8, 20, 22, 30, 37, 42); however not all studies have observed this finding (5, 7). Despite the reduced constant-load heart rate which occurred with CHO+Pro in the present study, we did not observe any evidence of enhanced exercise performance with CHO+Pro. There are a few potential explanations for this finding. Firstly, it is possible that the present study design was not adequately powered to detect meaningful differences in performance. Due to the large number of performance tests, and the comparatively lengthy timeline of the training protocol (which may have increased variability in performance measures), it is possible that a larger number of subjects was required to detect nutritionally-mediated changes in performance. It is also possible that CHO+Pro does not enhance performance during/following intensified training when individuals are already consuming adequate dietary protein. Witard et al. (44) manipulated dietary protein levels in subjects during one week of intensified training. Despite substantial increases in protein intake (3.0 g Pro·kg BW\(^{-1}\)), cycling time-trial performances following ICT and RVT were virtually identical to when subjects were consuming lower protein amounts, which were consistent with most current dietary recommendations (1.5 g Pro·kg BW\(^{-1}\)) (44). Similarly, preliminary data from three subjects in the present study shows that protein intake (independent of supplementation) was 1.6 g·kg BW\(^{-1}\) per day during ICT. These findings suggest that supplemental protein intake during/following exercise does not enhance exercise performance following intensified training, provided that dietary intake is adequate.

Although the decrement in exercising HR with CHO+Pro supplementation was not accompanied by improved cycling performance, it is worth noting that maintenance of power output during training may have been improved in the CHO+Pro condition.
Average training power ‘likely’ decreased following the ICT period, but the magnitude of the reduction was ‘possibly’ less with CHO+Pro. In addition, average training power during the RVT period was ‘possibly’ increased versus NT, with ‘very likely’ larger increases in the CHO+Pro condition. Berardi et al. (4) have reported similar findings during a short-term intervention, with a CHO+Pro supplement providing better maintenance of power output in a subsequent exercise trial on the same day. Rowlands and associates have also noted improvements in power output following four days of heavy training with CHO+Pro supplementation (35), but not all studies have reported this outcome (36). These results suggest CHO+Pro supplementation could allow better maintenance of self-selected training intensities, potentially contributing to long-term benefits in performance. However, this interpretation should be made cautiously, as the predominant factor in our noted differences between treatments was an 11 W (‘likely’) difference in power outputs during the NT phase. Thus, it cannot be directly concluded that power outputs during training were higher with CHO+Pro supplementation.

In conclusion, our results suggest that constant-load HR is attenuated in well-trained cyclists following 10 days of intensified training with CHO+Pro supplementation, and that this adaptation persists following 10 days of reduced volume training. Changes in serum albumin concentration were similar between CHO and CHO+Pro treatments, and changes in plasma volume were not assessed, so it is unclear what mechanism may explain the observed reduction in exercise HR. In addition, changes in performance measures following ICT and RVT were similar between treatments. Thus, it appears that overall tolerance to intensified training, and adaptations following a period of recovery are generally similar when CHO+Pro and CHO are consumed during and following
exercise. It is possible that potential differences between treatments could be magnified with a larger sample size. Future studies should also measure cardiac output and plasma volume changes during and following training to better understand possible cardiovascular adaptations that may be occurring.
Manuscript References


References


