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EFFECTS OF A RECOVERY BEVERAGE ON MUSCLE
GLYCOGEN RESYNTHESIS IN RESPONSE TO ROAD CYCLING

by

Andrew Reinert

B.S., University of Minnesota, 2000

Presented in partial fulfillment of the requirements

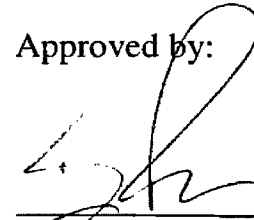
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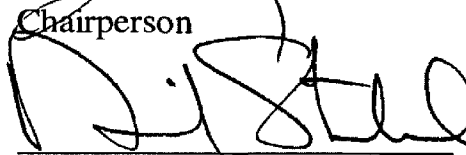
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Effects of a recovery beverage on muscle glycogen resynthesis in response to road cycling

Chairperson: Brent C. Ruby 

PURPOSE: The purpose of this study was to determine the effects of a recovery beverage on rates of muscle glycogen resynthesis in response to road cycling with supplied exogenous carbohydrate. **METHODS:** Eight recreational male cyclists, (25 ± 4.3 yr, 69 ± 5.2 kg, VO_2 peak = 66 ± 5.3 ml \cdot kg⁻¹ \cdot min.⁻¹) performed two similar 62 km outdoor training rides at self-selected intensities in a double-blind, randomized cross-over experiment. Duration (min), self-selected power output (watts, CycleOps PowerTap, Madison, WI), and 24-hour dietary intake from the first trial were duplicated during the second trial. Subjects received a food bar (38g CHO, 15g PRO, 5g FAT), and 28.6 ± 7.3 g of CHO in solution during each ride. A recovery beverage (CHO-PRO) or a placebo (PL) was administered 30 minutes post-exercise. At 2 hours post-exercise, a solid meal was given. Muscle biopsies were obtained from the vastus lateralis pre, post, and 4 hrs post-exercise. Blood samples were collected pre, post, 1, 2.5 and 4 hrs post-exercise. **RESULTS:** The rides were similar in duration, (122 ± 6 vs. 124 ± 5 min) and average power output (210 ± 28 vs. 212 ± 26 watts) for the PL and CHO-PRO trials, respectively. There were no significant differences in the rates of muscle glycogenolysis (44.8 and 40.6 mmol \cdot kg⁻¹ wet wt.⁻¹ \cdot hr.⁻¹ for PL and CHO-PRO, respectively). Rates of glycogen resynthesis were also similar for both recovery periods (4.0 ± 2.1 and 4.9 ± 2.9 mmol \cdot kg⁻¹ wet wt.⁻¹ \cdot hr.⁻¹, for the PL and CHO-PRO trials, respectively). **CONCLUSIONS:** The addition of a supplemental recovery beverage did not increase the rate of muscle glycogen resynthesis at 4 hours following ~ 2 hours of outdoor cycling when exogenous CHO was supplied before and during exercise.

Keywords: carbohydrate feedings, glycogenolysis, field trials

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Chapter One: Statement of the Problem

Introduction

Muscle glycogen is an essential energy substrate during prolonged, moderate to high intensity exercise^{1,2,15}. After an extended bout of exercise (2.5 hrs), muscle glycogen levels can be reduced to 25 percent of normal resting levels¹⁸. Rapid restoration of muscle glycogen after exercise is necessary for many athletes who perform multiple training or competitive sessions within a 24-hour period. It has been demonstrated that if carbohydrate (CHO) intake is inadequate during continuous days of prolonged exercise, muscle glycogen concentrations will be reduced and performance will be hindered^{9,17}. Several studies have shown that ingestion of a sufficient amount of CHO shortly after exercise helps restore muscle glycogen^{6-8, 10, 18-20, 22, 23, 26, 27, 29-33, 35}. Most of the prior studies have focused on maximizing muscle glycogen resynthesis rates by varying the amount^{6, 13, 20} timing^{19, 23, 29} and type of CHO feedings^{7, 10, 26} post-exercise. Recent focus has included additional proteins^{8, 18, 22, 30-33, 35} or a variety of amino acids^{22, 32} in addition to a CHO bolus to increase rates of glycogen recovery. Despite inconclusive results related to the addition of protein and various amino acids, manufacturers of recovery drinks have begun to include protein in their products.

Despite the wide variety of feeding and exercise protocols mentioned above, there is little known regarding rates of muscle glycogenolysis and subsequent glycogen resynthesis in response to field exercise trials under typical training intensities and nutritional conditions. Although the laboratory environment allows for precise control with the use of electronic cycle ergometers, the development of devices capable of

measuring power output at the rear hub of the bicycle expands the potential for field investigation. Moreover, past research has validated the reliability and accuracy of these devices, which can be integrated with a riders own bicycle⁵.

The Problem

Many athletes have multiple training sessions or competitive events within a 24-hour period that may result in serial muscle glycogen depletion. Muscle glycogen needs to be restored after exercise prior to subsequent exercise to avoid overtraining or in order to perform successfully in a competitive event. There is little known about self-selected endurance training in the field and its effect on muscle glycogen use and recovery under normal training conditions.

Purpose

To determine the efficacy of a supplemental recovery (CHO-PRO) in addition to a typical meal on the rates of muscle glycogen resynthesis during a 4 hour recovery period.

To determine the muscle glycogen depletion profile of a 62 km self-selected intensity, outdoor training ride in recreational cyclists.

Research Hypothesis

Muscle glycogen resynthesis will be increased during four hours of recovery from self-selected endurance cycling in the field with ingestion of a carbohydrate-protein recovery drink.

Significance of Study

This study will determine the effectiveness of a carbohydrate-protein 'recovery' drink in restoring muscle glycogen following a typical training ride of male cyclists. Secondly, this study will show glycogen depletion profiles of a typical outdoor training ride which will aid in determining appropriate feeding strategies for recreational cyclists.

Rationale for Study

There have not been any prior field studies using real training rides in which muscle glycogenolysis and resynthesis have been evaluated under normal training and nutritional conditions.

Most prior research required subjects to fast prior to the start of the exercise protocols. Subjects in the current study will be fed a typical breakfast 3 hours prior to the start of their exercise trials. This will mimic a more typical training session for athletes. Additionally, the feeding protocols during the recovery periods in past research have not been consistent with normal athlete eating patterns. The majority of studies used beverage solutions or bolus feedings without including normal foods and athlete would typically eat during the 4-hour post exercise period. The current study will use a liquid carbohydrate-protein recovery beverage 30 minutes after exercise in addition to a typical solid meal during the 4-hour post-exercise recovery period.

Limitations

1. Due to changes in weather, the outdoor environment that the trials are conducted may be different between trials. To limit the variability in weather, trials were not performed on days of harsh weather or with temperatures drastically different between a subject's trials.
2. Subjects' diets and activity prior to the days of the trials could effect initial muscle glycogen concentrations. The use of dietary recall and activity logs as well as pre-exercise muscle biopsies helped researchers control the variation between the two trials.
3. There is an inherent error with the use of any instrumentation. To limit this error, equipment was carefully calibrated and the testers were adequately trained.

Delimitations

1. All subjects in this study are trained males who cycle regularly. A more diverse subject pool would allow the results to be inferred to a greater population. Thus, the results of this study should only be applied to trained male cyclists.

2. The recovery period is 4 hours in duration. This may be shorter for some athletes with successive training sessions or perhaps longer for other individuals.

Definition of Terms

Activity log: A list of all physical activities performed by the subjects for three days prior to each trial.

¹³C-NMR: Nuclear magnetic resonance. A technique used to obtain physical, chemical, electronic and structural information about a molecule. Allows for a non-invasive measure of muscle glycogen concentration.

Diet recall: A list of everything eaten by the subjects for three days prior to each trial.

Glycemic index: The area under the 2 hour blood glucose response curve following the ingestion of a fixed portion of carbohydrate. The immediate effect on blood glucose levels as a result of ingesting specific CHO. The index gives us an idea of how fast specific CHOs are absorbed and converted to blood glucose.

High glycemic index: Foods that elicit a relatively high blood glucose response after ingestion.

Isocaloric: Containing the same number of calories.

Low glycemic index: Foods that elicit a relatively low blood glucose response after ingestion.

PowerTap: A specialty bicycle hub manufactured by Cycleops that measures power (watts), heart rate, velocity, time, and distance on bikes used in the field.

Recovery drink: A beverage intended to be ingested post exercise to aid in the recovery of muscle glycogen and hydration.

Stationary trainer: An exercise training device that attaches to the rear axle of a bicycle and provides resistance against pedaling; allowing a cyclist to train or test indoors on their own bicycle.

Trained cyclist: For this study we have used the standard of a person who typically rides their bike 100-400 miles per week and has a peak VO_2 of at least $50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

VO_2 peak: The peak amount of oxygen an individual can consume during a graded exercise test.

Chapter Two: Literature Review

Muscle glycogen

The role of muscle glycogen as an essential source of energy during prolonged exercise was established by several early studies. In the classic study by Bergstrom and Hultman (1966), four healthy subjects pedaled with a load of 300 kpm per minute on a cycle ergometer for 30 minutes². Biopsies were collected pre, post and one hour post-exercise by use of a Bergstrom needle to assess muscle glycogen. Immediately following the exercise, muscle glycogen levels were substantially lower in all subjects. They concluded that muscle glycogen is the main carbohydrate source for muscle activity and that it may be a limiting factor in work capacity. In a study by Ahlborg et al., a direct correlation between exercise duration to exhaustion and initial glycogen stores of the working muscles was found ($r = .68$)¹. Subjects cycled at an average of 710 kpm · min⁻¹ for an average duration of 127 minutes. They concluded that local glycogen stores determined the ability for one to perform long-term exercise. A similar study with trained and untrained cyclists found initial glycogen stores limited the capacity for prolonged strenuous work¹⁵. It was also found that glycogen stores could be almost depleted within 1 - 2 hours of intense exercise.

Early studies established muscle glycogen's role as a necessary fuel source during prolonged exercise; and recovering from glycogen-depletion is vital for subsequent exercise. In a study performed on mice, Danforth showed that muscle glycogen exerts feedback inhibition on its own synthesis¹¹. When muscle glycogen content was high, resynthesis was slow; when muscle glycogen content was low, glycogen resynthesis was rapid. A study by Bergstrom and Hultman confirmed that exercise with glycogen

depletion enhanced the resynthesis of glycogen³. The study involved two people on either side of a cycle ergometer who pedaled with one leg. Biopsies were performed on the quadriceps femoris of the exercised and the non-exercised legs immediately after exercise and again on days 1, 2, and 3 post-exercise. Subjects consumed a diet consisting primarily of carbohydrates (CHO) during the recovery period. The glycogen content of the exercised legs after two days on the CHO diet was approximately twice as high as the non-exercised legs. They suggested that a factor operated locally in the exercised muscle and persisted for at least three days. A study by Zachwieja et al. demonstrated similar results in subjects who performed single-legged cycling for 30 minutes with ten one-minute sprints and double-legged cycling for 30 minutes³⁴. Subjects were fed CHO equal to $0.7\text{g} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ during 6 hours of recovery. The leg that performed more work had lower muscle glycogen content following exercise and demonstrated a significantly higher rate of glycogen resynthesis during 6 hours of recovery compared to the other leg.

Diet

Additional studies by Hultman and Bergstrom looked at the role of diet on muscle glycogen resynthesis. In one study, they compared the effects of either a high-carbohydrate (HCHO), a low-carbohydrate (LCHO), or a starvation diet on muscle glycogen during ordinary work for 5-12 days¹⁶. Biopsies were performed throughout the study. The LCHO and starvation diets both caused a decrease in glycogen content of the muscle after five days. The HCHO diet caused muscle glycogen to rise. In a separate study performed at the same time, five subjects performed hard intermittent work to exhaustion on a cycle ergometer. Biopsies were taken pre, post, and four hours post-exercise, as well as every 2nd or 3rd day of the seven days of the recovery period.

Subjects fasted for 24 hours post-exercise and were then given either a primarily CHO or a primarily fat and protein diet. Subjects consuming the fat and protein diet did not restore glycogen to pre-exercise levels after five days. In contrast, subjects who consumed the HCHO diet restored muscle glycogen to pre-exercise levels or higher. Similar findings have been found by Bergstrom et al. and Piehl^{4,24}.

The early studies of muscle glycogen established the essential role of muscle glycogen for prolonged exercise and the importance of consuming carbohydrates before and after extended bouts of exercise. However, the focus of more recent research has been on improving muscle glycogen recovery by varying the amount, timing, and type of carbohydrate ingested post-exercise.

Amount of carbohydrate

The amount of CHO ingestion necessary to achieve maximal glycogen resynthesis has been extensively studied. In a study by Fallowfield et al., 12 men and 4 women ran at 70% VO_2 max for 90 minutes or to volitional fatigue¹³. Post-exercise and 2 hours post-exercise subjects were given enough 6.9% CHO solution to equal 1g CHO \cdot kg body weight⁻¹ (bw) or placebo. No biopsies were taken, but performance was measured four hours later by running to fatigue at intensities similar to the first run. Subjects who ingested CHO ran an average of 22.2 minutes longer than the placebo group (62 vs. 39.8 min). In a study by Ivy et al., after fasting for 14 hours, eight healthy males pedaled on a cycle ergometer at 62-75% VO_2 max for 2 hours²⁰. Subjects were given a placebo, 1.5 or 3.0g \cdot kg bw⁻¹ of a glucose polymer immediately post-exercise and again 2 hours post-exercise. The rate of CHO intake was 0.75 and 1.5g \cdot kg bw⁻¹ \cdot hr⁻¹ for the 1.5 and 3.0g treatments respectively. Biopsies were taken immediately after exercise and at 2 and 4

hours post-exercise. A rapid rate of muscle glycogen storage was found with ingestion of 0.75 and 1.5g CHO ·kg bw¹ ·hr⁻¹. Interestingly, the ingestion of 1.5g CHO ·kg bw¹ ·hr⁻¹ did not significantly raise the rate of muscle glycogen resynthesis compared to ingesting 0.75g CHO ·kg bw¹ ·hr⁻¹ (5.1 vs. 4.5mmol ·kg wet wt⁻¹ ·hr⁻¹).

Blom et al., compared feeding different amounts of CHO post-exercise in 27 fasted, healthy males⁶. Subjects pedaled on a cycle ergometer at 75% VO₂ max to exhaustion (78-113 min). Immediately after exercise and at 2 and 4 hours post-exercise subjects were given 0.35g, 0.70g, or 1.4g ·kg bw⁻¹ of glucose equaling a rate of CHO intake of 0.18, 0.35, and 0.70g CHO ·kg bw¹ ·hr⁻¹ respectively. Biopsies were taken pre, post, 2, 4, and 6 hours post-exercise. Average muscle glycogen was reduced by 80% during exercise. The 0.7 g CHO ·kg bw¹ ·hr⁻¹ rate resulted in higher insulin levels, however, glycogen resynthesis rates were similar between treatments of 0.35 and 0.7g ·kg ·bw¹ ·hr⁻¹ (5.8 vs. 5.7 mmol ·kg⁻¹ ·hr⁻¹), and noticeably higher than the 0.18g ·kg bw⁻¹ treatment (2.1 mmol ·kg⁻¹ ·hr⁻¹). They suggested a maximum rate of approximately 6 mmol ·kg⁻¹ ·hr⁻¹ of glycogen resynthesis may exist. Although the optimal amount of CHO to give athletes post-exercise is equivocal, Jentjens and Jeukendrup suggested the optimal rate of glycogen resynthesis occurs when CHO is consumed at ~1.2g ·kg⁻¹ ·hr⁻¹²¹.

Timing and frequency

The timing of CHO intake after exercise is important in restoring muscle glycogen. A study by Parkin et al. compared different CHO feeding times during 24 hours of recovery in six trained male cyclists²³. After pedaling on a cycle ergometer for 2 hours at 70% VO₂ max, subjects received five high-glycemic meals. The first three meals were consumed either during the first 2 hours post-exercise or after 2 hours post-exercise.

The next two meals were given 2 and 4 hours after consumption of the first three meals. Biopsies were taken immediately post-exercise, and at 8 and 24 hours post-exercise. Blood samples were collected pre-exercise, 30, 60, and 90 minutes after each meal. Rates of muscle glycogen resynthesis, blood insulin concentration, and blood glucose levels of subjects were similar regardless of the feeding protocol. It was concluded that delaying feeding time post-exercise by two hours did not affect the rate of glycogen resynthesis 8 and 24 hours after 2 hours of moderate exercise. Potentially, the similarity between trials may be due in part to the relatively long recovery periods of 8 and 24 hours. For shorter recovery periods (< 4 hours), the timing of CHO intake post-exercise has been shown to be a critical factor that influences the rate of resynthesis.

Ivy et al. showed delaying ingestion of $2\text{g CHO} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ by 2 hours post-exercise will result in a reduced rate of muscle glycogen storage¹⁹. Subjects given CHO immediately post-exercise resynthesized muscle glycogen 45% faster during the first 4 hours of recovery compared to subjects given CHO 2 hours post-exercise. They suggested the reduced rate of muscle glycogen storage was due to a reduced rate of muscle glucose uptake. Goodyear et al. showed that at 2 hours post-exercise both glucose transporter number and transport activity had dropped to non-exercised control values in rat skeletal muscle¹⁴. This finding provides a mechanism that supports the results of Ivy et al.¹⁹. In a study by Price et al. subjects' muscle glycogen was depleted to 25% of resting levels as a result of exercise²⁵. During the post-exercise period, subjects were provided with water only. Results demonstrated two apparent phases in the process of post-exercise muscle glycogen resynthesis. The first phase was described as insulin-independent and may last for 30 - 60 minutes post-exercise, resulting in a rapid rate of

glucose uptake and subsequent muscle glycogen resynthesis. The second phase was described as insulin-dependent and results in a slower rate of glycogen resynthesis. These findings strongly suggest that CHO feeding provided immediately post-exercise are crucial in maximizing muscle glycogen resynthesis for short recovery periods. Similar to the potential effects of timing, the type of carbohydrate ingested may also play an important role in determining the rate of glycogen recovery.

Type of carbohydrate

In a study by Costill et al. six trained male runners ran 16.1 km at 80% VO_2 max, followed by sprint intervals¹⁰. Biopsies were obtained from the gastrocnemius immediately post-exercise, 1, and 2 days post-exercise. Subjects consumed a diet of 70% simple sugars or complex carbohydrates during the recovery (2 meals per day). There were no significant differences between the two treatments after 24 hours of recovery. After 48 hours of recovery, subjects who consumed the diet of complex carbohydrates increased their muscle glycogen storage significantly more than subjects who consumed a diet of mainly simple sugars. In a study by Burke et al. five well-trained cyclists pedaled on a cycle ergometer for 120 minutes at 75% VO_2 max followed by four 30 second sprints⁷. Subjects consumed a high or low-glycemic index meal immediately post-exercise, 4, 8, and 21 hours post-exercise. Biopsies were taken from the vastus lateralis immediately post-exercise and 24 hours post-exercise. Subjects who consumed high-glycemic index foods and low-glycemic index foods increased their muscle glycogen concentration by 106.1 and 71.5 $\text{mmol} \cdot \text{kg}^{-1}$ wet weight, respectively. The results demonstrated a more rapid increase in muscle glycogen during 24 hours post-exercise with the consumption of high-glycemic index foods. The findings contradict those of

Costill et al. (1981) that suggested there were not any significant differences in glycogen resynthesis rates for 24 hours post-exercise between subjects who consumed diets of either complex or simple CHOs.

The mode of carbohydrate administration does not seem to affect the rate of muscle glycogen resynthesis. Reed et al. demonstrated similar glycogen resynthesis rates after consumption of either a liquid or solid CHO feeding²⁶. Subjects pedaled on a cycle ergometer for 2 hours and were given $3\text{g}\cdot\text{kg}^{-1}$ body weight of CHO in liquid or solid form immediately post-exercise and 2 hours post-exercise. The rate of CHO intake during the 4 hour recovery period was $0.75\text{g}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ for both forms of CHO. Muscle biopsies were taken immediately post-exercise, 2, and 4 hours post-exercise. Muscle glycogen resynthesis rates were 5.1 and 5.5 $\text{mmol}\cdot\text{kg wet weight}^{-1}\cdot\text{hr}^{-1}$ for the liquid and solid forms of CHO, respectively. During the 4 hours of recovery, blood glucose levels were similar between treatments, but plasma insulin levels were significantly higher for the liquid CHO treatment compared to the solid CHO treatment. They suggested the rate of muscle glycogen resynthesis is not limited by gastric emptying.

Additional proteins and amino acids

Recent research has attempted to maximize post-exercise muscle glycogen resynthesis with the addition of protein or amino acids to a CHO meal or beverage. Zawadzki et al. compared a CHO treatment with a CHO-protein (CHO-PRO) treatment during 4 hours of recovery from exercise³⁵. Nine male cyclists pedaled on a cycle ergometer at 60-80% VO_2 max for 2 hours. Subjects consumed 112g CHO, 40.7g PRO, or 112g CHO + 40.7g PRO immediately post-exercise and 2 hours post-exercise. Muscle biopsies were taken post-exercise and 4 hours post-exercise. Blood was collected every

30 minutes during the recovery period. The rate of muscle glycogen storage during the CHO-PRO treatment was significantly faster than either CHO or PRO treatments. The plasma insulin response for the CHO-PRO treatment was significantly greater than either the CHO or PRO treatments. The authors suggested the enhanced muscle glycogen storage during the CHO-PRO treatment was a result of the interaction of carbohydrate and protein on insulin secretion. However, it should also be mentioned that the underlying mechanism of the CHO-PRO treatment is unclear because it was not isocaloric compared to the CHO treatment. Van Loon et al. demonstrated increases in muscle glycogen recovery rates during 5 hours of recovery from intense cycling with the addition of protein and with higher CHO intake³². Subjects were given either a low dose of 0.8g CHO ·kg⁻¹·hr⁻¹ (LCHO), 0.8g CHO ·kg⁻¹·hr⁻¹ + 0.4g PRO ·kg⁻¹·hr⁻¹ (CHO-PRO) or a high dose of 1.2g CHO·kg⁻¹·hr⁻¹ (HCHO). Muscle glycogen resynthesis rates were 16.6, 35.4, and 44.8 mmol /kg dry muscle weight⁻¹ for the LCHO, CHO-PRO, and HCHO beverages respectively. The CHO-PRO beverage resulted in an enhanced plasma insulin response for the first 4 hours of recovery compared to an equal amount of CHO without added protein. The study also showed higher glycogen resynthesis rates when subjects were given 1.2 vs. 0.8g CHO·kg⁻¹·hr⁻¹.

In a similar study by Ivy et al. muscle glycogen resynthesis during 4 hours of recovery was studied using three beverages¹⁸. Seven trained male cyclists pedaled on a cycle ergometer at 65-75% VO₂ max for 2 hours followed by 1-minute sprints. Subjects were fed either a CHO-PRO beverage containing 80g CHO + 28g PRO + 6g fat, a HCHO beverage containing 108g CHO + 6g fat, or a LCHO beverage containing 80g CHO + 6g fat. The CHO-PRO and HCHO beverages each contained 378 kcals; the LCHO beverage

contained 294 kcals. Beverages were administered immediately post-exercise and 2 hours post-exercise. ^{13}C - nuclear magnetic resonance (NMR) scans were performed pre, post, 20, 40, 60 minutes post-exercise, and at 2, 3, and 4 hours post-exercise to measure muscle glycogen concentration of the quadriceps. There were no significant differences in the plasma insulin response among treatments; however, blood glucose was lower in the CHO-PRO treatment. Subjects in the CHO-PRO treatment had significantly faster muscle glycogen resynthesis rates compared to the HCHO and LCHO treatments. After 4 hours of recovery, 46.8% of the glycogen used in the CHO-PRO treatment had been restored, compared to 31.1% and 28.0% for the HCHO and LCHO treatments, respectively. The researchers concluded the mechanism by which CHO-PRO supplements increase muscle glycogen resynthesis is not related to an enhanced plasma insulin response. This finding conflicts with the previous studies by Zawadzki et al. and van Loon et al. and demonstrate an increased rate of glycogen resynthesis under isocaloric conditions^{32,35}. A recent study by Ruby et al. demonstrated increased rates of muscle glycogen resynthesis during 4 hours of recovery with the addition of a herbal extract, fenugreek, to a liquid CHO bolus²⁷. The fenugreek extract ($2.0 \text{ mg} \cdot \text{kg}^{-1} \text{ bw}$) provided a high concentration of a specific amino acid (4-hydroxy isoleucine) and does not add additional calories, keeping the trials isocaloric. Blood glucose and insulin levels were similar between trials. The increased rates of glycogen resynthesis were uniform in all subjects during the fenugreek trial, representing a 63% increase in glycogen resynthesis rates compared to CHO alone (10.6 ± 3.3 vs. $6.5 \pm 2.6 \text{ g} \cdot \text{kg wet wt}^{-1} \cdot \text{hr}^{-1}$ for the experimental and control trials respectively). Moreover, the increased rates of

glycogen recovery in the absence of an enhanced insulin response were in agreement with Ivy et al¹⁸.

The previously mentioned studies^{18, 27, 32, 35} have shown increases in muscle glycogen resynthesis rates with the addition of protein or amino acids to CHO feeding protocols. There have also been numerous conflicting studies showing no significant increases in muscle glycogen resynthesis when additional protein and/or essential amino acids have been provided in concert with a high CHO bolus. Tarnopolsky et al. demonstrated no additional increases in muscle glycogen resynthesis rates during 4 hours of recovery with protein and fat added to an isocaloric CHO beverage³⁰. Eight active males and eight active females cycled at 65% VO₂ max for 90 minutes. Immediately post-exercise and 1 hour post-exercise subjects were given beverages containing 1g PRO ·kg⁻¹ + 0.02g FAT ·kg⁻¹ + 0.75g CHO ·kg⁻¹ or 1g CHO ·kg⁻¹. Biopsies were performed post-exercise and 4 hours post-exercise. No statistical differences were found between the two trials. Additional studies using trained cyclists showed muscle glycogen recovery rates were not increased with the addition of protein or amino acids to CHO beverages^{8, 22, 31}.

Chapter Three: Methods

Subjects

Eight recreationally active male cyclists (mean \pm SD: age 25 ± 4 yrs, mass 69 ± 5 kg, VO_2 peak 65.6 ± 5.3 mL \cdot kg⁻¹ \cdot min⁻¹, body composition $10.8 \pm 2.8\%$ body fat) participated in this study. Prior to data collection, the research procedures were approved by the University Internal Review Board. Subjects were informed of all experimental procedures and risks associated with the study and provided written consent prior to participation.

Preliminary testing

Maximal exercise and steady state testing

Prior to the experimental trials, VO_2 peak was determined for each subject using a ramp protocol (25 W \cdot min⁻¹) on an electronically braked cycle ergometer (Velotron, Seattle, WA). Subjects fasted for 3 hours prior to the test. Expired gases were collected and analyzed at 15-second intervals during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT). Following the completion of the maximal exercise test, subjects were allowed to recover. The subjects own bicycle was outfitted with a Power-Tap Pro power meter (CycleOps, Madison, WI) and mounted on a stationary electronic trainer (CycleOps, Electronic). Subjects completed three steady state intensities (mean \pm sd: 152 ± 28 , 202 ± 25 , and 253 ± 29 watts, respectively) on the fluid trainer to establish individual regression equations for power (watts), and VO_2 and VCO_2 . This was completed to account for potential differences in calibration of individual PowerTaps and the cycle ergometer used for the maximal exercise test and to

more accurately calculate total energy expenditure and whole body substrate oxidation during the field trial rides.

Body composition

Body composition was determined using hydrodensitometry. Subjects fasted for 3 hours prior to the underwater weighing. Subjects were instructed to void prior to all measurements. Body weight was recorded on a dry weight scale (Befour Inc., Cedarburg, WI). Water temperature of the water tank was recorded to correct for water density. Subjects were then weighed in a lightweight swimming suit underwater using a calibrated underwater digital scale (Exertech, Dresbach, MN). Residual volume was estimated from known values based on the subjects' gender, age, and height. Body composition was calculated from body density²⁸.

Experimental Design Overview

All subjects participated in a placebo controlled, double-blind crossover experiment with at least 6 days between trials. Training logs and diet recall were kept by the subjects for 3 days prior to the first trial. The records were used to standardize the subjects' physical activity and diets before the second experimental trial.

The exercise consisted of subjects cycling on their own bike over a pre-determined 62 km outdoor course. A muscle biopsy was obtained from the vastus lateralis before and immediately after the ride. Subjects consumed either a recovery drink or placebo 30 minutes post-exercise. A standardized meal of spaghetti, tomato sauce, sourdough roll, and milk was given 2 hours post-exercise. A third biopsy was taken 4 hours after the immediate post-exercise biopsy. Blood samples were taken pre,

post, 1, 2.5, and 4 hours post-exercise. Throughout the recovery period, subjects remained in the lab during sitting quietly.

Experimental protocol

After an overnight fast, subjects reported to the lab at 0700. Subjects consumed a breakfast at 0715 consisting of a 100g plain bagel (265 calories, 53g CHO, 10g protein, 1.6g fat), 28g cream cheese (100 cal, 0 CHO, 10g fat, 2g protein), and 450 ml orange juice (220 cal, 54g CHO, 4g protein, 0 fat). Subjects remained in the lab. At 0930 a baseline blood sample was obtained from an antecubital vein in untreated tubes. The sample was allowed to clot for 15 minutes, and then spun for 10 minutes at 4000 rpm in a refrigerated centrifuge set at 4°C. Serum was removed from the sample, separated into three different vials, and stored at – 80°C for later analyses of insulin and glucose. Subsequent blood collections were handled similarly. Immediately after the blood collection, the leg was prepared for a muscle biopsy. A muscle biopsy was obtained using a suction-modified Bergstrom needle-biopsy technique from the midsection of the vastus lateralis muscle. Muscle samples were dissected of any visible connective tissue, cut into three pieces, and placed in separate vials. Muscle samples were immediately frozen in liquid nitrogen and stored at – 80°C until assayed for total muscle glycogen. Subjects voided if necessary. At 1000 subjects consumed a 250 calorie food bar (5g fat, 38g CHO, 15g protein). At 1015, subjects began the 62 km ride with 600 ml of water and 600 ml of a commercially available carbohydrate beverage (6% CHO solution) in separate bottles mounted to their bike. Subjects completed a pre-determined course at a self-selected intensity similar to a typical training ride. At 45 and 90 minutes into the

ride, subjects increased their intensity for 10 minutes at a self-selected power output and resumed normal pace when the interval was completed. Time, velocity, distance, heart rate and power output (watts) were displayed and recorded during the ride to a mounted cyclometer (Power Tap Pro, CycleOps, Madison, WI). Subjects were required to ride at similar intensities and duration for both rides. Total amounts of water and carbohydrate beverage consumed during the initial rides were standardized between rides.

Immediately after the ride, a post exercise blood sample was collected and a second biopsy was obtained from the vastus lateralis approximately 2 cm proximal to the first biopsy on the same leg. Blood and muscle samples were handled as described above. At 30 minutes post-exercise, 353g (360 ml) of a recovery beverage containing (40g CHO, 20g protein) or a similar flavored placebo was ingested. Additional blood samples were collected at 1, 2.5, and 4 hrs post-exercise. Subjects consumed a standardized meal consisting of 112g spaghetti (400 calories, 88g CHO, 14g protein, 2g fat) with 200ml tomato sauce (117 cal, 17 g CHO 3.3g protein, 4.2g fat, 80g sourdough roll (170 calories, 34g CHO, 7g protein, .5g fat), and 480 ml milk (180 calories, 26g CHO, 16g protein, 0g fat) at 2 hours post exercise. A final muscle biopsy was obtained at 4 hours post-exercise from the vastus lateralis approximately 2 cm proximal from the second biopsy on the same leg.

Muscle glycogen analysis

Muscle glycogen was analyzed using an enzymatic spectrophometric method after tissue preparation. Samples (25 ± 3.2 mg wet weight) were weighed upon removal from a -80°C freezer and placed in 1 ml, 1 N HCL solution and homogenized using a manual

mortar and pestle tissue grinder. Once homogenized, samples were incubated at 95.6°C for three hours. After the incubation, 0.5 ml, 1 N NaOH was added to 0.5 ml of tissue sample to normalize pH. Samples were analyzed in triplicate against known glycogen and glucose controls run simultaneously. Muscle glycogen concentrations were expressed in $\text{mmol} \cdot \text{kg}^{-1}$ wet weight of muscle. Muscle glycogenolysis for each trial was calculated from the equation: $(G_{\text{pre}} - G_{\text{post}}) / t$, where G_{pre} is muscle glycogen concentration pre-exercise, G_{post} is the muscle glycogen concentration immediately post-exercise, prior to feeding. Rates of muscle glycogen resynthesis for each trial were calculated from the equation: $\text{Rate} = (G_{4\text{ post}} - G_{\text{post}}) / t$, where $G_{4\text{ post}}$ is the muscle glycogen concentration 4 hours post exercise, and t is the time between biopsies.

Blood analysis

Blood samples were analyzed for glucose in duplicate using an enzymatic spectrophometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Insulin was also analyzed in duplicate using an enzymatic spectrophometric ELISA method (EIA2935, DRG International). Average intra-assay coefficient of variation for glucose and insulin was <5%.

Statistics

Descriptive data including mean; duration, power (watts), HR, exercise intensity (% VO_2 peak), estimated total energy expenditure (TEE) (kcal/ride), and CHO intake, between trials were analyzed using a 2-tailed, dependent t-test. Mean; power (watts) and HR of the first and second intervals between trials were analyzed using a 2 x 2 ANOVA

with repeated measures. Blood glucose and insulin concentrations were analyzed using a 2 x 5 (pre, post, 1, 2.5, and 4hrs post-exercise) ANOVA with repeated measures. Changes in muscle glycogen concentration (pre and post) were analyzed across each trial using a 2 x 2 ANOVA with repeated measures. Muscle glycogenolysis (pre – post) was analyzed between trials using a 2-tailed, dependent t-test. Rates of muscle glycogen resynthesis (4 hrs post-exercise – post-exercise) were analyzed between trials using a 2-tailed, dependent t-test. Statistical significance was established using an alpha level of $p < 0.05$.

Effects of a recovery beverage on muscle glycogen resynthesis in response to road cycling

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Running Title: Nutritional recovery from outdoor cycling.

PURPOSE: The purpose of this study was to determine the effects of a recovery beverage on rates of muscle glycogen resynthesis in response to road cycling with supplied exogenous carbohydrate. **METHODS:** Eight recreational male cyclists, (25 ± 4.3 yr, 69 ± 5.2 kg, VO_2 peak = 66 ± 5.3 ml \cdot kg⁻¹ \cdot min.⁻¹) performed two similar 62 km outdoor training rides at self-selected intensities in a double-blind, randomized cross-over experiment. Duration (min), self-selected power output (watts, CycleOps PowerTap, Madison, WI), and 24-hour dietary intake from the first trial were duplicated during the second trial. Subjects received a food bar (38g CHO, 15 g PRO, 5g fat), and 28.6 ± 7.3 g of CHO in solution during each ride. A recovery beverage (CHO-PRO) or a placebo (PL) was administered 30 minutes post-exercise. At 2 hours post-exercise, a solid meal was given. Muscle biopsies were obtained from the vastus lateralis pre, post, and 4 hrs post-exercise. Blood samples were collected pre, post, 1, 2.5 and 4 hrs post-exercise.

RESULTS: The rides were similar in duration, (122 ± 6 vs. 124 ± 5 min) and average power output (210 ± 28 vs. 212 ± 26 watts) for the PL and CHO-PRO trials, respectively. There were no significant differences in the rates of muscle glycogenolysis (44.8 and 40.6 mmol \cdot kg⁻¹ wet wt.⁻¹ \cdot hr.⁻¹ for PL and CHO-PRO, respectively). Rates of glycogen resynthesis were also similar for both recovery periods (4.0 ± 2.1 and 4.9 ± 2.9 mmol \cdot kg⁻¹ wet wt.⁻¹ \cdot hr.⁻¹, for the PL and CHO-PRO trials, respectively). **CONCLUSIONS:** The addition of a supplemental recovery beverage did not increase the rate of muscle glycogen resynthesis at 4 hours following ~ 2 hours of outdoor cycling when exogenous CHO was supplied before and during exercise.

Keywords: carbohydrate feedings, glycogenolysis, field trials

INTRODUCTION

Paragraph Number 1 Muscle glycogen is an essential energy substrate oxidized during prolonged, moderate to high intensity exercise^{1, 2, 15}. After an extended bout of exercise (2.5 hrs), muscle glycogen levels can be reduced to 25 percent of normal resting levels¹⁸. Therefore, to better prepare for subsequent training sessions or exercise bouts it is often necessary to restore muscle glycogen within a 24-hour period. Similarly, if carbohydrate (CHO) intake is inadequate during continuous days of prolonged exercise, muscle glycogen concentrations are reduced and performance may be impaired^{9, 17}. Several previous studies have shown that the ingestion of a sufficient amount of CHO shortly after exercise can rapidly restore muscle glycogen in depleted skeletal muscle^{6-8, 10, 18-20, 22, 23, 26, 27, 29-33, 35}. The majority of this past research has focused on maximizing the rates of muscle glycogen resynthesis by varying the amount^{6, 13, 20} timing^{19, 23, 29} and type of CHO feedings^{7, 10, 26} provided during a standardized post-exercise recovery period. Additional attempts to promote glycogen recovery have included the addition of protein^{8, 18, 22, 30-33, 35} or a variety of amino acids^{22, 32} to the high CHO bolus. Although the results associated with the addition of protein and various amino acids are inconclusive, several commercial recovery drinks have incorporated protein in their products.

Paragraph Number 2 Even with the wide variety of feeding and exercise protocols mentioned above, there is little known regarding rates of muscle glycogenolysis and subsequent glycogen resynthesis in response to field exercise trials under typical training intensities and nutritional conditions. Although the laboratory environment allows for precise control with the use of electronic cycle ergometers, the development of devices capable of measuring power output at the rear hub or bottom bracket of the bicycle

expands the potential for field investigation. Moreover, past research has validated the reliability and accuracy of these devices, which can be integrated with a riders own bicycle⁵.

Paragraph Number 3 The purpose of the present investigation was to determine the efficacy of a supplemental carbohydrate-protein (CHO-PRO) recovery beverage on rates of muscle glycogen resynthesis during 4 hours of recovery when subjects were provided with exogenous CHO prior to and during a 62 km road ride. We hypothesized that the use of the on board power meters would allow us to control exercise intensities similar to laboratory conditions.

METHODS

Paragraph Number 4 Subjects. Eight recreationally active male cyclists (mean \pm SD: age 25 ± 4 yrs, mass 69 ± 5 kg, VO_2 peak 65.6 ± 5.3 mL \cdot kg⁻¹ \cdot min⁻¹, body composition $10.8 \pm 2.8\%$ body fat) participated in this study. Prior to data collection, the research procedures were approved by the University Internal Review Board. Subjects were informed of all experimental procedures and risks associated with the study and provided written consent prior to participation.

Paragraph Number 5 Preliminary testing. Peak oxygen uptake (VO_2 peak) was determined for each subject using a ramp protocol (25 W \cdot min⁻¹) on an electronically braked cycle ergometer (Velotron, Seattle, WA). Subjects fasted for 3 hours prior to the test. Expired gases were collected and analyzed at 15-second intervals during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT). Following the completion of the maximal exercise test, subjects were allowed to recover. The subjects own bicycle was outfitted with a PowerTap Pro power meter (CycleOps, Madison, WI)

and mounted on a stationary electronic trainer (CycleOps, Madison, WI). Subjects completed three steady state intensities (mean \pm sd: 152 \pm 28, 202 \pm 25, and 253 \pm 29 watts, respectively) on the stationary trainer to establish individual regression equations for power (watts), and VO_2 and VCO_2 ($\text{L} \cdot \text{min}^{-1}$). This was completed to account for potential differences in calibration of individual PowerTap and the cycle ergometer used for the maximal exercise test and to more accurately estimate total energy expenditure and whole body substrate oxidation during the field trial rides.

Paragraph Number 6 Body composition was determined using hydrodensitometry. Subjects fasted for no less than 3 hours prior to the underwater weighing. Subjects were instructed to void prior to all measurements. Body weight was recorded on a dry weight scale (Befour Inc., Cedarburg, WI). Subjects were then weighed in a lightweight swimming suit underwater using a calibrated underwater digital scale (Exertech, Dresbach, MN). Residual volume was estimated from known values based on the subjects' gender, age, and height. Body composition was calculated from body density²⁸.

Paragraph Number 7 Experimental Trials. All subjects participated in a placebo controlled, double-blind crossover experiment with at least 6 days between trials. Training logs and dietary recall were kept by the subjects for 3 days prior to the first trial. The records were used to standardize the subjects' physical activity and diets prior to the second experimental trial.

Paragraph Number 8 After an overnight fast, subjects reported to the lab at 0700. Subjects consumed a breakfast at 0715 (100g plain bagel, 28g cream cheese and 450 ml orange juice; 107g CHO, 16g protein, and 11.6g of fat). Subjects remained in the lab. At 0930 a baseline blood sample was obtained from an antecubital vein and collected in

untreated vacutainer tubes. The sample was allowed to clot for 15 minutes at room temperature, and then centrifuged at 4°C for 10 minutes at 4000 rpm. Serum was removed from the sample, separated into three cryovials, and stored at – 80°C for subsequent analyses of insulin and glucose. Subsequent blood collections were handled similarly. Immediately after the blood collection, the leg was prepared for a muscle biopsy. A muscle biopsy was obtained using a suction-modified Bergstrom needle-biopsy technique from the midsection of the vastus lateralis muscle. Muscle samples were dissected of any visible connective tissue, cut into three pieces, and placed in separate cryovials. Muscle samples were immediately frozen in liquid nitrogen and stored at – 80°C until assayed for total muscle glycogen. Following the collection of the muscle biopsy, subjects were asked to void after which a measure of body weight was obtained using the scale described above. At 1000 subjects consumed a food bar consisting of 38g CHO, 15g protein, and 5g fat. At 1015, subjects began the 62 km ride with 600 ml of water and 600 ml of a commercially available carbohydrate beverage (6% CHO solution) in separate bottles mounted to their road bike. Subjects completed a pre-determined course at a self-selected intensity similar to a typical training ride. At minutes 45 and 90 into the ride, subjects increased their intensity for 10 minutes to a self-selected power output and then resumed their normal pace and power output when the interval was completed. Time, velocity, distance, heart rate (HR) and power (watts) were displayed and recorded during the ride with a mounted cyclometer (PowerTap Pro, CycleOps, Madison, WI). Subjects were provided with a ride script based on the first trial to ensure similar intensities for the second road cycling trial. The total amounts of water and carbohydrate beverage consumed during the initial rides were standardized

between rides. Immediately after each ride, a post exercise blood sample was collected and a second biopsy was obtained from the vastus lateralis approximately 2 cm proximal to the first biopsy on the same leg. Blood and muscle samples were handled as described above. At 30 minutes post-exercise, 353 g (360 ml) of a recovery beverage containing 40g CHO, 20g protein (CHO-PRO) or a similarly flavored placebo (PL) was ingested. Additional blood samples were collected at 1, 2.5, and 4 hrs post-exercise. Two hours post-exercise, subjects consumed a standardized meal (112g spaghetti, 200 ml tomato sauce, 80g sourdough roll, and 480 ml milk; 165g CHO, 40g protein, and 7g fat). A final muscle biopsy was obtained at 4 hours post-exercise from the vastus lateralis approximately 2 cm proximal from the second biopsy on the same leg using the above mentioned procedures.

Paragraph Number 9 Blood analysis. Blood samples were analyzed in duplicate for glucose in using an enzymatic spectrophometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Insulin was also analyzed in duplicate using an enzymatic spectrophometric ELISA method (EIA2935, DRG International).

Paragraph Number 10 Muscle glycogen analysis. Muscle glycogen was analyzed using an enzymatic spectrophometric method after tissue preparation. Samples (25 ± 3.2 mg wet weight) were weighed upon removal from a -80°C freezer. Samples were placed in 1 ml, 1 N HCL solution and homogenized using a manual mortar and pestle tissue grinder. Once homogenized, samples were incubated at 95.6°C for three hours. After the incubation, 0.5 ml, 1 N NaOH was added to 0.5 ml of tissue sample to normalize pH. Samples were analyzed in triplicate against known glycogen and glucose controls run simultaneously. Muscle glycogen concentrations were expressed in $\text{mmol} \cdot \text{kg}^{-1}$ wet wt.

muscle. Muscle glycogenolysis for each trial was calculated from the equation: $(G_{\text{pre}} - G_{\text{post}}) / t$, where G_{pre} is muscle glycogen concentration pre-exercise, G_{post} is the muscle glycogen concentration immediately post-exercise, prior to feeding and t is the time between biopsies (hours). Rates of muscle glycogen resynthesis for each trial were calculated from the equation: $\text{Rate} = (G_{4\text{ post}} - G_{\text{post}}) / t$, where $G_{4\text{ post}}$ is the muscle glycogen concentration 4 hours post exercise, and t is the time between biopsies.

Paragraph Number 11 Statistics. Descriptive data including mean; duration, power (watts), HR, exercise intensity (%VO₂ peak), estimated total energy expenditure (TEE) (kcal/ride), and CHO intake, between trials were analyzed using a 2-tailed, dependent t-test. Average power output (watts) and HR of the first and second intervals between trials were analyzed using a 2 x 2 ANOVA with repeated measures. Blood glucose and insulin concentrations were analyzed using a 2 x 5 (pre, post, 1, 2.5, and 4hrs post-exercise) ANOVA with repeated measures. Changes in muscle glycogen concentration (pre and post) were analyzed across each trial using a 2 x 2 ANOVA with repeated measures. Muscle glycogenolysis (pre – post) was analyzed between trials using a 2-tailed, dependent t-test. Rates of muscle glycogen resynthesis (4 hrs post-exercise – post-exercise) were analyzed between trials using a 2-tailed, dependent t-test. Statistical significance was established using an alpha level of $p < 0.05$.

RESULTS

Paragraph Number 12 Similarity between trials. Average; duration, power, HR, exercise intensity, estimated TEE, and CHO intake were similar between the PL and CHO-PRO trials (Table 1, Figure 2).

Paragraph Number 13 Serum glucose. Glucose concentrations were similar between trials at pre and post-exercise ($p>0.05$) (Figure 3). After ingestion of the placebo or recovery drink, glucose was significantly elevated at 1 hour post-exercise compared to post-exercise values for the CHO-PRO trial, but not for the PL trial. At 2.5 hours post-exercise, glucose was significantly higher for both trials compared to post-exercise values. At 4 hours post-exercise, glucose concentration returned to post-exercise values in the CHO-PRO trial, but was significantly elevated during the PL trial compared to post-exercise values. Between trials glucose was significantly lower for the PL trial at 1 hour post-exercise and significantly higher at 4 hours post-exercise compared to the CHO-PRO trial.

Paragraph Number 14 Serum insulin. Insulin concentrations were similar between trials at pre and post-exercise ($p>0.05$) (Figure 4). Insulin levels were significantly elevated at 1 hour post-exercise compared to post-exercise during the CHO-PRO trial, but not significantly different for the PL trial. At 2.5 and 4 hours post-exercise insulin was significantly higher compared to post-exercise values in both PL and CHO-PRO trials. Between trials, insulin levels were significantly higher at 1 hour post-exercise in the CHO-PRO trial and significantly lower at 4 hours post-exercise compared to the PL trial.

Paragraph Number 15 Muscle glycogenolysis. Both trials demonstrated a decrease in muscle glycogen (142 ± 28 and 54.8 ± 28 mmol \cdot kg⁻¹ wet wt.⁻¹ for pre and post-exercise, respectively) but, there were no significant differences in the rates of glycogen depletion between the trials (44.8 and 40.6 mmol \cdot kg⁻¹ wet wt.⁻¹ \cdot hr.⁻¹ for PL and CHO-PRO, respectively) (Figure 5).

Paragraph Number 16 Muscle glycogen resynthesis. For the measure of muscle glycogen resynthesis, a total of six subjects were used as two post-exercise samples were damaged in the preparation/storage process. Muscle glycogen resynthesis was significant (4 hours post-exercise vs. post-exercise) ($p < 0.05$), but muscle glycogen resynthesis was similar for both the PL and CHO-PRO trials during the 4-hour re-feeding period (4.0 ± 2.1 and 4.9 ± 2.9 $\text{mmol} \cdot \text{kg}^{-1} \text{ wet wt.}^{-1} \cdot \text{hr.}^{-1}$)

DISCUSSION

Paragraph Number 17 With the advent of reliable power meters that can be integrated into a rider's own bicycle, it is possible to conduct controlled cycling trials in the field. In the present study we evaluated rates of muscle glycogen resynthesis during four hours of recovery from a 62 km road ride when exogenous CHO was provided. The main finding in the current study was that inclusion of a CHO-PRO supplement ingested 30 minutes post-exercise, in addition to a standard meal two hours post-exercise, did not increase the rates of muscle glycogen resynthesis above the PL trial.

Paragraph Number 18 After administration of the CHO-PRO recovery beverage 30 minutes post-exercise, serum glucose and insulin were significantly higher ($p < 0.05$) at one hour post-exercise compared to PL. It seems reasonable to assume that the elevated insulin and glucose levels shortly after exercise would provide for increased glucose transport into the muscle¹⁴. However, at four hours post-exercise serum glucose and insulin were significantly higher in the PL compared to CHO-PRO trial resulting in similar glycogen storage rates between trials.

Paragraph Number 19 In the current study the difference in total CHO intake between the PL and the CHO-PRO trials was relatively small during the 4-hour recovery period.

The CHO-PRO drink contained 40g CHO, equaling $\sim 0.29\text{g CHO} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$ (vs. 0 for PL) for the first two hours of recovery for the CHO-PRO trial. The meal received at two hours post-exercise contained 165g CHO equaling $\sim 1.2\text{g} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$ for hours 2-4 of the recovery period. Therefore, the overall rate of CHO intake during the entire 4 hour recovery period was 0.60 and $0.75\text{g} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$ for the PL and CHO-PRO trials respectively. Our results are in agreement with Ivy et al.²⁰, showing rates of glycogen resynthesis were similar when glucose was ingested at either 0.75 or $1.5\text{g glucose} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$, respectively) during a 4-hour recovery period. Similarly, Blom et al. demonstrated similar glycogen resynthesis rates when glucose was ingested at rates of 0.35 or $0.7\text{g glucose} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$, during six hours of recovery⁶. Blom et al. also noted that when CHO was ingested at only $0.18\text{g glucose} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$, glycogen storage rates were decreased.

Paragraph Number 20 When post-exercise CHO intake is ingested at high rates ($>1.2\text{g} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$) glycogen resynthesis rates can be markedly increased. Following >90 minutes of cycling, van Loon et al. demonstrated rates of glycogen resynthesis nearly tripled when post-exercise CHO intake during the 5 hour recovery period was given at 1.2 vs. $0.8\text{g} \cdot \text{kg}^{-1}\text{bw} \cdot \text{hr}^{-1}$ ³². The feeding protocol in the present investigation represents a less aggressive approach to post-exercise re-feeding. Therefore, it is possible that the present findings may in part be explained by both the small CHO load and the small differences in total CHO.

Paragraph Number 21 Of greater importance is the potential interaction between the 4-hour recovery period and the provision of supplemental CHO immediately prior to and during the rides. The majority of past research has compared rates of muscle glycogen

resynthesis in subjects that were fasted prior to and during exercise. In the present study, glycogen resynthesis was evaluated under fed conditions that might be described as more commonplace for endurance athletes. Subjects received a breakfast (107g CHO) three hours prior to exercise and a food bar (38g CHO) immediately before the start of exercise. During the exercise subjects consumed 28.6 ± 7.3 g CHO per ride. Our results are similar to the data of De Bock et al., who demonstrated lower rates of glycogen resynthesis when subjects were fed before and during 120 minutes of cycling vs. placebo¹². Rates of glycogen resynthesis during the 4-hour recovery period were three times higher (11.0 ± 7.8 vs. 32.9 ± 2.7 mmol · kg⁻¹ dw · hr⁻¹) for the fed vs. fasted trials¹². Although we did not compare fasted vs. fed states in the present investigation, the relatively low rate of glycogen resynthesis demonstrated in our subjects is comparable to the rate of resynthesis of fed subjects shown by De Bock et al.

Paragraph Number 22 Another finding was that muscle glycogenolysis was consistent between the two rides (88.9 ± 20 and 81.6 ± 31 mmol · kg⁻¹ wet wt.⁻¹ · 2hr⁻¹ for PL and CHO-PRO trials, respectively) despite the fact that they exercise trials were completed outside of the laboratory environment. The average exercise intensity for the rides was 62% VO₂ peak for each trial (73% for the two 10 minute intervals). These results are similar to the data of Ivy et al. who demonstrated similar rates of muscle glycogenolysis (~ 84.7 mmol · kg⁻¹ wet wt.⁻¹ · 2hr⁻¹) in response to ~ 2.5 hours of cycling at 65-75% VO₂ peak with 1 minute intervals¹⁸. Although the subjects in the present study were fed before and during the exercise, and cycled outdoors, rates of muscle glycogenolysis were comparable for similar intensities and durations of cycling previously collected under laboratory conditions.

Paragraph Number 23 The practical considerations from the present investigation are important in that they clearly demonstrate that the common practices of supplemental feedings prior to and during exercise are likely to influence the demonstrated effectiveness of post-exercise re-feeding strategies to improve rates of muscle glycogen recovery.

Paragraph Number 24 While previous investigations have clearly suggested nutritional protocols to increase rates of muscle glycogen resynthesis^{6-8, 18-21, 27, 30, 32, 33, 35}, it has also been shown that supplemental feeding during exercise may attenuate the demonstrated benefits of re-feeding strategies as they relate to glycogen recovery¹². In the present study we have determined that the inclusion of a CHO-PRO beverage (adding a small percentage to the total CHO intake) to post-exercise re-feeding did not increase muscle glycogen resynthesis when exogenous CHO was supplied before and during exercise. Additionally, our data reveal that research under controlled field conditions is viable and can be used to evaluate the effectiveness of feeding strategies on exercise performance and recovery in authentic training conditions.

REFERENCES

1. Ahlborg, B., J. Bergstrom, L.-G Ekelund and E. Hultman. Muscle Glycogen and Muscle Electrolytes during Prolonged Physical Exercise. *Acta Physiol Scand.* 70:129-142, 1967.
2. Bergstrom, J. and E. Hultman. The effect of exercise on muscle glycogen and electrolytes in normals. *Scand J Clin Lab Invest.* 18:16-20, 1966.
3. Bergstrom, J. and E. Hultman. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature.* 210:309-310, 1966.
4. Bergstrom, J. and E. Hultman. Synthesis of muscle glycogen in man after glucose and fructose infusion. *Acta Med Scand.* 182:93-107, 1967.
5. Bertucci, W., S. Duc, V. Villerius, J. N. Pernin, and F. Grappe. Validity and Reliability of the PowerTap Mobile Cycling Powermeter when compared with the SRM Device. *Int J Sports Med.* 26, 2005.
6. Blom, P. C., A. T. Hostmark, O. Vaage, K. R. Kardel, and S. Maehlum. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med Sci Sports Exerc.* 19:491-496, 1987.
7. Burke, L. M., G. R. Collier, and M. Hargreaves. Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. *J Appl Physiol.* 75:1019-1023, 1993.
8. Carrithers, J. A., D. L. Williamson, P. M. Gallagher, M. P. Godard, K. E. Schulze, and S. W. Trappe. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. *J Appl Physiol.* 88:1976-1982, 2000.
9. Costill, D. L. and M. Hargreaves. Carbohydrate nutrition and fatigue. *Sports Med.* 13:86-92, 1992.
10. Costill, D. L., W. M. Sherman, W. J. Fink, C. Maresh, M. Witten, and J. M. Miller. The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. *Am J Clin Nutr.* 34:1831-1836, 1981.
11. Danforth, W. H. Glycogen Synthetase Activity in Skeletal Muscle. Interconversion of Two Forms and Control of Glycogen Synthesis. *J Biol Chem.* 240:588-593, 1965.
12. De Bock, K. Exercise in the fasted state facilitates fibre-type specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *J. Physiol* 564, 2005.
13. Fallowfield, J. L., C. Williams, and R. Singh. The influence of ingesting a carbohydrate-electrolyte beverage during 4 hours of recovery on subsequent endurance capacity. *Int J Sport Nutr.* 5:285-299, 1995.
14. Goodyear, L. J., M. F. Hirshman, P. A. King, E. D. Horton, C. M. Thompson, and E. S. Horton. Skeletal muscle plasma membrane glucose transport and glucose transporters after exercise. *J Appl Physiol.* 68:193-198, 1990.
15. Hermansen, L., E. Hultman, and B. Saltin. Muscle glycogen during prolonged severe exercise. *Acta Physiol Scand.* 71:129-139, 1967.
16. Hultman, E. and J. Bergstrom. Muscle glycogen synthesis in relation to diet studied in normal subjects. *Acta Med Scand.* 182:109-117, 1967.
17. Ivy, J. L. Muscle glycogen synthesis before and after exercise. *Sports Med.* 11:6-19, 1991.

18. Ivy, J. L., H. W. Goforth, Jr., B. M. Damon, T. R. McCauley, E. C. Parsons, and T. B. Price. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J Appl Physiol.* 93:1337-1344, 2002.
19. Ivy, J. L., A. L. Katz, C. L. Cutler, W. M. Sherman, and E. F. Coyle. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. *J Appl Physiol.* 64:1480-1485, 1988.
20. Ivy, J. L., M. C. Lee, J. T. Brozinick, Jr., and M. J. Reed. Muscle glycogen storage after different amounts of carbohydrate ingestion. *J Appl Physiol.* 65:2018-2023, 1988.
21. Jentjens, R. and A. Jeukendrup. Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med.* 33:117-144, 2003.
22. Jentjens, R. L., L. J. van Loon, C. H. Mann, A. J. Wagenmakers, and A. E. Jeukendrup. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. *J Appl Physiol.* 91:839-846, 2001.
23. Parkin, J. A., M. F. Carey, I. K. Martin, L. Stojanovska, and M. A. Febbraio. Muscle glycogen storage following prolonged exercise: effect of timing of ingestion of high glycemic index food. *Med Sci Sports Exerc.* 29:220-224, 1997.
24. Piehl, K. Time course for refilling of glycogen stores in human muscle fibres following exercise-induced glycogen depletion. *Acta Physiol Scand.* 90:297-302, 1974.
25. Price, T. B., D. L. Rothman, R. Taylor, M. J. Avison, G. I. Shulman, and R. G. Shulman. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *J Appl Physiol.* 76:104-111, 1994.
26. Reed, M. J., J. T. Brozinick, Jr., M. C. Lee, and J. L. Ivy. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. *J Appl Physiol.* 66:720-726, 1989.
27. Ruby, B. C., S. E. Gaskill, D. Slivka, and S. G. Harger. The addition of fenugreek extract (*Trigonella foenum-graecum*) to glucose feeding increases muscle glycogen resynthesis after exercise. *Amino Acids.* 28:71-76, 2005.
28. Siri, W. E. Body composition from fluid spaces and density: analysis of methods., 1961.
29. Siu, P. M., S. H. Wong, J. G. Morris, C. W. Lam, P. K. Chung, and S. Chung. Effect of frequency of carbohydrate feedings on recovery and subsequent endurance run. *Med Sci Sports Exerc.* 36:315-323, 2004.
30. Tarnopolsky, M. A., M. Bosman, J. R. Macdonald, D. Vandeputte, J. Martin, and B. D. Roy. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. *J Appl Physiol.* 83:1877-1883, 1997.
31. van Hall, G., W. H. Saris, P. A. van de Schoor, and A. J. Wagenmakers. The effect of free glutamine and peptide ingestion on the rate of muscle glycogen resynthesis in man. *Int J Sports Med.* 21:25-30, 2000.
32. van Loon, L. J., W. H. Saris, M. Kruijshoop, and A. J. Wagenmakers. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *Am J Clin Nutr.* 72:106-111, 2000.

33. Williams, M. B., P. B. Raven, D. L. Fogt, and J. L. Ivy. Effects of recovery beverages on glycogen restoration and endurance exercise performance. *J Strength Cond Res.* 17:12-19, 2003.
34. Zachwieja, J. J., D. L. Costill, D. D. Pascoe, R. A. Robergs, and W. J. Fink. Influence of muscle glycogen depletion on the rate of resynthesis. *Med Sci Sports Exerc.* 23:44-48, 1991.
35. Zawadzki, K. M., B. B. Yaspelkis, 3rd, and J. L. Ivy. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J Appl Physiol.* 72:1854-1859, 1992.

Table 1. Descriptive data for both trials (mean \pm SD)

Trial	Duration (min)	Power (watts)	HR (bpm)	Exercise Intensity (%VO₂ peak)	TEE (kcal/ ride)	CHO intake during ride (g)
PL	122.4 \pm 5.8	210 \pm 28	148 \pm 8	63.6 \pm 11.6	1728 \pm 292	28.6 \pm 7.3
CHO- PRO	123.8 \pm 5.2	212 \pm 26	148 \pm 8	63.8 \pm 10.6	1753 \pm 285	28.6 \pm 7.3

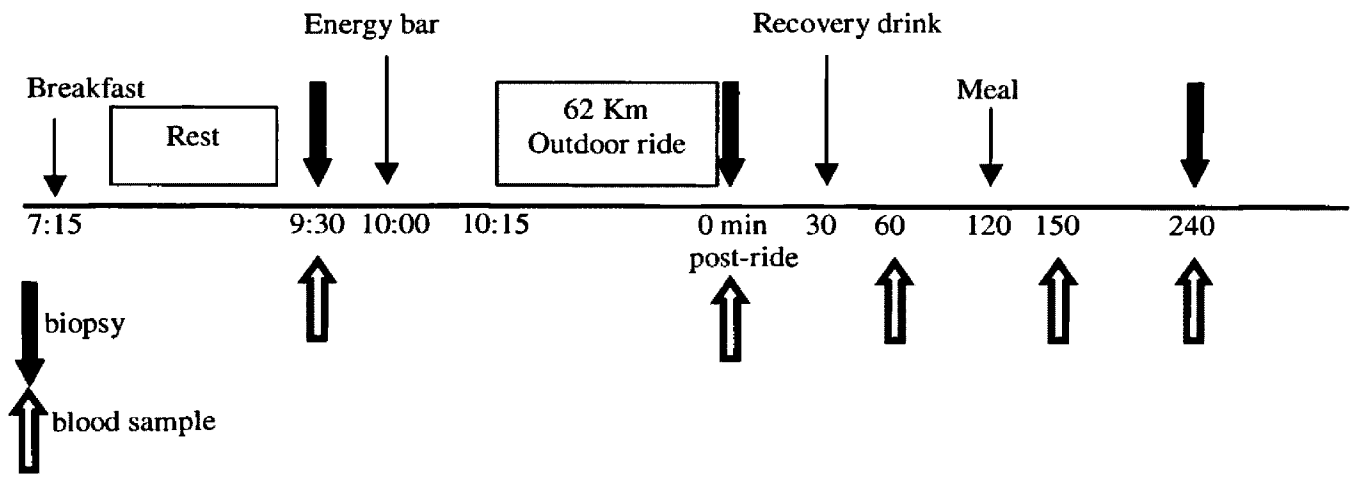


Figure 1. Experimental timeline of events and schedule.

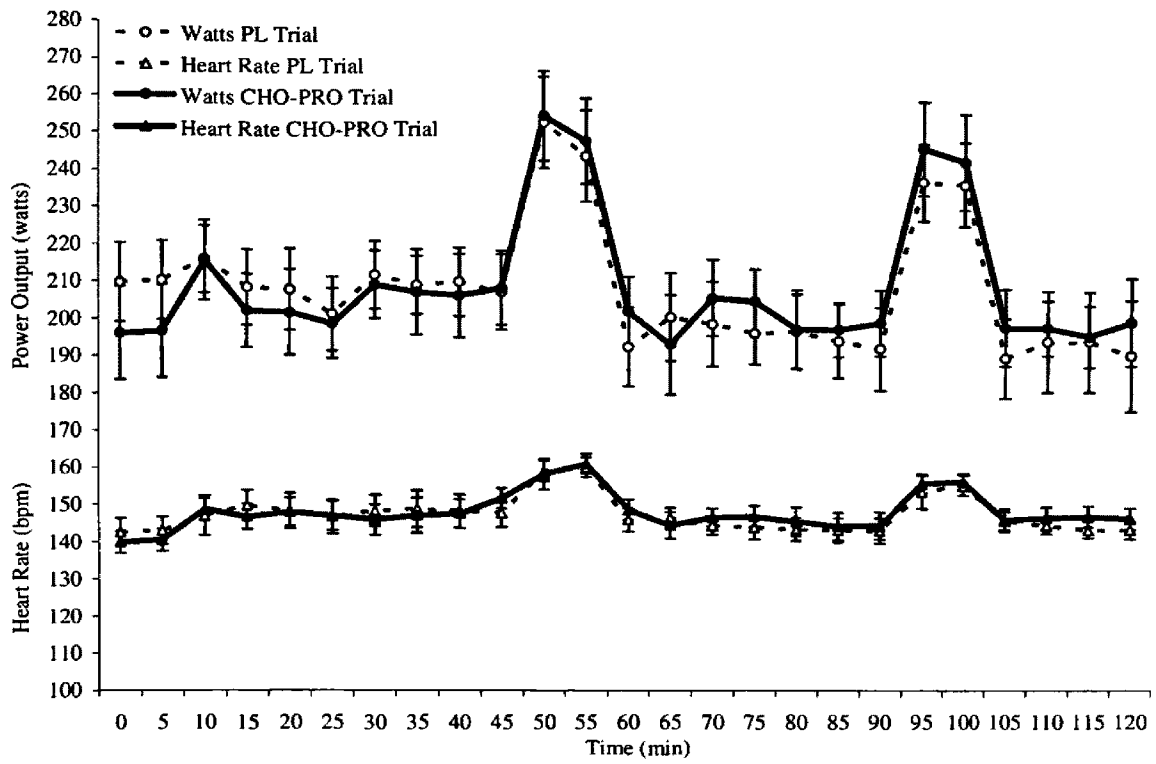


Figure 2. Mean heart rate and power for both trials (5 min averages).

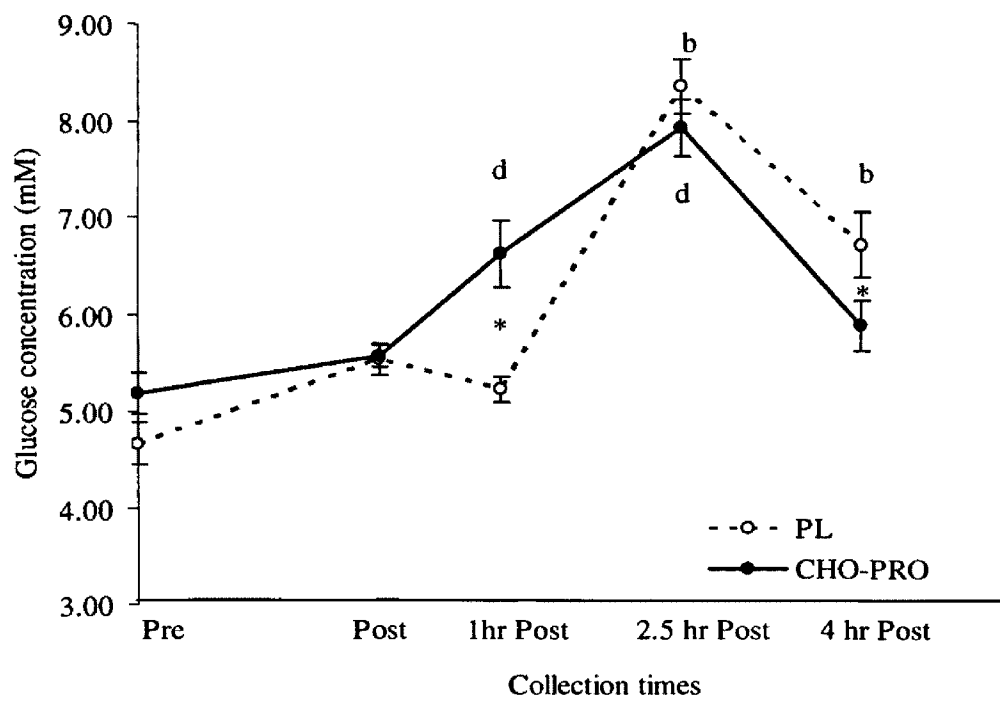


Figure 3. Serum glucose concentrations (mM) during the exercise and recovery periods.

*, $p < 0.05$, CHO-PRO vs. PL. *b*, $p < 0.05$ vs. post (PL); *d*, $p < 0.05$ vs. post (CHO-PRO).

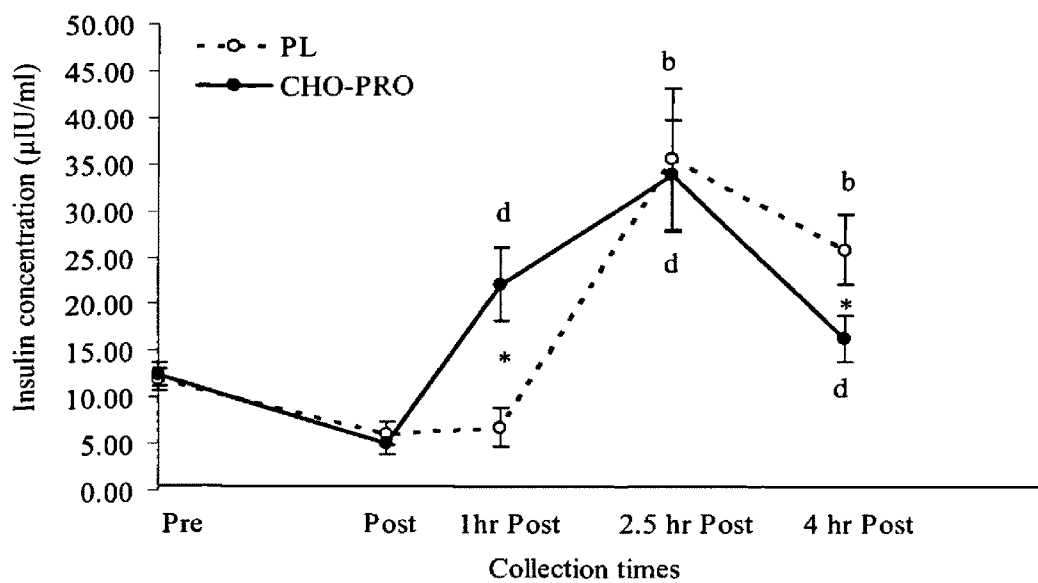


Figure 4. Serum insulin concentrations ($\mu\text{IU/ml}$). *, $p < 0.05$, CHO-PRO vs. PL. *b*, $p < 0.05$ vs. post (PL). *d*, $p < 0.05$ vs. post (CHO-PRO).

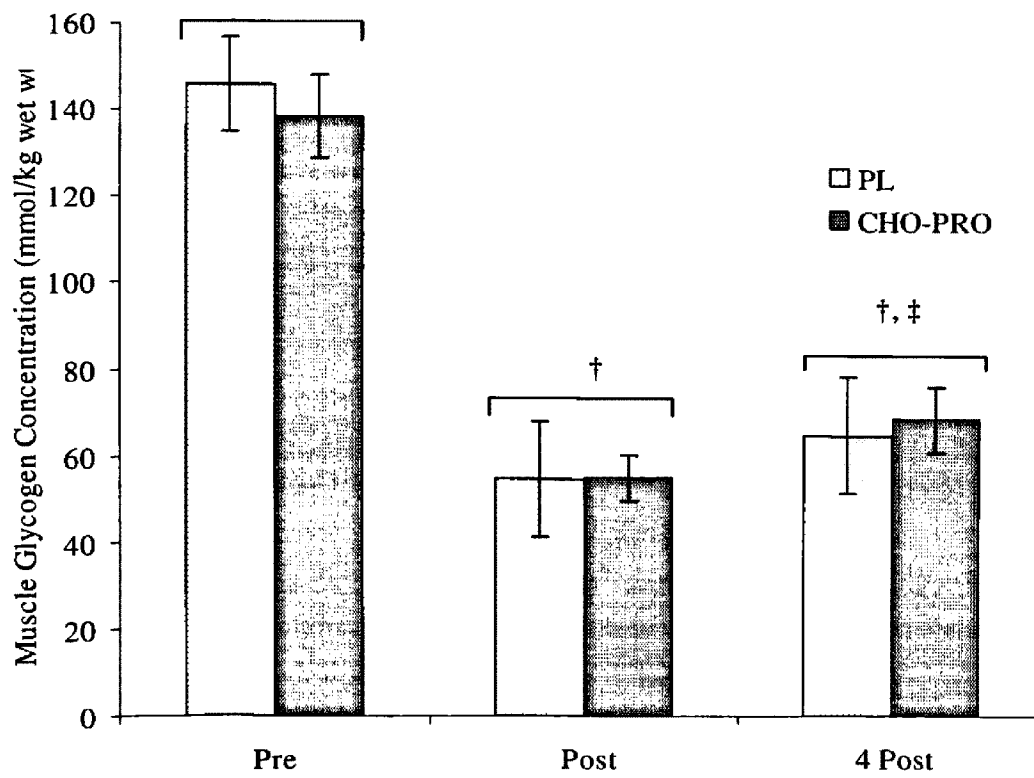


Figure 5. Muscle glycogen concentrations (mmol/kg wet wt) for pre, post and 4 hours post-exercise. †, $p < 0.05$ vs. pre ($n = 8$, main effect of time); ‡, $p < 0.05$ vs. post ($n = 6$, main effect of time).

INFORMED CONSENT

HUMAN PERFORMANCE LABORATORY
Dept of Health and Human Performance
UNIVERSITY OF MONTANA
Missoula, Montana

SUBJECT INFORMATION AND CONSENT FORM

Participant _____ ID# _____ Today's Date: _____
/_____/_____
(Print Name) Month Day
Year

Effects of exercise and meal replacement on glycogen resynthesis and performance

STUDY DIRECTOR(S): Brent Ruby, Ph.D. (406) 243-2117
University of Montana

This consent form may contain words that are new to you. If you read any words that are not clear to you, please ask the person who gave you this form to explain them to you.

PURPOSE OF THE RESEARCH

- You are being asked to take part in a research study to evaluate 1) the effects of road riding on the depletion of muscle glycogen (the stored form of carbohydrate in the muscle) and 2) the effects of different types of meals provided after an extended period of cycling exercise. Currently, it is thought that the consumption of easy to digest liquid and/or solid carbohydrate sources consumed immediately after exercise leads to a more rapid recovery and replenishment of your muscle glycogen (stored muscle carbohydrate). However, it is unclear whether traditional meals or the addition of a sports drink/food are a better approach to improve muscle recovery.

OVERALL PROCEDURES

This research will require 3 visits to the Human Performance Lab in McGill Hall, University of Montana. You have been asked to serve as a subject for this exercise study because you are a trained male cyclist between the ages of 18-40 and have a peak VO_2 of at least 50 ml/kg/min and have a history of road riding between 100-400 miles/week.

The different tests that will be done as part of this study include:

1. A measure of underwater weighing to estimate body composition.

2. A graded exercise test on your own bicycle (mounted to a stationary trainer) to measure the maximal rate of oxygen consumption your body can consume during intense exercise. This test will also allow us to estimate your ventilatory threshold (anaerobic threshold).
3. Two extended cycle exercise bouts with a monitored 4-hour recovery session. These two trials will include an extended bout of road cycling followed by a post exercise feeding schedule which will include a combination of liquid and solid food sources with or without a carbohydrate/protein sports drink or placebo. The order of the two trials will be random and you will be blinded as to what post exercise feeding you will be provided with.
4. During the course of each exercise trial you will have three muscle biopsies taken from the quadriceps muscle (pre, post, 4-hour post exercise). After the exercise trials, venous blood samples from your arm vein will also be collected to determine changes in blood sugar and insulin. These are safe techniques and standard laboratory practices for safety and sterility are followed. Both require a needle and are associated with a small amount of pain. These methods will be fully discussed with you prior to your participation.

The following testing sessions will be completed for this study.

- **Session 1: Maximal Exercise Test – Cycle Ergometer:**
This test will consist of riding your own road bicycle mounted on a stationary trainer to a maximal effort. You will be asked to increase the resistance of the bicycle each minute by changing your cadence and/or your gears. This will progress to fatigue. You will be encouraged to continue to ride until exhaustion. During the entire testing session on the cycle, you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen your body uses during the exercise. Your heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 45 minutes to 1-hour. You should not eat for a period of approximately 3 hours prior to the completion of this test.
- **Session 2: Body Fat Measurement – Underwater Weighing:**
This test session will require that you do not eat for a minimal of 4 hours prior to the testing. Prior to the test, your body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighting tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. Your underwater weight will be recorded within 2-4 seconds and you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided at your request. This test will take approximately 30 minutes.

- Sessions 3, 4: Extended Endurance Ride and Post-Exercise Muscle Carbohydrate Evaluation:

The order of these sessions will be randomized and blinded to you as a study participant. These trials will be separated by no less than 7-days so that both post exercise feeding protocols can be completed. You will report to the laboratory after a 12 hour fast (at approximately 6:00 AM). Upon arrival, you will be provided with a standardized breakfast that will include a small amount of juice, a bagel and a Gatorade food bar. After the consumption of breakfast, you will be allowed to rest (read, complete homework, or watch a movie) for a period of 3 hours. After the 3-hour rest, the following procedures will be completed prior to the road ride.

1) A resting blood sample will be obtained from an arm vein.

2) A muscle biopsy will be obtained from your front thigh muscle (vastus lateralis, approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the site be sterilized. After the site is cleaned, a small amount of lidocaine will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the site on the leg. After the area is treated with the lidocaine (approximately 3-5 ml, 1% lidocaine), a small incision (approximately 1/4 inch long) will be made through the skin and to a depth of approximately 3/4-1.5 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips and/or a single stitch and bandaid and wrapped with an ace bandage. A flexible ice pack will then be placed on the site for 10 minutes. The muscle biopsies will be used to evaluate alterations in muscle carbohydrate and fat stores in response to physical activity. Latex free bandages will be provided if subjects have a known allergy to latex. All of the muscle biopsies will be conducted by Dr. Brent Ruby, the study director.

3) The road ride exercise bout will include a 50-mile ride that includes several intervals similar to the intensities you would select during a typical training ride. During the ride, you will be followed by a chase vehicle that will bring additional water and provide mechanical assistance if you have a flat tire. It is expected that the total ride time will be approximately 2.5 to 3 hours in duration.

4) The post-exercise period will be initiated immediately after the road ride exercise bout is completed and you return to the laboratory. Immediately upon return to the laboratory, a second muscle biopsy will be obtained from the same area on the same leg to determine the extent of muscle carbohydrate loss. At this time, another blood sample will be obtained from an arm vein as indicated above. After the collection of the biopsy and blood sample, you will

consume either a sport drink containing a mixture of carbohydrate (sugar sources) and protein or a similarly flavored placebo beverage. After two hours of recovery, you will be provided with a high carbohydrate meal including common food items that you select from a list (bagels, potatoes, pasta, etc). At the 4-hour time point, a final muscle biopsy will be obtained as indicated above to determine how much muscle carbohydrate has been restored in the muscle. The total time commitment for each of these exercise session days will be approximately 11 hours (including the rest, exercise, and recovery periods).

LOCATION AND STUDY LENGTH.

The majority of the study will take place at the Human Performance Laboratory in McGill Hall (First Floor – enter main doors, go straight through lobby, then enter lab via first door to the right.) The road ride exercise periods will be conducted outdoors on road ride routes that you commonly ride during your normal training. The time commitments for each of the testing sessions have been indicated above. The time commitment for your participation in this study is expected to be approximately 25 hours in total.

PAYMENT

The main variable measured during the road rides is power (watts). Therefore, your road bike will be fitted with a specialized rear hub that allows power output to be quantified (a measure typically limited to laboratory equipment). As compensation for your time and participation in this study and upon completion of the study (for the completion of both endurance exercise trials) you will be allowed to keep the power system (retail value \$1200).

If you are not able to complete the entire study, you will be compensated as follows (\$15 for completion or partial completion of the body composition and/or maximal exercise test (even if you do not meet the exclusion criteria of a peak VO_2 of 50 ml/kg/min), \$50 for completion or partial completion of either of the road cycling trials). For example, if you complete the body fat and max test and one road cycling trial (but decide to drop out of the study before completing the second road cycling trial, you will receive \$65 for your time).

RISKS/DISCOMFORTS

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting. A researcher will be in close proximity to the cycle ergometer during the maximal exercise test to assure your safety in case of a loss of balance due to fatigue.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.

3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred, as with other moderately strenuous exercise activities. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. Equipment and trained personnel are available to deal with unusual situations should they arise. A trained CPR technician will be on hand at all times and the laboratory has standard emergency procedures should any potential problems arise.
4. You will be informed of any new findings that may affect your decision to remain in the study.
5. The muscle biopsy or blood draw techniques may cause some local and temporary discomfort. Should redness or swelling persist, you should seek appropriate medical attention and then notify the study director, Brent C. Ruby, (406) 243-2117 (office) and/or (406) 396-4382 (cell).
6. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped.

BENEFITS OF PARTICIPATING IN THIS STUDY

1. There is no promise that you will receive any benefit outside of the compensation described above as a result of taking part in this study.
2. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.

CONFIDENTIALITY

1. *Your records will be kept private and will not be released without your consent except as required by law.*
2. Only the researchers will have access to the files.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, your name will not be used.
5. All data, identified only by an anonymous ID #, will be stored in our laboratory.
6. Your signed consent form and information sheet will be stored in a locked office separate from the data.
7. The muscle tissue will be analyzed for muscle glycogen and other possible metabolic markers or mRNA associated with select muscle proteins. Blood samples will be analyzed for metabolic markers and hormones such as glucose, lactate, and insulin. All tissue (blood/muscle) remaining after the analyses for the aforementioned will be destroyed.

COMPENSATION FOR INJURY

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. *“In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Claim representative or University Legal Counsel.”*

VOLUNTARY PARTICIPATION/WITHDRAWAL

1. You have the right to request that a test be stopped at any time.
2. Your decision to take part in this research study is entirely voluntary.
3. You may refuse to take part in or you may withdraw from the study at any time without penalty or loss of benefits to which you are normally entitled. If you decide to withdraw from the study at any time, you will be provided with the compensation according to the schedule described above.
4. You may leave the study for any reason.

You may be asked to leave the study for any of the following reasons:

1. Failure to follow the study investigator’s instructions.
2. A serious adverse reaction, which may require evaluation.
3. The study director/investigator thinks it is in the best interest of your health and welfare.
4. The study is terminated.

QUESTIONS

- You may wish to discuss this with others before you agree to take part in this study.
- If you have any questions about the research now or during the study contact: Dr. Brent Ruby (406) 243-2117 (office), (406) 396-4382 (cell).
- If you have additional questions about being a research subject, you can also contact the Chair of the Internal Review Board at 243-6670.

SUBJECT'S STATEMENT OF CONSENT

I have read the above description of this research study. I have been informed of the risks and benefits involved, and all my questions have been answered to my satisfaction. Furthermore, I have been assured that a member of the research team will also answer any future questions I may have. I voluntarily agree to take part. I understand I will receive a copy of this consent form.

Printed (Typed) Name of Subject

Subject's Signature

Date

Witness Signature

Date

SUBJECT'S STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING THE STUDY

I voluntarily agree to allow my photograph to be taken during the data collection for this study. I also provide my consent allowing the researchers to use these photographs as part of further presentation of these data.

Printed (Typed) Name of Subject

Subject's Signature

Date