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The effects of post-exercise nutrient intake on hydration status and muscle soreness ratings in youth cyclists

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The Effects of Post-Exercise Nutrient Intake on

Hydration Status and Muscle Soreness Ratings in Youth Cyclists

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by Allison Taylor Cadematori

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PUBLIC PRESENTATION

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Introduction

Endurance Exercise

Endurance exercise training leads to a wide variety of physiological adaptations. These adaptations allow trained individuals to have smaller homeostatic disturbances with endurance exercise than untrained individuals. For example, endurance cycling results in positive adaptations to various physiological systems, including cardiovascular (increased stroke volume, cardiac hypertrophy), muscular (increased mitochondrial density, capillarization), and respiratory function (3,29,45). Collectively, these changes result in improvements in lactate threshold, movement economy, and peak aerobic capacity (VO_{2max}), which relate to enhanced cycling performance. In order to elicit the aforementioned physiological gains, endurance cyclists engage in heavy training, with high metabolic/caloric demands (29). Such training causes acute physiological changes (such as glycogen and fluid losses, and muscle damage), which must be ameliorated to enable high training quality in subsequent bouts of exercise. These issues will be discussed in further detail below, as well as nutritional strategies which may support recovery between heavy exercise sessions.

Endurance exercise utilizes, and in turn depletes, a variety of substrates. Endogenous carbohydrate reserves (such as liver and muscle glycogen) are the primary fuel for skeletal muscle in exercise lasting less than two hours (33). Muscle/liver glycogen levels can be substantially depleted during endurance exercise, especially in prolonged moderate- to high-intensity events. Muscle glycogen depletion is linked to general feelings of fatigue during exercise, and can contribute to the inability to continue performing exercise (1,11,15,16,22).

Exercise induced muscle damage (EIMD) and sarcolemmal disruption are known to occur with moderate to high intensity exercise, especially if the exercise is largely eccentric in nature or if the athlete is unaccustomed to the exercise. EIMD and the oxidative stress associated with endurance exercise results in increased levels of perceived soreness, decreased contractile force of the muscle, and can be detrimental to performance in subsequent exercise. (23,38).

In addition, fluid losses during endurance training can lead to exercise-induced dehydration due to perspiration and environmental factors (i.e. high ambient temperatures, humidity, etc.). When exercise-induced dehydration is greater than two percent of body weight, it causes significant impairments in subsequent endurance performance (13). Dehydration has also been shown to significantly impair cardiovascular function during endurance exercise, especially when an athlete becomes hyperthermic (24). Furthermore, electrolytes lost with sweat, such as potassium, may also become depleted during endurance exercise. This can also have negative effects on the athlete, as cellular potassium losses may decrease muscular contractile ability and increased perceived fatigue (28).

The effects of chocolate milk on recovery: refueling, repair, and rehydration

Post exercise nutrition for athletes is essential to promote overall recovery and the development of positive adaptations to training. Generally, nutritional approaches to recovery have emphasized three integrated approaches: refueling, repair, and rehydration (33). In recent years, chocolate milk has received a considerable amount of attention as a recovery beverage. As discussed below, this is because the carbohydrate, protein, and water/electrolyte content of chocolate milk appear to align effectively with strategies for refueling, repair, and rehydration, respectively.

<u>Refueling</u>

Post-exercise carbohydrate intake is important to promote effective muscle glycogen resynthesis. Muscle glycogen resynthesis rates appear to be enhanced by carbohydrate intake in dose response fashion, with maximal resynthesis rates achieved with ingestion rates of ~1.0-1.2 g/kgBW/hr (2). Some studies show that the co-ingestion of carbohydrate and protein (CHO+Pro) may have even greater benefits than carbohydrate alone, possibly due the influences of protein on insulin sensitivity (2). However, most evidence suggests that CHO+Pro supplements will augment glycogen resynthesis only when doses are below optimal rates, and glycogen replenishment rates are not likely to be increased with CHO+Pro when carbohydrate is administered at ≥ 1 g/kgBW/hr (2). Nevertheless, these findings indicate that CHO+Pro ingestion following exercise is an effective strategy for glycogen resynthesis following exercise, and will likely elicit equal or superior resynthesis rates to isocaloric carbohydrate ingestion, depending on the amount consumed.

Bovine milk is a beverage that naturally contains carbohydrate (from lactose) and protein (casein and whey), and the flavoring in chocolate milk adds additional carbohydrate content (often from sucrose). As a result, the macronutrient composition of chocolate milk (carbohydrate and protein content) is very similar to CHO+Pro beverages that have been shown to be effective for glycogen replenishment (2). Ferguson-Stegall *et al.* reported that chocolate milk consumption after heavy endurance exercise improved glycogen resynthesis versus a placebo, and similar levels to an isocaloric CHO recovery drink containing 2.5g CHO/kg/hr (8). In addition, a number of studies have reported that post-exercise chocolate milk consumption has resulted in enhanced time to fatigue in subsequent exercise, in comparison to carbohydrate only beverages (20,26,48). It has been speculated that this outcome was due to enhanced muscle glycogen resynthesis, but none of these studies directly measured glycogen levels (26,48).

<u>Repair</u>

Protein ingestion can influence protein turnover rates during recovery, which may be important for muscle repair (27,41). Specifically, Howarth and colleagues reported that skeletal muscle fractional synthetic rate (FSR) was elevated with CHO+Pro ingestion following exercise, compared to carbohydrate alone, regardless of the carbohydrate dose administered. As a result, CHO+Pro improved FSR and whole-body net protein balance, even when compared to an isocaloric carbohydrate only treatment (19). In addition, Moore et al reported that the co-ingestion of CHO+Pro may attenuate exercise-induced muscle protein breakdown by reducing the demand for endogenous amino acid release from the muscle. This could prevent the development of a catabolic muscular environment after exercise (33). These changes may have implications for post-exercise recovery in athletes. For example, D'Lugos et al found that supplemental protein during training and recovery has positive influences on skeletal muscle function and morphology after intensified endurance cycling training (6). In addition, several studies have reported that CHO+Pro ingestion following endurance exercise has resulted in lower levels of post-exercise muscle soreness, plasma CK (a marker of sarcolemmal disruption) and/or attenuated impairment in muscle function versus post-exercise carbohydrate ingestion (36,42).

Milk and/or chocolate milk has a protein content high enough to elicit increased protein balance and protein synthesis (8,30,32). Volterman and associates reported that protein balance was more positive when subjects consumed skim milk, versus carbohydrate/electrolyte drinks or water after exercise (49). In addition, post-exercise chocolate milk intake has been associated with decreased muscle soreness (35,37,38) and decreased sarcolemmal disruption after heavy exercise in some (4,36), but not all (9) studies.

Rehydration

Carbohydrate/electrolyte beverages have generally been shown to promote fluid retention after exercise. There is varying data about whether or not the addition of protein is beneficial for rehydration. Some studies have reported that beverages containing protein enhance hydration, due to the beneficial effects of protein on water and sodium absorption in the small intestine (51). However, other studies have suggested that high doses of protein intake may slow gastric emptying, which could be detrimental for rehydration (42).

Milk/chocolate milk contains moderate amounts of protein per serving (8 g per 8 ounces). Shirreffs, Watson, and Maughan reported that milk consumption after exercise which elicited dehydration of < 2% body weight, resulted in superior rehydration/fluid retention versus a carbohydrate-electrolyte sports drink, and versus plain water. The authors speculated that the electrolyte content of milk, particularly its high potassium content, was likely the cause of the positive fluid balance (43). This data aligns with a study by Watson, and colleagues, as well as two studies from Voltermann et al., which all reported that skim milk ingestion enhanced fluid retention versus carbohydrate-electrolyte sports drinks (50,51,52).

Chocolate milk and adolescent athletes

The carbohydrate, protein, and water/electrolyte content of chocolate milk appears to be well suited to supporting the refuel, repair, and rehydration principles of recovery discussed above. In addition, chocolate milk contains other nutrients that are commonly deficient in the adolescent diets, such as iron, calcium, and vitamin D (7,34,44).

Vitamin D deficiencies were found in 24.1% of teenage subjects in a 2014 study. Subjects were limited to an urban environment, however, the authors explained that it is likely that a smaller, yet still significant, proportion of teenagers are deficient in Vitamin D nationwide (11). Chocolate milk has 32% of the RDA of Vitamin D, making it a potentially large contributor to the overall Vitamin D levels of adolescents. Similarly, chocolate milk provides a significant proportion of the RDA for iron and calcium, indicating that this beverage can help support a variety of nutrient needs in youth athletes.

As chocolate milk is a commonly consumed, whole food, there are ethical advantages to its promotion use a recovery beverage, as compared to nutritional supplements (7). Additionally, the relatively low cost, accessibility, and taste make it a strong candidate for post-exercise nutrition in adolescents. Many studies, as discussed above, have analyzed the effects of chocolate milk on recovery. However, there is very little information regarding the effects of chocolate milk for post-exercise nutrition in adolescent athletes. This limitation is likely due to practical challenges of recruiting sufficiently large groups of competitive adolescent athletes. Adolescent athletes do not have the same metabolic demands as adults, and there is a need for evidence-based guidelines for this population that are not solely scaled down versions of guidelines designed for adults (7).

The JMU HPL and the Miller School of Albemarle Endurance Team

Over the past three years, the James Madison University (JMU) Human Performance Lab (HPL) has established a partnership with the Miller School of Albemarle's (MSA) Endurance Team, an elite high-school cycling program. As a result, the HPL has conducted physiological/ performance testing on many of the team's riders, to assist the team in developing optimal training and nutrition programs. Riders on the MSA Endurance Team have garnered multiple national championships, college scholarships, World Championship team selections, and professional cycling contracts. Due to their highly competitive status, the training loads of these adolescent cyclists are very high in comparison to traditional high school athletes. The highly competitive status of these athletes makes them ideal subjects in which to investigate the effects of chocolate milk for recovery, as they are able to complete the high intensity endurance exercise associated with depleted endogenous carbohydrate reserves, muscle damage, and dehydration.

Research Questions

1. Is perceived muscle soreness following heavy cycling exercise reduced following the consumption of chocolate milk (CM), in comparison to carbohydrate-electrolyte (CHO), or placebo (PL) beverages?
2. Is hydration status following heavy cycling exercise influenced by the consumption of chocolate milk (CM), in comparison to carbohydrate-electrolyte (CHO), or placebo (PL) beverages?

Hypotheses

1. Perceived muscle soreness ratings will be lower following CM ingestion, versus CHO and PL beverages.
2. Hydration status will be enhanced after CM ingestion, versus CHO and PL beverages.

Proposed Methods

Overview

This study will test the effects of altered post-exercise nutrient intake on recovery from exercise in youth cyclists. Data collection will occur from September 2018 through January of 2019. Following heavy cycling exercise, one of three different post-exercise recovery beverages (with varying macronutrient levels) will be administered, and muscle soreness ratings and hydration status will be assessed thereafter.

Each subject will complete the exercise and nutrition protocols described below on three different occasions, and will receive one type of nutritional treatment during each occasion. Nutritional treatments will be provided using a randomly counterbalanced design, with the treatments double-blinded. There will be approximately one week between each trial.

Subjects

Eight subjects (males and females ages 14-18) will be completing this protocol: 6 MSA athletes, and 2 non-MSA athletes from the Harrisonburg area. Parental consent and youth assent will be obtained prior to all testing procedures. All subjects must train at least seven hours a week and have a VO_2 peak of at least 50 mL/kg/min in order to be included in this study due to the rigorous nature of the training protocols. All testing procedures were approved by the Institutional Review Board of JMU.

Experimental Protocols

Pre-test measurements

Prior to the experimental trials, all subjects will complete preliminary testing in order to determine their VO_{2peak} and W_{max}. Subjects will begin cycling at a moderate workload and every two minutes the workload will be increased by 25W until volitional fatigue is reached. VO_{2peak} and W_{max} will be obtained from the highest workload achieved, and will be used to determine appropriate workloads for each athlete in the experimental trials.

Preliminary Exercise

Participants will complete a bout of cycling exercise lasting approximately 90 min. The first portion will be 25 min of constant load exercise: five min at 40% W_{max} and 20 min at 60% W_{max}. There will then be 60 min of high-intensity intervals, and five mi of cooldown at 40% W_{max}. 250 mL of water will be provided every 15 min and consumed as desired by the subject. An interval workout was selected for the exercise bout, as it has been shown to significantly deplete muscle glycogen (21) and similar protocols have been used in similar studies focused on adults (26,49).

The intervals will consist of two min at a high workload, followed by two min at 50% W_{max}. The high workload will be 90% W_{max} for as long as can be sustained, and will then be lowered by 10% of W_{max} as needed. Workload will be lowered if the subject cannot sustain a cadence of at least 60 rpm. The interval training will end when the subject cannot continue with a cadence of 60 rpm at 70% W_{max} during the high intensity intervals, or when 15 high intensity intervals are completed.

Nutritional Treatments

Subjects will be administered one treatment beverage option on each trial day immediately after exercise and two hours following exercise.

Treatments:

Chocolate Milk (CM): 11.8 mL/kgBW of low-fat chocolate milk, containing approximately 1.2 g CHO, 0.4 g Pro, 0.11 g fat, 9 mg sodium, and 21 mg potassium per kgBW)

Carbohydrate-electrolyte beverage (CHO): 1.72 g/kgBW of chocolate flavored Clif Shot gels, containing approximately 1.2 g CHO, 0 g Pro, 0.08 g fat, 3.1 mg sodium, and 4 mg potassium per kgBW) mixed with water to provide 11.8 mL/kgBW of beverage

Placebo (PL): 11.8 mL/kgBW of an artificially-flavored water beverage

During all trial days, subjects will eat a self-selected, standardized lunch at 12:15 pm that is typical of their regular, daily meal. The constituents of this meal will be recorded and replicated in subsequent trials.

Dependent Measurements

Muscle Soreness Ratings

Muscle soreness ratings will be obtained immediately prior to exercise, and 4 h nd 7.5 h post-exercise. Subjects will be asked to subjectively rate muscle soreness levels on a 100 mm visual analog scale, upon which 0 mm represents no muscle soreness and 100 mm represents impaired movement due to muscle soreness.

Hydration Status

Hydration status will be assessed via changes in body weight. Body weight will be measured immediately after consuming the first recovery beverage, 2 hours after consuming the 1st recovery beverage (before consuming the second beverage), and 4 hours after consuming the 1st recovery beverage. No food/fluids will be consumed during the recovery period, other than the treatment beverages. The weight of the 2nd recovery beverage will be subtracted from the final weight measurement, such that changes in body weight reflect fluid loss during the recovery period (i.e. smaller losses in weight reflect more fluid retention).

Diet/Exercise Controls

Subjects will be instructed to complete no heavy exercise in the day before each trial and to maintain consistent, regular exercise and dietary habits in the 48 hours prior to each trial day. Diet and exercise logs will be completed for the day prior to trials. Subjects will consume their last self-selected meal at least 12 hours before the start of EX1, consume a standardized snack at 8:00 pm the night before the trials, and consume a standardized morning snack 30 minutes prior to EX1. The night snack will be composed of a 500 mL bottle of water and a Clif bar (44 g CHO, 9 g PRO, 5 g fat). The morning snack will be composed of a 354 mL bottle of Gatorade (21 g CHO) and either two chocolate chip granola bars (18 g CHO, 1 g protein, 2 g fat) or one chocolate chip granola bar and one standard banana (27 g CHO, 1.3 g PRO).

Statistics

Means and standard deviations will be reported for each variable at each time-point. Changes in dependent measurements over time and between-treatments will be assessed using magnitude-based inferences, using methods described by Hopkins (17). All data will be log transformed to diminish the effects of non-uniformity. The smallest meaningful effect will be quantified as 0.2*SD for each variable. Publicly published spreadsheets will be utilized to determine the % likelihoods of treatment-effects (18). In addition, semantic descriptions of treatment effects will be 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% =most likely.

Abstract

PURPOSE: Muscle soreness levels and hydration status are important indicators of recovery from high intensity endurance exercise, and can be influenced by post-exercise nutrient intake. Chocolate milk has been reported to be an effective recovery beverage in adults, as it contains carbohydrate, protein, and water/electrolytes at levels that are appropriate for refueling, muscle repair, and rehydration. However, few studies have tested the efficacy of chocolate milk for post-exercise recovery in adolescents. **METHODS:** Eight competitive teenage cyclists (5 male, 3 female; 16.1 ± 1.1 y; 174.0 ± 10.7 cm; 138.4 ± 5.6 lbs; 61.8 ± 8.2 ml/kg/min) completed three experimental trials, in randomly counterbalanced order. Each trial consisted of a 90 min session of high-intensity cycling intervals, followed by the intake of two doses of a post exercise nutritional beverage. A different treatment beverage was consumed in each trial. Beverages were an artificially-flavored water placebo beverage (PL), a carbohydrate-electrolyte beverage (CHO), and chocolate milk (CM). Beverages were provided in double-blind fashion, and matched for fluid volume, and carbohydrate content (other than PL). Muscle soreness ratings were assessed (via a 100 mm visual analog scale) pre-exercise and 4 and 8 h post-exercise. Hydration status was measured via changes in body weight, assessed immediately after consuming the first beverage, and prior to consuming the second beverage (2 h later). Means and standard deviations were reported for each variable at each time-point. Changes in dependent measurements over time and between-treatments were assessed using magnitude-based inferences. **RESULTS:** Muscle soreness was generally increased 4 h post-exercise, and remained elevated 8 h post-exercise. Changes in soreness from pre-exercise to 4 h post-exercise were "likely" attenuated with CM versus CHO (89% likelihood of positive effect) and for CM versus PL (91% likelihood). Changes in soreness from pre-exercise to 8 h post-exercise were "likely" reduced with CM versus PL (87% likelihood), and also "likely" decreased for CHO versus PL (88% likelihood). Body weight "likely" decreased in the 2 h after beverage consumption, with no clear between-treatment effects. **CONCLUSIONS:** CM produced positive effects on muscle soreness in youth cyclists, in comparison to PL and CHO treatments. This supports prior findings in adults that CM is effective for post-exercise recovery. CM had similar effects on fluid retention (measured via changes in weight) versus PL and CHO beverages. Future studies of the effects of CM on post-exercise rehydration in adolescents should be conducted in which greater fluid losses from exercise are part of the methodology.

Introduction

Heavy endurance exercise utilizes a variety of energetic substrates. Endogenous carbohydrate reserves (such as liver and muscle glycogen), are the primary fuel in endurance exercise lasting less than two hours, and can be heavily depleted during endurance exercise (33). Muscle glycogen depletion is associated with fatigue during prolonged exercise (1,11,15,16,22). Exercise induced muscle damage (EIMD) and sarcolemmal disruption also occur with moderate to high intensity exercise. EIMD increases perceived soreness, decreases muscular contractile ability, and can impair performance in subsequent exercise (23,38). In addition, fluid losses during endurance training can lead to exercise-induced dehydration due to perspiration and environmental factors, and can significantly impair performance (13).

Appropriate post-exercise nutrition can promote effective recovery from heavy endurance exercise, and enhanced performance. Nutritional strategies to promote recovery emphasize three integrated approaches: refueling, repair, and rehydration (33). In recent years, chocolate milk (CM) has received considerable attention as a recovery beverage, as the carbohydrate, protein, and water/electrolyte content of CM appear to align effectively with these approaches. Post-exercise carbohydrate intake allows for effective refueling through muscle glycogen resynthesis. Some (2), but not all (24), studies show that the co-ingestion of carbohydrate and protein (CHO+PRO) increases rates of glycogen replenishment versus carbohydrate alone. CM in particular contains levels of carbohydrate and protein that are sufficient to promote effective rates of glycogen replenishment (2).

Protein ingestion can also influence protein turnover rates during recovery, which may be important for muscle repair (27,41). Post-exercise CHO+PRO ingestion, including CM, elicits higher rates of protein synthesis in skeletal muscle and total body protein balance than carbohydrate alone (8,19,30,32). In addition, CM intake may reduce markers of EIMD, including post-exercise muscle soreness (35,37,38), and sarcolemmal disruption (4,36). Furthermore, the fluid, electrolyte and protein content of CM appears to promote enhanced rehydration/fluid retention in comparison to sports drinks or water (43,50,51,52).

As CM is a commonly consumed, whole food, there are ethical advantages to its promotion as a recovery beverage in young athletes, as compared to nutritional supplements (7). Additionally, the relatively low cost, accessibility, and palatability of CM make it a strong candidate for post-exercise nutrition in adolescents. Numerous studies, as discussed above, have analyzed the effects of CM on recovery in adults. However, there is very little information regarding the effects of CM for post-exercise nutrition in adolescent athletes. This limitation is likely due to practical challenges of recruiting sufficiently large groups of competitive adolescent athletes. Adolescent athletes do not have the same metabolic demands as adults, and there is a need for evidence-based guidelines for this population that are not solely scaled down versions of guidelines designed for adults (7). Therefore, the purpose of the present study is to analyze the effects of CM compared to CHO and PL on muscle soreness and hydration status in adolescent athletes.

Conducted Methods

Overview

This studied tested the effects of altered post-exercise nutrient intake on recovery from exercise in youth cyclists. Data collection occurred from September 2018 through January 2019. Following a session of high-intensity cycling exercise, one of three different post-exercise recovery beverages (with varying macronutrient levels) was administered, and muscle soreness ratings and hydration status were assessed thereafter.

Each subject completed the exercise and nutrition protocols described below on three different occasions, and received a different nutritional treatment during each occasion. Nutritional treatments were provided using a randomly counterbalanced design, with the treatments double-blinded. There was approximately one week between each trial.

Subjects

Ten subjects were recruited to complete the study. Eight subjects (5 males and 3 females, aged 14-18) completed all testing: 6 MSA athletes, and 2 non-MSA athletes from the Harrisonburg area. Parental consent and youth assent were obtained prior to initiation of the study. All subjects met inclusion criteria which included completion of $\geq$ 7 hr/week of cycling training for the preceding 2 months, and a VO_{2peak} of $\geq$ 50 mL/kg/min. All testing procedures were approved by the Institutional Review Board of JMU.

Experimental Protocols

Pre-test measurements

Prior to experimental trials, all subjects completed a graded exercise test on a cycle ergometer (VeloTron, Racermate, Inc,) to determine their VO_{2peak} and W_{max}. Subjects began cycling at a moderate workload, workload was increased by 25W every two min until volitional fatigue. VO_{2peak} and W_{max} were obtained from the highest workload achieved, and were used to calculate workloads for each athlete in the experimental trials (i.e. workloads were based on % W_{max} for each subject).

High-Intensity Cycling Exercise

During each experimental trial, participants completed a bout of cycling exercise lasting approximately 90 min. The first portion was 25 min of constant load exercise: five min at 40% W_{max} and 20 min at 60% W_{max}. There was then a 60 min period of high-intensity intervals (described below), and a five min cooldown period at 40% $W_{max.}$ 250 mL of water was provided every 15 min and consumed as desired by the subject. An interval workout was selected for the exercise bout, as it has been shown to significantly deplete muscle glycogen (20) and similar protocols have been used in similar studies focused on adults (26,49).

The intervals consisted of two min at a high workload, followed by two min at 50% $W_{max.}$ The high workload was 90% W_{max} for as long as could be sustained, which was then lowered by 10% W_{max} as needed. Workload was lowered when the subject could not sustain a cadence of at least 60 rpm. The interval training period ended when the subject could not continue with a cadence of 60 rpm at 70% W_{max} during the high intensity intervals, or when 15 high intensity intervals were completed.

Nutritional Treatments

Subjects were administered one treatment beverage on each trial day. Beverages were provided immediately after exercise, and two hours following exercise.

Treatments:

Chocolate Milk (CM): 11.8 mL/kgBW of low-fat chocolate milk, containing approximately 1.2 g CHO, 0.4 g Pro, 0.11 g fat, 9 mg sodium, and 21 mg potassium per kgBW)

Carbohydrate-electrolyte beverage (CHO): 1.72 g/kgBW of chocolate flavored Clif Shot gels, containing approximately 1.2 g CHO, 0 g Pro, 0.08 g fat, 3.1 mg sodium, and 4 mg potassium per kgBW) mixed with water to provide 11.8 mL/kgBW of beverage

Placebo (PL): 11.8 mL/kgBW of an artificially-flavored water beverage

In addition to treatment beverages, subjects consumed a self-selected, standardized lunch at 12:15 pm (~ 4.5 hr post-exercise) that was typical of their regular, daily meal. The constituents of this meal were recorded for each subject during their first trial, and replicated in subsequent trials.

Dependent Measurements

Muscle Soreness Ratings

Muscle soreness ratings were obtained immediately prior to exercise, and 4 h and 7.5 h post-exercise. Subjects were asked to subjectively rate muscle soreness on a 100 mm visual analog scale, upon which 0 mm represented no muscle soreness and 100 mm represented impaired movement due to muscle soreness.

Hydration Status

Hydration status was assessed via changes in body weight. Body weight was measured immediately after consuming the first recovery beverage, 2 hours after consuming the 1st recovery beverage (before consuming the second beverage), and 4 hours after consuming the 1st recovery beverage. No foods/fluids were consumed during the recovery period, other than the treatment beverages. The weight of the 2nd recovery beverage was subtracted from the final weight measurement, such that changes in body weight reflected fluid loss during the recovery period (i.e. smaller losses in weight reflect more fluid retention).

Diet/Exercise Controls

Subjects were instructed to complete no heavy exercise in the day before each trial and to maintain consistent, regular exercise and dietary habits in the 48 h prior to each trial day. Diet and exercise logs were completed for the day prior to trials. Subjects consumed their last self-selected meal at least 12 h before the start of exercise, consumed a standardized snack at 8:00 pm the night before the trials, and consumed a standardized morning snack 20 min prior to exercise. The night snack was composed of a 500 mL bottle of water and a Clif bar (44 g CHO, 9 g PRO, 5 g fat). The morning snack was composed of a 354 mL bottle of Gatorade (21 g CHO) and either two chocolate chip granola bars (18 g CHO, 1 g PRO, 2 g fat) or one chocolate chip granola bar and a banana (27 g CHO, 1.3 g PRO).

Statistics

Means and standard deviations were reported for each variable at each time-point. Changes in dependent measurements over time and between-treatments were assessed using magnitude-based inferences, using methods described by Hopkins (17). All data was log transformed to diminish the effects of non-uniformity. The threshold for the smallest meaningful effect was quantified as 0.2*SD for muscle soreness (approximately 5 mm) and as 0.016*SD for body weight (equivalent to body weight changes associated with 100 mL of fluid loss). Publicly published spreadsheets were utilized to determine the % likelihoods of treatment effects (18). In addition, semantic descriptions of treatment effects 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% =most likely.

Body weight measurements obtained at 4 h post-exercise were omitted from data analysis, because increases in body weight were reported in some subjects. This outcome is not possible if no other fluid was consumed during this time period, indicating either measurement error or a lack of adherence to the instruction to abstain from fluid ingestion during this period. As such, body weight data from the 4 h post-exercise time were omitted from further analysis.

Results

Demographic Data

Demographic data for the subjects are shown in table 1. Values are reported for all eight subjects, and for male and female subjects separately.

Table 1. Demographic variables for subjects (mean ± SD).

	All (8)	**Males (5)**	**Females (3)**
Age (years)	16.1 ± 1.1	16.4 ± 1.1	15.7 ± 1.2
Height (cm)	174.0 ± 10.7	180.4 ± 5.3	163.3 ± 8.5
Weight (lb)	138.4 ± 5.6	130.7 ± 14.5	143.0 ± 9.2
Absolute VO_2Max (mL/min)	3900 ± 682	4353 ± 319	3146 ± 244
Relative VO_2Max (mL/kg/min)	61.8 ± 8.2	66.9 ± 5.1	53.3 ± 2.9

Dependent Measures

Muscle Soreness

Muscle soreness generally increased in the hours after the exercise bout, as reported in Table 2. The within-treatment differences show that there was a "very likely" increase in soreness ratings 4 h and 8 h after exercise in the PL trial, and there was a "likely" increase in soreness 4 h after exercise in the CHO trial. No clear changes in soreness were observed post-exercise in the CM trial.

Figures 1A and 1B present the differences in muscle soreness between-treatments. 1A shows treatment differences in the changes in soreness from pre-exercise to 4 h post-exercise, and Figure 1B depicts treatment differences in the changes in soreness from pre-exercise to 8 h post-exercise. Changes in muscle soreness from 4 h post to 8 h post are not shown, as there were no clear within-treatment or between-treatment effects between these timepoints.

Table 2. Changes in muscle soreness ratings from pre-exercise to post-exercise.

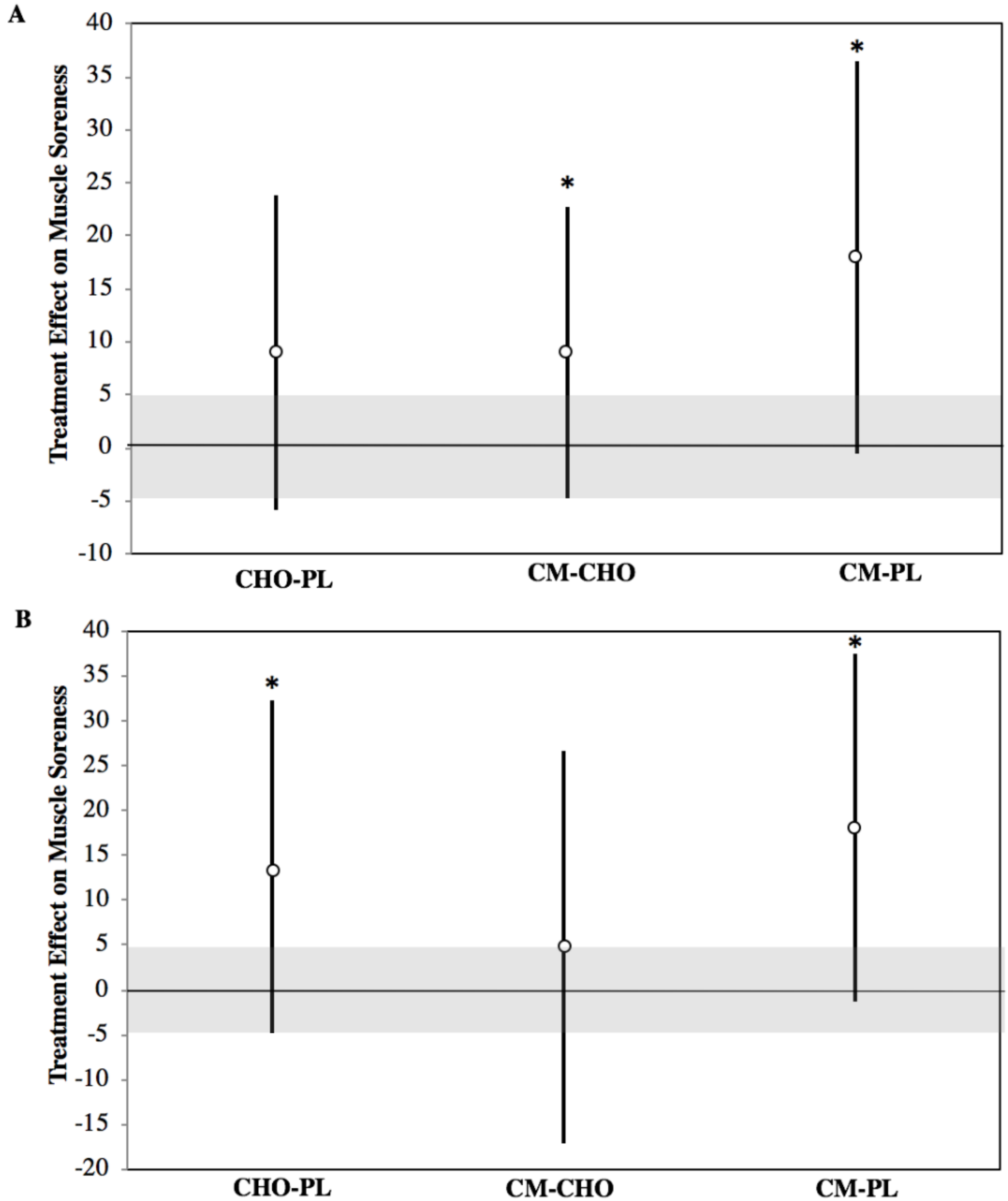
		Mean ± SD			**Within-Treatment Differences Mean, ± 90%CI (raw) % Likelihoods (+/triv/-), Inference**		
Variable	**Treat**	**Pre**	**4 h Post**	**8 h Post**	**Pre-4 h Post**	**Pre-8 h Post**	**4h-8 h Post**
Soreness	**PL**	44.1 ±23.1	67.4 ±22.2	68.3 ±19.6	23.3±15.3 96/3/1 Very Likely Positive	24.1±14.5 97/2/1 Very Likely Positive	0.9±3.2 11/88/1 Likely Trivial
	CHO	37.4 ±25.7	51.6 ±27.6	48.1 ±24.4	14.3±11.5 94/6/1 Likely Positive	10.8±16.4 48/39/13 Unclear	-3.5±11.1 38/29/33 Unclear
	CM	41.0 ±15.6	46.3 ±23.0	47.0 ±27.4	5.3±10.2 25/20/55 Unclear	6.0±12.9 55/26/19 Unclear	0.8±5.5 16/52/33 Unclear

Ratings scored on a 100 mm analog scale. Threshold for 'meaningful change' = 0.2*SD

Body Weight

Changes in body weight following beverage ingestion (reflecting fluid losses) are displayed in Table 3. Overall, body weights were "likely" decreased 2 h after beverage ingestion, in all trials. No clear treatment differences were observed.

Figure 1. Treatment differences in muscle soreness changes from pre-exercise to 4 h post-exercise (A), and from pre-exercise to 8 h post-exercise (B)



*Indicates a “likely” positive effect on muscle soreness

Table 3. Body weight changes following beverage consumption, and treatment effects.

		Mean ± SD		Within-Treatment Differences Mean, ± 90%CI (raw) % Likelihoods (+/triv/-), Inference	Between-Treatment Differences Mean, ± 90%CI (raw) % Likelihoods (+/triv/-), Inference		
Variable	**Treat**	**Post Bev 1**	**Pre Bev 2**	**Post Bev 1-Pre Bev 2**	**Experimental Treat Post Bev1-PreBev2**	**PL**	**CHO**
Body Weight (lb)	**PL**	139.7 ±17.4	139.1 ±17.8	-0.7±0.6 2/8/90 Likely Negative	**PL**		
	CHO	139.7 ±14.0	139.0 ±4.6	-0.7±0.5 1/6/93 Likely Negative	**CHO**	0.0±0.5 13/66/21 Unclear	
	CM	140.4 ±13.6	139.8 ±14.2	-0.6±0.7 2/11/87 Likely Negative	**CM**	0.2±0.7 39/44/16 Unclear	0.1±0.8 36/47/17 Unclear

Threshold for ‘meaningful change’ in weight = 0.16% (~100 ml fluid loss). Change scores may not reflect raw means in all cases, due to missing data for a single treatment trial.

Discussion

Several recent studies have examined the effects of CM as a post-exercise recovery aid (8,26,30,32,40,42,48,53). Although the results of these studies have been largely positive, the vast majority of studies have been limited to adult populations. The present investigation aimed to examine the effects of post-exercise CM ingestion on muscle soreness ratings and hydration status in adolescent cyclists. The primary findings of this study were that: a) CM ingestion "likely" attenuated changes in muscle soreness four hours after exercise compared to CHO and PL, as well as reducing changes in soreness eight hours after exercise versus PL, and b) CM had no clear effect on hydration status compared to CHO and PL.

Muscle Soreness

As shown in table 2, muscle soreness ratings generally increased with time after exercise. This aligns with data in multiple other studies, as muscle soreness generally increases in the 24 hours after high-intensity exercise (4,39,42). However, the present investigation analyzed muscle soreness in the 8 h after exercise, whereas most previous studies have primarily analyzed delayed onset muscle soreness (DOMS) in the first 48-72 hours (4,39). The short-term post-exercise soreness measured in the present study is a somewhat different phenomenon than DOMS; DOMS often occurs after heavy or novel exercise and peaks in the 24-48 h post exercise, whereas the short-term changes in muscle soreness following cycling in trained individuals is likely a more transient event. A 2012 paper by Goh et al. analyzed short-term post exercise muscle soreness in the four hours between high intensity endurance exercise bouts utilized the same analog scale to determine muscle soreness and provided a variety of treatments after the first exercise bout (EX1). Four hours after EX1, soreness was significantly increased regardless of treatment, which aligns with the increases in soreness found in the present study (10).

As depicted in Figure 1A-B, CM was found to have a “likely positive” effect on muscle soreness compared to CHO and PL in the first four hours, and compared to PL in the first 8 hours after exercise. This supports the hypothesis and aligns with previous findings, as CHO+PRO feedings (with macronutrient combinations similar to CM) have been found to attenuate muscle soreness (10,39,42). For example, Romano-Ely et al (2006) reported that muscle soreness ratings 24 h after heavy cycling exercise were attenuated by over 50% when subjects received carbohydrate-protein-antioxidant beverages, as opposed to a carbohydrate only beverage (39). It seems plausible that the beneficial effects of CM are related to its’ protein content, as no significant changes have been seen in muscle soreness ratings between CHO+PRO and CM treatments in other studies (42).

The causes of muscle soreness are multifactorial; it is usually linked to EIMD, oxidative stress, and sarcolemmal disruption (23,36,38,42). As the present study did not analyze markers of muscle disruption or function, it cannot be specifically determined what caused the changes in muscle soreness with CM. However, reduced muscle soreness is generally indicative of reduced muscle damage (23,38). Other studies have hypothesized that CHO+PRO similar to CM improves subsequent muscular function and is linked to decreased levels of biomarkers for muscular damage (42).

Contradictory to the present study, Cockburn et al (2012) observed a different time-course for the effects of milk/protein on muscle soreness. These investigators found milk ingestion to have unclear effects on muscle soreness in the first 24 hours after “muscle damaging exercise” (unilateral eccentric-concentric knee flexions). However, milk intake did result in reduced muscle soreness (versus a placebo) 48 h after exercise. Additionally, the aforementioned study found a “very likely decrease” in creatine kinase (CK; a marker of muscle damage) with milk intake versus placebo, suggesting that milk may attenuate muscle damage (4). The variations in these findings could be due to a variety of factors, including potential differences in the type/amount of muscle damage resulting from resistance versus endurance exercise. Further studies comparing CM/milk in both endurance and resistance exercise recovery would allow for more clarity regarding the effects of milk on muscle soreness, and the time-course of these changes after exercise.

Hydration Status

As depicted in Table 3, body weight generally decreased in the two hour period after beverage ingestion. This was expected, as subjects were instructed not to consume any other fluids or foods during this time, and thus weight loss reflected normal fluid losses from urination (and small amounts from perspiration). Due to the electrolyte and macronutrient composition of CM, it was predicted that CM would be more effective at supporting fluid retention than CHO and PL (42,43). However, no clear differences in body weight changes between treatments were observed. For between treatment differences, the 'trivial zone' of change was set to represent a 100 mL change in fluid (.22 lbs). This was decided as it is a meaningful amount of fluid and could a change of this magnitude could be detected on the utilized scale (which measured with a precision of 0.1 lb).

The findings of the current study contradict the results of several other studies. A 2008 study by Watson et al found that in the first two hours after exercise, urine output was approximately 100 mL (0.2 lbs) less for milk versus a carbohydrate-electrolyte treatment (52). Similarly, other studies conducted in adults (43) and children (50) have reported that post-exercise consumption of skim milk was more effective at rehydration/fluid retention than water and carbohydrate-electrolyte treatments. However, each of these studies were conducted in hot environments (34 - 35 °C) without fluid intake during exercise, in order to elicit meaningful fluid losses post-exercise (which equaled 1.5 – 2.0% body weight). By contrast, the present study was not designed to purposefully cause dehydration, as exercise was conducted at an average temperature of 22.1°C and subjects drank water ad libitum during exercise. These variations in methodology likely explain the differences in results.

Measurement error may have also influenced our body weight results. As explained in the methods section, body weight data was obtained at four hours post-exercise but not included in data analysis due to increased body weight values in some subjects at this timepoint. This outcome should not have been possible, as subjects were instructed not intake any outside food or beverages between measurements periods. Thus, this outcome suggests that there were issues with subject compliance to these instructions, or potentially or limitations with the accuracy of the scale utilized for the study.

Conclusion

In summary, post-exercise consumption of CM elicited “likely positive” effects on muscle soreness ratings in comparison to CHO and PL after exercise. As such, CM appears to be a useful beverage for post-endurance exercise nutrition in adolescents. Although CM was found to have unclear effects versus CHO and PL on rehydration, this outcome may have been related to the methodological approach taken in this study. Further investigation to determine the effects of CM versus other recovery treatments in adolescents, and in other exercise modalities/environments should be considered in the future.

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